HEPATOPROTECTIVE ACTIVITY OF ETHANOL EXTRACT OF BACOLEPIS NERVOSA AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT: Medicinal plants have a long standing history in many indigenous communities and continue to provide useful tools for treating various diseases. Our aim was to evaluate the hepatoprotective effect of ethanol extract of Bacolepis nervosa stem and leaves with a view to explore its use for the treatment of hepatotoxicity in human. Oral administration of ethanol extract of B. nervosa (150 and 300 mg/kg) effectively inhibited CCl₄ induced changes in the serum marker enzymes, cholesterol, serum protein and bilirubin in a dose dependent manner as compared to the normal and the standard drug silymarin treated groups. Hepatic steatosis, fatty infiltration, hydropic degeneration and necrosis observed in CCl₄ treated groups which were completely absent in histology of the liver sections of the animals treated with the extracts. The results suggest that the ethanol extracts of stem and leaves of B. nervosa possess significant potential as hepatoprotective agent.

Key words: Ethanol, Carbon tetrachloride, Hepatotoxicity, Albino rats

INTRODUCTION

Hepatic disease is a term for collection of conditions, diseases and infections that affect the cells, tissues, structures or functions of the liver. About 20,000 deaths are reported every year due to liver disorders (Bhardwaj et al., 2011). Hepatic damage is a global metabolic and epidemic disease, affecting essential biochemical activities in almost every age group (Dhiman et al., 2012). Excess consumption of certain drugs like antibiotics, chemotherapeutic agents, acetaminophen and exposure to some chemicals such as peroxidised oils, aflatoxin, CCl₄, alcohol, etc. make liver vulnerable to a variety of disorders viz., jaundice, hepatitis, etc. which are the two major hepatic disorders that account for high death rate (Nirmala et al., 2012). Treatment for these disorders is done by using drugs from different sources, including traditional herbal medicines. These traditional herbal medicines are believed to be better than other in treating liver disorders without any scientific proof. Therefore, it is necessary to explore and develop such herbal medicines scientifically.

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. It has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Dally, 1999). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. Liver diseases are some of the fatal diseases in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders.
The plant *Bacolepis nervosa* (Wight & Arn.) Decne. ex Moq. (Periplocaceae) is an endemic plant to Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu, India. This plant contains rich source of bioactive compounds such as phenolic compounds, flavonoids, steroids and alkaloids. The impact of this plant in various disease treatments should be considered to discover new drug molecule or its derived compounds. But no such literatures are revealed for its activity against treatment for hepatic diseases. Hence the present study focuses on evaluating the hepatoprotective activity of stem and leaf extracts of *Bacolepis nervosa*.

**MATERIALS AND METHODS**

**Plant material**
The whole plant of *Bacolepis nervosa* was collected from Kothagiri, Nilagiri Biosphere Reserve, Western Ghats, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

**Preparation of plant extract for hepatoprotective studies**
The stem and leaves of the plant were dried under shade and then powdered separately with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a Soxhlet apparatus using ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for hepatoprotective studies.

**Animals**
Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

**Acute Toxicity Studies**
Acute oral toxicity study was performed as per OECD- 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after that the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 …… 2000 mg/kg body weight.

**Experimental protocol**

**Induction of hepatotoxicity**
Carbon tetrachloride (CCl₄) 2.5 ml/kg body weight was dissolved in 7.5 ml of paraffin and administered intraperitoneally.

**Grouping of animals**
A total of 35 rats were taken and were divided into seven groups of 5 rats each, of which, six groups contained CCl₄ hepatic toxicity induced rats and the remaining one group contained normal rats.

- **Group - I**: Rats served as a normal control and were given normal saline by using an intragastric catheter tube (IGC).
- **Group - II**: Liver injured rats received normal saline for 14 days by IGC, at a dose of 2.5 ml/kg body weight and served as CCl₄ hepatic toxicity induced control.
- **Group – III**: Liver injured rats received stem extract of *B. nervosa* at the dose of 150 mg/kg body weight for 14 days by using an IGC.
- **Group – IV**: Liver injured rats received stem extract of *B. nervosa* at the dose of 300 mg/kg body weight for 14 days by using an IGC.
- **Group – V**: Liver injured rats received leaf extract of *B. nervosa* at the dose of 150 mg/kg body weight for 14 days by using an IGC.
- **Group – VI**: Liver injured rats received leaf extract of *B. nervosa* at the dose of 300 mg/kg body weight for 14 days by using an IGC.
- **Group – VII**: Liver injured rats received silymarin orally at the dose of 100 mg/kg body weight for 14 days by using an IGC.
All the treatments were given between 9.30 and 10.00 hrs in the morning. After 24 hrs of last treatment, the final body weight was recorded and the animals were sacrificed by decapitation. Blood was collected from each group of rats. Serum from the blood was separated by centrifugation at 3000×g for 10 minutes and stored at -20°C until used for various biochemical assays.

Biochemical Analysis
The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum protein (Lowry, 1951) and serum albumin was determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong (1934). Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw (1987). The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Gammaglutamyltransferase (GGT) was estimated by the method of Szasz (1969). Liver homogenates (10% W/V) were prepared in ice cold 10mM tris buffer (pH7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiorbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Okhawa. Enzymatic antioxidants, superoxide dismutase (SOD) (Mishra and Fridowich, 1972 Catalase (Aebi, 1974; Colowick, 1984) and non enzymatic antioxidant glutathione peroxidase (GPx) (Pagila and Valentine, 1967) glutathione reductase (GRD) (Goldberg and Spooner, 1983) and reduced glutathione (GSH) (Prins and Loos, 1969) were also assayed in liver homogenates.

Statistical Analysis
The data were expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. p<0.001, p<0.01 and p<0.05 were considered as statistical significance using SPSS software.

RESULTS
Body weight
The administration of CCl₄ caused a significant (p<0.05) decrease in the body weight of rats as compared with the control rats. The animals treated with stem and leaf extracts of B. nervosa (150 mg/kg and 300 mg/kg) also gained weight during the experimental period (Table 1).

Table 1: Effect of ethanol extracts of stem (BNS) and leaf (BNL) of Bacolepis nervosa on the body weight of rats before and after treatment in the normal, liver damaged and drug treated mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose(mg/kg) body weight</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Mean weight Gain (G↑) / Loss (L↓) (Gm)</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.9% Saline</td>
<td>198.56±9.36</td>
<td>214.56±11.24</td>
<td>16.00</td>
<td>8.05</td>
</tr>
<tr>
<td>II</td>
<td>Liver damaged Control</td>
<td>213.64±12.13</td>
<td>196.82±7.88</td>
<td>16.82</td>
<td>7.87</td>
</tr>
<tr>
<td>III</td>
<td>BNS(150)</td>
<td>193.62±8.13</td>
<td>208.13±9.27</td>
<td>14.51</td>
<td>7.49</td>
</tr>
<tr>
<td>IV</td>
<td>BNS(300)</td>
<td>208.54±10.54</td>
<td>217.13±9.42</td>
<td>8.59</td>
<td>4.11</td>
</tr>
<tr>
<td>V</td>
<td>BNL (150)</td>
<td>203.28±9.16</td>
<td>209.54±6.81</td>
<td>6.26</td>
<td>3.07</td>
</tr>
<tr>
<td>VI</td>
<td>BNL (300)</td>
<td>197.33±6.81</td>
<td>208.63±7.32</td>
<td>11.30</td>
<td>5.72</td>
</tr>
<tr>
<td>VII</td>
<td>Silymarin (100)</td>
<td>205.16±9.54</td>
<td>216.14±10.17</td>
<td>10.98</td>
<td>5.35</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n= 5 in each group.

*p < 0.05. Comparison made between normal control and liver injured rats

*p < 0.05. Comparison made between liver injured rats and drug treated groups

ns: not significant

Biochemical parameters
The results of serum biochemical parameters are presented in Tables 2 and 3. In the CCl₄ control group, significant (p<0.05; p<0.01) decreased levels of total protein, albumin and globulin were observed. But the group which received the drug of stem and leaf extracts at the dose of 300 mg/kg body weight showed a significant (p<0.05) increase in the reduced levels of protein. The elevation of SGOT, SGPT and ALP in CCl₄ intoxication was significantly (p<0.01) high when compared to the normal. The elevated levels of SGOT, SGPT and ALP in groups IV and VI animals (post treated with stem and leaf extracts, 300 mg/kg) were significantly (p<0.05; p<0.01) reduced as depicted in Table 2.
Table 3 shows the levels of total bilirubin, conjugated and unconjugated bilirubins and GGTP levels. When compared to normal control rats (Group I), the serum of CCl₄ treated rats (Group II) showed a significant elevation in the total bilirubin, conjugated and unconjugated bilirubins. In all the other groups treated with the stem and leaf ethanol extracts of B. nervosa (Group III to VI), the above said biochemical parameters were found to be decreased when compared to CCl₄ treated diabetic control rats (Group II). However, the decrease in the concentration of total bilirubin, conjugated and unconjugated bilirubins were found to be greater in the liver damaged rat group IV, followed by group VI, treated with stem and leaf extracts of B. nervosa respectively.

The effect of B. nervosa stem and leaf extracts on LPO, GPₓ, GRD, SOD, GSH and CAT activities are shown in Table 4. It showed that GPₓ, GRD, SOD, GSH and CAT activities were significantly ($p<0.001; p<0.01$) decreased in liver damage control group. On the other hand, the groups received with both stem and leaf extracts of B. nervosa showed a significant elevation in the activities of GPₓ, GRD, SOD, GSH and CAT groups compared to normal control (Group I). The results are well compared with silymarin standard drug-treated group (Group VII).

Table 2: Effect of ethanol extracts of stem (BNS) and leaf (BNL) of Bacolepis nervosa on the serum protein, albumin, globulin concentration and serum GOT, GPT and ALP enzyme activity in the normal, liver damaged and drug treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg body weight)</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Protein (g/dl)</td>
<td>Albumin (g/dl)</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>8.65±0.93</td>
</tr>
<tr>
<td>II</td>
<td>Liver damaged control</td>
<td>6.13±0.14**</td>
</tr>
<tr>
<td>III</td>
<td>BNS (150)</td>
<td>7.16±0.28</td>
</tr>
<tr>
<td>IV</td>
<td>BNS (300)</td>
<td>8.51±0.31a</td>
</tr>
<tr>
<td>V</td>
<td>BNL (150)</td>
<td>6.94±0.22</td>
</tr>
<tr>
<td>VI</td>
<td>BNL (300)</td>
<td>8.11±0.18a</td>
</tr>
<tr>
<td>VII</td>
<td>Silymarin (100)</td>
<td>8.54±0.28a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n= 5 in each group.

*a p < 0.05; ** p<0.01 Comparison made between normal control, liver injured rats and drug treated group.

*p < 0.05; **p<0.01 Comparison made between liver injured rats and drug treated. ns: not significant

Table 3: Effect of ethanol extracts of stem (BNS) and leaf (BNL) of Bacolepis nervosa on the serum total bilirubin, conjugated and unconjugated bilirubins and GGTP levels in the normal control, liver injured and drug treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg body weight)</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Bilirubin (mg/dl)</td>
<td>Conjugated (mg/dl)</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>0.86±0.04</td>
</tr>
<tr>
<td>II</td>
<td>Liver damaged Control</td>
<td>3.28±0.16***</td>
</tr>
<tr>
<td>III</td>
<td>BNS (150)</td>
<td>1.93±0.16a</td>
</tr>
<tr>
<td>IV</td>
<td>BNS (300)</td>
<td>1.08±0.07aa</td>
</tr>
<tr>
<td>V</td>
<td>BNL (150)</td>
<td>2.84±0.07aa</td>
</tr>
<tr>
<td>VI</td>
<td>BNL (300)</td>
<td>1.18±0.05a</td>
</tr>
<tr>
<td>VII</td>
<td>Silymarin (100)</td>
<td>0.93±0.07aa</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n= 5 in each group.

*p < 0.05; **p<0.01; ***p<0.001- Comparison made between normal control, liver injured rats and drug treated groups.

*a p < 0.05; **p<0.01- Comparison made between liver injured rats and drug treated. ns: not significant

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Table 4: Effect of ethanol extracts of stem (BNS) and leaf (BNL) of Bacolepis nervosa on serum LPO, GPx, GRD, SOD, CAT and GSH activity in the normal control, liver injured and drug treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg body weight)</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LPO (nano mole of MDA/mg protein)</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>2.163±0.012</td>
</tr>
<tr>
<td>II</td>
<td>Liver damaged Control</td>
<td>6.054±0.054**</td>
</tr>
<tr>
<td>III</td>
<td>BNS(150)</td>
<td>3.926±0.048*</td>
</tr>
<tr>
<td>IV</td>
<td>BNS(300)</td>
<td>2.613±0.036a</td>
</tr>
<tr>
<td>V</td>
<td>BNL (150)</td>
<td>4.184±0.036a</td>
</tr>
<tr>
<td>VI</td>
<td>BNL (300)</td>
<td>3.02±0.024a</td>
</tr>
<tr>
<td>VII</td>
<td>Silymarin (100)</td>
<td>2.418±0.013a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n= 5 in each group.

* p < 0.05; ** p < 0.01; *** p < 0.001 - Comparison made between normal control, liver injured rats and drug treated groups

* p < 0.05; aa p < 0.01 - Comparison made between liver injured rats and drug treated. ns: not significant

Histopathology

Liver section of the control group showed normal liver parenchyma with the central vein clearly seen along with the bile ducts and hepatic arteries. The hepatocytes were neatly arranged in anastomosing plates with the sinusoids radiating from the central vein (Plate 1A). Venous congestion, necrosis and mononuclear infiltration were the histopathological changes observed in the CCl₄ treated animals (Plate 1B). Histoarchitecture of both low and high dose of stem extract groups showed normal cellular architecture with well brought out central vein, well presented cytoplasm and prominent nucleus (Plate 1C & D). Mild necrosis with mononuclear cell infiltration and edema were the changes observed in rats administered with low dose leaf extract (Plate 1E). Histopathological examination of the liver tissue of rats pretreated with high dose leaf extract showed a high degree of protection when compared with liver damaged control (Plate 1F). The sections of liver taken from the animals treated with standard drug silymarin showed the normal hepatic architecture, which was similar to that of control (Plate 1G).

HISTOLOGY OF LIVER TREATED WITH BNL AND BNS EXTRACT
DISCUSSION
Liver damage induced by CCl₄ is commonly used model for the screening of hepatoprotective activity. The rise in serum levels of SGPT, SGOT, ALP and bilirubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages. Carbon tetrachloride induces hepatotoxicity by metabolic activation therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic functions. CCl₄ metabolically activated by CYP₄₅₀ dependent mixed oxidase in the endoplasmic reticulum to form a trichloromethyl free radical (CCl₃), which combined with cellular lipids and protein in the presence of oxygen to induce lipid peroxidation (Sureshkumar and Mishra, 2007). Assessment of liver toxicity was done by measuring the marker enzymes such as SGPT, SGOT and ALP. Ethanol extracts of stem and leaf of B. nervosa at the doses of 150 and 300 mg/kg body weight significantly restored the elevated levels of serum marker enzymes. The normalization of serum markers by the stem and leaf extracts of B. nervosa, suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl₄ induced leakages of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

Diminution of total protein and albumin induced by CCl₄ is a further indication of liver damage (Navarro and Senior, 2006). A depression in total protein is observed due to the disruption and disassociation of polyribosomes from endoplasmic reticulum following CCl₄ administration (Vetriselvan et al., 2011). Albumin is a single polypeptide chain. It is synthesized in liver where it amounts to 60% of hepatic protein synthesis though less than one third of hepatocytes appear to synthesize albumins at any one time. In any form of hepatocellular damage, there is an increase in the plasma acute phase proteins and a fall in the plasma concentration of albumin (Sies, 1993). The CCl₄ induced hepatotoxicity rats treated with stem and leaf extracts of B. nervosa significantly increased serum total protein towards the respective normal value, which indicates hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells (Rip et al., 1985; Tadeusz et al., 2001).

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract, there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes (Wolf et al., 1997). Administration with the stem and leaf extracts of B. nervosa significantly (p<0.05; p<0.01) decreased the level of bilirubin and increased the level of protein suggesting that it offered protection. γ-glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ-glutamyl transferase is generally elevated as a result of liver disease, since γ-glutamyl transferase is a hepatic microsomal enzyme. Serum γ-glutamyl transferase is most useful in the diagnosis of liver diseases. Change in γ-glutamyl transferase is parallel to those of amino transferases. The acute damage caused by CCl₄ increased the γ-glutamyl transferase level but, the same attains the normal, after treatment with the stem and leaf of B. nervosa due to its antioxidant activity.

Antioxidants or free radical scavengers are very important in protecting the cells against any damage induced by free radicals, which are produced continuously in cells either during phagocytosis or accidentally as by-product metabolites. Each biological system has certain antioxidant defense mechanisms against the aggregations of such free radicals. The balance of prooxidant-antioxidant systems must exist in the cells, while the disturbance of antioxidant prooxidant balance causes oxidative stress (Karan et al., 1999).
The body has an effective mechanism to prevent or neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH). When the balance between ROS production and antioxidant defences is lost, oxidative stress occurs which is through a series of events deregulates the cellular functions leading to various pathological conditions (Khan and Sultana, 2009). Any compound, natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Lipid peroxidation (LPO) has been postulated to the destructive process of liver injury due to CCl₄ administration. In the present study, an elevation in the levels of end products of lipid peroxidation was observed in the liver of CCl₄ treated rats. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment with stem and leaf extracts of *B. nervosa* significantly reversed these changes. Hence, it may be concluded that the mechanism of hepatoprotective effect by ethanol extracts of stem and leaf of *B. nervosa*, is due to their antioxidant effects.

Superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical (Kharpate et al., 2007). In the present study, it was observed that the stem and leaf extracts of *B. nervosa* significantly (*p*<0.05; *p*<0.01) increased the SOD activity in CCl₄ intoxicated rats thereby diminished CCl₄ induced oxidative damage.

Catalase (CAT) is widely distributed in all animal tissues and which decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Arum and Balasubramanian, 2011). Administration with stem and leaf ethanol extracts of *B. nervosa* increased the activity of CAT in CCl₄ induced liver damage in rats to prevent the accumulation of excessive free radical and protected the liver from CCl₄ intoxication. Glutathione peroxidase (GPx) is a seleno-enzyme, two third of which (in liver) (Zaltzber et al., 1999) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxides (Bishayee et al., 1995). Glutathione (GSH), extensively found in cells, protects cells from electrophilic attacks provided by xenobiotics such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, muscle, lungs, colon, liver, lymphocytes and brain (Orhan et al., 2007). In the present study, treatment with stem and leaf ethanol extracts of *B. nervosa* increased the activities of GPx and GSH in CCl₄ induced liver damaged rats.

**Histopathology**

Liver is the largest organ and it is the target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification (Larrey, 2003). Drug and chemical induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases (Watkins and Seef, 2006). CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl₄ are largely due to generation of free radicals (Shenoy et al., 2001). Carbon tetrachloride induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effect of drugs or medicinal plant extracts, by in vivo and in vitro techniques (Kiso et al., 1983, Allis et al., 1990).

In the present study, the ethanol extracts of stem and leaf of *B. nervosa* provided significant protection against the toxic effect of CCl₄ on liver. Preventive action of liver damage induced by the CCl₄ has widely been used as indicator of the liver protective in general (Clausion, 1989). CCl₄ produces an experimental damage that histologically resembles viral hepatitis (James and Pickering, 1976). Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures (Recnagel, 1983). The toxic metabolite CC1₃ radical is produced by microsomal oxidase system binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In view of this, the test drug mediated reduction in levels of SGOT and SGPT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CC1₄. This effect is in agreement with the commonly accepted view that serum level of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al., 1987).

Alkaline phosphate is the prototype of these enzymes that reflects the pathological alteration in biliary flow (Ploa and Hewitt, 1989). CCl₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The extract mediated suppression of the increased serum ALP activity with the concurrent depletion of raised bilirubin suggests the possibility of both the test drug being able to stabilize biliary dysfunction in rat liver during hepatic injury.
Hence, the histological studies reveal the changes, which take place during the damage and recovery. A similar effect has been reported due to treatment with extract of *Boussingaultia gracilis* (Lin et al., 1994), extract of *Cyperus rotundus* (Sureshkumar and Mishra, 2004) and extract of *Curcuma longa* (Deshpande et al., 2003).

In addition, the absence of necrotic lesions in liver samples of the extract treated group, suggests that the hepatoprotective action may be due to membrane stabilizing effects in hepatic cells. These findings appear similar to those of an earlier study (Sree Ramamurthy and Srinivasan, 1993) where it was reported that the pretreatment of rats with *Tephrosia purpurea* offered hepatoprotection due to a membrane stabilizing effect in hepatic cells. Silymarin, a standardized extract of *Silybum marianum* is also a potent hepatoprotective agent. It reverses hepatotoxic-induced alterations of biochemical parameters and has so far been the most thoroughly investigated of all the plant substances in prevented liver damage induced by carbon tetrachloride, D-gal N and paracetamol in rat models (Bahati et al., 2006). The present study thus demonstrated its hepatoprotective effect against CCl₄ induced hepatotoxicity in rats. Further studies are required to elucidate the mechanism of its hepatoprotective action.

**CONCLUSION**

From the overall results, it could be inferred that *B. nervosa* has hepatoprotective activity and it was assumed that it confers hepatoprotection probably as a result of the presence of both enzymic and non-enzymic antioxidants that could bring about free radical suppressing activity.

Further studies are required to elucidate the mechanism of its hepatoprotective action.

**REFERENCES**


