Antihyperglycemic and Hypolipidemic effects of *Tamarindus indica* L.: A Potential agent for treatment of metabolic syndrome

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**Abstract**

*Tamarind* (*Tamarindus indica* L.) is a tree-type of plant which is indigenous to tropical Africa but has become naturalized in North and South America from Florida to Brazil, and is also cultivated in subtropical China, India, Pakistan, Indochina, Philippines, Java and Spain. The purpose of the present study was to assess the pharmacological properties of different extracts from *Tamarindus indica* L. leaves extracts as potential agent for the treatment of diabetes and hyperlipidemia. In oral glucose tolerance test the blood glucose level was significantly (P<0.05) controlled in 0-90 min after glucose administration in normal and STZ induced diabetic rats. The blood glucose level of Streptozotocin induced diabetic rats were determined at 0,7,14 days of treatment after 14 days of treatment blood glucose levels were significantly (P<0.05) decreased. Hydroalcoholic extract of leaves at the dose of 200mg/kg significantly increased the serum insulin level. *Tamarindus indica* leaves extracts at all the dose level 100 and 200 mg/kg, after 14 day days of treatment significantly (P<0.05) increased hepatic glycogen level. On the other hand after 14 days of treatment lipid profile CHOL, HDL,TG,LDL,VLDL were significantly (P<0.05) improved in diabetic rats treated with Hydroalcoholic and aqueous extracts of *Tamarindus indica* leaves. The results suggested that the hydroalcoholic extracts having more antidiabetic, hypolipidemic potential in comparison with aqueous extracts of *Tamarindus indica* leaves.

**Key word:** *Tamarindus indica*, diabetes, hypolipidemic, glycogen, insulin, Streptozotocin

**Introduction**

Diabetes mellitus, a metabolic disorder of multiple etiologies, is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism that results from imperfections in insulin secretion or resistance¹. The developed countries such as India, China, and the U.S. are presently the countries with the leading number of diabetics. Furthermore, seven percent of the residents of the United States are diabetic. Though it is a non-communicable disease, but is considered to be one of the five leading causes of death world-wide². As diabetes aggravates and β-cell function deteriorates, the insulin level begins to fall below the body’s requirements and causes prolonged and more severe hyperglycemia. The

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sustained supra physiological glucose may induce toxicity to metabolic processes and to cells resulting in the long-term complications of diabetes. Experimental diabetes mellitus has been induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. The second method for creating diabetes in animals is injecting drugs such as alloxan or Streptozotocin (STZ). Oxidative stress is increased in experimental models of streptozotocin induced diabetes mellitus. The cytotoxic action of streptozotocin selectively destroys β-cells of pancreas by generating excess ROS and carbonium ion (CH$_3^+$) leading to DNA breaks by Alkylating DNA bases causing oxidative damage. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes. The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest. Therefore considerable focus has been given to an intensive search for novel type of antioxidants from numerous plant materials for management of diabetes without any side effects.

*Tamarindus indica* is a large tree 12-18 m. high, branches spreading belonging to family: Caesalpiniaceae Leaves: 5-12.5cm long, rachis slender channeled, stipulus linear, cadcous. Leaflets: Subsessile, 10-20 pairs, tolerably closely set on the rachis, 8-30 by 5-8 mm. oblong, obtuse, glabrous reticulately veined. It is a dicotyledonous plant. Seed coat is brownish black in colour though the kernel is white in colour. In this context, the present study was undertaken to verify the reported antidiabetic and hypolipidemic properties of *Tamarindus indica* in Streptozotocin induced diabetic rats.

**Materials**

**Drugs and Chemicals**

UV–visible spectrophotometer (UV-1601PC Shimadzu, Japan), Gluco-One glucose measurement strips (Dr. Morpene), One Touch blood glucometer, LINCO ELISA kit, Lipid profile kit were procured from (Span Diagnostics, Surat, India). Glibenclamide was obtained as a gift sample from (Bioplus life sciences, Bangalore, India). Streptozotocin obtained from (Himedia laboratories Pvt. Ltd, Mumbai). The rest of the chemicals utilized were of analytical grade and were obtained from Fisher scientific, Mumbai, India.

**Plant material**

The leaves of *T indica* were collected from local region around Raipur, Chhattisgarh, India in the month of November-December. Leaves were washed under running tap water followed by rinsed with distilled water for five minutes. Leaves of *T indica* were identified and authenticated by Dr. P.C. Panda. Principal scientist taxonomy division of “Regional Plant resource centre, Bhubaneswar, Odisha, India” (Voucher specimen no: B M -1).

**Animals**

Studies were carried out using Wistar albino rats of both sexes (150-200 g). They were housed in standard cages at room temperature 25±2°C and 50±5% relative humidity, under a light/dark cycle of 10/12 h, for 1 week before the experiment for acclimatization. Animals were provided with standard rodent pellet diet (Amrut,
India), and water *ad libitum*. The animals were deprived of food for 24 hours before experimentation, but had free access to drinking water. Experimental protocols were approved by Institutional Ethical Committee which follows guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The experiment were performed after the approval of Institutional Animal Ethical Committee (IAEC approval no. SBRL/IAEC/2012/11). Normoglycemic animals were selected for this experiment having the fasting blood glucose level 85 ± 5 mg/dl.

**Extraction**

Fresh leaves of *T indica* were washed under running tap water followed by rinsed with distilled water and dried under shade. The dried leaves were pulverized by an electrical blender the coarse powder was passed through sieve No.20. The powdered plant up to 500 g was extracted with boiling water for 10h. The resulted extracts were filtered through Whatman filter paper no 4 and concentrated by lyophilizer. The yield of extract was 5.34 % (w/w). Hydroalcoholic extraction was carried out by mixing the powdered (500 g) powder with ethanol and water (8:2) for 2 days. The resulted extract was filtered and concentrated by rotary evaporator under reduced pressure and low temperature. The yield of extract was 8.87 % (w/w).  

**Preliminary Phytochemical screening**

Preliminary phytochemical screening was carried out to find out the different constituents presents in leaves. 

**Acute oral toxicity study and selection of doses**

The acute toxicity study of both the extract HAETIL and ATIL were determined as per the Organization of Economic Co-Operation and Development (OECD) guideline no. 425 (Acute toxic class method). It was observed that the HAETIL and ATIL were not mortal up to 2000 mg/kg dose orally. Hence (100 and 200 mg /kg) was selected for this study.  

**Experimental procedure**

**Induction of diabetes**

Freshly prepared solution of STZ (55 mg/kg in 0.1 M citrate buffer pH 4.5) was injected intraperitoneally to overnight fasted rats. After 48h of STZ administration, fasting blood glucose levels were measured. The rats with stabilized fasting blood glucose levels between 150 and 250 mg/dl were considered diabetes and selected for study.

**Assessment of hypoglycemic activity in normal rats**

In the experiment, a total of 42 rats (6 normal and 36 Streptozotocin induced diabetic rats were used. The animals were assigned into seven groups (I-VII) of six animals each and received the following treatments: Group I: Normal control + distilled water, Group II: Diabetic control (Streptozotocin 55mg/kg) + distilled water, Group III: Diabetic + Glibenclamide (2.5 mg/kg) as standard drug, Group IV: Diabetic + HAETIL(100 mg/kg), Group V: Diabetic + HAETIL (200 mg/kg), Group VI: Diabetic + AETIL (100 mg/kg) And Group VII: Diabetic + AETIL (100 mg/kg) for 14 days. The effects HAETIL and AETIL on Streptozotocin induced diabetic rats were determined weekly once by body weight, blood glucose...
measurement on overnight fasted rats, lipid profile and histopathology of pancreas was studied at the end of the experiment\textsuperscript{11}. The freshly prepared solutions of test drugs were orally administered daily for 14 days. At the end of the experimental period, the animals were fasted overnight and blood was collected for various biochemical estimations. The animals were sacrificed by cervical decapitation. Organ like liver and pancreas dissected out immediately rinsed in ice cold saline and stored for further biochemical estimations.

**Sample collection**
At the end of 13 days the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in fresh vials containing sodium fluoride and potassium oxalate (anticoagulant agent) for the estimation of glucose. Plasma was separated for the estimation of insulin, lipid profile. Pancreas was dissected out.

**Biochemical measurements**

**Determination of blood glucose concentration**
Blood glucose analysis was done by GOD-POD method using Glucose Estimation Kit (Dr. Morpene, India and New Delhi).

**Determination of insulin concentration**
Serum insulin was determined using insulin ELISA kit (LINCO ELISA kit) by ELISA reader.

**Determination of Hepatic Glycogen Level**
Hepatic glycogen content was measured according to the anthrone-H_{2}SO_{4} methods, with glucose as the standard (Minzhu Briefly, liver tissue (<100 mg) was homogenized in three volumes of an ice-cold 30\% (w/v) KOH solution and dissolved in a boiling water-bath (100\textdegree C) for 20 min. Glycogen was resolubilized in distilled water and the glycogen concentration was then determined by treatment with an anthrone reagent (2 g anthrone/1 l of 95\% (v/v) H_{2}SO_{4}, and the absorbancy was measured at 620 nm\textsuperscript{[12,13]}. Aliquot was then calculated using the following equation:

\[ \mu g \text{ of glycogen in aliquot} = \frac{100xU}{1.11xS} \]

U: the optical density of the unknown test solution, S: the optical density of the 100 \mu g glucose standard

**Determination of lipid profile**
HDLC, Total Cholesterol, Triglyceride content in plasma was estimated by using a reagent kit, (Span Diagnostic Ltd. Surat India). VLDLC and LDL fractions were calculated as follows:

\[ \text{VLDLC} = \frac{\text{Triglyceride}}{5} \text{ and } \text{LDL} = (\text{Total cholesterol}) - (\text{HDL Cholesterol}) - (\text{Triglyceride}/5). \]

**Histopathology**
At the end of treatment, small pieces of tissues of pancreas were blotted and freed from blood, fixed in 10\% neutral buffered formalin for 48 h, trimmed and processed for paraffin embedment and 5 mm thickness of tissue sections were stained with haematoxylin and eosin for histopathological examination. Histological structures of pancreatic sections were examined using a light microscopy\textsuperscript{[14]}. The results are expressed as Mean ± SEM. One-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test were carried out to determine the significant differences. Mean of negative control group and standard treated groups
statistically significant if (P<0.01). Mean of negative control and HAETIL and AETIL treated groups statistically significant if (P<0.05). The analyses of the results were carried out using GRAPH PAD Prism 5.0 package software.

Results

Preliminary phytochemical screening
The results of preliminary phytochemical screening indicates that the leaves extracts contain carbohydrates, Flavonoids, proteins and glycosides and alkaloids.

Acute oral toxicity study
In acute toxicity study, HAETIL and AETIL treated animals did not show any changes in their behavioural pattern. There was no any significant difference body weight, food consumption as compared to vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded that HAETIL and AETIL safe up to 2000 mg/kg.

Effect of HAETIL and AETIL on body weight
STZ cause decrease in body as compared to normal animals during the study, Tabel.1. Diabetic control continued to decrease body weight till the end of the study while HAETIL and AETIL at the dose of (100 and 200 mg/kg) and Glibenclamide treated animals showed improvement in body weight as compared to diabetic control.

Effect of HAETIL and AETIL on oral glucose tolerance in normal and STZ-induced diabetic rats
HAETIL and AETIL were administered at the dose of (100 and 200mg/kg) 60 minutes prior to glucose loading blood glucose levels were measured at 0, 30, 60 and 90 min after glucose administration and AUC were determined. Animals treated with HAETIL and AETIL and glibenclamide treated group significant (P<0.05, P<0.01), Figure.1 represents effect of blood glucose level during glucose tolerance test.

Effect of HAETIL and AETIL on blood glucose level in STZ induced diabetic rats
The effect of repeated oral administration of HAETIL and AETIL on blood glucose level in STZ-diabetic rats at the dose of 100 and 200 mg/kg and standard glibenclamide for 14 days is presented in figure. 2. Blood glucose levels were measured at 1, 7 and 14th day of experiment and AUC was determined. Results indicated that there was significant (P<0.05) decreased of blood glucose level of HAETIL and AETIL treated groups were noticed. Standard treated group also showed a significant (P<0.01) decrease of blood glucose level.

Effect of HAETIL and AETIL on serum insulin
STZ caused a significant decrease in serum insulin of experimental rats. Administration of HAETIL and AETIL at the dose of 100 and 200 mg/kg and standard glibenclamide for 14 days HAETIL 200 mg/kg caused significant (P<0.05) increase in serum insulin level in diabetic animals. (Figure.3) represents effects of standard and test drug on serum insulin level.

Effect of HAETIL and AETIL on hepatic glycogen level
Glycogen content was significantly decrease (P<0.05) in diabetic control group compared to normal control. (Figure. 4) Administration of HAETIL
AETIL at the dose of 100 and 200 mg/kg and standard Glibenclamide for 14 days caused significant (P<0.05) increase in hepatic glycogen level in all groups with HAETIL, AETIL and standard drug treated group at significant (P<0.01) level.

**Effect of HAETIL and AETIL on lipid profile in STZ-induced diabetic rats**

Serum lipid profiles of rats were determined at the end of the experiment. It was observed that there was a significant (P<0.05) increase in serum Cholesterol, Triglyceride, LDL and VLDL and decrease of HDL level was noticed in STZ-induced group (Table.2). After treatment of HAETL, AETL at the dose 100 and 200 mg/kg for 14 days caused a significant decrease in serum cholesterol, Triglyceride, LDL and VLDL level. It also significantly (0.050 increased serum HDL level. Standard glibenclamide treated groups there was significant (0.01) decrease of Cholesterol, Triglyceride, LDL VLDL and significant increase of HDL was noticed.

**Effect of HAETL and AETIL on pancreas histology in rats**

Figure 5 (A-G) represents the photomicrographs of hematoxylin-eosin staining of pancreatic tissues section of control and experimental groups of rat. Figure 5(A) shows the pancreatic tissue of control rats exhibiting a concentric arrangement of the pancreatic cell (α and β) around the central vein and arteries. Figure 5(B) the section of pancreatic tissues of STZ-induced group of rat exhibiting distortion in the arrangement of β-cell around pancreas. Figure 5(C) Pancreatic tissues of Glibenclamide treated rats presenting normal pancreatic cells arrangement, Figure 5(D-E) demonstrates the section of pancreatic tissues of STZ induced groups of rats treated with hydroalcoholic extracts of *T. indica* leaves. Figure 5(F-G) demonstrates diabetic rats treated with aqueous extracts of leaves representing distortion in the arrangement of β-cell around pancreas.

![Figure 1](image-url)  
**Figure.1.** Effect of *Tamarindus indica* leaves extracts on oral glucose tolerance test (OGTT) in diabetic and normal albino rats. Each value is expressed as Mean ± S.E.M. (n=6).  
\(^a\)P<0.05 when compared to corresponding value of normal control.  
\(^b\)P<0.05 when compared to corresponding value of diabetic control.  
\(^c\)P<0.01 when compared to corresponding value of diabetic control.
Figure 2 Effect of *Tamarindus indica* leaves extract on blood glucose level in diabetic and normal albino rats. During 14 days of treatment. Each value is expressed as Mean ± S.E.M. (n=6).

\(^{a}P<0.05\) when compared to corresponding value of normal control.

\(^{b}P<0.05\) when compared to corresponding value of diabetic control.

\(^{c}P<0.01\) when compared to corresponding value of diabetic control.

Figure 3 Effect of *Tamarindus indica* leaves extracts on serum insulin level in STZ-induced diabetic and normal albino rats after 14 days of treatment. Each value is expressed as Mean ± S.E.M. (n=6).

\(^{a}P<0.05\) when compared to corresponding value of normal control.

\(^{b}P<0.05\) when compared to corresponding value of diabetic control.

\(^{c}P<0.01\) when compared to corresponding value of diabetic control.

Figure 4 Effect of *Tamarindus indica* leaves extracts on liver glycogen level in diabetic and normal albino rats after 14 days of treatment. Each value is expressed as Mean ± S.E.M. (n=6).

\(^{a}P<0.05\) when compared to corresponding value of normal control.

\(^{b}P<0.05\) when compared to corresponding value of diabetic control.

\(^{c}P<0.01\) when compared to corresponding value of diabetic control.
Table 1. Effect of *Tamarindus indica* leaves extracts on serum level of Total Cholesterol, HDL cholesterol, Triglycerides TG, LDL Cholesterol and VLDL Cholesterol in diabetic and normal albino rats after 14 days of treatment.

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Treatment</th>
<th>CHL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control (NC)</td>
<td>146.33 ±1.9</td>
<td>69.8±1.49</td>
<td>131±2.47</td>
<td>50.53±0.67</td>
<td>26.13±0.37</td>
</tr>
<tr>
<td>II</td>
<td>Streptozotocin (STZ)</td>
<td>212.83±1.77a</td>
<td>37.33±1.43a</td>
<td>199.66±2.47a</td>
<td>137.06±0.59a</td>
<td>39.93±0.34a</td>
</tr>
<tr>
<td>III</td>
<td>STZ+ Glibenclamide</td>
<td>166.16±1.179c</td>
<td>58±2.17c</td>
<td>159.33±2.45c</td>
<td>80.73±1.06c</td>
<td>32.1±0.33c</td>
</tr>
<tr>
<td>IV</td>
<td>STZ+ HAETIL (100mg/kg)</td>
<td>189.33±2.98b</td>
<td>40.5±1.23</td>
<td>169.16±2.05b</td>
<td>114.96±0.94b</td>
<td>34.36±0.21b</td>
</tr>
<tr>
<td>V</td>
<td>STZ+HAETIL (200mg/kg)</td>
<td>170.83±2.85b</td>
<td>53±2.32b</td>
<td>167.66±1.72b</td>
<td>92±3.85b</td>
<td>33.66±0.34b</td>
</tr>
<tr>
<td>VI</td>
<td>STZ+AETIL (100mg/kg)</td>
<td>192±3.82</td>
<td>40±2.51</td>
<td>179.33±1.74b</td>
<td>124.26±1.07</td>
<td>36.4±0.32b</td>
</tr>
<tr>
<td>VII</td>
<td>STZ+ AETIL(200mg/kg)</td>
<td>194.5±5.11b</td>
<td>39.33±3.34</td>
<td>183±2.75b</td>
<td>128.86±6.71</td>
<td>36.3±0.62b</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± S.E.M. (n=6), Evaluated by using one way ANOVA followed by Dunnett’s multiple comparison test.

*P<0.05 when compared to corresponding value of normal control, †P<0.05 when compared to corresponding value of diabetic control , ‡P<0.01 when compared to corresponding value of diabetic control.
Discussion

Medicinal plants are widely used by the population of developing countries as alternative therapy. In India, hundreds of plants are used traditionally for the management of diabetes mellitus. Unfortunately only a few of such Indian medicinal plants have received scientific scrutiny. The present study was therefore designed to study the antidiabetic and hypolipidemic effect of *Tamarindus indica*. Leaves extract against streptozotocin induced diabetic rats. The continuous treatment of *T indica* for a period of 14 days produced a significant (p<0.05) decrease in blood glucose level in diabetic rats which is comparable to that of standard and diabetic control group. An increase in blood glucose seen in the oral glucose tolerance test (OGTT) was significantly greater in the diabetic rats than in the non-diabetic rats. The level of plasma insulin was increased by glucose tolerance test in the non-diabetic rats, while it was not changed in diabetic rats. Oral administration of HAETIL and AETIL 100 and 200 mg/kg significantly improved the impaired glucose tolerance in the diabetic rats with change in plasma insulin level. Considering the above result, the hypoglycemic effect of the plant may involve its insulin-like action i.e., acting at peripheral level to increase cellular glucose uptake or increase glycogenesis. A number of different plants have been shown to exert hypoglycemic activity through stimulation of insulin release like glibenclamide that is reported to enhance the activity of β cells of pancreas resulting in increased secretion of large amount of insulin which in turn decreases blood glucose level. From the results it is assumed that the leaves extract of *T. indica* could be responsible for stimulation of insulin and the observed restoration of metabolic activity. The reversal of weight loss in extract-treated diabetic group indicates that the restorative effect of *T. indica* may be by the reversal of gluconeogenesis and glycogenolysis. The concentrations of lipids, such as cholesterol, triglycerides (TG) and HDLC, were significantly higher in diabetic rats than in the control group. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids. It was observed that *T. indica* is responsible for improving lipid profile.

Figure 5 (A-G) - (A) Pancreas of normal rats (control), (B) Pancreas of diabetic rats (control STZ induced), (C) Pancreas of diabetic rat standard drug glibenclamide treated, (D) Pancreas of diabetic rats treated with HAETIL 100 mg/kg, (E) Pancreas of diabetic rats treated with HAETIL 200 mg/kg, (F) Pancreas of diabetic rats treated with AETIL 100 mg/kg, (G) Pancreas of diabetic rats treated with AETIL 200 mg/kg.
In addition to its hypoglycaemic effect HAETL or AETIL were also able to improve some lipid metabolites including TC, TG, HDL and LDL cholesterol levels in diabetic rats. It has been reported that in diabetes alteration reported that diabetes are associated with profound alterations in lipid and lipoprotein profile\textsuperscript{19, 20}. By regulating plasma lipid level macro and micro vascular complications can be regulated\textsuperscript{21}. Thus, this result suggested that by preventing diabetes we can prevent dyslipidemia and diabetes related complications.

After treatment with HAETIL, AETIL blood glucose, TC, TG, LDL, VLDL significantly decreased and at the same time cardioprotective HDL lipid level was increased. This provides evidence in favor of the views that \textit{T. indica} could play an important role in treating diabetic/hyperlipidemic patients. Which could prevent or helpful in reducing the complications of lipid profile seen in some diabetic in which hyperglycemia and hypercholesterolemia quite often coexist. Many current oral hypoglycemic or hypolipidemic agents are synthetic drugs with certain adverse effect\textsuperscript{22}. Our study reveals the potential of \textit{T. indica} for use as a natural oral agent with both hypoglycemic and hypolipidemic effect.

Histopathological study of pancreatic cells indicates improvement of pancreatic cells architecture and destruction of pancreatic cells of diabetic untreated pancreatic cells.

**Conclusion**

From this study it can be concluded that hydroalcoholic and aqueous extracts of \textit{T. indica} leaves extracts has potential beneficial effects on blood glucose and lipid lowering potential in STZ-induced diabetic rat model. This was mediated via increasing secretion of insulin. Thus, lending support to its folkloric use in the management of diabetes mellitus. From preliminary phytochemical analysis it was found that the major chemical constituents of the \textit{T. indica} were tannins, polyphenolic compound and flavonoids so it is possible that the presence of tannins or flavonoids may be responsible for the observed antidiabetic activity. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.

**Acknowledgment**

The authors are grateful SBRL, Bhopal, India for providing necessary facilities to carry out this research work. Authors also thankful to Mr. B. Bhoi, Scientist Zydus Cadila Pharma, Ahmadabad, India for his valuable support during statistical works.

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