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ASSESSMENT OF ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF *Acacia suma* ROXB ROOT

Jitendra Debata[1]*, H. K. Sundeep Kumar[2]

1. *GNITC-School of Pharmacy, Ibrahimpatnam, Rangareddy-501506, Telangana, India.*
2. *Institute of Pharmacy and Technology, Salipur, Cuttack-754202, Odisha, India.*

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ABSTRACT

The present study was executed to study the antihyperglycemic and antihyperlipidemic activity in roots related to methanol extract of *Acacia suma* Roxb in rats. The test was performed in rats which are alloxan-induced hyperglycemic under treatment of the tested methanol extract at 200 and 400 mg/kg dose levels for 30 days and methanol extract. glibenclamide (2.5 mg/kg) was used as a standard drug for activity comparison. The parameters related to biochemical issues used in the study are plasma-insulin, blood glucose and lipid profiles. Standard experimental method was used in performing the experiment. The results of the test showed that the blood glucose level comes down significantly ($p<0.05$) in normoglycemic rats in a specific does, this happened when the hyperglycemic rats experiences the sudden decrease of blood sugar ($p<0.05$ to 0.001). The tested dose levels of the extract, significantly ($p<0.05$) increases glucose by rat hemidiaphragm which is isolated. It demonstrated the remarkable antidiabetogenic effect exerted by the methanol root extract of *Acacia suma*.

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Introduction

Diabetes mellitus (DM) is metabolic disorders that leads to a hyperglycemia because of overcome production or not efficient utilization of insulin. The WHO estimated that DM can influence almost 171 million people around the world and this number of population can be 366 million in 2030 [1]. Globally, it is the most serious disease and the largest endocrine disorder which considered to be one of the five leading causes of death in the world [2]. The highest rate in diabetic countries are India, China, and the U.S. The International Diabetes Federation predicts that the overall Indian people having diabetes can be about 50.8 million in 2010, and this number can reach to 87.0 million by 2030 [3].

In both type 1 and 2 diabetics, cardiovascular diseases increase two or three- fold morbidity compared to people with no diabets [4]. Hyperglycemia in the gield of diabetes is attributed to interchanging the metabolism of lipid and glucose and modifying enzyme level [5]. The liver which is the significant insulin dependent tissue, has an important position in the metabolism of glucose and lipid. Moreover, it can be strongly influenced during diabetes [6]. Liver has a role in oxidation, comprehension and metabolic conversion of free fatty acids, cholesterol synthesing, triglycerides and phospholipids. In DM, unusual reaction of lipids is not surprising. The usual reaction is the progression of overall and triglyceride, VLDL cholesterol, decreasing HDL cholesterol and the ascendance of small, dense LDL aces [7]. Unusual actions of lipids in diabetic people can increase the danger of atherogenesis [8]. From the beginning of the last century, there are some confirmations related to the properties of lipid-lowering of medicinal plants [9]. Herbal medicines obtaining from plant extracts are being used mostly to cure variety of clinical-related diseases such as liver disease [10], ischemia, acute hypertension, perfusion injury, hemorrhagic shock, diabetes mellitus atherosclerosis and cancer [11]. The World Health Organization suggested that this field needs more investigation [12].

The family Fabaceae (alternatively known as the Leguminosae) is one of the largest varieties of plants, with 730 genera and about 19,400 species [13]. The genus *Acacia* comprises about 1200 species, which can be found in tropical and subtropical regions, [14]. *Acacia suma* Roxb var. *Acacia polyacantha* Willd is a kind of tree with the size between 3.5 -20 meters in height, mostly seen in India [15].

Corresponding Author: Jitendra Debata. GNITC-School of Pharmacy, Ibrahimpatnam, Rangareddy-501506, Telangana, India. E-mail: jitendra_debata @ rediffmail.com

Ethnopharmacological data showed that the stem barks of *A. suma* is utilized in different traditional and domestic systems of medicine all around the world. The extract of the bark is used orally to cure gonorrhea, pneumonia, leprosy, malaria, and diabetes [16, 17]. Gessler et al. [17] talked about the antimalarial reactions of the stem bark. The hypoglycemic activities of various decoctions of bark also are mentiond by some other investigators [18]. Some other authors also talked about the presence of gallocatechins in the barks and an indole alkaloid N, N-dimethyl tryptamine in the leaves [19].

Materials and Methods

Plant Materials

The plant material (root) was collected from the Ganjam district of Odisha forests in June 2017 . After due authentication, fresh matured root was collected in bulk, cleaned thoroughly with distilled water, followed by shade drying for 14 days. The shade dried roots were coarsely powdered and preserved till further use.

Preparation of the extract

The fat was removed fromThe powdered material (600 g) by using petroleum ether and extracted with methanol for 48 h in a Soxhlet extractor. In order to yielding dried extractive, the liquid extract was put under vacuum. The percentage of this step was calculated by considering the dried plant material (yield: 7.7 %w/w). Preliminary phytochemical screening of the extract was executed by utilizing standard methods [20, 21].

Preparation of the test samples

The measured quantity of *Acacia suma* related to methanol extract and glibenclamide (2.5 mg/kg) was suspended in water with 20% Tween 20 and used as a test drug for oral administration.

Maintenance of Animals and approval of a protocol

Healthy albino Wistar rats of either sex, weighing 150–200 g body weight were collected from the institutional animal house for the study. The selected animals were housed in polypropylene cages in standard environmental conditions (temp: 20–25 $^{\circ}$C; relative humidity: 45-55 % under 12 h light/dark cycle), which were feeding some special food for 7 days to adapt to the laboratory atmosphere and water *ad libitum*. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of the Institute of Pharmacy and Technology (Regd. No. 1053/PO/Re/S/07/CPCSEA).

Acute toxicity study

These studies were conducted on Swiss albino mice as per the OECD guidelines 423 [22], for this the limitation of the does 2000 mg/kg, p.o. was used. The test was executed by Ganapaty et al. [23] and Shivhare et al. [24]. As soon as dosing, the animals were precisely monitored for 4 h. The changing in their behavior was observed for hyperactivity, ataxia, convulsion, salivation, tremors and sleep. Then they were observed for 14 days after drug administration to determine the mortality if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/kg, body weight, p.o.) of the methanol extract of *Acacia suma* was selected for antihyperglycaemic and antihyperlipidemic activity studies.

Determination of blood glucose levels

Fasting blood glucose concentration was measured, using a Gluco monitor, according to the glucose oxidase method. The process of blood sampling was done from the tip of tail in particular time [25, 26].

Screening for glucose lowering effects of test extract

The Screening for antihyperglycaemic activity was executed as per the standard process [27].

The multi-dose treated normoglycaemic animals

The animals were fasted for 12 hours, but they were allowed to drink water before or during the experiment. At the end of this period, the rats were divided into four groups. Group I was considered as control and received only vehicle (2 ml/kg) through the oral route. Group II received glibenclamide (2.5 mg/kg) and considerd as a reference control. Groups III and IV had the methanol extract which was tested at doses of 200 and 400 mg/kg,. The tested methanol extract, standard drug, and solvent were administered to the respective group once daily for 30 days. On the 0, 5, 10, 15, 20, 25 and 30th day of experiment the blood glucose level.

The multi-dose treated alloxan-induced diabetic animals

For 4h the animals received no food exept water *ad libitum* and they were injected intraperitoneally by a dose of 150 mg/kg of alloxan monohydrate.

Study of glucose utilization on isolated rat hemidiaphragm

By decapitation the selected healthy rats were killed and diaphragms were isolated quickly avoiding trauma and were segregated into two halves. The hemidiaphragms were then placed in culture tubes containing 2 ml tyrode solution with 2% glucose and incubated for 30 min at 37^{0}C in an atmosphere of 95% O_2 – 5% CO_2 with shaking. Six sets of similar experiments were performed. The diaphragms were exposed to, (I) corresponds to diabetic control (II) reference standard insulin (0.25 IU/ml), (III) methanol extract (50 mg/ml), (IV) methanol extract (100 mg/ml), (V) insulin (0.25 IU/ml + methanol extract (50 mg/ml)) and (VI) insulin (0.25 IU/ml + methanol extract (100 mg/ml)). The hemidiaphragms were taken out and weighed according to incubation. Moreover, the glucose content of the incubated medium was measured. The difference was calculated in this process [28].

Effect of methanol extract on serum lipid levels

After 30 days of treatment with the methanol extract, the animals were killed by decapitation under ether anesthesia. The serum supernatant was segregated by centrifugation. Also it was used for the determination of the lipid profile studies such as total lipids, phospholipids, total cholesterol, triglycerides, HDL, LDL, VLDL and free fatty acids [29].

Statistical analysis

Generally the data are expressed as mean ± SEM, for six animals in each group. The differences between groups were measured by one-way Analysis of Variance (ANOVA) followed by Dennett's Multiple Comparison test.

Results

The results of this study was a kind of evidence for the presence of alkaloids, tannins, terpenoids, flavonoids, and saponins.

Effects of methanol extract on blood glucose levels

Effect of methanol extract on multi-dose treated normoglycaemic animals

The results of methanol extract on the normoglycemic rats and their blood sugar level are depicted in Table 1. The result presents that there is a significant reduction ($p<0.05$ to $p<0.01$) in blood glucose level from 15th day onwards, and registered 23.37 and 33.24% reduction after 30 days, in animals treated with 200 and 400 mg/kg of the methanol extract. However, the standard drug glibenclamide on the same day reduces the blood glucose by 39.73% with $p<0.001$ when compared with the solvent control group.

Effect of methanol extract on multi-dose treated alloxan-induced diabetic rats

The results depicted in Table 2, reveals that, the extract reduces the blood glucose level to an extent of 45.04% and 56.82% at 200 mg/kg and 400 mg/kg body weight, respectively after the 30th day of the study, whereas the standard drug glibenclamide registered 68.77% of reduction at the same day of the study. However, the individual data show a statistical significance ranges between $p<0.05$ to $p<0.001$, throughout the experiment when compared with solvent control.

Effect of methanol extract on glucose utilization by isolated rat hemidiaphragm

The results of the study on glucose uptake by isolated rat hemidiaphragm are shown in Table 3, which reveals that the test extract at 200 mg/ml and 400mg/ml concentrations exhibited uptake of 4.73 and 5.83 mg/g/30min respectively, while only insulin showed 8.46 mg/g/30min. However, insulin and test extract combination responds to 8.86 and 10.35 mg/g uptake of glucose at the same time. The extent of glucose uptake differs significantly ranging from $p<0.05$ to $p<0.001$ when compared with the diabetic control group.

Effect of methanol extract on serum lipid profile

Table 4 illustrates the levels of serum lipid profile such as total lipids, total cholesterol, phospholipids, triglycerides, HDL, LDL, VLDL and free fatty acids on 30th day of the study. The diabetic rats showed significant ($p<0.01$) increase level of all tested lipid profiles except HDL, which showed decreased value in a significant ($p<0.05$) extent. The extract at both the dose levels showed a dose-dependent and significant ($P < 0.05$ to $p<0.001$) reduction in total lipids, triglycerides, LDL, VLDL, and free fatty acids, however a marked decrease in the levels of total cholesterol and phospholipids were also recorded, when compared to diabetic control group, while the HDL levels were approaching almost normal values when compared to without-treatment normal control group.

Table 1: Effect of me**thanol root extr**act of *Acacia suma* on blood glucose in multidose treated on normoglycemic rats in oral route.

Groups	Experimental groups	Blood Glucose Levels (mg/dl)							
		0th Day	5th Day	10th Day	15th Day	20th Day	25th Day	30th Day	% decrease at 30th Day
I	Solvent Control	97.53±3.50	99.45±3.35	97.55±3.26	98.25±4.25	97.45±4.34	96.62±3.35	97.10±4.20	-
II	Glibenclamide (2.5 mg/kg)	96.5±3.22	89.5±2.44	82.41±3.25*	62.5±3.65**	63.35±2.22**	63.24±2.45**	58.16±2.45**	39.73
III	Methanol extracts (200 mg/kg, p.o.)	98.45±4.33	96.45±3.77	91.24±4.35	86.35±3.57	85.14±4.55	81.55±4.44*	75.44±2.45*	23.37
IV	Methanol extracts (400 mg/kg, p.o.)	99.24±5.55	95.15±5.56	91.53±4.42	88.25±5.65*	83.45±4.35*	80.44±4.62**	66.25±4.22**	33.24

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test Multiple Comparison test.
*P<0.05, **P<0.01 as compared to solvent control group.

Table 2: Effect of methanol extract on multi-dose treated alloxan-induced diabetic rats

Groups	Experimental groups	Blood Glucose Levels (mg/dl)							
		0th Day	5th Day	10th Day	15th Day	20th Day	25th Day	30th Day	% decrease at 30th Day
I	Solvent Control	280.55±5.35	275.34±5.46	260.46±4.45	267.45±5.43	262.14±5.80	258.83±5.76	254.34±6.30	-
II	Glibenclamide (2.5 mg/kg)	283.44±6.53	186.55±6.10**	125.16±5.16**	103.34±7.45**	100.26±7.53**	91.73±6.35**	88.5±6.53**	68.77
III	Methanol extracts (200 mg/kg, p.o.)	279.18±5.14	247.5±6.52	210.63±7.12*	181.53±6.42**	175.23±7.62**	161.34±6.44**	153.43±7.32**	45.04
IV	Methanol extracts (400 mg/kg, p.o.)	280.55±5.51	214.33±5.17**	165.32±6.52**	145.25±6.45**	138.23±6.55**	135.34±7.56**	121.12±7.22**	56.82

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test Multiple Comparison test.
*P<0.05, **P<0.01 as compared to solvent control group.

Table 3: Effect of methanol root extract of *Acacia suma* on peripheral glucose-uptake by isolated rat hemi-diaphragm.

Experimental groups	Incubation medium	Glucose uptake (mg/g/30 min)
I	Tyrode solution with glucose (2 g%) + Diabetic control	4.67 ± 0.16
II	Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml)	8.45 ± 0.21**
III	Tyrode solution with glucose (2 g%) + Methanol extract (200 mg/ml)	4.73 ± 0.54
IV	Tyrode solution with glucose (2 g%) + Methanol extract (400 mg/ml)	5.83 ± 0.23*
V	Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml) + Methanol extract (200 mg/ml)	8.86 ± 0.36**
VI	Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml) + Methanol extract (400 mg/ml)	10.35 ± 0.41**

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test Multiple Comparison test.
*P<0.05, **P<0.01 as compared to solvent control group.

Table 4: Effect of root extracts of *Acacia suma* on serum lipid profile in alloxanised rats on 30th day of study.

Groups	Experimental groups	Serum Lipid Profile (mg/dl)							
		Total Lipids	Total Cholesterol	Phospholipids	Triglycerides	HDL	LDL	VLDL	Free Fatty Acids
I	Normal	110.35±5.25	73.34±5.44*	102.35±7.16	64.13±6.54	53.5±5.34	26.73±6.56	15.24±1.13	405.22±61.36
II	Diabetic control	385.17±15.35	183.14±13.25	211.36±13.24	183.04±17.17	32.25±2.35	63.56±6.35	42.4±1.82	1310.58±127.10
III	Glibenclamide (2.5 mg/kg)	142.36±13.24**	93.15±6.23*	124.43±6.22*	74.33±6.22**	53.23±5.25*	35.21±3.13*	17.73±1.72**	662.32±75.36**
IV	Methanol extracts (200 mg/kg, p.o.)	216.35±15.41**	165.13±14.17	184.32±16.52	111.38±11.45**	33.13±4.76	44.46±5.25	31.26±2.16**	915.08±70.26**
V	Methanol extracts (400 mg/kg, p.o.)	210.52±23.41**	143.04±20.24	171.23±14.25	96.35±12.22**	45.17±2.38	41.58±2.72*	22.37±2.18**	781.12±55.26**

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control group

Discussion

The present study aims at extensive evaluation of methanol extract of *Acacia suma* roots towards a mechanistic hypoglycemic potential upon 30 days of study. The data revealed a defined role of methanol extract in normoglycemic, and alloxan-induced diabetic rats, the methanol extract of roots of *Acacia suma*, found to possess dose- dependent suppression of glucose level, with prolonged hypoglycemia at a higher dose of 400mg/kg, which has almost the same effect as that of synthetic drug glibenclamide.

All these glucose-lowering effects can be because of the insulinotropic effect at the islet beta cell level as evidenced by the increased plasma insulin levels [30].

The activity of the extract of methanol in increasing the probability of using glucose by isolated rat hemidiaphragm, suggest that the extract may contribute to the insulinotropic effect or direct insulin-like activity and extra pancreatic effect [31].
Alloxan, a beta-cytotoxin, induces "chemical diabetes" by pancreatic cell damage mediated through the generation of cytotoxic oxygen free radicals. This first goal of these is DNA of pancreatic cells, causing DNA fragmentation [32]. This can damage many β-cells, [33, 34]. The results depicted in this study revealed that the sub-acute antidiabetic, hypoglycemic and insulinotropic effects of methanol extract were similar to those of glibenclamide. The possible mechanism, by which the plant extract mediates its antidiabetic action, is the potentiation of pancreatic secretion of insulin from existing residual β-cell of islets and due to enhanced utilization of blood glucose by peripheral tissues as well.
It has been reported that the increase in glucose levels in alloxan-induced diabetic rats is associated with dyslipidemia characterized by elevated serum triglycerides and total cholesterol levels. The improvement of blood glucose levels caused by most hypoglycaemic treatments is attributed to a reduction of serum triglycerides and total cholesterol [35, 36]. The significant reduction of LDL, VLDL, TC, TG, FFA, phospholipids & total lipids and progressive nature of HDL demonstrates that, the extract may have property to enhance the transcription of lipoprotein lipase similar to that of insulin.

Conclusion

The present study report clearly depicted that the *Acacia suma* methanol extract endowed with hypoglycaemic and antihyperglycaemic activity due to its possible action on pancreatic and extra-pancreatic site of glucose and lipid metabolism is evidenced by insulinotropic properties of the extract.

Abbreviation used

DM: Diabetes mellitus; VLDL: Very-low-density lipoprotein; HDL: High-density lipoproteins; LDL: Low-density lipoprotein; *A. suma*: *Acacia suma*; OECD: Organisation for Economic Co-operation and Development; p.o.: Per OS; IU: International Unit; DNA: Deoxyribonucleic acid; TC: Total Cholesterol; TG: Triglycerides; FFA: Free Fatty Acids.

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