IN VITRO AND IN VIVO ANTIDIABETIC ACTIVITY OF METHANOL EXTRACT OF *TERMINALIA CATAPPA* LINN BARK IN STZ INDUCED DIABETIC RATS

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ABSTRACT

The bark of *Terminalia catappa* was collected, authenticated, dried, powdered and extracted with petroleum ether, chloroform, ethylacetate and methanol. In vitro antidiabetic activity was conducted for extracts of *Terminalia catappa*. The methanol extract was found to be active after the results were compared with acarbose. In vivo antidiabetic activity was conducted for methanol extract, controle and standard on STZ induced diabetic rats. Measurement of blood glucose and biochemical parameters were conducted. The methanol extract shown significant antidiabetic activity at dose levels 1/5 of lethal dose. Histological studies of pancreas of rats were done. The results were compared with glibenclamide. Methanolic extract of *Terminalia catappa* bark exhibited significant antidiabetic activity in streptozotocin induced diabetic rats. This extract showed improvement in parameters like body weight and lipid profile as well as regeneration of \(\beta\)-cells of pancreas. Hence, it has value in treatment of diabetes.

KEYWORDS: Antidiabetic activity, Terminalia catappa, STZ, Extraction, Insulin.

INTRODUCTION

Diabetes mellitus is a chronic disease caused by deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced, such a deficiency results in
increased concentrations of glucose in the blood, which cause retinopathy, neuropathy, nephropathy, cardiovascular problems and damage to blood vessels. The number of people with diabetes from day to day multiplies worldwide. It is projected to become one of the world’s main disablers and killers within the next 25 years. The management of diabetes is a global problem until now and successful treatment is not yet discovered. There are many synthetic medicines developed for diabetes but they produce undesirable side effects. Hence it is required to shift towards the different indigenous plant and herbal formulations.

*Terminalia catappa* Linn is found throughout the warmer parts of India and called as Indian Almond, Malabar Almond and Tropical Almond. It is a medium sized tree with leaves clustered towards the ends of the branches. The various extracts of leaves and bark of the plant have been reported to be anticancer, anti-HIV reverse transcriptase, hepatoprotective, anti-inflammatory, hepatitis and aphrodisiac.[1-6] They are also reported to contain phytochemicals which are potent in treatment of diabetes. The various extracts of leaves and fruits are reported as potent antidiabetic drug.[7-9] Terminalia catappa is rich in tannins that are reported to be antidiabetic.

In view of alleged antidiabetic potential of leaves and fruits of *Terminalia catappa*, we have investigated effect of extracts of its bark on fasting blood glucose levels and serum biochemical analysis in streptozotocin induced diabetic rats.

**MATERIAL AND METHODS**

**2.1 Plant material**

The bark of *Terminalia catappa* was collected in October, 2013 from Warangal and authenticated at department of botany, Kakatiya University, Warangal, India. A voucher specimen of the plant has been deposited at the department of botany, Kakatiya university, Warangal, India.

**2.2 Preparation of extracts**

The bark was cut into pieces and shade dried at room temperature. The dried bark was subjected to size reduction to coarse powder. This powder was packed into soxhlet apparatus and extracted successively with petroleum ether, chloroform, ethylacetate and methanol (% yield 2, 1, 2.2 and 8). All the extracts were dried at room temperature and stored in refrigerator. The suspensions of extracts were prepared by using 0.5% Tween-80 in normal saline.
2.3 In vitro α-glucosidase inhibition activity

**Isolation of α-glucosidase enzyme from rat small intestine**

A male rat (200 g) was sacrificed by cervical dislocation. The small intestine was obtained and flushed several times with ice-cold NaCl (0.9% w/w). The intestine was cleaned from adipose tissue and cut longitudinally. The mucosa was scraped with a glass slide on an ice-cold glass surface. The obtained material containing α-glucosidase was homogenized with 20 ml of sodium phosphate buffer and stored at -25°C until used. Total protein content was determined by the Lowry method.\cite{10}

2.4 Determination of α-glucosidase inhibition

To all the test tubes 0.5 ml of Sodium phosphate buffer (80 Mm), pH 7.0 containing 37 mM sucrose was taken and to the test tubes 1 ml of various concentrations of test sample and standard was added. For the control and blank wells 1 ml of phosphate buffer pH 7.0 was added. The reaction was initiated by adding 50μl of crude enzyme to all the tubes except blank. All the samples were incubated at 37°C for 20 min. The reaction was then stopped by heating the test tubes at 95°C for 1.5 min. The liberated glucose was measured using commercial glucose kit. The percentage inhibition was calculated by using the following formula.

\[
\text{% inhibition} = 100 - \left[ \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100 \right]
\]

\[A_{\text{sample}} = \text{absorbance of the sample,}\]

\[A_{\text{control}} = \text{absorbance of the control}\]

The results of the in vitro α–glucosidase inhibition activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Terminalia catappa* are given in Table 1. Among the samples only methanol extract was show good inhibition activity (IC\textsubscript{50} 56.18 ± 0.5 μg/ml) and the results were comparable to standard, acarbose (IC\textsubscript{50} 38.05 ± 0.3 μg/ml).

Table 1: In vitro α-glucosidase inhibition activity of extracts of *Terminalia catappa*

<table>
<thead>
<tr>
<th>Name of the extract</th>
<th>IC\textsubscript{50}(μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>Not active</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Not active</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Not active</td>
</tr>
<tr>
<td>Methanol</td>
<td>56.18±0.5</td>
</tr>
<tr>
<td>Acarbose</td>
<td>38.05±0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n=3
2.5 In vivo antidiabetic activity

2.5.1 Animals
Wistar albino rats of either sex weighing 180-220 g (6 to 8 weeks) with no prior drug treatment were used for the present experiment. The rats were kept on feeding (Amrut laboratory animal feeds, Pranav Agro industries Ltd, Sangli) and provided water ad libitum. The animal experiment was approved by the Institutional Animal Ethical Committee (PIPS/IAEC/14/13/45).

2.5.2 Induction of diabetes
Streptozotocin was purchased from Himedia Laboratories Pvt Ltd, Mumbai, it was dissolved in sterilized citrate buffer PH 4.5. The wistar albino rats were fast over night and Streptozotocin 55 mg/kg b.w was administered intraperitoneally. After a period of 7 days blood glucose was estimated to confirm the diabetes. The rats were maintained for a period of 14 days to stabilize the diabetic condition. The rats with blood glucose level above 200 mg/dl were considered as diabetic and were taken for screening of antidiabetic activity.[11]

2.5.3 Sample collection
Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using commercial glucose kit.

2.5.4 Experimental design
All the animals were randomly divided into four groups with six animals in each group. Group A, B and C were served as vehicle control, diabetic control and glibenclamide respectively. Preliminary oral LD₅₀ dose of methanol extract of *Terminalia catappa* in rats were found to be 200 mg/kg. Group D was treated with one-fifth of LD₅₀ dose of methanol extract (40 mg/kg per day).

2.5.5 Assessment of extracts on streptozotocin induced diabetic rats
Diabetic rats were treated with glibenclamide and methanol extract of *Terminalia catappa* for 3 weeks. Blood samples were drawn at weekly intervals till the end of study (3 weeks). Fasting blood glucose estimation and body weight measurements were done on day 1, 7 and 21 of the study.

On day 21, blood was collected by retro-orbital plexus puncture under mild ether anesthesia from overnight fasted rats and fasting blood glucose was estimated. Serum was separated and
analysed for serum cholesterol, serum triglyceride, serum HDL, serum LDL, serum creatinine, serum urea and serum alkaline phosphatase.

The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5μ thickness were cut and stained by haematoxylin and eosin for histological examination. The photomicrographs of histological studies are presented in Fig. 2(A-D).

2.5.6 Statistical analysis
All the values of body weight, fasting blood glucose and biochemical estimations were expressed as mean ± S.E.M and analyzed for ANOVA and post hoc Duanet’s t-test. Differences between groups were considered significant at P<0.01 levels.

RESULTS AND DISCUSSION
In vitro antidiabetic activity of successive methanol extracts of *Terminalia catappa* was shown significant activity. Hence methanol extract of *Terminalia catappa* was selected for in vivo antidiabetic activity. The antihyperglycemic effect of methanol extract on the fasting blood glucose level of diabetic rats is shown in Figure 1 and 2. The treatment of diabetic rats for 3 weeks with methanol extract of *Terminalia catappa* led to fall in blood glucose level. The effect seems to reach maximum after 15 days of treatment and remains constant in third week.

Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 21 days (Table 2). The animals treated with methanol extract of *Terminalia catappa* showed stable body weight after 7 days of treatment. Serum cholesterol, serum triglyceride, serum LDL, serum creatinine, serum urea and serum alkaline phosphatase levels were decreased significantly by glibenclamide and methanol extract of *Terminalia catappa* after 21 days of treatment, while serum HDL levels were increased (Table 3).

Photomicrographs (Fig. 2) showed normal acini and normal cellular population in the islets of langerhans in pancreas of vehicle treated rats (A), extensive damage to the islets of langerhans and reduced dimensions of islets (B), restoration of normal cellular population size of islets with hyperplasia by glibenclamide (C) was shown. The restoration of normal
cellular population and enlarged size of β-cells with hyperplasia was shown by methanol extract (D).

The methanol extract of *Terminalia catappa* bark has good antidiabetic activity without significant change in body weight. This was also improve the conditions of diabetes mellitus as indicated by parameters like body weight along with serum creatinine, serum urea and serum alkaline phosphatase. The β-cells regeneration takes place by treatment with methanol extract of *Terminalia catappa* bark. Antidiabetic studies of Epicatechin and *Vinca rosea* extracts has also shown to act by β-cell regeneration. [12, 13]

In the present study, the damage of pancreas in streptozotocin treated diabetic control rats (Fig. 2B) and regeneration of β-cells by glibenclamide (Fig. 2C) was observed. The comparable regeneration was also shown by methanolic extract of *Terminalia catappa* bark (Fig. 2D). This effect may be due to the β-carotine, which is reported to be constituent of *Terminalia catappa*. The role of β-carotene in antidiabetic activity in rats had been reported previously.[14, 15] Photomicrographical data in this study shows the healing of pancreas by methanolic extract of *Terminalia catappa* bark.

![Graph showing blood glucose level](image)

**Fig. 1.** Comparative effect of methanol extract of bark of *Terminalia catappa* on blood glucose level in streptozotocin induced diabetic rats.
Fig. 2. Photomicrographs of rat pancreas stained by haematoxylin and eosin of (A) Untreated (B) Streptozotocin induced diabetic (C) Effect of glibenclamide and (D) Methanol extract of *Terminalia catappa*

Table 2: The effect of 3 week treatment with extracts of *Terminalia catappa* on body weight after streptozotocin induced diabetes in rats

<table>
<thead>
<tr>
<th>Gr. No</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Average body weight (g) ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>A</td>
<td>Vehicle control</td>
<td>0.2 ml (^a)</td>
<td>201.46±2.68</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control</td>
<td>0.2 ml (^a)</td>
<td>206.53±3.21</td>
</tr>
<tr>
<td>C</td>
<td>Glibenclamide</td>
<td>10</td>
<td>205.32±2.08</td>
</tr>
<tr>
<td>D</td>
<td>Methanol extract</td>
<td>40</td>
<td>206.68±2.05</td>
</tr>
</tbody>
</table>

Values are given in average body weight (g) ±SEM for groups of six animals each

\(^a\)vehicle (0.5% Tween-80 solution in normal saline)

\(^*\)P<0.05 as compared to vehicle control on corresponding day
Table 3: Effect of methanol extract of Terminalia catappa on serum profile in streptozotocin induced diabetic rats after 21 days of treatment

<table>
<thead>
<tr>
<th>Gr. No</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Serum cholesterol</th>
<th>Serum triglyceride</th>
<th>Serum HDL cholesterol</th>
<th>Serum LDL cholesterol</th>
<th>Serum creatinine</th>
<th>Serum urea</th>
<th>Serum alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Vehicle control</td>
<td>0.2 ml&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152.16±4.81</td>
<td>87.18±3.54</td>
<td>46.23±2.52</td>
<td>93.18±5.23</td>
<td>0.52±0.13</td>
<td>26.32±1.42</td>
<td>118.16±2.68</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control</td>
<td>0.2 ml&lt;sup&gt;a&lt;/sup&gt;</td>
<td>271.32±7.24</td>
<td>203.62±6.18</td>
<td>30.18±1.810</td>
<td>197.16±5.86</td>
<td>1.38±0.18</td>
<td>62.18±1.84</td>
<td>258.52±5.16</td>
</tr>
<tr>
<td>C</td>
<td>Glibenclamide</td>
<td>10</td>
<td>146.56±4.38&lt;sup&gt;*&lt;/sup&gt;</td>
<td>98.26±6.25&lt;sup&gt;*&lt;/sup&gt;</td>
<td>51.83±1.93&lt;sup&gt;*&lt;/sup&gt;</td>
<td>73.62±5.18&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.54±0.08&lt;sup&gt;*&lt;/sup&gt;</td>
<td>30.12±1.68&lt;sup&gt;*&lt;/sup&gt;</td>
<td>128.16±5.23&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>Methanol extract</td>
<td>40</td>
<td>148.21±5.62</td>
<td>102.46±7.21&lt;sup&gt;*&lt;/sup&gt;</td>
<td>48.26±1.91&lt;sup&gt;*&lt;/sup&gt;</td>
<td>76.56±4.83&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.52±0.16&lt;sup&gt;*&lt;/sup&gt;</td>
<td>31.68±1.21&lt;sup&gt;*&lt;/sup&gt;</td>
<td>130.62±5.81&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM for groups of six animals each.

<sup>a</sup>Vehicle (0.5% Tween-80 solution in normal saline)

<sup>*</sup>P<0.01 (Dunnet t-test), diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.
CONCLUSIONS
Methanolic extract of *Terminalia catappa* bark exhibited significant antidiabetic activity in streptozotocin induced diabetic rats. This extract showed improvement in parameters like body weight and lipid profile as well as regeneration of β-cells of pancreas. Hence, it has value in treatment of diabetes.

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REFERENCES


