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Protective effect of *Arque-Ajeeb* on acute experimental diarrhoea in rats

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Abstract

Background: Diarrhoea is a major health problem for children worldwide, accounting for 5–8 million deaths each year. *Arque-Ajeeb* (AA) is a compound formulation of Unani medicine. It is reputed for its beneficial effects in the treatment of diarrhoea and cholera, but the claim of its efficacy is yet to be tested. Therefore the present study has been planned to investigate the real efficacy of this drug in rats.

Methods: The effect of *Arque-Ajeeb* was investigated for antidiarrhoeal activity against charcoal-induced gut transit, serotonin-induced diarrhoea and PGE₂-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 *Arque-Ajeeb* (0.07 ml and 0.14 ml/kg, p.o.) respectively except the control group of PGE₂-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 PGE₂ (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 PGE₂ administration.

Results: *Arque-Ajeeb* (0.07 ml and 0.14 ml/kg) significantly inhibited the frequency of defaecation and decreased the propulsion of charcoal meal through the gastrointestinal tract, reduced the wetness of faecal droppings in serotonin-induced diarrhoea and also reduced the PGE₂-induced small intestine enteropooling.

Conclusion: *Arque-Ajeeb* may have potential to reduce the diarrhoea in rats. Thus the drug may prove to be an alternate remedy in diarrhoea.

Background

Diarrhoea is a major health problem especially for chil-

dren 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 [1]. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea [2].

Arque-Ajeeb (*Arque-e-Hayat, Zam Zam, Qulzum, Akseer-e-Azam*) is a compound formulation of Unani medicine containing *Satt-e-Podina* (plant extract of *Mentha arvensis* Linn), *Satt-e-Ajwain* (seeds extract of *Trachyspermum ammi* Linn) and *Kaphoor* (Camphor). It is reputed for its beneficial effects in treatment of diarrhoea [3] and cholera [4], but the claim of the efficacy is yet to be tested. The drug is said to possess antispasmodic, digestive [5] and antiftulant [6] properties. Therefore the present study has been planned to investigate the real efficacy of AA in rats. The goal of this study is to find out the effective dose of AA and its ameliorating potential in diarrhoea as a supporting evidence of its antidiarrhoeal property.

Methods

Drugs

Satt-e-Podina, *Satt-e-Ajwain* and *Kaphoor* were procured from Dawakhana Tibbia College, Aligarh Muslim University, Aligarh, India. Activated charcoal (Sarabhi M. Chemical Ltd. India), serotonin creatinine sulphate (ICN Biomedical, Ohio), PGE₂ (Astra-IDL, India) and Diphenoxylate hydrochloride (Iomotil) (Searle India Ltd.) were procured from their respective sources. Fresh solution of activated charcoal (10%) and diphenoxylate were prepared in distilled water and PGE₂ was prepared in 5% (v/v) ethanol in normal saline before administration.

Preparation of Arque-Ajeeb (AA)

All the three constituents viz: *Satt-e-Podina*, *Satt-e-Ajwain* and *Kaphoor* were mixed in equal (1:1:1 gm) ratio. After mixing, it turns into liquid form and known as *Arque-Ajeeb* [7]. It was diluted with distilled water 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.

Experimental design

Charcoal-induced gut transit

The method of Jabbar et al [8] was followed. Non-fasted animals were divided in to four groups of six each. Group

I and II were treated with normal saline (2 ml/rats) and standard (diphenoxylate hydrochloride, 5 mg /kg), while group III and IV were treated with AA (0.07 and 0.14 ml/kg) respectively. All the doses of control, standard and AA were administered orally and after 30 min activated charcoal solution (10 ml/kg) 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 15 min and another half animals were sacrificed after 30 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 ileocecal junction).

Serotonin-induced diarrhoea

The method of Jabbar et al [8] was followed. Non-fasted animals were divided in to four groups of six each. Group I and II were treated with normal saline (2 ml/rats) and standard (diphenoxylate hydrochloride, 5 mg /kg), while group III and IV were treated with AA (0.07 and 0.14 ml/kg). All the doses of control, standard and AA were administered orally and after 30 min serotonin (600 µg/kg) 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:

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

PGE₂-induced small intestine enteropooling

The method of Biswas et al [9] was followed. Non-fasted animals were divided in to four groups of six each. Groups I and II were treated with 1 ml of 5 % (v/v) ethanol in saline intraperitoneally, while the groups III and IV were treated with AA (0.07 ml and 0.14 ml/kg) orally. Group I was then administered with normal saline (1 ml/ rat) orally and utilized as control. Immediately afterwards, PGE₂ (100 µg/kg in 5 % v/v ethanol in normal saline) was administered orally to each rat except the group I which received only 5% ethanol in normal saline and control vehicle. After 30 min under deep ether anaesthesia all the animals were sacrificed. Small intestines (from pylorus to the ileocecal junction) of each animal were removed surgically and its contents was measured.

Table 1: Effect of AA on charcoal-induced gut transit changes

| Groups | Doses | Distance traveled by charcoal in cm (Mean \pm S.E.M.) | |
|------------------|-------------|---|---------------------|
| | | After 15 min | After 30 min |
| Control (saline) | 2 ml/rat | 49.46 \pm 4.07 | 97.95 \pm 0.9 |
| Diphenoxylate | 5 mg/ kg | 8.37 \pm 0.66*** | 15.92 \pm 0.71*** |
| Arque-Ajeeb | 0.07 ml/ kg | 23.42 \pm 1.81** | 33.67 \pm 1.70*** |
| Arque-Ajeeb | 0.14 ml/ kg | 19.99 \pm 0.20*** | 25.79 \pm 0.88*** |

p < 0.01 and *P < 0.001 statistically significant as compared to control value.

Table 2: Effect of AA on serotonin-induced diarrhoea

| Groups | Doses | % respondent | Mean latent period in hour \pm S.E.M. | Mean number of stools \pm S.E.M. | Purging indexes |
|------------------|-------------|--------------|---|------------------------------------|-----------------|
| Control (saline) | 2 ml/rat | 100 | 1.33 \pm 0.09 | 1.00 \pm 00 | 75.02 |
| Diphenoxylate | 5 mg/ kg | 16.67 | 5.83 \pm 0.15*** | 0.17 \pm 0.15** | 0.48*** |
| Arque-Ajeeb | 0.07 ml/ kg | 33.33 | 5.00 \pm 0.57** | 0.33 \pm 0.19 ^{NS} | 2.22*** |
| Arque-Ajeeb | 0.14 ml/ kg | 16.67 | 5.83 \pm 0.15** | 0.17 \pm 0.15** | 0.48*** |

p < 0.01 and *p < 0.001 statistically significant as compared to control value and ^{NS}p > 0.05 (non-significant).

Statistical analysis

All the values were expressed as mean \pm 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

Effect of AA on charcoal-induced gut transit changes

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 15 and 30 min respectively. But after 15 min 0.07 ml/ kg dose of AA was found significant (p < 0.01) and 0.14 ml/ kg dose of AA was also found significant (p < 0.001) as compared to control value. Both doses of AA (0.07 ml and 0.14 ml/ kg) were found significant (p < 0.001) after 30 min as compared to control value. The results of study are shown in Table 1.

Effect of AA on serotonin-induced diarrhoea

The study shows that standard and AA markedly reduces the number of respondents from 100% to 16.67%, 33.33% and 16.67% respectively. The mean latent period of standard was found highly significant (p < 0.001) and both doses of AA viz: 0.07 ml/kg (p < 0.01) and 0.14 ml/ kg (p < 0.001) were also found significant as compared to control value. The mean numbers of stools of standard and 0.14 ml/ kg dose of AA were found significant (p < 0.01) but 0.07 ml/ kg dose of AA was found non-significant (p > 0.05). However the standard and AA resulted

very low purging indexes (p < 0.001). The results of study are shown in Table 2.

Effect of AA on PGE₂-induced small intestine enteropooling

The fluid volume of the rat intestine was significantly increased by PGE₂, when compared with the untreated animals (control), which received only ethanol in normal saline and control vehicle (p < 0.001). Both doses of AA (0.07 ml and 0.14 ml/ kg) significantly inhibited PGE₂-induced small intestine enteropooling when compared with the PGE₂ treated group (p < 0.001). The results of study 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 [10]. The extrinsic nerves to the intestine appear to have only a minor role in modulating the peristaltic activity of the organ [11]. Earlier study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption [12]. Thus gut transit test with activated charcoal was carried out to find out the effect of AA on peristaltic movement. Our study also shows that AA suppressed the propulsion of charcoal meal in a dose dependent manner.

Table 3: Effect of AA on PGE₂-induced small intestine enteropooling

| Groups | Doses | Volume of intestinal fluid in ml (Mean ± S.E.M.) |
|-----------------------------|-------------|--|
| Control (ethanol in saline) | 1 ml/rat | 0.76 ± 0.15 |
| PGE ₂ | 100 µg/ kg | 3.15 ± 0.15*** |
| Arque-Ajeeb | 0.07 ml/ kg | 1.93 ± 0.18*** |
| Arque-Ajeeb | 0.14 ml/ kg | 1.18 ± 0.65*** |

*** p < 0.001 statistically significant (PGE₂ vs. control and AA vs. PGE₂).

The use of serotonin induced diarrhoea model in our study is also logical because serotonin itself is a diarrhoeogenic 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 [13], whereas in others (i.e. ileum), the indirect neural effect appears to predominate [14]. In the present study AA inhibited serotonin-induced diarrhoea in a dose dependent manner resulting in very low purging indexes.

In vitro, PGE₂ added to the serosal side of small intestine of animals inhibits sodium and chloride absorption from mucosal surface [15]. It causes stimulation of motility and conversion of small intestinal mucosa from absorption to secretion of water and electrolytes. PGE₂ also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes [16]. In the present study AA inhibited PGE₂-induced small intestine contents in a dose dependent manner.

Diphenoxylate, which was used as a standard drug to compare the antidiarrhoeal effects of AA, seems to exert stronger effects with similar action. Although further investigations are necessary to elucidate the mechanisms by which AA suppressed the charcoal-induced gut transit, serotonin-induced diarrhoea and PGE₂-induced small intestine enteropooling. These properties may explain the rational for the extensive and effective use of the AA as an antidiarrhoeal agent in Unani medicine.

Conclusion

The results of this investigation reveal that the AA contains pharmacologically active substance (s) with antidiarrhoeal properties. Thus we presume that AA 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.

Competing interests

None declared.

Authors' contributions

MAK – Participated in sequence alignment and experimental work.

NAK – Supervised the design and coordination of the study.

IAQ – Supervised the design, sequence alignment and coordination of the study.

GA – Supervised the design, sequence alignment and coordination of the study.

SZ – Participated in statistical analysis and the drafting of manuscript.

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