Evaluation of anti-diabetic and antioxidant activity of extract of *Calotropis gigantea* Linn. in streptozotocin-induced diabetic rats

Singh Kuldeep1*, Rao Ch V2, Hussain Zeashan3, Pahuja Ritu4

1. Department of Pharmacology, Faculty of Pharmacology and Pharmaceutical Science, Mahatma Gandhi Institute of Pharmacy, Junabganj, Kanpur Road Lucknow, Uttar Pradesh-227101, India. [Email: kuldeepsingh.pharma@gmail.com]
2. CSIR-National Botanical Research Institute, Ranapraptap Marg Lucknow, Uttar Pradesh -226001, India.
3. Director, Department of Pharmacology, Faculty of Pharmacology and Pharmaceutical Science, Mahatma Gandhi Institute of Pharmacy, Junabganj, Kanpur Road Lucknow, Uttar Pradesh-227101, India [Email: zeashanmgip@gmail.com]
4. All India Institute of Medical Sciences (AIIMS), Ansari Nagar East, Gautam Nagar, New Delhi, India. [Email: ritu.pahuja1987@gmail.com]

*Calotropis gigantea* L, belonging to family: Asclepiadaceae is also known as Sweat akand, is used in traditional medicine for treatment of various ailments. The present study showed that the efficacy of *Calotropis gigantea* leaves extract/fractions (EtOH, ChF, and BtF) for its antidiabetic and antioxidant activity. Since the effectiveness of *Calotropis gigantea* has been practicing among the ethnic population in and around eastern and southern regions of India. As in present study planned to investigate for the scientific pharmacological validation which can explore its antidiabetic effects.

**Keyword:** *Calotropis gigantea* Linn., Streptozotocin, Antidiabetic activity, Antioxidant assay, Histopathology.

**1. Introduction**

Diabetes is the metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia. The characteristic feature of diabetes mellitus is hyperglycemia due to defect in both action and secretion of insulin. Hyperglycemia as a common end point for all type of diabetes mellitus is followed by micro and macro vascular complications leading to cardiovascular disease, nephropathy, neuropathy and retinopathy. When the amount of glucose in the blood increases, e.g., after a meal, it triggers the release of the hormone insulin from the pancreas. Insulin stimulates muscle and fat cells to remove glucose from the blood and stimulates the liver to metabolize glucose, causing the blood sugar level to decrease to normal levels. In people with diabetes, blood sugar levels remain high. This may be because insulin is not being produced at all, is not made at sufficient levels, or is not as effective as it should be[1,2].

Type 2 diabetes is associated with increased oxidative stress. Free radicals are continuously produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Under physiological conditions, a wide range of antioxidant defenses protect against adverse effects of free radical production *in vivo*[3]. Oxidative stress results from
an imbalance between radical production and reduced activity of antioxidant defenses or both these phenomena. Hyperglycemia causes release of tissue damaging reactive oxygen species (ROS) that disturbs balance between radical production and protective antioxidant defense\cite{4}. It has been proposed that streptozotocin (STZ) acts as a diabetogenic agent owing to its ability to destroy pancreatic β-cells, possibly by a free radical mechanism\cite{5,6}. The level of lipid peroxidation in cell is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenging systems which are altered in diabetes\cite{7}.

In Indian system of medicine (Ayurvedic and Herbal) the usefulness *Calotropis gigantea* includes its antioxidant, hepatoprotective, insecticidal activities, etc. But the pharmacological and scientific evidence for its antidiabetic effect is yet to be proved. So based on above fact it can be evaluated for antidiabetic and antioxidant efficacy in streptozotocin (STZ) induced wistar rat strain.

2. Material and methods

2.1 Chemicals and reagents

Streptozotocin was purchased from Aldrich and Ascorbic acid, Nitro blue tetrazolium (NBT), sodium nitroprusside, dimethyl sulphoxide, potassium chloride and sodium chloride from Ranbaxy Laboratories Ltd., Mohali, India. Sulphanilic acids, sodium bicarbonate from E-Merck (India) Ltd, Mumbai, India. Disodium hydrogen phosphate, nutrient broths were obtained from Hi-Media Lab PVT. Ltd, Mumbai. All other chemicals used in the studies were analytical /laboratory grades procured from the following manufacturers, Loba chemie, ACROS Organics, Merck lab, S.D. Fine chemicals, Fluka.

2.2 Collection and Authentication

The fresh leaves (whole plant) of *Calotropis gigantea* us was collected during June 2011, from the ABS Botanical gardens kaaripatti Salem district, Tamilnadu. The plant species was identified and authenticated by taxonomist Dr. A. Balasubramanian. A voucher specimen was retained in the department for future reference.

The biochemical estimation was determined by using the commercially available standard diagnostic kits (Ecoline diagnostic kits and lab kits) manufactured by E-Merck Ltd. and Aldrich.

2.3 Preparation of the Extract

The collected fresh plant materials of *C. gigantea* were successively extracted with 95% ethanol by continuous hot percolation method using soxhlet apparatus. The solvent was removed under reduced pressure. The extract obtained was kept for drying and stored in vacuum desiccator.

2.4 Animals

Healthy, adult Wistar rats of both sexes (180-220 gm) were obtained from the Institutional Animal Ethics Committee, Mahatma Gandhi Institute of Pharmacy Uttar Pradesh. The animals were kept in a well ventilated room and the animals had exposed to 12 hrs day and night cycle with a temperature between 20±3 °C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this institution. All the experiments were performed after obtaining prior approval from IAEC Number 160/PO/a/12/CPSCA (30.4.2011).

2.5 Phytochemical Screening

The preliminary phytochemical studies were performed for testing different chemical groups in 95% ethanol extracts.

2.6 Induction of Diabetes

Non-Insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of 50 mg/kg streptozotocin. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hrs. The rats with permanent NIDDM (250-350 mg/dL) were used for the study.

2.7 Experimental Design

All the procedures for antidiabetic activity were performed in accordance with the Institutional Animal Ethical Committee constituted as per the
norms of CPCSEA, under the Ministry of Animal Welfare, Govt. of India, New Delhi, India. In order to assess the anti-diabetic activity, the animals were divided in five groups of six animals in each group[8, 9].

Group 1: Untreated Control
Group 2: Diabetic control
Group 3: Positive control (glibenclamide 10 mg/kg b.w i.p)
Group 4: EtOH extract of Calotropis gigantea (100 mg/kg, orally)
Group 5: EtOH extract of Calotropis gigantea (200 mg/kg, orally)
Group 6: ChF of Calotropis gigantea (100 mg/kg, orally)
Group 7: BtF of Calotropis gigantea (100 mg/kg, orally)

The test drug was administered for 21 days at a four different dose level 100, 200 mg/kg for ethanolic extract and 100, 100 mg/kg each of two successive fractions made in aqueous and given by orally. The blood was collected by sinus orbital under light diethyl ether anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Body weight, urine sugar, serum glucose was analyzed every week and fluid intake was analyzed every day. Total protein, albumin, creatinine, urea were also analyzed by serum. On the day of termination of the study, the animals were sacrificed, kidney were excised and stored in 10% buffered neutral formalin for histopathological studies[10-12].

2.8 In vitro antioxidant assay
The in vitro methods are based on the inhibition of free radical action. Samples are added to a free radical–generating system, inhibition of the free radical action is measured and this inhibition is related to antioxidant activity of the samples. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the endpoint that is used for the determination. Important is that all methods developed have strengths and limitations and a single measurement of antioxidant capacity usually is not sufficient. A number of different methods may be necessary to adequately assess in vitro antioxidant of a specific compound or antioxidant capacity of a biological fluid. Hence, all the extracts were tested of in-vitro antioxidant activity using several standard methods. In all these methods, the absorbance was measured against the corresponding blank solution and IC_{50} values were determined. IC_{50} is the concentration of the sample required to scavenge 50% of free radicals was calculated. The percentage inhibition was calculated by using the following formula[13, 14].

\[
\text{Radical scavenging activity (\%) = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100}
\]

2.9 Lipid peroxidation (LPO) assay
The test samples (100 µl) of different concentrations were added to 1 ml of egg lectin mixture, control was without test sample. Lipid peroxidation was induced by adding 10 µl FeCl_{3} (400 mM) and 10 µl L-ascorbic acids (200 mM). After incubation for 1 hr at 37 °C, the reaction was stopped by adding 2 ml of 0.25 N HCl containing 15% TCA and 0.375% TBA and the reaction mixture was boiled for 15 min then cooled, centrifuged and absorbance of the supernatant was measured at 532 nm.

2.10 Nitric oxide radical inhibition activity
The reaction mixture (6 ml) containing SNP (10 mm, 4 ml), PBS (1 ml) and 1 ml of extract in DMSO were incubated at 25 °C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrate was removed and 1 ml of sulphanalic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of NEDD was added, mixed and allowed to stand for 30 minutes in diffused light at room temperature. The absorbance of these solutions was measured at 540 nm using ELISA reader against corresponding blank solution. IC_{50} value obtained is the concentration of the sample required to inhibit 50% nitric oxide radical.

2.11 Scavenging of Hydrogen peroxide radicals
A solution of H_{2}O_{2} (20 mM) was prepared in PBS, (pH 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2 ml of H_{2}O_{2} solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a
blank solution that contained extracts in PBS without H$_2$O$_2$.

2.12 Scavenging of Superoxide radical by alkaline DMSO method
To the reaction mixture containing 1 ml of alkaline DMSO, 0.3 ml of the extracts in DMSO at various concentrations were added to 0.1 ml of NBT (0.1 mg) to give a final volume of 1.4 ml. The absorbance was measured at 560 nm.

2.13 Scavenging of hydroxyl radical by deoxyribose method
Various concentration of the extracts compound and standard in DMSO, were added to the reaction mixture containing deoxyribose and other chemical to make a total volume of 1.2 ml. The solutions were then incubated for 30 min at 37 °C. After incubation, ice-cold trichloro acetic acid and thiobarbituric acid in 0.25N Hydrochloric acid were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm.

2.14 Histopathological study
The kidney samples fixed were fixed for 48 hrs in 10% formal saline were dehydrated by passing successfully in different mixture of ethyl alcohol-water, cleaned in xylene and embedded in paraffin. Sections of kidney were prepared and then stained with hematoxylin and eosin dye, which was mounted in neutral deparaffinization xylene (DPX) medium for microscopic observations.

2.15 Statistical Analysis
All the data are expressed as mean ± SEM and analysed statistically using ANOVA followed Dunnett's test and compare with respective control group. A value of $P$<0.001 was considered as statistically significant.

3. Result
3.1 Phytochemical Studies
The preliminary phytochemical analysis of extract of *C. gigantea* showed the presence of glycoside, tannins, triterpenoids, saponins, Flavonoids, alkaloaid and protein.

3.2 Effect on body weight and fluid intake
Gradual increase in body weight in untreated control while the diabetic control continue to loose the weight. However, treated diabetic group gained 16%, 11%, 16%, 15% as compared to diabetic control and body weight of diabetic treated towards normal range $(P<0.001)$.

<table>
<thead>
<tr>
<th>S. No</th>
<th>GROUP</th>
<th>Body Weight (g)</th>
<th>Fluid intake g/animal/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>1.</td>
<td>Untreated control</td>
<td>194±1.88</td>
<td>220.5±1.839</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic control</td>
<td>202.66±2.33</td>
<td>168.5±2.513***</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetic+Glibenclamide(10mg/kg)</td>
<td>206.66±1.745</td>
<td>222.33±1.96***</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic+EtOH(100mg/kg)</td>
<td>209±1.932</td>
<td>220.16±1.078***</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetic+EtOH(200mg/kg)</td>
<td>197±2.176</td>
<td>213.6±1.476***</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetic+ChF(100mg/kg)</td>
<td>205.83±2.182</td>
<td>220.83±2.182***</td>
</tr>
<tr>
<td>7.</td>
<td>Diabetic+BtF(100mg/kg)</td>
<td>202.16±2.056</td>
<td>217.16±2.056***</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M (n=6). ***$P$<0.001 as compared to diabetic control
**$P$<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison test.

In chronic treatment with Ethanolic extract/fractions of CG significantly decrease the fluid intake as compared to diabetic control $(P<0.001)$. The change in body weight and fluid intake in all group of animals were given in Table 1.
**Fig 1:** Effect of treatment with ethanol extract/fractions of CG and standard drug Glibenclamide on change in body weights in normal and diabetic rats.

**Table 2:** Effect of administration of feeding the Ethanolic extract/fractions of CG leaves on serum glucose estimation in normal and diabetic rats.

<table>
<thead>
<tr>
<th>S.No</th>
<th>GROUP</th>
<th>Serum glucose (mg/dL)</th>
<th>0 day</th>
<th>7th day</th>
<th>14th day</th>
<th>21th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Untreated control</td>
<td></td>
<td>84.83±5.41</td>
<td>85.33±5.87</td>
<td>84.66±5.77</td>
<td>84.83±5.09</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic control</td>
<td></td>
<td>298.16±17.20</td>
<td>367.33±4.7**</td>
<td>413.83±16.61**</td>
<td>410±2.045**</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetic+Glubenclamide (10mg/kg)</td>
<td></td>
<td>280.33±2.44</td>
<td>205.33±1.145**</td>
<td>165±1.29***</td>
<td>114.83±1.302***</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic+EtOH (100mg/kg)</td>
<td></td>
<td>277.7±7.69</td>
<td>223.3±4.195**</td>
<td>164.66±1.406***</td>
<td>116.5±1.232***</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetic+EtOH (200mg/kg)</td>
<td></td>
<td>282.66±4.49</td>
<td>223.3±1.94**</td>
<td>164.16±1.406***</td>
<td>115±0.966***</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetic+ChF (100mg/kg)</td>
<td></td>
<td>290.83±5.46</td>
<td>221.5±3.51**</td>
<td>167±1.065***</td>
<td>115.83±0.945***</td>
</tr>
<tr>
<td>7.</td>
<td>Diabetic+BlF (100mg/kg)</td>
<td></td>
<td>285±533</td>
<td>219±2.63**</td>
<td>166.83±1.77***</td>
<td>115.3±0.881***</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± SEM (n=6).

**Fig 2:** Effect of treatment with ethanol extract/fractions of CG and standard drug (Glibenclamide) on change in serum glucose estimation in normal and diabetic rats.
3.3 Effect of CG on Serum glucose

The initial blood glucose levels of the diabetic rats selected for the study where in the range of 240-300 mg/dL. In the untreated control(diabetic) rats the blood glucose level increase to 379 mg/dL on the 7th day the glucose levels on the 14th and 21st day of the animals which survived where 410 mg/dL respectively. In the CG treated rats the blood glucose level suddenly decreased (P<0.001, P<0.01) thus the CG treatment restore the serum glucose levels almost nearer to normal value and comparable to that of positive control (P<0.001). The changes in Serum glucose estimation in all groups of animal were given in Table 2 and Figure 2.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Untreated control</td>
<td>86.5±0.76</td>
<td>54.83±1.138</td>
<td>54.80±0.693</td>
<td>18.38±0.40</td>
<td>8.793±0.40</td>
</tr>
<tr>
<td>2. Diabetic control</td>
<td>156.68±0.72</td>
<td>183.5±11.59</td>
<td>21.33±0.44</td>
<td>37.73±6.75</td>
<td>37.10±2.85</td>
</tr>
<tr>
<td>3. Diabetic + Glibenclamide (10mg/kg)</td>
<td>69.33±2.81</td>
<td>116.5±5.21</td>
<td>32.63±2.30</td>
<td>16.27±1.39</td>
<td>23.96±1.13</td>
</tr>
<tr>
<td>4. Diabetic + EtOH (100mg/kg)</td>
<td>126.88±0.25</td>
<td>64.16±1.53</td>
<td>35.45±3.66</td>
<td>52.38±1.51</td>
<td>13.82±0.22</td>
</tr>
<tr>
<td>5. Diabetic + EtOH (200mg/kg)</td>
<td>126.95±0.22</td>
<td>69.66±4.63</td>
<td>38.42±2.72</td>
<td>53.07±0.79</td>
<td>13.98±0.78</td>
</tr>
<tr>
<td>6. Diabetic + ChF (100mg/kg)</td>
<td>124.45±1.07</td>
<td>68.33±1.12</td>
<td>39.48±1.94</td>
<td>59.51±3.05</td>
<td>16.33±0.91</td>
</tr>
<tr>
<td>7. Diabetic + BtF (100mg/kg)</td>
<td>124.58±1.12</td>
<td>64.33±0.80</td>
<td>47.06±3.44</td>
<td>53.06±4.03</td>
<td>14.49±0.32</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01, as compared to diabetic control

3.4 Serum lipid profile and lipoprotein profile

Effect of extract/fraction of CG on the control and experimental animals. STZ diabetic rats group where found to have significantly increased HDL, LDL, VLDL, TG, TC, levels as compared to control group (P<0.001, P<0.01) HDL cholesterol was also reduced significantly in diabetic rats after treatment of ethanolic extract/fraction of CG. Positive control was significantly preventing the increasing the serum TC, TG, HDL, LDL, VLDL, as compared to diabetic group. Diabetic treated group was significantly increased in HDL cholesterol level as compared to diabetic group. (P<0.001, P<0.01). Thus the CG treatment restores all these changes near to normal value. The change in serum lipid and lipoprotein profile were tabulated in table 3.

3.5 Effect of Calotropis gigantea leaves on kidney markers

Effect of extract/fractions of CG on serum levels urea, creatinine, total protein, and albumin in control and diabetic rats. Urea, creatinine biochemical variable were significantly elevated in diabetic induced rats. (P<0.01) when compared to control animal (P<0.01). After oral administration of CG for 21 days the urea and creatinine level were significantly lowered in diabetic rats. (P<0.01) and the level of total protein and albumin were found to be reduced in diabetic animals (P<0.01) (P<0.001) when compared to control animal the lowered level of albumin and total protein reverted back significantly in CG treated diabetic rats (P<0.01), (P<0.001). The change in all serum parameter (urea, creatinine, total protein, albumin) in all groups of animals were given in table 4.

3.6 Effect of Calotropis gigantea leaves of extract/fractions on in vitro antioxidant parameter: Among the two successive fraction and extract tested for in vitro antioxidant activity.
Ethanolic extract exhibit potent antioxidant activity in nitric oxide inhibition, lipid per oxidation assay and IC₅₀ values were found to be 800.58±0.300, 48.50±0.381 respectively. The values were found to be comparable to those obtained for the standards used. However ethanolic extract was found to be moderate to low in inhibition of hydrogen peroxide, deoxyribose, and superoxide assay. The chloroform fraction showed poor antioxidant activity in lipid peroxidation and deoxyribose inhibition assay with IC₅₀ value 64.375±1.33, 71.875±0.625 and was found to be active in all other method. The n-butanol fraction showed good scavenging activity in nitric oxide, hydrogen peroxide, superoxide method with IC₅₀ value 764.54±0.658, 286.04±0.52, 248.91±0.583. In lipid per oxidation and deoxyribose assay shown moderate antioxidant activity with IC₅₀ value 45.50±0.125, 40.51±0.516 respectively. The changes in in vitro antioxidant method were given in table 5.

**Table 4:** Effect of ethanolic extract/fractions of CG leaves on serum urea, creatinine, total protein and albumin in Streptozotocin induced rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Untreated control</td>
<td>23.70±0.148</td>
<td>0.9±0.025</td>
<td>6.195±0.26</td>
<td>3.48±0.15</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic control</td>
<td>37.59±0.73</td>
<td>2.41±0.123</td>
<td>3.99±0.12</td>
<td>1.63±0.10</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetic+Glibenclamide (10mg/kg)</td>
<td>21.50±0.39*</td>
<td>1.35±0.114**</td>
<td>6.09±0.174***</td>
<td>3.26±0.083**</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic+EtOH (100mg/kg)</td>
<td>28.17±2.58*</td>
<td>1.20±0.413**</td>
<td>5.86±0.24***</td>
<td>2.99±0.407**</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetic+EtOH (200mg/kg)</td>
<td>30.32±1.77*</td>
<td>1.09±0.258**</td>
<td>5.46±0.29***</td>
<td>3.33±0.12**</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetic+ChF (100mg/kg)</td>
<td>28.46±1.58*</td>
<td>1.02±0.171**</td>
<td>5.26±0.342***</td>
<td>3.45±0.73**</td>
</tr>
<tr>
<td>7.</td>
<td>Diabetic+BtF (100mg/kg)</td>
<td>28.16±2.24*</td>
<td>1.3±0.314**</td>
<td>6.61±0.49***</td>
<td>3.57±0.096**</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01 as compared to diabetic control. ### P<0.001, ##P<0.01 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison test.

**Table 5:** In vitro antioxidant activity

<table>
<thead>
<tr>
<th>S. No</th>
<th>Extract/Compound</th>
<th>Hydrogen peroxide IC₅₀ Value ± S.E.M. (µg/mL)</th>
<th>Nitric oxide</th>
<th>Lipid-peroxidation</th>
<th>Deoxyribose</th>
<th>Superoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethanolic extract</td>
<td>330.1±0.076</td>
<td>800.58±0.300</td>
<td>48.50±0.381</td>
<td>45.65±0.046</td>
<td>298.87±0.590</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform fraction</td>
<td>388.83±0.600</td>
<td>&gt;1000</td>
<td>64.375±1.33</td>
<td>71.875±0.625</td>
<td>502±1.155</td>
</tr>
<tr>
<td>3.</td>
<td>n-butanol fraction</td>
<td>286.04±0.52</td>
<td>764.54±0.658</td>
<td>45.50±0.125</td>
<td>40.51±0.516</td>
<td>248.91±0.583</td>
</tr>
</tbody>
</table>

**Standard**

<table>
<thead>
<tr>
<th></th>
<th>Rutin</th>
<th>α-Tocopherol</th>
<th>Ascorbic acid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36.23±0.0145</td>
<td>_</td>
<td>_</td>
<td>1000.2±0.200</td>
</tr>
<tr>
<td></td>
<td>68.3±0.152</td>
<td>_</td>
<td>91.38±0.198</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>11.24±0.003</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

Means (-) not done, Average of three determinations
3.7 Histopathological Examination of Kidney

Histopathological examination of kidney

Fig 3a: Photomicrograph of normal rat kidney showing normal morphology

Fig 3b: Photomicrograph of diabetic untreated rat kidney showing fatty degeneration

Fig 3c: Photomicrograph of glibenclamide treated rat kidney showing near normal

Fig 3d: Photomicrograph of EtOH 100mg/kg treated rat kidney showing tubular epithelial damage messengial capillary proliferation
4. Discussion

Qualitative phytochemical screening and ethnobotanical survey on the ethanolic root extract of *Calotropis gigantea* leaves revealed the presence of certain phytoconstituents such as alkaloids, tannins, high amount of saponins, phytosterols, proteins, terpenoids and flavonoids[15, 16]. Phytochemical constituents such as glycoside, tannins, triterpenoids, saponins, alkaloid and protein may be linked to the antidiabetic activity. The secondary metabolites like tannins, phenol and flavonoids are likely responsible for the observed antidiabetic activity. Flavonoids and Phenol to possess a wide spectrum of biological actions including hypoazotemic, hypotensive, hypoglycaemic, oestrogenous, spasmylytic, chologogue, anti-inflammatory, antilipidemic and antioxidant activities. In present study the extract/fraction showed maximum amount of total phenol and flavonoids content[17].
A number of scientific reports indicate certain terpenoids, steroid, phenolic compound such as tannins, flavonoids have protective effect due to antioxidant properties after confirming of these constituent tested by standard test in CG extract/fractions. The present study observed that the CG extract/fractions showed the presence of constituents which has good to moderate antioxidant activity in different in vitro method. It could be relevant contributor for the synergistic activity of antioxidant of CG extract/fractions[15, 16].

The dose of ethanolic extract EtOH (100, 200 mg/kg), Chf, BtF (100 mg/kg) not only lowered the TC, TG, VLDL, LDL levels but also enhanced the cardio protective lipid HDL in normal and diabetic rats after 21 days of treatment. In present study the extract/fractions not only decrease the TC level but also enhance the HDL cholesterol significantly. High level of triglycerides and more importantly LDL cholesterol is major cause of coronary risk factors. Administration of leaves of extract/fractions to diabetic rats for 21 days lowered TC and LDL cholesterol level respectively. This is the important finding of this study as diabetes is associated with coronary complications[11].

Rise in whole kidney weight with in 72 hr of induction of STZ in experimental diabetic rats we have also observed an increased whole kidney weight in diabetic animals when compared with normal control animals. This is due to the glomerular cell proliferation accompanying glomerular enlargement in the early phase of STZ induced diabetes in rats. In our present study oral administration of CG significantly decrease the kidney weight to near normal value. This might be due to the protective effect of CG on glomerular cells in STZ induced diabetes in rats [18].

The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine which are considered as significant marker of renal dysfunction. The result showed a significant increase in the level of serum urea, creatinine in the diabetic rats when compared with respective control rats. The levels of urea and creatinine were significantly decreased after the treatment of STZ diabetic rats with CG extract/fractions. Reduction in serum total protein and albumin level was observed in diabetic rats and this is consistent with result[18].

The decrease in total protein and albumin may be due to the microproteinuria and albumiuria which are important clinical markers of diabetic nephropathy may be due to protein catabolism. The result of present study demonstrated that the treatment of diabetic rat with CG caused noticeable elevation in serum total protein and albumin levels as compared with normal levels. It has been established that insulin stimulates the incorporation of amino acids into proteins.

The abnormally high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots since insulin inhibits the hormones sensitive lipase. On the other hand glucagon, catecholamine and other hormones enhance lipolysis. The marked hyperlipidemia that characteristic the diabetic state may therefore be regarded as a consequence of the unlimited action of lipolytic hormones on the fat depot. In our study we have also observed higher level of cholesterol and triglycerides from kidney homogenate in STZ diabetic rats. The result of the study showed the continuous supplementation of CG for 21 days prevent elevation of tissue lipids in diabetic state[19].

Histopathology reports of kidney gave additional support to the study. Kidney sections of normal animals showed the normal architecture with well brought out central vein, well preserved cytoplasm and prominent nucleolus whereas the diabetic group section showed the presence of feathery degeneration, micro and macro cellular fatty changes and inflammatory cells around portal tract. The other groups showed good protection from STZ induced changes in the Kidney. The sections of normal rat kidney showed the normal nephro-morphology whereas the diabetic section showed fatty degeneration. The other groups showed the less pathological changes of the kidney.
5. Conclusion
The result of this investigation revealed that the extract of *C. gigantea* plant possesses significant antidiabetic and antioxidant activity in treating Streptozotocin-induced. The main constituent present in the extract was identified as flavonoids. From the previous literature it was reported that flavonoids and terpenoids are strong antioxidant and showed very potent antidiabetic activity. Hence in future study, fractionization is going on for the isolation of lead molecule from extract that will be responsible for potent antioxidant and anti-diabetic agents.

6. Conflict of interest
The authors declare that they have no conflict of interest.

7. Acknowledgement:
I wish to express my sincere gratitude to Shri Venkateshwara University for their encouragement to carry out research work.

8. References
