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Evaluation of Biological Activities of Methanolic Extract of Leaves of *Bruguiera gymnorhiza* (L.) Lam.: *In vivo* Studies using Swiss Albino Mice Model

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Abstract

Leaves of *Bruguiera gymnorhiza* (L.) Lam. are very popular traditional remedies used by tribal people of Bangladesh near to Sundarbans. The purpose of this work was to assess the pharmacological attributes of methanolic extract of leaves of *B. gymnorrhiza* employing mice model. Using the well-known tail tipping method, the *in vivo* glucose-lowering ability of methanolic extract of leaves of *B. gymnorrhiza* was investigated. The writhing experiment was performed to determine peripheral analgesic activity. The anti-diarrheal activity was determined utilizing the castor oil-induced diarrhea in the mice model. In consequence, oral treatment of the extract at 400 mg/kg b.w. dose caused significant suppression of acetic acid-induced writhing (58.33%). The findings were comparable to the corresponding standard diclofenac (50 mg/kg b.w.) that exhibited 86.67% inhibition of abdominal writhing. Besides, after 180 min of oral ingestion (400 mg/kg) of the extract, the maximum reduction in blood glucose (38.46%) was found, compared to standard glibenclamide (73.67%). Furthermore, when compared to conventional loperamide (65.06%), there was a 40.02% reduction in diarrheal feces at 400 mg/kg b.w. dose. The methanolic extract of leaves of *B. gymnorrhiza* has strong peripheral analgesic and anti-diarrheal efficacy, as well as modest glucose-lowering effect, according to these *in vivo* bioassays. Further studies are still recommended to isolate bioactive molecules in order to develop novel drug moieties.

Key words: *Bruguiera gymnorhiza*, antihyperglycemic, analgesic, antidiarrheal.

Introduction

From ancient times, plants have been utilized as a source of medicines to treat a variety of illnesses. Before the development of modern medicines, people anticipate the healing properties of medicinal plants (Alam *et al.*, 2020). Though a large quantity of synthetic medicines has been commercialized to treat illness, they are plagued with several serious side effects. On the contrary, plant-derived medicines have fewer side effects with more therapeutic effects (Emon *et al.*, 2020). Different parts of the plants with distinct medicinal value have been utilized by several medicinal practices like Ayurveda, Unani, traditional Chinese medicines, traditional Persian medicines etc. (Alam *et al.*, 2021). Medicinal plants are currently gaining a lot of attention because of their unique properties as a rich source of bioactive phytochemicals that could lead to the development of new medications (Emon *et al.*, 2021). Besides, the use of plants as medicine has been proven as a safer option as they are blessed with very few adverse effects. The widespread availability and inexpensive cost of these plants have led to their folkloric use among people (Rudra e*t al*., 2020). According to the

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World Health Organization, 80% of people around the world use herbal medicines as some component of their primary health care. Plant medications are expected to account for up to 25% of total drugs in established countries such as the United States, while they account for up to 80% of total drugs in fast-developing countries such as India and China (Ekor, 2014). Even in the modern era, plants remain a prospective medicinal source for pain, oxidative stress, cancer, diarrhea, depression, fever, and thrombosis, as well as other life-threatening disorders (Emon *et al.*, 2021; Ekor, 2014).

Bruguiera gymnorhiza (L.) Lam. (Family: Rhizophoraceae) is a small evergreen mangrove tree and is locally named "Kakra". This plant is commonly found in tropical and subtropical coastal regions growing to a height of 9-10 m. In Bangladesh, it is mostly seen in the Sundarbans. The leaves of *B. gymnorrhiza* are frequently used to cure diarrhea, diabetes, fever, liver disorders and intestinal worms (Uddin *et al.*, 2011). Only a few pieces of researches have been reported on *B. gymnorrhiza*, the medicinal plant of the Sundarbans to explore its pharmacological potential. Therefore, the current study was carried out as a part of research on bioactivity screening of the leaves of this medicinal plant in order to scientifically evaluate its antioxidant, analgesic and antidiarrheal activities.

Materials and Methods

Collection, drying, and extraction: The leaves of *B. gymnorrhiza* were collected from Khulna, Bangladesh. After collection, the plant samples were sun-dried for 20 days, and afterward, stove dried for 25 h at impressively low temperature (not more than 40 ^{o}C). The dried natural products were subsequently pulverized into a coarse powder in the phytochemical research laboratory of the State University of Bangladesh using a high-limit granulating machine. The powdered material (300 g) was taken in a cleaned, golden shading reagent bottle (2.5 L) and soaked in 2.0 L of methanol for 15 days with infrequent shaking. The entire blends were then sieved through a new cotton plug, lastly with a Whatman No.1 paper. The volume of the filtrate was then permitted to evaporate at the surrounding temperature until around 70% dissolvable was dissipated (Miah *et al*., 2018).

Study animals: Swiss Albino male mice weighing between 25-35 g and age between 4-5 weeks old were taken from Jahangirnagar University. The mice were housed in the animal house of State University of Bangladesh and fed standard food in a closely monitored environment. Natural changes were deliberately observed and preceding any test, the mice were permitted (4 days) to adapt to the new ecological conditions. The Federation of European Laboratory Animal Science Associations (FELASA) rules and proposals were followed to lessen the agony and worry of the exploratory mice. About the *in vivo* bioassays, the mice were isolated into four groups (Group I, II, III, and IV) of 3 animals in each group. The initial two groups (I and II) were filled in as the negative and positive control, while groups III and IV took care of 200-and 400 mg/Kg b.w. of unrefined concentrate (Kayser *et al*., 2019).

Drugs and chemicals: Glibenclamide, loperamide, diclofenac sodium and acetylsalicylic acid were the products of Beximco Pharmaceutical Ltd., Bangladesh. Morphine injections were taken from the Ganashasthaya hospital, Dhanmondi. The analytical quality of all the reagents was ensured.

Data analysis: All the findings were documented in triplicates (n=3) and those results were expressed as the mean ± standard error mean (SEM). One-way ANOVA and Post Hoc Dunnett's test were used in SPSS version 25.0 to examine the significance of variables between groups where the p values < 0.05 were considered to be statistically significant.

Evaluation of antihyperglycemic activity: Using the well-known tail tipping method, the *in vivo* glucose-lowering ability of the methanolic extract was investigated (Dürschlag *et al*., 1996). After a short-term fasting period, four groups of animals (n=3) were treated with 200 and 400 mg of methanolic extract orally, 5 mg glibenclamide, 1% Tween-80 with saline solution, per kg body weight individually. After 30 min of extract or medication

administration, all mice were given either a 10% glucose solution (2 g/kg b.w.) orally based on their assigned group, and blood glucose levels were monitored for 30 min, 60 min, 120 min, and 180 min.

The equation to calculate % reduction of blood glucose level is given below:

$$\%\ \text{Reduction of blood glucose level} = \frac{\text{Mean blood glucose level (control)} - \text{Mean blood glucose level (test)}}{\text{Mean blood glucose level (control)}} \times 100\%$$

Evaluation of peripheral analgesic activity: The acetic acid-induced writhing approach proposed by Kabir *et al.* (2021) was used to evaluate peripheral analgesic response employing intra-peritoneal acetic acid (0.1 ml) as the source of pain sensation (Kabir *et al.*, 2021). The control group received (n=3) 1% Tween 80 in saline, and the standard group (n=3) was provided diclofenac sodium (5 mg/kg b.w.), while the two test groups (n=3, each) received two different doses of methanolic extract individually (200 mg/kg or 400 mg/kg). The percent inhibition of writhing was calculated using the formula mentioned below:

$$\%\ \text{Inhibition of writhing} = \frac{\text{Mean no. of writhing (control)} - \text{Mean no. of writhing (test)}}{\text{Mean no. of writhing (control)}} \times 100\%$$

Evaluation of castor oil induced antidiarrheal activity: The antidiarrheal activity of the methanolic extract of leaves of *B. gymnorrhiza* was evaluated using the method of castor oil-induced diarrhea in mice (Saha and Paul, 2012). Each mouse was given 1.0 ml of a highly pure analytical grade castor oil to induce diarrhea. For each mouse, the number of fecal stools was counted. The anti-diarrheal activity of the samples was assessed by comparing the observations of the experimental and standard groups to those of the control group (n=3 each). The control group received an oral intake of 10 ml/kg of 1% tween 80 while the standard group received an oral intake of 2 mg/kg loperamide. Two sets of experimental groups labeled as MESF 200 and MESF 400 received methanolic extracts of 200 mg/kg and 400 mg/kg b.w., respectively. The result was counted after 4 h.

$$\%\ \text{Inhibition of number of diarrheal feces} = \frac{\text{Mean no. of of diarrheal feces (control)} - \text{Mean no. of of diarrheal feces (test)}}{\text{Mean no. of of diarrheal feces (control)}} \times 100\%$$

Results and Discussion

Antihyperglycemic activity analysis: Both glibenclamide (5 mg/kg) and methanolic extract of leaves of *B. gymnorrhiza* at 400 mg/kg b.w. dose exhibited a noteworthy effect after 180 min. However, after 180 min, the MESF at 400 mg/kg resulted in a maximal reduction of 38.46% in blood glucose, whereas at the same time glibenclamide showed a reduction in blood glucose by 73.67% during the same time laps. The results are displayed in table 1.

Peripheral analgesic activity analysis: The methanolic extract of leaves of *B. gymnorrhiza* also conveyed notable peripheral analgesia comparable to the standard diclofenac sodium. The extract at a dose of 400 mg/Kg b.w. inhibited acetic acid-induced writhing response by 58.33% ($p<0.001$), in where 86.67% reduction ($p<0.001$) was observed for the standard. The results are displayed in table 2.

Antidiarrheal activity analysis: The methanolic extract of leaves of *B. gymnorrhiza* exhibited a significant reduction of average diarrheal feces at a dose of 400 mg/kg ($p<0.0001$) and the results were

comparable to the standard antidiarrheal drug loperamide (2 mg/kg orally). The % inhibitions of diarrheal feces are 65.06%, 20.08% and 40.02% respectively for standard, MESF 200 and MESF 400. The results are displayed in figure 1.

Table 1. Effect of different doses of methanolic extract of leaves of *B. gymnorrhiza* on glucose-induced hyperglycemia in Swiss albino mice.

Test groups	Before treatment	After treatment				% Inhibition			
	0 min	30 min	60 min	120 min	180 min	30 min	60 min	120 min	180 min
Control	4.5±0.43	17.89±0.18	13.45±0.16	10.24±0.34	6.76±0.48				
Standard (5 mg/kg)	3.7±0.35	14.68±0.24	6.78±0.45**	3.16±0.46***	1.78±0.73***	17.94%	49.59%	69.14%	73.67%
MESF 200	4.8±0.26	16.49±0.38	12.18±0.46	8.87±0.19*	5.46±0.46*	7.83%	9.44%	13.38%	19.23%
MESF 400	5.1±0.34	15.78±0.16	11.56±0.27*	8.46±0.37*	4.16±0.15*	11.79%	14.05%	17.38%	38.46%

Values are expressed as Mean ± SEM (n=3); ***p<0.001, **p< 0.01, *p< 0.05 compared to negative control.

Table 2. Effect of methanolic extract of leaves of *B. gymnorrhiza* in the acetic acid-induced writhing test in Swiss albino mice.

Test groups	Writhing count			Number of writhing Mean ± SEM	% Writhing	% Inhibition of writhing
	M1	M2	M3			
Control (1% Tween-80)	21	19	20	20.00±0.58	100	-
Diclofenac sodium (50 mg/kg)	2	3	3	2.67±0.67***	13.33	86.67
MESF (200 mg/kg)	13	13	14	13.33±0.67**	66.67	33.33
MESF (400 mg/kg)	7	9	9	8.33±0.88**	41.67	58.33

Values are expressed as mean ± SEM (n=3); ***p<0.001, **p<0.01, *p<0.05 compared to negative control. M-1, M-2, M-3 = Mice 1, Mice 2, Mice 3, respectively.

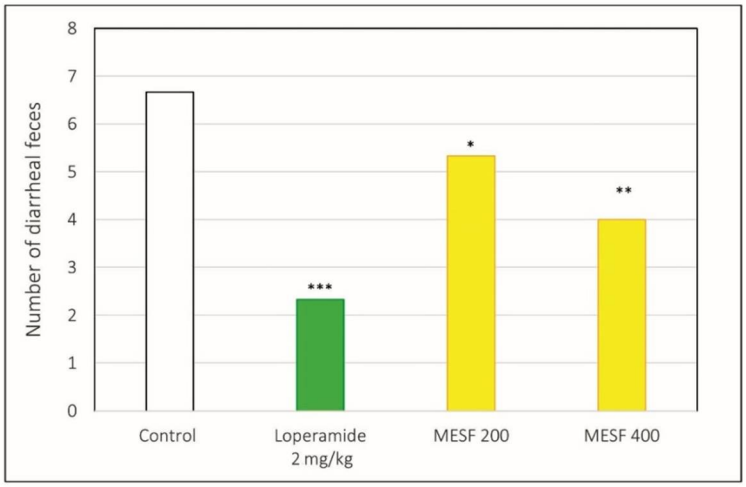

Figure 1. Effect of methanolic extract of leaves of *B. gymnorrhiza* in the castor oil diarrhea in Swiss albino mice. Values are expressed as Mean ± SEM (n=3); ***p<0.001, **p< 0.01, *p< 0.05 compared to control compared to negative control.

The methanolic extract of leaves of *B. gymnorrhiza* demonstrated a moderate reduction of blood glucose after glucose-induced hyperglycemia in Swiss albino mice. Increased production of insulin by the β-cells in the pancreas and expanded take-up of glucose by liver and muscle tissues are the two basic phenomena that could decrease sugar fixation in the blood (Michael *et al*., 2010). Phytoconstituents, for

example, alkaloids are characteristically hypoglycemic while flavonoids have been found to upgrade peripheral glucose take-up and cell glycolysis (Zheng *et al*., 2012; Brahmachari, 2011). Also, saponins were found to animate pancreatic *β*-cells in this way expanding insulin fixation in the blood (Hu *et al.,* 2014). Different heterocyclic compounds like indole, alkaloids (isocodonocarpine), flavonoids, and triterpenoids have been accounted for to be found richly in the different types of Rhizophoraceae family. In diabetic rats, the extract increased glucokinase activity, which in turn altered glycogen metabolism (Adisakwattana *et al*., 2005). Hence, the antihyperglycemic action of the *B. gymnorrhiza* could be ascribed due to the nearness of its phytochemicals.

On the other hand, the acetic acid-induced 'writhing' response for determining peripheral analgesia has been associated with the release of prostaglandins in the periphery *via* the COX pathway (Ahmed *et al*., 2006). The methanolic extract of leaves of *B. gymnorrhiza* was found to significantly ($p<0.001$) diminish the writhing response and therefore might contain phytochemicals interfering with the peripheral prostaglandin synthesis pathway (Ferdous *et al*., 2008). Phytochemicals like flavonoids have been additionally responded to target prostaglandin production which is involved in pyrexia (Narayana *et al*., 2001). In like manner, a few alkaloids and tannins are being connected with blocking pain and against nociceptive movement (Rao *et al*., 1998; Uche and Aprioku, 2008; Ramprasath *et al*., 2006). Hence, prospective phytochemicals from leaves of *B. gymnorrhiza* can be driving forces behind its usage as a pain reliever and can be considered as very prospective lead compounds to discover and develop novel therapeutics against pain-associated discomforts.

The antidiarrheal action of methanolic extract of leaves of *B. gymnorrhiza* has been accounted for without precedent for this investigation. This action means that its utilization in different stomach-related issues like cramps, constipation, indigestions, and so forth. One of the different mechanisms by which castor oil initiates diarrhea is electrolyte permeability *via* endogenous prostaglandin secretion. One of the expected explanation by which the crude extracts shows their antidiarrheal action by restraining prostaglandin secretion in the digestive tract is apparent by the way that the defecation after treatment were somewhat dry (Razan *et al*., 2016).

Conclusion

The present study revealed the antihyperglycemic, analgesic, and antidiarrheal effects of methanolic extract of leaves of *B. gymnorhiza.* Analgesic properties of this plant showed significant results which support the use of the plant leaves during chest pain by tribal people. Moreover, the antidiarrheal activity of the plant extract is noteworthy. Antihyperglycemic test also showed mild activity and thus consideration of bioactive extract of this plant leaves identified by the present study might be worthy in this manner.

Conflict of interest

The authors declare that they have no conflict of interest.

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