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ANTIDIARRHEAL ACTIVITY OF AQUEOUS LEAF EXTRACT OF *SWERTIA CHIRATA* IN RATS

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ABSTRACT

The effect of the aqueous extract of *Swertia Chirata hum* against diarrhea at 25,50 and 100 mg/kg body weight was evaluated by using gastrointestinal transit, diarrhea and enteropooling induced by castor oil models in female rats. The extract was positive for Ophelic acid, glycosides, amarogentin, chiratin, alkaloids, gentianine and gentiocrucine. The 25 mg/kg of the extract effectively ($P<0.05$) prolonged the onset time of diarrhea, decreased the fecal parameters (number, water content, fresh weight, total number of wet faces) with no episodes in the animals treated with 50 mg/kg and 100 mg/kg. The activity of small intestine Na+-k+ ATPase increased ($P<0.05$) while the nitric oxide, volume and mass of intestinal fluid as well as distance travelled by the charcoal meal decreased. The changing patterns were similar to reference drugs. Overall, the antidiarrheal activity of the aqueous leaf extracts of *Swertia chirata hum* may be due to the chemical constituents present in the extract.

KEYWORDS: chirata, enteropooling, feces.

INTRODUCTION

Diarrhea is one of the leading death causing diseases, especially in developing countries it has been a very concerning issue as it causes millions of deaths. Diarrhea is a gastrointestinal disorder which is characterized by an increarse in stool frequency and changed consistency. The incidence of diarrhea is still high, despite all the efforts of international organizations. As, antibiotics used against diarrhea sometimes cause side effects, so search for effective and safe agents from plant origin continues.

Swertia chirata hum is a 4 to5 feet long herb. It has bald, tiny and yellowish branches. The leaves are elliptical and do not have foot stalk. The fruit is ovate in shape and after ripping it gets blackish. The seeds are round in shape and yellow in color. It is usually found in Bhutan and Kashmir in huge amount. Locally, it is called chirata. Chirata tastes a little bitter. The flower can be seen in August and September. According to Unani medicine, chirata, strengthens heart and liver. It also increases eyesight and works as anti-inflammatory agent. In some areas of India chirata is used to cure Asthma. It is also used as tonic agent. According to ayurvedic medicine, chirata helps in digestion. Chirata is also said to be effective in and against Influenza, vomiting, asthma, itching, erosion, hairfall and diarrhea.

MATERIALS AND METHODS

Plant materials

The plant that is used in the experiment was gathered from a local market (Ipata) in Dhaka, Bangladesh. It was authenticated at the Department of Biology, University of Dhaka, Bangladesh.

Drugs and chemicals

Loperamide hydrochloride, atropine sulfate, and castor oil were products of Rangs International Ltd., Dhaka, Bangladesh; Laborate Pharmaceuticals, Punjab, India, and Wells international Ltd., Barcelona, Spain respectively. Adenosine 5'-triphosphate (disodium salt) was a product of RAK Chemical Co, Dhaka, Bangladesh. Nitric oxide assay kit was a product of Assay Designs Stressgen, Ann Arbor, MI, USA.

Animals

Healthy, female albino rats (*Rattus norvegicus*) weighing 137.40 ± 4.04 g were collected from the Animal House of the Department of Pharmacy, University of Dhaka, Bangladesh. All the animals were kept in clean aluminum cages placed in a well ventilated house conditions (temperature 25ºC, photoperiod 12 hours natural light, 12 hours dark and humidity 45-50%). The animals were allowed free access to rat feeds and clean tap water except while fasting was going on. The cages were cleaned on a daily basis. This study was carried out according to the guidelines of European Convention for the Protection of Vertebrate Animals and Other Scientific Purposes- ETS-123 (2005).

Preparation of extract

The leaves of *Swertia chirata hum* were separated from the stem, washed under running tap and dried in oven

(Uniscope Laboratory Oven, SM9053, Surgifriend Medicals, England) at 40ºC for 48 hours. The dried materials were pulverized using an electric blender (Mikachi MX 1830, Shangai, China) and stored in an air-tight container prior to extraction. A portion (30 g) of the powder was extracted in 1500 ml of cold distilled water for 48 hours. The extract was filtered (Whatman No. 1 filter paper) and the resulting filtrate evaporated to dryness on a water bath (Uniscope Laboratory Water Bath SM801A, Surgifriend Medicals, England) to give a yield of 15 g which correspond to a percentage yield of 50%. This was reconstituted separately in distilled water to give the required doses of 25, 50 and 100 mg/kg body weight used in the present study. The doses of 25 and 50 correspond respectively, to "a pinch" and "a spoon" of the plant powder estimated to be consumed as a remedy for an adult 70 kg man. The 100 mg/kg body weight dose which is a quadruple-fold of the least dose was used to account for cases of 'abuse' by the user.

Phytochemical screening

To detect the presence of alkaloids, steroids, saponins, phenolics, flavonoids, cardiac glycosides, tannins, cardenolides and dienolides Preliminary chemical tests were done on the extract according to the procedures described by Sofowora (1993).

Castor oil-induced diarrhea in rats

Diarrhea was induced in the rats using a modified method of Sunil et al (2001). The test animals were kept fastisting (without food, but water) for 18 hours before the start of the experiment. Each animal was placed in a cage, the floor of which was lined with blotting paper. Animals in the first group (negative control) were orally administered with 1 mL of distilled water while those in the second, third and fourth groups were respectively administered with the same volume (1 mL) of the extract corresponding to 25, 50 and 100 mg/kg body weight.

The fifth group (positive control), was orally administered with same volume (1 mL) of loperamide hydrochloride preparation corresponding to 2.5 mg/kg body weight. After 30 minutes of treatment, each animal was again administered orally with 1 mL of castor oil and the time between the administration of the oil and the appearance of the first diarrheal drop was noted. The severity of diarrhea was accessed every hour for a period of 6 hours by monitoring the diarrheal drops on the pre-weighed blotting paper placed under the individual rat cages. The total number of feces, diarrheal feces and total weight of feces excreted were expressed as average of six determinations and compared with the control groups. The percentage inhibition of diarrheal defecation in each group was also computed. At the end of the 6 hours of monitoring the diarrheal drops, the animals were sacrificed and small intestine homogenates prepared according to the procedure described by Akanji and Yakubu (2000). The assay of both the activity of Na+-K+ ATPase and nitric oxide concentration in the small intestine homogenates was done using the protocol described by Bewaji et al (1985) and Nathan (1992) respectively.

Castor oil-induced enteropooling, intestinal transit and intestinal fluid in rats

Intraluminal fluid was determined as described by Havagiray et al (2004). Briefly, fasted animals as previously described were randomly selected into five groups of six animals each. Animals in the negative control group received 1 mL of distilled water orally while those in the positive control group were orally administered with 1 mL of atropine sulphate corresponding to 0.6 mg/kg body weight. Animals in the test groups were administered with same volume of the extract corresponding to the doses of 25, 50 and 100 mg/kg body weight. Immediately after the administration, 1 ml of castor oil was also administered orally to each rat in all the groups. After 30 min, the rats were sacrificed using the procedure previously described by Akanji and Yakubu (2000). The small intestine was excised and the intestinal content was squeezed quantitatively into a measuring cylinder. The volume and mass of the intestinal content were obtained and the inhibition of intestinal content was also computed.

Gastrointestinal motility test

The method described by Gerald et al (2007) was adopted for the determination of the effect of the extract on gastrointestinal transit in the rats. Fasted animals (as previously described) were assigned into five groups of six rats each. The animals in the negative control group received 1 mL of distilled water orally while those in the positive control received 1 mL of atropine sulphate intramuscularly. Animals in the third, fourth and fifth groups received equal volume of the extract corresponding to 25, 50 and 100 mg/kg body weight. After 30 minutes, all the animals were again administered orally with 1 ml of charcoal meal (10% charcoal suspension in 5% agarose agar). At 30 min post administration of the charcoal meal, all the animals were sacrificed using the procedure described by Akanji and Yakubu (2000). The small intestine was removed and afterwards, the length of the small intestine and the distance travelled by charcoal meal through the organ was measured. The distance was expressed as a percentage of the length of the small intestine.

Statistical analysis

Data were expressed as the means ± SEM of 6 replicates. Statistical analysis was performed using One-way Analysis of Variance (ANOVA) and complemented with Student's t-test. The values were considered statistically significant at $P < 0.05$.

RESULTS

The aqueous extract of *Swertia chirata hum* was positive for ophelic acids, glycosides: amarogentinand chiratin; alkaloids, gentianine and gentiocrucine while saponins, tannins steroids, cardenolides and dienolides were not detected (Table I)

Table 1: some phytochemical constituents of aqueous extract of *Swertia chirata* hum

Phytochemical constituents	Result
Alkaloids	Present
Saponins	Not detected
Tannins	present
Glycosides	Present
Steroids	Not detected
Phenolics	Present
Cardenolides and dienolides	Not detected

The 25 mg/kg body weight of the extract significantly($p<0.05$) prolonged the onset time of diarrhea. In contrast, there was no episode of diarrhea seen in the 50 and 100 mg/kg body weight extract treated animals (Table II). Compared with animals treated with the distilled water, the extract significantly ($p<0.05$) declined the amount of feces in a dose related manner. On the contrary, in the animals administered with 25 mg/kg body weight of the extract, the total number of wet feces, fresh weight of feces and water content of feces decreased significantly in a manner similar to the loperamide treated animals.

Table 2: the effect of aqueous leaf extract of *Swertia chirata* on castor oil induced diarrheal rats.

Parameter/ doses	Lopeamide/ (mg/kg body weight	Water	Plant extract (mg/kg body weight		
	2.5	0	25	50	50
Onset time (mins)	233 ± 8.75b	63.55 ± 1.64a	194.50 ± 4.92c	Nil	Nil
Total number of feces	2.50 ± 0.54b	8.50 ± 0.54a	6.00 ± 0.00c	2.00 ± 0.00d	1.50 ± 0.00c
Number of wet feces	2.00 ± 0.00b	4.50 ± 0.55a	2.00 ± 0.00b	Nil	Nil
Fresh weight of feces(g)	1.31 ± 0.02b	1.69 ± 0.00a	1.03 ± 0.55c	Nil	Nil
Water content of feces (mL)	0.62 ± 0.02c	1.27 ± 0.03a	0.55 ± 0.00c	Nil	Nil
Inhibition of defecation (%)	55.56	0	55.56	100	100
Small intestine Na+-K+ ATPase activity (µmol Pi/mg protein/ hour	1322.73 ± 12.22a	951.85 ± 15.09b	1210.10 ± 14.44c	1330.04 ± 11.88a	1509.08 ±19.72d
Small intestine nitric oxide Concentration (µmol/L)	88.21 ± 8.00a	274.36 ± 7.10b	86.09 ± 11.08a	87.17 ± 8.10a	89.00 ± 7.18a
Values are mean ± SD (n=6); Values carrying different superscript along each rows are significant (<0.05) different from each other					

Table 3: Effect of aqueous extract of *Swertia chirata hum* on castor oil induced enteropooling in rats.

Parameter/ dose	Atropine sulfate (mg/kg body weight)	Water	Plant extract (mg/kg body weight)		
	0.60	0	25	50	100
Mass of intestinal fluid (g)	1.16 ± 0.05b	3.27 ± 0.28a	1.31 ± 0.05c	0.51 ± 0.01d	0.55 ± 0.00d
Volume of intestinal fluid (ml)	1.40 ± 0.17b	3.30 ± 0.10a	2.80 ± 0.21d	1.90 ± 0.13d	1.00 ± 0.11c
Inhibition of intestinal content (%)	64.62	0	59.94	84.39	83.17
Values are mean ± SD (n=6); Values carrying different superscript along each rows are significant (p<0.05) different from each other					

there was no episode in the animals administered with the 50 and 100 mg/kg body weight of the extract. Although the computed percentage inhibition of defecation climbed up in all the treated groups when these were compared with animal which were administered distilled water, it is mentionable, the 50 and 100 mg/kg body weight of the extract produced 100% inhibition of defecation. The activity of Na+-K+ ATPase activity in the small intestine also rocketed dose dependently in the extract treated animals exactly like the positive control animals. While, the concentration of nitric oxide was reduced significantly by the extract in this study similarly the reference drug does (**Table II**). The extract diminished the volume and mass of intestinal fluid of castor oil-induced enteropooling in rats. While the mass of intestinal fluid declined at 50 and 100 mg/kg body weight of the extract was more than the atropine sulphate, it was only the 100 mg/kg body weight of the extract that declined the volume of the intestinal fluid more than the reference drug treated animals. Usually,

the inhibition of intestinal fluid was higher in the extract and atropine sulphate treated animals (Table III). Although, the length of the small intestine in all the experimental animals was not significantly different from each other, the extract significantly reduced the distance travelled by the charcoal meal. These values were lower in the extract and atropine sulphate treated animals than in the distilled water control animals (Table IV).

Table 4: Effect of aqueous extract of *Swertia chirata hum* on charcoal meal transit time of rats.

Parameters	**Atropine sulphate (mg/kg body weight)**	**Water**	**Plant extract (mg/kg body weight)**		
	0.60	**0**	**25**	**50**	**100**
Length of intestine (cm^3)	85.00 ± 5.25a	84.15 ± 6.75a	86.00 ± 4.90a	85.17 ± 6.25a	85.20 ± 5.00b
Distance travelled by meal after 30 min(cm^3)	45.00 ± 3.28b	68.00 ± 0.00a	45.00 ± 0.00c	47.00 ± 1.10d	40.50 ±0.00c
Distance travelled by meal to length of small intestine (%)	52.90	80.80	52.30	55.30	49.90
Values are mean ± SD (n=6); Values carrying different superscript along each rows are significant (p<0.05) different from each other					

DISCUSSION

The use of herbal medicines in the treatment of diarrhea is a common matter in many countries including Bangladesh. Therefore, the need to substantiate or otherwise the folkloric claim of *swertia chirata* as an antidiarrheal agent using several models of diarrhea cannot be overemphasized. It can be seen from the result that there has been statistically significant reduction not only on the onset of diarrhea but also on its severity as revealed by the castor oil-induced diarrhea and enteropooling as well as charcoal meal gastrointestinal transit models in the present study. Castor oil has been widely used in diarrhea studies because it is capable of causing the body through its metabolite, ricinoleic acid, to produce autocoids and prostaglandins which are known inducers of diarrhea in animals (Greenbargena et al., 1978). Ricinoleic acid initiates diarrhea via several mechanisms such as: i. causing irritation and inflammation of the intestinal mucosa, leading to the release of prostaglanding which stimulates motility and secretory diarrhea (Pierce et al., 1971; Mbagwu and Adeyemi, 2008); ii. affecting electrolyte transports (by reducing active Na+ and K+ absorption) and smooth muscle contractility in the intestine via decreasing or inhibiting the activity of Na+- K+ ATPase in the small intestine and colon (Palombo, 2006); iii. increasing the volume of intestinal content by preventing the reabsorption of water; iv. interfering with oxidative metabolism and thus an effect on adenylate cyclase or mucosal adenosine 3', 5'-cyclic monophosphate content; and being cytotoxic to intestinal epithelial cells and causing histological abnormalities and mucosal permeability (Mascolo et al., 1993). These sequences of events may be related to the release of eicosanoids, prostaglandins, nitric oxide, platelet activating factor, cAMP and tachykinins by the intestinal mucosal, which consequentially could give rise to diarrhea. Therefore, the significantly ($p<0.05$) prolonged time of induction of diarrhea, decreased frequency of stool and fecal parameters (total number of feces, fresh weight, water content and number of wet feces) following the administration of the extract suggest antidiarrheal activity at this dose. This assertion was further corroborated with the increased inhibition of defecation. The same percentage of inhibition of defecation in the 25 mg/kg body weight of the extract and loperamide hydrochloride suggest that the antidiarrheal activity of the extract may proceed via the same mechanism as that of the reference drug, loperamide hydrochloride. The clinical effect of the extract as antidiarrheal agent was demonstrated at 50 and 100 mg/kg body weight where the typical parameters of diarrhea did not manifest in the animals. The extract might have exerted its antidiarrheal activity via secretory mechanism as evident from reduction in total number of wet faeces. Furthermore, this antidiarrheal activity could have resulted from the inhibitory activity of aqueous leaf extract of *swertia chirata* hum on prostanglandins synthesis, nitric oxide and platelet activating factors production, as inhibitors of prostaglandins and nitric oxide syntheses are known to delay diarrhea induced by castor oil (Capasso et al., 1994; Adzu et al., 2003; Tangpu and Yadav, 2004). Similar effects were reported in several studies by Qnais et al (2005), Akindele and Adeyemi (2006) and Appidi et al (2010) following the administration of aqueous leaf extracts of Juniperus phoenicia, Byrsocarpus coccineus and Hermania incana, respectively. Castor oil, the inducer of diarrhea in animals decrease or inhibit the activity of Na+-K+ ATPase in the small intestine and colon and thus affect electrolyte transports by reducing active Na+ and K+ absorption (Palombo,2006). Similarly, study by Capasso et al (1994) have implicated elevated nitric oxide in the pathogenesis of diarrhea, a disease which was prevented by the intraperitoneal injection of nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (2.5–50 mg/kg twice) to rats.

Therefore, the increase in the activity of Na+-K+ ATPase as well as decrease in the concentration of nitric oxide in the small intestine of extract treated animals may be one of the mechanisms by which the extract exhibits its antidiarrheal effect. The accumulation of intestinal fluids may be a resultant clinical effect of bowel function disturbance, in which case, there is impaired intestinal absorption, excessive intestinal secretion of water and electrolytes and a rapid bowel transit (Gurgel et al., 2001; Mbagwu and Adeyemi, 2008). The reduction in the parameters of enteropooling and consequent increase in the percentage inhibition of intestinal content of the animals suggest that the extract might have inhibited or reduced the massive secretion of water into the intestinal lumen. It is possible that the aqueous leaf extract of *swertia chirata* hum may be explored in managing secretory diarrhea. This anti-enteropooling effect of *swertia chirata* hum could be due to the presence of flavonoids in the extract, as the phytochemical have been reported to inhibit intestinal motility and hydroelectrolytic secretion (Perez et al., 2005). Atropine sulfate is known to produce an anticholinergic effect on intestinal transit whereas activated charcoal can prevent the absorption of drugs and other chemicals into the body by absorbing them on the surface of the charcoal particles (Venkatesan et al., 2005). Thus, the suppression or reduction in the intestinal propulsive movement of the charcoal meal by all the doses of the extract in the present study suggest among others that the extract was able to increase the time for absorption of water and electrolytes in a manner similar to the reference drug, atropine sulfate (Teke et al., 2007). It may also indicate a reduction in peristaltic activity and ultimately reduction in the gastrointestinal motility (Nwiniyi et al., 2004). This effect which suggests antidiarrheal activity may be attributed to the flavonoids since it has been reported to be able to inhibit fluid secretion in the small intestine thereby reducing the rate of flow in the gut. The extract appears to have acted on all parts of the intestine producing inhibitory effect on both the gastrointestinal propulsion and fluid secretion. The findings in this study are similar to the report by Maridass (2011) following the administration of 500 mg/kg body weight of ethanolic tuber extract of Eulophia epidendrae to castoroil-induced diarrheal rats. Previous studies have implicated a wide array of phytochemicals with antidiarrheal activity. These include tannins, alkaloids, saponins, flavonoids, sterols, terpenoids and reducing sugars (Galvez et al., 1993; Mukherjee et al., 1998; Otshudi et al., 2000; Shoba, 2001; Havagiray et al., 2004; Venkatesan et al., 2005). Flavonoids and saponins are known to inhibit the release of autocoids and prostaglandins thereby reducing the motility and secretion induced by castor oil (Veiga et al., 2001; Perez et al., 2005). Because many of these compounds might have antidiarrheal effects, it is difficult to suggest which of them is responsible for the desired effect. However, we suggest that alkaloids, saponins and flavonoids present in the extract of *swertia chirata* hum might be responsible for its antidiarrheal activity. In conclusion, aqueous leaf extract of *swertia chirata* hum has antidiarrheal activity made possible by the alkaloids, phenolics, flavonoids and saponins via reduction or inhibition of typical indices of diarrhea such as the fecal parameters, enteropooling, gastrointestinal motility and stimulation/enhancement of Na+-K+ ATPase activity and reduction in the nitric oxide concentration of the small intestine.

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