IMPACT OF COLEUS AROMATICUS EXTRACT ON CCL₄-MEDIATED OXIDATIVE DAMAGE IN RAT LIVER

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ABSTRACT

The antioxidant effect of the ethanolic extract of Coleus aromaticus, an indigenous ayurvedic medicinal plant used in India, was studied in rats with CCL₄-induced hepatotoxicity. Rats were divided into four groups: Groups 1 received control, Groups 2 received CCL₄, Groups 3 received CCL₄ and silymarin and Groups 4 received CCL₄ and ethanolic extract of Coleus aromaticus for 14 days. The results showed significantly elevated level of serum and tissue thiobarbituric acid reactive substances, and significantly lowered activities/levels of antioxidants such as superoxide dismutase, catalase, glutathione-S-transferase, glutathione reductase, glutathione peroxidase and reduced glutathione in CCL₄-treated rats compared with control rats. Administration of ethanolic extract of Coleus aromaticus to rats with CCL₄-induced liver injury significantly decreased the levels of serum and tissue thiobarbituric acid reactive substances and significantly elevated the activities of superoxide dismutase, catalase, glutathione-S-transferase, glutathione reductase, glutathione peroxidase and reduced glutathione in the tissues compared with unsupplemented CCL₄-treated rats. These findings suggest that ethanolic extract of Coleus aromaticus has a modulatory effect on CCL₄-induced hepatotoxicity in rats.

KEYWORDS: Antioxidant, Coleus aromaticus, lipid peroxidation and CCL₄.
INTRODUCTION
Antioxidants are substances which are capable of recording on preventing the process of fat oxidation is they prevent the oxidation of unsaturated fats. Oxidative cell injury induced by reactive species is protected by the antioxidant defence system, including enzymatic and non-enzyomatic components. Antioxidant strategies, including administration of pharmacological or dietary agent are based on two main mechanism: The inhibition of reactive species generation and the enhancement of Reactive species elimination. The agent the acting according to the latter mechanism include antioxidant enzyme, which catalyze reactive species degradation, Scavenges neutralizing Reactive species and agents that may enhance intrinsic antioxidant forces.\(^\text{[1]}\)

One of the most clearly defined hypotheses relating to the action of hepatotoxic agents is the lipid peroxidative damage of cellular mechanism. Lipid peroxidation may be looked upon as occurring in two steps. Some toxic event initiates lipid peroxidation and organic free radicals generated by the initiation process serve to propagate the reaction. The concept of lipid peroxidative damage was advanced by Mcmillan et al.\(^\text{[2]}\) as the principles mechanism of CCl\(_4\) induced liver injury and has found ample experimental support.

CCl\(_4\) induced liver damage has been through to depend on the formation of reactive intermediates such as tricloromethyl free radical\(^\text{[3]}\) and peroxyl radical.\(^\text{[4]}\) Antioxidant compounds can protect against lipid peroxidation, but prior presence is required for their protective action. These include vit E, ascorbic acid and GSH, which stop the propagation of the lipid peroxidative process.\(^\text{[5]}\) CCl\(_4\) – induced lipid peroxidation can be obtained by antioxidants such as pyrogallol and other polyols and sulfhydryl compounds such as cystamine. Antioxidants inhibit toxic oxidative reaction by removing metal catalysts oxygen species scavenging intiated, radicals (or) breaking the chain reaction of initated sequences.\(^\text{[6]}\)

Karpuravalli (Coleus aromaticus L.) with its distinctive smelling leaves is a common home remedy for infantile cough, cold and fever. They are useful in cephalagia, anorexia, dyspepsia, colic, diarrhea and cholera especially in children, halitosis, convulsions, epilepsy, chronic asthma, bronchitis, renal vesical calculi stroangury, hepatopathy and malarial fever. Juice is mixed with sugar is give to children in colic. Its also useful for gonorrhea, piles. Crushed leaves are used as a local application of the head in headache and relieve the pain and irritation caused by sting centipedes.\(^\text{[7]}\)
This study was designed to test the hypothesis that *Coleus aromaticus* would reduce CCl₄ induced toxicity by modulating lipid peroxidation and antioxidants.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *Coleus aromaticus* were collected from S.T.E.T Medical plant garden, Mannargudi, Thiruvarur District and authenticated by Botany Department of A.V.V.M. Sri Pushpam College, Poondi. After authentication the plant material were washed under running tap water.

**Preparation of Plant Extract**

*Coleus aromaticus* leaves were dried (without direct sunlight) and converted to powder form. The powder obtained was successively extracted in methanol and distilled water by using soxhlet apparatus. It was stored at 4°C until used when needed the residual extract was suspended in distilled water and used in the study.

**Animals**

A healthy swiss albino rats were housed in well ventilated hygienic atmosphere. Animals with 100 – 150g were used our study. Animals were fed with commercial rat feed (Saidurga feeds & foods, Bangalore) and tap water adlibitum. After randomization into various groups, the rats were acclimatized for a period of 2-3 days in the new environment before initiation of experiment.

**Chemicals**

All of the chemicals were of analytical grades and were obtained from Central Drug House Pvt. Ltd (New Delhi, India).

**Experiment design**

In the experiment, a total of 24 rats were used. The rats were divided in to following 4 groups of 6 each.

- **Group I**: Control
- **Group II**: CCl₄ treated (Intraperitoneal administration of CCl₄ at a dosage of 1.5ml/kg/body weight for 14 days).
Group III: CCl₄ and silymarin (Intraperitoneal administration of CCl₄ as the above mentioned dose along with oral administration of 25mg of silymarin/ml of paraffin/kg/body weight for 14 days).

Group IV: CCl₄ and *coleus aromaticus* treated (Intraperitoneal administration of CCl₄ as the above of 300mg of *coleus aromaticus* 1 ml of paraffin/kg/bodyweight for 14 days).

Sample Collection
After 14 days of herbal treatment, the blood sample were collected from the anaesthetized rats by puncturing the orbital sinus. After the collection of blood, it was allowed to stand for 10 mts.

Biochemical measurements
Tissue and plasma TBARS [8], SOD [9], CAT [10], GST [11], GR [12], GPx [13], GSH [14] were determined.

Statistical analysis
Results are expressed as mean ± SE from six observations.

RESULT
Table 1 shows the levels of TBARS in plasma and tissue. There was a significant elevating in the levels of TBARS in plasma, liver in CCl₄ treated group compared to normal. On treatment with *Coleus aromaticus* there was a significant decrease in the levels of TBARS when compared to CCl₄ treated group.

Table 2 the changes in the activity of superoxide dismutase, catalase and glutathione-s-transferase are given in table 2. The activities of SOD, CAT and GST were decreased significantly in CCl₄ treated groups compared to normal. *Coleus aromaticus* treatment significantly increased the activities of SOD, CAT and GST in liver compared to CCl₄ treated group.

Table 3 shows the changes in the levels of glutathione reductase, glutathione peroxidase and reduced glutathione in liver. There was a significant decrease in the levels of GR, GPX and GSH in tissue of CCl₄ treated rats when compared to normal treatment into *Coleus aromaticus* showed a significant increase in the levels of GR, GPX and GSH.
Table I: showing level of TBARS in plasma and liver.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>TBARS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma (mM/100ml)</td>
</tr>
<tr>
<td>1.</td>
<td>GP-I</td>
<td>0.140 ± 0.036</td>
</tr>
<tr>
<td>2.</td>
<td>GP-II</td>
<td>0.550 ± 0.0394</td>
</tr>
<tr>
<td>3.</td>
<td>GP-III</td>
<td>0.170 ± 0.037</td>
</tr>
<tr>
<td>4.</td>
<td>GP-IV</td>
<td>0.220 ± 0.037</td>
</tr>
</tbody>
</table>

(Values are mean ± S.E from 6 rats in each group).

Table II: showing level of superoxide dismutase, catalase and glutathione-s-transfarase in liver.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>SOD (U A/mg of protein)</th>
<th>Catalase (U B/mg protein)</th>
<th>GST (U C/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>GP-I</td>
<td>14.17 ± 2.83</td>
<td>56.17 ± 2.83</td>
<td>10.43 ± 3.65</td>
</tr>
<tr>
<td>2.</td>
<td>GP-II</td>
<td>5.603 ± 0.502</td>
<td>37.92 ± 3.53</td>
<td>5.710 ± 0.762</td>
</tr>
<tr>
<td>3.</td>
<td>GP-III</td>
<td>13.00 ± 3.63</td>
<td>54.10 ± 3.80</td>
<td>9.32 ± 1.10</td>
</tr>
<tr>
<td>4.</td>
<td>GP-IV</td>
<td>12.08 ± 3.86</td>
<td>52.68 ± 3.57</td>
<td>8.42 ± 2.75</td>
</tr>
</tbody>
</table>

(Values are mean ± S.E from 6 rats in each group).

U A Enzymes required for 50% inhibition of NBT min/mg protein
U B µ moles of H2O2 liberated/min/mg protein
U C µ moles of CDNB conjugate formed/min/protein
Table III: showing level of glutathione reductase (GR), glutathione peroxidase (GPx) and reduced glutathione (GSH) in liver of normal and experimental groups.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>GR (U^E/mg of protein)</th>
<th>GPx (U^C/mg protein)</th>
<th>GSH (mg/100g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>GP-I</td>
<td>18.70 ± 3.67</td>
<td>10.40 ± 4.22</td>
<td>5.21 ± 0.785</td>
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<td>2.</td>
<td>GP-II</td>
<td>14.01 ± 3.81</td>
<td>3.28 ± 1.02</td>
<td>3.04 ± 0.731</td>
</tr>
<tr>
<td>3.</td>
<td>GP-III</td>
<td>17.30 ± 3.57</td>
<td>8.24 ± 1.95</td>
<td>4.80 ± 1.24</td>
</tr>
<tr>
<td>4.</td>
<td>GP-IV</td>
<td>16.90 ± 3.87</td>
<td>7.82 ± 1.62</td>
<td>4.00 ± 1.16</td>
</tr>
</tbody>
</table>

(Values are mean ± S.E from 6 rats in each group).

U^E = nano moles of NADPH oxidized per minute.

U^C = µ moles of GSH utilized per minute
DISCIISSION
The levels of thiobarbituric acid reactive substances (TBARS) are significantly elevated in CCl_4 treated groups. Chronic administration of CCl_4 activates microsomal cytocheoms P-450 dependent mono oxygenase system resulting in the formation of trichloromethyl free radical and reactive oxygen species that initiate lipid peroxidation. [15] Increased free radical production and lipid peroxidation have been proposed as major cellular mechanism involved in CCl_4 toxicity. [16] It has been shown that free radical (or) reactive oxygen species (ROS) such as hydroxyl ethyl radicals, O_2^- radical, OH^- Radical, peroxy radical and hydrogen peroxide are implicated an CCl_4 induced lipid peroxidation. [17]

The most abundant oxidative free radical generated in living cells are superoxide anions and derivatives particularly the highly reactive and damaging hydroxyl radicals which induces peroxidation of cell membrane lipids. The end products of lipid peroxidation are known to induce cellular damage and have been known to be responsible for oxidative free radical induced human disease. [18]

The potential toxicity of oxygen has been attributed to formation of H_2O_2, recently, however, the case with O_2 can be reduced in tissue to the superoxide anion free radical (O_2^-) and the occurrence of super oxide dismutase in aerobic organism have suggested that the toxicity of O_2 is due to its conversion to superoxide is formed when reduced are reoxdized by molecular oxygen. Superoxide dismutases are a family of metallo enzyme which are known to catalyze dismutation of superoxide radical to H_2O_2 and molecular oxygen.

SOD acts as scavengers of free radicals and reduce the toxicity of oxygen. Oxygen toxicity may be caused by the superoxide free radical. Tissues are protected from superoxide by the specific enzyme superoxide dismutase. [19] The increased oxidative stress due to ROS generation may alter the antioxidant defence system of the tissues which may be an important factor in CCl_4 induced hepato toxicity. Liver showed increased LPO indicating increased oxidative stress with CCl_4 treatment.

Liver SOD activity diminished after CCl_4 administration probably due to increased O_2^- anions as O_2 anions have been shown to inhibit SOD activity. Decreased activity of SOD in CCl_4 treated (group II) rat liver may increase this susceptibility to oxidative injury. However, the over expression of the antioxidant molecules with leaves of Coleus aromaticus indicative of their ability to reactivate hepatocellular antioxidant defense in the liver.
Catalase is one of the important enzyme in the supportive team of defense against ROS. Catalase is hemoprotein containing four heme groups. This enzyme for catalyses the decompositions of H$_2$O$_2$ to H$_2$O and oxygen and thus protecting the cell from oxidative by H$_2$O$_2$ and OH$^-$. The generation of H$_2$O$_2$ may also lead to inactivation of this enzyme. Catalase, which acts as preventive antioxidant plays on important role in protection against the deleterious effect of lipid peroxidation. The inhibition of catalase activity may be due to enhanced production of O$_2^-$ and peroxyl radicals during the chronic administration of CCl$_4$. Increase the catalytic activity is essential and beneficial effect from increase in SOD activity is to be expected.

The finding in our study reveal that the activity of catalase was significantly lowered in rats treated CCl$_4$ (groups II). Group III and group IV rats shown increase activity of catalase than group II. Decreased activity of catalase in CCl$_4$ treated rat liver may increased their susceptibility to oxidative injury. However the over expression of the antioxidant molecule with crude drug of Coleus aromaticus in indicative of their hepatocellular antioxidant defense in the liver.

Glutathione-S-Transfuses another scavenging enzyme, binds to many different lipophilic compounds. So it would be expected to bind to lipophilic CCl$_4$ and act as an enzyme for GSH in conjugation reaction. GST is a member of a complex supergene-encoded family of detoxification enzyme found in wide variety of animal tissue. The decreased level of GST activity in CCl$_4$ drug may be due to the down regulation of glutathione-S-transferase subunits.

The depression of hepatic GST activity might will be an adaptive response to the increased production of oxidized glutathione in the tissue of CCl$_4$ hepatotoxic animals. Since the efflux of oxidized glutathione and glutathione-S-transferase used the same transport system. The decrease in hepatic GST activity may favour the excretion of oxidized glutathione, there by maintaining the thiol redox status in tissues. The moderate decline in GST activity in liver seen in this study might have been due to the decreased availability of GSH this is consistent with a reported study. Which showed a retection of GST activity in liver. These findings led to the conclusion that GSH and GSH dependent enzyme system may be directly related the pathogenic mechanism of hepatitis.
Glutathione reductase (GR) is the major component of the endogenous non-protein sulphydryl and it binds to reactive free radicals and may influence the physical properties of mucus, since its subunits are joined by disulphide bridges.\textsuperscript{[24]} GSH (reduced glutathione) is formed from GSSG (Oxidised Glutathione) by the action of an enzyme glutathione reductase which depends upon the availability of NADPH.

The pentose phosphate pathway in the erythrocyte proves NADPH for the reduction of oxidized glutathione reductase, a flavoprotein enzyme containing FAD. In turn reduced glutathione removes H$_2$O$_2$ from the erythrocyte in a reaction catalysed by glutathione peroxidase. This reaction is important since accumulator of H$_2$O$_2$ any decrease the life span of the erythrocytes by increase in the rats oxidation of hemoglobin to methamoglobin.\textsuperscript{[25]} The activities of GR in liver of the experimental animals after CCl$_4$ induction is declined then the control rats. The decreased activity is due to inactivation as a result of oxidative stress.

Glutathione peroxidase of which selenium is an integral component, provide a first line of defence against hyperperoxides before they can damage membranes and other cell component, thus selenium which is necessary for the digestion and absorption of lipids. The most important reactions of free radicals in aerobic cells involve molecular oxygen and its radical derivatives (Superoxide anion and hydroxyl radicals). Glutathione peroxides (GPX) has a well established role in protecting cells against injury.

GPX can also determine the chain reaction of lipid peroxidation by removing lipid hydroperoxides and H$_2$O$_2$ form the cell membrane.\textsuperscript{[26]} In our study, the activities of GPX is increased by the treatment with Coleus aromaticus extract. Ohata et al., \textsuperscript{[27]} have reported the decreased activities of SOD, CAT and GPX after the administration of single does of CCl$_4$. In our study decline in activities of GPX levels in CCl$_4$ administered rats (groups II) and recovery to normal in groups III & IV revealed that oxidative stress elicited by CCl$_4$ intoxication has been nullified due to the antioxidant effect of Coleus aromaticus.

GSH is a tripeptide consisting of glutamate, cysteine and glycine. It acts as an antioxidant both intracellularly and extracellularly in conjunction with various enzymatic process that reduced hydrogen peroxide and hydroperoxides as GSH is oxidized to GSSG and other mixed disulfides. GSH is produced in the liver and maintained at a higher concentration is most tissue.\textsuperscript{[28]}
GSH is a critical determinant of tissue susceptibility to oxidative damage. The depletion of GSH levels has been shown to be associated with enhanced CCl₄ toxicity.[29] In our study we observed a significant reduction in GSH levels in plasma and liver homogenates in CCl₄ treated rats.

CONCLUSION
In the present bring out the antioxidant activity on *Coleus aromaticus* against CCl₄ induced hepatotoxicity in rats. The use of *Coleus aromaticus* as chronic cough syrup seems to be effective. To rationalise use of the plant however, more work needs to be carried out at molecular level.

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REFERENCES