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EVALUATION OF ANTI-DIARRHEAL, CENTRAL NERVOUS SYSTEM (CNS), HYPOGLYCEMIC AND THROMBOLYTIC ACTIVITIES OF METHANOLIC EXTRACT OF *TERMINALIA CITRINA* LEAVES

Narhari Das*, Md. Sharif Hasan, Zobaer Al Mahmud and Sheikh Zahir Raihan

Department of Clinical Pharmacy & Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

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***Corresponding Author**
Narhari Das
Department of Clinical Pharmacy & Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

ABSTRACT

The present study was designed to investigate anti-diarrheal, central nervous system (CNS) depressant, hypoglycemic and thrombolytic effects of methanolic extracts of *Terminalia citrina* leaves. The anti-diarrheal activity was evaluated by castor oil induced method while central nervous system depressant activity and hypoglycemic activity were evaluated by phenobarbitone induced sleeping time method and by blood glucose level test and thrombolytic activity was evaluated using *in vitro* clot lysis model. The extract of *T. citrina* significantly ($p<0.01$) and dose-dependently reduced the percentage of diarrhea with 25.48% at the dose of 400 mg/kg body weight and didn't significantly prolong the phenobarbitone induced sleeping time. In a hypoglycemic evaluation of extract showed significantly ($p<0.01$) at 400 mg/kg body weight and showed significantly ($p<0.01$) having 10.74±1.27% clot lysis. The study has shown the extract of *T. citrina* exhibited high hypoglycemic properties, low anti-diarrheal and thrombolytic effect and no central nervous system depressant effect.

KEYWORDS: *Terminalia citrina*, Anti-diarrheal, Central nervous system (CNS) depressant, Hypoglycemic, Thrombolytic.

INTRODUCTION

Terminalia citrina (Bengali name: Haritaki, Family: Combretaceae) is a deciduous tree wide spread throughout the forest of Gazipur, Tangail, Sylhet, Chittagong, Rangamati and

Chittagong hill tracts of Bangladesh. Different parts of the plant are used for various ailments. The fruit is used in long-term fever, loss of appetite and as a sexual stimulant in Bangladesh;[1] diarrhea, helminths and other digestive disorders in Iran.[2] The leaves possess antinociceptive, anti-inflammatory, anxiolytic and anthelmintic activities.[3, 4] Its bark is diuretic and cardio tonic.[5] The Seed is used in stomach aches and intestinal diseases.[6] The plant is also used in asthma, diarrhea, boils, burns, constipation, migraine, dental disease, haemoptysis, dizziness, bleeding hemorrhoids, eye disease, gastric hyperacidity, anemia, arthritis, hoarse voice, dysentery, pyrexia, infections, traumatic cuts, cardiac diseases, cough, hepatomegaly, urolithiasis and for life longevity in Myanmar.[7] A detailed literature survey revealed that leaves of plant was reported to possess antioxidant properties[8] and 13 new furofuran lignin glucosides, terminalosides A−K (1−4, 6−12), 2-epiterminaloside D (5) and 6-epiterminaloside K (13), were characterized using various spectroscopic techniques having antiestrogenic activity.[9]

However, no detailed pharmacological study has been found in the literature. Therefore in the present investigation, we aimed to investigate the activity of anti-diarrheal, central nervous system (CNS) depressant, hypoglycemic and thrombolytic effects of methanolic extracts of *Terminalia citrina* leaves.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of *Terminalia citrina* were collected from Rangamati district, Bangladesh during the month of January 2013. The plants were mounted on paper and the sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession Number-38094).

Preparation of Methanolic Extract

The leaves of the plant were collected in fresh condition. The sun dried and coarse powder (1000 g) was extracted with methanol (4.0 L) in an air-tight flat bottomed container for 15 days at room temperature with occasional stirring. The extract was then filtered through a cotton plug followed by a Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40^{o}c temperature and with pressure of 25 bar to afford crude methanolic extract (50 g).

Chemicals and reagents

Pure Phenobarbitone sodium and Normal saline water were obtained from Incepta Pharmaceuticals and Beximco Pharmaceuticals Ltd, Bangladesh. Diazepam 10mg/2mL ampoule and Metformin were obtained from Square Pharmaceuticals Ltd, Bangladesh. DMSO was obtained from Merck, Germany. All other chemicals used were analytical grade.

Animals

Swiss-albino mice (20-25 g) of either sex, aged 4-5 weeks obtained from the Animal Resource Branch of Jahangirnagar University, Dhaka, Bangladesh were used for the experiment. They were kept in standard environmental condition and fed standard formulated rodent food and water. All experimental protocols were approved by Dhaka University, Faculty of Pharmacy Ethics Committee.

Phytochemical screening

The preliminary phytochemical tests were carried out according to the methods reported by Das.[4]

Acute toxicity test

The acute toxicity of *T. citrina* methanolic extract was determined in mice with a slight modification according to the method.[10] Thirty mice fasted for 12 h were randomly divided into five groups of six mice per group. Graded doses of the extract (100, 250, 500, 750 and 1000 mg/ kg p.o.) were separately administered to each group of mice once a day by means of bulbed steel needle. All mice were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period was recorded.

Castor oil-induced diarrhea

The method described with slight modification by Shoba and Thomas.[11] The animals were divided into control, positive control and test groups containing six mice in each group and fasted for 12 h. The first group received 1% Tween 80 in water as control. The second group received loperamide at a dose (50 mg/kg) orally as standard drug. The test groups received the extract at the doses of 200 and 400 mg/kg orally. After 30 min of above treatment, all the animals were orally administrated with 1 mL of castor oil orally and the watery fecal material and number of defecation was noted up to 4 h in the transparent metabolic cages with filter paper at the base.

Central nervous system (CNS) depressant activity

The methanolic extract of the leaves of *T. citrina* was assessed for an effect on the central nervous system (CNS) depressant using phenobarbitone-induced sleeping time test in mice.[12] Thirty min after the oral administration of crude methanolic extract at the doses 200 and 400 mg/kg, vehicle control (1% Tween-80 solution in saline, 0.1 mL/10g) and intraperitoneal injection of diazepam (1 mg/kg), all mice were injected with phenobarbitone (25 mg/kg). The animals were observed for the latent period (time between phenobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex).

Hypoglycemic activity

The test is performed using a slight modification.[13] The animals were fasted 12 h, weighed and randomly divided into four groups consisting of 6 mice in each group. At zero hour fasting blood glucose level from each group was measured from tail vein just prior to glucose administration by using Glucometer (Ez Smart-168) and Glucose oxidase-peroxidase reactive strips. To measure the blood glucose level, a tail tip of mice was cut with a sharp blade and a then little amount of blood was collected and exposed to the touch of glucose test strips. Within seconds, blood glucose level was visualized. Nebanol (Bacitracin) ointment was applied on the wound to avoid infection. Then, the samples were administered (0.1% saline for control, metformin for standard and crude extracts) using oral feeding needle. After 1 h, 2 h and 3 h, blood was collected in the same procedure and blood glucose level measured to see the hypoglycemic effect of the test sample in relative to control and standard groups.

Thrombolytic activity

In vitro thrombolytic potential of methanolic extract was evaluated with the method developed by Daginawala using streptokinase as the standard substance.[14] A total of 5 mL venous blood was drawn from ten healthy volunteers without a history of oral contraceptive or anticoagulant therapy. Blood from each volunteer was distributed in ten different pre-weighed sterile micro centrifuge tubes and incubated at 37°C for 45 min. After clot formation, serum was completely aspirated out without disturbing the clot formed and the weight of clot in each tube was measured. To each micro centrifuge tube containing pre-weighed clot, 100 µL aqueous solutions of extracts with the concentration of 10 mg/mL was added separately. Then, 100 µL of streptokinase (SK) and 100 µL of distilled water were separately added to the control tube as positive and negative controls, respectively. All the

tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, the released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (weight of released clot /clot weight) × 100

Statistical analysis

All values were expressed as the mean ± standard error of the mean (SEM) and the results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's t-test by using SPSS software Version (16.0) $P < 0.05$ compared to standard was considered to be statistically significant.

RESULTS

Phytochemical screening

The phytochemical screening test showed the presence of alkaloids, flavonoids, tannins, reducing sugar and carbohydrates in the leaves of *T. citrina*.

Acute toxicity test

In acute toxicity study, oral administration of graded doses (100, 250, 500, 750 and 1000 mg/kg p.o.) of the methanol extract of *T. citrina* to mice showed no significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group at 48 h after administration. *T. citrina* was safe up to a dose level of 1000 mg/kg body weight.

Anti-diarrheal activity study

The result of methanolic extract of *T. citrina* leaves on castor-oil induced diarrhea is shown in Table 1. Here, the methanolic extract at the dose of 200 and 400 mg/kg body weight, exhibited reduction of diarrhoeal feces by 16.02% and 25.48% ($p<0.01$), respectively compared to the reduction obtained by the standard drug loperamide (73.62% feces reduction).

Table 1: Effect of methanol extract of *T. citrina* leaves on castor oil (1ml/mice) induced diarrhea in mice.

Treatment	Dose(b.w.)	No. of diarrhoeal feces (Mean ± SEM)	Reduction of diarrhea (%)
Control (Saline)	10 ml/kg	17.66 ± 0.80	---
Standard (Loperamide)	50 mg/kg	4.66 ± 0.61**	73.61
Methanolic extract	200 mg/kg	14.83 ± 0.94	16.02
Methanolic extract	400 mg/kg	13.16 ± 0.79*	25.48

Values are expressed as Mean ± SEM (n=6). **p < 0.001, *p<0.01 compared to control (one-way ANOVA followed by Dunnett's test).

Central nervous system (CNS) depressant activity

In Phenobarbitone induced sleeping time test, the extract at a dose of 200 mg/kg and 400 mg/kg don't significantly prolonged the duration of sleeping time in test animals as compared to control. The overall result of central nervous system (CNS) depressant property of *T. citrina* is shown in Table 2.

Table 2: Effect of methanol crude extract of *T. citrina* on phenobarbitone sodium–induced sleeping time in mice.

Treatment	Dose(b.w.)	Onset of sleep (min)	Duration of sleep (min)
Control (Saline)	1% Tween-80 p.o.	16.83 ± 0.90	35.83 ± 1.49
Standard (Diazepam)	1 mg/kg i.p.	5.00 ± 0.36*	100.33 ± 2.23*
Methanolic extract	200 mg/kg p.o.	17.83 ± 0.65	32.66 ± 1.08
Methanolic extract	400 mg/kg p.o.	16.16 ± 0.70	30.33 ± 1.02

Each value represents the mean ± SEM (n=6). *p<0.05 compared with control (One way ANOVA followed by Dunnett's test).

Hypoglycemic activity

The results found from extracts of *T. citrina* were significant blood glucose lowering activity is shown in Table 3. The test was performed by taking the samples at the doses of 200 mg/kg and 400 mg/kg body weight. After 1 hour, the plasma glucose level at doses 200 mg/kg and 400 mg/kg were 5.00 ± 0.33 mmol/L (p<0.05) and 4.65 ± 0.25 mmol/L (p<0.01).

Table 3: Effects of extracts of *T. citrina* on blood glucose level induced in mice.

Group	Level of glucose (Mean ± SEM)			
	0 hour	1 hour	2 hour	3 hour
Control	5.98 ± 0.28	6 ± 0.15	5.9 ± 0.19	5.75 ± 0.17
Standard (Metformin)	6.05 ± 0.18	3.76 ± 0.13***	3.6 ± 0.15***	3.43 ± 0.11***
Methanol extract (200 mg/kg)	6.13 ± 0.22	5 ± 0.33*	4.71 ± 0.29**	4.43 ± 0.24***
Methanol extract (400 mg/kg)	6.06 ± 0.25	4.65 ± 0.25**	4.31 ± 0.21***	3.96 ± 0.11***

Each value represents the mean ± SEM (n=6). ***p<0.001, **p<0.01, *p<0.05 compared with control (One way ANOVA followed by Dunnett's test).

Thrombolytic activity

The thrombolytic activity of methanol extract of *T. citrina* had been evaluated. The addition of 100 µL streptokinase (SK), a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 62.69% clot lysis. Clots when treated with 100 µL sterile distilled water (negative control) showed only negligible clot lysis (3.63%). The mean difference in clot lysis percentage between positive and negative control was significant. After treatment of clots with 100 µL of the extract showed mild clot lysis (10.74%) occurred when compared with the negative control (water) as shown in Table 4.

Table 4: Effect of methanol extract of *T. citrina* on *in vitro* clot lysis.

Extract/ Drug	% clot lysis (Mean ± SEM)	P value (one way ANOVA followed by Dunnett's t-test) when compared to negative control(water)
Distilled water	3.63 ± 0.49	-------
Streptokinase	62.69 ± 1.73	<0.001
Methanolic extract	10.74 ± 1.27	<0.01

Each value is represented as mean ± S.E.M (n=10) and p< 0.001 was considered as highly significant.

DISCUSSION

The use of herbal medicine is a common practice in many countries, particularly in Asia[15] and Africa.[16] To search potent and better alternative drugs is the quest of science and scientists. Like the past still plant origin is a vast unrevealed source to conduct these assiduous researches. Focusing the demand of natural, effective anti-diarrheal, central nervous system (CNS) depressant, hypoglycemic and thrombolytic drugs of plant origin the current study was performed.[17]

In evaluation of anti-diarrhoeal activity by castor oil method, methanolic crude extract dose-dependently reduced diarrhoeal feces which were comparable to that obtained by the standard loperamide. Castor oil causes diarrhea due to its active metabolite, ricinoleic acid, which stimulates peristaltic activity in the small intestine, leading to changes in electrolyte permeability of the intestinal mucosa.[18, 19] The liberated ricinoleic acid also causes irritation

and inflammation of the intestinal mucosa leading to the release of endogenous prostaglandins.[20]

Several other mechanism have been reported to cause diarrhea by castor oil inducing inhibition of intestinal Na^+/K^+-ATPase activity, activation of adenylate cyclase or mucosal cAMP- mediated active secretion and platelet activating factor.[21] Diarrhea also results from an active intestinal secretion driven predominantly by a net secretion of sodium and potassium. Therefore, the decrease in the wetness of feces and the frequency of defecation observed with the extract prove the moderate anti-diarrhoeal activity of *T. citrina* leaves and this effect might be due to inhibition of prostaglandins biosynthesis.

The results of the hypoglycemic test indicated that the extracts suppressed the rise in blood glucose level after administration of the extract. The outcome may be due to enhancement of gluconeogenesis, which is characteristically activated at fasting state in diabetes animals or increase the disposal of glucose by enhanced insulin sensitivity.[22] The extracts of *T. citrina* reduced the blood glucose level. As β-cells were destroyed, it is clear that the observed hypoglycemic effect may be due to a potential secretion of insulin from the few existing β-cells or there is any extrapancreatic mechanism. It is also possible that increase peripheral glucose utilization helped in blood glucose reduction.[23] Significant percent glycemic reductions in fasting blood glucose level of all test groups were observed. The Significance of p value ($p<0.05$) was obtained for plant extract groups.

This study also displays the *in vitro* thrombolytic potential of crude methanolic extract of *T. citrina* leaves using human blood. The test model used is a newly developed cheap and simple technique which can be performed with limited facilities available in countries like Bangladesh. But this technique is validated, sensitive and reliable. The results show mild thrombolytic activity. This is an important finding which may have important implications in cardiovascular health. In addition, this finding may indicate the possibility of developing novel thrombolytic agent/s from the leaves of the plant. Plasmin, a natural fibrinolytic agent, lyses clot by breaking down the fibrinogen and fibrin contained in a clot. Streptokinase forms a 1:1 stoichiometric complex with plasminogen that can convert additional plasminogen to plasmin.[24] Moreover, phlorotannin, isolated from to marine brown algae, have a unique property of dissolution of intravascular blood clot via antiplasmin inhibition.[25] Since, phytochemical analysis showed that the crude extract of *T. citrina* contains tannin, alkaloid

and reducing sugar the clot lysis activity showed by the extract was due to the presence of these phytoconstituents which might form complexes with plasminogen to produce plasmin.

CONCLUSION

The overall results of the present study indicate the promising hypoglycemic activity, moderate anti-diarrheal and mild thrombolytic properties of the leaves of *T. citrina* which deserves further investigation to isolate the bioactive constituents responsible for these activities and to establish the mechanism of action. Currently, phytochemical investigation to isolate bioactive pure compounds is in progress in our laboratory.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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