HYPOGLYCEMIC AND HYPOLIPIDEMIC POTENTIALS OF ALANGIUM SALVIIFOLIUM (LINN. F). FLOWERS ETHYL ACETATE EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RAT MODELS

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ABSTRACT

The present study was carried out to evaluate the hypoglycemic and hypolipidemic activity of ethyl acetate extract flower extract of Alangium salviifolium (Linn. F). in STZ induced diabetic rats. Diabetes was induced into albino Wistar rats by intraperitoneal administration of STZ. Blood samples were collected from overnight fasted normal and diabetic rats on 1th, 5th, 10th and 15th days of treatment. Hypoglycemic activity was evaluated by measuring the serum glucose level and glycosylated haemoglobin level after dosing with ethyl acetate extract. Hypolipidemic activity was evaluated by measuring various biochemical parameter like total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein, high density lipoprotein and phospholipids. The results showed that the extracts 200 mg/kg and 400 mg/kg significantly (P < 0.001,p<0.01) reduced fasting blood glucose of STZ diabetic rats in a dose-related manner, when compared to control. The ethyl acetate extract has a significant recovery in the levels of parameters measured in lipid profile, when compared to control group. The present investigation established pharmacological evidence to support the folklore claim that it is a hypoglycemic and hypolipidemic agent.

KEY WORDS: Alangium sovifolium, flowers, Hypoglycemic activity, lipid profile

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INTRODUCTION

Diabetes mellitus (DM) is a serious metabolic disease which has several complications, including diabetic nephropathy, diabetic neuropathy, coronary heart disease and hypertension. It has been estimated that by the year 2010, the prevalence of DM worldwide will reach approximately 240 million. Patients with DM are more likely to develop and die from microvascular and macrovascular complications than the nondiabetic population. There is usually an association between coronary heart disease or atherosclerosis and dyslipidaemia. Dyslipidaemia is a frequent complication of DM and is characterized by low levels of HDL-cholesterol and high levels of LDL cholesterol and triglyceride. Several groups of hypoglycaemic drugs are currently available to treat DM. However, their toxic side effects and sometimes diminution in response after prolonged use are problematic. Management of DM to avoid these problems is still a major challenge. In the indigenous Indian system of medicine, a good number of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and the active principles isolated. However, search for new antidiabetic drugs were continues. India has one of the oldest, richest and diverse cultural traditions associated with the use of the plants and herbs for human, liver stock and plant health. Many of the ingredients of Indian cooking which have been handed down from ages contain medicinal properties. A vast ethnobotanical knowledge exists in India from ancient times. However, very few plants used by locals for medicines are subjected to scientific investigation. The need for conservation of medicinal plants and traditional knowledge, particularly in developing countries like India, taking into account the socio cultural and economic conditions is urgent. *Alangium salvifolium* (Linn. F) Wang. is commonly known as Ankolah, a well known medicinal plant in Ayurveda. It’s having synonyms like *Vangasena* and *Amruthaghrita*. Sushruta has mentioned *Ankolah* in the context of *Vasisth* and *Vagabatha* also followed the same. In *Nighantu* *A德拉sha*, *Ankolah* has mentioned as an emetic substance, although the *Brihatrayee* has not mentioned it as a *Vakama dravya* (emetic agent). Fruits of *Ankolah* are mentioned in *Ashmari cikitsa* (treatment of calculus) by Sushruta *Acharya* . *Acharya* has stated the properties of *Ankolah* flower (*Caraka. Sutrasasthana*-27/153). In another context *Acharya* has stated it as a flower for *visra* (poison), its system as a component of *Arunaghritha*. Sushruta has mentioned *Ankolah* in the context of *Musika Visa* (rat poison). *Acharya* Vagabatha also followed the same. In *Nighantu* Adrastra, *Ankolah* has mentioned as an emetic substance, although the *Brihatrayee* has not mentioned it as a *Vakama dravya* (emetic agent). Fruits of *Ankolah* are mentioned in *Ashmari cikitsa* (treatment of calculus) by Sushruta. *Acharya* has mentioned *Ankolah* fruit as a *gupta sneha* (internally possess unctuous substance). Chakra dutta and Vangasena has mentioned *Ankolah* as a *samgrahi* and remedy for *Alisara* (*diarrhoea*). *Acharya* Sodhala has mentioned *dhoopana* (fumigation) of *Ankolah* patram (leaf) can cure the poisoning effect of fish. Also root powder mixed with *tandulodaka* (rice water) can be applied in *Kamala* as snuffing. Rasaratnakar has opined oil prepared from *Ankolah* can be applied in umbilicus in case of early ejaculation. Various numbers of studies are available regarding the Pharmacognostical and pharmacological properties of *Ankolah* these all supports the classical uses of this important medicinal plant. Present review designed to file the all existing records concerning the Pharmacognostical and Phytochemical study of this important medicinal plant. Different text books and related information from the internet were collected and analysed. Review suggests that still assorted area of action of this plant yet to be explored to justify its traditional uses. It is a small tree 10-15 m height; branchlets apprisred - tomentose, sometimes spine tipped. Leaves variable, oblong - lanceolate, ovate or elliptic 5 -14 ×2 -2.5 cm, chartaceous, 3-5 nervet, base prominent, yellow, glabrous and glossy above, puberulous below, base obtuse, sub acute, margin entire, apex attenuate or sub acute, slightly retuse; petiole about 1 cm long, tomentose. Flowers to 2.5 cm long, 1.5 cm across in irregular axillaries cymes or clusters; bract ovate, 1 mm, deciduous; pedicel to 4 mm, jointed; buds terete, velvety.Calyx-tube cupular, 2.5 mm adnate to ovary, tomentose; lobes 10, triangular, ovate 0.5 mm sub equal. Petals 10, white, linearly oblong, 2.5 × 0.2 cm, calloge at base, tomentose andreflexed. Disc distinct. Stamens 20; filaments to 1 cm, with a fleshy and villous base, sub connate; anthers linear, to 1 cm, ovary turbinate to 2 mm, 1celled; ovule 1 per cell; style to 2 cm, glabrous, stigma capitale, obscurely fimbriate. Fruits berry globose, to 2 ×1cm, Crowned.

MATERIAL AND METHODS

Preparation of Alangium salvifolium flower ethyl acetate extract

Fresh flowers of *Alangium salvifolium* obtained from the Chandragiri, Chittoor district, were washed in tap water and then left to dry at room temperature for one week (7 days). The dried flowers were then ground to fine powder in a mixer. The dried flower powder was then extracted with ethyl acetate using a Soxhllet apparatus for 15hr. After filtration through cotton wool; the filtrate was concentrated at 650C by a rotavapor. The concentrate was then freeze dried to yield dried powder and were designated as *Alangium salvifolium* flower ethyl acetate extract.

Animal preparation

STZ- induced albino rat model

Male Wistar albino rats weighing between 200-250g were used in the study with the approval of the animal ethical committee of SVU, college of sciences S.V. University, Tirupati. Rats were housed in a 24 hrs light-dark cycle at 25 ± 2 °C. The animals were provided standard rat pellet feed and tap water. All animals were cared for in accordance with the principles and guidelines of the Institutional Animal Ethics Committee of department of zoology, SVU. Diabetes was induced in rats by tail vein injection of streptozotocin (50 mg/kg, i.v.) (Sigma chemicals) dissolved in normal saline. (One group of identical rats was kept without streptozotocin administration as normal control, group I). Forty eight hours after streptozotocin administration blood samples were drawn by retro orbital puncture and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood
glucose levels in the range of 270 to 300 mg/100 ml were selected for the studies. Following four groups of rats, were taken. Group I : normal control (NC) Group II: diabetic control (DC) –given (untreated rats) 0.5 ml of 5% Tween 80. Group III: diabetic rats given (200 mg/kg) ethyl acetate extract of *Alangium salvifolium* in 0.5 ml 5% Tween 80 Group IV: diabetic rats treated with (400 mg/kg) in 0.5ml 5% tween 80 (GT).The experiments were performed with subject to minimum pain to the experimenting animals. All the ethical considerations have been followed. The research was conducted in accordance with the ethical rules on animal experimentation, approved by Ethical committee, Department of Zoology, Sri Venkateswara University, Tirupati,(Approval No.49/2012-2013 / (i)/a/CPCSEA/IAEC/SVU/UKS-SVR. Dt.08-09-2012.

**Acute toxicity studies**
These studies were carried out to study the acute toxic effects and determine minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing between 20-30 g fasted overnight, were used for the study. Each extract was orally administered at doses of 30, 100, 300 and 3000 mg/kg body weight to separate groups of mice. Subsequent to administration of drug extracts, the animals were observed closely for the first three hours, for any toxic manifestations, like increased motor activity, salivation, clonic convulsions, coma and death. Subsequently observations were made at regular intervals for 24 hours. The animals were under further investigation up to a period of 1 week.  

**Experimental design**

**Oral glucose tolerance test**

The oral glucose tolerance test was performed in overnight fasted (16-h) normal animals. Rats divided into four groups were administered 200 mg/ kg bodyweight and 400 mg / kg body weight of ethyl acetate extract, respectively Glucose (2 g/kg) was fed 30 min after the administration of samples. Blood was withdrawn from the retro-orbital sinus at 0, 30, 60, 90 and 120 min of samples administration. Fastiging blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips (Accuchek, Roche Diagnostics, USA).

**Long term biochemical estimation in STZ- induced rat model**
The diabetic rats exhibiting blood glucose levels in the range of 270 to 300 mg/100 ml were selected for the studies. The treatments were continued daily for 25 days. Blood was collected by retro-orbital puncture for glucose estimation just before drug administration on the 1st day and 1 h after drug administration on days 0, 6, 12, 18, 24. Blood glucose (FBG) concentration of all the four experimental groups was determined by glucometer during different phases of the experiment by withdrawing blood from the retro orbital vein. For estimating serum lipid profile, serum was isolated from the blood collected on 25th day of *Alangium solvifolium* flower ethyl acetate extract treatment and serum total cholesterol (TC), triglyceride (TG) and HDL-cholesterol were estimated by using diagnostic kits (Erba Mannheim Cholesterol kit, Transasia Bio- Medicals Ltd., Daman). VLDL and LDL cholesterol were calculated as per Friedevald’s equation [30]: VLDL-cholesterol = Serum triglyceride- Cholesterol LDL-cholesterol = Serum total-cholesterol – VLDL-cholesterol – HDL-cholesterol. Results were expressed in mg/dl.

**Data and statistical analysis**
Results are expressed as mean ±Standard Error of Mean (SEM). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) using SPSS (version 10.0) and student’s ‘t’-test using Sigma Plot (version 8.0). The values of $P<0.05$ were considered as statistically significant.

**RESULT AND DISCUSSION**

**Table 1**

<table>
<thead>
<tr>
<th>Blood glucose concentration (mg/dl) OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>Group 2</td>
</tr>
<tr>
<td>Group 3</td>
</tr>
</tbody>
</table>

Values in each group are represented as means ± SD for 6 rats in each group

**Figure 1**

**Blood glucose concentration (mg/dl) OGTT**

Values in each group are represented as means ± SD for 6 rats in each group

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Table 2
Effect of long term evaluation of Alangium solvifolium flower ethyl acetate extract on blood glucose levels of STZ induced diabetic rat

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>0 day</th>
<th>6th day</th>
<th>12th day</th>
<th>18th day</th>
<th>24th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal control</td>
<td>88.56±4.66</td>
<td>89.43±3.44</td>
<td>81.24±2.78</td>
<td>81.20±7.11</td>
<td>84.16±2.32</td>
</tr>
<tr>
<td>Group 2</td>
<td>Diabetic control</td>
<td>284.96±4.91</td>
<td>295.21±5.71</td>
<td>300.68±1.17</td>
<td>300.40±6.068</td>
<td>297.18±9.26</td>
</tr>
<tr>
<td>Group 3</td>
<td>Extract treated with 200 mg/kg/bw</td>
<td>286.61±7.24</td>
<td>275.23±9.46</td>
<td>236.88±9.96</td>
<td>202.85±9.99</td>
<td>185.87±5.76</td>
</tr>
<tr>
<td>Group 4</td>
<td>Extract treated with 400 mg/kg/bw</td>
<td>279.47±6.32</td>
<td>205.27±7.60</td>
<td>198.01±8.51</td>
<td>140.42±8.93</td>
<td>116.67±6.27</td>
</tr>
</tbody>
</table>

Values in each group are represented as means ± SD for 6 rats in each group.

Table 3
Effect of treatment of Alangium salvifolium leaf extract on serum lipid profile (mg/dl) in Streptozotocin -induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Total Cholesterol</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal control</td>
<td>86.82±4.75</td>
<td>78.50±4.60</td>
<td>31.36±2.45</td>
<td>45.92±4.19</td>
<td>18.57±1.39</td>
</tr>
<tr>
<td>Groups 2</td>
<td>Diabetic control</td>
<td>219.49±5.05</td>
<td>164.55±3.77</td>
<td>20.43±2.80</td>
<td>170.88±3.05</td>
<td>37.98±2.72</td>
</tr>
<tr>
<td>Group 3</td>
<td>Extract treated 200 mg/kg/bw</td>
<td>142.49±4.93</td>
<td>120.90±1041</td>
<td>23.77±2.15</td>
<td>121.83±5.96</td>
<td>31.40±1.18</td>
</tr>
<tr>
<td>Group 4</td>
<td>Extract treated 400 mg/kg/bw</td>
<td>88.95±3.02</td>
<td>74.44±3.21</td>
<td>31.53±1.60</td>
<td>48.97±3.93</td>
<td>19.19±1.02</td>
</tr>
</tbody>
</table>

Values in each group are represented as means ± SD for 6 rats in each group.

Acute toxicity study revealed the nontoxic nature of the extract. There was no mortality and no toxic reactions found at any of the doses tested until the end of the study period. As per OECD guidelines, therapeutic range was considered between 1/10 to 1/5 times of LD<sub>50</sub>. Accordingly, 200 mg/kg and 400 mg/ kg BW doses of the extracts were selected for determination of pharmacological studies. The present study focused the scientific explanation about the hypoglycemic and hypolipidemic activity for both the extracts of flowers of Alangium salvifolium for the management of STZ induced diabetes. Experimental animals were made diabetic using STZ. STZ is a toxic glucose analogue, which selectively destroys insulin producing cells in the
pancreas when administered to rodents and many other animal species. This causes an insulin dependent diabetes mellitus in these animals, with characteristics similar to type-2 diabetes in humans. In diabetic rats, STZ led the elevation of fasting blood glucose level, which was maintained over a period of 24 days. Decrease in blood glucose level may be due to the regeneration of β-cells of the pancreas which was destroyed by STZ. Lipid play an important role in the pathogenesis of complications involved with diabetes mellitus. The elevated level of serum cholesterol, LDL, VLDL, triglycerides and reduced level of HDL possess to be a rises of factor for developing microvascular complication leading atherosclerosis and cardiovascular diseases like coronary heart disease. TC, TG, LDL and VLDL-cholesterol reduced significantly (P<0.05) whereas, the level of serum HDL-cholesterol was significantly increased. Alangium salvifolium flower extract treated group were showed the increased level of HDL as compare to normal treated group after 24 days. The abnormal high concentration of serum lipid in diabetic mainly due to increased mobilization of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase, insulin deficiency or insulin resistance may be responsible for dislipidimia. The present studies provide the introductory approach for the evaluation of its traditional preparations in order to scientifically validate the therapeutic use of Alangium salvifolium in the control of diabetes as well as maintenance of various biochemical parameters.

CONCLUSION

Our finding indicates that the ethyl acetate extract of Alangium salvifolium flowers may be useful for treatment of diabetes associated with hyperlipidemia. The extracts of Alangium salvifolium have potential to decrease blood glucose level as well as improving hyperlipidaemia and to reduce the complications associated with experimental diabetes. This study also supports the folklore usefulness of this plant in the treatment of diabetes. It can be concluded that the roots of this plant could be further investigated for antidiabetic bioactive principles.

CONFLICT OF INTEREST

Conflict of interest declared None.

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