Evaluation of Antidiabetic Activity of Ethanol Extracts of Leaves and Barks of *Alangium salvifolium* in Streptozotocin-Induced Diabetic Rats

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**Abstract**

*Alangium salvifolium* is a medicinal plant, used by herbalist for treating various diseases, one of which is diabetes mellitus in Central India. However, its antidiabetic activity has not been scientifically validated so far. The aim of the present study was to evaluate the antidiabetic activity of ethanol extracts of leaves and barks of *Alangium salvifolium* against streptozotocin (STZ)-induced diabetic rats. The ethanol extracts of leaves and barks of *Alangium salvifolium* at doses of 200 and 400 mg/kg body weight was administered orally to diabetic rats. The blood glucose levels were monitored at specific intervals and found significantly lowered the blood glucose level. Glibenclamide was used as a standard drug at a dose of 0.25 mg/kg. The effect of extracts on induced hyperlipidemia was analyzed where the extracts significantly lowered the elevated total cholesterol, triglycerides (TGL) and low density lipoprotein (LDL) level while increased the high density lipoprotein (HDL). Moreover, the decreased in body weight of rats after induction of diabetes, and increased in body weight of rats after treatment with extracts was observed. The experimental data exhibited that extract of leaves and barks of *Alangium salvifolium* has significant antidiabetic activity in streptozotocin-induced rats compared to standard drug. The ethanol extracts of leaves exhibited maximum antidiabetic activity as compared to barks extract.

**1 Introduction**

Diabetes mellitus often simply referred to as diabetes is a group of metabolic diseases in which a person has high blood sugar. Diabetes was first identified as a disease associated with "sweet urine," and excessive muscle loss in the ancient world. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine. It is a chronic disease that requires long-term medical attention both to limit the development of its devastating complications and to manage them when they do occur. Insulin is a hormone produced by the pancreas to control blood sugar. Diabetes can be caused by too little insulin in which pancreas does not make enough insulin, resistance to insulin that is muscle, fat, and liver cells do not respond to insulin normally, or both. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications, and ulceration. Thus, diabetes covers a wide range of heterogeneous diseases.

Diabetes was recognized with complete details and its types (Type 1 and Type 2 diabetes - that is insulin dependent and non insulin dependent) in the year, 1959. According to W.H.O estimates, by 2025 total 300 million of the worldwide population will be affected by diabetes. For every 21 seconds, someone is diagnosed with diabetes, an estimation given by American Diabetes Association. And, there are 20.8 million diabetics in US at present, which is roughly estimated as 7% of US population, out of this figure about 6.2 millions are unaware of the diabetes existence in there life. As we know this is one of the old diseases, existing in many individuals and still on rising charts. Hence, scientists are continuously working to relieve us from it, by discovering the relevant drugs and making new researches. The medicinal plants...
imparts chief role in controlling the diabetes, associated with minimum side effects compared to synthetic drugs.

*Alangium salvifolium*, commonly known as Sage Leaved Alangium, stone mango, hill sack tree and ancolah. *Alangium salvifolium* belongs to genus *Alangium*, family Alangiaceae. This family consists of twenty-two species out of which *Alangium salvifolium* is mainly used as medicine in India, China and Phillipines. It is the most versatile medicinal plants having a wide spectrum of biological activity.

*Alangium salvifolium* showed potent anticancer, diuretic, anti-inflammatory, antimicrobial, laxative, astringent, emollient, anthelmintic and antiepileptic activities. The plant was also reported for its anti fungal activity, anti microbial activity, cardiac activity and anti fertility activity. In Ayurveda almost all parts of the tree use for medicinal purposes. The roots and the fruits are used for the treatment of rheumatism, leprosy and hemorrhoid. Externally, it is used for the treatment of bites by rabbits, rats, and dogs. Root bark is an antidote for several poisons. Fruits are sweet, cooling and purgative and used as a poultice for treating burning sensation and haemorrhage. In the present study the antidiabetic activity of leaves and barks of *Alangium salvifolium* was explored to identify its medicinal properties.

2 Materials and methods

2.1 Plant material

The leaves and barks of *Alangium salvifolium* were collected from the forest area of Raipur (Chhattisgarh). The plant was authenticated by Taxonomist, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. A voucher specimen of the plant was preserved in the herbarium for further reference.

2.2 Preparation of extracts

500 gram of powdered of leaves and barks were successively extracted on a Soxhlet apparatus, employing petroleum ether, ethanol and distilled water respectively. The solvents of petroleum ether extract, ethanol extract and distilled water extract were removed by distillation and the last traces of solvent being removed under reduced pressure. The ethanol extract of leaves and barks of *Alangium salvifolium* were used for further pharmacological activity. The ethanol extract of leaves and barks of *Alangium salvifolium* were denoted as EASL and EASB respectively.

2.3 Acute toxicity study

Limit test at 2000mg/kg body weight was selected to perform acute toxicity study on selected according to the guidelines of the Organization for Economic Cooperation and Development (OECD)

Ratra et al. Evaluation of Antidiabetic Activity of *Alangium salvifolium*
The purpose of the study was to determine the dose needed to perform different pharmacological activities.

2.4 Antidiabetic activity

2.4.1 Oral glucose tolerance test (OGTT) of *Alangium salvifolium* extract

The oral glucose tolerance test was performed in overnight fasted (18 hours) normal rats. The rats were divided into seven groups (n = 6). Group I served as normal control rats, administered drinking water daily; Group II had glucose control rats; Group III rats were administered standard drug Gilbenclamide (0.5 mg/kg); Group IV rats were administered EASL (200 mg/kg); Group V rats were administered EASAL (400 mg/kg); Group VI rats were administered EASB (200 mg/kg); and Group VII rats were administered EASB (400 mg/kg). Glucose (2 g/kg) was fed to rats of Group II to Group VII, 30 minutes prior to the administration of the extracts and standard drug. Blood was withdrawn from the retro-orbital sinus after 0, 30, and 90 minutes of extract and standard drug administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase–peroxidase glucose estimation kit.

2.4.2 Induction of non-insulin dependent diabetes mellitus (NIDDM)

Non-insulin dependent diabetes mellitus was induced in overnight fasted adult Wistar strain albino male rats weighing 170 – 220 g by a single intraperitoneal injection of 60 mg/kg Streptozotocin, 15 minutes after i.p. administration of 120 mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours and then on day 7, after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as > 126 mg/dl. Only those rats that were found to have permanent NIDDM were used for the study.

2.4.3 Evaluation of antidiabetic activity of *Alangium salvifolium* extracts

The animals were segregated into seven groups of six rats each. The extract was administered for 28 days. Group I served as normal control rats, administered drinking water daily for 28 days; Group II had diabetic control rats, administered drinking water daily for 28 days; Group III rats were administered EASL (200 mg/kg); Group IV rats were administered EASL (400 mg/kg); Group V rats were administered EASB (200 mg/kg); Group VI rats were administered EASB (400 mg/kg); and Group VII rats were administered standard drug Gilbenclamide (0.5 mg/kg) for 28 days. The fasting glucose levels were determined on days 0, 7th, 14th and 28th of extract administration. During the experimental period, the rats were
weighed daily and the mean change in body weight was calculated.

2.4.4 Estimation of biochemical parameters
The biochemical parameters were determined on day 12 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were determined by the glucose oxidase method, using an auto-analyzer.

2.5 Statistical analysis
The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet’s test. A P < 0.05 value was considered as statistically significant.

3 Results
3.1 Acute toxicity study
Limit test at 2000 mg/kg body weight was selected to perform acute toxicity of ethanol extracts of leaves and barks of Alangium salvifolium on laboratory animals. In LD₅₀ studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed.

3.2 Selection of dose
The LD₅₀ of ethanol extracts of leaves and barks of Alangium salvifolium as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000 mg/kg. The pharmacological evaluations were carried out at doses of 200 to 400 mg/kg body weights.

3.3 Oral glucose tolerance effects of Alangium salvifolium
The effects of ethanol extracts of Alangium salvifolium on the plasma glucose level are shown in table 1. After administration of glucose in rats the rise in glucose level was observed in glucose control, extract treated and standard group. In rats treated with leaves and barks extracts of Alangium salvifolium, there was a significant reduction in plasma glucose level, while in glucose control rats the plasma glucose level increased. Meanwhile same results were observed in glibenclamide treated group.

3.4 Effect on non-insulin dependent diabetes mellitus of Alangium salvifolium
Induction of diabetes in experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of bark

Ratra et al. Evaluation of Antidiabetic Activity of Alangium salvifolium and root extracts of Alangium salvifolium, on serum glucose levels of normal and Streptozotocin-induced rats are shown in table 2.

Table 1: Effect of ethanol extracts of Alangium salvifolium on oral glucose tolerance test

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Normal Control</td>
<td>76.3±1.20</td>
</tr>
<tr>
<td>Glucose control</td>
<td>81.4±2.05</td>
</tr>
<tr>
<td>Glucose + Glibenclamide (0.5 mg/kg)</td>
<td>77.2±1.85</td>
</tr>
<tr>
<td>EASL (200 mg/kg)</td>
<td>75.7±1.72</td>
</tr>
<tr>
<td>EASL (400 mg/kg)</td>
<td>74.6±2.62</td>
</tr>
<tr>
<td>EASB (200 mg/kg)</td>
<td>75.9±3.18</td>
</tr>
<tr>
<td>EASB (400 mg/kg)</td>
<td>76.3±3.47</td>
</tr>
</tbody>
</table>

The animals treated with streptozotocin namely Group II, a significant increase in serum glucose level was observed on 0, 7th, 14th and 28th day when compared with normal group rats (Group I). The Group III received glibenclamide (0.5 mg/kg p.o.) showed significant decrease in serum glucose level when compared with diabetic control rats. After the oral administration of EASL in diabetic control rats, a significant reduction in blood glucose level was observed when compared with diabetic control rats. Moreover the administration of EASB in diabetic control rats, also significantly decreased the serum glucose level compared with diabetic control rats. The outcomes exhibited that EASL and EASB at dose of 400 mg/kg body weight significantly decreases the blood glucose level of diabetic rats on 7th day. While the EASL and EASB at dose of 200 mg/kg body weight significantly decreases the blood glucose level of diabetic rats on 14th day. From results it has been observed that the ethanol extracts of leaves exhibited maximum antidiabetic activity as compared to barks extract.

3.5 Anti-hyperlipidaemic activity of Alangium salvifolium
The outcomes of lipid profiles in control and experimental rats are exhibited in table 3. The rats of diabetic control showed significant increase in serum TGL, total cholesterol and LDL while increase in
HDL when compared with normal. The rat treated with glibenclamide also reduced TGL, total cholesterol, LDL, and increased HDL when compared with diabetic control group. The EASL and EASB showed significant decrease in total cholesterol, LDL, Triglycerides and significant increase in HDL when compared with diabetic control group. All these effects were observed on day 28. From result of lipid profile it has been observed that the leaves extract exhibited maximum antihyperlipidaemic activity on compared with barks extract. The present experimental result indicated that ethanol extracts of leaves and barks exhibited a potent blood glucose lowering properties in STZ diabetic rats.

Table 2: Effect of ethanol extracts of *Alangium salvifolium* on fasting plasma glucose level in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting plasma glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>78.2±2.58</td>
</tr>
<tr>
<td>Diabetic control <em>(Streptozotocin)</em></td>
<td>142.3±4.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EASL (200 mg/kg)</td>
<td>136.5±3.43</td>
</tr>
<tr>
<td>Diabetic + EASL (400 mg/kg)</td>
<td>139.2±2.62</td>
</tr>
<tr>
<td>Diabetic + EASB (200 mg/kg)</td>
<td>136.8±4.16</td>
</tr>
<tr>
<td>Diabetic + EASB (400 mg/kg)</td>
<td>140.3±2.71</td>
</tr>
<tr>
<td>Diabetic + Standard Glibenclamide (0.50 mg/kg)</td>
<td>138.6±4.87</td>
</tr>
</tbody>
</table>

Ethanol extract of *Alangium salvifolium* of leaves (EASL), Ethanol extract of *Alangium salvifolium* of barks (EASB), Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at <sup>a</sup>P<0.05 when compared with normal control group, <sup>*</sup>P<0.05 when compared with diabetic control group

Table 3: Determination of biochemical parameters after treatment with ethanol extracts of *Alangium salvifolium*

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipid Profile (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Normal control</td>
<td>84.2±2.32</td>
</tr>
<tr>
<td>Diabetic control <em>(Streptozotocin)</em></td>
<td>179.4±3.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EASL (200 mg/kg)</td>
<td>105.7±1.63&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EASL (400 mg/kg)</td>
<td>81.3±2.39&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EASB (200 mg/kg)</td>
<td>121.6±3.49&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EASB (400 mg/kg)</td>
<td>89.5±2.47&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Standard Glibenclamide (0.50 mg/kg)</td>
<td>86.1±2.82&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Ethanol extract of *Alangium salvifolium* of leaves (EASL), Ethanol extract of *Alangium salvifolium* of barks (EASB), Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at <sup>*</sup>P<0.05 when compared with normal control group, <sup>*</sup>P<0.05 when compared with diabetic control group
3.6 Effect on body weight by *Alangium salvifolium*

During the study, the body weights of rats before and after induction of diabetes, and after treatment were measured (Table 4). The results exhibited that decreased in body weight of rats after induction of diabetes, and increased in body weight of rats after treatment with extracts.

Table 4: Effect of ethanol extracts of *Alangium salvifolium* on changes in body weight in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in Body weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Induction</td>
</tr>
<tr>
<td>Normal control</td>
<td>169.8 ±1.32</td>
</tr>
<tr>
<td>Diabetic control (Streptozotocin)</td>
<td>178.2±4.25</td>
</tr>
<tr>
<td>Diabetic + EASL (200 mg/kg)</td>
<td>191.7±2.21</td>
</tr>
<tr>
<td>Diabetic + EASL (400 mg/kg)</td>
<td>186.1±4.27</td>
</tr>
<tr>
<td>Diabetic + EASB (200 mg/kg)</td>
<td>178.9±3.41</td>
</tr>
<tr>
<td>Diabetic + EASB (400 mg/kg)</td>
<td>192.5±3.47</td>
</tr>
<tr>
<td>Diabetic + Standard</td>
<td>184.6±3.89</td>
</tr>
</tbody>
</table>

Ethanol extract of *Alangium salvifolium* of leaves (EASL), Ethanol extract of *Alangium salvifolium* of barks (EASB)

4 Discussions

Streptozotocin, a mono functional nitrosourea derivative, derives diabetogenic activity due to its ability to induce oxidative stress and damage in β-cells. Streptozotocin can selectively attack pancreatic β-cells by producing free radicals of oxygen, nitrogen monoxide, and reducing intracellular NAD and NADP, which are crucial for the electron delivery and energy metabolism in β-cells.

Ratra et al. Evaluation of Antidiabetic Activity of *Alangium salvifolium* The diabetes was induced on rats after administration of streptozotocin. The EASL and EASB were screened for streptozotocin-induced antidiabetic activity. The EASL and EASB significantly reduced the blood glucose level in STZ-induced-diabetic rats as compared to the diabetic control group. Moreover the EASL and EASB increased the body weight of diabetic rats. The possible mechanism by which *Alangium salvifolium* brings about its hypoglycemic action in diabetic rat may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.26,27

Generally, it has been observed that hyperlipidemia is a complication associated with hyperglycemia. During study it was observed increase in total cholesterol, triglycerides, LDL, and decrease in HDL in streptozotocin induced diabetic rats as compared to normal animals. The EASL and EASB showed significant reduction in total cholesterol, LDL, Triglycerides and significant rise in HDL when compared with diabetic control group. The potent antidiabetic effect of the plant extract suggests the presence of potent antidiabetic active principles, which produced antihyperglycemic effect in diabetic rats. The outcomes of lipid profile confirmed the potent antidiabetic activity of leaves and barks of *Alangium salvifolium*.

In recent years, considerable interest has been directed towards the investigation of plasma lipids and lipoproteins pattern in diabetes mellitus due to the fact that abnormal lipid level leads to the development of coronary artery disease in diabetic patients.

In the present study, ethanol extract of bark and root of *Alangium salvifolium* had significantly decreased total cholesterol, triglycerides and LDL with increase in HDL which is having a protective function for the heart compared with diabetic control group.31-33

5 Conclusions

In present study, we selected *Alangium salvifolium* to evaluate its antidiabetic activity owing to its traditional uses. The findings of study exhibited that the oral administration of ethanol extract of leaves and barks of *Alangium salvifolium* exhibited significant antidiabetic effect in controlling the blood glucose level. Additionally, the extract decreased total cholesterol, triglycerides and LDL with increase in HDL at the end of the treatment. This confirms the potent antidiabetic effect of extracts. The ethanol extracts of leaves exhibited maximum antidiabetic activity as compared to barks extract. It can thus be concluded that this plant extract promises an effective breakthrough in its potential development as a powerful oral therapeutic agent for controlling and managing diabetes mellitus.

6 Conflict of interests

The authors declare that they have no competing interests.
7 Authors’ contributions

MR and RG designed overall experiments, and carried out animal study. All authors read and approved the final manuscript.

8 References

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