Assessment of In-vivo Anti-diabetic and Anti-diarrheal Effects of *Flemingia stricta* Roxb. Leaf.

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Abstract

Objective: To evaluate the anti-diabetic and anti-diarrheal activity of methanol extract of *Flemingia stricta* Roxb. (Fabaceae) leaf.

Methods: In anti-diabetic study, the extract was administered to alloxan-induce diabetic mice at two concentrations (200mg/kg and 400mg/kg body weight) for acute (12 hours) and prolong treatments (15 days) and blood glucose levels (Blood glucose level) of diabetic mice were monitored at intervals of hours and days throughout the duration of treatment. Antidiarrheal test was conducted by castor oil induced diarrhea and enteropooling as well as intestinal motility in mice at three different concentration (100mg/kg, 200mg/kg and 400mg/kg body weight).

Results: Treatment of alloxan induce diabetic mice with the extract caused a significant reduction in fasting blood glucose level of the diabetic mice both in acute (12 hours) and prolong treatment (15 days) and it was determined that the extract at both concentration (200mg/kg and 400mg/kg) showed the significant ($P<0.05$) hypoglycemic effect in comparison to the standard drug Metformin (10mg/kg). In the case of castor oil induced diarrheal test, enteropooling test and gastrointestinal motility test, the extract of *F. stricta* at 100mg/kg, 200mg/kg and 400mg/kg has given significant effect ($P<0.05$) in comparing to standard drug Loperamide (5mg/kg).

Conclusion: These result suggested that the methanol extract of *F. stricta* Roxb. possess promising anti-diabetic effect on alloxan-induced mice and significant antidiarrheal effect on castor oil induced diarrheal mice.

Keywords: *Flemingia stricta*; Anti-diabetic; Anti-diarrheal; Alloxan; Metformin; Loperamide; Castor oil.

Introduction

Plants are the great source of medicine. Plant derived medicine has tremendous efficacy as well as safe and cost effective. Therefore, traditionally phytomedicine has been used for centuries around the world [1]. Traditional medicine has always played a pivotal role in the health care systems all over the world. In developing countries, 80% people are still depending on traditional medicine [2]. Moreover, over 25% compounds of commonly used medicines are extracted from medicinal plants [3]. From this perspective, importance of medicinal plants is inevitable. *Flemingia stricta* Roxb. is a subshrubs of fabaceae family which is widely distributed in southeast Asian country such as Bangladesh, Bhutan, China, India, Indonesia, Laos, Philippines, Thailand, Vietnam [4,5]. It is generally found in Chittagong, Chittagong Hill Tracts and Sylhet area of Bangladesh. Moreover, it is traditionally known as Charchara (in Bangla); and Krangdunaduepay, SaiKheu (Marma); Keramkana (Tripura); Tamatamaking (Khumi) and Harsanga, Khaskura, Uskura (Chakma) in local tribes of Chittagong, Bangladesh [6]. This plant species has several medicinal properties. Therefore, it is traditionally used for the treatment of various diseases such as bone fracture, cough, goiter and polio, asthma, and polio [7-9].

Diabetes is characterized by the destruction of beta cell caused by the high blood glucose level which in turn decreases or ceases the production of insulin in the human body. The number of people suffering from diabetes is increasing day by day. As reported by International Diabetes Federation (IDF), approximately 425 million people are affected by diabetes in 2017 and will reach about 642 million in 2040 [10]. According to the study of researchers, diabetic patients will be increased in near future at an alarming rate in United States, China and India [11]. In United States, more than 30 million adults and children have diabetes. Besides, another individual is diagnosed with diabetes in every 21 seconds [12]. Due to the food habit and life style, it is also a major threat to a developing country like Bangladesh. By 2030, Bangladesh will have the 7^{th} highest number of diabetic patients [13, 14].

Diarrhea is defined as abnormally loose or watery stools which generally originated by virus, bacteria or parasitic organisms. In spite of great development in pharmaceutical field and discovery of anti-diarrheal agents, diarrheal diseases are still one of leading causes of mortality and morbidity around the world which are correlated with approximately 1.3 million deaths annually [15, 16]. According to the UNICEF, diarrheal diseases are accountable for about 8 percent of all deaths among children under age 5 in 2016 which means 1300 young children are dying every day or approximately 480,000 children in a year. Due to lack of education and hygiene awareness, developing countries in Africa and Asia are more vulnerable to diarrheal diseases [17, 18].

Hence, this experiment was designed to evaluate the in vivo anti-diabetic activity and anti-diarrheal activity of *F. stricta* Roxb leaf extract against alloxan induced diabetic mice as well as castor oil induced diarrhea in mice respectively.

Materials and Methods

Plant material

F. stricta Roxb leaves were collected from a local area (Bhatiary) of Chittagong district, Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Bangladesh.

Preparation of extract

The leaves were air dried under the shade and grounded. The ground (500 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

Animals

In the present study, Swiss albino mice (male), which weighed between 35-45gm were used. The animals were collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB) and housed in polypropylene cages under controlled conditions. The animals were exposed to alternative 12:12 h light and dark cycle at an ambient temperature of 25 ± 2ºC. Animals were allowed free access to drinking water and pellet diet, collected from ICDDRB Dhaka. Mice were acclimatized for 10 days in the laboratory environment prior to the study. All animal experiments were cared for in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23 revised 1985).

Materials and Chemicals

Castor oil (BDH Chemicals, UK), normal saline solution (Beximco Infusion Ltd., Bangladesh), Metformin (Square Pharmaceuticals Ltd., Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh), Castor oil (WELL's Heath Care, Spain), were procured and used in the experiment. For the induction of diabetes, Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and used in the experiment. Glucometer kit was purchased from Accu-Check active, Roche Diagnostic GmbH, Mannheim, Germany. All chemicals in this investigation were of analytical reagent grade.

Determination of median lethal dose (LD_{50})

The LD_{50} of the extract was determined using Swiss albino mice. The extract was administered intraperitoneally (i.p.) and the method of Miller and Tainter [19] was adopted. This involved the administration of different doses of the extract (100–1000 mg/kg) to groups of five mice each. The animals were observed for physical manifestation of signs of toxicity. Administration of the methanol leaf extract of *F. stricta* (1000mg/kg) did not produce any mortality in the animals.

Anti-diabetic Activity

Induction of diabetes

The animals (male mice) were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (130 mg/kg) in ice cold 0.9% saline (NaCl) solution. The animals were given 2 mL of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycemia. Seventy two hours later, Mice with blood glucose levels (Blood glucose level) above 26mmol/dL were considered diabetic and selected for the experiment.

Evaluation of anti-diabetic activity

The animals were randomly divided into four groups with five mice in each group and treated as follows:

Group I: Diabetic mice were administered with normal saline (10ml/kg) p.o.
Group II: Diabetic mice were given Metformin (10 mg/kg b.w.) p.o.
Group III: Diabetic mice were administered orally with *F. stricta* extract (200 mg/kg b.w.).
Group IV: Diabetic mice were administered orally with *F. stricta* extract (400 mg/kg b.w.).

The change in body weight and fasting blood glucose level of all the rats were recorded at regular intervals during the experimental period. For acute study, the Blood glucose level were monitored after 1, 3, 5, 7, 10 and 12 h of administration of a single dose of the extract and at the end of 1, 3, 5, 7, 10, 15 days for prolonged treatments. The Blood glucose level were monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were recorded [20].

Anti-diarrheal activity

Anti-diarrheal activity was performed by three tests which are castor oil-induced diarrheal test, Castor oil-induced enteropooling test and lastly gastrointestinal motility test.

Castor oil-induced diarrhea in mice

Castor oil-induced diarrhea was done according to the method of Soba et al. [21] and Uddin et al [22]. Mice were divided into five groups of five mice each. The animals were fasted for 18 h prior to the test. Group I was treated with normal saline (2 mL/kg), which served as control; while Group II received Loperamide (5 mg/kg). Both groups III, IV and V received methanol extract at 100mg/kg, 200 mg/kg and 400mg/kg. All doses were administered orally. After 1 h, all groups received 1 mL of castor oil orally. Then animals were placed in cages lined with adsorbent papers and observed for 4 h for the presence of diarrhea defined as watery (wet), unformed stool. The control group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percent inhibition of defecation was calculated as follows:

% Inhibition of defecation = [(A-B)/A] ×100
Where A indicated mean number of defecation caused by castor oil; B indicated mean number of defecation caused by drug or extract.

Castor oil-induced enteropooling

The castor oil-induced enteropooling was carried out according to the method of Robert et al [23]. Mice were divided into five groups and fasted for 18 h prior to the test. Saline water was given to Group I as control (saline 2 mL/kg body weight, orally); Group II received standard drug (Loperamide 5 mg/kg body weight, orally), and the rest groups (Groups III, IV and V) were given methanol extract (at 100mg/kg, 200 mg/kg and 400mg/kg body weight, orally). One hour later, all the mice were challenged with 1 mL of castor oil orally. After 1 h of castor oil received, the mice were sacrificed and the small intestine from the pylorus to the caecum was isolated. Then the intestinal contents were weighed and volume measured by graduated tube [23, 24].

Gastrointestinal motility test

This test was performed according to the method previously described using charcoal as a diet marker [25]. Animals were divided into five groups of five mice in each and fasted for 18 h before test. All groups received castor oil to produce diarrhea. One hour later, group I treated as control (saline 2 mL/kg body weight, orally); Group II received standard drug (Loperamide 5 mg/ kg body weight, orally) and the rest three groups received three different doses (100mg/kg, 200 mg/kg and 400mg/kg body weight, orally) of *F. stricta* methanol extract. After 1 h of drug administration, all animals were received 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, all animals were sacrificed, and the distance covered by the charcoal meal in the intestine from the pylorus to the caecum was measured and expressed as percentage of distance moved [26].

Statistical analysis

The data was expressed as mean ± standard error of mean (S.E.M.). Statistical comparisons were performed using one-way ANOVA followed by post-hoc Dunnett's test with the SPSS program (SPSS 20.0, USA). The values obtained were compared with the vehicle control group and were considered statistically significant when *P< 0.05.

Result

Anti-diabetic activity

There were observable changes in the body weight of treated and untreated (control) diabetic mice. Treatment of diabetic mice with the leaf extract of *F. stricta* and Metformin improved the weight gain compared to untreated diabetic mice whereas weight of control group was decreased. **(Table-1).**

Table-1: Effect of methanol leaf extract of *F. stricta* on body weights of alloxan-induced diabetic rats.

Group	Treatment (p.o)	Day 0	Day 3	Day 7	Day 10	Day 15
I	Saline (10ml/kg)	40.72±0.60	38.92±1.00	35.19±0.74	30.96±0.71	27.82±0.65
II	Metformin (10 mg/kg)	38.79±0.52	36.96±1.01	40.49±1.01*	43.38±0.84*	44.78±0.75*
III	FSM (200mg/kg)	39.74±0.32	36.56±0.39	31.69±1.19	35.67±0.85*	42.13±1.02*
IV	FSM (400mg/kg)	44.67±0.64*	37.64±0.46	34.58±0.59	39.00±0.65*	49.12±0.74*

Values are expressed as mean±SEM (n=5). *P<0.05 when compared with control group. FSM: *F. stricta* methanol extract.

In the case of acute treatment, a dose-dependent reduction in Blood glucose level was observed in alloxan-induced diabetic mice treated with methanol leaf extract of *F. stricta*. After a single dose of the extract give to the alloxan-induced diabetic mice, there was a significant (P<0.05) reduction in Blood glucose level of the diabetic mice within the period of acute study compared to control. The maximum effect was observed at 5 h for 200mg/kg dose and 7 h for 400mg/kg dose. However, the effect of extract was found significant compared to the standard drug, Metformin (**Table-2).**

Table-2: Anti-diabetic effect of methanol leaf extract of *F. stricta* on blood glucose level of alloxan-induced diabetic study. (Acute Treatment)

Group	Treatment	Blood glucose level(mmol/dl) in hours						
		0 hour	1 hour	3 hour	5 hour	7 hour	10 hour	12 hour
I	Saline (10ml/kg)	29.32±0.82	27.50±0.45	26.76±0.42	27.24±0.58	26.66±0.80	26.46±0.41	26.22±0.42
II	Metformin (10mg/kg)	27.96±0.84	24.80±0.54*	13.22±0.32*	9.22±0.31*	7.62±0.31*	6.42±0.26*	10.06±0.30*
III	FSM (200mg/kg)	31.24±0.49	27.06±0.52	16.46±0.91*	11.52±0.68*	16.22±0.63*	23.06±0.92	27.72±0.55
IV	FSM (400mg/kg)	30.80±0.81	26.32±0.76	14.22±0.57*	9.38±0.14*	8.56±0.24*	16.58±0.49*	23.64±0.61*

Values are expressed as mean±SEM (n=5). *P<0.05 when compared with control group. FSM: *F. stricta* methanol extract.

During prolonged study (15 days), the extract produced a sustained significant (P<0.05) reduction in Blood glucose level of the diabetic mice compared to control **(Table-3).** The effects

of the highest dose of the extract were near about to the standard drug, Metformin, 10 mg/kg, on day 15.

Table-3: Effect of methanol leaf extract of *F. stricta* on blood glucose level of alloxan-induced diabetic rats during prolonged treatment. (Prolonged Treatment)

Group	Treatment	Blood glucose level(mmol/dl) in hours						
		Day 0	Day 1	Day 3	Day 5	Day 7	Day 10	Day 15
I	Saline (10ml/kg)	28.54±0.26	27.86±0.38	28.80±0.41	30.14±0.53	30.76±0.36	31.46±0.53	32.46±0.47
II	Metformin (10mg/kg)	27.90±0.43	25.20±1.01	20.96±0.77*	18.10±0.49*	14.36±0.34*	8.62±0.24*	5.50±0.24*
III	FSM (200mg/kg)	30.46±0.42*	31.38±0.65*	27.96±0.41	26.44±0.83*	21.98±0.69*	16.60±0.59*	10.98±0.26*
IV	FSM (400mg/kg)	30.40±0.51	30.96±0.68*	27.30±1.02	25.28±0.35*	19.70±0.61*	13.94±0.52*	9.08±0.22*

Values are expressed as mean±SEM (n=5). *P<0.05 when compared with control group. FSM: *F. stricta* methanol extract.

Castor oil induced diarrhea

In castor oil induced diarrhea test, methanol extract showed considerable antidiarrheal effect in mice at three doses (100mg/kg, 200mg/kg and 400mg/kg). Methanol extract significantly inhibited the frequency of defecation when compared with untreated control mice (P<0.05). Three doses of the extract decreased the total number of wet feces produced upon administration of castor oil when compared to the control (saline) rats. The results are shown in **Table 4.**

Table 4: Effect of methanol leaf extracts of *F. stricta* in castor oil induced diarrhea on mice.

Groups	Treatment (p.o)	Total number of feces	% inhibition of defecation	Total number of diarrheal feces	% inhibition of diarrhea
I	Saline(2ml/kg)	16.2±0.95	--	14.8±1.36	--
II	Loperamide (5mg/kg)	4.6±0.66	71.61*	3.2±0.59	78.38*
III	FSM (100mg/kg)	8.4±0.65	23.46*	8.4±1.07	43.24*
IV	FSM (200mg/kg)	5.2±0.86	67.91*	7.4±1.07	50.00*
V	FSM (400mg/kg)	5±0.58	69.14*	5.2±1.16	64.86*

Values are expressed as mean±SEM (n=5). *P<0.05 when compared with control group. FSM: *F. stricta* methanol extract.

Castor oil induced enteropooling

The plant extract at three different doses showed noticeable effect in castor oil induced enteropooling test in the mice (**Table 5).** The intestinal volume was decreased by 17.69%, 20.99% and 38.44% for three different doses (100mg/kg, 200mg/kg and 400mg/kg) of methanol leaf extract. The values were statistically significant (P<0.05). The standard drug, Loperamide (5mg/kg), also significantly inhibited intestinal fluid accumulation (49.29%) (P<0.05) and the effect of doses of the extract was near about in comparison to the standard drug.

Table 5.Effect of methanol leaf extracts of *F. stricta* on castor oil induced enteropooling in mice.

Groups	Treatment (p.o)	Weight of Intestinal content (g)	% of inhibition	Volume of intestinal content (ml)	% of inhibition
I	Saline(2ml/kg)	1.63±0.12	--	0.84±0.04	--
II	Loperamide (5mg/kg)	0.61±0.07	62.55*	0.43±0.03	49.29*
III	FSM (100mg/kg)	1.03±0.02	37.21*	0.69±0.03	17.69*
IV	FSM (200mg/kg)	0.84±0.03	48.59*	0.67±0.02	20.99*
V	FSM (400mg/kg)	0.72±0.02	54.93*	0.52±0.02	38.44*

Values are expressed as mean±SEM (n=5). *P<0.05 when compared with control group. FSM: *F. stricta* methanol extract.

Gastrointestinal motility test

The gastrointestinal distance traveled by the charcoal meal in the mice significantly (P<0.05) lessened by three doses (100mg/kg, 200mg/kg and 400mg/kg) of methanol leaf extract compared with the control group. Loperamide (5 mg/kg) produced a marked decrease (45.91%) in the propulsion of charcoal meal through gastrointestinal tract.

Table 6. Effect of methanol leaf extracts of *F. stricta* on small intestinal transit in mice

Groups	Treatment (p.o)	Total length of Intestinal (cm)	Distance travel by marker (cm)	% of Inhibition
I	Saline(2ml/kg)	57.13±0.50	50.78±1.03	--
II	Loperamide (5mg/kg)	57.12±0.58	27.47±0.70	45.91*
III	FSM (100mg/kg)	53.98±0.61	46.20±1.28	9.03
IV	FSM (200mg/kg)	53.02±0.42	39.20±0.86	22.81*
V	FSM (400mg/kg)	55.26±0.32	32.40±0.92	36.20*

Values are expressed as mean±SEM (n=5). *P<0.05 when compared with control group. FSM: *F. stricta* methanol extract.

Discussion

The anti-diabetic activity of *F. stricta* leaf extract was carried out in alloxan induced diabetic mice. The methanolic leaf extract was demonstrated significant anti-diabetic activity in alloxan induced diabetic mice. There are a lot of reports implicating phytochemical compounds in plants as being responsible for their anti-diabetic activities [27-29]. Perhaps, presences of some phytochemical constituents are responsible for the observed significant activity of this plant extract either singly or in synergy, like standard drug Metformin or other anti-diabetic agents are effective in diabetic state and ineffective in severe diabetic state where pancreatic β-cells are completely destroyed [30]. The observed reduction in blood glucose levels of the diabetic mice by Metformin in this study displayed significant anti-diabetic activity as a standard drug. In addition, acute and prolong treatment with the methanol leaf extract of *F. stricta* for a period of 12 hours and 15 days respectively caused significant decrease in blood glucose levels of treated mice compared to untreated (control) diabetic mice.

Diarrhea is usually considered as a result of altered motility and fluid accumulation within the intestinal tract. The objective of diarrheal test was to determine the effect of methanol extract of *F. stricta* on castor oil induced diarrhea. Castor oil is a triglyceride characterized by a high content of the hydroxylated unsaturated fatty acid ricinoleic acid. About 90% of ricinoleate present in castor oil is mainly responsible for diarrhea production [31,32]. After oral ingestion of castor oil, ricinoleic acid is released by lipases in the intestinal lumen, and considerable amounts of ricinoleic acid are absorbed in the intestine [33,34]. Presence of ricinoleate in small intestine, the peristaltic activity of small intestine increases as a result of permeability of Na+ and Cl- changed in the intestinal mucosa. Secretion of endogenous prostaglandin is also stimulated by ricinoleate [35,36]. Prostaglandins of the E series are considered to be good diarrheogenic agents in experimental animals as well as in human beings. The inhibitors of prostaglandin biosynthesis are therefore considered to delay castor oil-induced diarrhea [37]. Many anti-diarrheal agents act by reducing the gastrointestinal motility and/or the secretions. In this experiment, the methanol extract of *F. stricta* exhibits significant ($P<0.05$) anti-diarrheal activity. The given effects of this plant extract at three different doses were noticeable considering with the standard drug, Loperamide at 5 mg/kg. There are many reports on anti-diarrheal activity of plant extract using this dose level [38]. Plant extract significantly reduced intestinal transit by the decrease in the distance traveled by charcoal meal.

From the above result, we found the extracts suppressed the propulsion of charcoal meal by increasing the absorption of water and electrolytes. From this point of view we can suggest that, *F. stricta* displays promising anti-diabetic and anti-diarrheal effects which can be a potential source of biologically important drug candidates.

Conclusion

In conclusion, the results of this experiment exhibits that methanol leaf extract of *F. stricta* possess significant anti-diabetic and anti-diarrheal properties. Hence, further investigations are required in order to elucidate the mechanism of this leaf extract.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

Authors wish to thank Botanist Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Bangladesh, who helped to identify the plant. We would like to express our gratitude to the authority of International Centre for Diarrheal Disease and Research, Bangladesh (ICDDRB) for providing the experimental mice. The authors are grateful to the Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh, for providing research facilities.

Funding

No fund was available.

Ethical approval

The set of rules followed for animal experiment were approved by the institutional animal ethics committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh according to governmental guidelines. Pharmacy P&D 59/04-10.

Consent for publication

All authors read and approved the final content of this manuscript for publication.

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