Antioxidant potentials of *Terminalia catappa* leaf extract in Streptozotocin induced diabetes in rats

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**ABSTRACT**

The objective of this study to evaluate the antioxidant capacity of the ethanolic extract of *Terminalia catappa* in vivo in Wistar albino rats. Streptozotocin (STZ) used as toxin it induces diabetes, damages the cell membrane and causes oxidative stress. The leaves (T. catappa) were extracted using 95 % ethanol by hot continuous percolation method. The extracts were concentrated by rotaevaporador. The residues extract were administrated orally to the STZ induced diabetes animals. After 45th day the animals were sacrificed and blood, liver tissues were collected and various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), were reduced in STZ alone treated animals with subsequent increase in LPO. The serum levels of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA), phospholipids, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and high density lipoprotein (HDL) was found out. Treatment with ethanolic extract of *T. catappa* at a dose of 300, 500 mg/kg once in a day has altered the levels of biochemical markers and brings back to near normal levels. Among which the dose 500 mg/kg having scavenging action to eradicate free radicals and maintained antioxidant status. The statistical data of P Values <0.001 were considered as level of significance.

**Key words:** Antioxidant, Catalase, Lipid peroxidation, Lipid profile, Reduced glutathione. Streptozotocin.

**INTRODUCTION**

Diabetes mellitus (DM) is a group of metabolic ailments described with hyperglycemia and its secondary complications in various organs. It is a challenge to individual’s health and their lifestyle (Anthony et al., 2008). Chronic complications of diabetes are associated with oxidative stress. The free radicals such as hydrogen peroxide (H$_2$O$_2$), nitric oxide (NO), superoxide anions, generated in diabetic patient causes oxidative injury inspite of the capacity to eradicate the free radicals and maintain the antioxidant levels. (Chandra et al., 2000). Commercially available antioxidant agents produce many side effects. *Terminalia catappa* Linn. (*T. catappa*) is one of the plants from the family Combretaceae and is native to Southeast Asia (Gao et al., 2004). *T. catappa* is a well-recognized tree in Ayurveda (Mininel et al., 2014). The juice of its fresh leaves is used in the preparation of medicinal lotion to treat leprosy and scabies and it is taken internally for stomach and headache (Mandloi et al., 2013). The concurrent pretreatment of the Chinese hamster ovary-K1 (CHO-K1) cells with the aqueous extract of *T. catappa* leaf considerably suppresses mitomycin C-induced micronuclei. It also inhibits lipid peroxidation (LPO) and hydrogen peroxide formation induced by TPA in human mononuclear leukocytes in a dose-dependent manner. (Liu et al., 1996). Lin et al., (1997) found that treatment with the aqueous extracts of *T. catappa* exhibited antihapatotoxic activity against carbon tetrachloride (CCl$_4$) induced toxicity in the rat. The current study is aimed to evaluate the antioxidant activity of plant *T. catappa* ethanolic extract in streptozotocin (STZ) induced diabetic rats.

**MATERIALS AND METHODS**

**Collection and extraction of *T. catappa***: The leaves of *T. catappa* were collected from Trichy and under shade dried and ground into powder and sieved. The powdered plant material was subjected to successively soxhlet extraction by with a solvent of 95% ethanol (600ml /100g of leaf powder) by hot continuous percolation method. The residues extract were concentrated by rotaevaporador. Finally the extract was concentrated by drying process at the temperature of 40-50°C.

**Selection of animals**: Wistar albino rats of either sex weighing 150-200 g (6 to 7 weeks) with no prior drug treatment were used for the experiment. The rats were kept in laboratory animal house at Periyar College of Pharmaceutical Sciences, Tiruchirappalli. The animals were given feed and water ad libitum. The experiment was approved by the Institutional Animal Ethical Committee.

**Induction of diabetes**: STZ was purchased from Himedia Laboratories Pvt Ltd, Mumbai. Hyperglycemia was induced...
by a single injection, and STZ was dissolved in sterilized citrate buffer pH 4.5. The Wistar albino rats were fast overnight and STZ 60 mg/kg was administered intraperitoneally. After 48h animals with elevated plasma glucose level above 200mg/dl were considered as diabetic. After the confirmation of disease, the animals were orally treated with a dose of various concentrations of ethanolic leaf extract of *T. catappa* 300 mg/kg b.w, and 500 mg/kg b.w) for 45 days.

**Experimental design:** Experimental animals were randomly divided into six groups with six animals, in each group 4th and 5th group treated with STZ and leaf extract (300 and 500 mg/kg of b.w) respectively in 3rd group treated with leaf extract (500 mg/kg of b.w) alone. In 6th group treated with standard drug (3mg/kg of b.w glibenclamide). In 2nd group as a control treated with (60mg/kg of b.w STZ). 1st group as a control.

Preliminary oral LD_{50} dose of ethanol extract of *T. catappa* in rats was found to be 5000mg/kg. One-tenth of the LD_{50} dose of ethanol extract 500 mg/kg per day was selected for treatment as maximum dose.

**Biochemical analysis:** Blood samples were collected by retro-orbital plexus puncture. The lipid profile and antioxidant levels were analysed in the blood and liver tissues respectively. A known weight of Liver tissues was homogenized in 0.1M ice-cold Tris – HCL buffer (pH 7.5). The concentration of LPO, (Gohel *et al.*, 1999), GSH, (Carlberg and Manervick., 1975), GPx (Rotruck *et al.*, 1973), SOD, (Misra and Fridovich,1979), CAT, (Sinha., 1972.) TC (Siedel *et al.*, 1983), TG (Foster and Dunn, 1973), LDL, VLDL, HDL, (Burstein *et al.*, 1970) FFA (Falholt *et al.*, 1973), phospholipids (Shirwaikar *et al.*, 2002) were estimated using standard laboratory procedures.

**Statistical analysis:** Statistical analysis was carried out by using one way ANOVA using Standard Statistical Software Package of Social Science (SPSS) version 12.0. P values <0.001 were considered as level of significance.

**RESULTS AND DISCUSSION**

Table 1 shows the concentration of SOD, CAT, GPx, LPO, and GSH in the liver of normal and experimental animals. The SOD value was decreased in STZ treated animals (group II), as compared to control animals (group I). The SOD values was increased and its attain a near normal level in *T. catappa* treated animals (group IV).

The remarkable reduction of CAT, GPx, GSH and increase in LPO was found in group II as compared to control animals. These antioxidant markers were near normal levels in liver tissue of *T. catappa* administered group V.

The level of lipoproteins including HDL, VLDL, LDL and FFA, phospholipids levels are shown in the Table 2. The concentration of HDL, LDL, and VLDL levels were increased in group II as compared to normal control animals. The concentration HDL, LDL, VLDL were decreased to normal level in group V when compared with the standard drug.

In diabetes the increased blood glucose level initiate the production of free radicals subsequently it affects cellular function. Cell membranes are damaged and it speed up the lipid peroxidation (Giugliano *et al.*, 1996). Peroxidation of lipid is very reactive and it causes the damages in protein, DNA molecule and finally causes the various diabetes mediated complications. (Gopalakrishnan and Dhanapal, 2013).

The tissue damage percentage predisposed by free radicals, depends on the balance between the generation of free radicals and endogenous antioxidant resistant mechanism (Davi *et al.*, 2005). In the present study, the SOD levels are increased after the treatment of ethanolic extract of *T. catappa* treated group becoming similar to the standard drug treated groups. The levels of CAT decreased in the

**Table 1:** Effect of ethanolic leaf extract of *T. catappa* leaves on antioxidant enzymes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
<th>GROUP IV</th>
<th>GROUP V</th>
<th>GROUP VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (μ mol/min/mg)</td>
<td>8.54 ± 0.80</td>
<td>4.49 ± 0.35</td>
<td>8.26 ± 0.80</td>
<td>5.47 ± 0.42</td>
<td>7.37 ± 0.64</td>
<td>7.02 ± 0.94</td>
</tr>
<tr>
<td>CAT (μ mol/min/mg)</td>
<td>75.08 ± 6.82</td>
<td>55.26 ± 5.46</td>
<td>75.26 ± 2.29</td>
<td>65.59 ± 3.94</td>
<td>73.14 ± 4.45</td>
<td>73.62 ± 4.95</td>
</tr>
<tr>
<td>GPx (μ mol/min/mg)</td>
<td>7.88 ± 0.70</td>
<td>4.10 ± 0.81</td>
<td>7.88 ± 0.93</td>
<td>6.77 ± 0.65</td>
<td>7.30 ± 0.38</td>
<td>7.18 ± 0.53</td>
</tr>
<tr>
<td>LPO (n mol/g)</td>
<td>75.00 ± 5.05</td>
<td>104.76 ± 8.94</td>
<td>74.40 ± 4.17</td>
<td>91.36 ± 3.31</td>
<td>82.32 ± 2.80</td>
<td>86.90 ± 7.02</td>
</tr>
<tr>
<td>GSH (mg/g)</td>
<td>19.03 ± 0.12</td>
<td>9.15 ± 0.31</td>
<td>18.18 ± 1.09</td>
<td>17.56 ± 0.73</td>
<td>14.07 ± 1.02</td>
<td>16.97 ± 0.65</td>
</tr>
</tbody>
</table>

Values are means ± SD for six rats in each group

* significant difference at P<0.001 compared with the group I. b significant difference P<0.001 compared with group II.

**Table 2:** Effect of ethanolic leaf extract of *T. catappa* leaves on lipid profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
<th>GROUP IV</th>
<th>GROUP V</th>
<th>GROUP VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL (mg/dL)</td>
<td>21.64 ± 3.37</td>
<td>102.15 ± 3.68</td>
<td>21.03 ± 1.09</td>
<td>35.03 ± 4.46</td>
<td>25.89 ± 3.44</td>
<td>26.53 ± 5.19</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>44.35 ± 2.86</td>
<td>23.85 ± 2.18</td>
<td>44.79 ± 2.05</td>
<td>37.96 ± 2.67</td>
<td>42.68 ± 2.53</td>
<td>41.98 ± 2.62</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>12.16 ± 0.45</td>
<td>24.28 ± 1.33</td>
<td>12.06 ± 0.42</td>
<td>17.80 ± 0.56</td>
<td>12.21 ± 0.54</td>
<td>12.47 ± 0.35</td>
</tr>
</tbody>
</table>

Values are means ± SD for six rats in each group

* significant difference at P<0.001 compared with the group I. b significant difference P<0.001 compared with group II.
### Table 3: Effect of ethanolic leaf extract of *T. catappa* leaves on Lipid profile in plasma and liver tissues

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
<th>GROUP IV</th>
<th>GROUP V</th>
<th>GROUP VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>76.16 ± 3.00</td>
<td>77.87 ± 2.90</td>
<td>80.98 ± 4.02</td>
<td>80.50 ± 3.86</td>
<td>80.98 ± 4.02</td>
<td>80.80 ± 3.86</td>
</tr>
<tr>
<td>(Plasma mg/dL)</td>
<td>150.29 ± 5.94</td>
<td>90.81 ± 5.04</td>
<td>102.64 ± 4.23</td>
<td>61.09 ± 0.18</td>
<td>61.09 ± 0.18</td>
<td>61.09 ± 0.18</td>
</tr>
<tr>
<td>(Liver mg/g wet tissue)</td>
<td>4.42 ± 0.21</td>
<td>4.40 ± 0.11</td>
<td>5.05 ± 0.26</td>
<td>6.10 ± 0.27</td>
<td>6.10 ± 0.27</td>
<td>6.10 ± 0.27</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>60.80 ± 2.26</td>
<td>60.30 ± 2.13</td>
<td>60.90 ± 2.83</td>
<td>60.90 ± 2.83</td>
<td>60.90 ± 2.83</td>
<td>60.90 ± 2.83</td>
</tr>
<tr>
<td>(Plasma mg/dL)</td>
<td>121.43 ± 6.66</td>
<td>89.02 ± 2.83</td>
<td>77.04 ± 2.74</td>
<td>77.04 ± 2.74</td>
<td>77.04 ± 2.74</td>
<td>77.04 ± 2.74</td>
</tr>
<tr>
<td>(Liver mg/g wet tissue)</td>
<td>3.74 ± 0.22</td>
<td>3.53 ± 0.09</td>
<td>4.40 ± 0.22</td>
<td>4.40 ± 0.22</td>
<td>4.40 ± 0.22</td>
<td>4.40 ± 0.22</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>58.88 ± 2.55</td>
<td>74.44 ± 2.97</td>
<td>59.61 ± 4.32</td>
<td>59.61 ± 4.32</td>
<td>59.61 ± 4.32</td>
<td>59.61 ± 4.32</td>
</tr>
<tr>
<td>(Plasma mg/dL)</td>
<td>91.44 ± 2.97</td>
<td>82.2 ± 0.48</td>
<td>108.2 ± 0.48</td>
<td>108.2 ± 0.48</td>
<td>108.2 ± 0.48</td>
<td>108.2 ± 0.48</td>
</tr>
<tr>
<td>(Liver mg/g wet tissue)</td>
<td>8.20 ± 0.25</td>
<td>8.2 ± 0.48</td>
<td>10.8 ± 0.48</td>
<td>10.8 ± 0.48</td>
<td>10.8 ± 0.48</td>
<td>10.8 ± 0.48</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>78.66 ± 2.79</td>
<td>77.33 ± 2.38</td>
<td>80.00 ± 3.37</td>
<td>80.00 ± 3.37</td>
<td>80.00 ± 3.37</td>
<td>80.00 ± 3.37</td>
</tr>
<tr>
<td>(Plasma mg/dL)</td>
<td>138.22 ± 7.80</td>
<td>97.51 ± 1.57</td>
<td>90.01 ± 3.37</td>
<td>90.01 ± 3.37</td>
<td>90.01 ± 3.37</td>
<td>90.01 ± 3.37</td>
</tr>
<tr>
<td>(Liver mg/g wet tissue)</td>
<td>21.42 ± 1.03</td>
<td>21.59 ± 1.80</td>
<td>23.11 ± 2.09</td>
<td>23.11 ± 2.09</td>
<td>23.11 ± 2.09</td>
<td>23.11 ± 2.09</td>
</tr>
</tbody>
</table>

Values are means ± SD for six rats in each group. * significant difference P<0.001 compared with group II.

In the present study, the level of GSH is decreased in the diabetic treated group, and the levels are normal in the ethanolic extract of *T. catappa* treated groups, and this extract treated group values are similar to the standard drug treated group. GSH is the intracellular free radical scavenger. Plasma antioxidant status is mediated by this enzyme and it also act as an important cofactor for numerous enzymes. In mesangial cells (Catherwood et al., 2002) and muscle cells (Sharpe et al., 1998, Hamilton et al., 2003) the level of GSH decreased in hyperglycemic condition. Decreased GSH concentration may reduce the resistance against the oxidative injury during the disease conditions including diabetes (Gopalakrishnan and Dhanapal, 2013). The amount of TG was significantly greater (P<0.001) in STZ treated rats as associated to normal control rats.

These components were present to attain a near normal level in plasma of *T. catappa* leaf extract treated rats. *T. catappa* alone treated rats and STZ+ glibenclamide treated rats. The concentration of LDL cholesterol was considerably (P<0.001) advanced in STZ treated rats as compared to normal control animals. These components were set up to achieve a near normal level in plasma of *T. catappa* treated rats STZ+ *T. catappa* treated rats and STZ+ glibenclamide treated rats. The concentration of HDL cholesterol was significantly (P<0.001) lower in STZ treated rats as compared to normal control animals. These constituents were found to attain a near normal level in plasma of *T. catappa* treated rats STZ+ *T. catappa* treated rats and STZ+ glibenclamide treated rats (P<0.001). Type 2 diabetes mellitus is followed by dyslipidemia (Harris and Crabb, 1982). The insufficient insulin may decreases the activity of lipoprotein lipase, causing disturbances in lipoprotein metabolism (Ranganathan and Kern, 2000). Chronic diabetes affect heart coronary vessels by the increasing level of TC.
TG, LDL and decreasing the level of HDL (Arvind et al., 2002). Therefore the primary treatment for diabetes is necessary to control the lipid profile values.

In serum TC, TG, LDL levels were elevated, and the HDL level decreased in the toxin treated group. The administration of Ethanolic extract of T. catappa leaves reduced the level of TC, TG, LDL, VLDL and increased the level of HDL. The present study states that the Ethanolic extract of T. catappa leaves exhibited the antidiabetic and its associated anti-oxidant activity in STZ induced animals. A noticeable increase in blood concentration of TC, TG, LDL and decreased HDL was detected with diabetic rats than normal control group which is often linked with hyperlipidemia. Hyperlipidemia certainly contributes to major risk factor for cardio vascular diseases (Umeh, et al., 2005, Nikkila, et al., 1973).

CONCLUSION

The results indicate that the Ethanolic extract of T. catappa may normalize the antioxidant and lipid levels in STZ induced diabetic rats, and T. catappa may be used for amelioration of antioxidant imbalances due to diabetes.

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