

VERSION – 01

***REPORT FOR HUMAN CLINICAL TRIAL PHASE III
TO STUDY THE SAFETY AND EFFECTIVENESS
OF OXY-POWDER®***

DOCUMENT NO.: MCERC/REPT-CLTR/OXY/1205/001



May 30th 2007

**TRIAL TO STUDY THE SAFETY AND EFFECTIVENESS
OF OXY-POWDER® IN TREATING CONSTIPATION AND IBS.**

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Contacts: Studies on Safety and Effectiveness of Oxy-Powder® in Treating Constipation

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Study Identifier: Mfair/oxy/2005-06
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Abstract

"The clinical trial has been conducted following Globally accepted Regulatory Guidelines and practices as follows":

- Human clinical trial was conducted following "Good Clinical Practices (GCP)" under supervision of an expert team following Helsinki's Declaration for protection of rights of the patients. The entire study was conducted based on globally accepted ICH 6E guidelines for determining Safety, Efficacy and Tolerability of Oxy-Powder® in Human subjects.
- The Animal toxicity studies were conducted in an ISO certified laboratory following WHO GCP guidelines and as per OECD tests. The tests were performed as per OECD guidelines enumerated below:
 - (a) 5 DAYS ACUTE ORAL TOXICITY TEST-OECD Guidelines Section 4, Test 420, 17-12 2001
 - (b) Repeated Dose 28 days Oral Toxicity Study in Rodents-Guidelines Section 4, Test 408, 21-09-1998
- *In vitro* antimicrobial tests indicated that Probiotic Lactobacillus bulgaricus was not affected in presence of Oxy-Powder® indicating thereby that Production of B Complex factors produced by this organism in gastrointestinal tract of patients suffering from constipation and IBS with related constipation shall continue to be produced in the normal course. The patient on the Oxy-Powder® treatment shall not suffer from B Complex Deficiency attributed to lethal effect of Oxy-Powder®.
- The capsules can be safely consumed by all vegetarians and non-vegetarians alike. The ingredients, including the outer inactive cover (Kosher Shell) are Vegetarian. None of the

non-vegetarian materials such as gelatin, egg product, meat or chicken products, sea food products, non-vegetarian oils and fats have been employed in the formulation or during processing.

Constipation is the slow movement of feces (stool or body wastes) through the large intestine resulting in infrequent bowel movements and the passage of dry, hard stools. The longer it takes for the stool to move through the large intestine, the more fluid is absorbed and the drier and harder the stool becomes. Constipation is annoying and uncomfortable, but fecal impaction (a collection of dry, hard stool in the colon or rectum) can be life threatening. Patients with a fecal impaction may not have gastrointestinal symptoms. Instead, they may have circulation, heart, or breathing problems. If fecal impaction is not recognized, the signs and symptoms will get worse and the patient could die. Irritable bowel syndrome (IBS), a functional gastrointestinal disorder characterized by the interplay of altered motility, abnormal visceral sensation, and psychosocial factors, is one of the most common reasons for referral to a gastroenterologist. It is associated with bouts of constipation and diarrhoea.

Multicentric Clinical Trial

‘Oxy-Powder®’ used universally as a dietary supplement for relieving constipation was taken up for the study under Clinical Trials Phase III in 40 constipation and 20 IBS patients in Open, Randomized, Comparative studies in 2 Centres. The Study protocol included exclusion and inclusion criteria, mode of administration of test and reference products, evaluation of effectiveness and safety and data analysis and management. The GCP guidelines were followed for the conduct of the studies. Reporting of AEs and SAEs has been emphasized. The duration of the study, post administration was 6 weeks. Primary objective of the clinical trial was to evaluate effectiveness and safety of Oxy-Powder® in treating constipation and IBS. The final results indicated that complete cure was obtained in 42.3% patients treated with Oxy-Powder® (as against 7.7% with Dulcolax), Improvement in 57.7% in patients treated with Oxy-Powder® (as against 76.9 % with Dulcolax), and 0% failure in patients treated with Oxy-Powder® (as against 15.4 % with Dulcolax), Thus, efficacy of Oxy-Powder® in treating constipation was significantly ($P<.05$) more than Dulcolax. This indicated that Oxy-Powder® was more efficacious in treating constipation than Dulcolax. As regards ADR’s in the Constipation group of patients, one patient in Oxy-Powder® administered group of 27 patients had severe diarrhoea on the 2nd day of treatment. He could not carry out his usual activities and felt dehydrated; hence he was withdrawn from the study

on 3rd day. Out of the remaining 26 patients, 2 patients had abdominal fullness after taking Oxy-Powder® for 2-3 days after which they were symptom- free. Remaining 24 patients had no ADR's. In the 13 Dulcolax administered group patients, 1 patient had mild abdominal pain which disappeared without medication. Remaining 12 patients had no ADR's. As regards ADR's in IBS + Constipation group of patients, none of the patients had any adverse events during the study period. The patients tolerated Oxy-Powder® quite well.

In Vitro (in a test tube) Microbiological Studies

Before commencing the clinical trials, the *in-vitro* effect of Oxy-Powder® on the normal Probiotic strains and colonies of microbes in the digestive tract was evaluated. The results showed that the Oxy-Powder® capsules with citric acid samples (for acidification required for liberation of nascent oxygen) were ineffective in killing the tests cultures *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus bifidigus*, *Enterobacter faecalis* and *Candida albicans* at the tested concentration indicating thereby that **the normal healthy flora of GI tract is not expected to be disturbed with the administration of Oxy-Powder®.**

Animal Toxicity Studies

Non-clinical safety studies involving acute and sub-chronic toxicity in rats (including histopathology) were also conducted. The acute toxicity profile suggested that **Oxy-Powder® was safe at 5000 mg/kg body weight dose level.** The Sub-chronic oral toxicity study was conducted to determine the toxicity profile of Oxy-Powder® when administered via oral route daily at the dose level of 1000 mg/kg body weight for male and female animals for 28 days in Sprague-Dawley rats.

Salient results of the study were as follows:

- 1) All the male and female animals from control and all the treated dose groups up to 1000 mg/kg survived throughout the dosing period of 28 days and the recovery period of 14 days.
- 2) No signs of intoxication were observed in male and female animals from different dose groups during the dosing period of 28 days and during the recovery period of 14 days.
- 3) Male and female animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days and the recovery period of 14 days.
- 4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days and the recovery period of 14 days.

- 5) Ophthalmoscopic examination, conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality.
- 6) Haematological analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment.
- 7) Biochemical analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment.
- 8) Functional battery observation tests conducted at termination revealed no abnormalities.
- 9) Urine analysis, conducted at the end of the dosing period in week 4 and at the end of recovery period in week 6, revealed no abnormality attributable to the treatment.
- 10) Organ weight data of male and female sacrificed at the end of the dosing period and at the end of the recovery period was found to be comparable with that of respective controls.
- 11) Gross pathological examination did not reveal any abnormality.
- 12) Histopathological examination did not reveal any abnormality.

Based on these findings, the “No Observed Effect Level (NOEL)” of Oxy-Powder® in Sprague Dawley rats via oral route, over a period of 28 days was found to be 1000 mg/kg body weight for male and female animals.

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Synopsis

Individual Study Table Referring to Part of the Dossier

Volume

Name of Sponsor Company Dr. Edward Group's
Global Healing Center, Inc.

Name of Finished Product Oxy-Powder®

Name of Active Ingredient 1. Ozonated Magnesium Oxides with Germanium-132 in
Oxy-Powder®
2. **Bisacodyl in Dulcolax**

(For National Authority Use only)

TITLE OF THE STUDY:

Studies on Efficacy & Safety of Oxy-Powder®

INVESTIGATORS:

CLINICAL:

1. Dr. Sharad Shah
2. Dr. Chetan Bhatt assisted By Dr. Aditi
3. Dr. Meena U. Shah
4. Dr. Deven Parmar

TOXICOLOGY:

1. Dr. Ranjit Bhide

MICROBIOLOGY:

1. Dr. Deepa Bhajekar

PUBLICATION (Reference): Proposed in *Journal of Alternative Medicine*

STUDIED PERIOD (Years)			
Clinical (date of first enrollment)	21-6-2006		
Clinical (date of last completed)	07-4-2007		
Non-Clinical	Commencement	Completion	Report Date
Toxicology	10-5-2006	22-6-2006	12-7-2006
Microbiology	22-6- 2006	01-6-2006	03-6-2006

STUDY OBJECTIVES:

Primary: The effectiveness and safety of Oxy-Powder® in treating constipation and IBS

Secondary: The *in-vitro* effect of Oxy-Powder® on the normal Probiotic strain and colonies in the digestive tract. Safety studies involving acute and sub-chronic toxicity in rats/ mice (including histopathology)

Study Center(s):

OVERALL: MAYFAIR CLINICAL EDUCATION & RESEARCH CENTRE, THANE.

Mayfair Clinical Education & Research Centre,

Mohan Mill Compound, opp. Lawkim, Ghodbunder Road, Thane-400 607, India.

Ph. No.: 91-22-2589 5856, Fax.: 91-22-2589 5854

1) NON-CLINICAL DATA

(a) Indian Institute of Toxicology, (FOR TOXICITY STUDIES)

‘KIM’ 2057, Sadashiv Peth, Vijaynagar Colony, PUNE-411 030

Tel: 91-020-24333185/ 26819961-62. Telefax: 91-020-26871429

E-mail : iit@pn2.vsnl.net.in

Contact: Prof. J. K. Lalla, PhD.

Contact: Dr. Ranjit Bhide

(b) MicroChem Laboratory, (FOR ANTI-MICROBIAL TESTING)

125, Vardhman Ind. Estate, Gokul Nagar, Thane (W) – 400 601.

Tel: 91-22-55772657. Telefax: 91-22-25304376/25390631.

E-mail : microchem@vsnl.net

Contact: Dr. Deepa Bhajekar

2) CLINICAL DATA

(c) Dr. Sharad C. Shah’s Clinic,

49, Hughes Road, Mumbai – 400 007.

Tel: 91-22-23632809.

Contact: Dr. Sharad C. Shah

(d) Bhatia General Hospital

J. Dadaji Road, Grant Road, Tardeo, Mumbai – 400 007.

Tel : 91-22-23071291-92

Contact: Dr. Chetan B. Bhatt. : 3071291, 3071292

Diagnosis and main criteria for inclusion

Diagnosis :

Patients suffering from constipation alone or constipation with IBS

❖ Main Criteria for inclusion :

1. 18 to 60 years of both sexes (60% females + 40% males)
2. Height & Weight conforming to Height/Weight chart of Life Insurance Corporation of India
3. Food habits: Vegetarians are eligible but Non-vegetarians preferred
4. Satisfying the Rome II Criteria for Constipation and IBS

Test product, dose and mode of administration

(A)Test product :Oxy-Powder® capsules, each containing 715.5 mg active ingredients

Dose and mode of administration :

For Cleansing: 4 capsules in the evening on an empty stomach with 240 mL water. If 3-5 bowel movements were not achieved the following day, the dosage was increased by adding two (2) capsules every night until 3-5 bowel movements were achieved the following day. Finalization of the dosage was considered as” day one (1)” of the seven (7) day cleanses. This dosage was continued for seven (7) consecutive days. After the seven (7) day cleanse, the maintenance dose was started.

Maintenance Dose: 4 capsules on alternate days for the next 5 weeks.

Batch number: 112809 **Mfr. Date:** Not Available **Expiry date:** Not Available

Duration of treatment: 6 weeks

(B) Reference therapy, dose and mode of administration

Reference therapy, Bisacodyl tablets (Dulcolax tablets of Cadila Health Care Ltd.)

Each tablet containing 5 mg drug was dispensed in a hard gelatin capsule, size ‘0’

Dose and mode of administration: 2 capsules in the evening on an empty stomach with 240 mL water. Treatment was followed as described for Oxy-Powder® capsules

Batch number G 1136 **Mfr. Date:** Jan.2006 **Expiry date:** Dec. 2009

Dose and mode of administration: 2 capsules in the evening on an empty stomach with 240 mL

water. Treatment was followed as described for Oxy-Powder® capsules

Criteria for evaluation:

Efficacy in patients :

Findings of i) Stool examination ii) Barium meal and iii) Colonoscopy

Relief of constipation and IBS, improvement of Quality of life

Safety: *In-vitro* findings of effectiveness of Oxy-Powder® on, normal Probiotic and pathogenic strains of microbes generally present in digestive tract. Safety studies involving acute and sub-chronic toxicity in rats (including histopathology)

(a) Recording of ADR's / SAEs, in patients, if any

Statistical methods

Student t test, Chi square or Fisher's exact test

Summary & Conclusions for Constipation Study

EFFICACY RESULTS

Type 1 patients

Summary

Efficacy	Drug	
	Oxy-Powder®	Bisacodyl (Dulcolax)
Complete cure	11 (42.3%)	1 (7.7%)
Improvement	15 (57.7%)	10 (76.9%)

Failure	0 (0%)	2 (15.4%)
	26 (100%)	13 (100%)

Conclusions

Efficacy of Oxy-Powder® in treating constipation was significantly ($P < .05$) more than Dulcolax. This indicated that Oxy-Powder® was more efficacious in treating constipation than Dulcolax.

Summary & Conclusions for IBS + Constipation Study

Type 2 patients Summary

Efficacy	Drug	
	Oxy-Powder®	Bisacodyl (Dulcolax)
Complete cure	4 (30.8%)	0 (0%)
Improvement	9 (69.2%)	5 (71.4%)
Failure	0 (0%)	2 (28.6%)
	13 (100%)	7 (100%)

Conclusions

Efficacy of Oxy-Powder® in treating IBS with constipation was significantly ($P < .05$) more than Dulcolax. This indicated that Oxy-Powder® was more efficacious in treating IBS with constipation than Dulcolax.

Safety Results - Adverse events

Type 1 patients

One patient in Oxy-Powder® administered group of 27 patients had severe diarrhoea on the 2nd day of treatment. He could not carry out his usual activities and felt dehydrated; hence he was withdrawn from the study on 3rd day. Out of the remaining 26 patients, 2 patients had abdominal fullness after taking Oxy-Powder® for 2-3 days after which they were symptom- free. Remaining 24 patients had no ADR's.

In 13 Dulcolax administered group patients, 1 patient had mild abdominal pain which disappeared without medication. Remaining 12 patients had no ADR's.

Type 2 patients (IBS+Constipation)

None of the patients had any adverse events during the study period.

Date of report: 30th May 2007

Part 1

SUMMARY REPORT ON ANIMAL TOXICITY STUDIES

(A) Acute Toxicity

PROJECT NO.12203

ACUTE ORAL TOXICITY OF OXY-POWDER® IN SPRAGUE DAWLEY RATS

Data Requirements

OECD Guidelines, Section 4, Test No.420, 17th December, 2001.

Study Director: Dr. R. M. Bhide, PhD

Testing Facility: Indian Institute of Toxicology

32/A/1, Hadapsar Industrial Estate,

Pune - 411 013.

India.

Sponsor: Mayfair Clinical Education and Research Centre, Mumbai

ACUTE ORAL TOXICITY OF OXY-POWDER®

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Mohan Mill Compound, Ghodbunder Road, Thane-400 607.

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STATEMENT OF COMPLIANCE

Project No. : 12203

Test Substance : Oxy-Powder®

Study Title : Acute Oral Toxicity of Oxy-Powder® in Sprague Dawley Rat

We hereby attest to the authenticity of the study and guarantee that the data is correct and accurate to the best of our knowledge and that the study was performed by the procedure described in the Indian Institute of Toxicology Standard Operating Procedures for the testing of chemicals. We hereby attest that this study was conducted in compliance with Protocol submitted to and approved by the sponsor. This study was performed in full compliance with the OECD Guideline for the Testing of Chemicals (No. 420, Section 4: Health Effects) "Acute Oral Toxicity - Fixed Dose Method" Adopted on 17th December 2001, Schedule "Y" in Drugs and Cosmetics (Eighth Amendment) Rules 1988, Ministry of Health and Family Welfare, Government of India and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Indian Institute of Toxicology Registration No.15/1999/CPCSEA).

Dr. R. M. Bhide, PhD – signed /- 12-7-2006

Study Director	Signature	Date
Dr. P. R. Tikhe, PhD	signed /-	12-7-2006

Quality Assurance Unit	Signature	Date
Mr. V. M. Bhide, M.B.A	signed /-	12-7-2006_

Director, Administration	Signature	Date
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STATEMENT OF QUALITY ASSURANCE UNIT

Project No. : 12203

Test Substance: Oxy-Powder®

Study Title: Acute Oral Toxicity of Oxy-Powder® in Sprague Dawley Rats

Quality Assurance Unit of the testing facility inspected the conduct of study on the following dates:

22/04/2006, Test substance preparation

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27/04/2006, Clinical observations

Raw data audit - 15/05/2006

Final report audit - 12/07/2006

No inspection led to findings which had to be reported to the management or would have impaired this study in any way.

Dr. P. R. Tikhe, PhD signed /- 12-7-2006

Quality Assurance Unit Signature Date

ARCHIVING

Project No.: 12203

Test Substance: Oxy-Powder®

Study Title: Acute Oral Toxicity of Oxy-Powder® in Sprague Dawley Rats

Indian Institute of Toxicology takes the responsibility of archiving Protocol, Raw data and all the relevant material generated during the study along with a copy of final report for a period of two years.

Mr. V. M. Bhide M.B.A signed/- 12-7-2006

Director, Administration Signature Date

PERSONNEL INVOLVED IN THE STUDY

Study Director: Dr. R. M. Bhide, PhD

Head Administration: Mr. V. M. Bhide M.B.A

Animal Care: Dr. S. D. Bhande T.V.'s

Technician: Mr. D. G. Shirsath B.Sc., Applied Toxicology

Quality Assurance: Dr. P. R. Tikhe, PhD

Audit

SUMMARY AND CONCLUSION

The study now reported was designed to determine the acute oral toxicity profile of **Oxy-Powder®** in Sprague Dawley rats.

The sighting study did not result in any signs of intoxication at the dose level of 2000 mg/kg body weight and the animal survived; therefore, one animal was treated with the higher dose

of 5000 mg/kg body weight. No signs of intoxication were observed in animals treated at the dose level of 5000 mg/kg body weight. Therefore the main study was continued at the dose level of 5000 mg/kg body weight.

The main study did not result in any signs of intoxication at the dose level of 5000 mg/kg body weight. All animals survived through the study period of 14 days.

Gross pathological examination did not reveal any abnormalities.

It was concluded that the acute toxicity study of **Oxy-Powder®** supplied by **Mayfair Clinical Education and Research Centre, Mumbai**, when administered via oral route in Sprague Dawley rats falls into the category 5 criteria of Globally Harmonised System (GHS).

Signed /-

DR. R.M.BHIDE

STUDY DIRECTOR

- 1) The results relate only to the items tested.
- 2) This report shall not be reproduced except in full, without the written approval of the laboratory.

PREFACE

GENERAL

Study Title : Acute Oral Toxicity of Oxy-Powder® in Sprague Dawley Rats

Sponsor : Mayfair Clinical Education and Research Centre, Mumbai

Monitoring Scientist: Dr. J. K. Lalla

Testing Facility : Indian Institute of Toxicology,

32/A/1, Hadapsar Industrial Estate,

Pune - 411 013.

Project No.: 12203

Schedule

Sighting study:

Date of treatment: 20/04/2006 and 22/04/2006

Date of termination 04/05/2006 and 06/05/2006

Main study:

Date of treatment: 24/04/2006

Date of termination 08/05/2006

Date of reporting : 12/07/2006

Guidelines

This study was performed in full compliance with the OECD Guideline for the Testing of Chemicals (No. 420, Section 4: Health Effects) "Acute Oral Toxicity - Fixed Dose Method" Adopted on 17th December 2001.

Schedule "Y" in Drugs and Cosmetics (Eighth Amendment) Rules 1988, Ministry of Health and Family Welfare, Government of India.

OBJECTIVES

The purpose of this study is to assess the Toxicological profile of **Oxy-Powder®** to a single administration via oral route to Sprague Dawley rats. The animals were observed for 14 days or more, depending on the occurrence of toxic symptoms. The results of acute toxicity study were useful for selection of doses for repeated dose toxicity study and may also provide preliminary information on the target organ of toxicity.

MATERIALS AND METHODS

TEST SUBSTANCE

Sponsor: **Mayfair Clinical Education and Research Centre, Mumbai**

Label on Sample: **Oxy-Powder®**

Characteristics of Sample :

Consistency - Solid (Capsule)

Colour - White

Disclaimer: The above physicochemical data of test substance is supplied by the Sponsor. All responsibility with regards to the accuracy and authenticity of this information remains with the Sponsor. The test lab is not responsible for any variations with the batch number supplied.

Preparation of Dose

Sighting study:

Dose : Treatment – Female: 2000 mg/kg body weight
: 5000 mg/kg body weight

Main study:

Dose : Treatment - Female - 5000 mg/kg body weight

Dose volume : 10 mL/kg.

Vehicle: Distilled water

Procedure: The test substance was suspended in distilled water to obtain 200.0 mg/mL and 500.0 mg/mL strength of suspensions. The test substance was administered in the dose volume of 10 mL/kg body weight. The formulation was prepared fresh on the day of dosing.

TEST SYSTEM

Species : Rat

Strain Sprague Dawley

Source : I.I.T. Animal house

Sex : Female

Age : 5 to 8 weeks

No. of animals per dose : **Sighting study:** One and **Main study:** Four

Acclimation: Five days prior to dosing.

Veterinary examination: Before allocation of animals to different doses after the completion of acclimation period.

Identification of animals: By cage number and individual marking on fur.

Diet: Pelleted feed *ad libitum* supplied by Nav Maharashtra ,Chakan Oil Mills Ltd., Pune.

Water: Aqua guard potable water in glass bottles *ad libitum*

Housing & Environment:

Sighting study: One animal per polypropylene cages provided with Bedding of husk.

Main study: Maximum 5 animals per polypropylene cages provided with bedding of husk. The temperature was maintained between 20 & 24 °C and relative humidity between 30 and 70%; 10-15 air changes per hour and 12 hours each of dark and light cycle was maintained.

Rationale for Selection of Sprague Dawley Rat as Test System

One of the recommended rodent species by the regulatory authorities for conducting preclinical toxicity studies among rodents, as it is a sensitive species for expression of toxic responses.

- 1) Rat is recommended rodent species for conducting acute toxicity studies as per OECD guidelines.
- 2) Availability of the historical control data at the facility.

Route of Administration and Reason for Choice

Oral route of administration is the proposed therapeutic route of administration in human being

Justification for Selection of Doses

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Sighting study:

Dose (mg/kg body weight)	No. of animals	Mortality
2000	1	0/1
5000	1	0/1

Results based on the sighting study, following dose was selected for the main study.

Main study:

Dose (mg/kg body weight)	No. of animals
5000	4

Randomization and Numbering of Animals

Eleven healthy female rats, acclimatized to laboratory conditions for 5 days prior to dosing, were used in this study. Animals were randomly assigned to the cages and the individual animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

Preparation of Animals

The rats were deprived of feed for 16 hours before and 3 hours after the administration of the test substance. Water was not withheld during this period.

EXPERIMENTAL PROCEDURE

The test substance, suspended in distilled water was administered by gavage to rats using a ball-tipped intubations needle (18 G) fitted on to a syringe as per SOP on Test Article/Substance (TA/S) administration – Gavage / Intubations, (SOP No.IIT/S-PSC/16.2).

Allocation of animals:

Sighting study:

Species/ strain	Group No.	Animal Nos.	Dose (mg/kg)	Concentration (mg/ml)	Route
		Female			
Rats/Sprague Dawley	I	1	2000	200	Oral
	II	1	5000	500	

Main study:

Species/	Group	Animal Nos.	Dose	Concentration	Route
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strain	No.	Female	(mg/kg)	(mg/ml)	
Rats/Sprague Dawley	I	4	5000	500	Oral

OBSERVATIONS

Clinical Signs of Intoxication

Observations of clinical signs were made at 10 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours and 6 hours after dosing on day 1 and once daily thereafter for 14 days at approximately the same time. Cage side observations included changes in the skin, fur, eyes and mucous membrane. It also included respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavioural pattern. Particular attention was directed to the observation of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma.

Mortality

Animals were observed twice daily for mortality

Body Weight

Individual animal body weight were recorded following the period of fasting on day 0, weekly thereafter and at termination on day 15. Changes in body weights were calculated and recorded.

Gross Pathology

Macroscopic examination was performed on animals found dead and animals sacrificed at the end of the observation period of 14 days.

Histopathology

Necropsy examination did not reveal any gross abnormality hence histopathological examination was not carried out.

RESULTS

Clinical Signs of Intoxication and Mortality (Table No. A, B & App. No. I, II)

Sighting study:

Group I - Animal treated at the dose level of 2000 mg/kg body weight did not result in any signs of intoxication during the study period of 14 days. Animal survived through the study period of 14 days.

Group II - Animal treated at the dose level of 5000 mg/kg body weight did not result in any signs of intoxication during the study period of 14 days. Animal survived through the study period of 14 days.

Main study:

Group I - Animals treated at the dose level of 5000 mg/kg body weight did not result in any signs of intoxication during the study period of 14 days. All animals survived through the study period of 14 days.

Body Weight (Table No. C & App. No. III)

Sighting study:

Group I (2000 mg/kg) - Percent body weight gain after 7 days and 14 days was found to be 14.46% and 32.93% respectively.

Group II (5000 mg/kg) – Percent body weight gain after 7 days and 14 days was found to be 18.77% and 36.19% respectively.

Main study: Group I (5000 mg/kg) - Percent body weight gain after 7 days and 14 days was found to be 15.41% and 30.88% respectively.

Gross Pathology Findings (Table No. D & App. IV)

Macroscopic examination of animals sacrificed at termination revealed no abnormalities.

Table No. A

Summary of Mortality Record

Under the conditions of the present study, the following mortality rates were recorded:

Sighting study:

Group No.	Dose (mg/kg body weight)	Mortality	
		Absolute	Relative %
I	2000	0/1	0
II	5000	0/1	0

Main study

Group No.	Dose (mg/kg body weight)	Mortality	
		Absolute	Relative %
I	5000	0/4	0

Table No. B

Summary of Clinical Signs of Intoxication

Sighting study:

Group No.	Dose mg/kg	Observed Signs	Total Number of Animals	Animal Nos.	Period of signs in days From - to
I	2000	Nil	1	1	1 - 14
II	5000	Nil	1	1	1 - 14

Main study:

Group No.	Dose mg/kg	Observed Signs	Total Number of Animals	Animal Nos.	Period of signs in days from - to
I	5000	Nil	4	2 - 5	1 - 14

Table No. C

Mean Body Weight and Percent Body Weight Gain

Sighting study:

Group No.	Dose (mg/kg body weight)	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
I	2000	124.50	142.50	14.46	165.50	16.14	32.93
II	5000	133.20	158.20	18.77	181.40	14.66	36.19

Main study:

Group No.	Dose (mg/kg body weight)	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
I	5000	130.08	150.00	15.41	170.05	13.39	30.88

Table No. D

Summary of Gross Pathology Findings

Sighting study:

SITE AND LESION OBSERVED	Group I II
NAD	1

Main study:

SITE AND LESION OBSERVED	Group I
NAD	2 – 5

(NAD = No Abnormality Detected)

Appendix No. I

Individual Animal - Mortality Record

Sighting study:

Group No.	Dose mg/kg	Animal No.	Mortality	
			Absolute	Relative %
I	2000	1	0	0
II	5000	1	0	0

Main study:

Group No.	Dose mg/kg	Animal No.	Mortality	
			Absolute	Relative %
I	5000	2	0	0
		3	0	0
		4	0	0
		5	0	0

Appendix No. II

Individual Animal - Clinical Signs of Intoxication

Sighting study:

Group No.	Dose mg/kg	Animal No.	Observed Signs	Period of signs in days from - to
I	2000	1	Nil	1 - 14
II	5000	1	Nil	1 - 14

Main study:

Group No.	Dose mg/kg	Animal No.	Observed Signs	Period of signs in days from - to
I	5000	2	Nil	1 - 14
		3	Nil	1 - 14
		4	Nil	1 - 14
		5	Nil	1 - 14

Appendix No. III

Individual Animal - Body Weight and Percent Body Weight Gain

Sighting study:

Group: I Dose: 2000 mg/kg body weight

Animal No.	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
1	124.5	142.5	14.46	165.5	16.14	32.93

MAYFAIR CLINICAL EDUCATION & RESEARCH CENTRE,
Mohan Mill Compound, Ghodbunder Road, Thane-400 607.

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Document No MCERC/REPTCLTR/OXY/1205/001 Study Identifier: Mfair/oxy/200506

Sighting study:

Group: II Dose: 5000 mg/kg body weight

Animal No.	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
1	133.2	158.2	18.77	181.4	14.66	36.19

Appendix No. III (Contd.)

Individual Animal - Body Weight and Percent Body Weight Gain

Main study:

Group I: Dose: 5000 mg/kg body weight

Animal No.	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
2	137.8	155.3	12.70	174.8	12.56	26.85
3	125.4	146.9	17.15	168.0	14.36	33.97
4	133.0	151.2	13.68	170.0	12.43	27.82
5	124.1	146.6	18.13	167.4	14.19	34.89

Appendix No. IV

Individual Animal - Gross Pathology Findings

Sighting study:

Group: I

Dose: 2000 mg/kg body weight

Animal No.	Fate	Gross Pathology Findings
1	TS	NAD

Group: II

Dose 5000 mg/kg body weight

Animal No.	Fate	Gross Pathology Findings
1	TS	NAD

Main study:

Group: I

Dose: 5000 mg/kg body weight

Animal No.	Fate	Gross Pathology Findings
2	TS	NAD
3	TS	NAD
4	TS	NAD
5	TS	NAD

[TS = Terminal sacrifice]

[NAD = No abnormality detected]

Part 2

SUMMARY REPORT ON ANIMAL TOXICITY STUDIES

(B) Sub Chronic Toxicity

PROJECT NO.12204

SUBCHRONIC ORAL TOXICITY STUDY (28 DAY)

OF OXY-POWDER®

IN THE SPRAGUE DAWLEY RATS

STUDY DIRECTOR: DR. R.M.BHIDE Ph.D.

TESTING FACILITY:

**INDIAN INSTITUTE OF TOXICOLOGY,
32/A/1, HADAPSAR INDUSTRIAL ESTATE,
PUNE - 411 013, INDIA.**

DATA REQUIREMENTS:

OECD GUIDELINE,

SECTION 4, TEST NO.408,

21 SEPTEMBER, 1998.

**SPONSOR: MAYFAIR CLINICAL EDUCATION AND
RESEARCH CENTRE, MUMBAI**

SUB CHRONIC ORAL TOXICITY OF OXY-POWDER®

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STATEMENT OF COMPLIANCE

Project No. : 12204

Test Substance : Oxy-Powder®

Title: Sub chronic Oral Toxicity Study (28 day) Of Oxy-Powder® in the Sprague Dawley Rats

We hereby attest to the authenticity of the study and guarantee that the data is correct and accurate to the best of our knowledge and that the study was performed by the procedure described in the Indian Institute of Toxicology Standard Operating Procedures. We hereby attest that this study was conducted in compliance with Protocol submitted to and approved by the sponsor. The study also complies with the Schedule Y in Drugs and Cosmetic Act (2nd Amendment) Rules, 2005, Ministry of Health and Family Welfare, Government of India, OECD Guideline for the testing of Chemicals No.408, “Repeated Dose 28 -day Oral Toxicity Study in Rodents” adopted on September 21st, 1998 and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Indian Institute of Toxicology Registration No.15/1999/CPCSEA).

Dr. R. M. Bhide Ph.D. Signed /- 12-7-2006

Study Director Signature Date

Dr. P. R. Tikhe Ph.D. ` Signed /- 12-7-2006

Quality Assurance Unit Signature Date

Mr. V. M. Bhide M.B.A. Signed /- 12-7-2006

Director, Administration Signature Date

STATEMENT OF QUALITY ASSURANCE UNIT

Project No. : 12204

Test Substance : Oxy-Powder®

**Study Title: Sub chronic Oral Toxicity Study (28 day) of Oxy-Powder®
in the Sprague Dawley Rats**

Quality Assurance Unit of the testing facility inspected the conduct of study on the following dates:

10-05-2006, Body weight data collection

11-05-2006, Test substance administration

25-05-2006, Food consumption data collection

08-06-2006, Necropsy

22-06-2006, Haematology technique

Raw data audit: 08-07-2006

Final report audit: 12-07-2006

No inspection led to findings which had to be reported to the management or would have impaired this study in any way.

Dr. P. R. Tikhe Ph.D. Signed /- 12-7-2006

Quality Assurance Unit Signature Date

ARCHIVING

Project No.: 12204

Test Substance Oxy-Powder®

Study Title: Sub chronic Oral Toxicity Study (28 day) Of Oxy-Powder® in the Sprague Dawley Rats

Indian Institute of Toxicology takes the responsibility of archiving the following items for a period of two years: Protocol, Raw data and a copy of Final Report, Wet tissue samples, Histology slides and blocks.

Mr. V. M. Bhide, M.B.A. Signed /- 12-7-2006

Director, Administration Signature Date

PERSONNEL INVOLVED IN THE STUDY

Study Director : Dr. R. M. Bhide Ph.D.

Head Administration: Mr. V. M. Bhide M.B.A.

Animal Care : Dr. S. D. Bhande B. V. Sc. & A. H.

Histopathology : Dr. S. K. Bokan M. V. Sc.

Technician : Dr. V. S. Jagdale M. V. Sc.

Biochemistry : Mr. N. G. Bidkar B.Sc.

Histology : Miss S. G. Tikhe B.Sc., D.M.L.T.

: Mr. S. N. Gaikwad B.Sc., D.M.L.T.

: Mrs. S. C. Gaikwad B.Sc., D.M.L.T.

Quality Assurance : Dr. P. R. Tikhe Ph.D.

Audit

SUMMARY AND CONCLUSION

The Sub chronic oral toxicity study was designed and conducted to determine the toxicity profile of **Oxy-Powder®** when administered daily for 28 days in Sprague Dawley rats. In acute toxicity test the compound was found to be non toxic at the dose level of 5000 mg/kg body weight. The dose has been selected on this basis and the justification provided on page 14 of this report.

Oxy-Powder® suspended in distilled water was administered to animals at the dose levels 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight. Two additional dose levels were added to the study as 0 mg/kg (Rev.) and 1000 mg/kg (Rev.), in order to study the reversibility or delayed occurrence of symptoms, if any. The control animals were administered with vehicle only.

Salient features of the study were as follows:

- 1) All the male and female animals from control and all the treated dose groups up to 1000 mg/kg survived throughout the dosing period of 28 days and the recovery period of 14 days.
- 2) No signs of intoxication were observed in male and female animals from different dose groups during the dosing period of 28 days and during the recovery period of 14 days.

- 3) Male and female animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days and the recovery period of 14 days.
- 4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days and the recovery period of 14 days.
- 5) Ophthalmoscopic examination, conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality.
- 6) Haematological analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment.
- 7) Biochemical analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment.
- 8) Functional battery observation tests conducted at termination revealed no abnormalities.
- 9) Urine analysis, conducted at the end of the dosing period in week 4 and at the end of recovery period in week 6, revealed no abnormality attributable to the treatment.
- 10) Organ weight data of male and female sacrificed at the end of the dosing period and at the end of the recovery period was found to be comparable with that of respective controls.
- 11) Gross pathological examination did not reveal any abnormality.
- 12) Histopathological examination did not reveal any abnormality

Based on these findings the no observed effect level (NOEL) of **Oxy-Powder®** supplied by **Mayfair Clinical Education and Research Centre, Mumbai**, in Sprague Dawley rat via oral route, over a period of 28 days was found to be 1000 mg/kg body weight for male and female animals.

Signed /—

DR. R.M.BHIDE

STUDY DIRECTOR

- 1) The results relate only to the items tested.
- 2) This report shall not be reproduced except in full, without the written approval of the laboratory.

PREFACE

General

Title: **Sub chronic Oral Toxicity Study (28 day) of Oxy-Powder® in the Sprague Dawley Rats**

Sponsor : **Mayfair Clinical Education and Research Centre, Mumbai**

Monitoring Scientist **Dr. J. K. Lalla**

Testing Facility **Indian Institute of Toxicology,**

32/A/1, Hadapsar Industrial Estate,

Project No. 12204

Test Substance **Oxy-Powder®**

Schedule

Date of receipt of animals for: 10/05/2006

Treatment (day 0)

Date of treatment (day 1): 11/05/2006

Date of termination of dosing : 07/06/2006

(Day 28)

Date of sacrifice (day 29) : 08/06/2006

Date of completion of recovery: 21/06/2006

Period 42 days

Date of sacrifice 43 Day : 22/06/2006

Date of reporting : 12/07/2006

Objective

The objective of this study is to assess the toxicological profile of **Oxy-Powder®** after administering at three dose levels by oral route for 28 consecutive days to Sprague Dawley rats to determine the target organ of toxicity and no observed effect level (NOEL). The study objective also includes the detection of delayed occurrence or reversibility of any signs / toxicity at the end of recovery period of 14 days

MATERIALS AND METHODS

TEST SUBSTANCE

Sponsor: Mayfair Clinical Education and Research Centre, Mumbai

Label on Sample : Oxy-Powder®

Characteristics of Sample:

Consistency – Solid (Capsule)

Colour – White

Disclaimer: The above physiochemical data of test substance is supplied by the Sponsor. All responsibility with regards to the accuracy and authenticity of this information remains with the Sponsor. The test lab is not responsible for any variations with the batch number supplied.

TEST SYSTEM

Species : Rat

Strain Sprague Dawley

Source : Indian Institute of Toxicology, Pune

Sex : Male and female

Age : 6 to 8 weeks

No. of animals per dose level: 5 per sex (dose groups sacrificed on day 29)

5 per sex (reversal groups sacrificed on day 43)

Acclimation : Seven days prior to dosing.

Veterinary examination: Prior to and at the end of the acclimation period.

Identification of animals : By cage number, animal number and individual marking on fur.

Diet : Pelleted feed supplied by Nav Maharashtra Chakan Oil Mills Ltd., Pune

Water: Aqua guard pure water in glass bottles *ad libitum*

Housing & Environment: The animals were housed 5 each, of the same sex in Polypropylene cages provided with bedding of husk.

The temperature was maintained between 20 & 24 °C and relative humidity between 30 and 70%; 10-15 air changes per hour and 12 hours each of dark and light cycle was maintained.

Dose: Male 0 mg/kg, 0 mg/kg (Rev.), 250 mg/kg, 500 mg/kg, 1000 mg/kg and 1000 mg/kg
(Rev.) body weight

Female 0 mg/kg, 0 mg/kg (Rev.), 250 mg/kg, 500 mg/kg, 1000 mg/kg and 1000 mg/kg (Rev.) body weight

Body weight at start of Study

Male Mean 96.66 g (= 100 %)

Minimum: 91.5 g (- 5.34 %)

Maximum: 100.5 g (+ 3.97 %)

Total No. of animals: 30

Female Mean 87.96 g (= 100 %)

Minimum: 81.7 g (- 7.12 %)

Maximum: 97.3 g (+ 10.62 %)

Total No. of animals: 30

METHODS

Randomization, Numbering and Grouping of Animals

Sixty rats i.e. 30 male and 30 female healthy animals were randomly divided into four groups of 5 animals per sex for dosing up to 28 days and 5 animals per sex as reversal groups for control and high dose i.e. 0 mg/kg, 0 mg/kg (Rev.) 250 mg/kg, 500 mg/kg, 1000 mg/kg and 1000 mg/kg (Rev.) body weight. Animals were allowed acclimation period of 7 days to laboratory conditions prior to the initiation of treatment. Rats were assigned five per sex per cage wise and the individual animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

Randomization was conducted as per the Indian Institute of Toxicology Standard Operating Procedure on Randomization of Study Animals (SOP No.IIT/S-PSC/13).

Route of Administration

Oral (gavage), once daily for 28 consecutive days.

Justification for Selection of Sprague Dawley rats for the Study

- 1) One of the rodent species recommended as test system for the use in toxicity studies,
- 2) This test system has been demonstrated to be sensitive to toxins,
- 3) Widely used throughout industry for the evaluation of toxicity of various products,
- 4) Historical data and evidence at the facility.

Justification for Selection of Route

The oral route was selected for use because,

Oral route is considered to be a proposed therapeutic route.

Dose Preparation

Oxy-Powder® suspended in distilled water was administered to animals at the dose levels of 250 mg/kg, 500 mg/kg and 1000 mg/kg in the dose volume of 10 mL/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only.

Justification for Dose Selection

In acute toxicity test the compound was found to be non toxic at the dose level of 5000 mg/kg body weight. The doses selected for the study were 0 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight.

OBSERVATIONS

Clinical Signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during the period of the study.

Body Weight

The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study and at termination to calculate relative organ weights. The group means body weights and percent body weight gain were calculated.

Food Consumption

The quantity of food consumed by groups consisting of five animals of each sex (or ten animals, 0 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg) and five animals of each sex (or ten animals, 0 mg/kg Rev. and 1000 mg/kg Rev.) was recorded weekly and the food consumption per animal was calculated for control and all the treated dose groups.

Ophthalmoscopy

The eyes of control and all the treated dose group animals were examined prior to the initiation of the dosing and in week 4 and in week 6 (for reversal group animals) of the study. Eye examination was carried out using a hand slit lamp after induction of mydriasis with 0.5% solution of tropicamide.

Functional Observations

Towards the end of the exposure period in week 4, sensory reactivity to stimuli of different types (auditory, visual and proprioceptive stimuli) and by grading different stimuli according to the Standard Operating Procedure for 'Conduct of functional observational battery' No.IIT/S-PSC/36, assessment of grip strength (Digital Grip Strength Meter, using Columbus Instrument) and motor activity assessment was conducted.

TERMINAL STUDIES

Laboratory Investigations

Following laboratory investigations were carried out on day 29 and on day 43 in animal's fasted over-night. Blood samples were collected from orbital sinus following morning using sodium heparin (200 IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Prothrombin time analysis was conducted using citrate bulb (100 µl of 3.8% solution of sodium citrate for 1 ml of blood). Blood samples were centrifuged at 3000 rpm for 10 minutes.

Haematological Investigations

Following haematological parameters were studied using Beckman Coulter Haematology analyzer.

Hb : Haemoglobin (g %)

RBC: Red Blood Corpuscles (x 10⁶ /c mm)

HCT: Hematocrit (%)

MCV: Mean Corpuscular Volume (µ m³)

MCH: Mean Corpuscular Haemoglobin (pg)

MCHC: Mean Corpuscular Haemoglobin Concentration (%)

Platelets (x10³ / µL)

WBC : White Blood Corpuscles (x 10³ / µL)

Analysis of following parameters was performed manually:

Rt. : Reticulocyte (%)

N : Neutrophils (%)

L : Lymphocytes (%)

E : Eosinophils (%)

M : Monocytes (%)

B : Basophil (%)

Pt. : Prothrombin time (Sec.)

Biochemical Investigations

Following biochemical parameters were studied using VeTEX (Veterinary Chemistry Expert) Clinical Chemistry auto-analyzer system.

Total Protein (g %)

BUN: Blood Urea Nitrogen (mg %)

ALT: Alanine Aminotransferase (IU/L)

AST: Aspartate Aminotransferase (IU/L)

AP: Alkaline Phosphatase (IU/L)

Blood Sugar (mg %)

Calcium (mg %)

Phosphorous (mg %)

γ GT: Gamma Glutamyl Transferase (U/L)

Bilirubin (mg %)

Albumin (g %)

Creatinine (mg %)

CPK: Creatine Phospho Kinase (IU/L)

Sodium (m mol/L)

Potassium (m mol/L)

Chloride (m mol/L)

Cholesterol (mg %)

Triglycerides (mg %)

LDH: Lactate dehydrogenase (IU/L)

Urine Analysis

Urine samples were collected in week 4 and in week 6

The following parameters were estimated using appropriate methodology as discussed below:

Volume (urine volume was collected from all male and female animals from each dose group using metabolic cages and collection duration was fixed at 16 hours.)

Appearance

Colour

pH = Multistix

Specific Gravity = Multistix

Proteins = Multistix

Glucose = Multistix

Ketones = Multistix

Bilirubin = Multistix

Urobilinogen = Multistix

Occult Blood = Multistix

Nitrite = Multistix

Results are reported according to the following convention:

Absent = 0

Trace = +

Small amount of analyte = ++

Moderate amount of analyte = +++

Large amount of analyte = ++++

Microscopy

Urine samples were centrifuged at 1000 rpm for 10 minutes. Following components were observed under the microscope:

P: Pus cells

E: Epithelial cells

C: Casts

R: RBC

Cr: Crystals

The presence and approximate frequency of these constituents are reported according to the following convention:

Grade	Description
0	None found in any field
1	Few found in some field
2	Few found in many field
3	Many found in many field

Necropsy

All the animals were sacrificed on day 29 except for reversal group animals which were sacrificed on day 43 i.e. post-dosing period of 14 days, using CO2 asphyxiation technique (body weights mentioned in the table section are fasting body weights, Appendix No. IX and X).

Necropsy of all animals was carried out and the weights of the following organs were recorded of Liver, kidneys, adrenals, epididymis, thymus, spleen, brain, heart, uterus and testes/ovaries. The organ weights were recorded as absolute values and their relative values (i.e. per cent of the body weight) were calculated.

Histopathology

Following tissue samples of organs from control and animals treated at the highest dose level of 1000 mg/kg, were preserved in 10% formalin and were subjected to Histopathological examination.

Adrenals, Aorta, Brain, Cecum, Colon, Duodenum, Epididymis, Gonads, Heart, Ileum, Jejunum, Kidneys, Liver, Lungs, Lymph nodes, Oesophagus, Prostate, Rectum, Sciatic nerve, Spleen, Sternum with bone marrow, Stomach, Seminal Vesicles, Spinal cord, Thymus, Thyroid / parathyroid, Trachea, Urinary Bladder and Uterus.

Following tissue samples of organs from low and intermediate dose groups' animals were preserved for histopathology examination. Liver, kidneys, adrenals, epididymis, thymus, spleen, brain, heart, uterus and testes/ovaries.

Statistical Analysis

All findings such as clinical signs of intoxication, body weight changes, food consumption, and haematology and blood chemistry were tabulated. Data on all parameters were evaluated by analysis (co-)variance followed by Student's t test and Cochran T-Test.

Histopathological observations were tabulated; evaluated and individual animal score is calculated according to degree and area by using software, LABCAT Module for Histopathology, Innovative Programming Associates, INC., Princeton, New Jersey.

RESULTS

Clinical Signs

Males

Group I (0 mg/kg): Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.1 to 5).

Group II (0 mg/kg, Reversal): Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.11 to 15).

Group III (250 mg/kg): Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.21 to 25).

Group IV (500 mg/kg): Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.31 to 35).

Group V (1000 mg/kg): Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.41 to 45).

Group VI (1000 mg/kg, Reversal): Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.51 to 55).

Females -

Group I (0 mg/kg) Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.6 to 10).

Group II (0 mg/kg, Reversal): Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.16 to 20).

Group III (250 mg/kg) Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.26 to 30).

Group IV (500 mg/kg) Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.36 to 40).

Group V (1000 mg/kg) Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.46 to 50).

Group VI (1000 mg/kg, Reversal) Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.56 to 60).

Mortality

Male and Female - All animals from control and all the treated dose groups survived throughout the dosing period of 28 days and the post-dosing recovery period of 14 days

Body Weight (App. I)

Male and Female - Animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days. During the post-dosing recovery period, animals from 1000 mg/kg reversal group exhibited normal body weight gain when compared with that of respective controls.

Food Consumption

Male and Female -

During the dosing period and the post-dosing recovery period the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Ophthalmoscopy

The eyes of control and all the treated dose group animals were examined prior to the initiation of the dosing and in week 4 and in week 6 (for reversal group animals) of the study. Ophthalmoscopic examination did not reveal any abnormality.

Functional Observations (App. II, III, IV and V)

Functional observation tests conducted at termination revealed no abnormalities.

Haematological Investigations (App. VI)

Males and Females

Haematological investigations conducted on day 29 and on day 43 (for reversal group animals) revealed following significant changes in the values of different parameters studied when compared with those of respective controls; However, the increase or decrease in the values obtained was within normal biological and laboratory limits. In other words, the effect was not dose-dependent.

Males:

MCHC: Decreased values were obtained for animals from 500 mg/kg ($P<0.05$) and 1000 mg/kg ($P<0.01$) dose groups, sacrificed on day 29 and

Total WBC: Increased values were obtained for animals from 500 mg/kg dose group, sacrificed on day 29 ($P<0.05$).

Females:

MCV: Increased values were obtained for animals from 500 mg/kg dose group, sacrificed on day 29 ($P<0.05$) and

MCV and MCH: Increased values were obtained for animals from 1000 mg/kg reversal group, sacrificed on day 43 ($P<0.05$).

Parameters	Laboratory range
MCV	44.5 to 69.0 (μm^3)
MCH	12.0 to 24.5 (pg)
MCHC	21.6 to 42.0 (%)
Total WBC	3.00 to 19.00 ($\times 10^3$ /uL)

Biochemical Investigations (App. VII)

Males and Females -

Biochemical investigation's conducted on day 29 and on day 43 (for reversal group animals), revealed following significant changes in the values of different parameters studied when compared with that of respective controls, however, the values obtained were within normal biological and laboratory limits or the effect was not dose dependent.

Males:

Blood Urea Nitrogen: Elevated levels were observed in animals from 500 mg/kg dose group, sacrificed on day 29 ($P < 0.05$),

LDH: Elevated levels were observed in animals from 1000 mg/kg dose group, sacrificed on day 29 ($P < 0.05$) and

Aspartate Aminotransferase: Decreased levels were observed in animals from 1000 mg/kg reversal group, sacrificed on day 43 ($P < 0.01$).

Females:

Alkaline Phosphatase: Elevated levels were observed in animals from 1000 mg/kg reversal group, sacrificed on day 43 ($P < 0.05$).

Parameters	Laboratory range
Alkaline Phosphatase	50 to 80 (IU/L)
BUN	20 to 50 (mg %)
AST	30 to 70 (IU/L)
LDH	300 to 400 (IU/L)

Urine Analysis (App. VIII)

Male and Female -

Urine analysis of control and treated animals in week 4 and reversal group animals in week 6, revealed no abnormality.

Organ Weights (App. XI, X)

Males and Females –

In comparison with respective controls on day 29, organ weight data of animals from different dose groups was found to be comparable.

In comparison with respective controls on day 43, organ weight data of animals from 1000 mg/kg reversal group was found to be comparable.

Necropsy (App. XI)

Gross pathological examination did not reveal any abnormality.

Histopathology (App. XI)

Histopathological examination revealed focal Lymphocytic infiltration and/or necrosis in liver, Lymphocytic infiltration in the kidneys, focal Lymphocytic infiltration in the heart, gliosis in the brain, interstitial Pneumonitis in the lungs, eosinophilic infiltration in uterus were observed in few male and female animals from control and 1000 mg/kg dose group animals with similar quality and quantity and are considered incidental, gender and physiology related and are covered in the background data of the pathology.

Normal Laboratory Ranges at Indian Institute of Toxicology (App. XII)

Haematology

Parameters	Laboratory range Rat	Laboratory range Mouse	Laboratory range Rabbit
Hb	11.1 to 18.0 (g %)	10.2 to 16.6 (g %)	9.3 to 19.3 (g %)
Total RBC	5.0 to 12.0 (x 10 ⁶ /cmm)	6.70 to 12.5 (x 10 ⁶ /cmm)	4.0 to 8.6 (x 10 ⁶ /cmm)
HCT	36.0 to 52.0 (%)	32.0 to 54.0 (%)	30.0 to 53.0 (%)
MCV	44.5 to 69.0 (µm ³)	31.0 to 62.0 (µm ³)	57.0 to 90.0 (µm ³)
MCH	12.0 to 24.5 (pg)	9.20 to 20.8 (pg)	16.0 to 31.0 (pg)
MCHC	21.6 to 42.0 (%)	22.0 to 35.5 (%)	22.0 to 38.7 (%)
Platelets	140 to 600 (x 10 ³ /uL)	150 to 500 (x 10 ³ /uL)	120 to 800 (x 10 ³ /uL)

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Mohan Mill Compound, Ghodbunder Road, Thane-400 607.

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Total WBC	3.00 to 19.00 (x 10 ³ /uL)	5.40 to 16.00 (x 10 ³ /uL)	2.0 to 15.0 (x 10 ³ /uL)
N	4 to 50 (%)	8 to 42.9 (%)	10 to 85 (%)
E	0 to 4 (%)	0 to 2.90 (%)	0 to 8 (%)
L	40 to 95 (%)	55 to 95 (%)	25 to 95 (%)
M	0 to 8 (%)	0 to 8 (%)	0.5 to 16.0 (%)
Pt	12 to 18 (Sec.)	12 to 18 (Sec.)	12 to 18 (Sec.)
APTT Activated Partial Thromboplastin Time (Sec.)	15 to 30 (Sec.)	15 to 30 (Sec.)	15 to 30 (Sec.)

Mice

Reticulocyte	4.0 to 7.0 % (Young) 1.0 to 3.0 % (Adult)
--------------	--

Rat

Reticulocyte	3.0 to 6.0 % (Young) 0.5 to 2.5 % (Adult)
--------------	--

Biochemistry

PARAMETERS	Laboratory range Rat	Laboratory range Mouse	Laboratory range Rabbit
Alkaline Phosphatase	50 to 80 (IU/L)	50 to 80 (IU/L)	50 to 80 (IU/L)
Cholesterol	50 to 70 (mg %)	50 to 70 (mg %)	50 to 70 (mg %)
Triglycerides	90 to 120 (mg %)	90 to 120 (mg %)	90 to 120 (mg %)
Potassium	3 to 5 (mmol / l)	3 to 5 (mmol/l)	3 to 5 (mmol/l)
Albumin	3 to 5 (g %)	3 to 5 (g %)	3 to 5 (g %)
Blood Sugar	70 to 110 (mg %)	70 to 110 (mg %)	70 to 110 (mg %)
Creatinine	0.8 to 1.2 (mg %)	0.8 to 1.2 (mg %)	0.8 to 1.2 (mg %)
Cholesterol	50 to 70 (mg %)	50 to 70 (mg %)	50 to 70 (mg %)
Gamma GT	5 to 25 (U/L)	5 to 25 (U/L)	5 to 25 (U/L)
CPK	50 to 70 (IU/L)	50 to 70 (IU/L)	50 to 70 (IU/L)

Sodium	130 to 150 (mmol/l)	130 to 150 (mmol/l)	130 to 150 (mmol/l)
Total Protein	6.6 to 8.6 (g %)	6.6 to 8.6 (g %)	6.6 to 8.6 (g %)
BUN	20 to 50 (mg %)	20 to 50 (mg %)	20 to 50 (mg %)
ALT	25 to 50 (IU/L)	25 to 50 (IU/L)	25 to 50 (IU/L)
AST	30 to 70 (IU/L)	30 to 70 (IU/L)	30 to 70 (IU/L)
Calcium	9 to 11 (mg %)	9 to 11 (mg %)	9 to 11 (mg %)
Phosphorus	3 to 5 (mg %)	3 to 5 (mg %)	3 to 5 (mg %)
Bilirubin	Up to 1 (mg %)	Up to 1 (mg %)	Up to 1 (mg %)
Potassium	3 to 5 (mmol/l)	3 to 5 (mmol/l)	3 to 5 (mmol/l)
Chlorides	95 to 106 (mmol/l)	95 to 106 (mmol/l)	95 to 106 (mmol/l)
LDH	300 to 400 (IU/L)	300 to 400 (IU/L)	300 to 400 (IU/L)
Urea	80 to 120 (mg %)	80 to 120 (mg %)	80 to 120 (mg %)

THE END

Part 3

**SUMMARY REPORT OF
ANTIMICROBIAL STUDIES ON OXY-POWDER®**

**PROJECT NO. 9165
ANTIMICROBIAL ACTIVITY TESTING
OF OXY-POWDER®
AGAINST 5 CULTURES**

**STUDY DIRECTOR
DR. (MRS) DEEPA BHAJEKAR, Ph.D.**

TESTING FACILITY

THE MICRO CHEM LABORATORY
125, VARDHMAN INDUSTRIAL ESTATE,
GOKUL NAGAR, THANE (W) 400 601
(Laboratory Certified by NABL for ISO / 17025)

SPONSOR: MAYFAIR CLINICAL, EDUCATION AND RESEARCH CENTRE,
MUMBAI

DATA REQUIREMENTS
AS SPECIFIED BY SPONSOR

ANTIMICROBIAL ACTIVITY TESTING OF OXY-POWDER®
AGAINST 5 CULTURES

SPONSOR: Mayfair Clinical Education and Research Centre, Mumbai

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STATEMENT OF COMPLIANCE

Project No.: 9165

Test Substance : Oxy-Powder®

Study Title: ANTIMICROBIAL ACTIVITY TESTING OF OXY-POWDER® AGAINST 5 CULTURES

We hereby attest to the authenticity of the study and guarantee that the data is correct and accurate to the best of our knowledge and that the study was performed by the procedure described in The Micro Chem. Laboratory Standard Operating Procedures. We hereby attest that this study was conducted in compliance with Protocol submitted to and approved by the sponsor.

The study also complies with the Schedule Y in Drugs and Cosmetic Act (2nd Amendment) Rules, 2005, Ministry of Health and Family Welfare, Government of India,

DR (MRS.) DEEPA BHAIKAR, Ph.D. Signed /- 03-06-2006

_____	_____	_____
Study Director	Signature	Date

Miss Nameeta Ganpule, MSc. Signed /- 03-06-2006

_____	_____	_____
Quality Manager	Signature	Date

STATEMENT OF QUALITY ASSURANCE UNIT

Project No.: 9165

Test Substance: Oxy-Powder®

Study Title :

ANTIMICROBIAL ACTIVITY TESTING OF OXY-POWDER® AGAINST 5 CULTURES

Quality Assurance Unit of the testing facility inspected the conduct of study on the following dates

22-05-2006: Preparation and sterilization of Media,

24-05-2006: Inoculation with relevant organism, transfer to sterile Petri Plates and boring of cups.

25-05-2006: Test solution preparation, addition to cups in LF unit and incubation.

29-05-2006: Incubation Result

31-05-2006: Raw data audit:

03-06-2006: Final report audit

No inspection led to findings which had to be reported to the management / sponsor or would have impaired this study in any way.

Miss Nameeta Ganpule, MSc signed /- 03-06-2006

_____	_____	_____
Quality Manager	Signature	Date

PERSONNEL INVOLVED IN THE STUDY

Study Director : DR (MRS.) DEEPA BHAJEKAR, Ph.D.

Microbiologist : Miss Niveda Prabhu MSc.

Quality Manager Audit : Miss Nameeta Ganpule, MSc.

Introduction:

(Ref: Normal Gastrointestinal Tract Flora by Charles Patrick Davis in

“Microbiology of the Gastrointestinal Tract” ed. by Sherwood L. Gorbach Medmicro Chapter 6)

The stomach is a relatively hostile environment for bacteria. It contains bacteria swallowed with the food and those dislodged from the mouth. Acidity lowers the bacterial count, which is highest (approximately 10^3 to 10^6 organisms/g of contents) after meals and lowest (frequently undetectable) after digestion. Some *Helicobacter* species can colonize the stomach and are associated with type B gastritis and peptic ulcer disease. Aspirates of duodenal or jejunal fluid contain approximately 10^3 organisms/ml in most individuals. Most of the bacteria cultured (streptococci, lactobacilli, *Bacteroides*) are thought to be transients. Levels of 10^5 to about 10^7 bacteria/mL in such aspirates usually indicate an abnormality in the digestive system (for example, achlorhydria or malabsorption syndrome). Rapid peristalsis and the presence of bile may explain in part the paucity of organisms in the upper gastrointestinal tract. Further along the jejunum and into the ileum, bacterial populations

begin to increase, and at the ileocecal junction they reach levels of 10^6 to 10^8 organisms/mL, with streptococci, lactobacilli, Bacteroides, and bifidobacteria predominating. Concentrations of 10^9 to 10^{11} bacteria / g of contents are frequently found in human colon and feces. This flora includes a bewildering array of bacteria (more than 400 species have been identified); nonetheless, 95 to 99 percent belong to anaerobic genera such as Bacteroides, Bifidobacterium, Eubacterium, Peptostreptococcus, and Clostridium. In this highly anaerobic region of the intestine, these genera proliferate, occupy most available niches, and produce metabolic waste products such as acetic, butyric, and lactic acids. The strict anaerobic conditions, physical exclusion (as is shown in many animal studies), and bacterial waste products are factors that inhibit the growth of other bacteria in the large bowel.

Although the normal flora can inhibit pathogens, many of its members can produce disease in humans. Anaerobes in the intestinal tract are the primary agents of intra-abdominal abscesses and peritonitis. Bowel perforations produced cancer, infarction, byappendicitis, surgery, or gunshot wounds almost always seed the peritoneal cavity and adjacent organs with the normal flora.

Anaerobes can also cause problems within the gastrointestinal lumen. Treatment with antibiotics may allow certain anaerobic species to become predominant and cause disease. For example, *Clostridium difficile*, which can remain viable in a patient undergoing antimicrobial therapy, may produce pseudo membranous colitis. Other intestinal pathologic conditions or surgery can cause bacterial overgrowth in the upper small intestine. Anaerobic bacteria can then deconjugate bile acids in this region and bind available vitamin B12 so that the vitamin and fats are malabsorbed. In these situations, the patient usually has been compromised in some way; therefore, the infection caused by the normal intestinal flora is secondary to another problem.

More information is available on the animal than the human micro flora. Research on animals has revealed that unusual filamentous microorganisms attach to ileal epithelial cells and modify host membranes with few or no harmful effects. Microorganisms have been observed in thick layers on gastrointestinal surfaces (Fig. 6-3) and in the crypts of Lieberkuhn. Other studies indicate that the immune response can be modulated by the intestinal flora. Studies of the role of the intestinal flora in biosynthesis of vitamin K and other host-utilizable products, conversion of bile acids (perhaps to co-carcinogens), and ammonia production (which can play a role in hepatic coma) show the dual role of the microbial flora in influencing the health of the host. Additional basic studies of the human bowel flora are necessary to define their effect on humans.

Probiotics (www.space-age.com/Probiotics.doc)

The micro ecology of the human gastrointestinal tract is incredibly complex, as there are at least five hundred different species of micro flora that are part of the normal intestinal flora. There are nine

times as many bacteria in the gastrointestinal tract as there are cells in the human body. The type and number of the gut bacteria play an important role in determining health and disease in the human body. **Probiotics** are beneficial micro organisms like *Lactobacillus*, *Bifidobacteria*, and *Streptococcus* in human intestinal tract. They manufacture vitamins, especially B vitamins like biotin, niacin, folic acid and B6 that detoxify chemicals and metabolize hormones. They empower enzymes that maximize food assimilation and digestion. A state of altered bacterial flora in the gut is known as **Dysbiosis**.

- **Probiotics boost immune response by inhibiting growth of pathogenic organisms**
- **Probiotics detoxify the intestinal tract by protecting intestinal mucosa levels**
- **Probiotics develop a barrier to food-borne allergies**
- **Probiotics neutralize antibiotic-resistant strains of bacteria**
- **Probiotics reduce cancer risk**
- **Probiotics reduce the risk of inflammatory bowel disease (IBS) and Diverticulosis**
- **Probiotics synthesize needed vitamins for healing**
- **Probiotics prevent Diarrhea by improving digestion of proteins and fats.**

Intestinal Flora / Probiotics Supportive Information

A large body of evidence over the past 75 years has demonstrated the preventive health value of eating foods fermented with *Lactobacilli* or *Bifidobacteria*. **These beneficial bacteria are referred to as Probiotics.** Probiotic bacteria are considered "friendly" bacteria. They are an essential component of a healthy gastrointestinal tract as they inhibit the growth of harmful bacteria, boost immune function, decrease infection in the digestive tract, and enhance digestion through enzyme production. There are numerous species of lactobacilli and many strains for each species. The most well known of these, *Lactobacillus acidophilus* and *Bifidobacteria* are normal inhabitants of the human digestive tract. Others, like *L. bulgaricus* and *L. Salivarius* are not. These organisms, though, still play an important role in maintaining the proper ratio of "friendly" organisms in the bowel by producing "bacteriocins" chemicals that destroy harmful (unfriendly) bowel organisms.

Acidophilus and *bifidobacteria* maintain a healthy balance of intestinal flora by producing organic compounds such as lactic acid, hydrogen peroxide and acetic acid. These compounds increase the acidity of the intestine and inhibit the growth of less desirable organisms that fair poorly in this acidic environment. By occupying an ecological niche in the intestine, they further limit the growth of opportunistic organisms.

A number of studies have demonstrated benefit of supplementation with Probiotics. The *Annals of Internal Medicine* published a study which showed that *Lactobacillus* ingestion reduced and prevented vaginal yeast infections in women. *Lactobacillus* has also demonstrated positive benefits in irritable bowel syndrome (IBS). In a recent study, increasing levels of *bifidobacteria* reduced the count of *Clostridium*, a pathogenic disease causing bowel organism. Lowering of the level of *Clostridium* reduced the amount of large bowel toxic chemicals believed to promote cancer. Also, the incidence of "traveller's Diarrhea," which is caused by pathogenic bacteria can be reduced by preventive use of probiotics. It is also important to utilize Probiotics after antibiotic use as re-colonizing the intestine may reduce post antibiotic infection in the digestive tract by fifty percent. The choice of Probiotics is very important. It should be manufactured using highly viable, stable strains of organisms that survive passage through the digestive tract and take up residence in the GI tract.

Side Effects: There are no known side effects with the use of probiotics.

References:

- De Simone C, Vesely R, Bianchi SB, et al. The role of probiotics in modulation of the immune system in man and in animals. *Int J Immunotherapy* 1993; 9:23–28.
- Rasic JL. The role of dairy foods containing bifido and acidophilus bacteria in nutrition and health. *N Eur Dairy J* 1983 4:80–88.
- Barefoot SF, Klaenhammer TR. Detection and activity of Lactacin B, a Bacteriocin produced by *Lactobacillus acidophilus*. *Appl Environ Microbiology* 1983; 45:1808–15.
- Hilton E, Isenberg HD, Alperstein P, et al. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for *Candida vaginitis*. *Ann Int Med* 1992; 116:353–57.
- Elmer GW, Surawicz CM, McFarland LV. Bio-therapeutic agents. *JAMA* 1996; 275(11):870–76.
- Scarpignato C, Rampal P. Prevention and treatment of traveller's Diarrhea: A clinical pharmacological approach. *Chemotherapy* 1995; 41:48–81.
- Golledge CL, Riley TV. "Natural" therapy for infectious diseases. *Med J Austral* 1996; 164: 94–95 [review].
- Newcomer AD, Park HS, O'Brian PC, et al. Response of patients with irritable bowel syndrome and lactase deficiency using unfermented acidophilus milk. *Am J Clinical Nutr* 1983; 38:257–263.
- D Bagchi and SK Dash, *Lactobacillus Acidophilus- Natural Antibiotics and Beyond*, Townsend Letter 78-82, 1996.

Diseases Caused by Overgrowth of Potential Pathogens

Bacterial Diarrhea

Enterotoxin-Mediated Diarrhoeal Diseases

Several enterotoxin-producing bacteria cause Diarrhea diseases (Table 95-1). The Diarrhea disease caused by *Vibrio cholerae* and enterotoxigenic strains of *E coli* has three main characteristics. First, there is intestinal fluid loss that is related to the action of an enterotoxin on the small bowel epithelial cells. Second, the organism itself does not invade the mucosal surface; rather, it colonizes the upper small bowel, adhering to the epithelial cells and elaborating the enterotoxin. The mucosal architecture remains intact with no evidence of cellular destruction. Bacteremia does not occur. Third, the fecal effluent is watery and often voluminous, so that the Diarrhea can result in clinical dehydration. The fluid originates in the upper small bowel, where the enterotoxin is most active.

TABLE 95-1 Toxin-Producing Bacteria Associated With Diarrheal Disease

Microorganism	Action of Toxin		
	Adenylate Cyclase	Cytotoxic	Guanylate Cyclase
<i>Vibrio cholerae</i>	+		
<i>E coli</i> (heat-labile toxin)	+		
<i>E coli</i> (heat-stable toxin)			+
<i>Shigella</i>		+	
<i>Staphylococcus aureus</i>		+	
<i>Clostridium perfringens</i>		+	

Cholera

The paradigm of the enterotoxigenic Diarrhea diseases is cholera in which stool volume can exceed 1 L / hour, with daily fecal outputs of 15 to 20 L if the patient is kept hydrated. Cholera is caused by *V cholerae*, which is usually ingested in contaminated water. Vibrios that survive passage through the stomach colonize the surface of the small intestine, proliferate, and elaborate the enterotoxin. Cholera toxin acts via adenylate cyclase to stimulate secretion of water and electrolytes from the epithelial cells into the lumen of the gut. The duodenum and upper jejunum are more sensitive to the toxin than the ileum is. The colon is relatively insensitive to the toxin and may still absorb water and electrolytes normally. Thus, cholera is an "overflow Diarrhea," in which the large volumes of fluid produced in the upper intestine overwhelm the resorptive capacity of the lower bowel. Cholera stool is described as resembling rice watery clear fluid flecked with mucus and is isotonic with plasma. Microscopy reveals no inflammatory cells in the fecal effluent; all that can be seen are small numbers of shed mucosal cells.

Enterotoxigenic *E. coli* Diarrhea

Certain strains of *E. coli* cause Diarrhea disease by elaborating enterotoxins. These strains produce two types of enterotoxins. One, called heat-labile toxin, similar in structure and in its mechanism of action to cholera toxin. The other, called heat-stable toxin appears to act via guanylate cyclase.

Enterotoxigenic *E. coli* strains are the most common cause of travelers' Diarrhea

Other Diarrhea causing toxins: Many strains of *Shigella* produce an enterotoxin, called Shiga toxin that causes secretion of fluid from the small intestine. Shiga toxin has a destructive, cytotoxic effect on the small-bowel epithelium, causing gross injury to the bowel surface. It does not activate adenylate cyclase. *E. coli* 0157:H7, the organism associated with consumption of undercooked chopped meat, also produces a Shiga-like toxin; it causes bloody Diarrhea and colitis. An organism that produces a different type of cytotoxic is *Vibrio parahaemolyticus*, a bacterium associated with seafood. Food-poisoning strains of *Staphylococcus aureus* and *Clostridium perfringens* both produce enterotoxins that are cytotoxic. The staphylococcal enterotoxin also has a direct effect on the vomiting center in the brain.

Gastrointestinal Disease Caused by Invasive Bacteria

Unlike the enterotoxigenic organisms, invasive bacteria exert their main impact on the host by causing gross destruction of the epithelial architecture; histological findings include mucosal ulceration and an inflammatory reaction in the lamina propria. The principal pathogens in this group are *Salmonella*, *Shigella*, *Campylobacter*, invasive *E. coli*, and *Yersinia*. The enteric viruses also invade intestinal epithelial cells, but the extent of mucosal destruction is considerably less than that caused by invasive bacterial pathogens.

***Salmonella* Enteritis**

Salmonella species are a common cause of food poisoning. The main site of attack is the lower ileum, where the salmonellae cause mucosal ulceration. They rapidly make their way through the epithelial surface into the lamina propria and enter the lymphatic stream and bloodstream. At least two virulence factors are associated with intestinal infection: one responsible for mucosal invasion, and the other causing secretion of fluid and electrolytes into the bowel.

***Shigella* Dysentery**

Shigella organisms cause bacillary dysentery, an invasive Diarrhea disease of the lower bowel in which the stool contains inflammatory exudates composed of polymorpho-nuclear leukocytes. The bacilli invade the epithelium of the colon and cause superficial ulceration. This invasive process depends on the presence of two virulence factors. The first mediates the initial penetration of the

mucosal surface by destroying the brush border; the bacteria are subsequently engulfed by invagination of the plasma membrane. The second virulence factor allows the organism to multiply within the mucosal tissue. Mucosal ulceration accompanied by an intense inflammatory response in the lamina propria results. The infection is usually restricted to the mucosa and lymph node involvement; Bacteremia is uncommon.

REFERENCES

- Finegold S (ed): Centennial symposium on anaerobes: A memorial to Andre' Veillon. Clin Infect Dis 18:5-245, 1994
- Goldin BR, Lichtenstein AH, Gorbach SL: The role of the intestinal flora. p. 500. In Shils M.E, Young VR (eds.): Modern Nutrition in Health and Disease. Lea & Febiger, Philadelphia, 1994
- Gorbach SL: Infectious Diarrhea and bacterial food poisoning. p. 1128 In Sleisenger MH, Fordtran JS (eds.): Gastrointestinal Diseases. WB Saunders, Philadelphia, 1993.
- Simon GL, Gorbach SL: Normal alimentary tract micro flora. p. 53. In Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL (eds.): Infections of the Gastrointestinal Tract, Raven Press, New York, 1995

SUMMARY AND CONCLUSION

SUMMARY: The objective of this study was to assess the *in-vitro* effect of **Oxy-Powder®** on the normal Probiotic strains and colonies of microbes generally present in the flora of human gig tract. The study was designed to include 3 strains of pathogenic bacteria namely *Escherichia coli*, *Staphylococcus aureus* and *Enterobacter faecalis*; one Probiotic organism *Lactobacillus bifidigus* and one species of a parasite, *Candida albicans*. The experimental work involved use of Cup plate technique in two sets (one for the test substance and the other for reference control) employing relevant media previously inoculated with the specific culture. **Positive control plate** comprising of inoculated medium in Petri plates was also incubated to indicate suitability of media to support the growth of cultures along with **Negative control** plates containing sterilized medium to validate sterility of the medium. All the operations were performed under sterile conditions to avoid entry of contaminating microbes. The procedure used (a)contents of one capsule of **Oxy-Powder®** (715.5 mg) dissolved in 60 mL sterile distilled water and (b)25 mg citric acid dissolved in 60 mL sterile distilled water as a control since citric acid present in **Oxy-Powder®** could also inhibit growth of microbes. Since **Oxy-Powder®** acted *in vivo* by liberation of nascent oxygen, **prepared** solution of **Oxy-Powder®** was added to the cups at five different intervals namely 0 minute, 15 minutes, 30 minutes,

45 minutes and 60 minutes. The plates were incubated at 37⁰C for 72-96 hours and recording growth every 24 hours.

CONCLUSION: The *in vitro* experiments did not exhibit inhibition at the given concentration of Oxy-Powder® against the five test cultures *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus bifidigus*, *Enterobacter faecalis* and *Candida albicans* at the given time intervals indicating thereby that even the Probiotic culture, *Lactobacillus bulgaricus* will also probably not be killed by Oxy-Powder® when the latter is used in the patients suffering from constipation and IBS.

NOTE: 1) The results relate only to the items tested.

2) This report shall not be reproduced except in full, without the written approval of the laboratory.

PREFACE

General

Title: **ANTIMICROBIAL ACTIVITY OF OXY-POWDER® AGAINST 5 CULTURES**

Sponsor: **Mayfair Clinical Education and Research Centre, Mumbai**

Monitoring Scientist: **Dr. J. K. Lalla**

Testing Facility: **THE MICRO CHEM LABORATORY**

125, VARDHMAN INDUSTRIAL ESTATE,

GOKUL NAGAR, THANE (W) 400 601

Project No. **9165**

Test Substance **Oxy-Powder®**

Schedule:

15-05-2006 Date of receipt Of Samples

22-05-2006, Preparation and sterilization of Media,

24-05-2006, Inoculation with relevant organism, transfer to sterile Petri Plates and boring of cups.

25-05-2006, Test solution preparation, addition to cups in LF unit and incubation.

29-05-2006, Incubation Results

31-05-2006 Raw data audit:

03-06-2006 Final report audit:

Objective

The objective of this study was to assess the *in-vitro* effect of **Oxy-Powder®** on the normal Probiotic strains and colonies of microbes generally present in the flora of human gig tract

MATERIALS AND METHODS

TEST SUBSTANCE

Sponsor: Mayfair Clinical Education and Research Centre, Mumbai

Label on Sample: Oxy-Powder®

Characteristics of Sample:

Consistency: Solid (Capsule)

Color: White

Disclaimer: The above physicochemical data of test substance is supplied by the Sponsor. All responsibility with regards to the accuracy and authenticity of this information remains with the Sponsor. The test laboratory is not responsible for any variations with the batch number supplied.

SUMMARY REPORT ON *IN-VITRO* ANTI BACTERIAL ACTIVITY OF OXY-POWDER® (4 ORGANISMS + 1 PARASITE)

The Microbiological testing was done by observing following conditions:

Test Organisms:

Pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter faecalis*, *Candida albicans*,

Probiotic organism: *Lactobacillus bifidigus*

Test Method: Cup plate technique.

Concentrations: 1 capsule dissolved in 60 ml sterile distilled water.

25 mg citric acid dissolved in 60 ml sterile distilled water.

1 (Citric acid was included since Oxy-Powder® generates O₂ and is active only in acidic medium)

Frequency of analysis:

0 minute, 15 minutes, 30 minutes, 45 minutes and 60 minutes.

Observations:

No inhibition was observed at the given concentration against the five test cultures *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus bifidigus*, *Enterobacter faecalis* and *Candida albicans* at the given time intervals.

Conclusion:

The results very clearly indicate that Oxy-Powder® is ineffective in killing the test cultures.

It is very encouraging to see that the Probiotic organisms *Lactobacillus bulgaricus* is also not killed by Oxy-Powder® indicating thereby the survival of Probiotic organisms even when Oxy-Powder® is acting in large intestine and colon region against constipation. Other normal physiological processes including generation of B complex factors by the Probiotic organisms shall continue in routine and would avoid administration of B complex factors externally to the patients suffering from constipation and undergoing treatment with Oxy-Powder®.

Signed /-

Prof. J. K. Lalla, Ph.D.

Study Director

VERSION – 01

REPORT FOR
CLINICAL TRIAL PHASE III (CONSTIPATION)
SAFETY AND EFFICACY OF OXY-POWDER®

DOCUMENT NO.: MCERC/REPT-CLTR/OXY/1205/001



May 30th 2007

**TRIAL TO STUDY THE SAFETY AND EFFECTIVENESS
OF OXY-POWDER® IN TREATING CONSTIPATION AND IBS.**

MAYFAIR CLINICAL EDUCATION & RESEARCH CENTRE, (A KALTHIA GROUP ORGANIZATION)

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Part – 4 A
CLINICAL STUDY REPORT
ON OXY-POWDER®
—CONSTIPATION—

CLINICAL STUDY REPORT ON OXY-POWDER®—CONSTIPATION

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ADMINISTRATIVE STATEMENTS

(A)GLOSSARY

ADVERSE EVENT: An unwanted effect caused by the administration of drugs. Onset may be sudden or develop over time

BASELINE: 1. Information gathered at the beginning of a study from which variations found in the study are measured. 2. A known value or quantity with which an unknown is compared when measured or assessed. 3. The initial time point in a clinical trial, just before a participant starts to receive the experimental treatment which is being tested. At this reference point, measurable values such as CD4 count are recorded. Safety and efficacy of a drug are often determined by monitoring changes from the baseline values.

BIAS: When a point of view prevents impartial judgment on issues relating to the subject of that point of view. In clinical studies, bias is controlled by blinding and randomization.

BLIND: A randomized trial is "Blind" if the participant is not told which arm of the trial he is on. A clinical trial is "Blind" if participants are unaware on whether they are in the experimental or control arm of the study; also called masked.

CENTRAL DRUGS STANDARD CONTROL ORGANIZATION (CDSCO): Regulatory authority under Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India responsible for (1)laying down standards of drugs, cosmetics, diagnostics and devices and (2) regulating market authorization of new drugs and clinical research in India.

CLINICAL: Pertaining to or founded on observation and treatment of participants, as distinguished from theoretical or basic science.

CLINICAL ENDPOINT: See Endpoint.

CLINICAL INVESTIGATOR: A medical researcher in charge of carrying out a clinical trial's protocol.

COMPLEMENTARY AND ALTERNATIVE THERAPY: Broad range of healing philosophies, approaches, and therapies that Western (conventional) medicine does not commonly use to promote well-being or treat health conditions. Examples include acupuncture, herbs, etc.

CONFIDENTIALITY REGARDING TRIAL PARTICIPANTS: Refers to maintaining the confidentiality of trial participants including their personal identity and all personal medical information. The trial participants' consent to the use of records for data verification purposes should be obtained prior to the trial and assurance must be given that confidentiality will be maintained.

CONTRAINDICATION: A specific circumstance when the use of certain treatments could be harmful.

CONTROL: A control is the nature of the intervention control.

CONTROL GROUP: The standard by which experimental observations are evaluated. In many clinical trials, one group of patients will be given an experimental drug or treatment, while the control group is given either a standard treatment for the illness or a placebo

CONTROLLED TRIALS: Control is a standard against which experimental observations may be evaluated. In clinical trials, one group of participants is given an experimental drug, while another group (i.e., the control group) is given either a standard treatment for the disease or a placebo.

DOUBLE-BLIND STUDY: A clinical trial design in which neither the participating individuals nor the study staff knows which participants are receiving the experimental drug and which are receiving a placebo (or another therapy). Double-blind trials are thought to produce objective results, since the expectations of the doctor and the participant about the experimental drug do not affect the outcome; also called double-masked study.

EFFICACY: (Of a drug or treatment). The maximum ability of a drug or treatment to produce a result regardless of dosage. A drug passes efficacy trials if it is effective at the dose tested and against the illness for which it is prescribed. In the procedure mandated by the FDA, Phase II clinical trials gauge efficacy, and Phase III trials confirm it

ELIGIBILITY CRITERIA: Summary criteria for participant selection; includes Inclusion and Exclusion criteria.

ENDPOINT: Overall outcome that the protocol is designed to evaluate. Common endpoints are severe toxicity, disease progression, or death.

FOOD AND DRUG ADMINISTRATION (FDA): The U.S. Department of Health and Human Services agency responsible for ensuring the safety and effectiveness of all drugs, biologics, vaccines, and medical devices, including those used in the diagnosis, treatment, and prevention of HIV

infection, AIDS, and AIDS-related opportunistic infections. The FDA also works with the blood banking industry to safeguard the nation's blood supply.

HYPOTHESIS: A supposition or assumption advanced as a basis for reasoning or argument, or as a guide to experimental investigation.

INCLUSION/EXCLUSION CRITERIA: The medical or social standards determining whether a person may or may not be allowed to enter a clinical trial. These criteria are based on such factors as age, gender, the type and stage of a disease, previous treatment history, and other medical conditions. It is important to note that inclusion and exclusion criteria are not used to reject people personally, but rather to identify appropriate participants and keep them safe.

IND: Investigational New Drug.

INFORMED CONSENT: The process of learning the key facts about a clinical trial before deciding whether or not to participate. It is also a continuing process throughout the study to provide information for participants. To help someone decide whether or not to participate, the doctors and nurses involved in the trial explain the details of the study.

INFORMED CONSENT DOCUMENT: A document that describes the rights of the study participants, and includes details about the study, such as its purpose, duration, required procedures, and key contacts. Risks and potential benefits are explained in the informed consent document. The participant then decides whether or not to sign the document. Informed consent is not a contract, and the participant may withdraw from the trial at any time.

INSTITUTIONAL REVIEW BOARD (IRB): 1. A committee of physicians, statisticians, researchers, community advocates, and others that ensures that a clinical trial is ethical and that the rights of study participants are protected. All clinical trials in the U.S. must be approved by an IRB before they begin. 2. Every institution that conducts or supports biomedical or behavioral research involving human participants must, by federal regulation, have an IRB that initially approves and periodically reviews the research in order to protect the rights of human participants.

INTENT TO TREAT: Analysis of clinical trial results that includes all data from participants in the groups to which they were randomized even if they never received the treatment.

IRRITABLE BOWEL SYNDROME: Irritable bowel syndrome (IBS), a functional gastrointestinal disorder characterized by the interplay of altered motility, abnormal visceral sensation, and psychosocial factors, is one of the most common reasons for referral to a gastroenterologist.

MCERC: Mayfair Clinical, Education and Research Centre, Thane.

OPEN-LABEL TRIAL: A clinical trial in which doctors and participants know which drug or vaccine is being administered.

PHASE II TRIALS: Controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks.

PHASE III TRIALS: Expanded controlled and uncontrolled trials after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather additional information to evaluate the overall benefit-risk relationship of the drug and provide an adequate basis for physician labelling.

PLACEBO: A placebo is an inactive pill, liquid, or powder that has no treatment value. In clinical trials, experimental treatments are often compared with placebos to assess the treatment's effectiveness.

PLACEBO CONTROLLED STUDY: A method of investigation of drugs in which an inactive substance (the placebo) is given to one group of participants, while the drug being tested is given to another group. The results obtained in the two groups are then compared to see if the investigational treatment is more effective in treating the condition.

PHARMACOVIGILANCE: Despite all the tests and precautions taken during the development and authorisation of a pharmaceutical product, medicines still sometimes produce side effects under certain conditions. Monitoring use and effects of a given medication to detect and prevent these adverse drug reactions (ADR) is the domain of Pharmacovigilance.

It is regarded as all post-authorisation scientific and data gathering activities relating to the detection, assessment, understanding and prevention of adverse events or any other product related problems.

PROTOCOL: A study plan on which all clinical trials are based. The plan is carefully designed to safeguard the health of the participants as well as answer specific research questions. A protocol describes what types of people may participate in the trial; the schedule of tests, procedures, medications, and dosages; and the length of the study. While in a clinical trial, participants following a protocol are seen regularly by the research staff to monitor their health and to determine the safety and effectiveness of their treatment.

RANDOMIZATION: A method based on chance by which study participants are assigned to a treatment group. Randomization minimizes the differences among groups by equally distributing people with particular characteristics among all the trial arms. The researchers do not know which treatment is better. From what is known at the time, any one of the treatments chosen could be of benefit to the participant.

RANDOMIZED TRIAL: A study in which participants are randomly (i.e., by chance) assigned to one of two or more treatment arms of a clinical trial. Occasionally placebos are utilized.

RISK-BENEFIT RATIO: The risk to individual participants versus the potential benefits. The risk/benefit ratio may differ depending on the condition being treated.

SERIOUS ADVERSE EVENT: A Serious adverse event is any untoward medical occurrence that at any dose a) results in death b) is life threatening c) requires hospitalization or prolongation of existing hospitalisation.

SIDE EFFECTS: Any undesired actions or effects of a drug or treatment. Negative or adverse effects may include headache, nausea, hair loss, skin irritation, or other physical problems. Experimental drugs must be evaluated for both immediate and long-term side effects.

SINGLE-BLIND STUDY: A study in which one party, either the investigator or participant, is unaware of what medication the participant is taking; also called single-masked study.

STATISTICAL SIGNIFICANCE: The probability that an event or difference occurred by chance alone. In clinical trials, the level of statistical significance depends on the number of participants studied and the observations made, as well as the magnitude of differences observed.

STUDY ENDPOINT: A primary or secondary outcome used to judge the effectiveness of a treatment.

STUDY TYPE: The primary investigative techniques used in an observational protocol; types are Purpose, Duration, Selection, and Timing.

(B) REGULATORY APPROVALS

The study protocol, the informed consent, and other information that required pre-approval were reviewed and approved by the Independent Ethics Committee (IEC) of Mayfair Clinical, Education and Research Centre and Institutional Review Board (IRB) of Bhatia General Hospital. A letter along with the Protocol and IEC approval letter was sent to Drugs Controller General of India at Central Drugs Standard Control Organization (CDSCO) office, New Delhi informing him about MCERC conducting Multicentric Clinical Trial, Phase III studies

In this study, Good Clinical Practices described under ICH E6 document, IEC notifications pertaining to Good Clinical Practice (GCP) Guidelines issued by CDSCO and Ethical Guidelines for Biomedical Research on Human Subjects, issued by Indian Council of Medical Research, have been followed.

Appendixes 2–4 contain the study protocol, and details pertaining to the IECs.

(C) INVESTIGATOR'S DECLARATION

Statement of Final Approval of Clinical Report

PROTOCOL NO.: MCERC/REP.CLTR/OXY/1207/001

We, the undersigned, have read and understood this report and hereby assure that the study was conducted in accordance with the approved protocol and SOP's in compliance with all the requirements regarding the obligations of investigators and all other pertinent requirements of the ICH (E-3 and E-6 Guidelines) 'Guidance on Good Clinical Practice', Declaration of Helsinki (Hong Kong, 1989) and national guidelines.. We further undertake that all the essential documents and the Investigational product pertaining to this study will be archived for a period of 3 years on submission of the report to the sponsor.

	Study Director	Study Monitor QA auditor and Clinical Pharmacologist	Investigator-1	Investigator-2	Statistician
Name	Prof. J. K. Lalla (PhD)	Dr.Meena U. Shah (MD)	Dr. Sharad Shah (MD)	Dr.Chetan Bhatt (MD)	Mr. Vinayak Deshpande, MSc
Sign					
Date					

(D) QA STATEMENT

Protocol No.: MCERC/REP.CLTR/OXY/1207/001

Title: Efficacy & Safety of Oxy-Powder®

Objective: To study the safety and efficacy of Oxy-Powder® in patients of chronic constipation and IBS.

Randomized, open, comparative study involving

- 1) 40 patients, both females and males [having non-vegetarians, and vegetarian food habits] suffering from constipation
- 2) 20 patients, both females and males (having non-vegetarians, and vegetarian food habits) suffering from IBS associated with constipation

The Quality Assurance Auditor of Mayfair Clinical, Education & Research Centre audited conduct of clinical Trial PROTOCOL NO,MCERC/REP.CLTR/OXY/1207/001 and verified that it was conducted in accordance with the Protocol approved by the IEC and the sponsor, Good Clinical Practice, Good Laboratory Practices and other applicable regulations and implemented internal standard operating procedures. The study commenced only after the Regulatory and other approvals were obtained.

The study was audited and data verified on the following dates:

06, 07, 08, 14, 16, 30 and 31 August 2006,
01, 11 and September 2006 and
13, 14 and 15 March 2007.

Signed /—

Dr. Mrs Meena U. Shah

QA Auditor Date: 30th May 2007

(E) APPROVAL OF REPORT BY STUDY DIRECTOR

Title of the Study:

Randomized, open, comparative trial to study Safety and Efficacy of Oxy-Powder® Marketed by Global Healing Centre, Inc. USA in treating constipation and IBS to be compared with Dulcolax.

I have read this report and confirm that to the best of my knowledge, it accurately describes the conduct and results of study.

Signed/—

Prof. J.K. Lalla, PhD Date: 30th May 2007

Study Director

**(F) APPROVAL OF STUDY REPORT BY CO-COORDINATOR AND MONITOR,
CLINICAL PHARMACOLOGIST, AND Q.A. DIRECTOR**

Title of the Study:

Randomized, open, comparative trial to study Safety and Efficacy of Oxy-Powder® Marketed by Global Healing Centre, Inc. USA in treating constipation and IBS to be compared with Dulcolax.

I have read this report and confirm that to the best of my knowledge, it accurately describes the conduct and results of study.

Signed/—

_____ ,

Dr. (Mrs.) Meena U. Shah Date: 30th May 2007

[Co-Coordinator, Monitor, Clin. Pharmacologist and Q.A. Director]

(G) APPROVAL OF STUDY REPORT BY BIOSTATISTICIAN

Title of the Study

Randomized, open, Multicentric, comparative trial to study Safety and Efficacy of Oxy-Powder® Marketed by Global Healing Centre, Inc. USA in treating constipation and IBS to be compared with Dulcolax.

I have read this report and confirm that to the best of my knowledge, it accurately describes the results of study.

Signed/—

Vinayak Deshpande Date: 30th May 2007

(Biostatistician)

Contributors to the Study

OVERALL

Prof. J.K. Lalla, Ph.D. – Study Director

CLINICAL TRIAL

- 1)Dr. Sharad C. Shah – Gastroenterologist and Clinical Investigator – Jaslok Hospital
- 2)Dr. Chetan B. Bhatt – Gastroenterologist and Clinical Investigator – Bhatia General Hospital
- 3)Chairperson and members – Independent Ethics Committee of MCERC
- 4)Chairperson and members – Institutional Review Board of Bhatia General Hospital
- 5)Dr. Meena U. Shah – Mayfair Clinical, Education and Research Centre
- 6)Dr. Deven Parmar – Mayfair Clinical, Education and Research Centre
- 7)Ms. Neeta Mote– CRA, Mayfair Clinical, Education and Research Centre
- 8)Mr. Kamlesh Mote– Documentation Assistant, Mayfair Clinical, Education and Research Centre

1.0 Introduction

1.1 Constipation:

Constipation is the slow movement of faeces (stool or body wastes) through the large intestine resulting in infrequent bowel movements and the passage of dry, hard stools. The longer it takes for the stool to move through the large intestine, the more fluid is absorbed and the drier and harder the stool becomes. Constipation is annoying and uncomfortable, but fecal impaction (a collection of dry, hard stool in the colon or rectum) can be life-threatening. Patients with a faecal impaction may not have gastrointestinal symptoms; instead they may have circulation, heart, or breathing problems. If fecal impaction is not recognized, the signs and symptoms will get worse and the patient could die. Chronic constipation is one of the most frequent gastrointestinal symptoms in the United States, accounting for nearly 2.5-2.7 million physician visits and 3900-90000 hospitalizations per year.

1.2 Irritable Bowel Syndrome (IBS):

Irritable bowel syndrome (IBS), a functional gastrointestinal disorder is characterized by the interplay of altered motility, abnormal visceral sensation, and psychological factors. It is one of the most common reasons for referral to a gastroenterologist. It is associated with bouts of constipation and diarrhoea.

1.3 Introduction to Rome II criteria:

At the 13th International Congress of Gastroenterology in Rome, Italy in 1988, a group of physicians defined criteria to more accurately diagnose constipation and IBS. Known as the "Rome Criteria," this set of guidelines that outlines symptoms and applies parameters such as frequency and duration make possible a more accurate diagnosis of Constipation and IBS.

The Rome Criteria were not widely accepted when originally presented, but were better received after their first revision. This second version, created in 1992 and known as Rome II, added a length of time for symptoms to be present and pain as an indicator. The second revision, known as Rome III, is currently underway

1.31 Rome II Criteria for Constipation:

Two or more of the following for at least 12 wk (not necessarily consecutive) in the preceding 12 mo:	
-	Straining during >25% of bowel movements;
-	Lumpy or hard stools for >25% of bowel movements;
-	Sensation of incomplete evacuation for >25% of bowel movements;
-	Sensation of anorectal blockage for >25% of bowel movements;
-	Manual maneuvers (digital evacuation, support of the pelvic floor) to facilitate >25% of bowel movements;
-	Less than 3 bowel movements per week;
Loose stools are not present, and there are insufficient criteria for irritable bowel syndrome	

Acceptable form of birth control being followed in female patients

1.32 Rome II criteria for IBS:

The Rome II diagnostic criteria of Irritable Bowel Syndrome always presumes the absence of a structural or biochemical explanation for the symptoms and is made only by a physician.

Irritable Bowel Syndrome can be diagnosed based on at least 12 weeks (which need not be consecutive) in the preceding 12 months, of *abdominal discomfort or pain that has two out of three of these features*:

1. Relieved with defecation; and/or
2. Onset associated with a change in frequency of stool; and/or
3. Onset associated with a change in form (appearance) of stool.

2.1 Symptoms that Cumulatively Support the Diagnosis of IBS:

1. Abnormal stool frequency (may be defined as greater than 3 bowel movements per day and less than 3 bowel movements per week);
2. Abnormal stool form (lumpy/hard or loose/watery stool);
3. Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);
4. Passage of mucus;
5. Bloating or feeling of abdominal distension.

2.2 Supportive Symptoms of IBS:

1. Fewer than three bowel movements a week
2. More than three bowel movements in a day
3. Hard or lumpy stools
4. Loose (mushy) or watery stools
5. Straining during a bowel movement
6. Urgency (having to rush to have a bowel movement)
7. Feeling of incomplete bowel movement
8. Passing mucus (white material) during a bowel movement
9. Abdominal fullness, bloating, or swelling

2.3 Red Flag symptoms which are NOT typical of IBS:

Pain that often awakens/interferes with sleep

Diarrhoea that often awakens/interferes with sleep

Blood in your stool (visible or occult)

Weight loss

Fever

Abnormal physical examination

3.1 **OXY-POWDER®**: Oxy-Powder® is marketed by Global Healing Centre, Inc. USA

Category: Dietary Supplement

Composition: Ozonated Magnesium Oxides: (685 mg per capsule)

A combination of USP grade Magnesium Oxide mixed with a small amount USP grade Magnesium Peroxide. This combination is then pressurized at subzero temperatures and Ozone gas is the stabilizing molecule. The final product contains Stabilized monatomic oxygen, which is released by an acid based catalyst i.e. HCl in the stomach.

Organic Germanium 132: (5.5 mg per capsule)

Does not contain the harmful form of Germanium (Germanium Dioxide)

Natural Citric acid: (25 mg per capsule)

Filled in Kosher certified “00” Vegetarian Capsules

Description:

Oxy-Powder® is a specifically designed compound which has been ozonated and stabilized to release beneficial free monatomic oxygen into the intestinal tract. The time-release delivery ensures that Oxy-Powder will provide an adequate amount of oxygen, slowly, for better utilization by the body. Oxy-Powder is a non-toxic, safe, effective and non-allergic. By using Oxy-Powder, the compaction from the small intestine, large intestine and colon gets oxidized safely and effectively. Organic Germanium – 132 has demonstrated in multiple scientific studies to be a powerful oxygen facilitator and immune system stimulant. Oxy-Powder is also harmless to the good bacteria in the intestinal tract and Natural citric acid has been added to facilitate oxygen release.

There is only one true way to clean the digestive tract. This is through an oxidation/reduction reaction or a clean raw food diet. As we age, we accumulate toxic substances in our digestive tract. The pancreas, the organ which produces the necessary enzymes to break down the food we eat, is limited. At birth our pancreas has a limited supply of enzymes. Some doctors say we only have enough enzymes to breakdown 1 cooked meal daily for 120 years. This means 80% of our diet should be raw fruits and vegetables.

With the poor diet, particularly non-vegetarian food i.e. eating high fat meals daily, which the body cannot utilize, tremendous strain is put on pancreas that secrete digestive enzymes by the time one is 30-40 years old. It can take the body up to 8 hours to break down proteins, and up to 48-72 hours to digest fats and carbohydrates. Thus, one constantly has undigested food particles in the intestinal tract. It has been estimated that the average person by the age of 40 has between 10-20 pounds of hard compacted faecal matter lodged in their intestinal tract. Human intestinal tract is 30-35 feet in length. In order to fully cleanse the digestive tract, the solid compaction gets into a liquid or gas, using time released oxygen and ozone (oxidation/reduction). By using Oxy-powder, the toxic residue gets oxidized from the small intestine, large intestine and colon, safely and effectively. This is important because a clean intestinal tract is the beginning of obtaining optimal health.

Oxy-Powder is harmless to the Probiotic (helpful) bacteria in the intestinal tract. When the intestinal tract is fully cleansed, the urine becomes cloudy and the stools become semi-solid. When this occurs, a maintenance dose of Oxy-Powder® is recommended. This helps provide oxygen directly into the bloodstream. By staying on a Maintenance Dosage of Oxy-Powder, the intestinal tract is kept clean and provides body with much needed oxygen.

OCCUPATIONAL SAFETY

Investigational product is not expected to pose significant occupational safety risk to site staff under normal conditions of use and administration. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions was available upon request from MCERC. End of Required Standard Wording.

However, precautions were expected to be taken to avoid direct contact with skin, eyes and generated aerosols or mists. In case of unintentional occupational exposure, it was the responsibility of the investigator to treat the patient or the staff in the normal course and report the incidence to the Study Director, MCERC.

Concomitant Medications and Non-Drug Therapies

Contraindications:

There are no contraindications reported when taking Oxy-powder with prescription medicines as long as the Oxy-Powder® is taken 6 hours before or 6 hours after the. Permitted Medications

Investigators were requested to record all concomitant medications taken during the study by the patient in the CRF with indication, dose information, and dates of administration.

Prohibited Medications

Laxatives, anti-diarrhoels and anti-spasmodics

3.1.1 GERMANIUM-132

Germanium is a metalloid element with atomic number 32 and atomic symbol Ge. Germanium is found in the earth's crust, in certain minerals and in living matter such as plants and the human body. Germanium is not an essential nutrient for humans. Organic germanium was first successfully synthesized by Dr. Kazuhiko Asai of Tokyo, Japan. Dr. Asai found that organic germanium protects against cancer by stimulating the production of interferon, a substance that stimulates the production of natural killer (NK) cells, which directly combat cancer cells.

Typical daily dietary intakes of germanium range from about 0.4 to 1.5 milligrams. Plant foods, such as wheat, vegetables, bran and leguminous seeds, are rich sources of germanium. Animal foods are low in germanium.

The main nutritional supplement form of germanium is known as Ge-132, Germanium-132, germanium sesquioxide or bis-carboxyethyl germanium sesquioxide. This is a synthetic organic product. It has not been found naturally.

ACTIONS

Bis-carboxyethyl germanium sesquioxide, Ge-132, may have antiproliferative activity. Ge-132 may also have antioxidant activity and hence, useful in treatment of cancer.

PHARMACOKINETICS

Organic Germanium compounds are rapidly absorbed and eliminated from the body without undergoing metabolic alteration. Within one hour of administration, 50% of the compound is in the gastrointestinal tract; after twelve hours, only 5% is there. Organic Germanium is reabsorbed by the vena portae. One hour after administration, 50% is in the vena portae; after 8 hours, this figure rises to 85%, and by twelve hours, it is quasi complexed. Serum plasma levels reach a maximum two hours after administration; in eight hours, Germanium is reduced by 80% of the maximum. Organic Germanium, administered orally, has also been shown to be absorbed by about 30%, distributed evenly throughout the body, leaving almost no residual concentration after twelve hours. It is excreted, unchanged metabolically, in the urine in twenty-four hours. Germanium is ubiquitously distributed in all organs - there are no specific target organs, and no differences in distribution patterns detected between sexes. Organic Germanium is also eliminated at quite a rapid, linear rate, of approximately 8% of the dose per hour, during the first eight hours. It is completely eliminated after three days, mainly via the kidneys (85%). Germanium is soluble in the interstitial fluids, and is not protein-bound. Germanium does not accumulate in any organ - no Germanium can be found in animals one week after their removal from treatment.

Human Toxicity Studies

In human clinical trials with healthy volunteers, as well as patients who have participated in all the studies described throughout this book, the toxicity of the particular organic Germanium compound was assessed. One of the outstanding features of organic Germanium is its virtual non-toxicity and its ability to be tolerated, in contrast to most highly toxic drugs. Organic germanium may cause minor side effects, including skin rash and diarrhea.

Organic Germanium - Oxygen Enricher and Antioxidant

Stress, anxiety, fear, all can promote oxygen deficiency within the body Organic Germanium enriches the body's oxygen supply and is also a potent antioxidant, properties which contribute to this trace element's widespread beneficial effects upon many inter-related metabolic process in the body

Organic Germanium Enriches Oxygen Supply

The structure of organic Germanium, a crystalline lattice network extensively bonded with negative oxygen ions, is said to actually substitute for oxygen, and to enable the attraction and elimination of acidifying hydrogen ions, which detoxifies the blood. In the electron transport scheme during oxidative metabolism, electrons are transferred along a set of electron acceptors, ending up, ultimately with the combination of hydrogen and oxygen to form water. However, when there is an oxygen deficiency, the loss of electrons can result in the accumulation of positive hydrogen ions, which lead

to blood acidification. Ge-132 has negatively charged oxygen ions, which can clear away these hydrogen ions, and thus detoxify the blood.

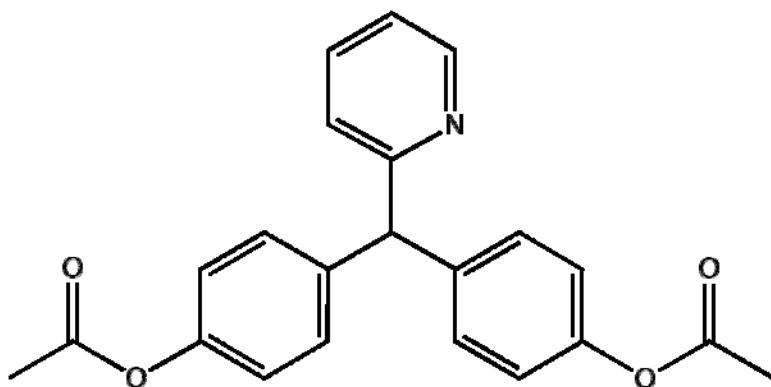
3.2 Bisacodyl (Dulcolax):

Bisacodyl is a popular non-prescription type of stimulant laxative. The drug reduces short-term constipation and is also used for preparing the colon or rectum for an examination or surgical procedure. This over-the-counter laxative can be taken by mouth or per rectum for treating constipation

Brand Name: Dulcolax, Fleet, Alophen, Correctol

Chemical Description: [4-[(4-acetyloxyphenyl)-pyridin-2-yl-methyl] phenyl] acetate

Structure of Bisacodyl:



WORKING:

Bisacodyl is a stimulant laxative. It causes muscles in the colon to contract and stools to pass. Bisacodyl works by stimulating colon movement (peristalsis). Constipation is generally relieved within 15 minutes to 60 minutes after administration of a suppository form and in six to twelve hours after oral administration

DOSAGE:

The drug is taken at night, if administered orally, and during morning if taken through the rectum. The drug is usually sold as 5mg tablets, 10mg suppositories, or 5mg pediatric suppositories

SIDE EFFECTS: This medication may cause stomachache, cramping, weakness, sweating, irritation of the rectal area, diarrhea, or dizziness. Notify your doctor if you experience: chest pain, fainting, rectal bleeding, lack of a bowel movement (especially after using this medicine). If you notice other effects not listed above, contact your doctor or pharmacist.

PRECAUTIONS: If you have any of the following conditions, do not take Bisacodyl without consulting your doctor: severe nausea, vomiting, stomach/intestinal problems, recent abdominal surgery, rectal bleeding, vitamin/mineral deficiencies, appendicitis, gastritis, an allergic reaction to this medication. Frequent use of laxatives can cause dehydration and loss of essential nutrients. Symptoms can include muscle cramps, muscle weakness or dizziness. Maintain adequate fluid intake while using this medication. Before using Bisacodyl, tell your doctor if you are pregnant. It is not known if Bisacodyl is excreted into breast milk. Consult your doctor.

Note: Nursing and pregnant women should consult a doctor before taking the drug.

DRUG INTERACTIONS: Tell your doctor of all prescription and nonprescription drugs you may use, especially of: potassium supplements, antacids, acid blockers (e.g., cimetidine, famotidine, and ranitidine), other laxatives. Do not start or stop any medicine without doctor or pharmacist approval

OVERDOSE: If overdose is suspected, contact your local poison control center or emergency room immediately. US residents can call the US national poison hotline at 1-800-222-1222. Canadian residents should call their local poison control center directly. Symptoms of overdose may include persistent diarrhea, nausea, vomiting and loss of appetite, muscle cramps, severe weakness, extreme thirst, or dry mouth.

NOTES: To help prevent constipation, drink 4 to 6 glasses of water daily, eat dietary fiber and/or leafy substances and exercise regularly.

MISSED DOSE: If you miss a dose, skip the missed dose. Do not double the dose

STORAGE: Store at room temperature between 59 and 86° F (between 15 and 30° C) away from moisture and sunlight. Do not store in the bathroom

USES: This medication is used to treat constipation or to clean out the intestinal tract before bowel examinations or bowel surgery.

HOW TO USE: Swallow the tablets whole with a full glass of water or juice. Do not crush or chew the tablets. The tablets should work within 6 to 10 hours. Do not take the tablets within one hour of taking any milk or dairy products. Severe stomach cramps and vomiting may occur. Bisacodyl should not be used longer than seven days without consulting your doctor. Prolonged use can lead to laxative dependence. Because this medication must be swallowed whole, do not give it to a child less than 6 years of age.

4.0 Rationale:

The effectiveness of the product in treating constipation and IBS in patients following

- ❖ Recording loss of weight
- ❖ The effectiveness of the product for Oxygen delivery in patients
- ❖ The effects Oxy-Powder has on the 4 microbes + 2 parasites present in the digestive tract including normal Probiotic strain and colonies in the digestive tract.
- ❖ Safety studies involving acute and sub-acute toxicity in rats/ mice (including histopathology)

4.1 Objectives of the study:

To find the effectiveness and safety of Oxy-Powder® in treating constipation

5.0 METHODOLOGY:

5.1 Conduct of study:

Randomized, open, Multicentric, comparative trial to study Safety and Efficacy of Oxy-Powder® Marketed by Global Healing Centre, Inc. USA in treating constipation to be compared with Dulcolax.

The present study was conducted to clinically evaluate safety and effectiveness of Oxy-Powder® in treating constipation and IBS–associated constipation precipitating loss of weight & improving oxygen delivery in patients of constipation and IBS–associated constipation.

5.1.1 Ethical Conduct of the Study:

This study was conducted in accordance with the International Conference on Harmonisation (ICH) Guidelines for GCP and all applicable regulatory requirements, including the Declaration of Helsinki

5.1.2 Subject Information and Consent:

Written informed consent was obtained from each subject before the performance of any study-specific procedures. The subject informed consent form used for this study is included in App.6

6.0 Investigators and Administrative Structure:

6.1 Investigators and Study Centres:

Subjects were enrolled in 2 centres. Following table presents the names of the investigators and their respective centres.

1	Dr.Sharad.C.Shah	Dr.Sharad.C.Shah's Clinic
2	Dr. Chetan B. Bhatt	Bhatia General Hospital

6.2 Study Administrative structure:

The study was sponsored by Dr. Edward Group III, CEO, and Global Healing Center, Inc. & contracted to Mayfair Clinical, Education & Research Centre, a Clinical Research Organisation (CRO) for conduct of the study.

7.0 Design and conduct of the study:

7.1 Study Design and Duration:

RANDOMIZED, OPEN, MULTICENTRIC, COMPARATIVE TRIAL

The study was done as Multicentric, Open, Randomized study comparing efficacy and safety of Oxypowder developed by Global Healing Centre Inc. USA with Dulcolax tablets containing 5 mg Bisacodyl manufactured by Cadila Health Care Ltd.

The duration of study was 6 weeks from the date of administration of the study products.

7.2 STUDY POPULATION

Any subject, who has given informed consent to participate in the clinical study and has met all the criteria required for inclusion into the clinical study, may take part in the research.

Subject participation in the research project is voluntary and refusal to participate will not indicate withdrawal from the clinical study. Refusal to participate will involve no penalty or loss of benefits to which the subject would otherwise be entitled.

No additional administration of investigational product beyond the dose detailed in the clinical study is permitted.

7.3 Number of Patients enrolled:

40 patients (females + males+ geriatric patients), suffering from constipation

20 patients (females + males), suffering from IBS.

8.0 PATIENT SELECTION

8.1 Inclusion Criteria for Constipation and IBS:

- ❖ **Patients suffering from constipation and IBS**
- ❖ **Age - 18 to 60 yrs**
- ❖ **Height & Weight conforming to Height/Weight chart of Life Insurance Corporation of India**
- ❖ **Sex – Patients belonging to both sexes were eligible**
- ❖ **Food habits-both non-vegetarians and vegetarian patients were eligible (More of the non-vegetarian patients were preferred).**
- ❖ **Satisfied Rome II Criteria for Constipation and IBS**

8.2 Exclusion Criteria for Constipation and IBS:

A subject was not considered eligible for inclusion in this study if any of the following conditions were observed in the patient

1. Evidence of Malignancy on colonoscopy / having diagnosed organic gig disorder
2. Pregnant and lactating women
3. Evidence of lactose intolerance to explain bowel symptoms
4. History of cardiac arrhythmias or heart disease
5. History of Glaucoma
6. History of urine retention
7. History of schizophrenia
8. History of substances of abuse/dependency
9. Intellectually unable or unwilling to complete daily gig ratings

8.3 Other Eligibility Criteria Considerations:

To assess any potential impact on subject eligibility with regard to safety, the investigator must refer to the following document(s) for detailed information regarding warnings, precautions, contraindications, adverse events, and other significant data pertaining to the investigational product(s) being used in this study.

9.0 Treatment and Timings:

Test Product (A) – Oxy-Powder® capsules marketed by Global Healing Centre, Inc. USA containing 715.5 mg Active in each capsule

Comparative Product (B) – Dulcolax tablets marketed by Cadila Healthcare Ltd, India containing 5 mg Bisacodyl in each tablet.

9.1 DOSAGE & ADMINISTRATION:

The patients were divided in two (2) groups as per the Randomization schedule. Group 1 was administered Product A while Group 2 was administered Product B

9.1.1 Test Product (A):

For first time, patients started with a seven-day initial cleansing procedure. After the initial cleanse, a maintenance dose was continued for keeping intestinal tract clean and deliver oxygen into the system. Patients were advised to take Oxy-Powder with plenty of potable water during the day and eat healthy diet.

(a) For Cleansing:

Patients were directed by the investigators to take four (4) capsules in the evening on an empty stomach with 240 mL water. If 3-5 bowel movements were not achieved the following day, the dosage was increased by adding two (2) capsules every night until 3-5 bowel movements were achieved the following day. The day on which the dosage was finalized was considered as “day one (1)” of the seven (7) day cleansing cycle. This dosage was continued for seven (7) consecutive days. After the seven (7) day cleansing cycle, the maintenance dose was started.

(b) Maintenance Dose:

Patients were given capsules supplies for seven (7) days with instructions that they had be taken as they did during cleansing cycle. For following 5 weeks, the same dosage was to be repeated by the patients every other day. They were assured that the capsules were not habit forming or harmful to the body and could be taken indefinitely.

9.2 Comparative Product (B):

Patients in this Group were directed by the investigators to take two tablets of Dulcolax (comparative formulation) as per the Randomization scheme with plenty of water every day, for first 7 days, in the evening on empty stomach. Thereafter, the subjects were instructed to take the same dose of Dulcolax tablets on alternate days on empty stomach in the evening for 6 weeks. Administration on day ‘0’, ‘3

weeks' and '6 weeks' was done on empty stomach (to enable measuring Oxygen levels) in the morning. Subjects were asked to report to the hospital on empty stomach in the morning for administration of Investigational products and Oxygen level in the blood was measured 15 minutes and 30 minutes after administration of tablets/ capsules. On the remaining days, the subjects were asked to take same dose of the Investigational product at their residence in the evening on empty stomach followed by Dinner two hours post-administration of the dose..

9.3 Study Population:

40 patients (females + males+ geriatric patients), suffering from constipation

9.4 Study Assessments and General Procedure for both Products:

Treatment period– 6 weeks

9.4.1 Study Procedure and Treatment:

After selection the patients were subjected to detailed medical History and clinical examination Patients then were subjected to the following pre-study evaluation :-

- I. Record of weight,
- II. Oxygen content of Blood This was done by using pulse oximeter,
- III. Stool examination,
- IV. Barium meal to rule out any Bowel organic lesion leading to constipation,
- V. Colonoscopy to rule out any colonic organic lesion and any faecal impaction,

After ruling out the presence of any organic lesion, the patients were included in the study.

They were instructed to take four capsules of Oxy-Powder® (Test formulation) or two tablets of Dulcolax (Comparative formulation), as per the Randomization scheme, with plenty of water, every day, for first 7 days, in the evening on empty stomach. Thereafter, subjects were instructed to take the same dose of either test or reference formulation on alternate days, on empty stomach, in the evening, for period of 6 weeks. Administration on day '0', '3 weeks' and '6 weeks' were done on empty stomach in the morning. Subjects were asked to report to the hospital with empty stomach; in the morning for administration of investigational products and Oxygen level in the blood were measured 15 minutes and 30 minutes after administration of tablets/capsules.

On the remaining days, the subjects were instructed to take investigational product at home at the same dose levels in the evening on empty stomach. They were told to take Dinner at least two hours post-administration of Drug.

Along with Oxypowder, drinking of lots pure water was suggested. This was not only healthy for the body but was necessary to aid the body in eliminating toxins from the bowel at a faster rate. Oxy-Powder works with stomach acid; if the level of HCl is below normal, it may hinder the effectiveness of the Oxy-Powder; hence it was suggested to take an organic lemon wedge squeezed into glassful of purified water with the Oxy-Powder® in the evening.

During the study, prescription medicines were not contraindicated as long as Oxypowder was taken 6 hours before or 6 hours after the medicine.

Laxatives, anti-diarhoeals and anti-spasmodics were prohibited during the study.

After starting the study medication, patients were initially called every day in the morning to check their daily weight for 7 days

After doing the oxygen content on Day 0, all the patients were asked to visit on 3rd week and the 6th week. They were called in the morning in fasting state.

Patients in Group 1 were administered Oxy-Powder® capsules and their oxygen levels were measured at 15 and 30 minutes post– administration of the dose. In case of Patients in Group 2, their oxygen levels were measured at 15 and 30 minutes after their arrival at the clinical site without administering Dulcolax, since they were comparative group.

On 3rd and the 6th week of treatment, the patients were inquired about their feeling of well being as a result of taking treatment and whether they experienced any adverse effects during the treatment period described to them by the investigators during counselling.

Stool analysis was repeated at the end of 6 weeks period.

During the course of treatment, the patients were instructed to report ADR's immediately to the investigator at the study centre. This information was also collected during personal interview the investigators had during their visits to the study centre on 7th day and at the end of 3rd week and the 6th week.

9.5 ENDPOINT(S):

In patients

- a) Findings of (i) Stool Examination (ii) Barium Meal and (iii) Colonoscopy
- b) Relief of Constipation and IBS resulting in improvement in “Quality of life” of the patient.

10.0 DEMOGRAPHY:

Demographic data including **Height, Weight, Age, and Sex** was collected for all the subjects in different groups at the time of their inclusion in the study simultaneous to recording of

their Medical History including their Food Habits, Smoking History, Details and Quantity of consumption of alcoholic drinks and other beverages in a day or frequency thereof and consumption of Tobacco in other forms or any Drug of Abuse.

As regards the symptom of constipation, duration of its existence, its severity, No. of Bowels / Week and type of stools were also recorded.

GRADING OF RESPONSE

The evaluated parameters were compared with those obtained with Dulcolax administered to the patients belonging to the constipation group.

11.0 EFFICACY:

a) Overall therapeutic response was graded individually by the investigator with each patient as ‘Excellent’, ‘Good’, ‘Fair’ & ‘Poor’.

b) Therapeutic efficacy was correlated and graded as ‘Complete Cure’, ‘Improvement’ and ‘Failure’.

12.0 SAFETY:

Severity of each **Adverse Event** was rated as ‘Mild’ (no limitation of usual activity), ‘Moderate’ (some limitation of usual activity), and ‘Severe’ (inability to carry out usual activities).

13.0 WITHDRAWAL FROM STUDY:

One patient in Oxypowder group had severe diarrhoea on the 2nd day of treatment. He could not carry out his usual activities and felt dehydrated. He voluntarily withdrew from the study on 3rd day. Remaining 26 patients continued with the study.

14.0 STATISTICS AND DATA HANDLING:

14.1 STATISTICAL ANALYSIS– DERIVED AND TRANSFORMED DATA:

Out of 40 patients, 39 patients completed the study & were included in the final analysis. The patients from two centres were pooled for analysis. Their demographic features were compared at baseline using appropriate tests (Student’s T-Test and Chi-square or Fisher’s exact test). Efficacy parameters in two treatment groups were compared by using Fisher’s exact test.

14.2 DATA ANALYSIS:

14.2.1 Hypotheses

Oxy-Powder® is both, safe and effective in patients with constipation

14.2.2 Sample Size Consideration

The study design had been examined and approved by a qualified statistician with respect to sample size.

14.2.3 Primary Comparisons of Interest

Oxy-Powder® is compared for effectiveness in relieving constipation vis-à-vis Dulcolax tablets in the same group.

14.2.4 Other Comparisons of Interest

Comparison of safety of Oxy-Powder® has been done via safety achieved post-administering of Dulcolax tablets.

14.2.5 Interim Analysis

Data from patients was collected regularly but analyzed at the end of the study for safety and effectiveness of the Oxy-Powder®.

14.2.6 Analysis Population

After the dropouts, it was assumed to have a minimum of 30 patients (out of 40) in constipation group completing the study. Number of patients who actually completed 6 weeks of study was 39. The population analysis for safety and effectiveness of Oxy-Powder® and Dulcolax has been done for these 39 patients at the end of the study.

14.2.7 Withdrawal

One patient who voluntarily withdrew from the study has not been included in the report

14.2.8 Missing Data

There has been no **Missing data** of any patient.

15.0 OTHER ISSUES:

Results of Barium Meal test conducted at the commencement of study at pre– dose administration indicated that none of the patients exhibited any signs of organic lesions.

In 7 patients completing the study, no organic lesions were seen in Barium meal test even 6 weeks post– dose administration at the completion of the study.

In the same 7 patients completing the study (of Oxy-Powder® as well as Dulcolax) , pre– dose colonoscopy as well as 6 weeks post dose administration colonoscopy studies showed similar findings in patients under study in having multiple spasmodic contractions in sigmoid and descending colon **but with slight reduction in impacted faecal material shown in post– administration colonoscopy.** This is perhaps one of the factors responsible for

efficacy of Oxy-Powder® in the treatment of constipation with and without IBS. Six weeks period of study was perhaps short to see greater reduction in impacted faecal material

The investigators recommended that since conduct of these two tests after 6 weeks of completion of the study did not show any significant differences that could lead to any conclusions, they should be dropped at the conclusion of the study. However, the patients should continue to be subjected to these two tests at the commencement of study.

These recommendations were forwarded to the sponsor by e-mail who gave his consent to follow the recommendations of the investigator and permitted waiving of conduct of these two tests at the end of 6 weeks period of the study in each of the rest of the patients.

16.0 ETHICS:

The design of the protocol conformed to the Declaration of Helsinki adopted by the 18th World Medical Assembly (WMA), Helsinki, Finland (1964) and all amendments. All the patients were informed of the nature and the purpose of the study and their consent to participate was obtained. They were also informed of their freedom to withdraw from the study at anytime and at any stage of the trial.

The study was conducted only after the protocol was approved by IEC of MCERC and IRB of Bhatia General Hospital.

Since Oxy-Powder® was marketed in the USA and other countries for almost a decade with no reported ADR's, and the present study was designed and conducted for global markets (other than India), the study director prudently and by way of abundant precaution informed Indian Regulatory Authority, Drugs Controller General of India at CDSCO, New Delhi, of intention of MCERC to conduct the clinical trial Phase I on Oxy-Powder® in Indian patients.

16.1 ETHICAL CONDUCT OF THE STUDY AND ETHICS APPROVAL

This study was conducted in accordance with "Good Clinical Practice" (GCP) guidelines and all other applicable regulatory requirements, including WMA's Declaration of Helsinki –VI on Ethical Principles for Medical Research Involving Human Subjects, as adopted by the 52nd WMA General Assembly, Edinburgh, October 2000.

The investigator was assigned the responsibility for ensuring that this protocol, the site's informed consent form, and any other information that will be presented to potential subjects (e.g. advertisements or information that supports or supplements the informed consent) were

reviewed and approved by the appropriate IEC/IRB. The investigator agreed to allow the IEC / IRB direct access to all relevant documents. MCERC provided the investigator, IEC and IRB with relevant document(s)/data that were needed for IEC/IRB review and approval of the protocol for conducting the study. After receiving copies of the IEC/IRB approval, the investigational product(s), blank copies of the approved informed consent forms, CRF's and any other information that the IEC/IRB had approved for presentation to potential subjects. were sent to the trial centre sites, No further amendments were made in the above formats by IEC/IRB

17.1 INFORMED CONSENT

Informed consent was obtained from each participant before the subject was permitted to participate in the study. The contents and process of obtaining informed consent was in accordance with all applicable regulatory requirements.

18.0 PROTOCOL COMPLIANCE:

There were three deviations in the protocol.

1) Due to non-availability of suitable patients, request was made to sponsor to reduce the number in constipation group to 40 in place of 60 (as described in the Protocol). This was necessary to avoid delay in completing the study. The sponsor gave his consent. Consequently, 27 patients were assigned to Oxy-Powder® group and remaining 13 patients to Dulcolax.

2) Age of the patients for enrollment was raised to 65 years to include geriatric patients in whom constipation is more prevalent. There was shortage of patients suffering from constipation in the age group specified in the protocol.

Permission from the sponsor was obtained to include patients suffering from constipation up to the age of 65 years.

3) During course of the study, both the investigators independently made following observation:

a) Pre- and post-colonoscopy showed multiple spasmodic contractions in sigmoid and descending colon with large amount of fecal impaction in entire colon.

b) In Barium meal test, no organic lesions were seen before and after Barium meal administration. These observations were made in case of type 1 and type 2 patients.

Both the investigators recommended that in view of these observations, post-colonoscopy and post-administration of Barium meal should not be done since they do not significantly contribute to the results of the study. Instead, they were causing discomfort discouraging the patients from continuing with the treatment. They recommended that both the tests must be done at the time of enrolling the patients.

These recommendations were forwarded to the sponsor who, while agreeing with the suggestions of the investigators permitted to waive conduct of post- colonoscopy as well as post administration of Barium meal.

19.0 RESULTS:

(a) DESCRIPTIVE:

(i) Demography:

The baseline demographic characteristics of patients were similar in two treatment groups of the study (P> .05). Summary of patient's characteristics including sex, age & weight is presented in Table 1

Table 1– Demographic Characteristics.

Drug & No. of Patients	Age (yrs) Mean (SD) Min. Max	Sex %		Wt. (Kg.) Mean (SD) Min. Max
		Male	Female	
A Oxypowder 27	37.77 (11.251) (20.58)	19 (70.4%)	8 (29.6%)	64.26 (12.841) (42.96)
B Dulcolax 13	40.62 (11.666) (22.61)	8 (61.5%)	5 (38.5%)	62.31 (14.419) (30.89)

(ii) Patient's living style

The food habits of the patients are given in Table 3. Both Non-Vegetarian and Vegetarian patients were included in the study.

Table 2 Food Habits.

Drug	Non veg (%)	Veg. (%)
A (Oxypowder) – 27	13 (48.1%)	14 (51.9%)
B (Dulcolax) – 13	7 (53.8%)	6 (46.2%)

In both the groups, some patients were smokers – Table 3.

Table 3 Smoking History

Drug	Yes	No
A (Oxypowder) – 27	4 (14.8%)	23 (85.2%)
B (Dulcolax) – 13	2 (15.4%)	11 (84.6%)

(iii) Medical History:

According to Rome II criteria for constipation, all patients were suffering from constipation for 12 weeks (3 months) or more. They were grouped as follows – Table 4

Table 4– Duration of constipation in months (Group wise)

<i>Groups</i>	<i>A (Oxypowder)</i>	<i>B (Dulcolax)</i>
3 months – 12 months	13 (48.1%)	6 (46.2%)
12 months – 24 months	6 (22.2%)	4 (30.8%)
> 24 months	8 (29.6%)	3 (23.1%)
Total	27 (100%)	13 (100%)

After grouping the patients according to duration of constipation, they were further grouped according to number of bowels passed in a week (Table 5). This was done to grade the patients in terms of severity of constipation (Table 6)

Table 5. No. Of Bowels in a Week for OP and DL on day 0 for Classifying Patients before study commenced

<i>Groups</i>	<i>A(Oxypowder)</i>	<i>B (Dulcolax)</i>
1 – 4	11 (40.7%)	8 (61.5%)
5 – 7	12 (44.4%)	4 (30.8%)
8 – 14	4 (14.8%)	1 (7.7%)
Total	27 (100%)	13 (100%)

Table 6 Severity of Constipation.

<i>Severity</i>	<i>A (Oxypowder)</i>	<i>B (Dulcolax)</i>
Mild	1 (3.7%)	- (0%)
Moderate	17 (63.0%)	8 (61.5%)
Severe	9 (33.3%)	5 (38.5%)
Total	27 (100%)	13 (100.0%)

(P > .05).

All above groups were statistically compared by using appropriate tests & there was no statistically significant difference between the two groups indicating they were comparable at the base line values

(IV) Study Medication:

Randomization:

The randomization of the patients was done as 2:1 i.e. 2 patients were given Oxypowder & 1 patient was given Dulcolax. Out of 40 patients, 27 patients received Oxypowder (A) & 13 patients received Dulcolax (B). Their disposition for the study is presented in Table 7.

Table 7 – Randomization

Oxypowder (A)	27
Dulcolax (B)	13
Total	40

(V) Non–Study Medication:

None of the patients received any medication other than the Test or the Reference formulation.

(VI) Patient Withdrawal:

One patient receiving Test formulation (Oxy-Powder®) withdrew on the second day of treatment as he had severe diarrhoea. He could not carry out his usual activities and felt dehydrated and hence withdrew from the study on the 3rd day. He withdrew mainly because of **Safety Reasons.**

(b)EFFICACY:

All the patients were checked for their daily weight for 7 days (0 + 6 days) & then on 3rd week & 6th week.

Table 8 shows comparison of their weights From Day 0 to day 42.

Table 8 – Comparison of weight (kg) (Day 0 – Day 42)

<i>Drug</i>	<i>No</i>	<i>Mean (\forall S.D.)</i>
A(OP) Day 0	27	65.19 (12.670%)
Day 1	27	64.96 (12.820%)
Day 2	27	64.89 (12.845%)
Day 3	26	65.10 (13.081%)
Day 4	26	65.13 (13.016%)
Day 5	26	65.00 (13.159%)
Day 6	26	65.02 (13.196%)
Day 21	26	65.23 (13.240%)
Day 42	26	65.33 (13.217%)
B (DL) Day 0	13	64. 00 (11.255%)
Day 1	13	63.65 (11.557%)

<i>Drug</i>	<i>No</i>	<i>Mean (\forall S.D.)</i>
Day 2	13	63.42 (11.310 %)
Day 3	13	63.50 (11.673%)
Day 4	13	63.31 (11.494%)
Day 5	13	63.19 (11.640%)
Day 6	13	63.23 (11.499%)
Day 21	13	63.23 (11.263%)
Day 42	13	63.81 (11.426%)

One patient from Oxypowder (A) groups was dropped from the study on 3rd day & hence not included in the final assessment.

As seen from the table, there is no reduction in the weight of the patients after 42 days as compared to base line (0 day) indicating administration of Oxypowder did not lead to reduction of weight in the patients.

Similarly there was no reduction in the weight in Dulcolax group.

In both groups, after starting administration of the study drug; frequency of stools

(Number of Bowels in a week) increased as shown in Table 9.

Table 9 – Number of Bowels in a week (grouped)

<i>Group</i>	<i>Day 0</i>		<i>Day 21</i>		<i>Day 42</i>	
	A (OP)	B (DL)	A (OP)	B (DL)	A (OP)	B (DL)
1 – 4	11 (40.7%)	8 (61.5%)	1 (3.8%)	1 (7.7%)	- (0%)	1 (7.7%)
5 – 7	12 (44.4%)	4 (30.8%)	10 (30.5%)	7 (53.8%)	6 (23.1%)	2 (15.4%)
8 14	4 (14.8%)	1 (7.7%)	12 (46.2%)	5 (38.5%)	19 (73.1%)	8) 61.5%)
> 14			3 (11.5%)	- (0%)	1 (3.8%)	2 (15.4%)
	27	13	26	13	26	13

Note: OP = Oxy-Powder®, DL= Dulcolax

As seen from Table 9 in Oxypowder group, 11 patients had frequency of stools as 1-4 / week on Day 0. After starting Oxypowder, frequency increased and on Day 42, 19(73.1%) patients had frequency of 8-14/ week.

Increase in the frequency of stools observed in A Group was more than in B Group.

Oxypowder releases nascent oxygen for its action and the objective of the study was to seen whether Oxypowder increases oxygen saturation of blood. As seen from Table 10, there was marginal increase in oxygen delivery after taking Oxypowder for 6 weeks (42 days) as compared to Day 0.

Table 10 – Oxygen content of blood (Day 0, Day 21, and Day 42)

<i>Drug</i>	<i>No</i>	<i>Mean (±S.D)</i>
Drug A (OP) Oxygen Content of Blood Day 0	27	98.85 (.907)
Oxygen Content of Blood Day 21 after 15 Min.	26	98.27 (.724)
Oxygen Content of Blood Day 21 after 30 Min.	26	99.46 (.508)
Oxygen Content of Blood Day 42 after 15 Min.	26	98.19 (.895)
Oxygen Content of Blood Day 21 after 30 Min.	26	99.50 (.510)
Drug B (DL) Oxygen Content of Blood Day 0	13	99.00 (.408)
Oxygen Content of Blood Day 21 after 15 Min.	13	98.54 (.519)
Oxygen Content of Blood Day 21 after 30 Min.	13	99.38 (.650)
Oxygen Content of Blood Day 21 after 15 Min.	13	98.46 (.967)
Oxygen Content of Blood Day 21 after 15 Min.	13	99.46 (.519)

During the study, the patients in both groups showed significant reduction in various symptoms as shown in Tables 11, 12, 13, 14, 15 ($P < .05$)

Table 11 (a) Straining During >25% of Bowel Movement (Day 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A(OP)	
NO	2 (7.4)
YES	25 (92.6)
Total	27(100.0)
B (DL)	
NO	1 (7.7)
YES	12 (92.3)
Total	13 (100.0)

Table 11(b) Straining During > 25% of Bowel Movement (Day 21).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	13 (50)
YES	13 (50)
Total	26 (100.0)
B (DL)	
NO	5 (38.5)
YES	8 (61.5)
Total	13 (100.0)

Table 11(c) Straining During > 25% of Bowel Movement (Day42).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	24 (92.3)
YES	2 (7.7)
Total	26 (100.0)
B (DL)	
NO	9 (7.7)
YES	4 (92.3)
Total	13 (100.0)

Table 12

Table12 (a) Lumpy Stools (Day 0).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	1 (3.7)
YES	26 (96.3)
Total	27 (100.0)
B (DL)	
NO	2 (15.4)
YES	11 (84.6)
Total	13 (100.0)

Table 12 (b) Lumpy Stools (Day 21).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	21 (80.8)
YES	5 (19.2)
Total	26 (100.0)
B (DL)	
NO	8 (61.5)
YES	5 (38.5)
Total	13 (100.0)

Table 12(c) Lumpy Stools (Day 42).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	24 (92.3)
YES	2 (7.7)
Total	26 (100.0)
B (DL)	
NO	11 (84.6)
YES	2 (15.4)
Total	13 (100.0)

Table 13(a) Sensation of incomplete evacuation for >25% of Bowel movement (Day 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	2 (7.4)
YES	25 (92.6)
Total	27 (100.0)
B (DL)	
NO	4 (30.8)
YES	9 (69.2)
Total	13 (100.0)

Table 13(b) Sensation of incomplete evacuation for >25% of Bowel movement (Day21).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	9 (34.6)
YES	17 (65.4)
Total	26 (100.0)
B (DL)	
NO	3 (23.1)
YES	10 (76.9)
Total	13 (100.0)

Table 13(c) Sensation of incomplete evacuation for >25% of Bowel movement Day 42.

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	21 (80.8)
YES	5 (19.2)
Total	26 (100.0)
B (DL)	
NO	6 (46.2)
YES	7 (53.8)
Total	13 (100.0)

Table 14(a) Sensation of Anorectal Blockage for >25% of Bowel Movement (Day 0).

<i>Drug</i>	<i>No. of patients(%)</i>
A (OP)	
NO	12 (44.4)
YES	15 (55.6)
Total	27 (100.0)
B (DL)	
NO	6 (46.2)
YES	7 (53.8)
Total	13 (100.0)

Table 14(b) Sensation of Anorectal Blockage for >25% of Bowel Movement (Day 21).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	25 (96.2)
YES	1 (3.8)
Total	26 (100.0)
B (DL)	
NO	13 (100.0)
Total	13 (100.0)

Table 14(c) Sensation of Anorectal Blockage for >25% of Bowel Movement (Day 42).

<i>Drug</i>	<i>No. of patients(%)</i>
A(OP)	
NO	25 (96.2)
YES	1 (3.8)
Total	26 (100.0)
B (DL)	
NO	11 (84.6)
YES	2 (15.4)
Total	13 (100.0)

Table 15 (a) Manual Manoeuvres to facilitate >25% of Bowel Movements (Day 0).

<i>Drug</i>	<i>No. of patients(%)</i>
A (OP)	
NO	16 (59.3)
YES	11 (40.7)
Total	27 (100.0)
B (DL)	
NO	13 (100.0)
Total	13 (100.0)

Table 15(b) Manual Manoeuvres to facilitate >25% of Bowel Movements (Day 21).

<i>Drug</i>	<i>No. of patients(%)</i>
A(OP)	
NO	26 (100%)
Total	26 (100.0)
B (DL)	
NO	13 (100.0)
Total	13 (100.0)

Table 15(c) Manual Manoeuvres to facilitate >25% of Bowel Movements (Day 42).

<i>Drug</i>	<i>No. of patients (%)</i>
A (OP)	
NO	26 (100.0)
Total	26 (100.0)
B (DL)	
NO	13 (100.0)

<i>Drug</i>	<i>No. of patients (%)</i>
Total	13 (100.0)

As seen from Table 11, in group A, 25 patients (92.6 %) had straining on Day 0 as compared to this on Day 42 only 2 (7.7%) patients had this symptom.

Similarly, in Table 12, in group A, 26 patients (96.3%) passed hard and lumpy stools on Day 0 where as on day 42 it was reported only in two patients (7.7%). Above parameters indicate that there was significant reduction in the symptoms ($P < 0.05$) in both groups.

Routine stool examination done before and after the drug treatment showed no abnormality in all 40 patients in this study.

Overall Efficacy:

Overall Efficacy was independently judged by the Investigators & the patients. It was graded as excellent, Good, Fair and Poor. The results were statistically analysed using Fisher's Exact Test.

Table 16–Overall Efficacy

(A) ASSESSMENT BY INVESTIGATORS

ASSESSMENT– InvestigatorS INVESTIGATORS INVESTIGATORS INVESTIGATORS INVESTIGATORS	<i>DRUG</i>		(P < 0.05)
	<i>A (OP)</i>	<i>B (DL)</i>	
Excellent	13 50.0%	1 7.7%	
Good	12 46.2%	6 46.2%	
Fair	1 3.8%	4 30.8%	
Poor	0 .0%	2 15.4%	
Total	26 100.0%	13 100%	(P<0.05)

(B) ASSESSMENT BY PATIENTS * DRUG CODE Cross tabulation

ASSESSMENT- PATIENTS	DRUG		
	A (OP)	B (DL)	
Excellent	12 46.2%	1 7.7%	
Good	13 50.0%	5 38.5%	
Fair	1 3.8%	5 38.5%	
Poor	0 0%	2 15.4%	
Total	26 100.0%	13 100.0%	(P < 0.05)

In group a out of 26 completed patients investigator assessment was excellent for 13 (50%) Good for 12(46.2%) Fair for 1 (3.8%), where as in group B out of 13 patients it was excellent for 1 (7.7%) Good for 6 (46.2%), Fair for 4 (30.8%) and poor for 2 (15.4%).

Efficacy of Oxypowder in treating constipation was significantly ($P < .05$) more than Dulcolax and hence this indicates Oxypowder was more efficacious in treating constipation than Dulcolax.

Overall efficacy was also judged as complete cure, Improvement and Failure. Table 17 shows that in group A, out of 26 patients 11 patients (42.3%) had complete cure, (57.7%) had improvement and there was no failure where as in group B, out of 13 patients, 1 patient (7.7%) had complete cure, 10 patients (76.9%) had improvement and 2 patients (15.9%) had failure.

Table 17 –

Comparison of Efficacy of Oxy-Powder® with Dulcolax

Efficacy	Drug(%)	
	A (OP)	B (DL)
Complete Cure	11 (42.3%)	1 (7.7%)
Improvement	15 (57.7)	10 (76.9%)
Failure	0 (0%)	2 (15.4%)
Total	26 (100%)	13 (100%)

(P < 0.05)

Results in above Table indicate that efficacy of Oxypowder is significantly more ($P < .05$) than Dulcolax in treating patients of constipation.

(c) SAFETY:

Adverse events

Regarding Adverse events, 1 patient in Oxypowder group had severe diarrhoea on the 2nd day of treatment. He could not carry out his usual activities and felt dehydrated and hence withdrew from the study on 3rd day. In the remaining 26 patients, 2 patients had abdominal fullness after taking Oxypowder for 2-3 days and then they were symptom– free. Remaining 24 patients had no ADR. In Dulcolax group, 1 patient had mild abdominal pain which disappeared without medication; remaining 12 patients did not have any ADR.

DISCUSSION AND SUMMARY

SUMMARY:

The present study titled

“**Multicentric Randomized, Open, Comparative study to evaluate the safety and efficacy of Oxy-Powder® in patients of chronic constipation and IBS**” was conducted to evaluate effectiveness of Oxypowder in treating **constipation** and **IBS with constipation** precipitating loss of weight & improving oxygen delivery in patients of constipation and IBS with constipation. The study was sponsored by Dr. Edward Group III, CEO, Global Healing Center, Inc. & contracted to Mayfair Clinical, Education & Research Centre (MCERC), a Clinical Research Organisation (CRO) for conduct of the study.

For evaluating effectiveness of Oxy-Powder® in patients of constipation, the study was conducted in Bhatia General Hospital and Sharad Shah’s Clinic, both located in Mumbai. However, for evaluating effectiveness of Oxy-Powder® in patients of IBS with constipation, the study was conducted in Bhatia General Hospital. The Study Protocol was carefully designed and got approved from the sponsor by MCERC. The respective investigators were Dr. Chetan Bhatt and Dr. Sharad Shah. The study commenced after MCERC obtained approvals from IEC of MCERC, IRB of Bhatia Hospital and other Regulatory authorities. The study proposed to enrol 60 patients according to inclusion and exclusion criteria given in the protocol but ultimately culminated with 40 patients (one patient out of these had ADR and withdrew from the study from 3rd day). The randomisation of the patients was done as 2:1 i.e. 2 patients were given Oxypowder & 1 patient was given Dulcolax, the comparative product. Thus, there were 27 patients in Oxy-Powder® group and 13 patients in Dulcolax group. All the patients were **counselled** before enrolment and their “**Informed Consent**” was taken. Their Medical History was recorded by the investigators. They were subjected to clinical examination and pre-study evaluation including recording of weight, oxygen content of blood using pulse

oxcimeter, stool examination, Barium meal (to rule out any Bowel organic lesion leading to constipation) and colonoscopy (to rule out any colonic organic lesion and any faecal impaction). Duration of the trial was 42 days i.e. 6 weeks out of which the first week administration of the treatment products was for bowel cleansing followed by 5 weeks of maintenance. The patients were instructed to take four capsules of Oxy-Powder® (Test formulation) or two tablets of Dulcolax (Comparative formulation), as per the Randomization scheme, with plenty of water every day for first 7 days, in the evening on empty stomach. Thereafter, subjects were instructed to take the same dose of either test of reference formulation on alternate days, on empty stomach, in the evening, for period of 6 weeks.

Administration on day '0', '3 weeks' and '6 weeks' were done on empty stomach in the morning. Subjects were asked to report to the hospital with empty stomach in the morning for administration of investigational products and Oxygen level in the blood were measured 15 minutes and 30 minutes after administration of tablets/capsules.

On the remaining days, the subjects were instructed to take investigational product at home at the same dose levels in the evening on empty stomach. They were told to take Dinner at least two hours post-administration of Drug. **Patients were emphatically told to report to the investigators any ADR's that they experience** during or after the study.

DISCUSSION OF THE RESULTS:

The Demographic Characteristics recorded in Table-1 indicate that among 27 patients enrolled in Oxy-Powder® (OP) group, there were 19 male patients (70.4%) and 8 female patients (29.6%) while in 13 patients in Dulcolax group (DL), there were 8 male patients (61.5%) and 5 female patients (38.5%). They conformed to the age and the weight as given in the protocol. 13(48.1%) in OP group were non-vegetarians and 14 (51.9%) were vegetarians as compared to 7(53.8%) non-vegetarians and 6(46.2%) vegetarians in DL group. Majority of patients in both the groups were non-smokers.

MEDICAL HISTORY: Their medical history indicated that in OP group, among the patients enrolled, 13 (48.1%) patients suffered from constipation problem for a period ranging between 3 months to 12 months, 6 (22.2%) between 12 months to 24 months and 8 (29.6%) for more than 24 months. In DL group, 6 (46.2%) patients suffered from constipation problem for a period ranging between 3 months to 12 months, 4 (30.8%) between 12 months to 24 months and 3 (23.1%) for more than 24 months.

These and the above figures indicate that enrolment was in full conformance with the requirements of the protocol.

As regards the severity of constipation based on number of bowels in a week, in OP group, 9(33.3%) patients suffered from **Severe constipation**, 17(63.0%) from **Moderate constipation** and 1(3.7%) from **Mild constipation**,

In DL group, 5(38.5%) patients suffered from **Severe constipation**, 8(61.5%) from **Moderate constipation** and none from **Mild constipation**,

IN OTHER WORDS, THE TWO GROUPS OF PATIENTS ENROLLED WERE EVENLY BALANCED WITH RESPECT TO SEVERITY OF CONSTIPATION.

POST ADMINISTRATION CHANGES:

As seen from the results recorded in Table 8, there is no reduction in the weight of the patients in OP group after 42 days of administration of Oxy-Powder® as compared to Base line (0 day) indicating that administration of Oxypowder did not lead to reduction of weight in the patients. Similarly, there was no reduction in the weight in Dulcolax group. In both the groups, after starting the investigating drug; frequency of stools (No. of Bowels in a week) increased as is shown by the results recorded in Table 9. Comparing the two groups, the percentage performance of Oxy-Powder® was better than Dulcolax on the 21st and the 42nd Day.

As stated in the “Introduction”, Oxypowder releases nascent oxygen for its action; the objective of this study was to see whether Oxypowder increases oxygen saturation of blood. As seen from Table 10, there was marginal increase in oxygen delivery after taking Oxypowder for 6 weeks (42 days) as compared to Day 0. It thus appears that the study period was little short and should have been extended approximately to 180 days to get more conclusive results.

During the study, the patients in both the groups showed significant reduction in various symptoms as shown in Tables 11, 12, 13, 14, 15 (Straining During >25% of Bowel Movement during 0-42 days, Lumpy Stools during 0-42 days, Sensation of incomplete evacuation for >25% of Bowel movement during 0-42 days, Sensation of Anorectal Blockage for >25% of Bowel Movement during 0-42 days and Manual Manoeuvres to facilitate >25% of Bowel Movements during 0-42 days). As can be seen from the results in Table 11 in Oxypowder group, 25 patients (92.6%) had straining on Day 0 as compared to only 2 (7.7%) patients on Day 42. Similarly, in Table 12, in the same group, 26 patients (96.3%) passed hard and lumpy stools on Day 0 where as on day 42, it was reduced only to two patients (7.7%). The above parameters indicate that there is significant reduction in the symptoms ($P<0.05$) in both groups. However, In this case also, the results were better in case of Oxypowder as compared to Dulcolax Routine stool examination done before and after the drug treatment showed no abnormality in all 40 patients in this study

EFFICACY JUDGED:

Efficacy was independently judged by the Investigators & the patients. It was graded as excellent, Good, Fair and Poor. The results were statistically analysed using Fisher's Exact Test. In Oxypowder group, out of 26 completed patients, investigator assessment was excellent for 13 (50%) Good for 12(46.2%) Fair for 1 (3.8%), where as in Dulcolax group, out of 13 patients, it was excellent for 1 (7.7%) Good for 6 (46.2%), Fair for 4 (30.8%) and poor for 2 (15.4%).

Overall efficacy was also judged as complete cure, Improvement and Failure. Table 17 shows that in Oxypowder group , out of 26 patients, 11 patients (42.3%) had complete cure, 15 patients (57.7%) had improvement and there was no failure where as in Dulcolax group, out of 13 patients, 1 patient (7.7%) had complete cure, 10 patients (76.9%) had improvement and 2 patients (15.9%) experienced failure

CONCLUSION:

Efficacy of Oxypowder in treating constipation was significantly ($P<.05$) higher than Dulcolax thus indicating that **Oxypowder** was **more efficacious** in treating constipation than Dulcolax.

No adverse event was reported other than one patient in Op group withdrawing from the study. Oxy-Powder® was well tolerated by all the patients under treatment of this product.

Appendix-1

Helsinki's Declaration -1964, World Medical Association

(Subsequently revised Tokyo 1975, Venice 1983, Hong Kong 1991, Edinburgh 2000)

Recommendations guiding physicians in biomedical research involving human subjects
Adopted by the 18th World Medical Assembly Helsinki, Finland, June 1964 and amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975 35th World Medical Assembly, Venice, Italy, October 1983 41st World Medical Assembly, Hong Kong, September 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996;

INTRODUCTION:

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The Health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which

might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the etiology and pathogenesis of disease.

In current medical practice, most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research, which ultimately must rest in part on experimentation involving human subjects. In the field of biomedical research, a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research, which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physician all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES:

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subjects or to others. Concern for the interests of the subject must always prevail over the interests of science and society.
6. The rights of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.
9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely given informed consent, preferably in writing.
10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case, the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with the national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

Whenever the minor child is in fact able to give consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.
2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutics methods.
3. In any medical study, every patient - including those of a control group, if any - should be assured of the best-proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.
4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.
5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).
6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to extent that medical research is justified by its potential diagnostic value for the patient.

II. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

(Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subject should be a volunteer - either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.
4. In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.

INDEPENDENT ETHICS COMMITTEE
Dr. (Mrs.) Savita Shahani

M.B.B.S., MD (Pharmacology)

12, Guldev Sagar,
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To,
Dr. J. K. Lalla
The Chief Investigator,
Mayfair Clinical, Education and research Centre,
Mohan Mill Compound, Ghodbunder Road,

02-05-06

Thane 400 607.

Subject: Your project entitled "Clinical phase III safety and Efficacy of Oxy Powder"
to be conducted by Mayfair Clinical, Education and Research Centre, Thane.

Dear Sir,

The independent Ethics Committee met on 01-05-06 for the above mentioned project proposal. The following documents were reviewed:

- 1- Study protocol (No. MCERC/PROT-CLTR/OXY/1205/001 version 02
- 2- Format of Patient Informed Consent Form
- 3- CV of the Principle Investigator
- 4- CV of the Co-Investigator

After reviewing the above documents, the Ethics Committee has decided to approve your above mentioned project, and the following has to be communicated in writing to the Ethics Committee:

- 1- Date of commencement of study.
- 2- Any serious adverse event observed and recorded during the study.
- 3- Any modification or deviation in the protocol or in the informed consent form done during the conduct of study.
- 4- New information that may affect adversely the safety of the subject or the conduct of the trial.
- 5- Project summary after the completion of the project.

You are expected to obtain appropriate permission if required from regulatory authorities.

Thanking you,

Yours faithfully,

hsc
Chairperson
Independent Ethics Committee

Recd.
27.05.06
✓

IEC– MCERC

APPENDIX– 2

IRB of Bhatia Hospital **APPENDIX– 3**

APPENDIX– 4

VERSION – 02

***PROTOCOL FOR
CLINICAL TRIAL PHASE III
SAFETY AND EFFICACY OF OXY-POWDER®***

DOCUMENT NO.: MCERC/PROT-CLTR/OXY/1205/001



December 20, 2005

MAYFAIR CLINICAL EDUCATION & RESEARCH CENTRE,

(A KALTHIA GROUP ORGANIZATION)

Mohan Mill Compound, Ghodbunder Road, Thane-400 607.

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Division:	Clinical Research	Document Number:	MCERC/PROT- CLTR/OXY/1205/001
Document Type:	Protocol	Study Identifier:	Mfair/oxy/2005-06
Site of Issue:	Thane, Maharashtra		
Classification:	Level 2	Document Date:	9.12.2005

Title: Efficacy & Safety of Oxy-Powder®

The effectiveness of the Oxy-Powder® in treating constipation and IBS.

Abstract:

Constipation is the slow movement of feces (stool or body wastes) through the large intestine resulting in infrequent bowel movements and the passage of dry, hard stools. The longer it takes for the stool to move through the large intestine, the more fluid is absorbed and the drier and harder the stool becomes. Constipation is annoying and uncomfortable, but fecal impaction (a collection of dry, hard stool in the colon or rectum) can be life threatening. Patients with a fecal impaction may not have gastrointestinal symptoms. Instead, they may have circulation, heart, or breathing problems. If fecal impaction is not recognized, the signs and symptoms will get worse and the patient could die.

Irritable bowel syndrome (IBS), a functional gastrointestinal disorder characterized by the interplay of altered motility, abnormal visceral sensation, and psychosocial factors, is one of the most common reasons for referral to a gastroenterologist. It is associated with bouts of constipation and diarrhoea.

‘Oxy-Powder®’ used universally as a dietary supplement for relieving constipation is being taken up for the study under Clinical Trials Phase III in 60 constipation and 20 IBS patients in Open, Randomized, Comparative study in 3 Centres. The Study protocol includes exclusion and inclusion criteria, mode of administration of test and reference products, evaluation of effectiveness and safety and data analysis and management. The GCP guidelines shall be followed for the conduct of the studies. Reporting of AEs and SAR has been emphasized. The duration of the study, post administration is 6 weeks.

Authors: Prof. J K Lalla, Ph.D., Dr Meena Shah, M.D., Dr Deven Parmar, M.D.

Compound Numbers/Keywords (if applicable): Oxy-Powder®

CONFIDENTIAL

Distribution:

1. Dr. Edward Group-Sponsor from US
2. Dr. Mahesh Patel-Consultant from US
3. Prof. J. K. Lalla, Ph.D. – Study Director
4. Dr. Sharad Shah – Gastroenterologist – Jaslok Hospital
5. Dr. Chetan Bhatt – Clinical Investigator – Bhatia Hospital
6. Dr. Philip Ibrahim – Clinical Investigator – Hinduja Hospital
7. Chairpersons – Hospital Ethics Committee
8. Dr. Meena Shah – Mayfair Clinical, Education and Research Centre
9. Dr. Deven Parmar – Mayfair Clinical, Education and Research Centre
10. CRA's of Mayfair Clinical, Education and Research Centre

Objective:

To study the safety and efficacy of Oxy-Powder® in patients of chronic constipation and IBS.

Randomized, open, comparative study.

Document Number: MCERC/PRTO-CLTR/OXY/1205/001

Study Identifier: Mfair/oxy/2005-06

Approval Date: dd Mmm yyyy

Author(s): Prof. J K Lalla, Ph.D., Dr Meena Shah, M.D., Dr Deven Parmar, M.D.

Sponsor Signatory:

Signature:

Date:

Dr. Edward Group III

CEO, Global Healing Center,
Inc.

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SPONSOR INFORMATION PAGE:

Title: **Randomized, open, comparative study To study the safety and efficacy of Oxy-Powder® in patients of chronic constipation and IBS.**

Document Number: MCERC/PRTO-CLTR/OXY/1205/001

Study Identifier: Mfair/oxy/2005-06

Mfair/oxy/2005-06

Investigator Protocol Agreement Page:

I agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by Mayfair. Not to implement any changes to the protocol without agreement from the sponsor and prior review and written approval from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- That I am thoroughly familiar with the appropriate use of the investigational product(s), as described in this protocol, and any other information provided by the sponsor including, but not limited to, the following: the current Clinical Investigator's Brochure / Investigator's Brochure (CIB/IB) or equivalent document, CIB/IB supplement (if applicable), and approved product label (if the product is marketed in this country and the label is not already provided as an equivalent to a CIB/IB).
- That I am aware of, and will comply with, "good clinical practices" (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the investigational product(s) and of their study-related duties and functions as described in the protocol.
- That I have been informed that certain regulatory authorities require the Sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the Sponsor or the investigational product, and more generally about his/her financial ties with the Sponsor. Mayfair will use and disclose the information solely for the purpose of complying with regulatory requirements.

MAYFAIR CLINICAL EDUCATION & RESEARCH CENTRE,
Mohan Mill Compound, Ghodbunder Road, Thane-400 607.

111

Document No MCERC/REPTCLTR/OXY/1205/001 Study Identifier: Mfair/oxy/200506
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Hence I:

- Agree to supply MAYFAIR with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children);
- Agree to promptly update this information if any relevant changes occur during the course of the study and for 1 year following completion of the study; and
- Agree that MAYFAIR may disclose any information it has about such ownership interests and financial ties to regulatory authorities.

Investigator Name: _____

Investigator Signature

Date

The following co-signature is required only when the investigator is not a [insert the appropriate wording such as “physician” or “dentist”]

Physician Name: _____

Physician Signature

Date

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GLOSSARY

ADVERSE EVENT: An unwanted effect caused by the administration of drugs. Onset may be sudden or develop over time

BASELINE: 1. Information gathered at the beginning of a study from which variations found in the study are measured. 2. A known value or quantity with which an unknown is compared when measured or assessed. 3. The initial time point in a clinical trial, just before a participant starts to receive the experimental treatment which is being tested. At this reference point, measurable values such as CD4 count are recorded. Safety and efficacy of a drug are often determined by monitoring changes from the baseline values.

BIAS: When a point of view prevents impartial judgment on issues relating to the subject of that point of view. In clinical studies, bias is controlled by blinding and randomization.

BLIND: A randomized trial is "Blind" if the participant is not told which arm of the trial he is on. A clinical trial is "Blind" if participants are unaware on whether they are in the experimental or control arm of the study; also called masked.

CLINICAL: Pertaining to or founded on observation and treatment of participants, as distinguished from theoretical or basic science.

CLINICAL ENDPOINT: See Endpoint.

CLINICAL INVESTIGATOR: A medical researcher in charge of carrying out a clinical trial's protocol.

COMPLEMENTARY AND ALTERNATIVE THERAPY: Broad range of healing philosophies, approaches, and therapies that Western (conventional) medicine does not commonly use to promote well-being or treat health conditions. Examples include acupuncture, herbs, etc.

CONFIDENTIALITY REGARDING TRIAL PARTICIPANTS: Refers to maintaining the confidentiality of trial participants including their personal identity and all personal medical information. The trial participants' consent to the use of records for data verification purposes should be obtained prior to the trial and assurance must be given that confidentiality will be maintained.

CONTRAINDICATION: A specific circumstance when the use of certain treatments could be harmful.

CONTROL: A control is the nature of the intervention control.

CONTROL GROUP: The standard by which experimental observations are evaluated. In many clinical trials, one group of patients will be given an experimental drug or treatment, while the control group is given either a standard treatment for the illness or a placebo

CONTROLLED TRIALS: Control is a standard against which experimental observations may be evaluated. In clinical trials, one group of participants is given an experimental drug, while another group (i.e., the control group) is given either a standard treatment for the disease or a placebo.

DOUBLE-BLIND STUDY: A clinical trial design in which neither the participating individuals nor the study staff knows which participants are receiving the experimental drug and which are receiving a placebo (or another therapy). Double-blind trials are thought to produce objective results, since the expectations of the doctor and the participant about the experimental drug do not affect the outcome; also called double-masked study.

EFFICACY: (Of a drug or treatment). The maximum ability of a drug or treatment to produce a result regardless of dosage. A drug passes efficacy trials if it is effective at the dose tested and against the illness for which it is prescribed. In the procedure mandated by the FDA, Phase II clinical trials gauge efficacy, and Phase III trials confirm it

ELIGIBILITY CRITERIA: Summary criteria for participant selection; includes Inclusion and Exclusion criteria.

ENDPOINT: Overall outcome that the protocol is designed to evaluate. Common endpoints are severe toxicity, disease progression, or death.

FOOD AND DRUG ADMINISTRATION (FDA): The U.S. Department of Health and Human Services agency responsible for ensuring the safety and effectiveness of all drugs, biologics, vaccines, and medical devices, including those used in the diagnosis, treatment, and prevention of HIV infection, AIDS, and AIDS-related opportunistic infections. The FDA also works with the blood banking industry to safeguard the nation's blood supply.

HYPOTHESIS: A supposition or assumption advanced as a basis for reasoning or argument, or as a guide to experimental investigation.

INCLUSION/EXCLUSION CRITERIA: The medical or social standards determining whether a person may or may not be allowed to enter a clinical trial. These criteria are based on such factors as age, gender, the type and stage of a disease, previous treatment history, and other medical conditions. It is important to note that inclusion and exclusion criteria are not used to reject people personally, but rather to identify appropriate participants and keep them safe.

INFORMED CONSENT: The process of learning the key facts about a clinical trial before deciding whether or not to participate. It is also a continuing process throughout the study to provide information for participants. To help someone decide whether or not to participate, the doctors and nurses involved in the trial explain the details of the study.

INFORMED CONSENT DOCUMENT: A document that describes the rights of the study participants, and includes details about the study, such as its purpose, duration, required procedures,

and key contacts. Risks and potential benefits are explained in the informed consent document. The participant then decides whether or not to sign the document. Informed consent is not a contract, and the participant may withdraw from the trial at any time.

INSTITUTIONAL REVIEW BOARD (IRB): 1. A committee of physicians, statisticians, researchers, community advocates, and others that ensures that a clinical trial is ethical and that the rights of study participants are protected. All clinical trials in the U.S. must be approved by an IRB before they begin. 2. Every institution that conducts or supports biomedical or behavioral research involving human participants must, by federal regulation, have an IRB that initially approves and periodically reviews the research in order to protect the rights of human participants.

INTENT TO TREAT: Analysis of clinical trial results that includes all data from participants in the groups to which they were randomized even if they never received the treatment.

IRRITABLE BOWEL SYNDROME: Irritable bowel syndrome (IBS), a functional gastrointestinal disorder characterized by the interplay of altered motility, abnormal visceral sensation, and psychosocial factors, is one of the most common reasons for referral to a gastroenterologist.

OPEN-LABEL TRIAL: A clinical trial in which doctors and participants know which drug or vaccine is being administered.

PHASE II TRIALS: Controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks.

PHASE III TRIALS: Expanded controlled and uncontrolled trials after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather additional information to evaluate the overall benefit-risk relationship of the drug and provide an adequate basis for physician labelling.

PLACEBO: A placebo is an inactive pill, liquid, or powder that has no treatment value. In clinical trials, experimental treatments are often compared with placebos to assess the treatment's effectiveness.

PLACEBO CONTROLLED STUDY: A method of investigation of drugs in which an inactive substance (the placebo) is given to one group of participants, while the drug being tested is given to another group. The results obtained in the two groups are then compared to see if the investigational treatment is more effective in treating the condition.

PHARMACOVIGILANCE: Despite all the tests and precautions taken during the development and authorisation of a pharmaceutical product, medicines still sometimes produce side effects under certain conditions. Monitoring use and effects of a given medication to detect and prevent these adverse drug reactions (ADR) is the domain of Pharmacovigilance.

It is regarded as all post-authorisation scientific and data gathering activities relating to the detection, assessment, understanding and prevention of adverse events or any other product related problems.

PROTOCOL: A study plan on which all clinical trials are based. The plan is carefully designed to safeguard the health of the participants as well as answer specific research questions. A protocol describes what types of people may participate in the trial; the schedule of tests, procedures, medications, and dosages; and the length of the study. While in a clinical trial, participants following a protocol are seen regularly by the research staff to monitor their health and to determine the safety and effectiveness of their treatment.

RANDOMIZATION: A method based on chance by which study participants are assigned to a treatment group. Randomization minimizes the differences among groups by equally distributing people with particular characteristics among all the trial arms. The researchers do not know which treatment is better. From what is known at the time, any one of the treatments chosen could be of benefit to the participant

RANDOMIZED TRIAL: A study in which participants are randomly (i.e., by chance) assigned to one of two or more treatment arms of a clinical trial. Occasionally placebos are utilized.

RISK-BENEFIT RATIO: The risk to individual participants versus the potential benefits. The risk/benefit ratio may differ depending on the condition being treated.

SERIOUS ADVERSE EVENT: A Serious adverse event is any untoward medical occurrence that at any dose a) results in death b) is life threatening c) requires hospitalization or prolongation of existing hospitalisation.

SIDE EFFECTS: Any undesired actions or effects of a drug or treatment. Negative or adverse effects may include headache, nausea, hair loss, skin irritation, or other physical problems. Experimental drugs must be evaluated for both immediate and long-term side effects.

SINGLE-BLIND STUDY: A study in which one party, either the investigator or participant, is unaware of what medication the participant is taking; also called single-masked study.

STATISTICAL SIGNIFICANCE: The probability that an event or difference occurred by chance alone. In clinical trials, the level of statistical significance depends on the number of participants studied and the observations made, as well as the magnitude of differences observed.

STUDY ENDPOINT: A primary or secondary outcome used to judge the effectiveness of a treatment.

STUDY TYPE: The primary investigative techniques used in an observational protocol; types are Purpose, Duration, Selection, and Timing.

1.0 PROTOCOL SUMMARY

1.1 Rationale of conducting this study:

1. The effectiveness of the product in treating constipation and IBS in patients
2. Recording loss of weight
3. The effectiveness of the product for Oxygen delivery in patients
4. The effects Oxy-Powder has on the 4 microbes + 2 parasites present in the digestive tract including normal Probiotic strain and colonies in the digestive tract.
5. Safety studies involving acute and sub-acute toxicity in rats/ mice (including histo-pathology)

1.2 OBJECTIVE(S):

Primary: The effectiveness and safety of Oxy-Powder® in treating constipation and IBS

Secondary: The *in-vitro* effect of Oxy-Powder® on the normal Probiotic strain and colonies in the digestive tract

Safety studies involving acute and sub-acute toxicity in rats/mice (including histopathology)

1.3 ENDPOINT(S):

Primary: In patients

- a) Finding of i) stool examination ii) Barium Meal and iii) Colonoscopy
- b) Relief of constipation and IBS, improvement of Quality of life

Secondary: *in-vitro* findings of effectiveness of Oxy-Powder® on normal Probiotic and pathogenic strain of microbes in digestive tract.

Safety studies involving acute and sub-acute toxicity in rats/mice (including histopathology)

1.4 Study Design: Open, Comparative, randomized study

1.5 Study Population:

1. 60 patients (60% females + 40% males, more number of non-vegetarians, less number of vegetarians) suffering from constipation
2. 20 patients (60% females + 40% males, more number of non-vegetarians, less number of vegetarians) suffering from IBS.

1.6 Study Assessments and Procedures:

1. 6 weeks duration study
2. Start with a current physical examination
3. Check height and weight daily for 7 days
4. Oxygen content in the blood (pulse oximeter) on day 0, week 3, week 6 in the morning, on empty stomach
5. Fasting Patient called to the hospital, capsule administered & O₂ level measured at 15 & 30 minutes post administration
6. Barium meal, colonoscopy with minimal cleaning done to see how fecal infarction is in the bowel before and after the study (0 & 6 weeks)

INVESTIGATIONAL PRODUCT(S):

1.7.1 Test Product: (A)

Oxy-Powder® marketed by Global Healing Centre, Inc. USA

Category:

Dietary Supplement

Composition:

Ozonated Magnesium Oxides: A combination of USP grade Magnesium Oxide mixed with a small amount USP grade Magnesium Peroxide. This combination is then pressurized at subzero temperatures and Ozone gas is the stabilizing molecule. The final product contains Stabilized monatomic oxygen, which is released by an acid based catalyst i.e. HCl in the stomach acid.

Per capsule dosage: 685 mg

Organic Germanium 132:

Does not contain the harmful form of Germanium (Germanium Dioxide)

Per capsule dosage: 5.5 mg

Natural Citric acid:

25 mg per capsule

Other Ingredients:

Kosher certified 00 Vegetarian capsules

1.7.2 Reference Product: (B)

Dulcolax tablets manufactured by Cadila Healthcare Ltd., India.

(Each tablet containing 5 mg Bisacodyl)

Category: Drug belonging to GI therapeutic group

2.0 BACKGROUND

Constipation is the slow movement of feces (stool or body wastes) through the large intestine resulting in infrequent bowel movements and the passage of dry, hard stools. The longer it takes for the stool to move through the large intestine, the more fluid is absorbed and the drier and harder the stool becomes.

Constipation is annoying and uncomfortable, but fecal impaction (a collection of dry, hard stool in the colon or rectum) can be life threatening. Patients with a fecal impaction may not have gastrointestinal symptoms. Instead, they may have circulation, heart, or breathing problems. If fecal impaction is not recognized, the signs and symptoms will get worse and the patient could die.

Chronic constipation is one of the most frequent gastrointestinal symptoms in the United States, accounting for nearly 2.5-2.7 million physician visits and 39000-90000 hospitalizations per year in the United States. Constipation may be stratified, with considerable overlap, into issues of stool consistency vs defecatory behavior.

Irritable bowel syndrome (IBS), a functional gastrointestinal disorder characterized by the interplay of altered motility, abnormal visceral sensation, and psychosocial factors, is one of the most common reasons for referral to a gastroenterologist. It is associated with bouts of constipation and diarrhea.

3.0 OBJECTIVE(S)

To study the safety and effectiveness of Oxy-Powder®

4.0 STUDY DESIGN

Open randomized comparative study

5.0 STUDY POPULATION

Any subject who has given informed consent to participate in the clinical study and has met all the criteria required for inclusion into the clinical study may take part in the research.

Subject participation in the research project is voluntary and refusal to participate will not indicate withdrawal from the clinical study. Refusal to participate will involve no penalty or loss of benefits to which the subject would otherwise be entitled.

No additional administration of investigational product beyond the dose detailed in the clinical study is permitted.

6.0 NUMBER OF SUBJECTS:

- a) 60 for constipation
- b) 20 for IBS

7.0 ELIGIBILITY CRITERIA:

- Patients suffering from constipation and IBS
- Age - 18 to 60 yrs
- Height & Weight conforming to Height/Weight chart of Life Insurance Corporation of India
- Sex – Patients belonging to both sexes are eligible
- Food habits-both non-vegetarians and vegetarian patients are eligible
- More of the non-vegetarian patients will be preferred.

7.1 INCLUSION CRITERIA

A subject will be eligible for inclusion in this study only if all of the following criteria are satisfied:

1. 18 to 60 years of both sexes (60% females + 40% males)
2. Food habits: Vegetarians are eligible but Non-vegetarians preferred
3. Satisfying the Rome II Criteria for Constipation and IBS

Introduction to Rome II criteria:

At the 13th International Congress of Gastroenterology in Rome, Italy in 1988, a group of physicians defined criteria to more accurately diagnose constipation and IBS. Known as the "Rome Criteria," this set of guidelines that outlines symptoms and applies parameters such as frequency and duration make possible a more accurate diagnosis of Constipation and IBS.

The Rome Criteria were not widely accepted when originally presented, but were better received after their first revision. This second version, created in 1992 and known as Rome II, added a length of time for symptoms to be present and pain as an indicator. The second revision, known as Rome III, is currently underway and shall be released in 2006.

Rome II Criteria for constipation:

Two or more of the following for at least 12 wk (not necessarily consecutive) in the preceding 12 mo:	
-	Straining during >25% of bowel movements;
-	Lumpy or hard stools for >25% of bowel movements;
-	Sensation of incomplete evacuation for >25% of bowel movements;
-	Sensation of anorectal blockage for >25% of bowel movements;

-	Manual maneuvers (digital evacuation, support of the pelvic floor) to facilitate >25% of bowel movements;
-	Less than 3 bowel movements per week;
Loose stools are not present, and there are insufficient criteria for irritable bowel syndrome	

Acceptable form of birth control being followed in female patients

Rome II criteria for IBS

The Rome II diagnostic criteria of Irritable Bowel Syndrome always presumes the absence of a structural or biochemical explanation for the symptoms and is made only by a physician.

Irritable Bowel Syndrome can be diagnosed based on at least 12 weeks (which need not be consecutive) in the preceding 12 months, of *abdominal discomfort or pain that has two out of three of these features*:

1. Relieved with defecation; and/or
2. Onset associated with a change in frequency of stool; and/or
3. Onset associated with a change in form (appearance) of stool.

Symptoms that Cumulatively Support the Diagnosis of IBS:

1. Abnormal stool frequency (may be defined as greater than 3 bowel movements per day and less than 3 bowel movements per week);
2. Abnormal stool form (lumpy/hard or loose/watery stool);
3. Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);
4. Passage of mucus;
5. Bloating or feeling of abdominal distension.

Supportive Symptoms of IBS:

1. Fewer than three bowel movements a week
2. More than three bowel movements a day
3. Hard or lumpy stools
4. Loose (mushy) or watery stools
5. Straining during a bowel movement
6. Urgency (having to rush to have a bowel movement)
7. Feeling of incomplete bowel movement
8. Passing mucus (white material) during a bowel movement

9. Abdominal fullness, bloating, or swelling

Red Flag symptoms which are NOT typical of IBS:

Pain that often awakens/interferes with sleep

Diarrhea that often awakens/interferes with sleep

Blood in your stool (visible or occult)

Weight loss

Fever

Abnormal physical examination

7.2 EXCLUSION CRITERIA FOR CONSTIPATION AND IBS

A subject will not be eligible for inclusion in this study if any of the following conditions are observed in the patient

10. Evidence of Malignancy on colonoscopy / having diagnosed organic GI disorder
11. Pregnant and lactating women
12. Evidence of lactose intolerance to explain bowel symptoms
13. History of cardiac arrhythmias or heart disease
14. History of Glaucoma
15. History of urine retention
16. History of schizophrenia
17. History of substances of abuse/dependency
18. intellectually unable or unwilling to complete daily GI ratings

7.3 Other Eligibility Criteria Considerations

To assess any potential impact on subject eligibility with regard to safety, the investigator must refer to the following document(s) for detailed information regarding warnings, precautions, contraindications, adverse events, and other significant data pertaining to the investigational product(s) being used in this study.

8.0 STUDY ASSESSMENTS AND PROCEDURES

8.1 Demographic and Baseline Assessments

Physical examination, stool examination, pulse oximeter, Barium meal and colonoscopy

8.2 Safety in Clinical Trials

Action to be taken if pregnancy occurs

During the study, if the female subject becomes pregnant, she will be withdrawn from the study. She will be considered as 'drop-out' in the report which shall be documented in the raw data.

INVESTIGATIONAL PRODUCT - OXY-POWDER®

9.0 GENERAL LITERATURE DATA

9.1 INTRODUCTION:

Oxy-Powder is a specifically designed compound which has been ozonated and stabilized to release beneficial free monatomic oxygen into the intestinal tract and body. The time-release delivery ensures that Oxy-Powder will provide an adequate amount of oxygen, slowly, for better utilization by the body. Oxy-Powder is a non-toxic, safe, effective and non-allergic. By using Oxy-Powder, the compaction from the small intestine, large intestine and colon is oxidized safely and effectively. Organic Germanium-132 has demonstrated in multiple scientific studies to be a powerful oxygen facilitator and immune system stimulant. Oxy-Powder is also harmless to the good bacteria in the intestinal tract. Natural citric acid has been added to facilitate oxygen release.

Oxygen Enhancement and Intestinal Cleansing Formula

There is only one true way to clean the digestive tract. This is through an oxidation/reduction reaction or a clean raw food diet. As we age, we accumulate toxic substances in our digestive tract. The pancreas, the organ which produces the necessary enzymes to break down the food we eat, is limited. At birth, our pancreas has a limited supply of enzymes. Some doctors say we only have enough enzymes to breakdown 1 cooked meal daily for 120 years. This means 80% of our diet should be raw fruits and vegetables.

With the poor diet, particularly non-vegetarian food i.e. eating high fat meals daily, which the body cannot utilize, most of us put tremendous strain on our pancreas and run out of enzymes by the time we are 30-40 years old. It can take the body up to 8 hours to break down protein, and up to 48-72 hours to digest fats and carbohydrates. So now, you see that you constantly have undigested food particles in the intestinal tract.

It has been estimated that the average person by the age of 40 has between 10-20 pounds of hard compacted fecal matter lodged in their intestinal tract. Our intestinal tract is 30-35 feet in length. In order to fully cleanse the digestive tract the solid compaction gets into a liquid or gas, using time released oxygen and ozone (oxidation/reduction). By using Oxy-Powder, the toxic residue is oxidized

from the small intestine, large intestine and colon, safely and effectively. This is important because a clean intestinal tract is the beginning of obtaining optimal health.

Oxy-Powder is harmless to the good bacteria in the intestinal tract. When the intestinal tract is fully cleansed, the urine will become cloudy and the stools will become semi-solid. When this occurs, a maintenance dose is recommended. This helps provide oxygen directly into the bloodstream. By staying on a Maintenance Dosage of Oxy-Powder, the intestinal tract is kept clean and provides body with much needed oxygen.

9.2 DOSAGE & ADMINISTRATION

For first time, it is recommended starting with a seven day initial cleanse. After the initial cleanse, a maintenance dose is to be continued for keeping your intestinal tract clean and deliver oxygen into the system. Patients are advised to take Oxy-Powder with plenty of purified water during the day and eat healthy diet. The exact number of capsules to take will vary depending on body weight, previous dietary habits, exercise patterns, and stress levels.

For Cleansing:

It is recommended to take four (4) capsules in the evening on an empty stomach with 240 mL water. If 3-5 bowel movements are not achieved the following day, the dosage is increased by adding two (2) capsules every night until 3-5 bowel movements are achieved the following day. Once the dosage is finalized, it is day one (1) of the seven (7) day cleanse. This dosage is continued for seven (7) consecutive days. After the seven (7) day cleanse, the maintenance dose is started.

Maintenance Dose:

The same dosage is to be taken same as that taken for seven (7) day cleanse, every other day. This can be taken indefinitely without becoming habit forming or harmful to the body.

Suggestions:

Drinking of lots of pure water is suggested. This is not only healthy for the body but will aid the body in eliminating toxins from the bowel at a faster rate.

Oxy-Powder works with stomach acid; if the level of HCL is below normal, it may hinder the effectiveness of the Oxy-Powder. It is suggested to take an organic lemon wedge squeezed into glassful of purified water with the Oxy-Powder® in the evening.

- These statements are based upon the literature reports on patient results, clinical observation, and customer feedback

9.3 DOSE RATIONALE

Dose of 4 capsules per day for first 7 days administered for cleansing followed by administration of 4 capsules on alternate days as maintenance dose is found to be effective.

9.4 OVERDOSE:

No toxicity has been reported in the literature caused by overdose of Oxy-Powder®

9.5 PRECAUTIONS:

This product causes watery and gaseous stools which could cause the patient to feel the urge to pass gas. If patient is not able to control bowels, he/she has to be careful when using this product. Close availability to a bathroom is recommended during the 7-day cleanse. Oxy-Powder will cause watery, gaseous stools. This is not diarrhea; this is the by-product of oxidation since a solid is turned into a liquid or gas. To help the cleansing process, it is advised to drink plenty of purified water daily while taking the Oxy-Powder; even though there has never been a documented case of dehydration or electrolyte imbalances.

9.6 OCCUPATIONAL SAFETY

Investigational product is not expected to pose significant occupational safety risk to site staff under normal conditions of use and administration. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions either will be provided to the investigator (where this is required by local laws) or is available upon request from MCERC. However, precautions are to be taken to avoid direct skin contact, eye contact, and generating aerosols or mists. In the case of unintentional occupational exposure, treat as if the substance contains the active pharmaceutical even though it is absent from placebo formulations, and notify the monitor. Precaution will be taken to avoid direct contact with the investigational product. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions will be provided to the investigator.

9.7 CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

Contraindications:

There are no contraindications reported when taking Oxy-powder with prescription medicines as long as the Oxy-Powder® is taken 6 hours before or 6 hours after the permitted medications.

All concomitant medications taken during the study will be recorded in the CRF with indication, dose information, and dates of administration.

Prohibited Medications

Laxatives, anti-diarrhoeals and anti-spasmodics

10.0 STUDY DESIGN:

Multicentric, Open, Comparative, Randomized, Phase III, Clinical trial comparing Oxy-powder developed by Global Healing Centre Inc., USA with Dulcolax tablets (containing 5 mg Bisacodyl) manufactured by Cadila Health Care Ltd.

10.1 STUDY DURATION:

6 weeks from the date of administration of study products.

11.0 EVALUATIONS

11.1 *IN-VITRO* EVALUATION OF INVESTIGATIONAL PRODUCT:

1. Toxicological studies:
 - a) Acute: 7 days
 - b) Sub-acute: 28 days
2. Anti-microbial activity against 4 bacteria (including a Probiotic strain) and 1 parasite from amongst the colonies present in GIT

11.2 PRE-STUDY EXAMINATION OF INCLUDED SUBJECTS FOR THE FOLLOWING:

1. Height and weight
2. Oxygen content of blood
3. Bowel scan
4. Barium meal X-ray
5. Colonoscopy with minimal cleansing to see how mucoid plaque is present in the bowel.
6. Stool examination for dead parasites, toxic compounds etc.

11.3 PARAMETERS FOR POST-ADMINISTRATION EVALUATIONS IN 'INCLUDED' SUBJECTS FOR THE FIRST SEVEN DAYS:

1. Height and Weight check every day
 2. Oxygen content of blood On Day '0', '3 Week' And '6 Week' both for test and control group.
- The subjects from both the groups shall be called in the morning on empty stomach. Group administered "Oxy-Powder®" shall be administered capsules and their Oxygen levels shall be determined at '15 min' and '30 min.' post administration of the dose. In Dulcolax group of patients, based on the enrollment days, they shall be called exactly on the days as decided for Oxy-Powder® on

days '0', '3 weeks' and '6 weeks'. Since they are comparative group, their Oxygen level shall be measured without administering Dulcolax, '15 min' and '30 min' after their arrival.

3. Colonoscopy (after completion of the study at the end of 6 weeks)
4. Barium meal X-ray (after completion of the study at the end of 6 weeks)
5. Stool analysis (at the end of 3 weeks and at the end of 6 weeks)
6. The effectiveness of the product in treating constipation
7. The effectiveness of the product in body detoxification
8. The effectiveness of the product for Oxygen delivery and amount of oxygen delivered per capsule.

12.0 TREATMENT OF INVESTIGATIONAL PRODUCT:

Subject shall be instructed to take four capsules of Oxy-Powder® (Test formulation) or two tablets of Dulcolax (Reference formulation), as per the Randomization scheme, with plenty of water, every day, for first 7 days, in the evening on empty stomach. Thereafter, subjects shall be instructed to take the same dose of either test or reference formulation on alternate days, on empty stomach, in the evening, for 6 weeks. Administration on day '0', '3 weeks' and '6 weeks' shall be done on empty stomach (to enable measuring Oxygen levels) in the morning. Subjects shall be asked to report to the hospital with empty stomach; in the morning for administration of Investigational Products and Oxygen level in the blood shall be measured 15 minutes and 30 minutes after administration of tablets/ capsules.

On the remaining days, the subjects shall take Investigational product at home at the same dose levels in the evening on empty stomach. Dinner shall follow at least two hours post-administration of capsules.

13.0 Blinding

Treatment Assignment

Subjects will be assigned to study treatment in accordance with the randomization schedule.

14.0 HANDLING AND STORAGE OF INVESTIGATIONAL PRODUCT:

Investigational product must be dispensed or administered according to procedures described herein.

Only subjects enrolled in the study may receive investigational product, in accordance with all applicable regulatory requirements. Only authorized site staff may supply or administer investigational product. All investigational products must be stored in a secure area with access limited to the investigator and authorized site staff and under physical conditions that are consistent with investigational product-specific requirements.

14.1 Product Accountability

The investigator is responsible for investigational product accountability, reconciliation, and record maintenance. In accordance with all applicable regulatory requirements, the investigator or designated site staff must maintain investigational product accountability records throughout the course of the study. This person(s) will document the amount of investigational product received from MCERC, the amount supplied and/or administered to and returned by subjects, if applicable.

14.2 Assessment of Compliance

A diary card will be given to the patients during the entire course of the clinical trial which will record the number of capsules/ tablets taken and number of stools passed. Diary card will be issued at day '0', week 3 and week 6 after collecting the previously completed diary filled every day from the concerned patient. Patient shall be required to contact his physician.

15.0 SUBJECT COMPLETION AND WITHDRAWAL

15.1 Subject Completion

All subjects completing six weeks of treatment will be included in the final report.

15.2 Subject Withdrawal from Study

If a subject who has consented to participate in research withdraws from the clinical study for any reason other than lost to follow-up, the subject will be asked to give consent for withdrawal with or without the reason for withdrawal. Such subject shall be taken as 'drop-out'.

15.3 Screen and Baseline Failures:

Not applicable since the study is non-invasive

16.0 ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

16.1 Definition of an AE

Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE **include**:

- Significant or unexpected worsening or exacerbation of the condition/indication under study. See Section 10.3., “Lack of Efficacy”, for additional information.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequel of a suspected interaction.
- Signs, symptoms, or the clinical sequel of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Significant failure of expected pharmacological or biological action. See Section 10.3., “Lack of Efficacy” for additional information.

16.2 Lack of Efficacy

“Lack of efficacy” per se will not be reported as an AE. The signs and symptoms or clinical sequel resulting from lack of efficacy will be reported if they fulfill the AE or SAE definition (including clarifications).

The signs and symptoms, clinical sequel resulting from lack of efficacy, or both will be reported. In this study, failure of expected pharmacological action also constitutes an AE and will be reported as such in addition to the signs and symptoms.

Examples of an AE **does not include** a/an:

- ❖ Medical or surgical procedure (e.g., endoscopy, appendectomy); that leads to the condition of an AE.
- ❖ Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- ❖ Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- ❖ The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject’s condition.

For MCERC clinical studies, AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

16.3 Definition of a SAE

A serious adverse event is any untoward medical occurrence that, at any dose:

a) Results in death.

b) Is life threatening.

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c) Requires hospitalization or prolongation of existing hospitalization.

NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in disability/incapacity, or

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly/birth defect.

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

16.4 Disease-Related Events or Outcomes Not Qualifying as SAEs

16.5 Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs and SAEs

Abnormal laboratory findings (e.g., clinical chemistry, hematology, urinalysis) or other abnormal assessments (e.g., [Insert relevant examples of assessments applicable to the study, such as ECG's, X-rays, vital signs, etc.] that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE, as defined in Section 10.1. ("Definition of an AE") or SAE, as defined in Section 10.2. ("Definition of a SAE"). Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will **not** be reported as AEs or SAEs. The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

As defined by the protocol in Section 10.2., "Definition of a SAE", all Grade 4 laboratory abnormalities will be reported as SAEs.

Time Period, Frequency, and Method of Detecting AEs and SAEs

16.6 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the CRF. It is not acceptable for the investigator to send photocopies of the subject's medical records to MCERC in lieu of completion of the appropriate AE/SAE CRF pages. However, there may be instances when copies of medical records for certain cases are requested by MCERC. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to MCERC.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

AEs and subject-completed questionnaires are independent components of the study. Responses to each question in the questionnaires will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer. The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

16.7 Evaluating AEs and SAEs

16.7.1 Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the investigator's clinical judgment. The intensity of each AE and SAE recorded in the CRF should be assigned to one of the following categories:

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomfoting to interfere with normal everyday activities.

Severe: An event that prevents normal everyday activities.

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 10.2., "Definition of a SAE".

16.7.2 Assessment of Causality

The investigator is obligated to assess the relationship between investigational product and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the CIB/IB and/or Product Information, for marketed products, in the determination of his/her assessment.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to MCERC. However, it is very important that the investigator always make an assessment of causality for every event prior to transmission of the SAE CRF to MCERC. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE CRF accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

The investigator will provide the assessment of causality as per instructions on the SAE form in the CRF.

16.7.3 Follow-Up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information to MCERC on the subject's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE/SAE CRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, Histopathological examinations, or consultation with other health care professionals.

MCERC may request that the investigator perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obligated to assist. If a subject dies during participation in the study or during a recognized follow-up period, MCERC will be provided with a copy of any post-mortem findings, including histopathology.

New or updated information will be recorded on the originally completed "SAE" CRF, with all changes signed and dated by the investigator. The updated SAE CRF should be resent to MCERC within the timeframes outlined in Section 10.9.

17.0 Prompt Reporting of SAEs to MCERC Required Standard Wording:

SAEs will be reported promptly to MCERC as described in the following table once the investigator determines that the event meets the protocol definition of an SAE.

17.1 Timeframes for Submitting SAE Reports to MCERC

Type of SAE	Initial SAE Reports		Follow-up Information on a Previously Reported SAE	
	Time Frame	Documents	Time Frame	Documents
All SAEs	24 hrs	"SAE" CRF pages	24 hrs	Updated "SAE" CRF pages

	Initial SAE Reports		Follow-up Information on a Previously Reported SAE	
Type of SAE	Time Frame	Documents	Time Frame	Documents
	24 hrs		24 hrs	

17.2 Completion and Transmission of the SAE Reports

Once an investigator becomes aware that an SAE has occurred in a study subject, she/he will report the information to MCERC within 24 hours as outlined in Section 10.9., “Prompt Reporting of SAEs to MCERC. The SAE CRF will always be completed as thoroughly as possible with all available details of the event, signed by the investigator (or designee), and forwarded to MCERC within the designated time frames. If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying MCERC of the event and completing the form. The form will be updated when additional information is received.

The investigator will always provide an assessment of causality at the time of the initial report as described in Section 10.7.2., “Assessment of Causality”.

Facsimile transmission of the “SAE” CRF is the preferred method to transmit this information to the project contact for SAE receipt. In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the "SAE" CRF sent by overnight mail. Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE CRF within the timeframes outlined in Section 10.9., “Prompt Reporting of SAEs to MCERC”. MCERC will provide a list of project contacts for SAE receipt, fax numbers, telephone numbers, and mailing addresses.

The following pages of the CRF must accompany the SAE forms that are forwarded to MCERC: “Demography”, “Medical History”, “Concomitant Medications”, “Study Medication Records”, and “Form D” (if applicable).

17.3 Regulatory Reporting Requirements for SAEs

The investigator will promptly report all SAEs to MCERC in accordance with the procedures detailed in Section 10.9., "Prompt Reporting of SAEs to MCERC." MCERC has a legal responsibility to notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the appropriate project contact for SAE receipt is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the Institutional Review Board (IRB)/Independent Ethics Committee (IEC).

This protocol may be filed under an Investigational New Drug (IND) application / dietary supplement with the US Food and Drug Administration (FDA). A given SAE may qualify as an IND Safety Report if the SAE is both attributable to the investigational product and unexpected. In this case, all investigators filed to the IND (and associated IND's for the same compound) will receive an Expedited Investigator Safety Report (EISR), identical in content to the IND Safety Report submitted to the FDA.

EISR are prepared according to MCERC policy and are forwarded to investigators as required. An EISR is prepared for a SAE that is both attributable to investigational product and unexpected. The purpose of the EISR is to fulfill specific regulatory and Good Clinical Practice (GCP) requirements, regarding the product under investigation.

When a site receives from MCERC an Initial or Follow-up EISR or other safety information (e.g., revised Clinical Investigator's Brochure/Investigator's Brochure), the responsible person according to local requirements is required to promptly notify his or her IRB or IEC.

Expedited Investigator Safety Reports (EISR) are prepared according to MCERC policy and are forwarded to investigators as necessary. An EISR is prepared for a SAE that is both attributable to investigational product and unexpected. The purpose of the EISR is to fulfill specific regulatory and Good Clinical Practice (GCP) requirements, regarding the product under investigation.

An investigator who receives an EISR describing a SAE or other specific safety information from MCERC will file it with the Investigator Brochure and will notify the IRB or IEC, if appropriate according to local requirements.

17.4 Post-study AEs and SAEs

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE detection period defined in Section 10.5., "Time Period, Frequency, and Method of Detecting AEs and SAEs", of the protocol. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the investigator will promptly notify MCERC

17.5 SAEs Related to Study Participation

An SAE considered related to study participation (e.g., procedures, invasive tests, a change in existing therapy), even if it occurs during the pre- or post-treatment period, will be reported promptly to MCERC (see Section 10.9., "Prompt Reporting of SAEs to MCERC ").

18.0 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

18.1 Hypotheses

Oxy-Powder® is both, safe and effective in patients with constipation and constipation in presence IBS.

18.2 Treatment Comparisons of Interest

18.3 Primary Comparisons of Interest

This dietary supplement is to be compared for effectiveness in relieving constipation and IBS in two groups of patients versus Dulcolax tablets in another two groups.

1.1 Other Comparisons of Interest

Comparison of safety of this product shall be done with safety of administering Dulcolax

18.5 Interim Analysis

Data shall be collected regularly but analyzed at the end of the study for safety and effectiveness of the Oxy-Powder®.

18.6 Sample Size Considerations

The study design has been examined and approved by a qualified statistician with respect to sample size.

18.7 Analysis Populations

After the drop outs, it is assumed to have a minimum of 35 patients (out of 60) in constipation group and 15 patients (out of 20) in constipation + IBS group.

18.8 Data Sets

Data set 1: for Constipation group

It shall be prepared at the end of the study, 1 each for Oxy-Powder® and Dulcolax

Data set 2: for IBS with Constipation group

This shall be prepared separately at the end of the study, 1 each for Oxy-Powder® and Dulcolax

18.9 Withdrawal

The patients voluntarily withdrawn from the study (dropouts) shall be documented but shall not be included in the report.

1.0 Missing Data

Missing data not recorded by the patient shall be submitted separately by Mayfair

18.11 Statistical Analysis - Derived and Transformed Data

The pooled included in the report shall be statistically analysed for establishing its validity.

18.12 Other Issues

Any other issues arising during conduct of the study informed by the investigator / CRA shall be notified to the Sponsor.

1.1 Efficacy Analyses

Keeping in view, the evaluated parameters shall be compared with that obtained with Dulcolax administered to the group of patients.

1.2 Safety Analyses (Adverse Events and Serious Adverse Events)

Any AEs and SAEs shall be reported immediately.

1.3 Clinical Laboratory Evaluations

Colonoscopy, Barium Meal X-ray, Stool analysis, Oxygen levels

1.4 Other Safety Measures

Observations on side effects including Diarrhea.

1.5 Health Outcomes Analyses

Loss of weight: Patient feels more fit.

19.0 REGULATORY AND ETHICAL CONSIDERATIONS

- Approval of Ethics Committee of Hospitals where study is conducted
- IRB of MCERC

- Approval of DCGI for conducting study
- Other regulatory agencies e.g. USFDA etc.

19.1 REGULATORY AUTHORITY APPROVAL

MAYFAIR will obtain approval to conduct the study from the appropriate regulatory agency e.g. Drugs Controller General of India, in accordance with any applicable regulatory requirements prior to a site initiating the study in India.

19.2 ETHICAL CONDUCT OF THE STUDY AND ETHICS APPROVAL

This study will be conducted in accordance with "good clinical practice" (GCP) and all applicable regulatory requirements, including, where applicable, the [insert the appropriate date] version of the Declaration of Helsinki.

The investigator (or sponsor, where applicable) is responsible for ensuring that this protocol, the site's informed consent form, and any other information that will be presented to potential subjects (e.g., advertisements or information that supports or supplements the informed consent) are reviewed and approved by the appropriate IEC/IRB. The investigator agrees to allow the IEC/IRB direct access to all relevant documents. The IEC/IRB must be constituted in accordance with all applicable regulatory requirements. MCERC will provide the investigator with relevant document(s)/data that are needed for IEC/IRB review and approval of the study. Before investigational product(s) and CRF's can be shipped to the site, MCERC must receive copies of the IEC/IRB approval, the approved informed consent form, and any other information that the IEC/IRB has approved for presentation to potential subjects.

If the protocol, the informed consent form, or any other information that the IEC/IRB has approved for presentation to potential subjects is amended during the study, the investigator is responsible for ensuring the IEC/IRB reviews and approves, where applicable, these amended documents. The investigator must follow all applicable regulatory requirements pertaining to the use of an amended informed consent form including obtaining IEC/IRB approval of the amended form before new subjects consent to take part in the study using this version of the form. Copies of the IEC/IRB approval of the amended informed consent form/other information and the approved amended informed consent form/other information must be forwarded to MCERC promptly.

IEC/IRB approval of the consent forms must be obtained in addition to the approval given for the clinical study. Regulatory review and approval may be required in some countries before IEC/IRB approval can be sought.

19.3 INFORMED CONSENT

Informed consent will be obtained before the subject can participate in the study. The contents and process of obtaining informed consent will be in accordance with all applicable regulatory requirements.

Subjects who do not wish to participate in the PGX research may still participate in the clinical study. For each subject, informed consent must be obtained prior to any blood being taken for research. The informed consent for the research must be obtained **in addition to** the subject's consent to participate in the clinical study.

20.0 INVESTIGATOR REPORTING REQUIREMENTS

As indicated earlier, the investigator (or sponsor, where applicable) is responsible for reporting SAEs to the IEC/IRB, in accordance with all applicable regulations. Furthermore, the investigator may be required to provide periodic safety updates on the conduct of the study at his or her site and notification of study closure to the IEC/IRB. Such periodic safety updates and notifications are the responsibility of the investigator and not of MCERC

21.0 STUDY MONITORING

In accordance with applicable regulations, GCP, and MCERC procedures, MCERC monitors will contact the site prior to the subject enrollment to review the protocol and data collection procedures with site staff. In addition, the monitor will periodically contact the site, including conducting on-site visits. The extent, nature and frequency of on-site visits will be based on such considerations as the study objective and/or endpoints, the purpose of the study, study design complexity, and enrollment rate.

During these contacts, the monitor will:

- Check the progress of the study.
- Review study data collected.
- Conduct source document verification.
- Identify any issues and address their resolution.

This will be done in order to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements.

The investigator agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues. At study closure, monitors will also conduct all activities described in Section 23 "Study and Site Closure."

The monitor will also review subject-completed health outcomes questionnaire(s) for extraneous written comments that could indicate possible AEs. Information collected in the CRF and in the subject-completed health outcomes questionnaire(s) are independent components of this study. Except for header section information (e.g., subject number, treatment number, visit date) and other information as defined in the standard clarification agreement (SCA), neither the monitor nor the investigator will attempt to reconcile responses to individual questions/items recorded on the subject-completed health outcomes questionnaire(s) or health outcomes portions of diary cards (if applicable) with other data recorded in the CRF's. Subject-completed health outcome questionnaires generally serve as the source document; therefore, unless otherwise specified elsewhere, no other source document is available for data validation.

22.0 QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, MCERC may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

23.0 STUDY AND SITE CLOSURE

Upon completion of the study, the monitor will conduct the following activities in conjunction with the investigator or site staff, as appropriate: [Insert relevant activities, including but not limited to the following, if they are applicable to the study:

- Return of all study data to MCERC data queries.
- Accountability, reconciliation, and arrangements for unused investigational product(s).
- Review of site study records for completeness.
- Return of treatment codes to MCERC In addition, MCERC reserves the right to temporarily suspend or prematurely discontinue this study either at a single site or at all sites at any time for reasons including, but are not limited to, safety or ethical issues or severe non-compliance. If MCERC determines such action is needed, MAYFAIR will discuss this with the Investigator (including the

reasons for taking such action) at that time. When feasible, MCERC will provide advance notification to the investigator of the impending action prior to it taking effect.

MAYFAIR will promptly inform all other investigators and/or institutions conducting the study if the study is suspended or terminated for safety reasons, and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IEC/IRB promptly and provide the reason for the suspension or termination.

If the study is prematurely discontinued, all study data must be returned to MCERC. In addition, arrangements will be made for all unused investigational product(s) in accordance with the applicable MCERC procedures for the study.

Financial compensation to investigators and/or institutions will be in accordance with the agreement established between the investigator and MCERC

24.0 RECORDS RETENTION

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable backup of these reproductions and that an acceptable quality control process exists for making these reproductions.

MCERC will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations of MCERC standards/procedures; otherwise, the retention period will default to 15 years. The investigator must notify MCERC of any changes in the archival arrangements, including, but not limited to, the following: archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

25.0 PROVISION OF STUDY RESULTS AND INFORMATION TO INVESTIGATORS

When a clinical study report is completed, MCERC will provide the major findings of the study to the investigator.

In addition, details of the study treatment assignment will be provided to the investigator to enable him/her to review the data to determine the outcome of the study for his/her subject.

MCERC may list and summarize the PGX research results from coded samples by subject number in the clinical study report. In this event, the investigator and study staff would have access to the research results and would be able to link the results to a particular subject. The investigator and study staff would be directed to hold this information confidentially.

26.0 INFORMATION DISCLOSURE AND INVENTIONS

26.1 Ownership:

All information provided by MCERC and all data and information generated by the site as part of the study (other than a subject's medical records) are the sole property of MCERC. All rights, title, and interests in any inventions, know-how or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of MCERC and are hereby assigned to MCERC.

If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed between MCERC and the study site, that contract's ownership provisions shall apply rather than this statement.

This includes the results of the PGX assessments included in the study.

26.2 Confidentiality:

All information provided by MCERC and all data and information generated by the site as part of the study (other than a subject's medical records) will be kept confidential by the investigator and other site staff. This information and data will not be used by the investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the investigator or site staff; (2) information which it is necessary to disclose in confidence to an IEC or IRB solely for the evaluation of the study; (3) information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in the next paragraph. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

26.3 Publication:

For multicentric studies, the first publication or disclosure of study results shall be a complete, joint multicentric publication or disclosure coordinated by MCERC. Thereafter, any secondary publications will reference the original publication(s).

Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a “Publication”), the investigator shall provide MCERC with a copy of the proposed Publication and allow MCERC a period of at least thirty (30) days [or, for abstracts, at least five (5) working days] to review the proposed Publication.

Proposed Publications shall not include either MCERC confidential information other than the study results or personal data on any subject, such as name or initials.

At MCERC’s request, the submission or other disclosure of a proposed Publication will be delayed a sufficient time to allow MCERC to seek patent or similar protection of any inventions, know-how or other intellectual or industrial property rights disclosed in the proposed Publication.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement is executed, that contract’s publication provisions shall apply rather than this statement.

It is mandatory to inform and register with the Association of publishers of Medical General, the Clinical trial details, before commencement of the study.

Ref: The International Committee of Medical Journal Editors (ICMJE) Policy dtd. May 23, 2004,

27.0 DATA MANAGEMENT

Subject data are collected by the investigator or designee using the Case Report Form (CRF) defined by MCERC Subject data necessary for analysis and reporting will be entered / transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable MCERC standards and data cleaning procedures. Database freeze will occur when data management quality control procedures are completed. Original CRF’s will be retained by MCERC, while the investigator will retain a copy.

Appendix–5

MAYFAIR CLINICAL, EDUCATION & RESEARCH CENTRE, THANE

Clinical Study Report– Constipation

Randomization Schedule

No. of Patients: 40 A = Oxy-Powder® B = Dulcolax

Sr. No.	Drug	Sr. No.	Drug
1	A	21	B
2	B	22	A
3	A	23	B
4	B	24	A
5	B	25	A
6	A	26	B
7	A	27	A
8	B	28	A
9	A	29	A
10	B	30	A
11	A	31	A
12	A	32	B
13	B	33	B
14	A	34	A
15	A	35	A
16	A	36	A
17	A	37	A
18	B	38	B
19	A	39	A
20	A	40	A

CASE REPORT FORM

DOCUMENT NO.: MCERC/PROT-CLTR/OXY/1205/001

Version 02, December 20, 2005

CLINICAL TRIAL PHASE III

SAFETY AND EFFICACY OF

OXY-POWDER®

Sponsor

Mayfair Clinical, Education and Research Centre, Thane

Confidentiality Statement:

The information provided in this document is strictly confidential and is available for review to Investigators, potential investigators and appropriate Ethics committees. No disclosure should take place without written authorization from the Sponsor except to the extent necessary to obtain Informed Consent from potential subjects.

Subject No. :

Procedures at each Visit:

Procedures	Visit 1 Day 1	Visit 2 Day ----

Remarks:

MAYFAIR CLINICAL EDUCATION & RESEARCH CENTRE,
Mohan Mill Compound, Ghodbunder Road, Thane-400 607.

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Document No MCERC/REPTCLTR/OXY/1205/001 Study Identifier: Mfair/oxy/200506

CENTRE	BHATIA HOSPITAL
PRINCIPAL INVESTIGATOR	DR. SHARAD SHAH'S CLINIC
CO INVESTIGATOR	DR. ADITI

INSTRUCTIONS FOR THE INVESTIGATORS:

1. Please enter all the data in black ball-point pen only.
2. Please enter the Subject no. on the Case Record Form (CRF)
3. Please complete all entries in CRF and do not leave any blank spaces. Please (x) in the appropriate boxes. If data is not applicable, please enter NA, or if not done, enter ND
4. Do not rub, erase or use correction fluid for incorrect entries. For correction, if any, strike out the incorrect answer and enter the correct entry alongside, initial the correction with date.
5. Use only standard abbreviations, if required.
6. In case of any further query, kindly contact:

Mayfair Clinical, Education and Research Centre, Thane
Tel: 91-22-2589 5856 Fax: 2589 5854 E-mail: ijklalla@mayfaircro.com

Dr. J.K. Lalla: Res: 91-22-28701161 Mob: 98205 23127
Dr. (Mrs.) Meena Shah Res: 91-22-22021296 Mob: 98203 17747

Subject No. :

SCREENING VISIT (VISIT 0)

DATE: -----

SUBJECT CONSENT TAKEN: YES

NO

☐☐

(CONSENT FORM ENCLOSED)

Signature: _____ **Date:** _____
Subject

Signature: _____ **Date:** _____

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Investigator

DEMOGRAPHICS

Subject No. :

Subject Initials: _____

Gender: Male Female
(Please cross 'X')

Date of Birth: _____
DDMMYY

Height: _____ cm

Weight: _____ kg

***LMP:** _____
DDMMYY

Not applicable

(* Last Menstruation Period)

Birth control measures taken: Give in brief

Educational Status:

School dropout: YES NO

If 'Yes' give details: _____

High School

College

Graduate

Post graduate

(If the subject has dropped out somewhere in between e.g. between Std. X and Std. XII then the box corresponding to the last education completed has to be crossed.)

Subject No.:

SUBJECT HISTORY

(Please cross 'X' in the appropriate box)

Constipation alone

Constipation with IBS

For Constipation with or without IBS, give following details:

1.Food habits: Vegetarian

Non-vegetarian

2.Duration of Constipation:Weeks and Months

3.No. of bowel movements in a week:

4.Severity of disease: Mild Moderate: Severe:

5.Straining during bowel movement: Yes No

6.Urgency for passing stools: Yes No

7.Type of Constipation / IBS: Intermittent Persistent

8.Lumpy or hard stools Yes No

9.Loose or watery stools Yes No

10.Sensation of incomplete evacuation: Yes No

11.Sensation of anorectal blockage: Yes No

12.Passing mucus during bowel movement Yes No

13.Abdominal fullness / Yes No

14.Smoking History: Yes No

Diagnosis:

Subject No.:

[III] HISTORY OF SIGNIFICANT ILLNESS

(Mention any significant/major illness or surgery)

Sr. No.	Condition	Date of Onset/occurrence	Comment (for a past illness, date when the event stopped can also be mentioned here)
		<div> <div><input type="text"/></div> <div><input type="text"/></div> <div><input type="text"/></div> <div><input type="text"/></div> <div><input type="text"/></div> <div><input type="text"/></div> </div> <div> <div>DD</div> <div>MM</div> <div>YY</div> </div>	

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[IV] HISTORY OF MEDICATIONS USE (PREVIOUS AND ONGOING)

Drugs	Condition	Dose	Duration (Years/Months)	Continued		If 'No' period since discontinued
				Yes	No	

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Subject No.:

FAMILY HISTORY:

[V] CLINICAL EXAMINATION

VITAL SIGNS

Pulse

--	--	--	--	--	--	--	--	--	--

beats/minute

Blood Pressure:

(RIGHT ARM SITTING POSITION)

Systolic

--	--	--	--	--	--	--	--	--	--

mm Hg

Diastolic

--	--	--	--	--	--	--	--	--	--

mm Hg

Respiratory rate:

--	--	--	--	--	--	--	--	--	--

/min

Any abnormal finding (s) present? (please cross X)

	NO	YES	COMMENTS
Respiratory System			
Cardiovascular System			
Abdominal System			
Central Nervous System			
Others			

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Subject No.

[VI] SELECTION CRITERIA

(Please (X) Yes or No)

- | | YES | NO |
|---|--------------------------|--------------------------|
| 1. Subject aged 18-60 years | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. Rome II criteria for Constipation | <input type="checkbox"/> | <input type="checkbox"/> |
| Rome II criteria for IBS | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. Subject signing informed consent document. | <input type="checkbox"/> | <input type="checkbox"/> |

Has the subject met the selection criteria:

Yes

☐

No

☐

(Note: If any of the shaded boxes is ticked (✓) the subject will be excluded from the study)

Investigator's name:

Investigator's signature and Date:

Subject No.

Day

CLINICAL LABORATORY INVESTIGATIONS

PARAMETERS FINDING NORMAL RANGE

1. Oxygen content of blood after 15 minutes
2. Oxygen content of blood after 15 minutes
3. Stool examination
4. Barium meal
5. Colonoscopy

STUDY DRUG SHEET

Randomization code no.:

Document No MCERC/REPTCLTR/OXY/1205/001 Study Identifier: Mfair/oxy/200506

Study drug dispensed:

Test (Oxy-Powder®)

Reference (Dulcolax tablets)

Administration instructions:

For cleansing:

TEST:

Take four capsules in the evening on empty stomach with plenty of water for seven days (from day 1, every day up to 7th day)

REFERENCE:

Take two capsules in the evening on empty stomach for seven days (from day 1, every day up to 7th day)

For maintenance:

TEST:

Take four capsules in the evening on empty stomach with plenty of water every alternate day for next 5 weeks (from day 8 to day 42)

REFERENCE:

Take two capsules in the evening on empty stomach with plenty of water every alternate day for next 5 weeks (from day 8 to day 42)

VISIT 2

SUBJECT EVALUATION

(Please cross 'X' in the appropriate box)

∴

Subject No.:

Constipation alone: Constipation with IBS:

For Constipation with or without IBS, give following details:

1. Severity of disease: Mild Moderate: Severe:

2. Straining during bowel movement: Yes No

3. Urgency for passing stools: Yes No

4. Type of Constipation / IBS: Intermittent Persistent

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- 5.Lumpy or hard stools Yes ☐ No ☐
- 6.Loose or watery stools Yes ☐ No ☐
- 7.Sensation of incomplete evacuation Yes ☐ No ☐
- 8.Sensation of anorectic blockage: Yes ☐ No ☐
- 9.Passing mucus during bowel movement Yes ☐ No ☐
- 10.Abdominal fullness / bloating / swelling Yes ☐ No ☐
- 11.Red flag symptoms Yes ☐ No ☐
- (Page 21 of protocol)

ADVERSE EVENTS – after 1 week

Were there any adverse events? Yes ☐ No ☐

Please complete all sections below, cross appropriate number(s)

Adverse Events, specify, Please list ONE event per column. Give diagnosis if possible	Description of AE 1.	Description of AE 2.	Description of AE 3.						
Serious 1 No 2 Yes* (if yes fill the Serious Adverse Events form)	<table border="1"><tr><td>1</td><td>2</td></tr></table>	1	2	<table border="1"><tr><td>1</td><td>2</td></tr></table>	1	2	<table border="1"><tr><td>1</td><td>2</td></tr></table>	1	2
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Reason (several statements are possible) 1.Results in death 2.Life threatening 3.In subject hospitalization or prolongation of existing 4.Congenital anomaly/birth defect 5.Persistent or significant disability incapacity 6. Important medical event	<div>1 2 3 4</div> <div>5 6</div>	<div>1 2 3 4</div> <div>5 6</div>	<div>1 2 3 4</div> <div>5 6</div>
A DATE & TIME Start A Date & Time STOP C. 1. Continuous 2:Intermittent If ongoing update at next visit	A <div> </div> <div> </div>	A <div> </div> <div> </div>	A <div> </div> <div> </div>
	B <div> </div> <div> </div>	B <div> </div> <div> </div>	B <div> </div> <div> </div>
	<div>1 2</div>	C <div>1 2</div>	C <div>1 2</div>
Severity 1.Mild: awareness of symptoms but interfere with routine activities 2.Moderate: discomfort enough to interfere with routine activities. 3.Severe: Impossible to perform routine activities.	<div>1 2 3</div>	<div>1 2 3</div>	<div>1 2 3</div>

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Relation to study design** 1. Certain. 2. Probable. 3. Possible. 4. Unlikely 5. Unclassified-conditional 6. Unclassified-inaccessible	<table border="1"> <tr> <td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td> </tr> </table>	1	2	3	4	5	6	<table border="1"> <tr> <td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td> </tr> </table>	1	2	3	4	5	6	<table border="1"> <tr> <td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td> </tr> </table>	1	2	3	4	5	6																								
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Action taken (several statements are possible) 2. None 3. Dose of study drug reduced 3. Study drug discontinued and restarted 4. Study drug discontinued permanently 5. Remedial drug therapy, specify on concomitant medication page 6. Other (specify below) 7. Hospitalization required or prolonged	<table border="1"> <tr><td>1</td><td></td></tr> <tr><td>2</td><td></td></tr> <tr><td>3</td><td></td></tr> <tr><td>4</td><td></td></tr> <tr><td>5</td><td></td></tr> <tr><td>6</td><td></td></tr> <tr><td>7</td><td></td></tr> </table>	1		2		3		4		5		6		7		<table border="1"> <tr><td>1</td><td></td></tr> <tr><td>2</td><td></td></tr> <tr><td>3</td><td></td></tr> <tr><td>4</td><td></td></tr> <tr><td>5</td><td></td></tr> <tr><td>6</td><td></td></tr> <tr><td>7</td><td></td></tr> </table>	1		2		3		4		5		6		7		<table border="1"> <tr><td>1</td><td></td></tr> <tr><td>2</td><td></td></tr> <tr><td>3</td><td></td></tr> <tr><td>4</td><td></td></tr> <tr><td>5</td><td></td></tr> <tr><td>6</td><td></td></tr> <tr><td>7</td><td></td></tr> </table>	1		2		3		4		5		6		7	
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ACTION TAKEN 1. Resolved 2. Improved 3. Unchanged 4. Worsened 5. Death 6. Insufficient follow-up	<table border="1"> <tr> <td>1</td><td>2</td><td>3</td> </tr> <tr> <td>4</td><td>5</td><td>6</td> </tr> </table>	1	2	3	4	5	6	<table border="1"> <tr> <td>1</td><td>2</td><td>3</td> </tr> <tr> <td>4</td><td>5</td><td>6</td> </tr> </table>	1	2	3	4	5	6	<table border="1"> <tr> <td>1</td><td>2</td><td>3</td> </tr> <tr> <td>4</td><td>5</td><td>6</td> </tr> </table>	1	2	3	4	5	6																								
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ADR WAS EXPECTED? 1. Yes 2. No	<table border="1"> <tr> <td>1</td><td>2</td> </tr> </table>	1	2	<table border="1"> <tr> <td>1</td><td>2</td> </tr> </table>	1	2	<table border="1"> <tr> <td>1</td><td>2</td> </tr> </table>	1	2																																				
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ADVERSE EVENTS

*Any Serious Adverse Events seen: Yes No

☐☐

If yes, please fill the form of Serious Adverse Event and notify the sponsor and Ethics Committee of the institute within 24 hrs.

Time & Date of notification to sponsor

--	--	--	--	--

--	--	--	--	--	--	--	--

D D M M Y Y

Time & Date of notification to Ethics Committee

--	--	--	--	--

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D D M M Y Y

****Relationship to the drug in the investigational device.**

1.Certain: a clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definite pharmacologically or phenomenologically, using a satisfactory rechallenge procedure is necessary.

1.Probable/ likely: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to reasonable response or withdrawal (dechallenge). Rechallenge information is not required to fulfill this criterion.

2.Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drug or chemicals. Information on drug withdrawal may be lacking or unclear.

3.Unlikely: a clinical event, including laboratory test abnormality, reported as an adverse reaction about which more data is essential for a proper assessment or the additional data are under examination.

4.Unclassified / inaccessible: A report suggesting an adverse reaction, which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

INVESTIGATOR'S SIGNATURE AND DATE

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“EVALUATION AND ADR AND FINDINGS OF LAB INVESTIGATIONS” PAGES TO BE COPY PASTED FOR THE END OF 3 WEEKS AND END OF 6 WEEKS FOR EVERY SUBJECT.

SUBJECT COMPLETION STATUS

Did the subject complete the study period?

YES

☐

NO

☐

1. To be completed only for subjects prematurely terminated

Primary reasons for termination:

☐☐

Adverse Event

☐

Subject Non-compliance

☐

Consent Withdrawn

☐

Subject lost to follow up

☐

Death

☐

Protocol violation

☐

Other, specify

II. To be completed in all cases of death

Date of Death: DD MM YY

Cause of Death:

OVERALL THERAPEUTIC EFFECTIVENESS

ASSESSMENT BY THE INVESTIGATOR

EXCELLENT

GOOD

FAIR

POOR

ASSESSMENT BY THE PATIENT:

EXCELLENT

GOOD

FAIR

POOR

SAFETY

SIDE EFFECTS:

EXCELLENT NONE

GOOD MILD

FAIR MODERATE

POOR SEVERE

OVERALL EFFICACY RESPONSE

COMPLETE CURE

IMPROVEMENT

FAILURE

INVESTIGATOR'S SIGNATURE

Statement:

I certify that the entries on all pages of the case report form accurately and completely represent results of the examination, tests and evaluations performed on the dates specified. I was personally familiar with the clinical presentation and progress of the study subject.

Investigator's name:

Investigator's Signature and Seal

Date

Patient Informed Consent

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MOHAN MILL COMPOUND, GHODBUNDER ROAD, THANE-400 607.

PATIENT INFORMED CONSENT

1. I, _____ aged _____ years, have been given complete information on the project _____ investigating Oxy Powder Capsule.

• **Oxy Powder capsules – Brief Information**

Oxy powder, a dietary supplement is marketed by Global Healing Centre, Inc. USA. Each capsule of Oxy powder contains 685 mg Ozonated Magnesium Oxides, 5.5 mg Organic Germanium 132 and 25 mg of Natural Citric acid. It has laxative property. Oxy-Powder is a non-toxic, safe, effective and non-allergic. Oxy-Powder is also harmless to the good bacteria in the intestinal tract. No toxicity has been reported in the literature caused by overdose of Oxy Powder. To help cleansing process it is advised to drink plenty of purified water daily while taking the Oxy-Powder; even though there has never been a documented case of dehydration or electrolyte imbalances.

• **Dulcolax Tablets – Brief Information**

Dulcolax tablets containing Bisacodyl USP 5 mg are used to treat constipation or to clean out the intestinal tract before bowel examinations or bowel surgery. This medication may cause stomach ache, cramping, weakness, sweating, and irritation of the rectal area, diarrhoea, or dizziness if taken more than the dose recommended by the Doctor.

The above information has been explained to me (a) The medicinal properties, (b) safety, (c) side effects and (d) uses of the Oxy Powder Capsules & Dulcolax Tablets. I have clearly understood explanation given by the Doctor. I have also been explained the (e) benefits, (f) possible risks and potential side effects as above associated with consumption of Oxy Powder Capsules & Dulcolax Tablets proposed to be studied.

2. I understand that it is my responsibility to ask questions and seek clarifications on any points not clearly understood by me. I have asked all such queries to the Doctor and have received answers to my satisfaction.

3. I understand about my rights to withdraw from the study anytime without giving any reason.

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4. I have been explained not to conceal or distort any information pertaining to my medical history which might impair or affect my health or my participation in the Study. Due to my hiding any information, I shall not hold the Doctor/ Hospital /Centre responsible if any damage is caused to my health.

5. I recognize that the Oxy Powder Capsules & Dulcolax Tablets formulation shall be administered to me for “test and research purposes” and has not been manufactured by Mayfair Clinical Education & Research Centre Pvt. Ltd. but supplied by the Sponsor of the Project. It is manufactured by Oxygen Research LLC. It is available on the counter as a Dietary supplement in US.

6. I accept that I have to undergo the tests and procedures during conduct of the Study:

- Ingesting the Oxy Powder Capsules & Dulcolax Tablets as directed with water **truthfully and honestly** and strict check by the Study Co-ordinator.
- a current physical examination
- checking of weight daily for 7 days
- Oxygen content in the blood (pulse oximeter) on day 0, week 3, week 6 in the morning, on empty stomach
- O₂ level measured at 15 & 30 minutes post administration
- Barium meal, colonoscopy with minimal cleaning in the bowel before and after the study (0 & 6 weeks)
- Stool examination before and after the completion of the study.

I accept all the conditions described above. I shall abide by all the rules, regulations and code of conduct of in and outside the hospital. I shall maintain complete confidentiality of the Study.

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MOHAN MILL COMPOUND, GHODBUNDER ROAD, THANE-400 607.

For contact of Patient:

Name of the Patient: _____ Address: _____

Signature of patient: _____

Date: _____ Mobile _____
(DD/MM/YY) Email: _____

Tel: _____ Fax: _____

Signature of the witness _____ Date _____
(DD/MM/YY)

Name of witness: _____

**REPORT FOR
CLINICAL TRIAL PHASE III
SAFETY AND EFFICACY OF OXY-POWDER®
(CONSTIPATION-IBS)
VERSION 01**

DOCUMENT NO.: MCERC/REPT-CLTR/OXY/1205/001 ghanima



May 30th 2007

**TRIAL TO STUDY SAFETY AND EFFECTIVENESS OF OXY-POWDER® IN TREATING
IBS with related constipation.**

MAYFAIR CLINICAL EDUCATION RESEARCH CENTRE,
(A KALTHIA GROUP ORGANIZATION)

Mohan Mill Compound, Ghodbunder Road, Thane-400 607.

Ph. No. 91-022-2589 5856 Fax. 91-022-2589 5854

e-mail jkallalla@mayfaircro.com / info@mayfaircro.com

Part-4 B

CLINICAL STUDY REPORT

ON OXY-POWDER®

CONSTIPATION—IBS

CLINICAL STUDY REPORT ON OXYPOWDER-CONSTIPATION+IBS

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5	ADMINISTRATIVE STATEMENTS a) Glossary (b) Regulatory Approvals c) Statement of final approval of the report director, statistician, investigators, etc.	
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7	4.0 RATIONALE 4.1 OBJECTIVES OF THE STUDY	

8	<p>5.0 METHODOLOGY</p> <p>5.0.1 Conduct (including Ethical conduct) of Study</p> <p>5.0.2 Subject Information and Consent</p> <p>5.0.3 Investigator& Study Centre</p> <p>5.1.1Study Design, and Duration</p> <p>5.2 Procedure for Conduct of study</p> <p>5.2.1 Study Population</p> <p>5.2.2 No. of Patients enrolled</p> <p>5.3–5.4 Patient selection and inclusion Criteria</p> <p>5.4.1 Rome II Criteria for IBS</p> <p>5.4.2 Symptoms that cumulatively support the diagnosis of IBS</p> <p>5.5 Supportive symptoms of IBS</p> <p>5.6 Red flag symptoms which are not typical of IBS</p> <p>5.7 Diagnosis of IBS</p> <p>5.8 Subject Exclusion Criteria</p> <p>6.0 Study Procedure and Treatment</p> <p>7.0Treatment and Timings</p> <p>7.1 Dosage and Administration</p> <p>7.2 Study Assessment</p> <p>7.3 End Points</p> <p>8.0 Demography</p> <p>Grading of Response</p> <p>9.0 Efficacy</p> <p>10.0 Safety</p> <p>11.0 Withdrawal from study</p> <p>12.0 Statistics and data handling</p> <p>13.0 Other Issues</p> <p>14.0 Ethics</p> <p>15.0 Ethical Conduct and Approval of Studies</p> <p>15.1 Informed Consent</p>	
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	18.1 Screen and Baseline failures	
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	22.0 Data Management	
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25	Comparison of Weight (kg) (Day 0-Day 42)	
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37	Abdominal fullness(a)Day 0 (b) Day 21 and (c) Day 42)]	
38	Overall Efficacy-(A) Assessment By Investigators (B) Assessment By Patients	
39	Comparison of Efficacy of Oxy-Powder® with Dulcolax	

DETAILED CLINICAL REPORT ON OXYPOWDER CONSTIPATION– IBS:

1.0 DETAILS OF THE FOLLOWING TOPICS HAVE ALREADY BEEN DESCRIBED

ABOVE UNDER “Clinical Report On Oxy-Powder®–Constipation”:

Abstract–(Pages 2,3), Synopsis–(Pages 8–14), Glossary– (Pages 90–95), Regulatory Approvals–(Page 96), Investigators Declaration–(Page 97), QA statement– (Page 98),Signature of study Director / study coordinator–(Page 99), Signature of study Co–coordinator, Monitor, QA Director–(Page 100), Signature of Statistical Investigator–(Page 101),Contributors to the Study–(Page 102), Introduction–(Pages103–113), Subject Information and Consent–(Pages114–115)

Since they are common to both clinical trials (constipation and constipation–IBS), these are not being repeated here.

4.0 Rationale:

The present study was conducted to evaluate effectiveness of Oxypowder in treating IBS with constipation precipitating loss of weight & improving oxygen delivery in patients of IBS with constipation

4.1 Objective of the study

Primary objective of the present study was to evaluate effectiveness& safety of oxy-powder in treating IBS with constipation

5.0 METHODOLOGY

5.1 Conduct (including Ethical conduct) of study (Page 114)

5.1.1. Study Design and Duration.

The study was done as Multicentric open randomized comparing safety and effectiveness of Oxypowder developed by Global Healing Centre Inc. USA with Dulcolax tablets containing 5 mg Bisacodyl manufactured by Cadila Health Care Ltd. Duration of the study was 6 weeks from the date of administration of study products.

5.1.2 Subject Information and Consent

5.2 Procedure for Conduct of Study

The study was conducted in the out-patient setting in Bhatia Hospital. 20 patients of either sex were selected for the study. The subjects were included after obtaining their informed consent. Necessary Regulatory permission from Drug controller General of India was obtained and the study was cleared by Independent Ethics Committee of Mayfair and Institutional Review Board of Bhatia Hospital Medical Research Society, Mumbai. The study was conducted in accordance with Current Good Clinical Practice (GCP) Guidelines.

5.3 Patient Selection

5.4 Subject inclusion

The subjects were included only if the following criteria were met with:

- 1) Age 18 to 60 yrs.
- 2) Food habits. Both Vegetarians and Non-Vegetarians were included; however, non-vegetarians were preferred.
- 3) Acceptable form of birth control was followed in female patients
- 4) The patients were selected when they satisfied Rome II criteria for IBS (Irritable bowel syndrome) with constipation. The criteria were as follows:-
(The Rome II diagnostic criterion of irritable Bowel Syndrome always presumes the absence of a structural or biochemical explanation for the symptoms and is made only by a physician.)

5.4.1 Rome II criteria for IBS

The Rome II diagnostic criteria of Irritable Bowel Syndrome always presumes the absence of a structural or biochemical explanation for the symptoms and is made only by a physician.

Irritable Bowel Syndrome can be diagnosed based on at least 12 weeks (which need not be consecutive) in the preceding 12 months, of *abdominal discomfort or pain that has two out of three of these features*:

1. Relieved with defecation; and/or
2. Onset associated with a change in frequency of stool; and/or
3. Onset associated with a change in form (appearance) of stool.

5.4.2 Symptoms that Cumulatively Support the Diagnosis of IBS

1. Abnormal stool frequency (may be defined as greater than 3 bowel movements per day and less than 3 bowel movements per week);
2. Abnormal stool form (lumpy/hard or loose/watery stool);
3. Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);
4. Passing of mucus in the faeces.
5. Bloating with abdominal distension.

5.5 Supportive Symptoms of IBS

1. Fewer than three bowel movements a week
2. More than three bowel movements in a day
3. Passing hard or lumpy stools
4. Loose (mushy) or watery stools
5. Straining during a bowel movement
6. Urgency (having to rush to have a bowel movement)
7. Feeling of incomplete bowel movement
8. Passing mucus (white material) during bowel movement
9. Abdominal fullness, bloating, or swelling

5.6 Red Flag symptoms which are NOT typical of IBS

Pain that often awakens/interferes with sleep

Diarrhoea that often awakens/interferes with sleep

Blood in your stool (visible or occult)

Weight loss

Fever

Abnormal physical examination

5.7 Diagnosis of IBS

The diagnosis of irritable Bowel Syndrome was based on at least 12 weeks (which need not be consecutive) in the preceding 12 months of abdominal discomfort or pain that had two out of three of the following features:

3. Relieved with defecation; and/or
4. Onset associated with a change in frequency of stool; and/or

5. Onset associated with a change in form (appearance) of stool.

Since the patients selected were those suffering from IBS with constipation, following symptoms that supported the diagnosis of IBS with constipation were considered:

1. Abnormal stool frequency – Defined as less than 3 bowel movements per week

2 Abnormal stool form – lumpy/hard stools

3 Abnormal stool passages –

- straining During > 25% of Bowel movements
- sensation of incomplete evacuation for > 25% of Bowel Movements
- sensation of anorectal blockage for > 25% of Bowel movements
- manual Manoeuvres to facilitate > 25% of Bowel movements

6. Passage of Mucus during bowel movement.

7. Abdominal fullness or bloating

The patients who had following Red Flag symptoms which are not typical of IBS were excluded from the study –

Pain that often awakens/interferes with sleep

Blood in your stool (visible or occult)

Weight loss

Fever

Abnormal physical examination

5.8 Subject exclusion

Following patients were excluded from the study:

1. Evidence of Malignancy on colonoscopy / having diagnosed organic GI disorder
2. Pregnant and lactating women
3. Evidence of lactose intolerance to explain bowel symptoms
4. History of cardiac arrhythmia's or heart disease
5. History of Glaucoma
6. History of urine retention
7. History of schizophrenia
8. History of substances of abuse/dependency
9. Intellectually unable or unwilling to complete daily GI ratings.

6.0 Study procedure and Treatment

After selection, the patients were subjected to detailed medical History and clinical examination

Patients then were subjected to following pre-study evaluation :-

- ❖ Record of weight
- ❖ Oxygen content of Blood. This was done by using pulse oximeter.
- ❖ Stool examination
- ❖ Barium meal to rule out any Bowel organic lesion leading to constipation
- ❖ Colonoscopy to rule out any colonic organic lesion and any faecal impaction.

After ruling out any organic lesion, the patients were included in the study.

7.0 Treatment and Timings

Test Product (A) – Oxy-Powder® capsules marketed by Global Healing Centre, Inc. USA containing 715.5 mg Active in each capsule

Comparative Product (B) – Dulcolax tablets marketed by Cadila Healthcare Ltd, India containing 5 mg Bisacodyl in each tablet.

7.1 DOSAGE & ADMINISTRATION

The patients were divided in two (2) groups as per the Randomization schedule. Group1 was administered Product A while Group 2 was administered Product B

7.1.1 Test Product (A)

For first time, patients started with a seven-day initial cleansing procedure. After the initial cleanse, a maintenance dose was continued for keeping intestinal tract clean and deliver oxygen into the system. Patients were advised to take Oxy-Powder with plenty of potable water during the day and eat healthy diet.

(a) For Cleansing

Patients were directed by the investigators to take four (4) capsules in the evening on an empty stomach with 240 mL water. If 3-5 bowel movements were not achieved the following day, the dosage was increased by adding two (2) capsules every night until 3-5 bowel movements were achieved the following day. The day on which the dosage was finalized was considered as “day one (1)” of the seven (7) day cleansing cycle. This dosage was continued for seven (7) consecutive days. After the seven (7) day cleansing cycle, the maintenance dose was started.

(b) Maintenance Dose:

Patients were given capsules supplies for seven (7) days with instructions that they had be taken as they did during cleansing cycle. For following 5 weeks, the same dosage was to be repeated by the patients every other day. They were assured that the capsules were not habit forming or harmful to the body and could be taken indefinitely.

(c) Comparative Product (B)

Patients in this Group were directed by the investigators to take two tablets of Dulcolax (comparative formulation) as per the Randomization scheme with plenty of water every day, for first 7 days, in the evening on empty stomach. Thereafter, the subjects were instructed to take the same dose of Dulcolax tablets on alternate days on empty stomach in the evening for 6 weeks.

Administration on day '0', '3 weeks' and '6 weeks' was done on empty stomach (to enable measuring Oxygen levels) in the morning. Subjects were asked to report to the hospital on empty stomach in the morning for administration of Investigational products and Oxygen level in the blood was measured 15 minutes and 30 minutes after administration of tablets/ capsules.

On the remaining days, the subjects were asked to take same dose of the Investigational product at their residence in the evening on empty stomach followed by Dinner two hours post-administration of the dose..

Administration on day 0, 3 weeks and 6 weeks were done on empty stomach in the morning. Subjects were asked to report to the hospital in a fasting state in the morning for administration of investigational products and Oxygen level in the blood were measured 15 minutes and 30 minutes after administration of tablets/capsules.

On the remaining days, the subjects were instructed to take investigational product at home at the same dose levels in the evening on empty stomach. They were told to take Dinner at least two hours post-administration of Drug.

Along with Oxypowder, drinking of large quantity of potable water was recommended. This was not only healthy for the body but was necessary to aid the body in eliminating toxins from the bowel at a faster rate. Oxy-Powder works with stomach acid; if the level of HCl is below normal, it may hinder the effectiveness of the Oxy-Powder, and hence, it was suggested to take an organic lemon wedge squeezed into glassful of potable water with Oxypowder in the evening.

During the study, prescription medicines were not contraindicated as long as Oxypowder was taken 6 hours before or 6 hours after the medicine. Consumption of Laxatives, anti-diarhoeals and anti-spasmodics were prohibited during the study.

After starting the study medication, patients, were initially called every day in the morning to check their daily weight for 7 days.

After doing the oxygen content on Day 0, all the patients were asked to visit on 3rd wk and 6th wk. They were called in morning on empty stomach. Group administered "Oxy-Powder®" were administered capsules and their oxygen levels were determined at '15 min. and '30 min. Post administration of the dose. In Dulcolax group of patients, since they were comparative group, their Oxygen levels were measured without administering Dulcolax, '15 min. and '30 min. after their arrival. On 3rd week and 6th week patients were subjected to detailed symptoms to evaluate the effect of the study drug. Stool analysis was repeated at the end of 6 weeks.

7.2 Study Assessment (Page 118)

7.3 End Points (Page 121)

8.0 Demography

Demographic data including **Height, Weight, Age and Sex** was collected for all the subjects in different groups at the time of their inclusion in the study simultaneous to recording of their Medical History including their Food Habits, Smoking History, Details and Quantity of consumption of alcoholic drinks and other beverages in a day or frequency thereof and consumption of Tobacco in other forms or any Drug of Abuse.

As regards the symptom of constipation–IBS, duration of its existence, its severity, No. of Bowels / Week and type of stools were also recorded.

GRADING OF RESPONSE

The evaluated parameters were compared with those obtained with Dulcolax administered to the patients belonging to the constipation group.

9.0 Efficacy

- a) Overall therapeutic response was graded individually by the investigator with each patient as **‘Excellent’, ‘Good’, ‘Fair’ & ‘Poor’**.
- b) Therapeutic efficacy was correlated and graded as **‘Complete Cure’, ‘Improvement’ and ‘Failure’**.

10.0 Safety

Severity of each **Adverse Event** was rated as ‘**Mild**’ (no limitation of usual activity), ‘**Moderate**’ (some limitation of usual activity), and ‘**Severe**’ (inability to carry out usual activities).

11.0 Withdrawal from Study

There was no withdrawal of patients in either of the treatment in constipation–IBS category. All 20 patients enrolled continued with the study.

12.0 Statistics and data handling

12.1 Statistical Analysis– Derived and Transformed Data

Out of 20 patients enrolled, all 20 patients completed the study & were included in the final analysis. Their demographic features were compared at baseline using appropriate tests (Student’s t test and Chi-square or Fisher's exact test). Efficacy parameters in two treatment groups were compared by using Fisher’s exact test.

12.2 Data Analysis

12.2.1 Hypotheses

Oxy-Powder® is both, safe and effective in patients with constipation-IBS

12.2.2 Sample Size Consideration

The study design had been examined and approved by a qualified statistician with respect to sample size.

12.2.3 Primary Comparisons of Interest

Oxy-Powder® is compared for effectiveness in relieving constipation–IBS vis-à-vis Dulcolax tablets in the same group.

12.2.4 Other Comparisons of Interest

Comparison of safety of Oxy-Powder® has been done vis-à-vis safety achieved post-administering of Dulcolax tablets.

12.2.5 Interim Analysis

Data from patients was collected regularly but analyzed at the end of the study for safety and effectiveness of the Oxy-Powder®.

12.2.6 Analysis Population

After any drop outs, it was assumed to have a minimum of 15 patients (out of 20) in constipation-IBS group completing the study. Number of patients who actually completed 6 weeks of study was 20. The population analysis for safety and effectiveness of Oxy-Powder® and Dulcolax has been done for these 20 patients at the end of the study.

12.2.7 Withdrawal

No patient withdrew from the study until completion of study; all 20 patients have been included in the report.

12.2.8 Missing Data

There has been no **Missing data** of any patient.

13.0 OTHER ISSUES

Results of Barium Meal test conducted at the commencement of study, pre-administration of the dose indicated that none of the patients exhibited any signs of organic lesions. In 7 patients completing the study, no organic lesions were seen in Barium meal test even 6 weeks post- dose administration at the completion of the study.

In the same 7 patients completing the study (of Oxy-Powder® as well as Dulcolax) , pre-dose colonoscopy as well as 6 weeks post dose administration colonoscopy studies showed similar findings in patients under study in having multiple spasmodic contractions in sigmoid and descending colon **but with slight reduction in impacted faecal material shown in post- administration colonoscopy.** This is perhaps one of the factors responsible for efficacy of Oxy-Powder® in the treatment of constipation with and without IBS. Six weeks period of study was perhaps short to see greater reduction in impacted faecal material

The investigator recommended that since conduct of these two tests after 6 weeks of completion of the study did not show any significant differences that could lead to any conclusions, they should be dropped at the conclusion of the study. However, the patients should continue to be subjected to these two tests at the commencement of study.

These recommendations were forwarded to the sponsor by e-mail who gave his consent to follow the recommendations of the investigator and permitted waiving of conduct of these two tests at the end of 6 weeks period of the study in each of the rest of the patients.

14.0 Ethics

The design of the protocol conformed to the Declaration of Helsinki adopted by the 18th World Medical Assembly (WMA), Helsinki, Finland (1964) and all amendments. All the patients were informed of the nature and the purpose of the study and their consent to participate was obtained. They were also informed of their freedom to withdraw from the study at anytime and at any stage of the trial. The study was conducted only after the protocol was approved by IEC of MCERC and IRB of Bhatia General Hospital. Since Oxy-Powder® was marketed in the USA and other countries for almost a decade with no reported ADR's, and the present study was designed and conducted for global markets (other than India), the study director prudently and by way of abundant precaution informed Indian Regulatory Authority, Drugs Controller General of India at CDSCO, New Delhi, of intention of MCERC to conduct the clinical trial Phase III on Oxy-Powder® in Indian patients.

15.0 Ethical conduct of the study and Ethics Approval

This study was conducted in accordance with "Good Clinical Practice" (GCP) guidelines and all other applicable regulatory requirements, including WMA's Declaration of Helsinki –VI on Ethical Principles for Medical Research Involving Human Subjects, as adopted by the 52nd WMA General Assembly, Edinburgh, October 2000.

The investigator was assigned the responsibility for ensuring that this protocol, the site's informed consent form, and any other information that will be presented to potential subjects (e.g. advertisements or information that supports or supplements the informed consent) were reviewed and approved by the appropriate IEC/IRB. The investigator agreed to allow the IEC / IRB direct access to all relevant documents. MCERC provided the investigator, IEC and IRB with relevant document(s)/data that were needed for IEC/IRB review and approval of the protocol for conducting the study. After receiving copies of the IEC/IRB approval, the investigational product(s), blank copies of the approved informed consent forms, CRF's and any other information that the IEC/IRB had approved for presentation to potential subjects. were sent to the trial centre sites, No further amendments were made in the above formats by IEC/IRB

15.1 Informed Consent

Informed consent was obtained from each participant before the subject was permitted to participate in the study. The contents and process of obtaining informed consent was in accordance with all applicable regulatory requirements.

16.0 Protocol Compliance

There were two deviations in the protocol.

1) Age of the patients for enrollment was raised to 65 years to include geriatric patients in whom constipation is more prevalent. There was shortage of patients suffering from constipation in the age group specified in the protocol.

Permission from the sponsor was obtained to include patients suffering from constipation up to the age of 65 years.

2) During course of the study, both the investigators independently made following observation:

a) Pre- and post-colonoscopy showed multiple spasmodic contractions in sigmoid and descending colon with large amount of fecal impaction in entire colon.

b) In Barium meal test, no organic lesions were seen before and after Barium meal administration.

These observations were made in case of type 1 and type 2 patients.

Both the investigators recommended that in view of these observations, post-colonoscopy and post-administration of Barium meal should not be done since they do not significantly contribute to the results of the study. Instead, they were causing discomfort discouraging the patients from continuing with the treatment. They recommended that both the tests must be done at the time of enrolling the patients.

These recommendations were forwarded to the sponsor who, while agreeing with the suggestions of the investigators, permitted to waive conduct of post-colonoscopy as well as post administration of Barium meal.

17.0 Handling and Storage of Investigational Product

Investigational products were dispensed and administered according to procedures described herein.

Only subjects enrolled in the study received investigational product, in accordance with all applicable regulatory requirements. Only authorized site staff supplied and administered investigational products. All investigational products were stored in a secured area with access limited to the investigator and authorized site staff and under physical conditions that are consistent with investigational product's specific requirements.

17.1 Product Accountability

The investigator is responsible for investigational product accountability, reconciliation, and record maintenance. In accordance with all applicable regulatory requirements, the investigator and the designated site staff maintained investigational product accountability records throughout the course of the study. The authorized person(s) documented the amount of investigational product received from MCERC, the amount supplied and administered to the subjects.

18.0 Assessment of Compliance

A diary card was given to the patients during the entire course of the clinical trial which will record the number of capsules/ tablets taken and number of stools passed. Diary card was issued at day 0, week 3 and week 6 after collecting the previously completed diary filled every day from the concerned patient. Patient was required to contact his physician in the event of any difficulties / queries the patient had.

18.1 Screen and Baseline Failures

Not applicable since the study is non-invasive

19.0 Study Monitoring

In accordance with applicable regulations, GCP guidelines and MCERC procedures, MCERC monitors contacted the site prior to the subject enrollment to review the protocol and data collection procedures with site staff. In addition, the monitor periodically contacted the site, including conducting on-site visits. The extent, nature and frequency of on-site visits were based on such considerations as the study objective and/or endpoints, the purpose of the study, study design complexity, and enrollment rate.

During these contacts, the monitor

- Checked the progress of the study.
- Reviewed study data collected.
- Conducted source document verification.
- Identified any issues and address their resolution.

This was done in order to verify that the:

- Data was authentic, accurate, and complete.
- Safety and rights of subjects were being protected.

- Study was conducted in accordance with the currently approved protocol (and any amendments), GCP guidelines, and all applicable regulatory requirements.

The investigators agreed to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

At study closure, monitors conducted all activities described in Section, "Study and Site Closure."

The monitor also reviewed subject-completed health outcomes questionnaire(s) for extraneous written comments that could indicate possible AEs. Information collected in the CRF and in the subject-completed health outcomes questionnaire(s) are independent components of this study. Except for header section information (e.g., subject number, treatment number, and visit date) and other information as defined in the standard clarification agreement (SCA), neither the monitor nor the investigator attempted to reconcile responses to individual questions/items recorded on the subject-completed health outcomes questionnaire(s) or health outcomes portions of diary cards with other data recorded in the CRF's. Subject-completed health outcome questionnaires generally serve as the source document; therefore, unless otherwise specified elsewhere, no other source document is available for data validation.

20.0 Quality Assurance

To ensure compliance with GCP guidelines and all applicable regulatory requirements, MCERC regularly conducted Quality Assurance audit. It was open to Regulatory agencies to conduct regulatory inspection of this study at any time during or after completion of the study. The investigator and institution agreed to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

21.0 Study and Site Closure

Upon completion of the study, the monitor conducted the following activities in conjunction with the investigator or site staff, as appropriate:

- ❖ Return of all study data to MCERC Data queries.
- ❖ Accountability, reconciliation, and arrangements for unused investigational product(s).
- ❖ Review of site study records for completeness.
- ❖ Return of treatment codes to MCERC

22.0 Data Management

Subject data was collected by the investigators or designees using the Case Report Form (CRF) framed by MCERC in consultation with the sponsor and the investigators. Subject data necessary for analysis and reporting was entered into a validated database. Clinical data management was performed in accordance with the 'applicable MCERC standards' and data cleaning procedures. Original CRF's are retained by MCERC.

23.0 Statistical analysis

The data generated on the subjects was classified in different forms to obtain meaningful results on statistical analysis. Three different types of tables supplied to the statistician included:

T1—subject wise medical history including history of a) surgery undergone,

b) illness, c) medication, duration of constipation and IBS if applicable and its severity

T2—Baseline data including subject number and code, subject's age, sex, weight, food habits, smoking history

T3— subject wise clinical investigations (Oxygen content of the blood, stool examination at 0 and 42 weeks, Efficacy evaluation

by the investigators and side effects experienced, if any

The results were statistically analyzed using SAS statistical software.

24.0 Records Retention

Following closure of the study, the investigators maintained all site study records in a safe and secure location. The records were maintained to allow easy and timely retrieval, when needed (e.g. audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by local laws/regulations or institutional policy, some or all of these records were maintained in a format other than hard copy in an electronic form. The investigator also assured that all reproductions were legible, were a true and accurate copy of the original and met accessibility and retrieval standards, including re-generating a hard copy, when required. Furthermore, the investigator also ensured that there was an acceptable backup of these reproductions and that an acceptable quality control process existed for making these reproductions.

MCERC informed the investigators of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time would meet the strictest standards applicable to that site for the study, as dictated by any institutional requirements or local laws or

regulations, or MCERC standards/procedures which shall not exceed 1 year from the date of submission of the report

25.0 Provision of Study Results and Information to Investigators

After the completion of the clinical study report, Study Director of MCERC provided copy of the same to the investigators along with the details of the study treatment assignment to enable them to review the data to determine the outcome of the study for the benefit of their subjects.

. The investigator and the study staff were directed to hold the information confidentially

26.0 Information disclosure and inventions

26.1 Ownership

All information provided by MCERC and all data and information generated by the site as parts of the study (other than a subject's medical records) are the sole property of MCERC

All rights, title, and interests in any inventions, know-how or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of MCERC, and are hereby assigned to MCERC.

26.2 Confidentiality

All information provided by MCERC and all data and information generated by the site as part of the study (other than a subject's medical records) have been kept confidential by the investigators and other site staff. This information and data will not be used by the investigators or other site personnel for any purpose other than conducting the study. These restrictions do not apply to:

- (1) Information which becomes publicly available through no fault of the investigator or site staff;
- (2) Information which it is necessary to disclose in confidence to an IEC or IRB solely for the evaluation of the study;
- (3) Information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or
- (4) Study results which may be published as described in the next paragraph.

26.3 Publication

For Multicentric studies, the first publication or disclosure of study results shall be a complete, joint Multicentric publication or disclosure coordinated by MCERC Thereafter, any secondary publications will cite reference the original publication(s).

Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a “Publication”), the investigators shall provide MCERC with a copy of the proposed Publication and allow MCERC a period of at least thirty (30) days. The proposed Publications shall not include either MCERC’s confidential information other than the study results or personal data of any subject, such as name or initials.

At MCERC’s request, the submission or other disclosure of a proposed Publication will be delayed for a sufficient time to allow MCERC to seek patent or similar protection of any inventions, know-how or other intellectual or industrial property rights disclosed in the proposed Publication.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement is executed, that contract’s publication provisions shall apply rather than this statement.

It is mandatory to inform and register with the Association of publishers of Medical General, the Clinical trial details, before commencement of the study.

[Ref: The International Committee of Medical Journal Editors (ICMJE) Policy dtd. May 23, 2004].

20.0 RESULTS

(a) DESCRIPTIVE

(i) Demography:

The baseline demographic characteristics of patients were similar in two treatment groups of the study ($P > .05$). Summary of patient’s characteristics including sex, age & weight is presented in Table 18

Table18–Demographic Characteristics

Drug & No. of Patients	Mean Age (yrs) (√SD) Max Min.	Sex (%)		Mean Wt. (kg.) (√SD) Max Min.
		Male	Female	
A 13	37.08 23,60 (11.347)	12 (92.3%)	1 (7.7%)	66.962 50,80 (8.69)
B 7	31.83 23,41 (6.047)	7 (100%)	0 (0%)	61.357 56.5, 66 (3.70)

(ii) Patients living style

The food habits of the patients are given in Table 19. Both Non-Vegetarian and Vegetarian patients were included in the study.

Table -19 – Food Habits.

<i>Drug</i>	<i>Non veg (%)</i>	<i>Veg. (%)</i>
A – 13	10 (76.9%)	3 (23.1%)
B – 7	5 (71.4%)	2 (28.6%)

SMOKING:

In both groups, some patients were smokers – Table 20.

Table20– Smoking History

<i>Drug</i>	<i>Yes</i>	<i>No</i>
A – 13	5 (38.5%)	8 (61.5%)
B – 7	0 (0%)	7 (100%)

MEDICAL HISTORY:

According to Rome II criteria for IBS, all patients selected was suffering from IBS along with constipation for 12 weeks (3 months) or more. They were grouped as follows – Tables 21-23.

Table 21– Duration of constipation in months (As per Groups)

<i>Groups</i>	<i>A</i>	<i>B</i>
3 months – 12 months	3 (23.1%)	4 (57.1%)
12 months – 24 months	6 (46.2%)	2 (28.6%)
> 24 months	4 (30.8%)	1 (14.3%)
Total	13 (100%)	7 (100%)

Table 22 – Number of bowels in a week (As per Groups)

<i>Groups</i>	<i>A</i>	<i>B</i>
1 – 4	1 (7.7%)	0 (0%)
5 – 7	3 (23.1%)	4 (57.1%)
8 – 14	6 (46.2%)	2 (28.6%)
> 14	3 (23.1%)	1 (14.3%)
Total	13 (100%)	7 (100%)

Table 23–Severity of Constipation.

<i>Severity</i>	<i>A</i>	<i>B</i>
Mild	0 (0%)	0 (0%)
Moderate	11 (84.6%)	7 (100%)
Severe	2 (15.4%)	0 (0%)
Total	13 (100%)	7 (100%)

(P > .05).

All above groups were statistically compared by using appropriate tests & there was no statistically significant difference between the two groups indicating they were comparable at the base line values

All the patients were checked for their daily weight for 7 days (0 + 6 days) & then on 3rd week & 6th week.

(IV) Study Medication

Randomization:

The randomization of the patients was done as 2:1 i.e. 2 patients were given Oxypowder & 1 patient was given Dulcolax. Out of 20 patients, 13 patients received Oxypowder (A) & 7 patients received Dulcolax (B). Their disposition for the study is presented in Table 24.

Table 24 – Randomization

Oxypowder (A)	13
Dulcolax (B)	7
Total	20

(V) Non-Study Medication:

None of the patients received any medication other than the Test or the Reference formulation.

(VI) Patient Withdrawal

No patient withdrew from the study until completion of study; all 20 patients have been included in the report.

(b)EFFICACY:

All the patients were checked for their daily weight for 7 days (0 + 6 days) & then on 3rd week & 6th week.

Table 25 shows comparison of their weights From Day 0 to day 42.

Table 25 – Comparison of weight (kg) (Day 0 – Day 42)

<i>Drug</i>	<i>No</i>	<i>Mean (S.D.)</i>
A		
Day 0	13	66.96 (8.69)
Day 1	13	66.96 (8.69)
Day 2	13	66.96 (8.69)
Day 3	13	66.88 (8.72)
Day 4	13	66.85 (8.72)
Day 5	13	66.92 (8.70)
Day 6	13	66.92 (8.70)
Day 21	13	67.08 (8.79)
Day 42	13	67.15 (8.55)
B		
Day 0	7	61.35 (3.70)
Day 1	7	60.78 (3.13)
Day 2	7	60.78 (3.13)
Day 3	7	60.78 (3.13)
Day 4	7	60.64 (3.19)
Day 5	7	60.64 (3.19)
Day 6	7	60.79 (3.13)
Day 21	7	60.43 (2.93)
Day 42	7	60.57 (3.25)

Table 26 – Number of Bowels in a week (grouped)

<i>Group</i>	<i>Day 0</i>		<i>Day 21</i>		<i>Day 42</i>	
	A	B	A	B	A	B
1 – 4	1 (7.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
5 – 7	3 (23.1%)	4 (57.1%)	2 (15.4%)	2 (28.6%)	1 (7.7%)	2 (28.6%)
8 –14	6 (46.2%)	2 (28.6%)	8 (61.5%)	4 (57.1%)	11 (84.6%)	5 (71.4%)
> 14	3 (23.1%)	1 (14.3%)	3 (23.1%)	1 (14.3%)	1 (7.7%)	0 (0%)
Total	13 (100%)	7 (100%)	13 (100%)	7 (100%)	13 (100%)	7 (100%)

As seen from Table 26 all patients in both groups, had increase in the frequency of stool after 42 days of treatment.

Oxypowder releases nascent oxygen for its action and the objective of the study was to seen whether Oxypowder increases oxygen saturation of blood. As seen from Table 27, there was marginal increase in oxygen delivery after taking Oxypowder for 6 weeks (42 days) as compared to Day 0

Table 27– Oxygen content of blood (Day 0, Day 21, and Day 42)

<i>Drug</i>	<i>No</i>	<i>Mean (\pm S.D)</i>
A		
Oxygen Content of Blood Day 0	13	99.15 (.689)
Oxygen Content of Blood Day 21 after 15 Min.	13	98.69 (.630)
Oxygen Content of Blood Day 21 after 30 Min.	13	99.92 (.277)
Oxygen Content of Blood Day 42 after 15 Min.	13	98.77 (.599)
Oxygen Content of Blood Day 42 after 30 Min.	13	100.00 (.000)
B		
Oxygen Content of Blood Day 0	7	99.43 (.535)
Oxygen Content of Blood Day 21 after 15 Min.	7	98.14 (1.069)
Oxygen Content of Blood Day 21 after 30 Min.	7	99.86 (.378)
Oxygen Content of Blood Day42 after 15 Min.	7	98.57(1.272)
Oxygen Content of Blood Day42 after 30 Min.	7	100.00 (.000)

Since all the patients of IBS had constipation, various symptoms of constipation were compared on 3 week and 6 week to their baseline values (day 0)

Tables 28, 29, 30, 31, 32 show that their is significant reduction in all the symptoms in both groups (P> .05)

Table 28–(a) Straining During >25% of Bowel Movement (Day 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
YES	13 (100%)
B	
YES	7 (100%)

(b)Straining During > 25% of Bowel Movement (Day 21).

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	7 (53.8)
YES	6 (46.2)
Total	13 (100.0)
B	
NO	3 (42.9)

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<i>Drug</i>	<i>No. of patients (%)</i>
YES	4 (57.1)
Total	7 (100.0)

(c) Straining During > 25% of Bowel Movement (Day 42).

<i>Drug</i>	<i>No. of patients</i>
A	
NO	12 (92.3)
YES	1 (7.7)
Total	13 (100.0)
B	
NO	6 (85.7)
YES	1 (14.3)
Total	7 (100.0)

Table29 (a)– Lumpy Stools (Day 0).

<i>Drug</i>	<i>No. of patients</i>
A	
NO	1 (7.7)
YES	12 (92.3)
Total	13 (100.0)
B	
NO	1 (14.3)
YES	6 (85.7)
Total	7 (100.0)

Lumpy Stools (b) (Day 21).

<i>Drug</i>	<i>No. of patients</i>
A	
NO	13 (100.0)
B	
NO	5 (71.4)
YES	2 (28.6)
Total	7 (100.0)

Lumpy Stools (c) (Day 42).

<i>Drug</i>	<i>No. of patients</i>
A	
NO	13 (100.0)
B	
NO	6 (85.7)
YES	1 (14.3)
Total	7 (100.0)

Table30–(a) Sensation of incomplete evacuation for >25% of Bowel movement (Day 0).

<i>Drug</i>	<i>No. of patients</i>
A	
YES	13 (100.0)
B	
YES	7 (100.0)

(b)Sensation of incomplete evacuation for >25% of Bowel movement (Day21).

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	2 (15.4)
YES	11 (84.6)
Total	13 (100.0)
B	
NO	2 (28.6)
YES	5 (71.4)
Total	7 (100.0)

(c) Sensation of incomplete evacuation for >25% of Bowel movement (Day 42).

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	10 (76.9)
YES	3 (23.1)
Total	13 (100.0)
B	
NO	4 (57.1)

<i>Drug</i>	<i>No. of patients (%)</i>
YES	3 (42.9)
Total	7 (100.0)

Table 31–(a) Sensation of Anorectal Blockage for >25% of Bowel Movement (Day 0).

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	8 (61.5)
YES	5 (38.5)
Total	13 (100.0)
B	
NO	3 (42.9)
YES	4 (57.1)
Total	7 (100.0)

(b)Sensation of Anorectal Blockage for >25% of Bowel Movement (Day 21).

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	13 (100.0)
B	
NO	6(85.7)
YES	1 (14.3)
Total	7 (100.0)

(c)Sensation of Anorectal Blockage for >25% of Bowel Movement (Day 42).

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	13 (100.0)
B	
NO	7 (100.0)

Table 32–(a) Manual Manoeuvres to facilitate >25% of Bowel Movements (Day 0).

<i>Drug</i>	<i>No. of patients</i>
A	
NO	9 (69.2)
YES	4 (30.8)
Total	13(100.0)
B	
NO	7 (100.0)

(b) Manual Manoeuvres to facilitate >25% of Bowel Movements (Day 21).

<i>Drug</i>	<i>No. of patients</i>
A	
NO	13 (100.0)
B	
NO	7 (100.0)

(c) Manual Manoeuvres to facilitate >25% of Bowel Movements (Day 42).

<i>Drug</i>	<i>No. of patients</i>
A	
NO	13 (100.0)
B	
NO	7 (100.0)

As seen from Table 28 in group A all 13 patients had straining on Day 0 as compared to this on Day 42 only 1 (7.7%) patient had these symptoms.

Similarly in Table 29, in group A 12 patients (92.3%) passed hard, and lumpy stools on Day 0 where as on day 42 none of the patients had this symptoms Table 30, 31 & 32 also show significant reduction in symptoms in both groups ($P < .05$)

Effect of Oxypowder was evaluated on typical symptoms of IBS with constipation. i.e.

1.Abdominal discomfort or pain

- (a) Relieved with defecation – Table (33)
- (b) Onset associated with a change in frequency of stool – (Table 34)
- (c) Onset associated with change in form of stool – (Table 35)

2.Passing mucus during bowel movement – (Table 36)

3.Abdominal Fullness (Table 37)

Table 33–(a) ABDOMINAL PAIN RELIEVED WITH DEFECATION (DAY 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	3 (23.1)
YES	10 (76.9)
TOTAL	13(100.0)
B	
YES	7 (100.0)

(b)ABDOMINAL PAIN RELIEVED WITH DEFECATION (DAY 21)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
YES	13 (100.0)
B	
YES	7 (100.0)

(c)ABDOMINAL PAIN RELIEVED WITH DEFECATION (DAY 42)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	3 (23.1)
YES	10 (76.9)
TOTAL	13(100.0)
B	
NO	2 (28.6)
YES	5 (71.4)
TOTAL	7 (100.0)

Table 34–(a) ABDOMINAL PAIN ONSET ASSOCIATED WITH A CHANGE IN FREQUENCY OF STOOLS (DAY 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	3 (23.1)
YES	10 (76.9)
TOTAL	13(100.0)
B	
NO	2 (28.6)
YES	5 (71.4)
TOTAL	7 (100.0)

(b) ABDOMINAL PAIN ONSET ASSOCIATED WITH A CHANGE IN FREQUENCY OF STOOLS (DAY 21)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	11 (84.6)
YES	2 (15.4)
TOTAL	13(100.0)
B	
NO	7 (100.0)

(c) ABDOMINAL PAIN ONSET ASSOCIATED WITH A CHANGE IN FREQUENCY OF STOOLS (DAY 42)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	13 (100.0)
B	
NO	7 (100.0)

Table35–(a) ABDOMINAL PAIN ONSET ASSOCIATED WITH A CHANGE IN FORM OF STOOLS (DAY 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	6 (46.2)
YES	7 (53.8)
TOTAL	13(100.0)
B	
NO	2 (28.6)
YES	5 (71.4)
TOTAL	7 (100.0)

(b) ABDOMINAL PAIN ONSET ASSOCIATED WITH A CHANGE IN FORM OF STOOLS (DAY 21)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	12 (92.3)
YES	1 (7.7)
TOTAL	13(100.0)
B	
NO	6 (85.7)
YES	1 (14.3)
TOTAL	7 (100.0)

(c) ABDOMINAL PAIN ONSET ASSOCIATED WITH A CHANGE IN FORM OF STOOLS (DAY 42)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	13 (100.0)
B	
NO	6 (85.7)
YES	1 (14.3)
TOTAL	7 (100.0)

Table36– (a) PASSING MUCUS DURING BOWEL MOVEMENT (DAY 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	10 (76.9)
YES	3 (23.1)
TOTAL	13(100.0)
B	
NO	5 (71.4)
YES	2 (28.6)
TOTAL	7 (100.0)

(b) PASSING MUCUS DURING BOWEL MOVEMENT (DAY 21)

<i>Drug</i>	<i>No. of patients (%)</i>
A NO	13 (100.0)
B NO	7 (100.0)

(c)PASSING MUCUS DURING BOWEL MOVEMENT (DAY 42)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	13 (100.0)
B	
NO	6 (85.7)
YES	1 (14.3)
TOTAL	7 (100.0)

Table37--(a) ABDOMINAL FULLNESS (DAY 0)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	2 (15.4)
YES	11 (84.6)
TOTAL	13 (100.0)
B	
YES	7 (100.0)

(b) ABDOMINAL FULLNESS (DAY 21)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	9 (69.2)
YES	4 (30.8)
TOTAL	13(100.0)
B	
NO	6 (85.7)
YES	1 (14.3)
TOTAL	7 (100.0)

(c) ABDOMINAL FULLNESS (DAY 42)

<i>Drug</i>	<i>No. of patients (%)</i>
A	
NO	12 (92.3)
YES	1 (7.7)
TOTAL	13(100.0)
B	
NO	6 (85.7)
YES	1 (14.3)
TOTAL	7 (100.0)

As seen from Table 33, 34 and 35 there was significant reduction in Abdominal pain after the treatment in both groups.

As seen from Table 37, in group A 11 patients (84.6%) had abdominal fullness on Day 0, where as on Day 42, only 1 (7.7%) patient had this symptom indicating significant reduction ($P < .05$)

All above the parameters indicate that Oxypowder significantly reduced the symptoms of IBS with constipation.

Routine Stool Examination was done before and after the drug treatment, which was normal in all 20 patients.

Efficacy was independently judged by the Investigator & patient and it was rated as excellent, Good, Fair and Poor. It was statistically analysed using Fisher's Exact Test

Table 38– EFFICACY TABLES

(A) ASSESSMENT BY INVESTIGATOR

Efficacy Grade	<i>DRUG</i>		
	<i>A</i>	<i>B</i>	
Excellent	6 46.2%	0 0%	
Good	5 38.5%	3 42.9%	
Fair	2 15.4%	2 20.6%	
Poor	0 .0%	2 28.6%	
Total	13 100.0%	7 100%	(P < 0.05)

(B) ASSESSMENT BY PATIENTS

Efficacy Grade	<i>DRUG</i>		
	<i>A</i>	<i>B</i>	
Excellent	6 46.2%	0 0%	
Good	5 38.5%	3 42.9%	
Fair	2 15.4%	2 20.6%	
Poor	0 0%	2 28.6%	
Total	13 100.0%	7 100%	(P < 0.05)

In group A out of 13 completed patients investigator assessment was excellent for 6 (46.2%) Good for 5(38.5%) Fair for 2 (15.4%), where as in group B out of 7 patients it was Good for 3 (42.9%), Fair for 2 (28.6%) and poor for 2 (28.6%).

Efficacy of Oxypowder in treating IBS with constipation was significantly ($P < .05$) more than Dulcolax and hence this indicates Oxypowder was more efficacious in treating IBS with constipation than Dulcolax.

Table 39–Overall Efficacy

EFFICACY	DRUG	
	A	B
Complete cure	4 (30.8%)	0 (0%)
Improvement	9 (69.2%)	5 (71.4%)
Failure	0 (0%)	2 (28.6%)
Total	13 (100%)	7 (100%)

Overall efficacy was also judged as complete cure, Improvement and Failure. Table 39 shows that in group A, out of 13 patients 4 patients (30.8%) had complete cure, 9 patients (69.2%) had improvement and there was no failure where as in group B out of 7 patients, none of the patients had complete cure, 5 patients (71.4%) had improvement and 2 patients (28.6%) had failure Above Table indicates efficacy of Oxypowder is significantly more ($P < .05$) than Dulcolax in treating patients of IBS with constipation.

SAFETY:

ADR's

Regarding Adverse events, none of the patients had any adverse events during the study period.

DISCUSSION AND SUMMARY

SUMMARY:

The present study titled

“Multicentric Randomized, Open, Comparative study to evaluate the safety and efficacy of Oxy-Powder® in patients of chronic constipation and IBS” was conducted to evaluate effectiveness of Oxypowder in treating constipation and IBS with constipation precipitating loss of weight & improving oxygen delivery in patients of constipation and IBS with constipation The study was

sponsored by Dr. Edward Group III, CEO, Global Healing Center, Inc. & contracted to Mayfair Clinical, Education & Research Centre (MCERC), a Clinical Research Organisation (CRO) for conduct of the study.

For evaluating effectiveness of Oxy-Powder® in patients of constipation, the study was conducted in Bhatia General Hospital located in Mumbai. However, for evaluating effectiveness of Oxy-Powder® in patients of IBS with constipation, the study was conducted in Bhatia General Hospital. The Study Protocol was carefully designed and got approved from the sponsor by MCERC. The respective investigators were Dr. Chetan Bhatt and Dr. Sharad Shah.

The study commenced after MCERC obtained approvals from IEC of MCERC, IRB of Bhatia Hospital and other Regulatory authorities. The study proposed to enrol 60 patients according to inclusion and exclusion criteria given in the protocol but ultimately culminated with 40 patients (one patient out of these had ADR and withdrew from the study from 3rd day). The randomization of the patients was done as 2:1 i.e. 2 patients were given Oxypowder & 1 patient was given Dulcolax, the comparative product. Thus, there were 13 patients in Oxy-Powder® group and 7 patients in Dulcolax group. All the patients were **counselled** before enrolment and their “**Informed Consent**” was taken. Their Medical History was recorded by the investigators. They were subjected to clinical examination and pre-study evaluation including recording of weight, oxygen content of blood using pulse oximeter, stool examination, Barium meal (to rule out any Bowel organic lesion leading to constipation) and colonoscopy (to rule out any colonic organic lesion and any faecal impaction). Duration of the trial was 42 days i.e. 6 weeks out of which the first week administration of the treatment products was for bowel cleansing followed by 5 weeks of maintenance. The patients were instructed to take four capsules of Oxy-Powder® (Test formulation) or two tablets of Dulcolax (Comparative formulation), as per the Randomization scheme, with plenty of water every day for first 7 days, in the evening on empty stomach. Thereafter, subjects were instructed to take the same dose of either test or reference formulation on alternate days, on empty stomach, in the evening, for period of 6 weeks.

Administration on day '0', '3 weeks' and '6 weeks' were done on empty stomach in the morning. Subjects were asked to report to the hospital with empty stomach in the morning for administration of investigational products and Oxygen level in the blood were measured 15 minutes and 30 minutes after administration of tablets/capsules.

On the remaining days, the subjects were instructed to take investigational product at home at the same dose levels in the evening on empty stomach. They were told to take Dinner at least two hours

post-administration of Drug. **Patients were emphatically told to report to the investigators any ADR's that they experience** during or after the study.

DISCUSSION OF THE RESULTS:

The Demographic Characteristics recorded in Table-1 indicate that among 27 patients enrolled in Oxy-Powder® (OP) group, there were 19 male patients (70.4%) and 8 female patients (29.6%) while in 13 patients in Dulcolax group (DL), there were 8 male patients (61.5%) and 5 female patients (38.5%). They conformed to the age and the weight as given in the protocol. 13(48.1%) in OP group were non-vegetarians and 14 (51.9%) were vegetarians as compared to 7(53.8%) non-vegetarians and 6(46.2%) vegetarians in DL group. Majority of patients in both the groups were non-smokers.

MEDICAL HISTORY: Their medical history indicated that in OP group, among the patients enrolled, 13 (48.1%) patients suffered from constipation problem for a period ranging between 3 months to 12 months, 6 (22.2%) between 12 months to 24 months and 8 (29.6%) for more than 24 months. In DL group, 6 (46.2%) patients suffered from constipation problem for a period ranging between 3 months to 12 months, 4 (30.8%) between 12 months to 24 months and 3 (23.1%) for more than 24 months.

These and the above figures indicate that enrolment was in full conformance with the requirements of the protocol.

As regards the severity of constipation based on number of bowels in a week, in OP group, 9(33.3%) patients suffered from **Severe constipation**, 17(63.0%) from **Moderate constipation** and 1(3.7%) from **Mild constipation**,

In DL group, 5(38.5%) patients suffered from **Severe constipation**, 8(61.5%) from **Moderate constipation** and none from **Mild constipation**,

IN OTHER WORDS, THE TWO GROUPS OF PATIENTS ENROLLED WERE EVENLY BALANCED WITH RESPECT TO SEVERITY OF CONSTIPATION.

POST ADMINISTRATION CHANGES:

As seen from the results recorded in Table 8, there is no reduction in the weight of the patients in OP group after 42 days of administration of Oxy-Powder® as compared to Base line (0 day) indicating that administration of Oxypowder did not lead to reduction of weight in the patients. Similarly, there was no reduction in the weight in Dulcolax group. In both the groups, after starting the investigating drug; frequency of stools (No. of Bowels in a week) increased as is shown by the results recorded in Table 9. Comparing the two groups, the percentage performance of Oxy-Powder® was better than Dulcolax on the 21st and the 42nd Day.

As stated in the “Introduction”, Oxypowder releases nascent oxygen for its action; the objective of this study was to see whether Oxypowder increases oxygen saturation of blood. As seen from Table 10, there was marginal increase in oxygen delivery after taking Oxypowder for 6 weeks (42 days) as compared to Day 0. It thus appears that the study period was little short and should have been extended approximately to 180 days to get more conclusive results.

During the study, the patients in both the groups showed significant reduction in various symptoms as shown in Tables 11, 12, 13, 14, 15 (Straining During >25% of Bowel Movement during 0-42 days, Lumpy Stools during 0-42 days, Sensation of incomplete evacuation for >25% of Bowel movement during 0-42 days, Sensation of Anorectal Blockage for >25% of Bowel Movement during 0-42 days and Manual Manoeuvres to facilitate >25% of Bowel Movements during 0-42 days). As can be seen from the results in Table 11 in Oxypowder group, 25 patients (92.6%) had straining on Day 0 as compared to only 2 (7.7%) patients on Day 42. Similarly, in Table 12, in the same group, 26 patients (96.3%) passed hard and lumpy stools on Day 0 where as on day 42, it was reduced only to two patients (7.7%). The above parameters indicate that there is significant reduction in the symptoms ($P<0.05$) in both groups. However, In this case also, the results were better in case of Oxypowder as compared to Dulcolax Routine stool examination done before and after the drug treatment showed no abnormality in all 40 patients in this study

EFFICACY JUDGED:

Efficacy was independently judged by the Investigators & the patients. It was graded as excellent, Good, Fair and Poor. The results were statistically analysed using Fisher's Exact Test. In Oxypowder group, out of 26 completed patients, investigator assessment was excellent for 13 (50%) Good for 12(46.2%) Fair for 1 (3.8%), where as in Dulcolax group, out of 13 patients, it was excellent for 1 (7.7%) Good for 6 (46.2%), Fair for 4 (30.8%) and poor for 2 (15.4%).

Overall efficacy was also judged as complete cure, Improvement and Failure. Table 17 shows that in Oxypowder group , out of 26 patients, 11 patients (42.3%) had complete cure, 15 patients (57.7%) had improvement and there was no failure where as in Dulcolax group, out of 13 patients, 1 patient (7.7%) had complete cure, 10 patients (76.9%) had improvement and 2 patients (15.9%) experienced failure.

CONCLUSION:

Efficacy of Oxypowder in treating constipation was significantly ($P<.05$) higher than Dulcolax thus indicating that **Oxypowder** was **more efficacious** in treating constipation than Dulcolax.

No adverse event was reported other than one patient in Op group withdrawing from the study right in the beginning. Oxy-Powder® was well tolerated by all the patients under treatment of this product.

----- *THE END OF REPORT* -----