ABSTRACT

The Antidiabetic activity of *Kigelia africana* (Lam.) (Family: Bignoniaceae) and *Tabebuia rosea* (Bertol) DC. (Family: Bignoniaceae) were investigated in Alloxan induced diabetic albino rats. A comparison was made between both the plant extracts and a known antidiabetic drug Glibenclamide (5 mg/kg body weight). The dried leaves of *Kigelia africana* and *Tabebuia rosea* were subjected to extraction by continuous hot percolation using methanol as solvent and were subjected to standardization using pharmacognostical and phytochemical screening. Dose selection was made on the basis of acute oral toxicity study (200 mg/kg body weight) as per OECD and CPCSEA guidelines. Oral administration of extracts of *Kigelia africana* (200 mg/kg) and *Tabebuia rosea* (200 mg/kg) for 7 days resulted in a significant reduction in blood glucose levels. Alloxan induced diabetic rat model was used for the evaluation of antidiabetic activity. Activity is more for *Tabebuia rosea* in comparison with *Kigelia africana*. *Tabebuia rosea* methanolic extract (TRME) and *Kigelia africana* methanolic extract (KAME) showed significant (p<0.001) antidiabetic activity. The blood glucose levels of these plant extracts on seventh day of the study were TRME (108.2±4.1) KAME (127.5±2.5) in comparison of diabetic control (414.2±5.0). In glucose loaded rats, TRME exhibited glucose level of (129± 3.55) after 30 min and (91± 1.366) after 90 min, whereas the levels in KAME treated animals were (126.5±4.12) after 30 min and (88±3±0.647) after 90 min. These extracts also prevented body weight loss in diabetic rats. The drug has the potential to act as an antidiabetic drug.

Keywords: *Kigelia africana*, *Tabebuia rosea*, antidiabetic activity, Alloxan, Acute oral toxicity.

INTRODUCTION

MATERIALS AND METHODS

Animals

Healthy adult albino rats of Wistar strain of either sex between the age of 2-3 months and weighing 150-200 grams were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12 hours light and 12 hours dark cycle, 25 ±5°C and 40-60% humidity). They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg. no. (1447/PO/a/11/CPCSEA).

Chemicals

Alloxan monohydrate, Glibenclamide, Dextrose, Tween-80, Auto analyzer (Analytical technological limited) and One-touch (Horizon). All the other chemicals and reagents used were of analytical grade.

Plant Material

Fresh leaves were collected from Chittoor district, Andhra Pradesh, India and authenticated by Dr. K. Madhava Chetty, Professor, Department of Botany S.V. University, Tirupathi, Andhra Pradesh, India.

Preparation of Plant Extraction

The collected leaves were shade dried and powdered in a grinder mixture to get coarse powder. The powdered leaves were defatted with petroleum ether and later extracted with methanol. The extract was evaporated to dryness, gave a residue of 40% w/w.

Phytochemical Screening:

A preliminary phytochemical screening of methanolic extracts of *Kigelia africana* and *Tabebuia rosea* was carried by using standard procedures.

Acute Oral Toxicity Studies

Acute oral toxicity studies of the extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted and received from Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India. Administration of the stepwise doses of extracts of *Kigelia africana* from 40 mg/kg body weight up to the dose 2000 mg/kg body weight caused no considerable signs of toxicity in the tested animals. One tenth of upper limit dose were selected as the level for examination of anti-diabetic activity.

Experimental model

Alloxan monohydrate was first weighed individually for each animal according to their weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 120 mg/kg body weight intraperitoneally. After 1 hour of Alloxan administration, the animals were given feed ad libitum, and 5% dextrose solution was also given in a feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation and after 72 hours blood glucose was measured by One-touch glucometer. The diabetic rats (glucose level 200-300 mg/dl) were separated and divided into six different groups for experimental study, with each group containing six animals.

Experimental Design

Different groups of rats were used to study the effects of KAME and TRME. The rats were divided into six groups each consisting of six rats.

Group-I: Normal/control animals received 1% tween 80, 1 ml per orally.

Group-II: Alloxan (120 mg/kg body weight) induced diabetic animals received in 1% tween 80, 3 ml/kg body weight per orally.

Group-III: Alloxan (120 mg/kg body weight) induced diabetic animals received Glibenclamide 5mg/kg body weight per orally.

Group-IV: Alloxan (120 mg/kg body weight) induced diabetic animals received TRME 200mg/kg body weight per orally.

Group-V: Alloxan (120 mg/kg body weight) induced diabetic animals received KAME 200mg/kg body weight per orally.

Significant hyperglycemia was achieved within 48 hrs after Alloxan (120mg/kg b.w. i.p.) injection induced diabetic rats with more than 200mg/dl of blood glucose were identified as to be diabetic and used for the study.

In acute study all the surviving diabetic animals and normal animals were fasted overnight Blood samples were collected from the fisted animals prior to the treatment with above scheduled and after administration, at each day up to 7 days.
Alkaloids, Tannins, Flavonoids, Carbohydrates, and control, there was severe hyperglycemia
not significantly decreased. The methanolic extracts of
and Flavonoids in and
and KAME significantly (p<0.01) decreased fasting blood serum glucose
levels in diabetic rats on 3rd and 7th days as compared to initial (0 hr) blood serum glucose levels. When TRME and KAME were compared for their antidiabetic activity in comparison to active control, particularly Glibenclamide, the results showed that their potential was lesser but significant (**p<0.01) than the standard drug at subacute level.

Acute Oral Toxicity Studies
In acute toxicity study, none of the studied methanolic extracts
of leaves showed any significant toxicity sign when observed for
the parameters during the first 4 hours and followed by daily
observations for 14 days and mortality was also not observed. The
drug was found to be safe at the tested dose level of 2000 mg/kg b.
w. One-tenth of this dose level was taken as effective dose. All the
extracts were experimented at the same dose of 200 mg/kg b. w.
In order to ascertain a scientific base for the usefulness of this plant in
the treatment of diabetes. It was decided to evaluate experimental
design of antidiabetic activity by Alloxan-induced model.

Oral glucose tolerance test
The effect of different extracts on glucose tolerance test in normal
rats is shown in Table-1. At 30 min after glucose administration,
the peak of blood glucose level increased rapidly from fasting value and
then subsequently decreased. The methanolic extracts of Kigelia africana and Tabebuia rosea exhibited remarkable blood glucose
lowering effect at 90 min. africana and Tabebuia rosea exhibited
remarkable blood glucose lowering effect at 90 min.

Table 1: Effect of Methanolic extract of Kigelia africana and Tabebuia rosea on blood glucose level in Oral glucose tolerance test in normal rats.

<table>
<thead>
<tr>
<th>Sample</th>
<th>0 min</th>
<th>30 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>86 ± 1.065</td>
<td>127.2 ± 4.23</td>
<td>83.17 ± 1.24</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>80.50 ± 0.5</td>
<td>127 ± 2.5</td>
<td>92.17 ± 0.94***</td>
</tr>
<tr>
<td>TRME</td>
<td>80.33± 0.33</td>
<td>122.5± 0.12***</td>
<td>90.33± 2.12***</td>
</tr>
<tr>
<td>KAME</td>
<td>80.83± 0.47</td>
<td>127.2± 0.284***</td>
<td>88.83± 0.6007***</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM. n=6 animals in each group
Statistical significant test for comparison was done by ANOVA,
followed by Dunnett’s t-test. The blood glucose values of groups III, IV, V and VI are compared with control animals, values ***p<0.001,
**p<0.01, *p<0.05.

Alloxan induced diabetic model
As expected in the diabetic control, there was severe hyperglycemia
as compared to the normal animals. Compared to the diabetic
control, the TRME and KAME lowered the elevated blood glucose
levels only in subacute treatment Table-2. It was observed that the
standard drug glibenclamide lowered the blood glucose level significantly, bringing it nearly back to normal, whereas TRME and
KAME significantly (p<0.01) decreased fasting blood serum glucose
levels in diabetic rats on 3rd and 7th days as compared to initial (0 hr) blood serum glucose levels. When TRME and KAME were compared for their antidiabetic activity in comparison to active control, particularly Glibenclamide, the results showed that their potential was lesser but significant (**p<0.01) than the standard drug at subacute level.

Table 2: Effect of Methanolic extract of Kigelia africana and Tabebuia rosea on blood glucose level of alloxan induced diabetic albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood Glucose Levels (mg/dl)</th>
<th>0 day</th>
<th>3rd day</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>83.67 ± 2.48</td>
<td>84.17 ± 1.39</td>
<td>2.15</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>32.33 ± 1.65</td>
<td>369.7 ± 4.24</td>
<td>144.2 ±</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide</td>
<td>277.3 ± 8.27</td>
<td>127 ± 5.03</td>
<td>107.2 ±</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>TRME</td>
<td>5.22 ± 0.51</td>
<td>129.8 ± 4.11</td>
<td>108.2±4.1</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>KAME</td>
<td>295.3 ± 3.95</td>
<td>277.3 ± 6.57 **</td>
<td>127.5±2.5</td>
<td></td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM. n=6 animals in each group
Statistical significant test for comparison was done by ANOVA,
followed by Dunnett’s t-test. The blood glucose values of groups III, IV, V and VI are compared with control animals, values ***p<0.001,
**p<0.01, *p<0.05.
Table 3: Effect of Oral administration of the methanolic extracts of *Kigelia african* and *Tabebuia rosea* on serum profile in experiment rats after 7 days.

<table>
<thead>
<tr>
<th></th>
<th>TG</th>
<th>TP</th>
<th>LDH</th>
<th>Chol</th>
<th>Creat</th>
<th>ALP</th>
<th>AST</th>
<th>ALT</th>
<th>Alb</th>
<th>BUN</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>86.17±1.88</td>
<td>6.45±0.16</td>
<td>2192±80</td>
<td>139.3±2.98</td>
<td>0.53±0.03</td>
<td>114.7±1.61</td>
<td>2.04±0.99</td>
<td>2.75±0.11</td>
<td>0.763±0.073</td>
<td>4.145±1.45</td>
<td>43.5±1.09</td>
<td>32.17±0.60</td>
</tr>
<tr>
<td>DC</td>
<td>107.0±2.68</td>
<td>5.16±0.14</td>
<td>1485±15.0</td>
<td>274.5±1.94</td>
<td>1.30±0.11</td>
<td>315.3±8.83</td>
<td>1.70±4.67</td>
<td>2.75±0.29</td>
<td>60.80±0.89</td>
<td>32.17±0.60</td>
<td>109.5±2.98</td>
<td>1.765±0.073</td>
</tr>
<tr>
<td>Gli</td>
<td>92.17±1.70</td>
<td>6.27±0.08</td>
<td>1944±26.15</td>
<td>1447±3.21</td>
<td>0.55±0.03</td>
<td>130.3±3.99</td>
<td>61.17±1.53</td>
<td>3.66±0.15</td>
<td>1.118±0.05</td>
<td>1.376±0.05</td>
<td>1.376±0.05</td>
<td>72.83±2.98</td>
</tr>
<tr>
<td>TRME</td>
<td>93±2.12</td>
<td>6.27±0.13</td>
<td>1911±22.85</td>
<td>159.5±1.24</td>
<td>0.52±0.06</td>
<td>133.5±2.64</td>
<td>62.17±2.04</td>
<td>3.66±0.15</td>
<td>1.118±0.05</td>
<td>1.376±0.05</td>
<td>1.376±0.05</td>
<td>72.83±2.98</td>
</tr>
<tr>
<td>KAM</td>
<td>93.17±2.61</td>
<td>5.96±0.12</td>
<td>1995±13.29</td>
<td>171.2±2.04</td>
<td>0.79±0.04</td>
<td>135±3.16</td>
<td>74±2.56</td>
<td>141.7±1.83</td>
<td>3.62±0.16</td>
<td>41±0.730</td>
<td>84.17±1.515</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6. Statistical significance test for comparison was done by ANOVA, followed by Dunnett’s t-test. ***p<0.001, **p<0.01, *p<0.05.
Table 4: Effect of the methanolic extracts of *Kigelia africana* and *Tabebuia rosea* on body weight after treatment in diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Average body weight (g) ±SEM</th>
<th>Initial value</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>170 ± 1.36</td>
<td>185.8 ± 2.15</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>141.3 ± 1.99</td>
<td>118.3 ± 2.09</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide</td>
<td>180 ± 4.5***</td>
<td>203.8 ± 3.18***</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>TRME</td>
<td>180 ± 4.5***</td>
<td>201.3 ± 2.30***</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>KAME</td>
<td>169.8 ± 2.4***</td>
<td>172 ± 4.06***</td>
<td></td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s t-test. The Average body weight values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

Body Weight Measurement
In the present study, diabetic rats had lower body weights and high blood glucose level as compared to normal rats. In spite of increased food consumption, loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics. As shown in Table-4, treatment with TRME and KAME improved the average body weights of rats, which indicates that control over polyphagia and muscle wasting resulted due to hyperglycemic condition.

Histopathology

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**Normal Control Liver**

**KAME Treated Liver**

**TRME Treated Liver**
During diabetic perglycemic potential by various concentration of TC, TG, LDL and carbohydrates, 12, 13 uniappan BP, Perumal SM, Kandasamy M. 12, 13 Analysis of plasma lipids and most reliable marker and standard diagnosis practices for (HbA1c) is directly proportional to the decreased level of Flavonoids, Lignins, Proteins. Preliminary phytochemical screening revealed that KAME showed antidiabetic activity in normal structural intactness with their nucleus, e) Group IV (TRME) hepatocytes are normal. Occasional dilated central veins are seen along with occasional focal areas of necrosis.

REFERENCES
12. E. R. Briones, S. T. J. Mao, P. J. Palumbo, W. M. Ofallon, W. Chenoweth, B. A. Kottke, Analysis of plasma lipids and estimating the degree of protein glycation during diabetes mellitus8. Proglycation is a non-enzymatic reaction between excess glucose present in the blood and free amino groups on the globin component of haemoglobin. Measurement of HbA1c level provides information of long term glycemic status and to correlate with various complications related to Diabetes mellitus. On oral administration of TRME and KAME, the TRME, is more significantly decreased the Hb1c level possibly due to norglycemic control mechanisms in experimental rats which also reflect the decreased protein glycation condensation reactions and the reports obtained is concordant with the previous result9.

A marked increase in serum concentration of TC, TG, LDL and decreased HDL was observed with diabetic rats than normal control group which is often linked with hyperlipidaemia. Hyperlipidaemia certainly contributes to major risk factor for cardio vascular diseases10, 11. During diabetic state, insulin deficiency contributes to derangements of various metabolic and regulatory mechanisms in body. At normal state insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses triglycerides and prevents mobilization of free fatty acids12, 13. However, insulin deficiency inactivates the lipoprotein lipase which promotes liver conversion of free fatty acids into phospholipids and cholesterol and finally discharged into blood which resulted into elevated serum phospholipid level14, 15. My result of this study reveals that the administration of TRME and KAME not only lowered TC, TG and LDL, but also enhanced the cardioprotective lipid HDL.

