

**ROLE OF POTASSIUM CHANNELS, NITRIC OXIDE PATHWAY AND ADRENERGIC RECEPTORS IN ANTIDIARRHOEAL EFFECT OF MEBARID**

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**ABSTRACT**

Effect of Mebarid an Ayurvedic antidiarrhoeal formulation was evaluated on potassium channels, nitric oxide pathway and adrenergic receptors. Antidiarrhoeal effect of Mebarid was studied along with Glibenclamide (potassium channel blocker), Isosorbide dinitrate (nitric oxide donor) and Yohimbine ( $\alpha_2$  adrenergic receptor blocker). Glibenclamide and Isosorbide dinitrate has reduced the antidiarrhoeal activity of Mebarid. Antidiarrhoeal activity of Mebarid was not changed by Yohimbine. It shows that potassium channels and nitric oxide pathway play important role in the antidiarrhoeal effect of Mebarid while  $\alpha_2$  adrenergic receptors are not involved in the antidiarrhoeal effect of Mebarid.

**Keywords:** Mebarid, potassium channels, nitric oxide pathway, adrenergic receptors.

**INTRODUCTION**

Diarrhoea is one of the principal causes of death in the malnourished infants. It causes dehydration, electrolyte imbalance and malnutrition. Severe diarrhea can be life threatening. It is the passage of more than three unformed stools in 24 hours<sup>1, 2</sup>. Diarrhoea involves both an increase in the motility of the gastrointestinal tract, along with increased secretion, and a decrease in the absorption of fluid and thus a loss of electrolytes and water<sup>3, 4</sup>. Present study was planned to assess the role of potassium channels, nitric oxide pathway and adrenergic receptors in the antidiarrhoeal effect of Mebarid.

**MATERIALS AND METHODS****Drugs**

i) Mebarid Syrup – SG Phyto Pharma (P) Ltd. ii) Yohimbine – Sigma Chemicals Ltd. iii) Glibenclamide – Sigma Chemicals Ltd. iv) Isosorbide dinitrate – Sigma Chemicals Ltd.

**Composition of Mebarid**

Each 10 ml of Mebarid contains i) Ajmoda (100 mg), ii) Bael (100 mg), iii) Lodhara (100 mg), iv) Dadim (100 mg), v) Badishep (100 mg), vi) Daruhalahad (100 mg), vii) Jaiphal (50 mg), viii) Sunth (50 mg), ix) Ativish (50 mg), x) Kuda (50 mg), xi) Sugar (q.s.).

**Animals**

Swiss albino mice of either sex, weighing 20 – 25 gm obtained from VIPER, Pune, were used for the experiments. They were kept in standard environmental condition, fed standard food and water ad libitum. All experiments were performed after an overnight fast. The Institutional Animal Ethical Committee of Government College of Pharmacy, Aurangabad, Maharashtra, India (GCPA/IAEC/2011/235, 11/03/2011), approved the study.

**Acute toxicity study**

Mebarid was studied for acute oral toxicity as per revised OECD guidelines number 423. Mebarid was devoid of any toxicity up to 20 ml/kg in albino mice by oral route. Hence for further studies 2.5 ml/kg doses of Mebarid was used<sup>5</sup>.

**Effect of Yohimbine, Glibenclamide and Isosorbide dinitrate on antidiarrhoeal activity of Mebarid on castor oil induced diarrhoea.**

Groups of six mice each were treated as outlined below:

Group 1 (Control group): Distilled water 10 ml/kg, p.o.,

Group 2 (Test group): Mebarid 2.5 ml/kg, p.o.,

Group 3 (Test group): Glibenclamide 1 mg/kg, p.o. (given 30 min prior to the administration of Mebarid 2.5 ml/kg, p.o.,

Group 4 (Test group): Isosorbide dinitrate 150 mg/kg, p.o. (given 30 min prior to the administration of Mebarid 2.5 ml/kg, p.o.

Group 5 (Test group): Yohimbine 1 mg/kg, s.c. given 30 min prior to the administration of Mebarid 2.5 ml/kg, p.o.,

After 30 min, castor oil (0.2 ml/mouse) was administered to each mouse. The animals were then placed under separate glass funnels, with the floor lined with blotting paper, for observation for 4 h. The parameters observed were: onset of diarrhoea, total weight of faecal output, total weight of wet faeces, total number of faecal output, and number of wet faeces<sup>6,7,8</sup>.

**Statistics**

The results of all experiments were reported as mean  $\pm$  S.E.M. Statistical analysis was carried out using Student's 't'-test. A level of significance of  $P < 0.05$  was regarded as statistically significant.

**RESULTS****5.1.5 Effect of Glibenclamide, Isosorbide dinitrate, and Yohimbine on antidiarrhoeal activity of Mebarid in mice.**

In the course of observation for 4 h after castor oil administration, all the mice in control group produced copious diarrhoea. Pretreatment of mice with the Mebarid caused a significant dose dependent delay in the onset of copious diarrhoea, decrease in the frequency of purging (reduction of number of wet stools and total no of stools), weight of wet stools, and total weight of stools.

Mebarid showed 56.09% inhibition of diarrhoea at dose of 2.5 ml/kg. Along with glibenclamide (1 mg/kg), isosorbide dinitrate (150 mg/kg), and yohimbine (1 mg/kg), Mebarid showed 47%, 45.45%, 54.54% inhibition of diarrhoea at dose of 2.5 ml/kg, respectively.

**Table 1: Effect of Mebarid with Glibenclamide (GLIB), Isosorbide dinitrate (ISDN) and Yohimbine (YOHI) on castor oil (0.2 ml) induced diarrhoea in mice**

Group	Dose (/kg)	Onset of diarrhoea (min)	Total weight of stools (g)	Weight of wet stools (g)	Total number of stools	Number of wet stools	% Inhibition
Control		53 ± 2.11	0.372 ± 0.01	0.35 ± 0.01	13.33 ± 0.33	11 ± 0.36	
Mebarid	2.5 ml	83 ± 2.09	0.177 ± 0.006	0.16 ± 0.006	6.16 ± 0.30	4.83 ± 0.30	56.09
Mebarid +GLIB	2.5 ml 1 mg	76 ± 1.43	0.205 ± 0.006	0.19 ± 0.006	7.00 ± 0.36	5.83 ± 0.40	47
Mebarid + ISDN	2.5ml 150 mg	73 ± 2.36	0.208 ± 0.007	0.196 ± 0.008	7.16 ± 0.40	6.00 ± 0.366	45.45
Mebarid + YOHI	2.5 ml 1 mg	84 ± 4.39	0.17 ± 0.006	0.161 ± 0.007	5.83 ± 0.47	5.00 ± 0.63	54.54

Values are mean ± standard error of mean. Each value represents average of six determinations. P < 0.05 vs. control, student's 't' test.

## DISCUSSION

In epithelial cells of small intestine, K<sup>+</sup> channels provide the driving force for electrogenic transport processes across the plasma membrane, and they are involved in cell volume regulation. K<sup>+</sup> channels hyperpolarize the membrane voltage, thereby fueling electrogenic transport mechanisms such as Na<sup>+</sup>-coupled reabsorption of nutrients or luminal Cl<sup>-</sup> secretion<sup>9</sup>. Fine tuning of salt and water transport and of K<sup>+</sup> homeostasis occurs in colonic epithelia cells, where K<sup>+</sup> channels are involved in secretory and reabsorptive processes<sup>10</sup>. Glibenclamide, a potassium channel blocker has decreased the antidiarrhoeal activity of Mebarid, implying the involvement of potassium channels in the antidiarrhoeal effect of Mebarid.

Nitric oxide (NO) is known to have a protective effect on the gastrointestinal tract. NO has been reported to play an important role in castor oil-induced diarrhoea<sup>11</sup>. Pretreatment with NO synthase inhibitors from l-arginine prevent castor oil-induced diarrhoea and decrease the intestinal fluid accumulation and Na<sup>+</sup> secretion induced by castor oil. Thus, NO is one of the mediators of the intestinal secretion and diarrhoea induced by castor oil<sup>12, 13</sup>. Antidiarrhoeal effect of Mebarid on castor oil induced diarrhoea was reduced by Isosorbide dinitrate (NO donor), indicating that the antidiarrhoeal effect of Mebarid mediated by l-arginine NO pathway.

Sympathetic stimulation has the opposite effect of parasympathetic stimulation. Stimulation of the enteric nerves by the sympathetic system inhibits GI activity<sup>14, 15</sup>. Yohimbine ( $\alpha_2$  adrenergic receptor antagonist), did not produced significant effect on the observed antidiarrhoeal activity of Mebarid in this study. This precludes interference with this receptor in explaining the effectiveness of Mebarid in diarrhoea.

## CONCLUSION

Mebarid produced antidiarrhoeal effect may be via potassium channels and nitric oxide pathway, but not through  $\alpha_2$  adrenergic receptors.

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