Exploring the Hepatoprotective and Antioxidant Potential of *Ficus racemosa* Extract Against Ethanol – Induced Oxidative Stress in Wistar Albino Rats

Aarthi B.L. and Sendhilvadivu M.*

Department of Zoology, Queen Mary’s College, Chennai 600 004, Tamil Nadu, India

*Corresponding Author

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**Abstract:** The utilization of indigenous plants for the treatment of various ailments has gained substantial attention due to their therapeutic benefits. The present study aimed to investigate the hepatoprotective and antioxidant potential of *Ficus racemosa* against ethanol induced liver damage in male Wistar albino rats. In the present study, Group I served as Control. Ethanol is orally administered to Group II rats, which resulted in significant decrease in the levels of antioxidant enzymes such as GST, GPx, SOD and CAT. These antioxidant enzymes play a pivotal role in combating oxidative stress. Elevated levels of lipid peroxidation were also observed in the serum and liver tissue after Ethanol administration by estimating the level of TBARS. Treatment with *Ficus racemosa* leaf extract (Group III) led to a substantial increase in the levels of antioxidant enzymes, when compared with ethanol induced untreated rats. Silymarin is used as a standard reference drug which was administered to Ethanol induced Group IV rats. The results revealed that, the administration of *Ficus racemosa* extract possibly mitigate the damage caused by oxidative stress thus, suggesting its role as an excellent antioxidant in treating liver disorders.

**Keywords:** Albino rats, Antioxidant enzymes, Ethanol, *Ficus racemosa*, Oxidative stress

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**Introduction**

Alcohol consumption has been consistently increasing throughout the world. Alcohol is one of the leading causes of death and disability globally (Girish *et al.*, 2010). Consumption of excessive alcohol for a prolonged time can lead to variety of sociomedical and public health issues around the world (Nowak and Relja, 2020). Ethanol is a fat-soluble, non-electrolyte and a psychoactive substance present in majority of the alcoholic beverages. According to the National Institute on Alcohol Abuse and Alcoholism, most of the ethanol in the body is broken down in the liver by the action of Alcohol dehydrogenase (ADH) enzyme which converts the ethanol into toxic compound...
called Acetaldehyde. The enzymes cytochrome P450 2E1 (CYP2E1) and Catalase help break down alcohol to acetaldehyde. However, CYP2E1 is only active after a person has consumed large amounts of alcohol, and catalase metabolizes only a small fraction of alcohol in the body (Edenberg, 2007). Acetaldehyde is short lived and gets converted into Acetate by another enzyme called Aldehyde dehydrogenase (ALDH). Acetate is broken down into water and carbon dioxide for easy elimination. Alcohol induced hepatic toxicity is largely influenced by oxidative stress, lipid peroxidation, inflammation and the formation of toxic by products (Chen et al., 2020). Chronic ethanol intoxication results in increased lipid peroxidation, production of reactive oxygen species and Oxidative stress which leads to cell membrane destruction and cell damage. To counteract these oxidants, cells have several antioxidant enzymes such as Glutathione S Transferase (GST), Glutathione peroxidase (GPx), Super oxide dismutase (SOD) and Catalase (CAT).

India has a rich traditional system of medicine. The use of herbal medicines for the treatment of various health issues continues to expand rapidly across the world and there is a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries (Chaughule and Barve, 2023). *Ficus racemosa* is native to India, Australia and Southeast Asia. It is common in South India and distributed widely from the outer Himalayan ranges, Punjab, Khasia Mountain, Chota Nagpur, Bihar, Orissa, West Bengal, Rajasthan, Deccan (Joy et al., 2001). *Ficus racemosa* has multiple pharmacological benefits which include antidiabetic, antioxidant, antidiarrhoeal, hypolipidemic, antifilarial and hepatoprotective actions (Yadav et al., 2015).

The objective of the present study was to investigate the hepatoprotective and antioxidant potential of *Ficus racemosa* leaf extract against ethanol induced oxidative stress in albino rats.

**Materials and Methods**

**Experimental Animals:**
Healthy adult Wistar albino rats (*Rattus norvegicus*) weighing 150-200 g were obtained from Mass Biotech, Chengalpet, Tamil Nadu, India. Animals were maintained under standard laboratory conditions at 22±4°C, relative humidity 30 to 70% with a 12 h :12 h dark : light cycle. The rats were fed with standard laboratory rat pellet diet and water *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics Committee (2084/PO/RcBt/S/19/CPCSEA).

**Plant Material and Preparation of Plant extract:**
The leaves of *Ficus racemosa* (Athi) were collected at Queen Mary’s College, Chennai and were authenticated by a taxonomist. The collected leaves were shade dried and milled to fine powder. The aqueous extract of *Ficus racemosa* was prepared by adding desired amount of powder to the boiling water in the water bath and brought down to one fourth of its original volume (Abubakar et al., 2020). The concentrated extract was filtered and 2 ml of the prepared extract was orally administered to Group III rats.

**Acute Toxicity Study:**
Acute toxicity study was carried out according to the OECD guidelines, Rule No. 423. Adult male Wistar albino rats were divided into four groups of three animals each and they were orally administered with *Ficus racemosa* leaf extract at doses of 50, 100, 200 and 2000 mg/kg and the animals were observed for any allergic and toxic symptoms for 14 days.

**Experimental Design:**
The rats were randomly divided into four groups of six animals each, after acclimatization to the laboratory conditions. Ethanol and extracts were orally administered to the rats. Group I served as Control which gets free access to standard laboratory pellet diet and water *ad libitum*. While, Group II (Ethanol induced group) were exposed to 50 % (v/v) ethanol at a dose of 2 ml/kg for
3 weeks to induce Oxidative stress. The rats in the treatment group (Group III) were first orally administered with 50% Ethanol and further treated with *Ficus racemosa* leaf extract (200 mg/kg), respectively for 30 days. The ethanol intoxicated Group IV rats were orally administered with standard reference drug Silymarin (100 mg/kg) for 30 days. Behavioural changes, food and water intake were monitored daily throughout the study. Body weights of the animals were monitored weekly and blood samples were collected from retro orbital plexus puncture under isoflurane anaesthesia and used for various biochemical analysis. At the end of the experiment, animals were euthanized and liver of each animal was dissected out and weighed. Tissue extracts were prepared by homogenizing with phosphate buffer solution. The liver tissue homogenate was centrifuged and the supernatant obtained was collected and used for Oxidative stress analysis.

**Phytochemical analysis:**

A preliminary phytochemical screening was performed in *Ficus racemosa* leaf powder to find out the presence of active phytoconstituents.

**Oxidative stress Analysis:**

**Estimation of Serum and Liver TBARS:**

The level of Thiobarbituric acid reactive substances (TBARS) was estimated according to the method of Ohkawa *et al.* (1979) using Malondialdehyde (MDA) as a standard.

**Estimation of Antioxidant enzymes:**

Glutathione S Transferase (GST) in serum and liver tissue homogenate was measured according to the method of Habig *et al.* (1974). The level of Glutathione peroxidase (GPx) was determined according to the method of Rotruck *et al.* (1973). Super oxide dismutase (SOD) was determined according to the method of Beauchamp and Fridovich (1971). The level of Catalase (CAT) was determined according to the method of Chance and Maehly (1954).

**Statistical Analysis:**

The results are expressed as mean ± standard deviation (S.D). Differences in antioxidant enzymes and the level of TBARS were determined by one way – ANOVA followed by Post hoc Tukey’s test. Differences with P < 0.001 were considered statistically significant.

**Results**

**Phytochemical analysis of Ficus racemosa leaf extract:**

The phytochemical screening on *Ficus racemosa* leaf powder confirmed the presence of Tannins, Saponin, Proteins and Glycosides (Table 1).

**Acute Toxicity:**

During the observation period, rats were given graduated doses of *Ficus racemosa* aqueous extract (50, 100, 200, 2000 mg/kg) which results no significant changes in breathing, behavior, sensory, tactile responses, gastrointestinal and

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Contents</th>
<th>Present (+)/Absent (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>-</td>
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<tr>
<td>5</td>
<td>Proteins</td>
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<td>Terpenoid</td>
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<tr>
<td>8</td>
<td>Quinone</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Cardiac Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Phenol</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2: Effect of *Ficus racemosa* and Silymarin on the status of Antioxidant enzymes GST, GPx, SOD and CAT in serum of ethanol induced experimental animals

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>GROUP I</th>
<th>GROUP II*</th>
<th>GROUP III**</th>
<th>GROUP IV**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td>0.73 ± 0.14</td>
<td>0.05 ± 0.04</td>
<td>0.61 ± 0.12</td>
<td>0.66 ± 0.10</td>
</tr>
<tr>
<td>GPx</td>
<td>1.27 ± 0.32</td>
<td>0.04 ± 0.03</td>
<td>1.00 ± 0.24</td>
<td>1.02 ± 0.26</td>
</tr>
<tr>
<td>SOD</td>
<td>0.36 ± 0.06</td>
<td>0.05 ± 0.02</td>
<td>0.25 ± 0.07</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>CAT</td>
<td>0.78 ± 0.13</td>
<td>0.07 ± 0.04</td>
<td>0.76 ± 0.10</td>
<td>0.78 ± 0.11</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD for six rats. *Comparisons are made between Control rats (Group I) and **Comparisons are made between Ethanol induced group (Group II). The values are statistically significant at p < 0.001. [GST-Glutathione S Transferase, GPx- Glutathione peroxidase, SOD- Super oxide dismutase, CAT-Catalase] Units : GST – min/mg of protein, GPx – μg of reduced glutathione utilized/mg of protein/min, SOD – Units/mg of protein, CAT – μ mole of H₂O₂ consumed / mg of protein.

Table 3: Effect of *Ficus racemosa* and Silymarin on the status of Antioxidant enzymes GST, GPx, TBARS, SOD and CAT in liver tissues of experimental animals

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>GROUP I</th>
<th>GROUP II*</th>
<th>GROUP III**</th>
<th>GROUP IV**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td>0.21 ± 0.07</td>
<td>0.03 ± 0.01</td>
<td>0.20 ± 0.05</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>GPx</td>
<td>0.30 ± 0.06</td>
<td>0.04 ± 0.02</td>
<td>0.23 ± 0.05</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>SOD</td>
<td>0.26 ± 0.03</td>
<td>0.09 ± 0.17</td>
<td>0.26 ± 0.03</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>CAT</td>
<td>0.80 ± 0.07</td>
<td>0.12 ± 0.04</td>
<td>0.59 ± 0.07</td>
<td>0.71 ± 0.08</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD for six rats. *Comparisons are made between Control rats (Group I) and **Comparisons are made between Ethanol induced group (Group II). The values are statistically significant at p < 0.001. [GST-Glutathione S Transferase, GPx- Glutathione peroxidase, SOD- Super oxide dismutase, CAT-Catalase] Units : GST – min/mg of protein, GPx – μg of reduced glutathione utilized/mg of protein/min, SOD – Units/mg of protein, CAT – μ mole of H₂O₂ consumed/mg of protein.

Fig. 1: Per cent change of activity levels of serum antioxidant enzyme viz. GST, GPx, SOD and CAT in control compared with Ethanol, Treatment groups – *Ficus racemosa* and Silymarin compared with Ethanol group.

Effect of *Ficus racemosa* leaf extract on Serum and Hepatic Antioxidant enzymes – GST, GPx, SOD and CAT:

The data on antioxidant enzymes viz. Glutathione S Transferase (GST), Glutathione peroxidase (GPx), Super oxide dismutase (SOD) and Catalase (CAT) are presented in Tables 2 and 3. A significant decrease (P < 0.001) in the serum and cutaneous effects and no signs of toxicity is found such as irritability, abnormal body posture, abnormal vocalization, convulsions, seizures, tremors, increased or decreased urination or defecation. There was no mortality and no signs of abnormality observed up to a dose range of 2000 mg/kg after 24 h of extract administration in the acute toxicity sample.
Fig. 2: Per cent change of activity levels of liver antioxidant enzyme viz. GST, GPx, SOD and CAT in control compared with Ethanol, Treatment groups – Ficus racemosa and Silymarin compared with Ethanol group.

Table 4: Effect of Ficus racemosa and Silymarin on serum and liver tissue TBARS level of ethanol induced experimental animals

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>GROUP I</th>
<th>GROUP II*</th>
<th>GROUP III**</th>
<th>GROUP IV**</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>0.13 ± 0.03</td>
<td>0.37 ± 0.12</td>
<td>0.13 ± 0.03</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>TBARS</td>
<td>0.05 ± 0.02</td>
<td>0.26 ± 0.10</td>
<td>0.04 ± 0.03</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD for six rats. *Comparisons are made between Control rats (Group – I) and **Comparisons are made between Ethanol induced group (Group –II). The values are statistically significant at p < 0.001. [TBARS – Thiobarbituric acid reactive substance] Unit: μ moles / mg protein

Fig. 3: Per cent change in the levels of Thiobarbituric acid reactive substances (TBARS) in Control compared with Ethanol induced group and Ficus racemosa and Silymarin compared with Ethanol group.

hepatic antioxidant enzymes were observed in ethanol induced rats proving that it is a hepatotoxicant. However, the concentration of antioxidant enzymes was significantly improved and increased in rats treated with Ficus racemosa leaf extract. Figures 1 and 2 represent the
percentage of changes in the activity levels of antioxidant enzymes in blood and liver tissue samples. The hepatoprotective potential of *Ficus racemosa* is observed similar to the action of reference drug Silymarin.

**Effect of *Ficus racemosa* extract on Serum and Hepatic TBARS level:**

The effect of *Ficus racemosa* leaf extract on serum and hepatic TBARS level is presented in Table 4. Administration of ethanol in rats significantly increased (P < 0.001) the level of TBARS which is shown in Figure 3 where the percentage changes in the levels of TBARS in ethanol induced animals are at peak. However, the treatment with *Ficus racemosa* leaf extract significantly decreased the level of lipid peroxidation caused by ethanol which is clearly indicated by depletion in the level of TBARS enzyme in serum and liver samples. It is similar to the action of Silymarin which results in significant decrease in the values of TBARS.

**Discussion**

Ethanol is a hepatotoxicant which possibly induce liver damage by formation of free radicals such as reactive oxygen species (ROS) which causes lipid peroxidation and elevation in MDA content while reducing the levels of GST, GPx, SOD and CAT (Lieber, 1997). Liver is the target organ for ethanol metabolism and hence it is more prone to ethanol intoxication. Daily administration of 20% ethanol significantly increased liver enzymes and cause oxidative stress by increase in the lipid peroxidation and reduction in the catalase and glutathione peroxidase activity in liver homogenate (Ganapathi et al., 2021). Hepatocyte, by nature contains enzymatic and non-enzymatic molecules to combat oxidative stress. Among the antioxidant enzymes, Super oxide dismutase (SOD) is the first enzyme which defends the reactive oxygen species and converts the super oxide anions into hydrogen per oxide and oxygen. The generated hydrogen peroxide is neutralized by Catalase (CAT) and glutathione peroxidase (GSH-Px) enzymes. Thereby it causes protective action in hepatocytes against free hydroxyl radicals (Comporti et al., 2010).

Glutathione S Transferases (GSTs) are a family of enzymes that play a crucial role in the detoxification of reactive oxygen species (ROS) and other harmful compounds within the cells (Cummins et al., 2011). Their primary function is to catalyze the conjugation of glutathione, a tripeptide composed of three amino acids (cysteine, glycine, and glutamic acid) with various electrophilic substrates. This conjugation process enhances the solubility of these compounds, making them easier to eliminate from the body (Lushchak, 2012). GSTs are involved in neutralizing ROS, such as hydrogen peroxides and lipid peroxides, which are generated during oxidative stress. By catalyzing the conjugation of glutathione to these ROS, GSTs help transform them into less harmful compounds, reducing their potential to cause cellular damages. In the present study, treatment with *Ficus racemosa* leaf extract elevates the level of Glutathione S Transferase (GST) thereby combating Oxidative stress.

Glutathione peroxidase (GPx) is a vital antioxidant enzyme that plays a crucial role in protecting cells from oxidative stress. Its primary function is to reduce hydrogen peroxide and lipid peroxides to less harmful compounds, thus preventing oxidative damage to cellular components (Lubos, 2011). GPx provides protection against oxidative stress by reducing H$_2$O$_2$ and lipid hydroperoxides, GPx helps prevent cellular damage and protects against oxidative stress-related injuries. Its activity is particularly essential in tissues and cells exposed to high levels of ROS, such as the liver and erythrocytes (Kurutas, 2016). The present findings suggest that the active phytoconstituents present in *Ficus racemosa* increases the production of GPx enzyme and helps in protection against the oxidative damage caused by ethanol.

Super oxide dismutase (SOD) is a crucial antioxidant that plays a central role in counteracting oxidative stress by neutralizing superoxide radicals, which are highly reactive and...
harmful oxygen-derived free radicals. By converting superoxide radicals into hydrogen peroxide and oxygen, SOD prevents the accumulation of super oxide radicals and reduces their potential to initiate oxidative damage (Younus, 2018). This action helps protect cells and tissues from oxidative damage by elevating the level of SOD in blood and liver tissues and the hepatoprotective action of *Ficus racemosa* is similar to the action of reference drug Silymarin.

Catalase is a vital antioxidant enzyme that plays a critical role in protecting cells from oxidative stress by facilitating the breakdown of hydrogen peroxide into water and molecular oxygen (Nandi *et al*., 2019). In situations of increased oxidative stress, such as exposure to environmental toxins or during inflammation, Catalase activity can be upregulated to enhance the detoxification of accumulated hydrogen peroxide and mitigate potential oxidative damage (Pizzino *et al*., 2017). The administration of ethanol may result in dysregulation or deficiency of catalase, which is associated with various pathological conditions. Treatment with *Ficus racemosa* increases the production of Catalase which in turn, mitigates oxidative stress.

TBARS are a group of compounds formed during oxidative stress as a result of lipid peroxidation – a process where reactive oxygen species (ROS) attack and damage the lipids (fats) in cell membranes. TBARS are commonly used as a marker to assess the extent of lipid peroxidation and oxidative stress within cells and tissues (Ayala, 2014). The accumulation of TBARS indicates the degradation of cell membrane lipids, which can lead to altered membrane fluidity, impaired cellular functions and cellular damage. Elevated levels of TBARS are associated with various pathological conditions (Su *et al*., 2019). On account of that, the elevated levels of TBARS are found in ethanol intoxicated animals and subsequent treatment with *Ficus racemosa* extract suppressed the level of TBARS which is similar to the effect of Silymarin.

**Conclusion**

The use of herbal medicine in treating various diseases has gained recognition due to its holistic and naturalistic approach. Herbal remedies offer a promising avenue for managing health conditions while minimizing the potential side effects. Hence, the treatment with *Ficus racemosa* leaf extract showed protective effect in ethanol induced hepatotoxicity. The phytoconstituents present in *Ficus racemosa* could be the possible reason for mitigating the oxidative stress. As we continue to explore the therapeutic potential, the utilization of *Ficus racemosa* and its antioxidant rich constituents presents an exciting opportunity for the development of novel interventions that harness the power of nature to enhance well – being and combat the adverse effects of oxidative stress caused due to alcoholism.

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**References**


