# Efficacy and safety of Entoban for the treatment of chronic diarrhea

**Sadia Shakeel[1]*, Khan Usmanghani[1] and Hafiz Muhammad Asif[2]**
[1]Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan
[2]University College of Conventional Medicine, Faculty of Pharmacy & Alternative Medicine, Islamia University of Bahawalpur, Pakistan

**Abstract**: The current randomized clinical trial was conducted to assess the safety and effectiveness of Entoban for treating patients of chronic diarrhea. The study enrolled 150 patients fulfilling the inclusion criteria, among them 95 were males and 55 were females. Written informed consent was obtained from all study participants. Metronidazole tablets (400 mg) were used in a control group for 7-10 days. The test group received Entoban capsule 400mg every 8 hours for five days. Primary outcome of the study was daily bowel frequency evaluation; the secondary outcome was evaluation of clinical symptoms including abdominal pain, distention, stool consistency and sensation of incomplete evacuation. The study is registered at (https://register.clinicaltrials.gov) having registration number NCT02642250. In an intention-to-treat (ITT) analysis, it has been observed that 39(84.78%) in test group and 37(78.72%) in control group showed complete improvement. Participants in the test group exhibited a marked reduction in symptoms; the symptom score was decreased from 3 (maximum) to 1 (minimum) or 0 (absent) in most of participants. Major difference was observed regarding side effects reported between two treatment groups (p value <0.0001). Entoban possesses considerable therapeutic efficacy for the treatment of chronic diarrhea analogous with the conventional Metronidazole therapy.

**Keywords**: Clinical trial, Entoban, efficacy, herbal drug, safety.

## INTRODUCTION

Diarrhea is the third most frequent disease that affects people of all ages (Acheson and Allos, 2001). In spite of the drop in global mortality rate due to the illness, diarrhea still accounts for more than 2 million deaths per annum (Black *et al*., 2003). About two-thirds of the total annual deaths in Pakistan are currently of children under five, diarrhea being the major cause of these deaths. In 1990, 27% of children under five deaths have been associated with this disease and for children 1-11 months, the association increased to 40%. According to UNICEF (United Nations Children's Fund), diarrhea kills 1.5 million children under five years every year (Agtini *et al*., 2005).

Diarrhea is a sign of increased water content, whether due to impaired absorption and / or secretion of active ions, organic substrates (Musher and Musher, 2004; Weinstein *et al*., 2008). That different drugs are prescribed to treat chronic diarrhea (Li and Vaziri, 2012). An empirical mode of treatment of antibiotics is also considered viable when the occurrence of infection is elevated in the society. However, the resistance will be responsible as the main factor for treatment failure (Mylonakis *et al.,* 2001). Traditional herbal medicines have proven to be safe and effective and being utilized to cure many disorders, including GI ailments (J. Calixto, 2000). Herbal dosage form design have been shown to heal acute as well as chronic diarrheal diseases (Shaikh and Hatcher, 2005). Among the various phytochemicals tannins and flavonoids are considered to be accountable for antidiarrheal action by means of rising colonic water and electrolyte reabsorption. Since a number of the active components are toxic potentially, an investigation is required to ensure the safety of herbal drugs (Palombo, 2006). The current study was directed to a polyherbal formulation Entoban in which herbs having outstanding activity to treat problems associated with gastrointestinal tract are employed. The clinical efficacy of Entoban is proven in animal studies. Entoban gave evidence of good tolerance and the absence of detrimental effects on the functional state of the vital organs of the experimental animals in acute and sub chronic oral toxicity test (Sadia *et al.*, 2015). Entoban was considered safe for human consumption according to determined amounts of heavy metals (Shakeel *et al.,* 2015). The current study was planned to compare herbal formulation Entoban with Metronidazole for the management of chronic diarrhea.

## MATERIALS AND METHODS

### *Study design and setting*

The present study was a controlled, randomized and multicenter clinical trial conducted at Sharafi hospital in Karachi, at Nawaz Salik Hospital in Rawalpindi and Victoria Hospital in Bahawalpur for the period of January 2014 to December 2015. Patients which were clinically diagnosed based on clinical history; clinical presentation and stool DR of patients were enrolled. Consolidated standards of reporting trials (CONSORT) check list and flow chart were applied. The trial was registered at http://www.ClinicalTrial.org, a service of the US National Institutes of Health (registry No.NCT02642250).

**Corresponding author:* e-mail: zohad2012@gmail.com

***Inclusion and exclusion criteria***

Male or female between the age of 05-60 years, having three or more irregular stools for each day, course of illness more than 1 month and willing to participate were eligible for inclusion in study. A patient with history of kidney or liver failure, those having concomitant infection and patients treated with a medication against diarrhea in the five days prior to the study were also excluded. The pregnant women and lactating women were not eligible for study participation. An informed consent by the patient in writings was obtained prior to initiate the study. Assurance was given to the confidentiality of information provided, including personal information.

***Randomization and study protocol***

A block-randomization procedure, with a block size of 4, was adopted to assign participants either to treatment with allopathic or herbal therapy. The study was unblinded because the number of drugs and the dosing regimens differed between the 2 treatment groups. However, the statistician was blinded while performing the comparative analysis of data. Metronidazole tablets (Flagyl) in strength of 400 mg manufactured by Sanofi-aventis Pakistan limited was used in a control group for 7-10 days. The test group received Entoban capsule 400mg every 8 hours for five days. Entoban was prepared by Herbion Pakistan (Pvt.) Limited, Karachi, Pakistan from herbal drugs namely *Myrtus communis, Butea frondosa, Aegle marmelose, Berberis aristata, Holarrhena antidysenterica* and *Quercus infectoria.*

A comprehensive proforma, soliciting required demographic characteristics, presenting complaints, general examination, stool consistency, frequency, patient's weight and treatment option was filled for every patient by skilled healthcare personnel. All lab investigations were conducted by trained laboratory technician.

***Primary and secondary outcomes***

Primary outcome of the study was daily bowel frequency evaluation; the secondary outcome was evaluation of clinical symptoms including abdominal distention and pain, stool consistency and sensation of incomplete evacuation.

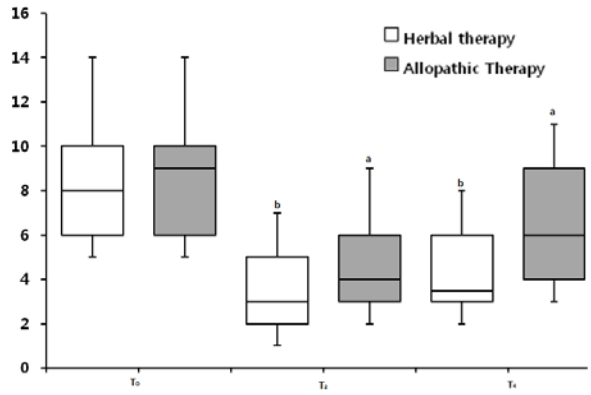
The details of relevant diarrheal symptoms (e.g., abdominal pain, anorexia, flatulence, nausea, vomiting, rectal urgency, incontinence and bloating) were obtained for every patient, by means of scoring system (absent, 0; mild, 1; moderate, 2; and severe, 3). Scores for each of the symptoms could range from 0, no symptoms, to a maximum of 3, severe symptoms. The follow-up information about improvement of symptoms and appearance of any side effects was recorded in the relevant file of each patient. The stool DR was executed at baseline, after 2 weeks and 4 weeks of treatment. Adverse reactions were evaluated by physical examination and taking history on daily basis every 3 days until the completion of study.

***Data analysis***

The filled questionnaires were entered into Statistical Package for Social Sciences (SPSS 20.0) for analysis to compare the effect of two drugs. Patients' characteristic data was expressed as the mean ± standard deviation (SD). A $\chi 2$ test using a $2 \times 2$ contingency table was used to check for a statistically significant difference in the cure rate as well as in the proportions of other categorical variables between 2 treatment groups, such as age, gender, occupation, and marital status. A Wilcoxon signed-rank test was applied to analyze the intensity of symptoms at baseline ($T_0$), after 2 ($T_2$) and 4 ($T_4$) weeks of treatment, expressed through median values and interquartile ranges (IQRs) ($p<0.05$ was considered significant).

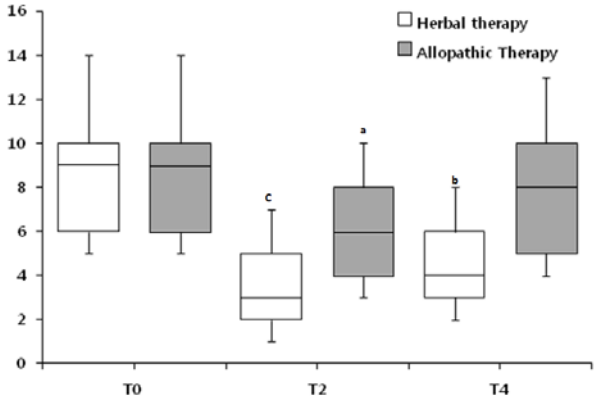
## RESULTS

Overall 150 patients were enrolled in the study but 10 in the test group and 7 in the control group did not receive the allocated treatment due to unknown reasons. Further 13 were dropped out during the treatment and 8 discontinued intervention due to side effects in control group. In test group, 15 were dropped out during the treatment and 4 discontinued intervention due to side effects. Overall 47 and 46 in control and test group retained till the completion of study. Both treatment options receiving Entoban and Metronidazole were evaluated for diarrheal symptoms and there was not any significant difference observed with respect to gender, age, duration of disease, and symptom scoring, in addition to daily bowel frequency (3.89±1.05 in test group, 3.41±1.35 in control group; p=0.54). Mean and standard deviation of the ages of participants for test and control groups were 25±11.86 years and 23±13.76 years, respectively. Mean weight of all the participants was 56±9.7kg (table). The skewness and kurtosis of the data were 0.67 and 0.16, respectively. At the 2$^{nd}$ week of treatment, mean bowel frequency was significantly lower in the test group than in the control group (1.88±1.24 vs 2.64±1.12, p<0.05). This difference was confirmed at the 4$^{th}$ week (1.39±0.92 in the test group vs 2.19±1.05 in the control group; p<0.05). The study revealed that 39(84.78%) in test group and 37(78.72%) in control group showed complete improvement. Participants in the test group with complete improvement exhibited significant decreases in overall GI symptoms from baseline ($T_0$) with a median of 8 and an IQR of 6 to 10, to week 2 ($T_2$) with a median of 3 and an IQR of 2 to 5 and to 1 month after treatment ($T_4$) with a median of 4 and an IQR of 3 to 6 (fig. 1). A significant decrease in symptoms was observed for participants in the test group with no improvement, also from $T_0$ with a median of 9 and an IQR of 6 to 10, to




$_a p < .001$. $_b p < .0001$.

**Fig. 1**: Overall severities of symptoms at baseline ($T_0$), two weeks after treatment ($T_2$) and one month after treatment ($T_4$) by herbal and allopathic therapy in patients who show complete improvement. Horizontal bar: median; box: 25–75th interquartile range; vertical lines: range of values.




$_a p<0.01$. $_b p <0 .001$. $_c p<0.0001$.

**Fig. 2**: Overall severity of symptoms at baseline ($T_0$), two weeks after treatment ($T_2$) and one month after treatment ($T_4$) by herbal and allopathic therapy in patients with no improvement. Horizontal bar: median; box: 25-75th interquartile range; vertical lines: range of values.

$T_2$ with a median of 3 and an IQR of 2 to 5 and to $T_4$ with a median of 4 and an IQR of 3 to 6 (fig. 2). The intensity of individual symptoms in the test group was monitored and statistically significant improvement was recorded after treatment (table). Participants in control group with improvement exhibited a statistically significant reduction in the overall diarrheal symptom score, from $T_0$ with a median of 9 and an IQR of 6 to 10, to $T_2$ with a median of 4 and an IQR of 3 to 6, and to$T_4$ with a median of 4 and an IQR of 3 to 7. No significant improvement in symptoms was observed, however, for the participants with no recovery, showing scores from $T_0$ a median of 9 and an IQR of 6 to 10, to $T_2$ a median of 6 and an IQR of 4 to 8, and to $T_4$ a median of 8.5 and an IQR of 5 to 10. In control group, the intensity of individual symptoms was recorded in the course of treatment.

There was a significant difference observed as regards the side effects between two treatment groups (p value < 0.0001) (table). Patients in control group reported more side effects as compared to test. Around 20% patient reported adverse effects in test group however in control group 55.31% reported adverse effects. The major adverse effects reported in control group were anorexia (14.89%), metallic taste (10.63%), dizziness (8.51%) and vomiting (4.25%). Among test group the major adverse effects reported were metallic taste (6.52%), anorexia and headache (4.34%).

## DISCUSSION

According to the WHO, mass population (65%-80%) in developing countries are using herbal medicine for primary health care needs for curative and preventive treatment (Oliveira *et al.,* 2006). Herbal medicines international trade has increased enormously and pharmaceutical manufacturing industries are developing and producing standardized herbal phototherapeutic agents with assuring safety, efficacy and quality (B. J. Calixto, 2000). Plant derived drug is the mainstay of about 75-80% of the global populace for primary health care; owing to the general belief that herbal drugs are devoid of any side effects besides being economical and easily accessible (Kamboj, 2000; Pal and Shukla, 2003). The present study was planned to assess the safety and effectiveness of herbal formulation Entoban which consists of *Berberis aristata, Querecus infectoria, Helicteres isora, Symplocos racemosa* and *Holarrhena antidysenterica* used for the treatment of gastrointestinal infections (Shakeel *et al.,* 2015). *Holarrhena antidysenterica Wall* has shown a pronounced antibacterial activity and its bark is utilized for anti-diarrheal and astringent activity (Voravuthikunchai *et al*., 2004). The antidiarrheal effect of the alkaloids from *H. antidysenterica* is due to the inhibition of production of watery stools or fluid (Kavitha *et al*., 2004). *Berberis aristata* (BA) has profound antibacterial activity and used in the treatment of diarrhea (Potdar *et al*., 2012). Shamkuwar revealed that aqueous extract of *Berberis aristata* treated mice, considerably reduced the stimulation time of diarrhea, amount of wet stools and frequency of stools in the diarrhea produced by magnesium sulphate. These consequences point toward that BA produces its antidiarrheal effect all the way through decreasing intestinal secretions and antispasmodic effect by restraining the intestinal motility (Shamkuwar and Pawar, 2013). Qualitative facts of past studies indicated that BA has important antisecretory effects in opposition to diarrhea due to enterotoxigenic *Escherichia coli* and *Vibrio cholerae* (Asgari *et al*., 2012). Extracts of the galls of *Q. infectoria* have high potential as an antibacterial agent (Basri and Fan, 2005). *Symplocos*

**Table**: Distribution of side effects by treatment option

| Side effects reported | Treatment option | | Total |
|---|---|---|---|
| | Herbal | Allopathic | |
| Yes | 9(19.56%) | 26(55.31%) | 35(37.63%) |
| No | 37(80.43%) | 21(44.68%) | 58(62.36%) |
| Types of side effects | Treatment option | | |
| | Herbal | Allopathic | |
| Anorexia | 2(4.34%) | 7(14.89 %) | |
| Metallic taste | 3(6.52%) | 5(10.63%) | |
| Headache | 2(4.34%) | 3(6.38%) | |
| Vomiting | 1(2.17%) | 2(4.25%) | |
| Dizziness | 0 | 4(8.51%) | |
| Dark or reddish-brown urine | 1(2.17%) | 0 | |
| Mouth or tongue irritation | 0 | 2(4.25%) | |
| Any other | 0 | 3(6.38%) | |

Pearson chi square value 26.04 and p value < 0.0001

*racemosa* possess antimicrobial activity (Devmurari, 2010). Mazumdera reported that chloroform extract of the roots of *Aegle marmelos* (Correa), have considerable antidiarrheal activity similar to loperamide (Mazumder *et al*., 2006). Thus Entoban possesses antimotility and antisecretory activity due to the presence of different phytochemicals including tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoids (Sadia *et al*., 2015). In this study Metronidazole has been used as a control drug to treat diarrhea. Fred reported that treatment with Metronidazole in sufferers of diarrhea, resulted in 76% clinical cure and clinical symptoms reappeared in 15% of the patients (Zar *et al*., 2007).

In this study, participants showing complete and no improvement in the test group exhibited a marked reduction in the symptoms; the symptom score was decreased from 3 (maximum) to 1 (minimum) or 0 (absent) in most of the participants. Research has shown that Metronidazole produces side effects including nausea, abdominal pain, loss in weight, vomiting, diarrhea, headache, metallic taste in the mouth and dizziness (Rossi, 2013). Similar adverse effects were reported by the participants in current study. There was a significant difference observed as regards the side effects between two treatment groups (p value < 0.0001). It is revealed from the literature that herbal preparations are comparatively safe then allopathic medicines owed to a balance of naturally occurring ingredients. Among these some have synergistic effects on others that consequences in increased activity whereas some adjust the effects of others thereby dropping down the rate of undesirable side-effects (J. Calixto, 2000). Tomoo Kuge conducted a clinical trial to assess the effectiveness and acceptability of Seirogan, a plant based drug used to cure diarrhea and reported that 27% subjects receiving Seirogan reported adverse events. Among them the most frequent adverse events were somnolence and altered taste (Kuge *et al*., 2003). This was in compliance with our study the major adverse effects reported by Entoban was metallic taste (6.52%). Entoban revealed high cure rates of chronic diarrhea with little or not any side effects as compared to Metronidazole. Furthermore Entoban improves the well-being off over all sign and symptoms of diarrhea and has better compliance (Siddiqui and Usmanghani, 2015).

## CONCLUSION

Entoban possesses considerable therapeutic significance for the cure of chronic diarrhea and symptoms associated that are comparable with those of the standard, conventional therapy. Entoban is a better tolerated drug as there were considerably more side effects accrue due to Metronidazole by comparison to Entoban.

## ACKNOWLEDGEMENTS

We are thankful to Mr. Nadeem Khalid, CEO and Mr. Zeeshan A Sheikh of Herbion Pakistan Private Limited for providing the test and control drugs for the clinical trials.

## REFERENCES

Acheson D and Allos BM (2001). Campylobacter jejuni infections: Update on emerging issues and trends. *Clin. Infect. Dis.,* **32**(8): 1201-1206.

Agtini MD, Soeharno R, Lesmana M, Punjabi NH, Simanjuntak C, Wangsasaputra F and Santoso H (2005). The burden of diarrhoea, shigellosis and cholera in North Jakarta, Indonesia: Findings from 24 months surveillance. *BMC Infectious Diseases,* **5**(1); 89.

Asgari Z, J Selwyn B, Vonville H and L DuPont H (2012). A systematic review of the evidence for use of herbal medicine for the treatment of acute diarrhea. *J. Nat. Prod.,* **2**(1): 1-8.

Basri D and Fan S (2005). The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents. Indian *J. Pharmacol.,* **37**(1): 26.

Black RE, Morris SS and Bryce J (2003). Where and why are 10 million children dying every year? *The Lancet,* **361**(9376): 2226-2234.

Calixto J (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J. Med. Biol. Res.,* **33**(2): 179-189.

Devmurari V (2010). Antibacterial evaluation and phytochemical screening of Symplocos racemosa Roxb. *Int. J. Pharm. Tech. Res.,* **2**(2): 1359-1363.

Kamboj V (2000). Herbal medicine. *Curr Sci-Bangalore,* **78**(1): 35-38.

Kavitha, D., Shilpa, P., and Devaraj, S. N. (2004). Antibacterial and antidiarrhoeal effects of alkaloids of Holarrhena antidysenterica WALL. *Indian J Exp Biol*. **42**(6): 589-594.

Kuge T, Shibata T and Willett MS (2003). Multiple-Dose Escalation, Safety and Tolerability Study of Wood Creosote, the Principal Active Ingredient of Seirogan, an Herbal Antidiarrheal Medication, in Healthy Subjects. *J. Clin. Pharmacol.,* **43**(3): 284-290.

Li Z and Vaziri H (2012). Treatment of chronic diarrhoea. *Best Pract & Res Clin Gastroenterol,* **26**(5), 677-687.

Mazumder R, Bhattacharya S, Mazumder A, Pattnaik A, Tiwary P and Chaudhary S (2006). Antidiarrhoeal evaluation of Aegle marmelos (Correa) Linn. root extract. *Phytother. Res.,* **20**(1): 82-84.

Musher DM and Musher BL (2004). Contagious acute gastrointestinal infections. *N. Engl. J. Med.,* **351**(23): 2417-2427.

Mylonakis E, Ryan ET and Calderwood SB (2001). Clostridium difficile–associated diarrhea: A review. *Arch. Int. Med.,* **161**(4): 525-533.

Oliveira WP, Bott RF and Souza CR (2006). Manufacture of standardized dried extracts from medicinal Brazilian plants. *Drying Technol.,* **24**(4): 523-533.

Pal, S. K., and Shukla, Y. (2003). Herbal medicine: current status and the future. *Asian Pac. J. Cancer Prev.,* **4**(4): 281-288.

Palombo EA (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother. Res.,* **20**(9): 717-724.

Potdar D, Hirwani R and Dhulap S (2012). Phyto-chemical and pharmacological applications of Berberis aristata. *Fitoterapia,* **83**(5): 817-830.

Rossi S (2013). Australian Medicines Handbook (2013 ed.). Adelaide: The Australian Medicines Handbook Unit Trust: ISBN 978-0-9805790-9-3.

Sadia S, Sheikh Z, Bano S and Usmanghani K (2015). Evaluation of Acute and Sub-Chronic Oral Toxicity of Entoban: A Polyherbal Drug on Experimental Mice. *J. Med. Diagn Meth.,* **4**(4):187-190.

Shaikh BT and Hatcher J (2005). Complementary and alternative medicine in Pakistan: Prospects and limitations. *Evid. Based Complement. Alternat. Med*. **2**(2): 139-142.

Shakeel S, Gul S, Zahoor A, Khan SS, Sheikh ZA, Naveed S and Usmanghani K (2015). Standardization of Biomarkers Gallic Acid and Berberine in Polyherbal Formulation Entoban Capsules by High-Performance Thin-Layer Chromatography Densitometry. *J. Planar Chromatog- Mod. TLC.,* **28**(5): 386-390.

Shakeel S, Sheikh ZA, Usmanghani K, Gul S and Naveed S (2015). Determination of the heavy metal content of Entoban herbal medicinal product. *Journal of Chinese Pharmaceutical Sciences,* **24**(11): 764-769.

Shamkuwar P and Pawar D (2013). Antidiarrhoeal and antispasmodic effect of Berberis aristata. *Inter. J. Pharmacog Phytochem Res.,* **5**(1): 24-26.

Siddiqui MI and Usmanghani K (2015). Comparison of allopathic and herbal medicine for the treatment of Entamoeba histolytica: A double blind clinical trial. *J. Med. Plan. Res.,* **9**(9): 301-309.

Voravuthikunchai S, Lortheeranuwat A, Jeeju W, Sririrak, T, Phongpaichit S and Supawita T (2004). Effective medicinal plants against enterohaemorrhagic Escherichia coli O157: H7. *J. Ethnopharmacol.,* **94**(1): 49-54.

Weinstein RA, Said MA, Perl TM and Sears CL (2008). Gastrointestinal flu: Norovirus in health care and long-term care facilities. *Clin. Infect Dis.,* **47**(9): 1202-1208.

Zar FA, Bakkanagari SR, Moorthi K and Davis MB (2007). A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile associated diarrhea, stratified by disease severity. *Clin. Infect Dis.,* **45**(3): 302-307.