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ASSESSMENT OF ANALGESIC AND ANTIDIARRHOEAL ACTIVITIES OF DIFFERENT FRACTIONS OF CRUDE EXTRACT OF *STEPHANIA JAPONICA* STEM

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ABSTRACT: The different fractions of crude methanol extract of stem of *Stephania japonica* (Thunb.) Miers (Menispermaceae) was screened for its analgesic and antidiarrhoeal activities. The different fractions of crude extract produced significant writhing inhibition in acetic acid induced writhing in mice at the dose of 250 and 500 mg/kg body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. When tested for its antidiarrhoeal effects on castor oil induced diarrhea in mice, it increased mean latent period and decreased the frequency of defecation significantly at the dose of 250 and 500 mg/kg body weight comparable to the standard drug loperamide at the dose of 50 mg/kg of body weight. The overall results suggest the analgesic and antidiarrhoeal activities of the different fractions of crude extract. Among all the fractions Ethyl acetate soluble fraction showed most significant analgesic activity and Petroleum ether soluble fraction showed the most significant antidiarrhoeal activity.

INTRODUCTION: The history of medicinal plants in remedy of different diseases is well established. Various species of different family of plant and other sources contribute in the development of present therapeutic processes.

Stephania japonica (Thunb.) Miers (Menispermaceae) locally known as Aknodi, Akonadi, Akondi, Fuit pata, etc., is a climbing shrubs distributed in widespread north from Eden, South Coast. also Qld and from India, throughout Asia to southern Pacific etc. Leaves and roots are bitter and astringent; used in fever, diarrhoea, urinary diseases and dyspepsia. Leaves are mounted on abscess and kept for bursting. Leaves are macerated in a glass of water and are taken after mixing with molasses to cure urethritis. Leaves are also given for gastritis in Khagrachari. Root paste is taken for vertigo and dysentery; root mixed with root juice of *Flemingia stricta* is taken for asthma; root paste is warmed and rubbed in hydrocele.

Ethanolic extract of the leaf possesses wide range of good antibacterial and antifungal properties. However, no other biological activity has yet been reported. The objective of the present study was to investigate the analgesic and antidiarrhoeal activity of the different fraction of the crude extracts of stem of *Stephania japonica* (*S. japonica*).

MATERIALS AND METHODS

Plant material collection and extraction: The stem of *Stephania japonica* was collected from Savar road side in December, 2010. One voucher specimen has been deposited in Bangladesh national Herbarium (DACB accession No. 35492). The plant sample (after cutting into small pieces) was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried plant material was then ground in coarse powder using high capacity grinding machine in the Phyto-

chemical Research Laboratory, Faculty of Pharmacy, and University of Dhaka. About 900 gm of the powdered sample was taken in a clean, round bottomed flask (5 liters) and soaked in 4.5 liters of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 14 days accompanying routine shaking and stirring.

The whole mixture was then filtered through cotton followed by Whatman No. 1 filter paper and the filtrate thus obtained was then air dried to solid residue in different beaker. The weight of the crude extract obtained from the stem of *S. japonica* was found 14 gm.

Solvent-Solvent Partition of Crude extract: Modified Kupchan Partition [1]:

1. **Preparation of Mother Solution:** 10 gm of dried methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This is the mother solution, which was partitioned off successively by four solvents of different polarity. In subsequent stages each of the fractions was analyzed separately for the detection and identification of compounds having different biological & pharmacological activities.

2. **Partitioning with Petrolium ether:** The mother solution was taken in a separating funnel. 100 ml of the petrolium ether was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice; petrolium ether fractions were collected together evaporated in Rota evaporator.

3. **Partitioning with Carbontetrachloride:** To the mother solution left after washing with petrolium ether, 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with CCl_4 (100 ml X 3). The CCl_4 fractions were collected together and evaporated. The aqueous fraction was preserved for the next step.

4. **Partitioning with Chloroform:** To the mother solution that left after washing with petrolium ether and CCl_4, 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with $CHCl_3$ (100 ml X 3). The $CHCl_3$ soluble fractions were collected together and evaporated. The aqueous methanolic fraction was preserved as aqueous fraction.

5. **Partitioning with Ethyl acetate:** To the mother solution that left after washing with petrolium ether, CCl_4 and $CHCl_3$, was then taken in a separating funnel and extracted with Ethyl acetate (100 ml X 3). The Ethyl acetate soluble fractions were collected together and evaporated. The aqueous methanolic fraction was preserved as aqueous fraction

Drugs: Diclofenac Sodium (Opsonin Chemical Industries Ltd, Bangladesh), Loperamide (Square Pharmaceuticals Ltd, Bangladesh)

Animals: Young Swiss-albino mice of either sex, weighing 25-35 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the tests. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12 h light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Pharmacological studies:

1. **Analgesic activity:** Analgesic activity of the different fraction of crude methanol extract of *S. japonica* was tested using the model of acetic acid induced writhing in mice [2, 3]. The experimental animals were randomly divided into four groups, each consisting of five animals.

   Group I was treated as' control group' which received 1% (v/v) Tween-80 in water by per oral (p.o.) route at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at the dose of 250 and 500 mg/kg of body weight respectively.

Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

2. **Antidiarrhoeal activity:** Antidiarrhoeal activity of the different fraction of methanol extract of *S. japonica* was tested using the model by castor oil induced diarrhoea in mice [4]. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment.

   The test animals were randomly chosen and divided into four groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug loperamide at the dose of 50 mg/kg of body weight; group III and group IV were test group and were treated with the extract at the dose of 250 and 500 mg/kg of body weight respectively.

   Control vehicle, standard drug and the extracts were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in five hours study after the castor oil administration.

   Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (5 h). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones.

Statistical analysis: Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

RESULTS:

1. **Analgesic activity: Table 1 & 2** show the analgesic effect and significance of the different fractions of methanol extract of *S. japonica* on acetic acid induced writhing in mice. The different fractions of methanolic extract of *S. japonica* stem i.e. Ethyl acetate (EAJS), chloroform (CLJS) carbontetra-chloride (CTJS) and Petroleum ether (PEJS) fractions were subjected to determine the analgesic activity by acetic acid induced writhing method on mice.

   The standard group showed analgesic activity with writhing inhibition of 62% compared to the control group. The different fractions of methanolic extract of the stem such as Ethyl acetate (EAJS), chloroform (CLJS) carbontetrachloride (CTJS) and Petrolium ether (PEJS) showed analgesic activity with writhing inhibition of 26%, 18%, 12% and 15% at 250 mg/kg dose respectively and 53%, 53%,32% and 35% at 500 mg/kg dose respectively.

2. **Antidiarrhoeal activity: Table 3** shows the effect of different fractions of *Stephania japonica* stem. The different fractions of methanolic extract of *S. japonica* stem i.e. Ethyl acetate (EAJS), chloroform (CLJS) carbontetrachloride (CTJS) and Petroleum ether (PEJS) fractions were subjected to determine the mean latent period of castor oil induced diarrhoeal episode activity in the dose of 250 mg/kg and 500 mg/kg body weight on mice.

   The standard group showed mean latent period of 2.21 hours compared to control group of 0.76 hours (**table 4**). The different fractions of methanolic extract of the stem Ethyl acetate (EAJS), chloroform (CLJS) carbon-tetrachloride (CTJS) and Petroleum ether (PEJS) showed antidiarrhoeal activity with mean latent period of 0.91 hr, 0.924 hr, 0.95 hr and 1.21 hr at 250mg/kg dose respectively and 1.33 hr, 1.25 hr, 1.35 hr, 2.082 hr at 500 mg/kg dose respectively.

TABLE 1: ANALGESIC ACTIVITY OF DIFFERENT FRACTION OF *S. JAPONICA* STEM ON ACETIC ACID INDUCED WRITHING OF MICE

Animal group	Writhing Count					Mean writhing	% of writhing	% of inhibition
	M-1	M-2	M-3	M-4	M-5			
Control	34	39	30	32	35	34	100	0
Standard	10	15	13	12	15	13	38	62
EAJS (250mg/kg)	25	30	26	20	30	25	74	26
EAJS (500mg/kg)	14	15	19	17	17	16	47	53
CFJS (250mg/kg)	28	30	27	29	28	28	82	18
CFJS (500mg/kg)	14	14	18	18	17	16	47	53
CTJS (250mg/kg)	27	29	32	30	34	30	88	12
CTJS (500mg/kg)	19	27	22	23	24	23	68	32
PEJS (250mg/kg)	30	31	30	28	27	29	85	15
PEJS (500mg/kg)	20	23	25	18	23	22	65	35

EAJS=Ethyl Acetate fraction of *japonica* stem; **CFJS=**Chloroform fraction of *japonica* stem; **CTJS=**Carbon Tetrachloride fraction of *japonica* stem; **PEJS=**Pet Ether fraction of *japonica* stem; M-1= Mice No.1; M-2= Mice No.2; M-3= Mice No.3; M-4= Mice No.4; M-5= Mice No.5

TABLE 2: STATISTICAL EVALUATION

Animal group	SD	SE	t-test (value of p)
Control	1.8	0.9	
Standard	1.9	0.95	16.15 P<0.001
EAJS (250mg/kg)	5.1	2.55	7.5 P<0.001
EAJS (500mg/kg)	1.94	0.97	13.6 P<0.001
CFJS (250mg/kg)	3.7	1.87	2.89 P<0.05
CFJS (500mg/kg)	2.05	1.10	13.3 P<0.001
CTJS (250mg/kg)	2.2	1.1	2.81 P<0.05
CTJS (500mg/kg)	2.6	1.3	7.24 P<0.001
PEJS (250mg/kg)	3.16	1.58	2.76 P<0.05
PEJS (500mg/kg)	1.11	0.56	11.43 P<0.001

SD = Standard deviation; SE = Standard error.

TABLE 3: EFFECT OF DIFFERENT FRACTIONS OF *S. JAPONICA* STEM ON THE LATENT PERIOD OF CASTOR OIL INDUCED DIARRHOEAL EPISODE IN MICE

Animal group	Latent period (hr)					MLP (hr)	SD	SE	t-test (p-value)
	M-1	M-2	M-3	M-4	M-5				
Control	0.58	0.83	0.91	0.66	0.81	0.76	0.11	0.06	
Standard	2.38	2.28	2.10	1.72	2.57	2.21	0.28	0.14	10.7 p<.001
EAJS (250mg/kg)	0.81	0.87	0.92	0.97	1.00	0.91	0.06	0.03	2.7 p<.05
EAJS (500mg/kg)	1.2	1.5	1.3	1.4	1.25	1.33	0.1	0.05	8.14 p<.001
CFJS (250mg/kg)	0.82	0.90	0.90	1.00	1.00	.924	0.06	0.03	7.83 p<.001
CFJS (500mg/kg)	1.3	1.25	1.40	1.20	1.10	1.25	0.1	0.05	7.00 p<.001
CTJS (250mg/kg)	0.75	1.00	0.85	0.95	1.20	0.95	0.07	0.04	2.7 p<.05
CTJS (500mg/kg)	1.4	1.25	1.60	1.20	1.30	1.35	0.14	0.07	6.6 p<.001
PEJS (250mg/kg)	0.9	0.95	1.50	1.70	1.00	1.21	0.31	0.16	2.6 p<.001
PEJS (500mg/kg)	2.1	2.16	2.30	1.90	1.95	2.082	0.14	0.07	14.4 p<.001

MLP=Mean Latent Period

TABLE 4: EFFECT OF VARIOUS FRACTIONS *STEPHANIA JAPONICA* STEM ON CASTOR OIL INDUCED DIARRHOEA IN MICE

Animal group	Stool count (in 4hr)					MNS (4hr)	SD	SE	t-test (p-value)
	M-1	M-2	M-3	M-4	M-5				
Control	10	9	11	8	9.5	9.5	1	0.5	10.7 p<.001
Standard	2.5	3.5	3	2	4	3.0	0.7	0.35	2.7 p<.05
EAJS (250mg/kg)	8	8.5	7.5	9.0	8.5	8.0	0.2	0.1	8.3 p<.001
EAJS (500mg/kg)	5	4.5	5.5	5	5.5	5.0	0.37	0.185	3 p<.05
CFJS (250mg/kg)	7.5	7.5	8	8.5	8	8.0	0.37	0.185	7.2 p<.001
CFJS (500mg/kg)	6	5	5.5	6	5	5.5	0.45	0.225	3.6 p<.01
CTJS (250mg/kg)	7.5	7.0	7.5	8.0	8.0	7.6	0.37	0.185	7 p<.001
CTJS (500mg/kg)	5.0	5.5	5	5.5	6.5	5.5	0.55	0.275	4 p<.01
PEJS (250mg/kg)	6.5	6.0	7.0	7.5	8.0	7.0	0.7	0.35	8.7 p<.001
PEJS (500mg/kg)	3.5	2.5	2.5	4.5	4.5	3.5	0.94	0.47	10.7 p<.001

MNS: Mean No. of Stool

DISCUSSION: To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness. Analgesic activity of the different fractions of methanol extract of *S. japonica* was tested by acetic acid induced writhing model in mice.

Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [5]. Increased levels of PGE_2 and $PGF_2\alpha$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid [6].

The different fractions of methanol extract of *S. japonica* produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 1 & 2).

On the basis of this result it can be concluded that the ethyl acetate soluble fraction of *S. japonica* posses most significant analgesic activity. Antidiarrhoeal activity of the different fraction of the methanol extract of *S. japonica* were tested by using the model of castor oil induced diarrhoea in mice [4] (Table 3 & 4).

Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect.

The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine.

The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase [7] or release prostaglandin [8]. The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool.

On the basis of the result of castor oil induced diarrhoea, it can be concluded that the various fractions of methanol extract of *S. japonica* posses antidiarrhoeal activity.

In conclusion, it could be suggested that the various fractions of crude extract of *S. japonica* posses analgesic and antidiarrhoeal effect. However, further studies are necessary to find out the active principles responsible for these activities.

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