Antihyperlipidemic Effect of Solanum incanum on Alloxan Induced Diabetic Wistar Albino Rats

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Abstract

The effect of orally administered aqueous fruit extract of Solanum incanum on serum lipid profile of Wistar Albino rats were determined. Twelve male and female Wistar Albino rats were randomly assigned into four groups of three rats each, following acclimatization to laboratory and handling conditions. Diabetes was induced with a single dose of alloxan (120 mg/kg) body weight and plasma glucose was taken 72 h after induction to confirm diabetes. The normal control was not induced. Animals in group A (normal control) and B (diabetic) were administered 0.5 ml of normal saline respectively. Group C was administered with 10 mg/kg weight of glibenclamide and group D was administered 500 mg/kg body weight of aqueous Solanum incanum extract. Extract administration lasted for fourteen days. Water and feeds were allowed ad libitum. After the two weeks treatment with the plant extract, blood samples were collected by cardiac puncture for lipid profile analysis by standard methods and enzyme kits. At the end of week two, the lipid profile of all groups were significantly different. The result on lipid profile showed that the extract treated group was significantly lower (P>0.05) in TC, TAG and VLDL as compared to diabetic control but significantly higher (P<0.05) in HDL and LDL as compared to diabetic control. The glibenclamide treated group was significantly lower (P>0.05) in TAG, I-ID L, and VLDL as compared to the diabetic control but significantly higher (P<0.05) in TC and LDL as compared to diabetic control. However, the extract was observed to have a high significant level of TC, TG, HDL, LDL and VLDL compared to the Normal control. Result on weight showed a significant difference at week and two, the lipid profile of all groups were significantly different. The result on lipid profile showed that the extract treated group was significantly lower (P>0.05) in TC, TAG and VLDL as compared to diabetic control but significantly higher (P<0.05) in TC, LDL as compared to diabetic control, however, the extract was observed to have a high significant level of TC, TG, HDL, LDL and VLDL compared to the Normal control.

Keywords: Diabetes; Hyperlipidemia; Solanum incanum; Glibenclamide; Alloxan monohydrate

Introduction

Background of the study

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism [1]. Experimental diabetes in animals provided a considerable insight into the physiologic and biochemical derangements of the diabetes state. Studies have shown that individuals with diabetes have a higher incidence of liver and kidney function abnormalities, as well as formation of free radicals due to glucose oxidative, non-enzymatic glycosylation of proteins and subsequent oxidative degradation of glycated proteins, leading to a decline in antioxidant defense mechanisms and damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin [2]. It is a long term chronic condition that causes high blood sugar levels. It is one of the common metabolic disorders with micro and macrovascular complications that results in significant morbidity and mortality. Diabetes mellitus results from defects in insulin secretion, insulin sensitivity or both and include a group of metabolic disorders characterized by hyperglycemia and abnormalities in carbohydrates, fats and protein metabolism [3]. It is considered as one of the five leading causes of death in the world [4,5]. It is recognized as a global epidemic by the world health organization [6].

Diabetes mellitus occur throughout the world and research has shown that its prevalence was higher and common in developed countries than in the developing countries in the mid ‘90s [7]. Globally as of 2010, an estimated 285 million people had diabetes [8]. In 2013, according to International Diabetes Federation, an estimated 381 million people had diabetes [9]. Its prevalence is increasing rapidly and by 2030, this number is estimated to almost double [10]. It is worry some to note that the major part of this numerical increase is expected to occur in developing countries where there is rapid urbanization, nutrition transition and increasingly sedentary lifestyles [11]. Patients with uncontrolled diabetes mellitus usually experience heart failure which indicates that hyperglycemia maybe responsible for the disease [12]. Hyperglycemia produces symptoms of polyuria, polydipsia and polyphagia. It is also associated with long term damage and failure of various organs such as eyes, kidney, liver, nerves, heart and blood vessels. Diabetes mellitus is associated with alteration in the plasma lipid and lipoprotein profile [13].

Current interest in the search for appropriate agents in the management of diabetes is therefore focused on traditional medicinal plants. Natural herbs are plants or plants part used for its scent, flavor or therapeutic properties. Herbal medicines maintain and improve health and they do not go through the testing that chemotherapeutic agents go through. Secondly, the plants by means of secondary metabolism, contain a variety of phytochemical ingredients that are thought to act on a variety of targets by various modes and mechanisms [14] as expected from its pathogenicity. Unlike chemotherapeutic agents, however, herbal remedies have been reported to be nontoxic, accessible...
and affordable and represent the first line of treatment available for many of the world’s population [15,16]. The interest in the use of herbal remedies could be attributed to the drawbacks in the use of the chemotherapeutic agents such as cost, hypoglycemia, weight gain, gastrointestinal disturbances and liver toxicity [17].

However of the several traditional plants reported for the treatment of diabetes [18-20] scientific interest and research reports have concentrated mostly on the screening of plants for blood sugar lowering effect to the neglect of investigation into their site and mechanism of action and for possible development into antidiabetic drugs and antihyperlipidemic drugs.

In indigenous medicine, *Solanum incanum* has a wide range of utilization from weight reduction to treatment of several ailments including asthma, allergic disease, swollen joint pains, gastro-esophageal reflux disease, constipation and dyspepsia. Scientific studies have supported the traditional use of this plant (*Solanum incanum*) in treating inflammation, asthma, glaucoma, diabetes and excessive weight gain [21]. The fruit is easily eaten as snack and it has been reported to be high in phytochemicals like Saponins, flavonoids, tannins and ascorbic acid [22]. Studies have shown that dyslipidemia associated non-communicable diseases like diabetes and obesity are on the increase in the developing world and a continuous study is required to identify indigenous plant materials that can mitigate against, or at least useful in the management of dyslipidemia [23-25]. Hence, the investigation of the influence of the consumption of *Solanum incanum* fruit on plasma lipid profile is of interest.

**Materials and Methods**

**Materials**

**Materials/Apparatus:** Fresh fruits of *Solanum incanum*, mortar and pestle, distilled water, glass bottle, ASTM 60 mesh, water bath, thermometer, Accu-check glucometer, test strips, cages for rats, weighing balance, micro pipette, centrifuge, dessicator, spectrophotometer, glucose, 15 male and female albino rats of wistar strain, were used for this study.

**Chemicals/Reagents utilized:** Alloxan monohydrate (St. Louis, MO., USA), Glibenclamide (Daonil; Aventis Pharma. Ltd., India), Cholesterol reagent (Teco Diagnostics., USA), Triglyceride GPO reagent (Teco Diagnostics., USA) and HDL Cholesterol reagent (Agappe Diagnostics., Switzerland) were obtained from a distribution company in Makurdi.

**Location of the study:** The entire study was carried out in Federal University of Agriculture Makurdi, Benue State. The plant extraction, acclimatization of rats, induction of diabetes, treatment of diabetes, biochemical analysis and statistical analysis were all carried out in the aforementioned institution.

**Plant sample collection:** Fresh fruits of *Solanum incanum* (bitter garden egg) were obtained from the university research farm of the Federal University of Agriculture, Makurdi after which it was washed and dried for further use.

**Experimental animals:** Healthy adult male and female wistar albino rats weighing about 140-180 g were obtained from the College of Health Sciences Animal House, Benue State University Makurdi and allowed to acclimatize for 30 days. The animals were housed in standard well ventilated cages at room temperature and provided with water and top feed growers pellets rat diet and water (Figure 1).

**Methodology**

**Experimental procedures for plant sample preparation:** The collected fresh fruits of *Solanum incanum* were washed and sliced into small pieces, partially sundried for one week to extract the powdered form of the plant after which it was shade dried for another one week. The dried pieces were then pounded gently into powder using mortar and pestle while the extract was then prepared from the powder [26-35].

**Experimental procedures for aqueous extract preparation:** 200 g of the powder was dissolved in 1600 ml of distilled water in a glass bottle for a period of 48 hours with intermittent vigorous shaking. The solution was filtered with ASTM 60 mesh size while the filtrate was then collected and evaporated at 45°C using water bath. The dried concentrate (extract) was then stored in a sealed transparent bottle for subsequent use.

**Experimental procedures for diabetes induction:** Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (120 mg/kg body weight) after 18 hours fast [26] while 5% glucose solution was administered orally so as to prevent the drug induced hypoglycemic effect of alloxan [27]. After 72 hours of alloxan injection, blood samples were collected by tail snip method to determine the blood glucose concentrations to confirm the development of diabetes mellitus. Rats with fasting blood glucose concentration of greater than 126 mg/dl [28] were considered hyperglycemic and were selected for the study [36-58].

**Experimental design:** The animals were randomly divided into four groups each containing three rats while each rat was marked using black stain; Group A received a mark on the head, Group B received a mark on the body, Group C received a mark on the tail, while Group D received a mark on both tail and ear. Each cage was identified by a label comprising the cage number, the dose of alloxan/treatment to be received by the animal, and the numbers and weight of the animals in each cage.
**Non-Diabetic group:**

**Group A:** (NORMAL CONTROL/non-diabetic rats): Were administered 0.5 ml normal saline only. On the 7th and 14th days of treatment, the blood glucose levels of the rats were determined using accu-check glucometer, the animals were then weighed to determine the effect of the plant extract on their body weights. The results obtained were then expressed in g of body weight and mg/dl of blood respectively.

**Diabetic groups:**

**Group B:** (NEGATIVE CONTROL/untreated diabetic rats): Served as diabetic control; receiving 0.5 ml normal saline/day/ rat.

**Group C:** (POSITIVE CONTROL/diabetic rats): Were administered Glibenclamide (10 mg/kg b.wt./day) in 0.5 ml normal saline as a fine aqueous suspension orally.

**Group D:** (TEST CONTROL /diabetic rats): Were administered aqueous extract of Solanum incanum (500 mg/kg b.wt./day) as a fine aqueous suspension orally in 0.5 ml normal saline [39-77].

**Percentage yield of extract:** The percentage yield of the plant sample can be calculated using the formula below:

\[
\text{Percentage Yield} = \left( \frac{\text{Weight of dried extract}}{\text{Weight of original sample}} \right) \times 100
\]

Weight of dried extract/weight of original sample x 100

Weight of dried sample = 9.53 g, weight of original sample = 200 g

9.53/200 X 100 = 4.765%

**Collection and treatment of sample:** The extracts were reconstituting in saline water and administered orally on daily basis. The extract group was treated with 500 mg/kg, while the diabetic control and the normal control were given 0.5 ml of saline water for a period of 14 days. At the end of 14 days, the fasting blood glucose level of all the animals were taken, the animals were weight and the animals were anaesthetized using chloroform and bled by cardiac puncture 24 h after the last treatment. The blood sample was collected in specimen bottles, allowed to clot and the serum separated by centrifugation for 10 min, and then stored at 37°C and subjected to biochemical analysis [78-89].

**Lipid profile Test Procedures**

**Determination of serum total cholesterol:** The principle: The enzymatic reaction sequence employed in the assay of cholesterol is as follows

Cholesterol Esters C. Esterase Cholesterol + Fatty acid

\[
\text{Cholesterol} + \text{O}_2 \rightarrow \text{C. Esterase Cholesterol} \rightarrow 2\text{H}_2\text{O} + 4\text{-Aminoantipyrine} + \text{p-HBS H. Peroxidase Quinoneimine} + 2\text{H}_2\text{O}_2
\]

(Reagent)

Cholesterol esters then hydrolyses to produce cholesterol. Hydrogen peroxide is then produced from the oxidation of cholesterol Oxidase. In a coupled reaction catalysed by peroxidase, quinoneimine dye colored red is formed from 4- aminoantipyrine, p-HBS and hydrogen peroxide. The absorption at 520 nm of the solution of this dye is proportional to the concentration of cholesterol in the sample. The routine procedures include:

Reagent were prepared according to instructions via Label. Test tubes were labelled: blank, standard, control, rat’s blood sample etc. 1.0 ml of reagent was pipetted to all test tubes and pre-warmed at 37°C for at least two minutes. 0.01 ml (10 µl) of sample was added to respective tubes, properly mixed, and returned to 37°C. All tubes were incubated at 37°C for ten minutes. The spectrophotometer was zeroed with the blank reagent at 520 nm. Absorbance of all tubes were read and recorded [90-105].

**Determination of triacylglyceride (TAG):** The enzymatic reaction sequence employed in the assay of TAG is as follows:

TAG Lipase Glycerol + Fatty Acids

Glycerol+ ATP Glycerol Kinase Glycerol -l-phosphate + ADP

Glycerol-l-phosphate + O_2 GIP Oxidase DAP + H_2O_3

H_2O_3 + 4AAP + DHBS Poxidase Quinoneimine Dye+ 2H_2O

The procedure involves hydrolysis of TAG by lipase. The glycerol concentration is then determined by enzymatic assay coupled with Trinder reaction that terminates in the formation of quinoneimine dye. The amount of the dye formed, determined by its absorption at 520 nm, is directly proportional to the concentration of TAG in the samples. The routine procedures include [106-110].

The triacylglyceride reagent was reconstituted according to instructions via label. Tubes were labelled: blank, standard, control, rat’s blood sample etc. 1.0 ml of reagent was pipetted to all tubes and pre-warmed at 37°C for 4 minutes. 0.01 ml (10 µl) of sample was to respective tubes, properly mixed and returned to 37°C. All tubes were incubated at 37°C for five (5) minutes. Spectrophotometer was zeroed with the reagent blank at 520 nm. The absorbance of all tubes was read and recorded (Teco Diagnostics, 2012).

**Determination of direct HDL cholesterol (HDLc):** The principles of biochemical analysis include: The chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) of serum are precipitated by phosphotungstic acid and magnesium ions. After centrifugation, High density lipoproteins (HDL) are in the supernatant. HDL content of the supernatant is measured by an enzymatic method. The routine procedures include [111-116].

**Precipitation:** The High density lipoprotein reagent was reconstituted according to instructions via label. Tubes were labeled: blank, standard, control, rat’s blood sample etc. 300 µl of sample and 300 µl of HDL reagent was pipetted into a test tube, it was properly mixed and allowed to stand for 10 min at room temperature, it was mixed again and centrifuged for 10 min, at 4000 rpm. After centrifugation, the clear supernatant was separated from the precipitate within 1 hour and the HDL cholesterol concentration was determined using the cholesterol reagent.

The tubes were labeled: blank, standard, control, rat’s blood sample, 1000 µl of cholesterol reagent was pipetted into each tube except the tube labeled standard, 50 µl of HDL supernatant was pipetted into the test tube labeled standard, it was mixed and incubated for five minutes at 37°C. The absorbance of the standard and sample was measured against the reagent blank.

**Determination of VLDL and LDL cholesterol by calculation:** The very low density lipoprotein was obtained by calculation using the formula: TG/5 (mg/dl). While Low density lipoprotein was calculated using the formula:

\[
\text{LDL} = \text{TC} - \text{TG}/5 \text{ (In mg/dl)}
\]

**Biochemical analysis:** The serum levels of total cholesterol and triglyceride and HDL were determined by Teco diagnostic, (2012) while the serum levels of LDL was measured according to the protocol of Friedewald [29].
**Statistical analysis:** Data was expressed as mean ± standard deviation. Comparative analyses between and amongst variables were done using analysis of variance (ANOVA). A post hoc comparison (LSD) test was performed to further ascertain significant differences between means. Statistical significance was set at P<0.05. All statistics were done using SPSS (Table 1).

**Results**

**Effect of Solanum incanum on plasma glucose level of wistar albino rats**

Mean values in the same column not followed by the same letter are significantly different at 0.05 level of significance.*Mean values of C (glibenclamide treated) and D (plant extract treated) are significantly different at 0.05 level of significance from those of B (diabetic control).†Mean values of C (glibenclamide treated) are significantly different at 0.05 level of significance from those of D (plant extract treated). Mean values of B (diabetic control), C (glibenclamide treated) and D (plant extract treated) are significantly different at 0.05 level of significance from those of A (normal control) (Figures 2 and 3).

Key: WAT: Weeks after treatment; A: Normal control; B: Diabetic control; C: Glibenclamide treated; D: *Solanum incanum* treated.

**Effect of Solanum incanum on body weight of wistar albino rats**

Mean values in the same column not followed by the same letter are significantly different at 0.05 level of significance.*Mean values of C (glibenclamide treated) and D (plant extract treated) are significantly different at 0.05 level of significance from those of B (diabetic control).†Mean values of C (glibenclamide treated) are significantly different at 0.05 level of significance from those of D (plant extract treated). Mean values of B (diabetic control), C (glibenclamide treated) and D (plant extract treated) are significantly different at 0.05 level of significance from those of A (normal control) (Table 2 and Figures 4, 5).

Key: A: Normal control; B: Diabetic control; C: Glibenclamide treated; D: *Solanum incanum* treated.

**Effect of Solanum incanum on lipid profile of wistar albino rats**

Mean values in the same column not followed by the same letter are significantly different at 0.05 level of significance.*Mean values of C (glibenclamide treated) and D (plant extract treated) are significantly different at 0.05 level of significance from those of B (diabetic control).†Mean values of C (glibenclamide treated) are significantly different at 0.05 level of significance from those of D (plant extract treated). Mean values of B (diabetic control), C (glibenclamide treated) and D (plant extract treated) are significantly different at 0.05 level of significance from those of A (normal control) (Table 3 and Figure 6).

Key: A: Normal control; B: Diabetic control; C: Glibenclamide treated; D: *Solanum incanum* treated; TC: Total Cholesterol; TAG: Triacylglycerol; HDL: High Density Lipoproteins; LDL: Low density Lipoproteins; VLDL: Very Low Density Lipoproteins.

**Discussion**

Dyslipidemia is a metabolic disorder that constitutes a crucial risk factor of atherosclerosis and cardiovascular diseases. Diabetes progresses with alteration in the serum lipid profile which can result in dyslipidemia [30]. It has been demonstrated that insulin deficiency in diabetes mellitus leads to accumulation of lipids such as total cholesterol and triglycerides in diabetic patients (Sharma et al.). In uncontrolled diabetes mellitus, increase in total cholesterol, triglyceride, LDL, VLDL cholesterol with decrease in HDL cholesterol which contributes to coronary artery disease have been observed [31].

The relationship between diabetes and hyperlipidemia is a well recognised phenomenon. The increased risk of coronary artery...
in the lipid profile in alloxan-induced diabetic Wistar Albino rats after 1st and 2nd weeks of treatment as shown in Table 4, suggesting that the extract may be used to reduce heart failure a view shared by Okutan [12]. It is thought that this reduction in lipid profile level by the plant extract may be related to its phytochemical constituent such as Saponins, flavonoids and phenolic compounds [38], which confers hypolipidemic activity on Solanum incanum.

My present findings indicated that lipid profile total cholesterol (TC), Triglycerol (TAG), and Very Low Density Lipoprotein (VLDL) was significantly decreased (P<0.05) in diabetic rats treated with Solanum incanum extract as compared to the non-treated group but significantly increased (P<0.05) in Density Lipoprotein (HDL) and Low Density Lipoprotein as compared to the diabetic group as shown in Table 4.

These findings support the work of Chinedu 2013 [39] which demonstrated that Solanum aethiopicum, a member of the Solanaceae family decreased the raised serum total cholesterol (TC) levels in treated rats. The only contradicting evidence between my findings and that of Chinedu 2013 [39] is that while LDL increased (P<0.05) in my work, Chinedu [39] showed a reduction in LDL. An increase in low density lipoprotein (LDL) may be due to increased body Weight in the rats treated with the extract, as initial approaches to lowering the levels of lipids in subjects with diabetes should include glycemic control, diet weight loss and exercise [32]. These findings also agrees with the work of Arulmozhi [40] who reported that Solanum nigrum, a member of the Solanaceae family increased low density lipoprotein (LDL) levels. While HDL increased (P<0.05) in my work, Arulmozhi [40] reported a reduction in HDL. Also, while TC, VLDL and TAG reduced (P<0.05) in my work, Arulmozhi [40] reported an increase in TC, VLDL and TAG.

The glibenclamide treated group was significantly lower (P<0.05) in TAG, HD, L, and VLDL as compared to the diabetic control but significantly higher (P<0.05) in TC and LDL as compared to diabetic control as shown in Table 4. The extract was significantly lower (P<0.05) in TC and LDL as compared to glibenclamide treated group but significantly higher (P<0.05) in TAG, HDL and VLDL as compared to glibenclamide treated group as shown in Table 4. The glibenclamide treated group was significantly lower (P<0.05) in TAG, HDL and VLDL as compared to the extract treated group but significantly higher (P<0.05) in TC and LDL as compared to extract treated group as shown in Table 4. However, the extract and the standard drug was observed to have high significant level of TC, TAG, HDL, LDL and VLDL compared to the Normal control as shown in Table 4.

A reduction in triglycerol level may be due to decreased lipogenesis increased lipolytic activity by inhibition of hormone sensitive lipase or the lipidemic enzymes or activation of some serum lipase as have been proposed for some anti-diabetic plants [41,42], exhibiting hypoglycemic activity as observed in this present study. The extract showed a significant decrease (P<0.05) on the plasma glucose level as shown in Table 4. Loss of body weight is a major consequence of disease in subjects with diabetes can be partially explained by the lipoprotein abnormalities associated with diabetes mellitus [32]. Hypercholesterolemia and hypertriglyceridemia are independent major risk factors that alone or together can accelerate the development of coronary artery disease [33].

Derangements in lipid metabolism which result in hyperlipidemia have been reported in the pathology of various human diseases such as diabetes and atherosclerosis [34]. It has been well established that reduction of total cholesterol or low density lipoprotein cholesterol (LDLc) could lead to decreased risk of atherosclerosis and coronary heart diseases [35].

When rats are injected with alloxan, they provide an animal model of insulin dependent diabetes mellitus [36]. The intraperitoneally administration of alloxan (120 mg/kg.wt) selectively destroys the insulin producing beta-cells of the Islet of Langerhans in the pancrease [37]. In this present study, there was severe hyperglycemia in the experimental rats as a result of alloxan induction in albino rats.

The result of the present findings demonstrated that aqueous fruit extract of Solanum incanum induced a significant reduction (P>0.05) as shown in Table 4. Loss of body weight is a major consequence of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC (mg/dl)</th>
<th>TAG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>140.37 ± 26.65*</td>
<td>100.50 ± 26.96*</td>
<td>44.43 ± 4.33*</td>
<td>111.33 ± 21.57*</td>
<td>20.10 ± 5.38*</td>
</tr>
<tr>
<td>B</td>
<td>309.80 ± 39.17**</td>
<td>179.87 ± 29.50**</td>
<td>47.07 ± 7.69*</td>
<td>225.77 ± 58.17**</td>
<td>35.97 ± 5.90**</td>
</tr>
<tr>
<td>C</td>
<td>339.20 ± 28.99***</td>
<td>129.80 ± 72.05***</td>
<td>34.27 ± 21.51***</td>
<td>284.60 ± 55.03***</td>
<td>25.96 ± 14.41***</td>
</tr>
<tr>
<td>D</td>
<td>301.77 ± 67.27***</td>
<td>165.57 ± 28.23***</td>
<td>69.87 ± 34.09***</td>
<td>254.13 ± 69.16***</td>
<td>33.65 ± 5.65***</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.002</td>
<td>0.18</td>
<td>0.266</td>
<td>0.017</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD, n=4. **Table 3: Effect of Solanum incanum on serum lipid profile of Wistar Albino Rats.**
Cholesterol metabolism must be investigated in order to confirm the oxidase or any superoxide generally enzymes which are involved in extract on LDL oxidation, HMG CoA synthase, macrophages, NADPH observed pharmacological activities. The direct or indirect effect of the isolate and characterize the actual compound(s) responsible for the effect on lipid profile result.

**Conclusion**

In conclusion, the result of the present study shows that indeed the aqueous fruit extract of *Solanum incanum* can protect against hypercholesterolemia and hypertriglyceridemia. The mechanism(s) of its antilipidemic action may be related to its strong antioxidant, antilipidemia, and protection against endothelium dysfunction as also suggested by Arulmozhi [40].

**Recommendations**

The following recommendations should be considered in subsequent research works: Research on the effect of *Solanum incanum* on lipid profile that will extend to 5th or 6th weeks of study period is required in order to observe adequate changes in the lipid profile of alloxan-induced diabetic Wistar Albino rats. Research on the effect of *Solanum incanum* on lipid profile that will involve different doses should also be carried out in order to investigate the changes occurred if they are dose-dependent. Subsequent researches should also investigate the effect of the duration of acclimatization on the body weight of Wistar Albino rats as overweight may also pose an effect on lipid profile result.

I also recommend that further work need to be carried out to isolate and characterize the actual compound(s) responsible for the observed pharmacological activities. The direct or indirect effect of the extract on LDL oxidation, HMG CoA synthase, macrophages, NADPH oxidase or any superoxide generally enzymes which are involved in cholesterol metabolism must be investigated in order to confirm the actual mechanism(s) of action of *Solanum incanum* whether it is related to its strong antioxidant, antihyperlipidemic and protection against endothelium dysfunction.

**References**


83. Kang ralkar VA, Patil SD, Bandivadekar RM (2010) Oxidative Stress and


85. K cannot be determined from the image.


APPENDIX

Table 4.1: Statistical Analysis of Treatment with *Solanum Incanum* Extract on Fasting Plasma Glucose Level of Alloxan-Induced Diabetic Wistar Albino Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before Induction (mg/dl)</th>
<th>72 Hours After Induction (mg/dl)</th>
<th>1 WAT (mg/dl)</th>
<th>2 WAT (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>75.67±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>114.00±10.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.00±3.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.67±19.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>78.66±4.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137.67±10.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.67±7.02&lt;sup&gt;h&lt;/sup&gt;</td>
<td>82.67±3.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>73.33±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.67±16.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.00±2.00&lt;sup&gt;h&lt;/sup&gt;</td>
<td>90.67±17.47&lt;sup&gt;a&lt;/sup&gt;††</td>
</tr>
<tr>
<td>D</td>
<td>73.67±3.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.67±15.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.00±1.00&lt;sup&gt;h&lt;/sup&gt;</td>
<td>78.67±6.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.154</td>
<td>0.982</td>
<td>0.006</td>
<td>0.737</td>
</tr>
</tbody>
</table>
Table 4.2: Statistical Analysis of Treatment with *Solanum Incanum* Extract on Body Weight of Alloxan-Induced Diabetic Wistar Albino Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 WAT (g)</th>
<th>2 WAT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>137.0±24.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>140.3±8.50&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>200.0±6.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>199.6±24.68&lt;sup&gt;b&lt;/sup&gt;†</td>
</tr>
<tr>
<td>C</td>
<td>200.0±20.88&lt;sup&gt;b&lt;/sup&gt;††</td>
<td>193.0±19.08&lt;sup&gt;c&lt;/sup&gt;††</td>
</tr>
<tr>
<td>D</td>
<td>205.0±17.52&lt;sup&gt;a&lt;/sup&gt;†</td>
<td>218.0±13.08&lt;sup&gt;a&lt;/sup&gt;†</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 4.3: Statistical Analysis of Treatment with *Solanum Incanum* Extract on Fasting Serum Lipid Profile of Alloxan-Induced Diabetic Wistar Albino Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC (mg/dl)</th>
<th>TAG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>140.3±26.65&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100.5±26.96&lt;sup&gt;d&lt;/sup&gt;</td>
<td>44.4±4.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111.3±21.57&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.1±5.39&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>309.8±39.17&lt;sup&gt;b&lt;/sup&gt;†</td>
<td>179.8±29.50&lt;sup&gt;b&lt;/sup&gt;†</td>
<td>47.0±7.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>225.7±58.17&lt;sup&gt;c&lt;/sup&gt;†</td>
<td>35.9±5.90&lt;sup&gt;c&lt;/sup&gt;†</td>
</tr>
<tr>
<td>C</td>
<td>339.2±28.99&lt;sup&gt;a&lt;/sup&gt;††</td>
<td>129.8±72.05&lt;sup&gt;c&lt;/sup&gt;††</td>
<td>34.2±21.51&lt;sup&gt;c&lt;/sup&gt;††</td>
<td>284.6±55.03&lt;sup&gt;a&lt;/sup&gt;††</td>
<td>25.9±14.41&lt;sup&gt;b&lt;/sup&gt;††</td>
</tr>
<tr>
<td>D</td>
<td>301.7±67.37&lt;sup&gt;c&lt;/sup&gt;†</td>
<td>165.5±28.23&lt;sup&gt;b&lt;/sup&gt;†</td>
<td>69.8±34.09&lt;sup&gt;a&lt;/sup&gt;†</td>
<td>254.1±69.16&lt;sup&gt;b&lt;/sup&gt;†</td>
<td>33.6±5.65&lt;sup&gt;a&lt;/sup&gt;†</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.002</td>
<td>0.180</td>
<td>0.266</td>
<td>0.017</td>
<td>0.180</td>
</tr>
</tbody>
</table>

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Figure 1.1: Bitter garden egg (*Solanum incanum*)
Figure 3.1: Wistar Albino Rats
Lipid Profile formulas

\[
\text{Triacylglycerol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of Standard (mg/dl)}
\]

\[
\text{Total cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}
\]

\[
\text{HDL Cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times N \times 2
\]

Where, \(2= \text{dilution factor of the sample, } N= \text{standard concentration}\)

\[
\text{LDL Cholesterol (mg/dl)} = \text{Total Cholesterol} - (\text{HDL Cholesterol} + \text{Triacylglycerides/5})
\]

\[
\text{VLDL Cholesterol} = \text{Triacylglycerides/5}
\]