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Antidiarrhoeal and hypoglycemic effects of *Synedrella nodiflora*

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Abstract

The present study was designed to investigate antidiarrhoeal and hypoglycemic potential of the methanolic extract of *Synedrella nodiflora* (SN) (Asteraceae) leaves. The extract studied for antidiarrhoeal property using castor oil induced diarrheal model in mice. At the doses of 200 and 400 mg/kg body weight, the extract showed the antidiarrhoeal activity considerably 58.97% and 73.91% inhibition after 4h. Hypoglycemic effect was evaluated in normal and alloxan induced diabetic rat. The intraperitoneal administration of plant extract at a dose of 150 and 300 mg/kg body weight was given to fasting glucose loaded rat with regard to normal control during 1 hr. study period and in alloxan induced (110 mg/kg body weight) diabetic rat in comparison with reference drug Metformin Hydrocloride (100 mg/kg) during 3 days study period. Considerable drop in elevated blood glucose level was observed in the normoglycemic and alloxan induced diabetic rat. At a dose of 150 and 300 mg/kg the extract showed glucose level reduction of 57.87% and 66.83% in alloxan induced rat while 72% was found for Metformin after 3 days. Altogether, these results suggest that the MeOH extract could be used as a potential antidiarrhoeal agent along with its hypoglycemic potentiality. This is the first report of antidiarrhoeal and hypoglycemic potential of *Synedrella nodiflora.*

Keywords: *Synedrella nodiflora*, antidiarrhoeal, alloxan;

Introduction

Diarrhea is an alteration in the normal bowel movement, characterized by increased frequency of bowel sound and movement, wet stool, and abdominal pain (Guerrant et al., 2001). Clinically it is used to describe increased liquidity of stool, usually associated with increased stool weight and frequency (Suleiman et al., 2008). Oral rehydration therapy (ORT) has been identified as a key factor in the decline of child mortality rate due to diarrh-

ea, although it does not reduce the volume or duration of diarrhea (Subbotina et al., 2003). Treatment with pharmacological agents that are pathogen specific or that suppress severe symptoms would be of benefit to patients suffering from prolonged diarrhea (Takahashi et al., 2001).

Diabetes mellitus is one of the common metabolic disorders. Almost 1.3% of the population suffers from this disease throughout the world (Ghosh et al., 2004) and number of diabetics is increasing by 6% per year (Resmi, 2001). Diabetes is still not completely curable by the present anti diabetic agents. Insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks like insulin resistance (Piedrola et al., 2001), anorexia, brain atrophy and fatty liver in chronic treatment (Pari et al., 1999). Hence, the search for safer and more effective hypoglycemic agents has continued.

Synedrella nodiflora (L) Gaertn. belongs to the family Asteraceae. It is a small, annual weed of cultivation, native to tropical America, found in the plains of India and in the Andamans. The methanol extract showed the presence of steroids, reducing sugars, phenolic compounds, saponins and tannins. Benzene and chloroform extracts showed the presence of steroids. Petroleum ether (40 0 – 60 0 C) extracts showed the presence of steroids and triterpenoids (Rathi et al., 2006).

Asteraceae family consists of herbs which are known to accumulate substantial amount of flavonoids and to display anti-inflammatory, antioxidant, antimicrobial, analgesic and antipyretic properties (Odom et al., 2000). In Ghana, *S. nodiflora* (L) Gaertn. weed is used for the treatment of epilepsy and pain (Idu et al., 2007). In Malaysia and Indonesia, the plant is used for headaches, earaches, stomach aches and rheumatism (Sumi et al., 2011).

Literature reviews indicated that no studies combining the antidiarrhoeal and antidiabetic activity of the leaves of *S. nodiflora* have so far been undertaken. Taking this in view and as a part of our ongoing research on Bangladeshi medicinal plants, the present study aimed to evaluate the antidiarhoeal and hypoglycemic activity of the methanolic leaves extract of *S. nodiflora*.

Materials and Methods

Plant Material

The plant *S. nodiflora* were collected from Rajshahi in the month of March 2009 and identified by Dr. M.A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh. A voucher specimen for this collection has been maintained in the Bangladesh National Herbarium (34479), Dhaka, Bangladesh.

Preparation of the extract

The leaves of plant were first washed with water to remove adhering dirt and then dried at 45°C for 36 hrs in an electric oven, then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The dried powdered material (1kg) was taken in a clean, flat bottomed glass container and soaked in methanol for seven days.

The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. The total filtrate was concentrated to dryness, *in vacuo* at 40^0C to render the methanol extract (390 g) of brownish red color.

Drugs and chemicals

The active drugs metformin and loperamide were the generous gift samples from Square Pharmaceuticals Ltd., Bangladesh. Castrol oil and alloxan were obtained from Merck, Germany. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. All chemicals used were of analytical reagent grade.

Animals

Young Long-Evans rats of either sex weighing about 80-120gm were used for the experiment. The rats were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDRB formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann et al., 1983).

Acute toxicity

The 50% lethal dose (LD_{50}) of the SN in rats was estimated by the up and down method (Bruce, 1985). Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.

In vivo anti-diarrheal activity

Castor oil-induced diarrhea

The experiment was performed according to the method described by Shoba & Thomas (Shoba et al., 2001). Briefly, Rats fasted for 24 h were randomly allocated to four groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhea were selected for the final experiment. Group I received 1% CMC (10 ml/kg, *p.o.*), groups III and IV received orally the crude extract (200 and 400 mg/kg), respectively. Group II was given loperamide (10 mg/ kg, *p.o.*) in suspension. After 60 min, each animal was given 0.5 ml of castor oil, each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, observed for 4 h and the characteristic diarrheal droppings were recorded.

Antidiabetic activity

The experiment was designed by the following method (Nahar et al., 2010). Animals were alienated into five groups and for every group six animals were taken. Group I: (Normal control) rats served as positive control received physiological saline (0.9% NaCl; 5ml/kg.

p.o). Group IIL: (Diabetic Control) Intraperitoneally injected Normal saline treated Alloxan induced Diabetic rat. Group III: rats were administered Metformin Hydrochloride (100 mg/kg/day) at a period of 24 hr for 3 successive days and served as standard. Group IV and V: rats were received intraperitoneal injection of *S. nodiflora* (150 and 300mg/kg/day) at a hiatus of 24 hr for three consecutive days. Blood glucose was measured on 1st, 2nd and 3rd day.

Preparation of Alloxan solution

At first body weight of rats were measured. Then necessary amount of Alloxan was measured according to the body weight by following the dose of 110 mg of Alloxan per 1000 gm of body weight. Then calculated quantity of Alloxan was dissolved in 0.1 ml of sterile normal saline water.

Induction of Alloxan

The rats were injected Alloxan monohydrate, dissolved in sterile normal saline water at a dose of 110mg/kg body weights intraperitoneally once a day. Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin discharge; therefore the rats were treated with glucose solution orally. After few days rats with moderate diabetes having glycosuria and hyperglycemia that is blood glucose level go beyond normal level were chosen.

Preparation of dosage of active drug and plant extract

Metformin hydrochloride

Metformin hydrochloride was in microcrystalline form and freely soluble in water. The dosage was prepared in solution form with sterilized water in such a concentration that each 0.1ml contained metformin hydrochloride according to the dose of 100 mg/kg/day, seeing as metformin is effective in such dose in case of humans.

S. nodiflora

The crude extract obtained from trunk was dissolved in 99% DMSO to prepare the solution where each 0.1 ml contained *S. nodiflora* according to the dose of 150 and 300 mg/kg/day. 0.1 ml of the tested solution was administered everyday during treatment to achieve required dose of respective agents.

Biochemical Assay

Fasting blood glucose level was evaluated in normal and diabetic rats from the tail vein by strip technique (Bioland Glucometer, Germany). At first it is done just prior to extract administration of first day then it is continued for 3 days just one hour after the administration of plant extract.

Table 1. Effect of *S. nodiflora* extract on castor oil-induced diarrhea in rat**.**

Treatment and doses	Number of Faeces				No. of faeces in 4 h	% inhibition of defaecation
	1st Hour	2nd Hour	3rd Hour	4th Hour		
Control 1% CMC (10 ml/kg, *p.o.*)	6.5 ± 0.61	9.0 ±0.57	11.33±0.80	15.34±0.60	42.17	00
Loperamie (10 mg/ kg *p.o.*)	2.16 ±0.30	1.50±0.42*	0.50±0.22**	0.30±0.10**	4.46**	89.42
SN (200 g/kg/Day)	7.50 ±0.56	5.0±0.63*	2.83±0.94**	1.70±0.25**	17.03**	58.97
SN(400 mg/kg/Day)	5.30±0.56	3.07±0.63*	1.73±0.94**	0.90±0.20**	11.0**	73.91

Values are presented as mean ± SEM, *P< 0.01 and **P<0.001 ANOVA followed by Dunnet T test Compaired to Control group.

Results

Acute toxicity

Oral administration of graded doses of SN (500 – 5000 mg/kg, body weight) did not cause any death in the different dose groups. The LD_{50} value for oral administration of the plant extract was found to be greater than 5000 mg/kg.

In vivo antidiarhoeal activity

In the castor oil induced diarrheal mice, the methanolic extract of *S. nodiflora* at the dose of 200and 400mg /kg b. wt. significantly lessen the total number of faces in a dose dependent manner (Table 1).

Effect on blood glucose level

Alloxan (110 mg/kg body weight) administration resulted in significant elevation of glucose level. Administration of *S. nodiflora* at a dose of 150 and 300mg/kg body weight administered for three days were able to correct this aberration significantly (p<0.001). The

Table 2: Effect of *S. nodiflora* on Fasting blood glucose (FBG) level in normal and Alloxan induced diabetic rat.

Group	1st Day	2nd Day	3rd Day
Control (Non Diabetic)	5.1±0.17	5.3 ±0.11	5.0 ± 0.14
Control Diabetic	19.11 ± 0.96	19.34 ± 0.49	19.75 ± 0.73
Metformin Hcl 100mg/kg/Day	12.46 ± 0.67**	8.75 ± 0.31**	5.53 ± 0.27**
SN 150 mg/kg/Day	15.54± 0.57**	10.75± 0.88**	8.32± 0.88**
SN 300 mg/kg/Day	12.31 ± 0.97**	8.76 ± 0.61**	6.55 ± 0.38**

Values are presented as mean ± SEM, **P< 0.001 ANOVA followed by Dunnet T test Compaired to Control group (Diabetic Control).

results of all the formulations tested are presented in Table 2. Before treatment schedule, fasting blood glucose level in all animals was within normal range. After treatment with Alloxan, the fasting blood glucose level was significantly changed and it was significantly (P<0.001) reduced by 3 days treatment with methanolic extract of *S.nodiflora* that is comparable to the standard Metformin HCl. On the progression of treatment with methanolic extract of *S. nodiflora* (150 and 300mg/kg/day) fasting blood glucose level reduced at 8.32± 0.88 mmol/L and 6.55 ± 0.38mmol/L respectively on 3rd day.

Discussion

Several mechanisms have been previously proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na^+, K^+- ATPase activity to reduce normal fluid absorption (Nell et al., 1984), activation of adenylate cyclase or mucosal cAMP mediated active secretion (Capasso et al., 1994), stimulation of prostaglandin formation (Galvez et al., 1993), platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil (Mascolo et al., 1996). However, it is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (Gaginella et al., 1975). Since the methanol extract of the leaves of *S. nodiflora* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action via antisecretory mechanism which was also evident from the reduction of total number of wet faeces in the test groups in the experiment. Again, flavonoids present in the plant extract (Wijaya et al., 2011) are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil (Hasan et al., 2009).

Alloxan is the most frequently employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is escalating evidence that alloxan caused diabetes by rapid exhaustion of a cells, by DNA alkylation and gathering of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a fall in insulin release there by a drastic diminution in plasma insulin concentrate-on leading to stable hyperglycemic states (Siddaiah et al., 2011).

The research on Antidiabetic activity in alloxanised rats, administration of ethanolic extract of *S. nodiflora* of 150 and 300mg/kg body weight administered for 3 days was able to correct this anomaly significantly (p<0.001). Significant reduction of blood glucose was observed from the 3rd day of the study. The comparable effect of the experimental extract with Metformin HCl may suggest similar mode of action since alloxan permanently destroys the pancreatic ß cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extract possesses extra pancreatic effect. On the progression of treatment with methanolic extract of *S. nodiflora* (150 and 300 $mgkg^{-1}day^{-1)}$ fasting blood glucose level reduced to 8.32± 0.88 mmol/L and 6.55 ± 0.38mmol/L respectively on 3rd day. These observations suggest that the experimental extract might acquire insulin like effect on peripheral tissues either by promoting glucose consumption metabolism or inhibiting hepatic gluconeogenesis since alloxan treatment causes permanent destruction of ß cells (Pari et al., 2002).

In conclusion, the results of the present study indicate that the MeOH extract exhibits interesting anti-diarrheal and antidiabetic activity which may be due to the presence of phenolic compounds and flavonoids in the extract. Now our next aim is to explore the isolation and characterization of lead compound liable for aforementioned activity from this plant.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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