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Effect of antidiarrhoeal formulation on acute experimental diarrhoea in rats

M Aleem Khan[1], Naeem A Khan[1], Iqbal A Qasmi[1], Ghufran Ahmad[1] and Shadab Zafar[2,*]

[1]Department of Ilmul Advia (Pharmacology), Faculty of Unani Medicine, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India; [2]Medical Officer-in-Charge, Unani Dispensary, Municipal Corporation of Delhi, Jhatikra, New Delhi-110043, India

SUMMARY

Aqueous extract of antidiarrhoeal formulation (ADF) containing *Holarrhena antidysentrica, Aegle marmelos* and *Punica granatum* was investigated for antidiarrhoeal activity against charcoal-induced gut transit, serotonin-induced diarrhoea and PGE_2-induced small intestine enteropooling in rats. The control, standard and test groups of experimental animals were administered with normal saline (p.o.), diphenoxylate hydrochloride (5 mg/kg, p.o.) and ADF (250 mg and 500 mg/kg, p.o.) respectively except the control group of PGE_2-induced small intestine enteropooling which received only 5% ethanol in normal saline (i.p.). Charcoal (10 ml/kg, p.o.) and serotonin (600 μg/kg, i.p.) were administered after 30 min, while PGE_2 (100 μg/kg, p.o.) was administered immediately afterwards. The distance traveled by charcoal in small intestine was measured after 15 and 30 min of charcoal administration, diarrhoea was observed every 30 min for six hour after serotonin administration and the volume of intestinal fluid was measured after 30 min of PGE_2 administration. Oral administration of ADF significantly inhibited the frequency of defaecation and decreased the propulsion of charcoal meal through the gastrointestinal tract, reduced the wetness of faecal dropping in serotonin-induced diarrhoea and also reduced the PGE_2-induced small intestine enteropooling. ADF may have potential to reduce the diarrhoea in rats.

Key words: Antidiarrhoeal formulation; Gut transit; Diarrhoea; Enteropooling

INTRODUCTION

Diarrhoea is a major health problem especially for children under the age of 5 years and up to 17% of all death in the indoor pediatrics patients is related to diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children less than 5 year especially in developing countries (Fauci *et al.*, 1998). According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea (Park, 2000).

In Unani system of medicine many single and compound formulations have long been used for the treatment of diarrhoea. But the claim of these drugs in management of diarrhoea has not been scientifically explored. Seeds of *Inder-jaw talkh* (*Holarrhena antidysentrica* Wall) are reported for its astringent (Nadkarni, 1989) and styptic (Ali, 1999) property and it is also used in diarrhoea and dysentery (Ghani, 1917). Reports on fruit pulp of *Bel* (*Aegle marmelos* Corea) shows that it has astringent and styptic property (Ghani, 1921) and effective against diarrhoea and dysentery (Ali, 1993). Flowers of *Gulnar farsi* (*Punica granatum*

*Correspondence: Shadab Zafar, Medical Officer-in-Charge, Unani Dispensary, Municipal Corporation of Delhi, Jhatikra, New Delhi-110043, India. E-mail: shadab_zafar@rediffmail.com

Linn) are also reported for astringent and styptic properties (Lubhaya, 1984) and it is also beneficial in the treatment of diarrhoea and dysentery (Haleem, 1948). In the literature of Unani medicine all these drugs are reported for the above similar properties and actions, hence the present investigations were undertaken to determine the real efficacy of these drugs in the form of compound formulation namely antidiarrhoeal formulation.

MATERIALS AND METHODS

Plants material

Plants material used in ADF includes seeds of *Holarrhena antidysentrica*, fruit pulp of *Aegle marmelos* and flowers of *Punica granatum* were procured from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh and authenticated at pharmacognosy section of the Department of Ilmul Advia (Pharmacology) where the voucher specimens of these drugs are deposited.

Preparation of antidiarrhoeal formulation (ADF)

All the three constituents (ratio 1:1:1) used in ADF were mixed and pulverized by a grinder in the form of coarse powder and the aqueous extract of this powder was prepared with the help of soxhlet's apparatus. The yield of aqueous extract was found to be 10% w/w in terms of dried starting material. Fresh solution of ADF in distilled water was prepared before administration.

Drugs and chemicals

Activated charcoal (Sarabhi M. Chemical Ltd. India), Serotonin creatinine sulphate (ICN Biomedical, Ohio), PGE_2 (Astra-IDL, India) and Diphenoxylate hydrochloride (Searle India Ltd.) were procured from their respective sources. Fresh solution of activated charcoal (10%) and diphenoxylate hydrochloride were prepared in distilled water and PGE_2 was prepared in 5% (v/v) ethanol in normal saline before administration.

Animals

Wistar albino rats (150 - 200 gm) of either sex were procured from Laboaid animal house, Meerut, India and provided food and water *ad libitum*. All the animals were maintained under laboratory conditions for an acclimatization period of seven days before performing the experiments. All studies were carried out using six rats in each group. The experiments were performed between 09:00 and 17:00 hrs. The animal ethics committee of Aligarh Muslim University approved the study protocol.

Charcoal-induced gut transit

The method of Jabbar *et al.* (1999) was followed. The experiment includes four groups (pre-treatment/post-treatment): (I) Control (normal saline, 2 ml/rats)/charcoal (10 ml/kg); (II) standard (diphenoxylate, 5 mg/kg)/charcoal (10 ml/kg); (III) ADF (250 mg/kg)/charcoal (10 ml/kg) and (IV) ADF (500 mg/kg)/charcoal (10 ml/kg). All the doses of control, standard and ADF were administered orally in non-fasted animals and after 30 min charcoal suspension was administered orally in each group.

All the animals of each group were divided into two subgroups. Under deep ether anaesthesia half animals of each group were sacrificed after 30 min and another half animals were sacrificed after 60 min of charcoal administration. Small intestines of each animal were removed surgically and the distance traveled by charcoal was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileocoecal junction).

Serotonin-induced diarrhoea

The method of Jabbar *et al.* (1999) was followed. The experiment includes four groups (pre-treatment/post-treatment): (I) Control (normal saline, 2 ml/rats)/serotonin (600 mg/kg); (II) standard (diphenoxylate, 5 mg/kg)/serotonin (600 mg/kg); (III) ADF (250 mg/kg)/serotonin (600 mg/kg) and (IV) ADF (500 mg/kg)/serotonin (600 mg/kg). All the doses of

control, standard and ADF were administered orally in non-fasted animals and after 30 min serotonin was administered intraperitoneally in each group.

After the administration of serotonin suspension each animal were kept in a separate cage and examined every 30 min for the presence of diarrhoea up to 6 hr. Diarrhoea was defined as the presence of fluid in the stool, which stained the absorbent paper placed beneath the cage. The total number of respondents and the number of stools passed during the 6 hrs period were recorded for each rat. The purging index (PI) was calculated by the following formula:

$$PI = \frac{\% \text{ respondents} \times \text{average number of stool}}{\text{average latent period}}$$

PGE_2-induced small intestine enteropooling

The method of Biswas *et al.* (2002) was followed. The experiment includes four groups (pre-treatment/post-treatment): (I) Control (5% ethanol, 1 ml/rats)/normal saline (1 ml/rat); (II) 5% ethanol (1 ml/rats)/ PGE_2 (100 mg/kg); (III) ADF (250 mg/kg)/PGE_2 (100 mg/kg) and (IV) ADF (500 mg/kg)/PGE_2 (100 mg/kg). Normal saline, PGE_2 and ADF were administered orally and the ethanol was administered intraperitoneally in non-fasted animals.

After 30 min under deep ether anaesthesia all the animals of each group were sacrificed. Small intestines (from pylorus to the ileocoecal junction) of each animal were removed surgically and its contents was measured.

Statistical analysis

All the values were expressed as mean ± SEM. Student's *t*-test was used to analyze significance of the two means. Probability level of less than 5% was considered as statistically significant.

RESULTS

In the animals of standard group the total length traveled by activated charcoal was found to be highly significant ($P < 0.001$) as compared to control value at 30 and 60 min respectively. Similarly the effect of 250 and 500 mg/kg doses of ADF were also found highly significant ($P < 0.001$) as compared to control value at 30 and 60 min respectively. The results of study are shown in Table 1.

The study shows that standard and both doses of ADF markedly reduces the number of respondents from 100% to 16.67%, 16.67% and 16.67% respectively. The mean latent period of standard ($P < 0.001$) and 250 and 500 mg/kg doses of ADF ($P < 0.001$) were found significant and the average number of stools of standard and both doses of ADF were also found significant ($P < 0.01$) as compared of control value. Standard and both doses of ADF were also resulted a very low purging indexes. The results of study are shown in Table 2.

The fluid volume of the rat intestine was significantly increased by PGE_2, when compared with the untreated animals (control), which received only ethanol in normal saline and control vehicle ($P < 0.001$). Both doses of ADF (250 and 500 mg/kg) significantly inhibited PGE_2-induced small intestine enteropooling when compared with PGE_2 treated

Table 1. Effect of ADF on charcoal-induced gut transit

Groups	Doses	n	Length traveled by charcoal in cm (Mean ± SEM)	
			After 30 min	After 60 min
I	2 ml/rat	6	49.46 ± 4.07	97.95 ± 0.9
II	5 mg/kg	6	8.37 ± 0.66***	15.92 ± 0.71***
III	250 mg/kg	6	10.04 ± 0.84***	17.78 ± 0.75***
IV	500 mg/kg	6	9.6 ± 0.9***	16.81 ± 0.88***

***$P < 0.001$ statistically significant as compared to control value. n, number of animals in each group.

Table 2. Effect of ADF on serotonin-induced diarrhoea

Groups	Doses	n	% respondent	Mean latent period in hour ± SEM	Mean number of stools ± SEM	Purging index (PI)
I	2 ml/rat	6	100	1.33 ± 0.09	1.00 ± 00	75.02
II	5 mg/kg	6	16.67	5.83 ± 0.15***	0.17 ± 0.15**	0.48
III	250 mg/kg	6	16.67	5.66 ± 0.30***	0.17 ± 0.15**	0.50
IV	500 mg/kg	6	16.67	5.83 ± 0.15***	0.17 ± 0.15**	0.48

**$P < 0.01$ and ***$P < 0.001$ statistically significant as compared to control value. n, number of animals in each group.

Table 3. Effect of ADF on PGE_2-induced small intestine enteropooling

Groups	Doses	n	Volume of intestinal fluid in ml (Mean ± SEM)
Control (ethanol in saline)	1 ml/rat	6	0.78 ± 0.18
PGE_2	100 μg/kg	6	3.18 ± 0.17***
ADF	250 mg/kg	6	1.97 ± 0.15***
ADF	500 mg/kg	6	1.10 ± 0.55***

***$P < 0.001$ statistically significant (PGE_2 vs. control and ADF vs. PGE_2). n, number of animals in each group.

group ($P < 0.001$). The results are shown in Table 3.

DISCUSSION

The gastrointestinal tract is innervated by both the parasympathetic and sympathetic fibers of the autonomic nervous system. The peristaltic movement of gastrointestinal tract is myogenic in character and is mainly initiated by the local reflexes and can occur without neural connections to the brain or the spinal cord (Shahriar *et al.*, 2000). The extrinsic nerves to the intestine appear to have only a minor role in modulating the peristaltic activity of the organ (Pierce *et al.*, 1971). Earlier study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption (Levy, 1982). Thus gut transit test with activated charcoal was carried out to find out the effect of ADF on peristaltic movement. Our study also shows that ADF suppressed the propulsion of charcoal meal in a dose dependent manner.

The use of serotonin-induced diarrhoea model in our study is also logical because serotonin itself is a diarreogenic hormone, which causes contraction of intestinal smooth muscle by two mechanisms, a direct action on smooth muscle and a neurally mediated action. In specific portion of the intestine (i.e. duodenum), the direct action predominates (Drakontides and Gershon, 1968), whereas in others (i.e. ileum), the indirect neural effect appears to predominate (Day and Vane, 1963). In the present study ADF inhibited serotonin-induced diarrhoea in a dose dependent manner resulting in very low purging indexes.

In vitro, PGE_2 added to the serosal side of small intestine of animals inhibits sodium and chloride absorption from mucosal surface (Declusin *et al.*, 1974). It causes stimulation of motility and conversion of small intestinal mucosa from absorption to secretion of water and electrolytes. PGE_2 also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes (Jaffe, 1979). In the present study ADF inhibited PGE_2-induced small intestine contents in a dose dependent manner.

Diphenoxylate, which was used as a standard drug to compare the antidiarrhoeal effects of ADF, seems to exert stronger effects with similar action. Tannins are responsible for protein denaturation producing protein tannate, which reduces secretion from intestinal mucosa (Tripathi, 1994). Fruits pulp

of *Aegle marmelos* as one of the component of ADF also contains 18 - 22% tannins (Anon, 1985), which may produce antisecretory activity. It is also suggested that *Aegle marmelos* has strong antidiarrhoeal activity (Shoba and Thomas, 2001). We have recently reported that aqueous extract (150 and 300 mg/kg) of ADF has antidiarrhoeal activity against barium sulphate-induced small intestinal transit and castor oil-induced diarrhoea in rats (Khan *et al.*, 2004). Thus the reported claim about these drugs may be validated by this study. Although further investigations are necessary to elucidate the mechanisms by which ADF suppressed the charcoal-induced gut transit, serotonin-induced diarrhoea and PGE_2-induced small intestine enteropooling.

CONCLUSION

The results of this investigation reveal that aqueous extract of ADF contains pharmacologically active substance (s) with antidiarrhoeal properties. Thus we presume that ADF can be developed for the treatment of diarrhoea. But to reach any conclusive decision additional models of diarrhoea and more detailed phytochemical studies are necessary to identify the active principle and exact mechanism of action.

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