Screening of Antidiabetic and Antioxidant Potential of Tectona Grandis Bark Ethanol Extract

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ABSTRACT

Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine but it is necessary to establish the scientific basis for the therapeutic actions of herbal plant medicines with the purpose of discovering new bioactive compounds for diabetes mellitus. Tectona grandis bark ethenol extract was studied for the OGTT study and acute and sub-acute effects on alloxan induced diabetic rats. Blood glucose levels, Serum lipid profiles and histological study of pancreas were performed. This extract’s DPPH radical scavenging potential was also studied. After administration of ethanol extract of bark of Tectona grandis, reduction of fasting blood glucose levels took place from 60 min (OGTT), blood glucose lowering potential percentage was 16 % at 6 hr (Acute study) and after 15 days of treatment it was 54 % (Sub acute study). This extract also showed better activity in quenching DPPH radical. Oxidative stress has been suggested as a contributory factor in the pathogenesis of diabetes. Alloxan causes diabetes through its ability to destroy the insulin producing β cells of the pancreas. The Tectona grandis bark ethenol extract has potent in vitro antioxidant potential and marked antihyperglycemic activity, which may be due to the presence of flavonoids and quinones.

KEYWORDS: Tectona grandis; antidiabetic; antioxidant; alloxan induced diabetic rats; hyperglycemic.

INTRODUCTION

Diabetes mellitus is a disease characterized by glycosuria, hyperglycemia and a disturbance in carbohydrate, fat and protein metabolism and water and electrolyte balance. The World Health Organization predicts that the number of cases worldwide for diabetes is now 150 million and will be doubled in coming years [1].

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. Increased oxidative stress has been postulated in the diabetic state[2].Antioxidants have been shown to reduce the risk of diabetes onset, improve glucose disposal and improve some of the associated complications [3].

Medicinal plants are used in several countries to manage DM and are thought to be less toxic than allopathic hypoglycemic drugs like the biguanides, sulphonylureas or insulin therapy. Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. These are gaining popularity because of several advantages such as often fewer side effects, better patient tolerance, relatively less expensive and acceptance due to long history of use. Plants are often less prone to the emergence of drug resistance.

Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. Tectona grandis Linn (Verbenaceae) tree commonly known as Sagvan, found throughout the India. It is a huge tree, bark ash colored. The wood has a characteristic aromatic odor. The roots are useful in anuria. The bark is useful in bronchitis, hyperacidity, diabetes, leprosy and skin diseases. The flowers are useful in leprosy, skin diseases, burning sensation and diabetes. Leaves are useful in inflammation, leprosy, in skin diseases [4] and in wound healing [5]. The ethanol extract of this plant is used in the treatment of anemia [6].

The literature screened in the process of the proposed work indicates that the selected plant contain classes of chemical constituents which have shown antidiabetic and antioxidant activities. Literature survey revealed that Tectona grandis bark ethanol extract has no scientific claims for anti-diabetic and antioxidant activity. Phytochemical and pharmacological investigations of this plant may yield useful information and material for better management for preventing the production of the free radicals and diabetes.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar albino rats weighing between 170-200 gm were used for the antidiabetic studies, whereas Wistar albino rats of either sex were used for determination of acute toxicity study. The animals were housed in groups of 5 per cage with free access to commercial rat pallet diet (Lipton India ltd., Mumbai, India) and water ad libitum. The animal room was maintained at 25°C ± 2°C with timed lighting on from 6 am to 6 pm and relative air humidity of 30 to 60%. The Institutional Animal Ethics Committee (CPCSEA/1/15/2007) approved the study.
Chemicals
All chemicals and solvents used were of analytical grade from Merck Ltd., Mumbai, India and Sigma Aldrich Co., USA. Glibenclamide was obtained from Hoechst India as in form of Daonil tablet.

Collection of Plant material
The bark of Tectona grandis was collected from local areas of Kolhapur (Maharashtra) and Belgaum (Karnataka) India. The specimen was authenticated from Dr.S.R.Yadav, Prof., Dept. of Botany, Shivaji University, Kolhapur (Maharashtra) India. The voucher specimen (KLEU/Pharm/07/15) was retained in the Herbarium of Department of Pharmacognosy, K.L.E. University, College of Pharmacy, Belgaum (Karnataka) India.

Preparation of plant extract
The collected plant material was washed thoroughly in water, chopped, shade dried at room temperature, reduced to a coarse powder in a mechanical grinder and passed through a 40 # sieve for desired particle size. The powder obtained was extracted with 95% ethanol in a soxhlet apparatus. The extract was concentrated under reduced pressure and dried. The yield of Tectona grandis bark ethanol extract was 3 % (w/w). The obtained extract was stored in a refrigerator at 2-8°C until usage.

Preliminary phytochemical investigations
Preliminary phytochemical investigation revealed the presence of Lapachol-a naphthaquinone (root) [7], napthoquinone derivatives (heart wood) [8] and tannins (Leaves) in Tectona grandis plant.

Experimental design
Screening of Tectona grandis bark ethanol extract for anti-diabetic action was done in rats by conducting glucose tolerance test (GTT) study and evaluating their effects (Single dose and Multidose treatment study) on blood glucose level and serum lipid profiles in alloxan diabetic rats.

Acute toxicity study
Determination of LD₅₀ for extracts is done by OECD guidelines for fixing the dose for biological evaluation. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The LD₅₀ of the extract as per OECD guidelines 2001, falls under 5mg, 50 mg, 300 mg and 2000 mg/kg bw with no signs of acute toxicity at respective doses. The biological evaluation of extract is carried out at 1/10 doses of LD₅₀ [9].

Oral glucose tolerance test (OGTT) [10]
Fasting blood glucose level of each rat was determined at zero time after overnight fasting with free access to water. Rats were divided into three groups containing six rats each. The first group of animals were received 1 ml of 1% gum acacia suspension orally (Control animals). Remaining groups received Glibenclamide (2.5 mg/kg - standard) and Tectona grandis bark ethanol extract (200 mg/kg), by oral route using an orogastric tube respectively. Glucose (2 gm/kg) was orally administered 30 min. after the administration of extracts or Glibenclamide or gum acacia suspension. Blood samples were collected from the tail vein under ether anaesthesia just prior to and 30, 60, 120 and 240 min after glucose loading. Glucose levels were estimated using glucose-oxidase-peroxidase reactive strips and a glucometer (Sugar-check, Wockhardt Ltd, Mumbai, India).

Effect of Tectona grandis bark ethanol extract on blood glucose levels in alloxan induced diabetic rats [Single dose (Acute) treatment] [11]
A single intraperitoneal injection of 120 mg/kg of alloxan monohydrate was employed to induce diabetes in overnight fasted male Wistar albino rats weighing 170-200gm. After 72 hr, animals with blood glucose levels higher than 250 mg/dl were considered diabetic and were included in the study. Animals were divided into four groups including six rats each. Group I: Normal control rats administered 1 ml of 1% gum acacia suspension; Group II: Diabetic control rats administered 1 ml of 1% gum acacia suspension; Group III: Diabetic rats administered Glibenclamide (2.5 mg/kg) and Group IV: Diabetic rats administered Tectona grandis bark ethanol extract (200 mg/kg) orally. Blood samples were collected from the tail vein prior to and at 30 min, 60 min, 2, 4, and 6 h intervals after the administration of the extract and blood glucose levels were estimated using glucometer.

Effect of Tectona grandis bark ethenol extract on blood glucose levels and serum lipid profiles in alloxan induced diabetic rats [Multi dose (sub-acute) treatment] [11]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose concentration (mg/dl) (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In fasting</td>
</tr>
<tr>
<td>Normal control (1% gum acacia)</td>
<td>93.5 ± 3.4</td>
</tr>
<tr>
<td>Glibenclamide (2.5 mg/kg)</td>
<td>95.0 ± 2.8</td>
</tr>
<tr>
<td>Tectona grandis bark ethanol extract (200 mg/kg)</td>
<td>87.50 ± 4.2</td>
</tr>
</tbody>
</table>

Significantly different from control: *P < 0.01
n= no of animals in each group
Diabetes was induced in overnight fasted adult male wistar albino rats weighing 170-200gm by a single intraperitoneal injection of 120 mg/kg of alloxan monohydrate. After 72 hr, animals with blood glucose levels higher than 250 mg/dl were considered diabetic and were included in the study. Animals were divided into four groups including six rats each. Group I: Normal control rats administered 1 ml of 1% gum acacia suspension; Group II: Diabetic control rats administered 1 ml of 1% gum acacia suspension; Group III: Diabetic rats administered Glibenclamide (2.5 mg/kg) and Group IV: Diabetic rats administered Tectona grandis bark ethanol extract (200 mg/kg). These rats were given the same doses of the extract once daily for 15 days in this study. Blood samples were collected from the tail vein of nonfasted rats on days 0, 5, 10 and 15 of extract administration and blood glucose levels were estimated using glucometer. Serum lipid profiles on day 15 were measured by an autoanalyzer.

Histopathological studies

Pancreatic tissues from rats of all groups of Multi dose (Sub acute) treatment were subjected to histopathological studies. The whole pancreas from each animal was removed after sacrificing the animal under anesthesia and was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin (H and E) for histological examination.

In Vitro Antioxidant –DPPH free radical scavenging activity

The free radical scavenging activity of Tectona grandis bark ethanol extract was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). For DPPH assay, the method of Blois was adopted. The capacity of Tectona grandis bark ethanol solvent extract to scavenge the lipid-soluble DPPH radical was monitored at an absorbance of 517 nm. Ethanol bark extract (1 ml) of Tectona grandis, at different concentration was allowed to react with DPPH. Thirty minutes later, the absorbance was measured at 517 nm. The percent inhibition of absorbance was calculated for each concentration relative to a blank absorbance using the spectrophotometer. The DPPH scavenging capacity of the extracts is compared with that of BHT (Butylated hydroxytolune). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All determinations are carried out at least three times, and in triplicate. IC_{50} value in the tested compound is, the concentration required to scavenge 50% DPPH free radical. Percentage inhibition was calculated as DPPH radical scavenging activity.

$\% \text{ inhibition} = \frac{1 - \text{Abs sample}}{\text{Abs control}} \times 100$

Where, Abs control is the absorbance of initial conc. of DPPH radical; Abs sample is the absorbance of DPPH radical + sample Extract/standard.

Table 2: Effect of Tectona grandis bark ethanol extract on the blood glucose levels in alloxan-diabetic rats (Single dose treatment/acute study)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose concentration (mg/dl) (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hour</td>
</tr>
<tr>
<td>Normal control (1% gum acacia)</td>
<td>85.25±2.6</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>287.5±5.2</td>
</tr>
<tr>
<td>Glibenclamide (2.5 mg/kg)</td>
<td>297.3±7.2</td>
</tr>
<tr>
<td>Tectona grandis bark ethanol extract (200 mg/kg)</td>
<td>273.3±5.0</td>
</tr>
</tbody>
</table>

*P < 0.01 Significant, compared to normal, *P < 0.05 & **P < 0.01 Significant, compared to diabetic control.

n= no of animals in each group

Table 3: Effect of Tectona grandis bark ethanol extract on the blood glucose levels in alloxan-diabetic rats (Multidose treatment/sub-acute study)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fasting blood glucose concentration (mg/dl) (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
</tr>
<tr>
<td>Normal control (1% gum acacia)</td>
<td>85.25±2.6</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>287.5±5.2</td>
</tr>
<tr>
<td>Glibenclamide (2.5 mg/kg)</td>
<td>299.3±6.9</td>
</tr>
<tr>
<td>Tectona grandis bark ethanol extract (200 mg/kg)</td>
<td>270.3±6.0</td>
</tr>
</tbody>
</table>

*P < 0.01 & **P < 0.05 Significant, compared to normal, *P < 0.01 & **P < 0.05 Significant, compared to diabetic control.

n= no of animals in each group
Statistical analysis
Values are presented as mean ± S.E.M. Statistical difference between treatments and the controls were tested by one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test using the “Stat” statistics computer program. A difference in the mean values of P<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Acute toxicity study
Acute toxicity study revealed no mortality or any toxic reactions with oral administration of ethenol extract of bark of *Tectona grandis* even at the highest dose (2000mg/kg).

The biological evaluation of extract is carried out at 1/10 doses of LD₅₀[9].

Table 4: Effect of *Tectona grandis* bark ethanol extract on the Serum profile in Alloxan-diabetic rats after 15 days of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TGL mg/dl</th>
<th>HDL mg/dl</th>
<th>VLDL mg/dl</th>
<th>LDL mg/dl</th>
<th>TOTAL CHOLESTEROL mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (1% gum acacia)</td>
<td>85.25±1.5**</td>
<td>37.00±1.5*</td>
<td>19.00±0.73*</td>
<td>16.25±0.4*</td>
<td>55.50±1.6**</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>123.5±2.4</td>
<td>30.00±1.4</td>
<td>27.75±1.3</td>
<td>34.42±3.7</td>
<td>83.00±2.0</td>
</tr>
<tr>
<td>Glibenclamide (2.5 mg/kg)</td>
<td>92.25±8.0**</td>
<td>51.50±1.9**</td>
<td>21.00±2.6*</td>
<td>19.00±1.9*</td>
<td>58.50±2.7**</td>
</tr>
<tr>
<td><em>Tectona grandis</em> bark ethanol extract (200 mg/kg)</td>
<td>99.00±4.7*</td>
<td>40.83±1.9**</td>
<td>25.00±1.9</td>
<td>22.00±5.3*</td>
<td>61.00±5.5**</td>
</tr>
</tbody>
</table>

*P < 0.05 & **P < 0.01 Significant, compared to diabetic control.

n=6: no of animals in each group
An administration of *Tectona grandis* bark ethanol extract was found to reduce blood glucose level in alloxan induced diabetic rats in single dose study. *Tectona grandis* bark ethanol extract exhibited significant (P<0.05) antihyperglycemic efficacy from 1 hr after its oral administration, the effect lasted up to 6 hrs when compared with normal rats and diabetic control rats. Blood glucose lowering potential percentage of *Tectona grandis* bark ethanol extract was 16 % at 6 hr after administration, while the standard drug Glibenclamide (2.5mg/kg) caused 20 % reduction of blood glucose at the same time interval when compared with diabetic control rats (Table 2 and Figure 2).

**Effect of *Tectona grandis* bark ethanol extract on blood glucose in alloxan induced diabetic rats [Multi dose (sub acute) treatment]**

Since diabetes is a chronic disorder requiring long-term therapy, there is a need to assess the effect of putative hypoglycemic/anti-hyperglycemic agents for a longer duration also. In order to determine the sub acute effects, *Tectona grandis* bark ethanol extract was administered throughout 15 days consecutively. The blood glucose level of each animal was monitored on 0th, 5th, 10th and 15th days after the administration of the test samples. As shown in the Table 3 and figure 3 initial antidiabetic activity was observed on 5th day and continued to increase in all groups during the experimental period. During the Multidose treatment period, administration of ethanol extract of bark of *Tectona grandis* (200 mg/kg/day) caused a significant decrease of 28%, 38% and 51% in blood glucose levels on 5th, 10th and 15th day intervals, respectively, when compared with diabetic control group. In this study the difference observed between the initial and final blood glucose levels under investigation reveals a significant elevation in blood glucose in diabetic control group at the end of 6 hrs (single dose treatment study) (Figure 2) or at the end of 15 days (multi dose treatment study) experimental period. Administration of extract to alloxan induced diabetic rats showed a significant decrease in the blood glucose level in both single dose and multi dose treatment study. The hypoglycemic effect comparable to glibenclamide suggested that the active fractions may act by regenerating the β cells in alloxan-induced diabetes [14]. Alloxan causes diabetes through its ability to destroy the insulin producing β cells of the pancreas. In vitro studies have shown that alloxan is selectively toxic to the pancreatic β cells, causing cell necrosis. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of β cells [15].

**Effect of *Tectona grandis* bark ethanol extract on serum lipid profiles in alloxan induced diabetic rats [Multi dose (sub-acute) treatment]**

Hyperlipidemia is a common complication of alloxan-induced diabetes mellitus in experimental animals. Observations of hypoglycemic effect of ethanol extract of bark of *Tectona grandis* in alloxan-induced hyperlipidemic rats are shown in Table 4 and figure 4. In serum lipid profiles study on day 15, diabetes which is induced by alloxan lead to a significant changes in levels of Serum cholesterol, triglyceride, LDL, VLDL, and HDL. When compared to control (healthy rats), serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) increased and high density lipoprotein cholesterol (HDL) decreased clearly in hyperlipidemic rats. After the treatment of ethanol extract of bark of *Tectona grandis* (200 mg/kg) and Glibenclamide in hyperlipidemic rats for 15 consecutive days, there was a significant decrease in serum lipids (TC, TG, LDL and VLDL), while there was an marked increase in HDL. This provided evidence in favor of the view that *Tectona grandis* could play an important role in treating diabetic/hyperlipidemic patients. Many current oral hypoglycemic or hypolipidemic agents are synthetic drugs with certain adverse side effects [16]. Our study reveals the potential of *Tectona grandis* bark for use as a natural oral agent with both hypoglycemic and hypolipidemic effects.

**Histopathological studies**

Histopathological examination of pancreas of these animals showed (Figure 5) comparable regeneration of Islets of Langerhans and β cells by ethanol extracts of *Tectona grandis* bark and Glibenclamide, which were earlier, necroses by alloxan. Figure 5(A-D) depicts the islets of the pancreas of rats of different groups. Photomicrographs (A) of the normal healthy control group showed normal acini and normal cellular population of the islets of Langerhans. However, in the alloxan only treated rats, there was extensive damage of the islets of Langerhans and they appeared to be irregular (B). Treatment of diabetic rats with glibenclamide showed moderate expansion of cellular population and size of islet cells (C). However, ethanol extract (200 mg/kg) treated-diabetic rats showed partial restoration of normal cellular population and size of islet cells (D). In our studies, damage of pancreas was observed in alloxan-treated diabetic control rats (Figure 5B). The glibenclamide-treated group showed regeneration of β cells [Figure 5 C]. The comparable regeneration was also shown by ethanol extracts of *Tectona grandis* bark (Figure 5D). Photomicrographs reinforce healing of pancreas by the ethanol extracts of *Tectona grandis* bark as a plausible mechanism of their antidiabetic activity. The antidiabetic activity of *Tectona grandis* may be due to the presence of tannins and quinones. Over 150 plant extracts and some of their active principle including flavonoids, quinones and tannins are known to be used for the treatment of diabetes [17].

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**Table 5: DPPH scavenging activity of ethanol extract of bark of *Tectona grandis***

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Ethanol Tectona grandis bark extract</th>
<th>BHT (Butylated hydroxytolune)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7.95</td>
<td>20.07</td>
</tr>
<tr>
<td>50</td>
<td>20.01</td>
<td>38.17</td>
</tr>
<tr>
<td>100</td>
<td>33.93</td>
<td>47.71</td>
</tr>
<tr>
<td>250</td>
<td>52.68</td>
<td>90.25</td>
</tr>
<tr>
<td>500</td>
<td>60.83</td>
<td>97.21</td>
</tr>
<tr>
<td>1000</td>
<td>79.52</td>
<td>95.22</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>238 µg /ml</td>
<td>107 µg /ml</td>
</tr>
</tbody>
</table>

Data represents mean ± S.E.M. of triplicate analysis.
In Vitro Antioxidant – DPPH free radical scavenging activity

Several concentrations ranging from 10-1000 µg/ml of the ethanol extract of bark of Tectona grandis tested for their antioxidant activity by DPPH model. It has been observed that free radicals were scavenged by the Tectona grandis bark ethanol extract in a concentration dependent manner in this DPPH assay (Table 5). The ethanol extract of bark of Tectona grandis showed DPPH radical scavenging activity with an IC₅₀ value of 238 µg/ml when compared with Standard BHT (Butylated hydroxytolune) IC₅₀ value of 107 µg/ml. Oxidative stress has been suggested as a contributory factor in the pathogenesis of diabetes. Diabetes, by itself, increases the production of tissue damaging reactive oxygen species (ROS) by glucose autoxidation and/or no enzymatic protein glycosylation [18]. Diabetic patients are exposed to oxidative stress and complications of diabetes seem to be mediated by oxidative stress. Hyperglycemia is one of the main causes of oxidative stress in type 2 diabetes. Under hyperglycemia, the increased blood level of various reducing sugars promotes protein glycation and advanced glycation end products (AGEs). Reactive oxygen species (ROS) are formed in this process and trigger tissue damage. Recently, the progressive deterioration of β cell function in type 2 diabetes has been accounted for in the oxidative stress-induced tissue damage [19]. The 1, 1-diphenyl -2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds. Plants provide a rich source of antioxidants, which include tocochromerols, Vitamin C, phenolic compounds, carotenoids [20], flavonoids, terpenoids, anthraquinones, steroids, strychnine and eugenol alkaloids [21]. From the present results, it may be postulated that Tectona grandis bark ethanol extract reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principals, so it can be concluded that the Tectona grandis bark ethanol extract has potent in vitro antioxidant potential which is attributed due to the presence of quinones and tannins like constituents present therein.

CONCLUSION

Tectona grandis Linn. bark ethanol extract shown to have hypoglycemic and antioxidant action. It is conceivable that antioxidant/ free radical scavenging activity of Tectona grandis bark ethanol extract is one of the mechanism associated with antidiabetic effect. The other mechanism is regeneration and moderate expansion of cellular population and size of islet of Langerhans and β cells by ethanol extracts of Tectona grandis bark. However, ethanol extract
(200 mg/kg) treated-diabetic rats showed partial restoration of normal cellular population and size of islet cells. These results seem to confirm the alleged antidiabetic activity by the traditional medicine. However the extract should further be subjected to bioactivity-guided drug discovery to isolate the lead compound responsible for antidiabetic activity and possible mechanisms(s) of action.

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