Antidiarrhoeal activity of antidiarrhoeal Unani formulation in rats

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Aqueous extract of antidiarrhoeal formulation (ADF) containing Holarrhena antidysenterica Wall, Aegle marmelos Correa ex Koen and Punica granatum Linn. was investigated for antidiarrhoeal activity against charcoal induced gut motility and serotonin induced diarrhoea in experimental rats. The control, standard and test groups of experimental animals were administered with distilled water, Lomotil and ADF (150 mg and 300 mg/kg) orally. Charcoal and serotonin were administered after 30 min in each group of first and second experiment. The distance traveled by charcoal in small intestine was measured after 30 and 60 min of charcoal administration and diarrhoea was observed every 30 min for six hours after serotonin administration. ADF causing significant reduction in the distance traveled by charcoal and serotonin induced diarrhoea. Thus ADF may have the potential to reduce the diarrhoea in rats.

Keywords: Antidiarrhoeal Activity, Diarrhoea, Medicinal Plants

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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 patient is related to diarrhoea. World wide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children of less than 5 year’s age, especially in developing countries. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea.

In Unani System of Medicine, many single and compound formulations have been used for the treatment of diarrhoea. But the claim of these drugs in the management of diarrhoea has not been scientifically explored. Seeds of Inder-jaw talkh (Holarrhena antidysenterica (Linn.) Wall.) are reputed for its astringent and styptic property and are also used in diarrhoea and dysentery. Reports on fruit pulp of Bel (Aegle marmelos Correa ex Koen.) show that it has astringent and styptic property and effective against diarrhoea and dysentery. Flowers of Gulnar farsi (Punica granatum Linn.) are reported for astringent and styptic properties and are also beneficial in the treatment of diarrhoea and dysentery. In Unani medicine literature, all these drugs are reported for similar activities, hence the present investigation was undertaken to determine the efficacy of these drugs (Fig. 1-6) in the form of compound formulation namely antidiarrhoeal formulation.

Methodology

Seeds of Holarrhena antidysenterica, 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.

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

Activated charcoal (Sarabhai M. Chemical Ltd. India), serotonin, creatinine sulphate (ICN biomedical, Ohio), lomotil (diphenoxylate 2.5 mg + atropin sulphate 0.025 mg, Searle India Ltd.), were procured from the respective sources. Fresh solutions of charcoal (10%), serotonin and lomotil were prepared in distilled water before administration.

Wistar albino rats (150-200 gm) were procured from Laboaid animal house, Meerut 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 - 17:00 hrs. The Animal Ethics Committee of Aligarh Muslim University approved the study protocol.

Charcoal induced gut motility experiment included four groups (pre-treatment/post-treatment): (I) control (distilled water, 2 ml/rats)/charcoal (10 ml/kg) (II) Standard (lomotil 7 mg/kg)/charcoal (10 ml/kg) (III) ADF (150 mg/kg)/charcoal (10 ml/kg) and (IV) ADF (300 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 sub-groups. 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. The small intestine of each animal was removed surgically and the distance traveled by charcoal suspension was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileocecal junction).

Serotonin induced diarrhoea experiment also included four groups (pre-treatment/post-treatment): (I) control (distilled water, 2 ml/rats)/serotonin (600 µgm/kg) (II) Standard (lomotil, 7mg/kg)/serotonin (600 µgm/kg) (III) ADF (150 mg/kg)/serotonin (600 µgm/kg) and (IV) ADF (300 mg/kg)/serotonin (600 µg/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 was kept in a separate cage and examined every 30 min for the presence of diarrhoea up to 6 hrs. Diarrhoea was defined as the presence of fluid in the stool which stained the absorbent paper placed beneath the cage. Total number of stools passed during the 6 hrs period were recorded for each rat. The purging index (Pl) was calculated by the following formula:

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\text{Pl} = \frac{\text{Percentage at respondents} \times \text{Average number of stool}}{\text{Average latent period}}
\]

All the values were expressed as mean ± S.E.M. 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 and Discussion

In standard groups, the distance traveled by charcoal was found highly significant (P<0.001) at 30 and 60 min respectively as compared to control value. The effect of 150 mg/ kg dose of ADF was found moderately significant (P<0.01) after 30 min and highly significant (P<0.001) after 60 min. But the effect of 300 mg/ kg dose of ADF was also found highly significant (P<0.001) after 30 and 60 min respectively as compared to control value (Table 1).

Standard and ADF markedly reduced the number of respondents from 100% to 16.67%, 33.33% and 16.67% respectively. The mean latent period of standard at 150 and 300 mg/ kg doses of ADF were found highly significant (P<0.001) as compared to control value. The mean number of stool of standard and 300 mg/ kg dose of ADF was found non-significant (P>0.05). Standard and both doses of ADF had also resulted in very low purging indexes (P<0.001). The results of the study are shown in Table 2.

The gastrointestinal tract is innervated by both para-sympathetic 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. The extrinsic nerves to the intestine appear to have only a minor role in modulating the peristaltic activity of the organ. Activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption. Gut motility test with activated charcoal was carried out to find the effect of aqueous extract of ADF on peristaltic movement. ADF was found to suppress the gut motility in a dose dependent manner.

The use of serotonin induced diarrhoea model is also logical because serotonin being a diarrhoegenic hormone 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 whereas in others (i.e. ileum), the indirect neural effect appears to predominate. In the present study, ADF inhibited serotonin induced diarrhoea in a dose dependent manner resulting in very low purging indexes.
Tannins are responsible for protein denaturation producing protein tannate which reduces secretion from intestinal mucosal. 16 Fruit pulp of Aegle marmelos contains 18-22% tannins 17 which may produce antisecretory activity. Since Aegle marmelos has strong antidiarrhoeal activity, 18 the reported claim finds support from this study.

Lomotil used as a standard drug to compare the antidiarrhoeal effects of ADF has been suggested to exert stronger effect with similar mechanism. Further investigations are necessary to elucidate the mechanisms by which ADF suppressed the charcoal induced gut motility and serotonin induced diarrhoea. Further a better strategy for in depth analysis can be made to extend it to other ratios of plant extracts up to maximum effective dose so that the best mechanism of action.

Aqueous extract of ADF was found to contain pharmacologically active substances with antidiarrhoeal properties. It is suggested that to develop ADF for the treatment of diarrhoea, additional models of diarrhoea, isolated intestinal segments and more detailed phytochemical studies are necessary to identify the active principle and exact mechanism of action.

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