Role of Antioxidants in Mercuric Chloride Induced Renal Damage Treated With the Ethanolic Extract of *Tabernaemontana Coronaria* in Wistar Albino Rats.

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**Abstract.** Plants and plant-derived products are part of the healthcare system since ancient human civilization. Herbal medicines have been used for many years. The present study was carried out to evaluate the effect of ethanolic extract of *Tabernaemontana coronaria* in renal oxidative damage induced by mercuric chloride (HgCl₂) in rats. HgCl₂ (3 mg/kg) was administered intraperitoneally to the rats and the extract was fed orally for 21 days. Results indicated that the levels of enzymic and non-enzymic antioxidants were decreased in the Group II rats and reverted back to the normal level in the treatment groups. Our findings showed that treatment with ethanolic extract of *Tabernaemontana coronaria* offers imperative protection from HgCl₂-induced nephrotoxicity. The deterioration of antioxidant enzymes and histological damage caused by HgCl₂ are markedly improved by treatment with the ethanolic extract of *Tabernaemontana coronaria*. These observations may be attributed partially to the antioxidant effect of ethanolic extract of *Tabernaemontana coronaria* and suggest that it may be a clinically valuable agent in the prevention of acute renal failure caused by inorganic mercury intoxication.

**Keywords:** *Tabernaemontana coronaria*, Nephrotoxicity, Mercuric chloride.

1. **Introduction**

Mercury is one of the most common heavy metals, used for more than 3000 years in medicines (as disinfectants, vaccines), industries (fluorescent lamps, batteries, thermostats, thermometers), gold mining and therapeutically as a cathartic, diuretic, anti-inflammatory and in dental amalgams (Clarkson *et al.*, 2003). The general populations are exposed to methyl mercury through the diet: the main source is fish consumption (Coccine *et al.*, 2000). Organic mercury has a lesser insult on kidneys than inorganic mercury. It is a transition metal and promotes the formation of reactive oxygen species. Mercuric chloride (HgCl₂) is an inorganic mercury compound with ionic mercury. Short term exposure to mercuric chloride may cause tubular necrosis, interstitial pneumonitis and gastrointestinal ulcerations. By contrast, long term exposure can cause membranous nephropathy and central neurotoxicity (Aymaz *et al.*, 2001).

Aerobic organs employ a battery of defense mechanisms such as antioxidant enzymes (SOD, Catalase, and glutathione peroxidase) to mitigate or prevent oxidative tissue damage. The diet derived antioxidants like ascorbic acid, Vitamin E, carotenoids, polyphenols and α-lipoic acid also served as primary lines of defense in destroying free radicals (Wang and Luo, 2007). The general drugs given for nephrotoxicity include diuretics (like suresomide and torsemide) and steroids (like prednizole and β-metasona). Since these medicines have certain serious side effects, there is an urgent need to systematically evaluate plants for their activities. Such research could also lead to new discovery or advance the use of indigenous herbal medicines for orthodox treatment. *Tabernaemontana coronaria* R.Br. (syn. *Ervatamia coronaria*) is a glabrous, evergreen, dichotomously branched shrub, belonging to the family Apocynaceae. This species has been extensively investigated and a number of chemical constituents such as alkaloids, triterpenoids, steroids, flavanoids, phenyl propanoids and phenolic acids were isolated from leaves, roots and stems of the plant (Malaya Gupta *et al.*, 2004). In this communication, the present study is investigated to study the role of...
antioxidant enzymes in mercuric chloride induced renal damage treated with the ethanolic extract of *Tabernaemontana coronaria* in Wistar albino rats.

2. Materials and Methods

Adult male albino rats weighing about 150-200 g were used for the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>Control animals</td>
</tr>
<tr>
<td>II</td>
<td>Mercuric chloride induced rats</td>
</tr>
<tr>
<td>III</td>
<td>Nephrotoxic rats treated with plant extract (200mg/kg bwt)</td>
</tr>
<tr>
<td>IV</td>
<td>Nephrotoxic rats treated with plant extract at (400mg/kg bwt)</td>
</tr>
<tr>
<td>V</td>
<td>Control animals treated with plant extract only (200mg/kg bwt)</td>
</tr>
<tr>
<td>VI</td>
<td>Control animals treated with plant extract only (400mg/kg bwt)</td>
</tr>
</tbody>
</table>

The animals were weighed and dosed through oral intragastric tube everyday. The test drug was fed orally for 21 days. A single intraperitoneal injection of freshly prepared Mercuric chloride (3 mg/kg body weight) was given on the 18th day. The experiment was terminated in overnight fasted rats at the end of 21 days. The rats were sacrificed by cervical dislocation after giving mild anesthesia using Chloroform. Blood was collected and serum was separated. Kidneys were immediately dissected out, washed and stored in 0.9% ice cold saline for various biochemical evaluations (Enzymic – SOD, Catalase, GPX and non-enzymic antioxidants Vit C & GSH), LPO and also in 10% formalin for histopathological studies.

3. Results and Discussion

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Control (Group I)</th>
<th>HgCl₂ control (Group II)</th>
<th>HgCl₂+ plant extract treated (200mg/kg) (Group III)</th>
<th>HgCl₂+ plant extract treated (400mg/kg) (Group IV)</th>
<th>Plant extract alone treated (200mg/kg) (Group V)</th>
<th>Plant extract alone treated (400mg/kg) (Group VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (µg of GSH/mg of protein)</td>
<td>39.33±0.335 a</td>
<td>20.18±0.187 b</td>
<td>32.62±0.364 c</td>
<td>34.51±0.447d</td>
<td>37.60±0.437e</td>
<td>38.78±0.420a</td>
</tr>
<tr>
<td>Superoxide dismutase (Units/g tissue)</td>
<td>1.69±0.314 a</td>
<td>1.13±0.166 b</td>
<td>1.281±0.253 a</td>
<td>1.45±0.279 a</td>
<td>1.588±0.272 a</td>
<td>1.656±0.368 a</td>
</tr>
<tr>
<td>Catalase (µ moles of H₂O₂ utilized/ min/mg/protein)</td>
<td>23.19±0.161 a</td>
<td>10.22±0.251 b</td>
<td>17.52±0.273 c</td>
<td>20.25±0.291 d</td>
<td>20.55±0.454 d</td>
<td>22.38±0.314 e</td>
</tr>
<tr>
<td>GST(µmoles of CDNB Conjugate formed/mg of protein)</td>
<td>8.51±0.228 a</td>
<td>4.31±0.389 b</td>
<td>8.063±0.113 a</td>
<td>8.35±0.174 a</td>
<td>8.506±0.254 a</td>
<td>8.51±0.362 a</td>
</tr>
<tr>
<td>Lipid peroxidation (n moles/gm of tissue)</td>
<td>14.68±0.317 a</td>
<td>29.49±0.403 b</td>
<td>19.59±0.413 c</td>
<td>16.25±0.410 d</td>
<td>14.53±0.450 a</td>
<td>14.42±0.323 a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Values are taken as a mean of six individual experiments.
Values not sharing a common superscript letter differ significantly (DMRT)

3.1. Concentration of Lipid Peroxidation

The effect of the ethanolic extract of *Tabernaemontana coronaria* in lipid peroxidation is given in table 1. The elevated levels of lipid peroxidation in HgCl₂ induced nephrotoxicants were reduced significantly to near normal levels upon treatment with *Tabernaemontana coronaria* extract. The plant extract alone treated rats did not show any significant change. Similar result was observed by Sivaprasad *et al.*, 2003.

3.2. Activities of Antioxidant Enzymes Glutathione Peroxidase, SOD, GST and Catalase

Administration of HgCl₂ alone in group II animals caused a significant decrease in the levels of antioxidant enzymes GPX, SOD, GST and catalase when compared with group I animals (Table 1). Group V
and group VI animals which received Tabernaemontana coronaria alone (200mg & 400mg/kg respectively) did not show any significant variation in the above parameters when compared with group I control animals. Rats given concomitant doses of HgCl₂ and Tabernaemontana coronaria showed a marked rise in the levels of above mentioned enzymes compared to group II animals. Our results were in agreement with the findings of Samipillai and Jagadeesan., 2009.

Table 2. The Concentration of Vitamin C and Reduced Glutathione in Kidney of Control and Experimental Groups

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Control (Group I)</th>
<th>HgCl₂ control (Group II)</th>
<th>HgCl₂+ plant extract treated (200mg/kg) (Group III)</th>
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<th>Plant extract alone treated (400mg/kg) (Group VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mg/g of protein)</td>
<td>1.64±0.273 a</td>
<td>0.72±0.022 b</td>
<td>1.30±0.032 a</td>
<td>1.44±0.121 a</td>
<td>1.63±0.273 a</td>
<td>1.65±0.269a</td>
</tr>
<tr>
<td>Glutathione (μg/mg protein)</td>
<td>48.23±0.393a</td>
<td>22.40±0.327 b</td>
<td>40.30±0.345 c</td>
<td>44.38±0.335 d</td>
<td>48.02±0.153 a</td>
<td>48.15±0.072a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D
Values are taken as a mean of six individual experiments
Values not sharing a common superscript letter differ significantly (DMRT)

3.3. Activities of Non Enzymic Antioxidants—Vitamin C and Glutathione

Table 2 represents the level of Vitamin C and Glutathione in control and experimental rats. It was observed that the level of vitamin C and glutathione were reduced in HgCl₂ induced nephrotoxic rats (group II) when compared with control animals (group I). By the administration of Tabernaemontana coronaria the elevated levels of Vitamin C and glutathione were restored to near normal values in group III and group IV animals. No significant changes were observed in Tabernaemontana coronaria alone treated animals in group V and group VI when compared with control animals. These results are supported by the results given by Lieber, 1997.

Figure 1: Histopathology of Kidney

Group I: Kidney of control animal showing normal histology.

Group II: Kidney of Mercuric chloride intoxicated rats showing focal renal tubular atrophy.

Group III: Kidney of nephrotoxic animal treated with ethanolic extract of T. coronaria (200mg/kg) showing normal histology.

Group IV: Kidney of nephrotoxic animal treated with ethanolic extract of T. coronaria (400mg/kg) showing normal histology.

Group V: Kidney of normal animal treated with plant extract T. coronaria (200mg/kg) showing normal histological structure.

Group VI: Kidney of normal animal treated with plant extract T. coronaria (400mg/kg) showing normal histological structure.
4. Conclusion

In conclusion, the findings reported in the study indicate that the oral administration of *Tabernaemontana coronaria* to mercuric chloride intoxicated rats exhibited significant nephroprotective effect. Although promising results have been obtained, more concerted efforts are still needed for the isolation, characterization and biological evaluation for the active principles of the extract.

5. Acknowledgement

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6. References


