Assessment of antihyperglycemic and antihyperlipidemic activity of *Spondias mangifera* willd root

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**ABSTRACT**

The present study was designed to investigate the antihyperglycemic and antihyperlipidemic activity of methanol extract of roots of *Spondias mangifera* Willd in rats. The test was performed in both normoglycemic and alloxan induced hyperglycemic rats under treatment of the tested methanol extract at 200 and 400 mg/kg dose levels for 30 days, and glibenclamide (2.5 mg/kg) was used as standard drug for activity comparison. The biochemical parameters used in the study are blood glucose, plasma-insulin and lipid profiles determination was performed using standard experimental procedure. The test result revealed that in normoglycemic rats the blood glucose level reduces to a significant (p<0.05) extent in a dose-dependent manner, while the hyperglycemic rats showed a progressive fall of blood sugar level in a significant extent (p<0.05 to 0.001). The extract at the tested dose levels, significantly (p<0.05) increases the peripheral utilization of glucose by isolated rat hemi-diaphragm.  

It was concluded that, the remarkable antidiabetogenic effect exerted by the methanol root extract of *Spondias mangifera*.

1. INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production or inefficient insulin utilization. The World Health Organization predicted that DM affects approximately 171 million people worldwide and the number is expected to reach to 366 million in 2030 [1].

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Globally, it is the most common serious and largest endocrine disorder and considered to be one of the five leading causes of death in the world [2]. The three countries with the largest number of people with diabetes are India, china, and U.S. The International Diabetes Federation estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030 [3].In both type 1 and 2 diabetics, cardiovascular diseases increases two or three fold morbidity and mortality rate compared to non-diabetic persons [4]. Hyperglycemia in the diabetics is associated with alteration of glucose and lipid metabolism and modification in liver enzymes level [5]. Liver is an important insulin dependent tissue which plays a pivotal role in glucose and lipid metabolism and is severely affected during diabetes [6]. Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and...
triglycerides. In DM, lipid abnormalities are almost the rule. Typical finding are elevation of total and VLDL cholesterol, triglyceride concentration, lowering of HDL cholesterol and a predominance of small, dense LDL particles [10]. Lipid abnormalities in patients with diabetes are likely to play important role in the development of atherogenesis [8]. From the beginning of last century, evidence of lipid lowering properties of medicinal plants has also been documented [9]. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases including liver disease [10], ischemia, perfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer with relatively little knowledge regarding their modes of action [11]. The World Health Organization (WHO) has recommended that this area warrants further evaluation [12].

*Spondias mangifera* Willd. (Family- Anacardiaceae) is a tree up to 10.5 m high with straight columnar trunk and smooth ash grey coloured bark having characteristic pleasant smell of its wood [13]. About seventeen species of *Spondias* are in record, being native of Indo-Malaysia, South Eastern Asia and tropical America. In India, it is cultivated in Punjab, Maharashtra, Odisha, West Bengal and Assam for its edible fruits [14]. All plants emit foetid turpentine like odour when broken or bruised; smell however, varies from species to species and very characteristic [15]. Ethno medicinally, its bark used as tonic, refrigerant and for treatment of articular and muscular rheumatism and also, in diarrhoea and dysentery [16-18]. Bark pounded to paste used against stomach ache [19]. Leaves glabrous, aromatic, acidic and astringent, used as flavouring agent while its juice applied as drops against ear ache [17,20]. The tribal women of Malaysia and India use internally hot aqueous root extract for regulating menstrual anomaly and paste prepared from roots used externally as massage balm for remission of muscular pain [17,18,21]. Juice, extracted from ripened fruits, popularly used as home remedy for biliousness [22].

Only a few pharmacological or biological test reports have been reported on this plant in the literature like methanol extracts of dried fruit (200 mg/ml) and ethanol (95%) extracts of dried leaf of *S. mangifera* possess antitumor promoting activity [23,24]. Various extract of dried seed of the plant is also reported to have antipyretic, antihistamine, antispasmodic and hypotensive activity at various dose levels [25]. Similarly, Valsaraj et al. 1997; also reports the antibacterial activity of ethanol (80%) extract of dried stem bark [26] and 70% methanol extract of *Spondias mangifera* stem bark was studied in *vitro* for total antioxidant activity. It also reported hypoglycemic, diuretic and laxative activity of different extracts of bark [27,28] and root for hypoglycemic activity [29]. Only a few phytochemical have been reported on this plant in the literature. Cycloartanone 24-methylene, daucosterol, lignoceric acid, stigmaster-4-en-3-one, β-amyrin, oleanolic acid and β-sitosterol was isolated from fruits and aerial parts of the plant, and also glycine, cystine, alanine and leucine present in fruits [30, 31].

It is also used in pharmaceuticals, neutraceuticals and in cosmetics. Ginger contains a fusion of an aroma oils both volatile (zingiberene) and non-volatile (oleoresin) oils and phenolic compounds (gingerol and shogaol, zingerone and paradol) [2]. Gingerol has less solubility, low bioavailability and rapidly eliminated from the body. To overcome of these problems and extend the retention time of gingerol, it combined with polymer and phospholipid. Phytosome is a patented technology, which improves the absorption and bioavailability of lipid compatible molecular complexes. The objective of the present work wasto prepare the complex of phytosome loaded of gingerol (GPLC)using soya lecithin as a phospholipid and chitosan as a polymer and compare it with phytosome of gingerol (GP).

2.MATERIAL AND METHODS

**Plant Materials**

The plant material (root) was collected from the forests of Paschim-Medinipur district of West Bengal during July 2017 and authenticated by the taxonomists of the Botanical Survey of India, Shibpur, Howrah, West Bengal, India. A voucher specimen has been kept in our research laboratory for further reference. After due authentication, fresh matured root were collected in bulk, cleaned thoroughly with distilled water, followed by shade drying for 14 days. The shade dried roots were coarsely powdered in an electrical grinder and preserved till further use.

**Preparation of the extract**

The powdered material (500 g) was defatted with petroleum ether and extracted with methanol for 48 h in a soxhlet extractor. The liquid extract was concentrated under vacuum to yield dried extractive. The percentage yield was calculated with respect to the dried plant material (yield: 8.7 %w/w). Preliminary phytochemical screening of the extract was performed using standard methods [32, 33].

**Preparation of the test samples**

The measured quantity of methanol extract of root of *Spondias mangifera* and glibenclamide (2.5 mg/kg) was suspended in 20% Tween 20 in distilled water and used as test drug for oral administration.

**Maintenance of Animals and approval of 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°C; relative humidity: 45-55 % under 12 h light/dark cycle), fed with standard rodent diet for one week in order to adapt to the laboratory conditions and water *ad libitum*. All experimental protocols were approved by the
Institutional Animal Ethics Committee (IAEC) of Netaji Subhas Chandra Bose Institute of pharmacy (Regd. No. 1502/PO/a/11/CPCSEA).

Acute toxicity study
The acute toxicity studies were conducted on Swiss albino mice as per the OECD guidelines 423, [34] where the test dose limit of 2000 mg/kg, p.o., was used. The test was carried out as suggested by Ganapaty et al., [35] and Shivhare et al. [36]. Immediately after dosing, the animals were closely observed for the initial 4 h after the administration and then once daily during the following days. The behavioral changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors and sleep. They were then kept under observation up to 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 Spondias mangifera was selected for antihyperglycaemic and antihyperlipidemic activity studies.

Determination of blood glucose levels
Fasting blood glucose concentration was measured, using a Gluco monitor, based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns (37, 38).

Screening for glucose lowering effects of test extract
The Screening for antihyperglycaemic activity was performed as per the standard procedures (39).

In multi dose treated normoglycaemic animals
The animals were fasted for 12 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), the rats were then divided into four groups. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route, Group II received glibenclamide (2.5 mg/kg) and served as reference control. Groups III and IV received the tested methanol extract at a dose of 200 and 400 mg/kg, respectively, in a similar manner. The tested methanol extract, standard drug and solvent were administered to respective group once daily for 30 days. Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was measured on 0, 5, 10, 15, 20, 25 and 30th day of treatment.

In multi dose treated alloxan induced diabetic animals
The animals were kept fasting for 24 h with water ad libitum and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. The animals were provided standard laboratory diet ad libitum after one hour. Under mild anesthesia the blood was withdrawn (0.1 ml) from the tip of the tail of each rat and the blood glucose level was checked before and after 48 h of introduction of alloxan monohydrate. Rats having the blood glucose level above 225 mg/dl were selected for antidiabetic study. The diabetic animals were segregated into four groups. Group I comprised as the diabetic rats (controls) which received only vehicle (2 ml/kg, p.o.). Group II received glibenclamide (2.5 mg/kg, p.o.). Animals of Group III and IV (diabetic rats) received the methanol extract of at 200 and 400 mg/kg, p.o respectively, for 30 days. The blood glucose level was measured on 0, 5, 10, 15, 20, 25 and 30th day of treatment.

Study of glucose utilization on isolated rat hemidiaphragm
The selected healthy rats were killed by decapitation and diaphragms were isolated quickly avoiding trauma and were divided into two halves. The hemidiaphragms were then placed in culture tubes containing 2 ml tyrode solution with 2g% glucose and incubated for 30 min at 37°C in an atmosphere of 95% O₂ – 5% CO₂ 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)). Following incubation, the hemidiaphragms were taken out and weighed. The glucose content of the incubated medium was measured. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium (40).

Effect of methanol extract on serum lipid levels
At the end of 30 days of treatment with the methanol extract, the animals were sacrificed by decapitation under ether anesthesia and blood samples were collected from test, standard and solvent treated groups including normal animal as reference. The serum supernatant was separated out by centrifugation and was subjected for the determination of the lipid profile studies such as total lipids, phospholipids, total cholesterol, triglycerides, HDL, LDL, VLDL and free fatty acids (41).

Statistical analysis
All the data is expressed as mean ± SEM, for six animals in each group. The differences between groups were evaluated by one way Analysis of Variance (ANOVA) followed by Dennett’s Multiple Comparison test. P<0.05 was considered significant.

3.RESULTS AND DISCUSSION
Preliminary phytochemical tests of the methanol extract of Spondias mangifera root revealed 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 blood sugar level of normoglycemc rats are depicted in Table1. The test
result indicates that, there is a significant reduction (p<0.05 to p<0.01) in blood glucose level from 15th day onwards, and registered 22.58 and 32.24% reduction at the end of 30 days, in animals treated with 200 and 400 mg/kg.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Experimental groups</th>
<th>0th Day</th>
<th>5th Day</th>
<th>10th Day</th>
<th>15th Day</th>
<th>20th Day</th>
<th>25th Day</th>
<th>30th Day</th>
<th>% decrease at 30th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Solvent Control</td>
<td>98.35±3.70</td>
<td>99.54±3.35</td>
<td>96.5±3.06</td>
<td>98.17±4.37</td>
<td>97.62±4.65</td>
<td>96.62±4.65</td>
<td>98.10±4.65</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Glibenclamide</td>
<td>96.5±3.13</td>
<td>88.5±2.54</td>
<td>81.5±3.35</td>
<td>61.5±3.75**</td>
<td>64.33±2.27*</td>
<td>62.5±2.45*</td>
<td>57.16±2.45**</td>
<td>40.76</td>
</tr>
<tr>
<td>III</td>
<td>Methanol extracts (200 mg/kg, p.o.)</td>
<td>97.45±4.23</td>
<td>95.35±4.98</td>
<td>90.14±4.75</td>
<td>85.25±5.57*</td>
<td>84.14±4.35*</td>
<td>80.35±4.74*</td>
<td>75.44±3.45*</td>
<td>22.58</td>
</tr>
<tr>
<td>IV</td>
<td>Methanol extracts (400 mg/kg, p.o.)</td>
<td>99.24±5.25</td>
<td>96.14±5.26</td>
<td>92.23±4.52</td>
<td>87.55±5.25*</td>
<td>85.45±4.35*</td>
<td>82.54±4.12*</td>
<td>67.24±4.52*</td>
<td>32.24</td>
</tr>
</tbody>
</table>

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 1: Effect of methanol root extract of S. mangifera on blood glucose in multidose treated on normoglycemic rats in oral route.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Experimental groups</th>
<th>0th Day</th>
<th>5th Day</th>
<th>10th Day</th>
<th>15th Day</th>
<th>20th Day</th>
<th>25th Day</th>
<th>30th Day</th>
<th>% decrease at 30th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Solvent Control</td>
<td>286.55±5.6</td>
<td>280.34±5.16</td>
<td>258.66±4.45</td>
<td>269.25±5.78</td>
<td>262.14±5.80</td>
<td>258.83±5.76</td>
<td>254.34±6.30</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Glibenclamide</td>
<td>283.55±6.2</td>
<td>187.66±6.24*</td>
<td>127.16±7.16*</td>
<td>105.84±7.55*</td>
<td>104.26±7.33*</td>
<td>105.84±7.55*</td>
<td>92.83±6.35**</td>
<td>68.43</td>
</tr>
<tr>
<td>III</td>
<td>Methanol extracts (200 mg/kg, p.o.)</td>
<td>279.38±5.2</td>
<td>249.5±6.82</td>
<td>212.43±5.22*</td>
<td>182.53±6.32*</td>
<td>176.13±7.42*</td>
<td>162.44±6.74*</td>
<td>154.83±7.72*</td>
<td>44.58</td>
</tr>
<tr>
<td>IV</td>
<td>Methanol extracts (400 mg/kg, p.o.)</td>
<td>280.35±5.5</td>
<td>216.23±5.27*</td>
<td>166.42±6.62*</td>
<td>147.25±6.45*</td>
<td>139.23±6.56*</td>
<td>136.44±7.56*</td>
<td>122.16±7.22*</td>
<td>56.42</td>
</tr>
</tbody>
</table>

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 root extract of S. mangifera on blood glucose in multidose treated in alloxan induced diabetic rats in oral route.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Incubation medium</th>
<th>Glucose uptake (mg/g/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tyrode solution with glucose (2 g%) + Diabetic control</td>
<td>4.68 ± 0.16</td>
</tr>
<tr>
<td>II</td>
<td>Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml)</td>
<td>8.46 ± 0.21**</td>
</tr>
<tr>
<td>III</td>
<td>Tyrode solution with glucose (2 g%) + Methanol extract (200 mg/ml)</td>
<td>4.83 ± 0.55</td>
</tr>
<tr>
<td>IV</td>
<td>Tyrode solution with glucose (2 g%) + Methanol extract (400 mg/ml)</td>
<td>5.87 ± 0.25*</td>
</tr>
<tr>
<td>V</td>
<td>Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml) + Methanol extract (200 mg/ml)</td>
<td>8.96 ± 0.36**</td>
</tr>
<tr>
<td>VI</td>
<td>Tyrode solution with glucose (2 g%) + Insulin (0.25 IU/ml) + Methanol extract (400 mg/ml)</td>
<td>10.37 ± 0.42**</td>
</tr>
</tbody>
</table>

Table 3: Effect of methanol root extract of S. mangifera on peripheral glucose-uptake by isolated rat hemi-diaphragm.
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 44.58% and 56.42% at 200 mg/kg and 400 mg/kg body weight respectively at the end of the 30th day of the study, whereas as the standard drug glibenclamide registered 68.43% of reduction at the same day of the study. However the individual data shows a statistical significance ranges between p<0.05 to p<0.001, throughout the experiment when compared with solvent control.

Table 4: Effect of root extracts of S. mangifera on serum lipid profile in alloxanised rats on 30th day of study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental groups</th>
<th>Serum Lipid Profile (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Lipids</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>111.55±6.75</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>389.27±20.25</td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide (2.5 mg/kg)</td>
<td>141.38±12.24**</td>
</tr>
<tr>
<td>IV</td>
<td>Methanol extracts (200 mg/kg, p.o.)</td>
<td>218.35±15.51**</td>
</tr>
<tr>
<td>V</td>
<td>Methanol extracts (400 mg/kg, p.o.)</td>
<td>211.82±26.51**</td>
</tr>
</tbody>
</table>

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.

Effect of methanol extract on glucose utilization by isolated rat hemidiaphragm

The results of study on glucose uptake by isolated rat hemidiaphragm are shown in Table 3, which reveals that the test extract at 200 mg/ml and 400 mg/ml concentration exhibited uptake of 4.83 and 5.87 mg/g/30min respectively, while only insulin showed 8.46 mg/g/30min. However, insulin and test extract combination respond to 8.96 and 10.37 mg/g uptake of glucose at the same time. The extent of glucose uptake differ significantly ranges from p<0.05 to p<0.001 when compared with diabetic control group.

Effect of methanol extract on serum lipid profile

The Table 4 illustrate 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 decrease 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 been recorded, when compared to diabetic control group, while the HDL levels were approaching almost normal values when compared to without treatment normal control group.

4. DISCUSSION

In the present study aims at extensive evaluation of methanol extract of Spondias mangifera 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 Spondias mangifera, found to possess dose dependent suppression of glucose level, with prolonged hypoglycemia at higher dose of 400mg/kg, which is almost same effect as that of synthetic drug glibenclamide.

All these glucose lowering effect of the extract may possibly due to the insulinotropic effect at the islet beta cell level as evidenced by the increased plasma insulin levels which can be comparable with that of standard glibenclamide and all these parameters are widely accepted as markers of insulinotropic effect (42). The activity of the methanol extract in increasing peripheral utilization of glucose by isolated rat hemidiaphragm, suggest that the extract may contribute to the insulinotropic effect or direct insulin like activity and extra pancreatic effect. (30).

Alloxan, a beta-cytotoxin, induces “chemical diabetes” by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation (43). This damages a large number of β-cells, resulting in decrease in endogenous insulin release, which leads to decreased utilization of glucose by the tissue (44). The results depicted in this study revealed that the sub-acute antiabetic, hypoglycemic and insulinotropic effects of methanol extract were similar to those of glibenclamide. The possible mechanism, by which the plant extract mediates its antiabetic action, is 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 dislipidemia characterized by elevated serum triglycerides and total cholesterol levels. The improvement of blood glucose levels caused by most hypoglycaemic treatments is associated with a reduction of serum triglycerides and total cholesterol (45, 46). The significant reduction in the levels of LDL, VLDL, TC, TG, FFA, phospholipids & total lipids and increase in the levels of HDL demonstrates that, the extract may have property to enhance the transcription of lipoprotein lipase similar to that of insulin. The above results indicated that the novel approach of GPLC drug delivery system combined the advantages of chitosan with GP, which showed better effects of promoting oral absorption and prolonging retention time of complex of gingerol loaded phytosome than gingerol phytosome or blank complex of methanol extract endowed with hypoglycaemic and antihyperglycaemic activity due to its possible action on pancreatic and extra-pancreatic site of glucose and lipid metabolism as evidenced by insulinotropist properties of the extract.

4. CONCLUSION

In the present study report clearly depicted that the Spondias mangifera methanol extract endowed with hypoglycaemic and antihyperglycaemic activity due to its possible action on pancreatic and extra-pancreatic site of glucose and lipid metabolism as evidenced by insulinotropist properties of the extract.

CONFLICTS OF INTEREST

The authors do not have any conflict of interest.

REFERENCE

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