Antidiabetic activity of alcoholic leaves extract of *Alangium lamarckii* Thwaites on streptozotocin–nicotinamide induced type 2 diabetic rats

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**Article info**

**ABSTRACT**

**Objective:** To investigate antidiabetic potential of alcoholic leaves extract of *Alangium lamarckii* (*A. lamarckii*) on streptozotocin–nicotinamide induced type 2 diabetic rats. **Methods:** Oral glucose tolerance test was done by inducing hyperglycemic state via administration of glucose in water (2 g/kg). Single dose of alcoholic leaves extract of *A. lamarckii* (250 and 500 mg/kg, p.o.) were administered to normoglycemic, hyperglycemic rats. Type 2 diabetes was induced by single intraperitoneal injection of nicotinamide (110 mg/kg) followed by streptozotocin (65 mg/kg). The study also included estimations of blood plasma glucose, lipid profile, liver glycogen, body weight and antioxidant status in normal and diabetic rats. **Results:** Administration of alcoholic extract of *A. lamarckii* at two dosage 250 and 500 mg/kg, p.o. did not showed any significant change in blood glucose level of normoglycemic rats (*P* > 0.05), whereas, oral glucose tolerance test depicted reduction in blood glucose level (*P* < 0.05). The streptozotocin–nicotinamide induced diabetic rats, significantly decreased the blood plasma glucose level (*P* < 0.001) comparable to glibenclamide (10 mg/kg), restored the lipid profile and showed improvement in liver glycogen, body weight and antioxidant status in diabetic rats. **Conclusions:** Present finding demonstrated the significant antidiabetic activity of alcoholic leaves extract of *A. lamarckii*.

**1. Introduction**

Diabetes mellitus (DM) is a group of metabolic disorder characterized by elevated blood glucose level resulting from the defects in insulin secretion, insulin action, or both[1]. The world prevalence of diabetes among adults is expected to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% ie. about 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries[2]. However, among the two major types of diabetes ie. type 1 and type 2, type 2 DM is the commonest form of diabetes constituting 90%–95% of the diabetic population. It was also documented that the number of people diagnosed with type 2 DM globally is estimated to be at 2%–3% of the world population and is rising at a rate of 4%–5% per year[3]. Currently available oral hypoglycemic drugs for the treatment of DM have characteristic profile of adverse effects. Hence, research is focused to screen the medicinal plant that are used traditionally for the treatment of DM to find a newer lead drug molecule from phytoconstituents with more potential and lesser side effects than the existing hypoglycemic agents[4]. Many review articles research paper appeared in the journals and book showed that many plant used in the traditional system of medicine for the treatment of DM proved to be scientifically effective[5,6]. *Alangium lamarckii* Thwaites (Syn. *A. salviifolium*)(*A. lamarckii*) belongs to family Alangiaceae is found commonly in tropical forest of South India and occasionally grown in garden. The root, root bark, seeds and leaves of the plant is used in Indian system of medicine. The root and root bark of the plant are used as antihelmenthic and purgative, whereas fruits are used as cooling, nutritive and tonic. Leaves of *A. lamarckii* are useful for curing diabetes[7,8]. Decoction of bark has been used as an emetic in India[9]. Methanol extract of *A. salviifolium* flowers have shown to have antibacterial activity against both gram–positive and gram–negative bacteria[10]. Methanolic extract of root of *A. salviifolium* have shown analgesic and anti-inflammatory activities in albino mice[11]. The lyophilized powder extract

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of pulverized wood of *A. salviifolium* showed good antifungal activity against *Candida albicans* [12]. Ethanolic extract obtained from leaves of *A. salviifolium* have showed wound healing potential in rats by incision, excision; dead space (granulation) wound models [13]. *Alangium A and B* from root bark and akoline, lamarkine, alangine, akharkantine from bark, have been reported [14]. The present study was undertaken to scientifically evaluate the antidiabetic potential of alcoholic extract of *A. lamarckii* leaves in type 2 diabetic rats.

### 2. Materials and methods

#### 2.1. Chemicals

Streptozotocin (STZ) was obtained from Sigma–Aldrich Co., St. Louis, USA. Solvents were purchased from SD Fine Chemicals Ltd., Mumbai, India. All the chemicals used were of analytical grade. For estimation of blood glucose and other biochemical tests, kits were obtained from Span diagnostic Ltd, India.

#### 2.2. Plant material

The leaves of *A. lamarckii* Thwaites were collected from Panakudi, Tirunelveli District in the month of July 2009, identified and authenticated by Prof. V. Chelladurai, Research Botanist (Retd.), Palayamkottai, Tamilnadu, India. For future reference the voucher specimen (Specimen number–COG/AL/09) and the prepared herbarium was deposited at the Department of Pharmacuetics, Banaras Hindu University, Varanasi (U.P), India.

#### 2.3. Preparation of extract

The leaves of *A. lamarckii* were freed from foreign matter [15], dried under shade and were crushed to coarse powder from where 250 g was subjected to extraction by cold maceration method using 2 L ethanol (95%) as an extraction menstrum for 7 days. The alcoholic extract of *A. lamarckii* (AAL) was concentrated under reduced pressure to dryness. The yield of alcoholic extracts was 11.2% w/w. Extract was preserved in a desiccator till further use.

#### 2.4. Animals

Healthy male albino rats of Charles foster strain (150–200 g) were obtained from the Central Animal House (Reg. No. 542/02/a/b/CPSEA), Banaras Hindu University, Varanasi, India. All animals were maintained under standard environment conditions (22–28°C, 60%–70% relative humidity, 12 h dark : light cycle) and were fed with standard rat feed (Mona Laboratory Animal Feeds, Raman Dairy Vikas Udyog, India) and allowed to drink water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. All the experimental procedures conducted after the approval of ethical committee (No. Dean/2009–10/579) and were in strict accordance with institutional animal ethical committee guidelines for the care and use of laboratory animals.

#### 2.5. Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Co–operation and Development guidelines 425. Nulliparous and non pregnant healthy female rats were used for this study. After the oral administration of AAL in overnight fasted rats, animals were observed individually for 48 h and their behavioral and neurological changes such as tremors, convulsions, salivation, diarrhoea, sleep, lacrimation and feeding behavior in drug treated rats were observed for sign of acute toxicity [16].

#### 2.6. Normoglycemic study

Fasted rats were divided into 4 groups consisting of 6 animals in each group. Group I rats received vehicle only. Group II and III rats received AAL at the doses of 250 and 500 mg/kg, p.o, suspended in CMC (0.5% w/v) in a single dose. Group IV received Glibenclamide (10 mg/kg, p.o.) as standard drug dissolved in distilled water. Blood samples were collected by retro–orbital puncture method just prior to and at 1, 2, 4 and 6 h after dosing and glucose was estimated [17].

#### 2.7. Oral glucose tolerance test

Overnight fasted animals were separated in 4 groups of 6 rats each. Animals of all groups were administered with glucose (2 g/kg) orally by means of gastric intubation. Animal in group second and third were treated orally with ethanolic extract at a dose of 250 and 500 mg/kg, p.o. and group fourth (positive control) with glibenclamide (10 mg/kg), 30 min before the oral administration of glucose orally. Control animals were administered with equal volume of water only, blood sample were withdrawn from the retro orbital plexus of eye of each animals just after oral glucose administration (0, 30, 60, 90 and 120 min) after glucose challenge.

#### 2.8. Induction of STZ–nicotinamide induce diabetes in rats

Diabetes was induced in overnight fasted rats by single intraperitoneal injection (i.p.) of STZ (65 mg/kg) prepared in citrate buffer pH 4.5, 15 min after the i.p. injection of nicotinamide (110 mg/kg) prepared in normal saline according to previously described method [18]. Rats with marked hyperglycemia (fasted blood glucose level greater than 200 mg/dL) after one week of administration of STZ were selected and used for this study.

The rats were divided into five groups of six rats in each group:

- **Group I (NC):** Normal rats treated with vehicle alone;
- **Group II (DC):** Diabetic rats treated with vehicle alone;
- **Group III (D+AAL 250):** Diabetic rats treated with AAL at the dose of 250 mg/kg;
- **Group IV (D+AAL 500):** Diabetic rats treated with AAL at the dose of 500 mg/kg;
- **Group V (D+Glib 10):** Diabetic rats treated with glibenclamide at the dose of 10 mg/kg;

All rats except normal and diabetic control group were administered single dose of drug (orally) daily for 14 days. Normal and diabetic control group rat received equal volume of vehicle only. The day of administration of first
dose was considered the zero day of treatment. Blood samples were collected by retro-orbital plexus of eye under light ether anesthesia and fasting blood glucose levels were determined by glucose oxidase method on day 0th, 7th, and 14th with commercially available biochemical kit. Body weight of rats was also taken on day 0th and 14th. On 14th day, plasma lipid profiles were estimated using biochemical kits and liver glycogen levels were estimated using anthrone reagent[19]. Lipid peroxidation in liver was estimated by measuring thiobarbituric acid reactive substances (TBARS) using the methods of Nehius and Samuelson[20]. Catalase (CAT) was estimated by the method of Sinha[21] and the activity of superoxide dismutase (SOD) was assayed by the method of Kakkar et al[22]. Protein content in tissue homogenate was measured by the method of Lowry et al[23].

2.9. Statistical analysis

Values were represented as mean ± standard error of mean (SEM). Two-ways ANOVA followed by Bonferroni post test was performed for normoglycemic, oral glucose tolerance test and evaluation of blood glucose of STZ–nicotinamide induced diabetic rats. One–way ANOVA followed by Tukey’s multiple comparison test was applied for the statistical analysis of the rest of parameters. GraphPad Prism (version 4) software was used for all statistical analysis. P values <0.05 were considered significant.

3. Results

Acute toxicity study showed that AAL upto 5 000 mg/kg body weight did not show any sign of acute toxicity and was found to be safe. In normoglycemic study, AAL at two doses ie, 250 and 500 mg/kg orally did not reduce the plasma glucose in rats. However, the rats treated with glibenclamide, 10 mg/kg showed a marked reduction in glucose level (P<0.001) as compared to control 1 h after its administration (Figure 1). Administration of AAL at two doses 250 and 500 mg/kg orally half an hour prior to glucose load showed improved glucose tolerance in normal rats. Maximum effect was observed 30–60 min after the glucose load in rat treated with AAL 250 mg/kg (P<0.001) while in case of AAL 500 mg/kg, maximum effect was observed 30–90 min after glucose load (P<0.001). Glibenclamide (10 mg/kg) showed a significant decrease in plasma glucose level when compared to vehicle control animals (Figure 2).

Induction of diabetes in the experimental rats was confirmed by the presence of a high fasting plasma glucose level estimated on 0th day of administration of AAL. The effect of AAL on fasting plasma glucose level of STZ–nicotinamide induced animals are presented in Figure 3. The one week treatment of diabetic rats with 250 mg/kg orally did not significantly reduced the plasma glucose level (P>0.05), however continuation of treatment upto two week significantly reduced the plasma glucose level. Administration of AAL 500 mg/kg orally showed significant lowering of fasting plasma glucose level (P<0.001) in diabetic rats and was comparable to standard drug treated rats.

Table 1 shows the level of plasma lipid profile such as triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL). Plasma TG, TC, and LDL level were significantly elevated and HDL level was decreased in diabetic rats when compared with control rats. Treatment with AAL reversed the diabetes induced hyperlipidemia. A significant reduction of TG, TC, LDL(P<0.001) and increase in HDL level was observed after the treatment with AAL(P<0.01).

Figure 1. Effect of AAL on normal rats. *P<0.001 compared to normal control (Two–way ANOVA followed by Bonferroni post test).

Figure 2. Effect AAL on oral glucose tolerance test in normal rats. *P<0.05, **P<0.001 compared to normal control (Two–way ANOVA followed by Bonferroni post test).

Figure 3. Effect of AAL on the blood glucose level of streptozotocin–nicotinamide induced diabetic rats. △P<0.001 compared to normal control, *P<0.001 compared to diabetic control (Two–way ANOVA followed by Bonferroni post test).

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Effect of AAL on body weight and liver glycogen is shown
Table 1
Effect of AAL on lipid profile of streptozotocin–nicotinamide induced diabetic rats (mean ± SEM, n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>TG (mg/dL)</th>
<th>TC (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>80.70±5.43</td>
<td>76.53±4.82</td>
<td>37.94±1.00</td>
<td>22.44±5.43</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>160.99±5.50 △</td>
<td>122.38±7.99 △</td>
<td>22.53±2.05 △</td>
<td>67.64±7.62 △</td>
</tr>
<tr>
<td>III</td>
<td>D + AAL (250 mg/kg)</td>
<td>140.99±4.58</td>
<td>104.33±6.31</td>
<td>25.18±1.99</td>
<td>50.95±5.18</td>
</tr>
<tr>
<td>IV</td>
<td>D + AAL (500 mg/kg)</td>
<td>117.44±6.87 **</td>
<td>83.99±4.90 **</td>
<td>32.44±2.37 **</td>
<td>28.06±4.91 **</td>
</tr>
<tr>
<td>V</td>
<td>D + Gliben (10 mg/kg)</td>
<td>109.36±6.32 **</td>
<td>80.62±4.78 **</td>
<td>34.06±1.55 **</td>
<td>24.68±5.52 **</td>
</tr>
</tbody>
</table>

△P<0.001 compared to normal control, * P<0.01, ** P<0.001 compared to diabetic control (One-way ANOVA followed by Tukey’s Multiple Comparison test).

Table 2
Effect of AAL on body weight and liver glycogen in streptozotocin–nicotinamide induced diabetic rats (mean ± SEM, n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Liver glycogen (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0th Day</td>
<td>14th Day</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>176.66±6.41</td>
<td>181.83±6.01</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>174.16±8.07</td>
<td>131.16±7.12 △</td>
</tr>
<tr>
<td>III</td>
<td>D + AAL (250 mg/kg)</td>
<td>178.83±4.72</td>
<td>142.66±4.92</td>
</tr>
<tr>
<td>IV</td>
<td>D + AAL (500 mg/kg)</td>
<td>171.50±3.51</td>
<td>157.83±3.32 *</td>
</tr>
<tr>
<td>V</td>
<td>D + Gliben (10 mg/kg)</td>
<td>175.83±8.50</td>
<td>162.66±8.73 *</td>
</tr>
</tbody>
</table>

△P<0.001 compared to normal control, * P<0.05, ** P<0.001 compared to diabetic control (One-way ANOVA followed by Tukey’s Multiple Comparison test).

Table 3
Effect of AAL on TBARS, SOD and CAT in streptozotocin–nicotinamide induced diabetic rats (mean ± SEM, n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>TBARS (μmol/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (μ mol. H2O2 consumed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal control</td>
<td>22.87±2.67 △</td>
<td>1.02±0.09</td>
<td>250.67±8.06</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>52.06±4.59 △</td>
<td>0.37±0.06 △</td>
<td>167.52±11.15 △</td>
</tr>
<tr>
<td>III</td>
<td>D + AAL (250 mg/kg)</td>
<td>44.46±4.86</td>
<td>0.54±0.08</td>
<td>204.13±12.57</td>
</tr>
<tr>
<td>IV</td>
<td>D + AAL (500 mg/kg)</td>
<td>33.23±2.70 **</td>
<td>0.71±0.07 *</td>
<td>225.48±10.77 **</td>
</tr>
<tr>
<td>V</td>
<td>D + Gliben (10 mg/kg)</td>
<td>32.09±2.60 **</td>
<td>0.77±0.07 *</td>
<td>232.00±12.28 **</td>
</tr>
</tbody>
</table>

△P<0.001 compared to normal control, * P<0.05, ** P<0.01 compared to diabetic control (One-way ANOVA followed by Tukey’s Multiple Comparison test).

in Table 2. The body weight of the diabetic rats showed significant reduction after the administration of STZ–nicotinamide compared to normal control rats (P<0.001). The treatment of diabetic rats with AAL at a dose 500 mg/kg for two weeks showed a significant increase in body weight (P<0.05). The effect of AAL at 500 mg/kg dose level on body weight was comparable to that of glibenclamide. Administration of AAL at dose 500 mg/kg significantly increased the glycogen level which was equal to that of glibenclamide (P<0.001).

There was a significant reduction of SOD, CAT and elevation in TBARS level were observed in diabetic rats compared to control animals (P<0.001). The administration of AAL (500 mg/kg) significantly increased the SOD (P<0.05) and CAT (P<0.01), respectively and reduced TBARS level (P<0.01) (Table 3).

4. Discussion

In the present study type 2 diabetes was induced in male albino rats of Charles Foster strain via single intraperitoneal injection of STZ, which acts by selectively destroying beta cells of pancreatic islet along with nicotinamide, which has protective effect thus, causing only minor damage to cells [18]. The present study was conducted to assess the type 2 antidiabetic activity of AAL in STZ–nicotinamide induced diabetic rats. The ability of AAL to effectively control increased blood glucose level in diabetic rats may be attributed to its antihyperglycemic effect as normoglycemic study revealed that AAL did not cause any reduction of blood glucose level. However, AAL was administered to glucose loaded normal fasted rats resulting in hypoglycemia which suggest that, animals treated with extract have better glucose utilization capacity suggesting its mechanism being similar to biguanides. Biguanides do not increase insulin secretion. They promote tissue glucose uptake and reduce hepatic glucose output, thereby producing antihyperglycemic effect and not hypoglycemic effect [24]. Phytochemical analysis of A. lamarckii has showed the presence of various classes of chemical compound viz. alkaloids, terpenoids, steroids, tannins, phenols etc. [25]. Several authors reported that alkaloids, flavonoids, steroids/terpenoids, phenolic compounds are known to be bioactive antidiabetic principles [26-28]. The present antidiabetic study of alcoholic extract may be due to synergistic effect of different classes of compounds.

DM is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary heart disease [29]. Hyperlipidemia is a recognized complication of DM characterized by elevated levels of...
cholesterol, triglycerides and phospholipids, and changes in lipoprotein composition[30]. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to impairment of insulin secretion at diabetic state. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities[24]. In present study AAL significantly reduced the lipid profile in diabetic rats indicates its hypolipidemic activity and may also decrease the risk of vascular disease and related complications[31].

STZ-induced diabetes is characterized by severe loss in body weight which was observed in the present study, AAL at dose level 500 mg/kg and glibenclamide administration have showed significant improvement in body weight (P<0.05) of diabetic rats when compared with untreated—diabetic ones, thought that AAL treated rats did not normalize the body weight completely. The decrease in body weight observed in diabetic control might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source[32]. The treated groups enhanced glucose metabolism and thus, improved the body weight in STZ—diabetic rats. Liver play an important role in maintenance of blood glucose level by regulating its metabolism. The conversion of glucose into glycogen in liver depends on concentration of glucose and availability of insulin which stimulates glycogen synthesis, which occur in presence of enzyme glycogen synthase and glycogen phosphorylase[33]. The reduced glycogen store in diabetic rats may be attributed to reduced activity of glycogen phosphorylase and increased activity of glycogen phosphorylase[34]. Treatment of diabetic rats with AAL and glibenclamide significantly restored the level of hepatic glycogen which may be due to increased secretion of insulin from residual pancreatic beta cells attributed to stimulation of glycogen synthase and inhibition of glycogen phosphorylase.

The increased concentration of lipid peroxidation due to hyperglycemia induces oxidative damage by increasing peroxy and hydroxyl radicals[35]. The increased lipid peroxidation in the plasma and tissues of diabetic animals may be due to the observed remarkable increase in the concentration of TBARS and MDA as a main product of lipid peroxidation in the plasma and liver[36—44]. Administration of AAL decreased significantly TBARS in treated diabetic rats when compared with diabetic rats. Oxidative stress in diabetes is associated with decrease in the antioxidant status, which can increase the deleterious effects of free radicals. The SOD and CAT are the two major scavenging enzymes that remove free radicals in vitro. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion and hydrogen peroxide in biological systems, which generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation[45]. The result of increased activities of SOD and CAT suggest that AAL contains a free radical scavenging activity. The increased activity of SOD accelerates dismutation of O$_2^{•−}$ to hydrogen peroxide, which is removed by catalase[46].

In conclusion, the present study indicates that AAL have significant antidiabetic activity in STZ—nicotinamide induced diabetes. The antidiabetic activity may be due to improvement in glucose tolerance, restoration of liver glycogen and antioxidant activity of AAL can reduce the risk of secondary complications associated with diabetes. Further studies are needed to know the exact mechanism by which AAL bring back blood glucose towards normal level and bio—activity guided fractionation may unveil the constituent/s responsible for the anti—diabetic activity of AAL.

Conflict of interest statement

The authors report no conflict of interest.

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References


