Terminalia chebula attenuates ischemia reperfusion induced progressive nephropathy in diabetic rats
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ABSTRACT
Terminalia chebula is called “king of medicines” in ayurveda and has been used traditionally to treat various diseases. Ischemia causes rapid progressive nephropathy in diabetic rats. The present study was undertaken to investigate the effect of T chebula on unilateral ischemia induced renal damage in diabetic rats. The 4 week treatment with ethanolic extract of T chebula fruit (200 mg/kg & 400 mg/kg) showed reduction in damage caused by ischemia; decreased oxidative stress and improved renal histological appearance of kidney section. From results it was concluded that T chebula attenuates the renal damage caused by ischemia and can be beneficial in diabetic nephropathy.

Keywords: Diabetic nephropathy, ischemia & reperfusion, Terminalia chebula.

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1. INTRODUCTION

_Terminalia chebula_ is called as "king of medicines" in ayurveda and has been used traditionally to treat various diseases. In modern era it has been used as cytoprotective (Lee, Ryu, Choi, Lee, No, Kim, & Ahn, 1995) antidiabetic (Kumar, Arulselvan, Kumar, & Subramanian, 2006), renoprotective (Rao, & Nammi, 2006), antioxidant (Naik, Priyadarsini, Naik, Gangabaghirathi, & Mohan, 2004) and α glucosidase inhibitor (Gao, Huang, Xu, & Kawabata, 2007). However, no reports were available on protective effect of _T. chebula_ against ischemic renal damage in diabetes, thus the aim of the present study is to investigate the effect of _T. chebula_ in ischemia induced renal damage in diabetic rats.

2. RESULTS AND DISCUSSION

Nephropathy in diabetic patients follows a well outlined course, starting with microalbuminuria through proteinuria, azotaemia and end stage renal disease (ESRD). Renal ischemia and reperfusion model was used in this study as it causes rapid progressive nephropathy in diabetic rats. Short period of ischemia caused substantial loss in renal function, resembling human renal disease causing mesangial cell expansion, basal membrane thickening, tubulointerstitial fibrosis (Wongmekiat, et al., 2007). Hyperglycemia is considered as a high risk factor for the development of diabetic nephropathy (Kumar, & Subramanian, 2008). In present study blood glucose level of diabetic and diabetic nephropathy animals was increased to almost 5 folds of normal range. Treatment groups showed a significant reduction in blood glucose level; prominent effect was observed by TC 4 (115.2±5.13) groups. _T. chebula_ was able to control the elevated blood glucose level supporting its antihyperglycemic effect; the effect may be due to presence of phytoconstituents such as alkaloids, glycosides and flavonoids resulting in insulinogenic effect (Kumar, & Subramanian, 2008).

Hyperglycemia causes glycation of hemoglobin; glycation itself generate the oxygen derived free radicals; further increasing the risk of proteinuria and renal failure. Strict control of glucose causes a subsequent reduction in GHb levels leading to reduction in inflammatory mediator’s (Krishnamurti, & Steffes, 2001). _T. chebula_ showed a prominent effect on reduction of glycosylate hemoglobin; TC 4 group brought elevated level to almost normal range (Table 1). The above result may be due to high degree of control of augmented hyperglycemia by _T. chebula_ leading to reduction in glycation. Increased oxidative stress play a major role in the etiology of diabetic complications (Ha, & Kim, 1999); ischemia leads to increased oxidative stress and decrease in antioxidant potential of cells. A reduction in mean level of plasma antioxidant concentration (Catalase, GSH and SOD level) and elevated lipid peroxidation (MDA) were observed in the diseased group (DC, NeC, DNC) as compared to normal control (NC) groups. Table 1 show that _T. chebula_ at dose of 400 mg/kg showed a higher degree of elevation in catalase enzyme concentration and GSH level. _T. chebula_ at both doses showed significant reduction in lipid peroxidation where as TC 4 elevated the SOD level and brought it to almost normal (22.67±1.26). Significant result was observed in groups treated with extract supporting previous work in this respect (Lee, Won, Kim, Lee, Jun, & Lee, 2005).

Increment in urinary albumin, urinary nitrogen urea, creatinine, urinary protein and glucose excretion was usually observed in diabetic nephropathy indicating progressive damage to glomerular and tubular cells resulting in decline in GFR (Rossier, Hommel, Smidt, & Parving, 1994). In present study urine creatinine, protein, albumin excretion, urea nitrogen and glucose excretion were highly increased in diseased group (DC, NeC and DNC group). After the treatment regimen; group showed a significant effect on urinary excretion profile; TC 4 decreased elevated urine creatinine and glucose to almost 50 % as compared to diseased group (Table 1). Results of our study attribute to the renoprotective effect of our drugs and also beneficial effect in diabetic nephropathy.

Diabetes mellitus induces pernicious effects in structure of kidney; diabetic nephropathy is an integrated entity that includes various pathological lesions (Nishi, Ueno, Hisaki, Iino, Iguchi, Oyama, Imai, Arakawa, & Geiyo, 2000). Histopathological profile of ischemic kidney showed damaged proximal convoluted tubules, mesangial cells, dilated tubules and glomerular necrosis (Figures 1 b-d); treatment with _T. chebula_ exhibited improvement in renal architecture over diseased control groups as evident from histopathological studies (Figures 1 e-f). Extracts were able to diminish the necrosis of glomerular, proximal convoluted tubule, mesangial cell, endothelial cells, but neither of treatment regimens was able to normalize the renal damage. Most promising result was seen in kidney of TC 4 group; the observed effect may be due to renoprotective effect of _T. chebula_.

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**Table 1**: Results of biochemical analysis of normal control and test groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Glucose (mg/dL)</th>
<th>Glucose Excretion (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Urea (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>115.2±5.13</td>
<td>5.4±1.2</td>
<td>0.9±0.1</td>
<td>12.6±2.1</td>
</tr>
<tr>
<td>NeC</td>
<td>70.6±1.5</td>
<td>0.3±0.1</td>
<td>1.1±0.2</td>
<td>12.6±2.1</td>
</tr>
<tr>
<td>DNC</td>
<td>70.6±1.5</td>
<td>0.3±0.1</td>
<td>1.1±0.2</td>
<td>12.6±2.1</td>
</tr>
<tr>
<td>DC</td>
<td>70.6±1.5</td>
<td>0.3±0.1</td>
<td>1.1±0.2</td>
<td>12.6±2.1</td>
</tr>
</tbody>
</table>

**Notes**: NC: Normal control, NeC: Nephropathy control, DNC: Diabetic control, DC: Diabetic.

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**Figures 1**: (a) Normal control; (b-d) Diseased control; (e-f) Treatment groups.
3. Experimental

3.1. Procurement and authentication of plants

Dried fruits of *T. chebula* were purchased from local market, Mandsaur, M.P.; India; identified by Prof. Gyanendra Tiwari, Scientist, Government College of Horticulture, Mandsaur (M.P.). A voucher specimen (BRNCP/TC/009/2009) was deposited in the herbarium of Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur (M.P., India).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>NC</th>
<th>DC</th>
<th>NeC</th>
<th>DNC</th>
<th>TC 2</th>
<th>TC 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG (mg/dl)</td>
<td></td>
<td>111.8±2.78</td>
<td>500.8±19.70</td>
<td>104.7±4.08</td>
<td>502.5±8.85</td>
<td>120.5±2.54£££***</td>
<td>115.2±5.13£££***</td>
</tr>
<tr>
<td>GHB (% HbAlC)</td>
<td></td>
<td>5.17±0.60</td>
<td>12.83±0.70</td>
<td>4.50±0.43</td>
<td>13.00±0.89</td>
<td>9.17±0.31*p</td>
<td>5.33±0.56£££***</td>
</tr>
<tr>
<td>UUN (mg/dl)</td>
<td></td>
<td>66.33±2.23</td>
<td>108.54±5.85</td>
<td>105.53±2.88</td>
<td>114.33±3.75</td>
<td>81.67±1.67£££***</td>
<td>78.33±1.54£££££££***</td>
</tr>
<tr>
<td>UC (mg/dl)</td>
<td></td>
<td>10.00±0.57</td>
<td>38.67±1.54</td>
<td>36.50±1.60</td>
<td>40.83±0.60</td>
<td>26.83±1.95£££***</td>
<td>17.63±1.29£££££££***</td>
</tr>
<tr>
<td>UP (mg/24hrs)</td>
<td></td>
<td>20.00±0.93</td>
<td>129.71±5.37</td>
<td>89.50±1.97</td>
<td>131.50±5.39</td>
<td>82.33±3.58£££***</td>
<td>71.33±1.87£££***</td>
</tr>
<tr>
<td>UAG (mg/24hrs)</td>
<td></td>
<td>2.92±0.07</td>
<td>10.06±0.28</td>
<td>9.81±0.37</td>
<td>10.85±0.40</td>
<td>9.59±0.10£**</td>
<td>6.50±0.10£££££££***</td>
</tr>
<tr>
<td>UG (mg/24hrs)</td>
<td></td>
<td>0.0±0.0</td>
<td>345±76.42</td>
<td>0.0±0.0</td>
<td>359±71.54</td>
<td>1945±76.63£££***</td>
<td>1073±52.17£££***</td>
</tr>
<tr>
<td>GSH (nmol/mg)</td>
<td></td>
<td>42.67±3.02</td>
<td>17.33±1.58</td>
<td>22.33±2.17</td>
<td>14.50±0.62</td>
<td>22.33±0.67**</td>
<td>40.67±0.71£££***</td>
</tr>
<tr>
<td>MDA (nmol/mg)</td>
<td></td>
<td>14.67±0.89</td>
<td>31.00±2.56</td>
<td>31.50±1.34</td>
<td>32.50±1.50</td>
<td>25.83±0.70£££***</td>
<td>17.83±0.47£££££££***</td>
</tr>
<tr>
<td>SOD (U/mg)</td>
<td></td>
<td>25.50±1.52</td>
<td>9.50±0.56</td>
<td>10.00±0.58</td>
<td>8.67±0.71</td>
<td>14.17±0.79£**</td>
<td>22.67±1.26£££££££***</td>
</tr>
<tr>
<td>CAT (nmol H2O2 cons/min/mg)</td>
<td></td>
<td>308.7±6.08</td>
<td>204.5±6.79</td>
<td>205.0±8.03</td>
<td>199.0±8.78</td>
<td>228.8±3.26£**</td>
<td>283.7±5.57£££££££***</td>
</tr>
</tbody>
</table>

*N=6; where *p < 0.05, **p < 0.01, ***p < 0.001 vs diabetic control (DC); †p < 0.05, ‡p < 0.01, §p < 0.001 vs nepropathy control (NeC); $p < 0.00, **p < 0.01, ***p < 0.001 vs diabetic nephropathy control (DNC); Value expressed in mean ± SEM*

Table 1. Effect of ethanolic extract of *T. chebula* fruit on biochemical alteration during study period.

Figure 1: Effect of extract on histopathology of kidney (H and E X 100); (a) NC - normal glomeruli, (b) DC - diabetic control (c) NeC - nepropathy control (d) DNC - diabetic nephropathy control (e) TC 2 - treated with *T. chebula* extract (200 mg/kg) and (f) TC 4 - treated with *T. chebula* extract (400 mg/kg).
3.2 Preparation of plant extracts
Fruits of *T. chebula* (3 kg) were coarsely powdered; defatted with petroleum ether (60°C-80°C); dried and subjected to extraction with absolute ethanol as solvent. The extraction was carried out for a period of 72 h. The extract obtained was dried in vacuum (yield 18 %).

3.3. Animals
Healthy adult wistar albino rats of either sex weighing between 200 – 250 gm of either sex were procured from animal house of B.R.N.C.P. and maintained as per OECD guideline and Institutional animal ethical committee (IAEC reg. no. 918/AC/05/CPCSEA).

3.4. Experimental design

3.4.1. Induction of diabetes and ischemia
Fasted rats were given single dose of streptozotocin (50 mg/kg in 0.1M citrate buffer pH 4.5; i.p.) and Wongmekiat et al., method was used to induce renal ischemia (Wongmekiat, Thamprasert, & Lumlertgul, 2007). The animals were randomly divided into the six groups (n=6) and treated for 28 days. Group I served as normal control (NC); group II served as diabetic control (DC); group III served as nephropathy control (NeC); group IV served as diabetic nephropathy control (DCN); group V served as diabetic nephropathy control (DC); group III served as nephropathy control (NeC); group IV served as diabetic nephropathy control (DCN); group V served as treatment group – treated with 200 and 400 mg/kg *T. chebula* fruit extract respectively. Glycosylated hemoglobin (GHB), urinary urea nitrogen (UUN), urine creatinine (UC), urine protein (UP), urinary albumin excretion (UAEx) and urinary glucose (UG) were assessed using commercially available diagnostic kits; Blood glucose (BG) level was assessed using glucose strips. Sharma et al., method was used to estimate glutathione (GSH), superoxide dismutase (SOD), catalase and malondialdehyde (MDA) levels (Sharma, Kulkarni, & Chopra, 2006).

3.4.2. Histopathological studies
Kidney were collected and immediately fixed in 10% formalin, dehydrated in a graded ethanol (50–100%) series, cleared in xylene, and embedded in paraffin. Sections (4-5 μm) were prepared and stained with hematoxylin and eosin (HE) dyes for photomicroscopic observations.

3.4.3. Statistical analysis
All values are presented as mean ± SEM. Statistical analysis of data was performed by one way analysis of variance (ANOVA) followed by “Dunnett’s test”; p value < 0.05 was considered as statistically significant.

4. CONCLUSION
The treatment using *T. chebula* can prevent diabetic nephropathy caused by ischemia in diabetic rats. Further studies are in progress to establish mechanism of action(s) and isolation of active constituents responsible for its action.

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5. REFERENCES