ORIGINAL ARTICLE

Antidiabetic and antihyperlipidemic effects of *Premna spinosa* bark in experimental animal models

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ABSTRACT

The purpose of the study is to evaluate the antidiabetic and hyperlipidemic potential of stem bark extract of *Premna spinosa* (Lamiaceae), by using streptozotocin (STZ)-nicotinamide (NA)-induced diabetic and triton-induced hyperlipidemic models in albino rats. The blood glucose, total cholesterol, and triglyceride levels were determined in STZ-NA-induced diabetic and triton-induced hyperlipidemic rats, as per the respective protocols. It was found that there is the dose dependent and significant reduction in foregoing parameters on the administration of extract from *Premna spinosa* stem bark at the doses of 200, 400, and 800 mg/kg body weight to diabetic and hyperlipidemic rats. From these observed results it may be inferred that the stem bark of *Premna spinosa* possesses remarkable antidiabetic and antihyperlipidemic properties.

Key words: Diabetes mellitus, hyperlipidemic, oxidative stress, premna spinosa, streptozotocin

INTRODUCTION

Currently, almost 4% population are affected by diabetes mellitus and hyperlipidemia globally and it is expected that this metabolic disorder will be increased to more than 5.4% in 2025.[1] The common cause of these disorders is due to abnormalities in carbohydrate, lipid, and lipoprotein metabolism. As per the clinician report, these metabolic disorders also affect insulin production, atherosclerosis resistance and utilization also causes destruction of beta cells of the pancreas.[2,3] There are many synthetic medicines were administered for the prevention and cure of diabetes, however, long-term use of these medications is not effective for normalizing the plasma sugar level either due resistance or severe adverse effect. Hence, safe and effective alternative remedies are required for the treatment of diabetes mellitus. *Premna spinosa* L. belongs to the family Lamiaceae, commonly known as *Gandana Saga* in Odia, is a small shrub/small tree used to treat many aliments since from ancient time. The different parts of the *Premna spinosa* such as root, root bark, leaf, stem, stem bark, and wood have traditionally been used in India to treat diabetes, inflammation, hyperglycemic conditions.[4] Reports found that pharmacological properties of the *Premna spinosa* are owing to the occurrence of the chemical constituents, namely, alkaloids such as premnine, flavonoids like luteolin, sterols such as lanosterol and triterpenes.[5-7] *Premna spinosa* is reported to possess hypolipidemic, anti-inflammatory, and immuno-modulatory activities.[8-10] On the limelight of the traditional usage of these plants, the present work plans to explore the antidiabetic and

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antihyperlipidemic properties of the stem bark extract of *Premna spinosa*.

MATERIALS AND METHODS

Plant extract preparation

The fresh stem bark from *Premna spinosa* L.(Lamiaceae) was hand-picked from the Utkal University campus in May 2021 and authenticated by Dr. Puspanjali Mishra, Botany Department, Utkal University, Bhubaneswar, Odisha and a voucher specimen (Ref. No. RC/CNU-069) was preserved at the same institute for reference. The collected stem bark was washed through tap water and after that, these are rinsed thoroughly with distilled water to clean dirt and dust after that the barks were dried under shade. The shade dried barks were coarsely powdered with the help of an electric grinder. The coarse powder is stored in an air-tight glass container until further use. The powdered bark (100 g) was extracted with aqueous ethanol (90%) by hot continuous percolation method with the help of a Soxhlet apparatus. The obtained residue was extracted again with the same solvent two times. After that, the bark extracts were filtered and dried using a rotary vacuum evaporator. The dry extract Ethanol extract of *Premna spinosa* bark (EEPS) was preserved at-20°C.

Experimental animals

The male albino rats of Wistar strain with body weight of 225–250 g were used in this experiment. All the animals were housed in the polypropylene cage for 7 days for proper acclimatization before the study. The experiment was carried out after getting CPCSEA, IAEC approval of Centurion University of Technology and Management, Ramachandrapur, Jatni, Bhubaneswar vide IAEC Reg. No.:2024/PO/Re/S/18/CPCSEA.

Acute toxicity

Oral acute toxicity of EEPS was performed by using albino rats weighing about 175–200 g with different dose level as per OECD guideline 425.[11]

Chemicals and drugs

The chemicals used during the experiments are purely analytical grade with high strength such as Sodium Chloride, Triton WR1339, streptozotocin (STZ), nicotinamide (NA) from Himedia Lab, Mumbai, whereas Atorvastatin from Ranbaxy Lab. Besides, the biochemical estimation kits such as glucose, cholesterol, and triglyceride kits were purchased from the Crest biosystem.

Experimental design

Oral glucose tolerance test

This study was conducted on rats which were not given food overnight. The experimental rats were classified into five groups ($n = 6$). Group I got isotonic saline, Group II received the reference agent gliclazide at the dose of 25 mg per kg. Groups III, IV, and V were given EEPS at the doses of 200, 400, and 800 mg/kg p. o, respectively. After providing the glucose solution (2.5 g/kg), the blood was collected from the sublingual vein at 30, 60, and 120 min. Glucometer and strip were used for measuring both fasting and postprandial glucose levels.

Antidiabetic activity

Diabetes was invoked in 12 h starved rats by a single intraperitoneal dose of NA of 120 mg/kg followed by fresh STZ solution in citrate buffer at the dose of 60 mg per kg. The next day of induction, blood sugar levels were measured, rats with plasma glucose levels >200 mg/dl were chosen for the next treatments.[12] Such rats were classified into five groups ($n = 6$).

Group I: negative control receiving isotonic saline without induction of NA and STZ (normal or nondiabetic control).

Group II: positive control receiving isotonic saline with NA and STZ (diabetic/disease control).

Group III: treatment group receiving with EEPS 200 mg/kg once a day with NA and STZ.

Group IV: treatment group receiving with EEPS 400 mg/kg once a day with NA and STZ.

Group V: reference control received with gliclazide 25 mg/kg daily with NA and STZ.

All the animals were treated orally for 28 consecutive days. During the experimental period, fasting blood was periodically collected from each group for estimation of plasma glucose levels.

Induction of hyperlipidemia using triton WR-1339

For inducing hyperlipidemia, the rats were classified into five groups ($n = 6$). The rats of Groups II, III, and IV were induced with single intraperitoneal dose (200 mg per kg) of triton WR-1339. Group I was considered negative control, in which only administered with normal saline, whereas group II got Triton WR-1339, 200 mg/kg and called as triton control group. Group III received reference atorvastatin at the dose of 7.2 mg/kg. Groups IV and V got EEPS at the doses of 400 and 800 mg/kg, respectively after induction of triton WR-1339. Body weight of rats was periodically monitored. Blood sample was obtained from each animal in the interval of 0, 18, 24, 40, and 48 h dose treatment for estimation of cholesterol and triglycerides using separated serum.

Statistical analysis

All the values were presented as mean ± standard error of the mean. The statistical significance was determined using one-way analysis of variance subsequently with Dunnett's test. $P < 0.05$ was observed as statistically significant.

RESULTS

Effect of EEPS on oral glucose tolerance test

It was found that EPSS (200, 400, and 800 mg/kg) and gliclazide (25 mg/kg), showed decreases in blood sugar levels from the diabetic control group at 30, 60, and 120 min, values are shown in Table 1.

Effect of EEPS diabetic rat induced by nicotinamide and streptozotocin

After administration of STZ, the blood glucose level was measured, it was found to in the range of 240–293 mg/dl in each rat, this range is considered as severe diabetes. When standard drug and test substances were administered, then their blood glucose level decreased significantly. From the results, it was found that blood sugar levels in the standard group decrease from 196 ± 10.81 to 144 ± 3.41 mg/dl, whereas in the case of OD dose of bark extract of *Premna spinosa* the blood sugar level decreases from 241.5 ± 7 to 209.83 ± 1.7 mg/dl. When the frequency of dosage increases then the rate of blood sugar decreases i.e., 229.8 ± 7.4–201.5 ± 4.31 mg/dl in BD dose and 228.5 ± 7.7-202.4 ± 4.8 mg/dl in QD dosage on the 28th day, respectively, in a significant manner, shown in Table 2. From the statistical analysis, it was found that EEPS has the antidiabetic effect ($P < 0.05$) as compared to disease control. It is found that on the 28th day, there was a significant reduction of blood glucose in STZ-induced diabetic rats.

Effect of EEPS in hyperlipidemia rat induced by using triton WR-1339

From the experiment, it was found that the mean body weight of groups I and II were not significantly changed, similarly in the treated groups no significant changes were observed in rat body weight, as shown in Table 3.

When the serum cholesterol was evaluated, EEPS shows a slight reduction in serum cholesterol content as compared to positive control in 18 and 24 h. When analyzed on day 0, there was no alteration in serum cholesterol content, but after induction of triton, there was a significant hike of serum cholesterol in hours 18 and 24. In comparison with reference group, EEPS shows a comparably significant decline in serum cholesterol content as compared with the disease (triton) control group [Table 4].

Similarly, serum triglyceride level in each group was examined, it was found that there were no significant changes occurring among all groups at 0 h. At 18 and 24 h, it was found that both EEPS and reference atorvastatin showed very little decline in serum triglycerides as compared with positive control as shown in Table 5.

DISCUSSION AND CONCLUSION

From the literature review, it was found that experimental diabetic Mellitus induced by NA and STZ, leads to severe diabetic conditions in which various organs get affected a lot because of the action of STZ on the β-cell of the pancreas.[13] The free radical generation characteristics of STZ, able to cause abnormalities in the beta cell and produce diabetic mellitus, further increasing blood sugar. Many authors propose the mechanism of blood sugar induction, STZ-stimulated beta-cell death is due to alkylation of DNA by intercalating with six position of guanine nucleoside of DNA. It was also hypothesized that this compound contributes to genetic damage which causes DNA fragmentation. In addition, the STZ-induced DNA damage decreases the cellular NAD + and also decreases ATP generation, and inhibits insulin production.[14,15] In this experiment study, after using bark extract of *Premna spinosa* for a certain period the blood sugar abnormalities

Table 1: Effect of EEPS on oral glucose tolerance test

Time (min)	Normal control	Diabetic control	Gliclazide (25 mg/kg)	EEPS (200 mg/kg)	EEPS (400 mg/kg)	EEPS (800 mg/kg)
0	75.83±7.10	72.5±3.21	70.21±3.81	78.5±3.51	71.1±3.20	72.2±3.7
30	77.66±6.15	139.5±6.01[a]	84.9±5.11	128.2±7.06*	115±4.20*	112.8±4.80*
60	76.4±4.43	129.3±6.5[a]	87.5±6.51*	120.4±4.81*	109±2.50*	104.6±2.83*
120	76.38±3.62	120.5±4.21[a]	79.16±4.20*	111.8±4.20*	81.6±4.21*	95.1±4.80*

*$P \leq 0.05$ diabetic control versus treated groups, [a]$P \leq 0.05$, normal control versus diabetic control, Values are presented as mean±SEM (n=6). SEM: Standard error of the mean, EEPS: Ethanol extract of *Premna spinosa* bark

Table 2: Effect of EEPS on blood sugar level in the streptozotocin-nicotinamide-induced anti-diabetic model

Duration	Normal control	Diabetic control	Gliclazide (25 mg/kg)	EEPS (200 mg/kg)	EEPS (400 mg/kg)	EEPS (800 mg/kg)
Week 0	89.56±6.5	257±4.25[a]	196±10.81*	241.5±70*	229.8±7.41*	228.5±7.72*
Week 2	96.5±5.21	277.5±2.21[a]	162±2.31*	217.3±2.13*	219.66±6.50*	227±6.31*
Week 4	96.41±5.41	297.5±6.51[a]	144±3.41*	209.83±1.73*	201.5±4.31*	202.4±4.80*

*$P \leq 0.05$ diabetic control versus treated groups, [a]$P \leq 0.05$, normal control versus diabetic control, Values are presented as mean±SEM (n=6). SEM: Standard error of the mean, EEPS: Ethanol extract of *Premna spinosa* bark

Table 3: Effect of EEPS on body weight in triton-induced hyperlipidaemic rats

Treatment groups	Body weight (g)		
	Day 0	Day 1	Day 2
Normal control	240.17±3.71	259±4.98	257.34±6.8
Triton control (200 mg/kg)	238.5±3.45	253.4±3.65	259.45±5.71
Atorvastatin (7.2 mg/kg)	242.2±2.3	258.3±4.7	255.7±5.52
EEPS (800 mg/kg)	238.53±4.95	253.5±3.84	254.73±5.41

Values are presented as mean±SEM (n=6). SEM: Standard error of the mean, EEPS: Ethanol extract of *Premna spinosa* bark

Table 4: Effect of EEPS on total cholesterol in triton-induced hyperlipidemic rats

Treatment groups	Serum total cholesterol (h)				
	0	18	24	40	48
Normal control	62.8±4.17	35.6±4.65	37.61±2.74	47.50±3.38	44.97±1.54
Triton (200 mg/kg)	64.85±5.21	247.55±29.41[a]	209.62±29.71[a]	58.12±3.34[a]	52.13±3.12[a]
Atorvastatin (7.2 mg/kg)	65.81±5.18	125.71±15.41*	112.75±18.13*	61.85±5.01*	59.85±4.21*
EEPS (800 mg/kg)	63.9±2.71	179.8±12.81*	143.65±12.81*	59.82±3.89*	54.98±2.31*

*P≤0.05 diabetic control versus treated groups, [a]P≤0.05, normal control versus diabetic control, Values are presented as mean±SEM (n=6). SEM: Standard error of the mean, EEPS: Ethanol extract of *Premna spinosa* bark

Table 5: Effect of EEPS on serum triglyceride in triton-induced hyperlipidemic rats

Treatment groups	Serum triglyceride (mg/dl), h				
	0	18	24	40	48
Normal control	51.14±4.95	59.61±10.16	87.86±16.60	91.35±13.13	81.91±6.35
Triton control (200 mg/kg)	61.41±2.10	1131.35±45.8	650.71±74.30	87.89±4.21	63.91±4.41
Atorovastatin (7.2 mg/kg)	41.68±4.95	662.72±150.31	356.34±105.01	76.15±7.12	67.5±5.81
EEPS (800 mg/kg)	72.25±10.33	901.81±44.35	504.34±73.82	91.78±19.59	75.01±13.50

Values are presented as mean±SEM (n=6). SEM: Standard error of the mean, EEPS: Ethanol extract of *Premna spinosa* bark

were significantly restored by decreasing the blood sugar level.

In the oral glucose tolerance test or glucose-loaded hyperglycemia study, EEPS shows a significant antihyperglycemic effect at the given doses of 400 and 800 mg/kg body weight, indicating the test extract might have enhanced the peripheral utilization of glucose.[16] However, our experimental finding supported that dosage of EEPS in OD, BD and QD enhance the glucose utilization at 60 and 120 min. When diabetic rats were treated with both EEPS and gliclazide, we found that there is a significant decrease in plasma glucose level, which indicates bark extract of *Premna spinosa* has antidiabetic activity due to revering action of oxidative stress or increase of plasma insulin level. In the hyperlipidemic model, the EEPS showed very less significant reduction of cholesterol and triglyceride in plasma when compared with the positive control, indicating that our test substance having some ability of interference of cholesterol synthesis process could support to reduce plasma hyperlipid level.

From the previously reported phytochemical analysis, it was found that *Premna spinosa* stem bark contains alkaloids (premnine), flavonoids (luteolin), sterol (lanosterol), and triterpenes.[5-7] These alkaloids and flavonoids are rich with antioxidant properties which could reverse the STZ-induced oxidative impact in diabetic rats.[17] To the best of the authors' comprehension, this is the first scientific account of the antidiabetic potential of this plant. Form the present experiments it may be inferred that, the stem bark of *Premna spinosa* possesses significant effectiveness against metabolic disorders like diabetes mellitus hence, further exploration on this plant leading to isolation and molecular mechanism studies are required to reap its exact usefulness for the therapeutic management of human diabetes mellitus.

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Conflicts of interest

There are no conflicts of interest.

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