Chapter 6

Investigation on the organoprotective effect of *Pseudarthria viscosa*

6.1. Protective effect of *Pseudarthria viscosa* against hepatotoxicity induced by CCl₄ and paracetamol

6.2. Protective effect of *Pseudarthria viscosa* against cisplatin induced nephrotoxicity

6.3. Protective effect of *Pseudarthria viscosa* against ethanol induced gastric ulcer
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6.1 PROTECTIVE EFFECT OF PSEUDARTHRIA VISCIDA AGAINST HEPATOTOXICITY INDUCED BY CCl₄ AND PARACETAMOL

6.1.1 Introduction

The liver has an essential role in detoxifying harmful substances entering the circulatory system. It plays a significant role in the metabolism of fats, carbohydrates and proteins. Any variation in structure or function the liver may result in portal hypertension, jaundice, and increased bleeding, which may lead to metabolic changes that affect other organs too (Ibrahim et al., 2008). Though most of the xenobiotics are detoxified with the help of metabolizing enzymes, bioactivation is not uncommon (Jaeschke et al., 2002). In such conditions, enhancement of toxicity of several xenobiotics could cause damage to vital organs and the first causality is liver. CCl₄ is a lethal hepatotoxin which cause liver cell damage in experimental animals (Klingensmith and Mehendale, 1982). CCl₄ metabolism in the liver mainly mediated by cytochrome P450 (CYP) enzymes, that results in the formation of the trichloromethyl radical (CCl₃˙); initiate a chain reaction that causes destruction to vial macromolecules (Weber et al., 2003). Paracetamol on reacting with CYP enzymes produce a toxic metabolite, N-acetyl-p-benzoquinoneimine cause liver damage (Udem et al., 1997). To withstand the oxidative stress and hepatotoxicity, it is necessary to have incorporation of proven antioxidants in treatment regimen. There is a clear shift happening towards considering herbal derivatives for treating chronic liver diseases. In several instances the efficacy of conventional drugs is in question. (Chattopadhyay,2003). Side effects and exorbitant prices of many of these drugs paved way for such an attitude change by patients, physicians, etc. Hence, in this study, we evaluated the hepatoprotective activity of P. viscida against CCl₄ as well as paracetamol induced toxicity.
6.1.2 Materials and Methods

6.1.2.1 Animals
Male Wistar Rats (220-240 g) were purchased from the Small Animal Breeding Station, Mannuthy, Kerala. The animals were maintained under standardized environmental conditions and fed with standard rat feed (SaiDurga Feeds, Bangalore) and water ad libitum. All the animal experiments conducted in the present study had prior permission from Institutional Animal Ethics Committee (IAEC) and followed guidelines of Animal Ethics Committee, Government of India.

6.1.2.2 Experimental design

6.2.2.1 Protective effect of *P. viscida* against CCl₄ induced hepatotoxicity
There were five groups having 6 animals each. Group I consisted of Normal animals. Group II comprised of CCl₄ (2 ml/Kg body weight) alone control group. Group III formed animals pre-treated with standard drug sylimarin (100 mg/kg) for 7 days before inducing hepatotoxicity by intraperitonial injection of CCl₄ exposure. Group IV and V were pre-treated with 200 mg/kg and 400 mg/kg body weight respectively of *P. viscida* for 7 days before inducing hepatotoxicity by intraperitonial injection of CCl₄ exposure.

6.2.2.2 Protective effect of *P. viscida* against paracetamol induced hepatotoxicity
There were four groups having 6 animals each. Group I consisted of Normal animals. Group II comprised of CCl₄ (2 ml/Kg body weight) alone control group. Group III and IV were pre-treated with 200 mg/kg and 400 mg/kg body weight respectively of *P. viscida* for 7 days before inducing hepatotoxicity by intraperitonial injection of CCl₄ exposure. On completion of 24 hours of the administration of CCl₄, animals of each group were sacrificed after giving ether anaesthesia.
6.1.2.3 Determination of serum markers and endogenous antioxidant levels for carbon tetrachloride and paracetamol induced liver toxicity

Blood was collected and serum separated was used to evaluate the activities of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), bilirubin and total proteins following standard procedures by using commercially available kits (Span Diagnostic Ltd). The 25% liver homogenate in tris buffer (pH 7) was subjected to further analysis to evaluate the effect of PVM in protecting liver tissues from oxidative stress. Reduced glutathione (GSH) was determined by the method described by Moron et al. (1979). Glutathione peroxidase (GPx) was measured by the method described by Hafeman et al. (1974). Estimation of Superoxide dismutase (SOD) was done by the method of Mc Cord and Fridovich (1969). Lipid peroxidation was measured as nmoles MDA/mg protein, following the method by Okhawa et al (1979).

6.1.2.4 Histopathology

After sacrificing the animals a small portion liver was excised washed in phosphate buffered saline; fixed in 10% formaldehyde solution and then embedded in wax. Sections were taken and stained with hematoxylin and eosin.

6.1.2.5 Statistical analysis

The values were expressed in Mean ± SD, for 10 animals in each group. All groups were analysed for one way ANOVA by Dunnetts test using GraphPad InStat software. The groups with ’p’ value less than 0.05 were considered statistically significant.

6.1.3 Result

6.1.3.1 Protective effect of P. viscida against CCl₄ induced hepatotoxicity

Serum GOT, GPT, ALP and Bilirubin level in animals with CCl₄ induced hepatotoxicity are presented in Table 6.1.1. Treatment with P. viscida was found effective in reducing the level of serum GOT, GPT, ALP and Bilirubin
in a very significant manner (p< 0.01), in animals with CCl₄ induced hepatotoxicity compared with CCl₄ alone control group. SOD, GPx and GSH levels were significantly (p< 0.01) elevated in P. viscida treated animals compared to control group (Table 6.1.2). Increased level of MDA was found reduced significantly (p< 0.01) in P. viscida treated animals in dose dependent manner. Histopathology analysis showed (Figure 6.1.1) an increased level of tissue necrosis in control group and a lesser level of damage was found in animals treated with P. viscida. This was evident from an increased level of protection to liver parenchyma. There was only a mild level of fatty change in P. viscida treated groups compared to control group.

### 6.1.3.2 Protective effect of P. viscida against paracetamol induced hepatotoxicity

The effect of P. viscida on serum GOT, GPT, ALP and Bilirubin in animals with paracetamol induced toxicity in comparison to control group which is summarized in Table 6.1.3. Serum GOT, GPT, ALP and Bilirubin levels were significantly lower (p< 0.01) in P. viscida treated groups even after getting exposed to higher dose of paracetamol in comparison to control group. Table6.1.4 present the level of SOD, GPx, GSH and MDA in liver tissue homogenate of animals in different groups. There was significant (p< 0.01 and p< 0.05) increase in SOD, GPx, GSH etc. in P. viscida treated animals in dose dependent manner. MDA level was very high in paracetamol alone group and was found significantly (p< 0.01) reduced in P. viscida treated animals. Histopathology of liver samples (Figure 6.1.2) showed, extensive level of liver necrosis along with significant level of fatty changes, in control group. P. viscida treatment provided partial protection to the liver tissues compared to control group.

### 6.1.4 Discussion

There are several chemical agents which are known to cause oxidative stress related hepatotoxicity. Carbon tetrachloride is known to cause free radical
induced toxicity to liver. Its metabolism in liver starts with the formation of the trichloromethyl radicals (CCl₃⁻) in the presence of CYP enzymes. Trichloromethyl radicals on reaction with oxygen produce trichloromethylperoxy (CCl₃OO⁻) which is a highly reactive free radical. These two radicals act in tandem and cause damages to macromolecules like nuclear components, protein, carbohydrates etc. which leads to cell destruction (Weber, et al 2003). The oxidative stress becomes too heavy that the components of the endogenous antioxidant mechanism such as GSH get sharply depleted, which was evident in CCl₄ (Lima et al., 2005). However, there was an elevated level of GSH in P. viscida treated animals which could be attributed to antioxidant property of P. viscida. The hepatocyte destruction in control animals was evident from the fact that serum showed elevated level of enzymes such as GOT, GPT and ALP in serum (Klingensmith and Mehendale, 1982). However, there was reduced levels of serum liver markers in P. viscida due to the lowering oxidative stress in hepatocytes. SOD has a very significant role in scavenging superoxide free radicals (McCord, 1976) and the elevated level of SOD in P. viscida treated animals show improved levels of protection from oxidative stress. Extensive evidence demonstrates that CCl₄ is causative in the production of highly reactive trichloromethyl radical in the liver, which could promote free radical-mediated lipid peroxidation of membrane phospholipids thereby causing damage to the cell membrane. Elevated level of MDA indicated increased level of lipid peroxidation (Poli et al., 1987). However, there was marked reduction in lipid peroxidation level of P. viscida compared to control group (Brunt and Tiniakos, 2005).

Paracetamol, a widely used antipyretic agent, is mainly metabolized in the liver. Its higher dosages could cause severe hepatic necrosis in experimental animals and humans (Ohkawa et al., 1979). Electrophilic metabolite of paracetamol, N-acetylparabenoquinonimine, conjugates with cellular
glutathione (GSH) causing sharp deterioration in GSH level (Muriel et al., 2002). However the treatment with P. viscida showed an improved level of GSH in liver tissues. Paracetamol toxicity in liver drastically increased the liver marker enzymes such as GOT, GPT and ALP in serum. The liver enzyme level in serum becomes quantitative markers to evaluate the extent and type of hepatocellular damage (Vaishwanar and Kowale, 1976). GSH, a thiol-based antioxidant plays an important role in the cellular defence cascade against oxidative damage. It protects the living system from heavy assault of reactive oxygen species (Kelly, 1999). SOD also plays a significant role by scavenging the dangerous superoxide free radicals (McCord et al., 1976). There was improvement in the SOD level in P. viscida treated animals compared to control group. GPx with the support of its cofactor GSH, catalyses the reduction of hydrogen peroxide to water and oxygen, and thereby limit the possibility of formation of hydroxyl radical, a highly toxic reactive oxygen species (Hsu et al., 2002). Deficiency of GSH leads to oxidant damage and increased level of lipid peroxidation, which in turn leads to cell damage (Scholz, 1989). Treatment with P. viscida improved the level of GSH and GPx; at the same time reduced the level of lipid peroxidation.

To conclude, the present study showed that P. viscida is effective in protecting hepatocytes from toxicity induced by carbon tetrachloride and paracetamol. Treatment with P. viscida showed remarkable improvement in the endogenous antioxidant level. Further studies to be conducted to understand the exact mechanism behind the hepatoprotective activity of P. viscida which would help in the incorporation of P. viscida in modern health care regimens.
### Table 6.1.1  Effect of *P. viscida* extract on serum GOT, GPT, ALP and bilirubin against CCl₄ induce toxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>233.61 ± 15.61</td>
<td>147.41 ± 11.21</td>
<td>155 ± 18.93</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>CCl₄ Control</td>
<td>817.02 ± 21.55ᵃ</td>
<td>379 ± 24.33ᵃ</td>
<td>388 ± 62.131ᵃ</td>
<td>0.82 ± 0.09ᵃ</td>
</tr>
<tr>
<td>Silymarin</td>
<td>582.4 ± 14.81ᶜ</td>
<td>189.4 ± 11.14ᶜ</td>
<td>233 ± 40.07ᶜ</td>
<td>0.55 ± 0.081ᶜ</td>
</tr>
<tr>
<td><em>P. viscida</em> 200mg/kg</td>
<td>645 ± 9.26ᶜ</td>
<td>221.33 ± 7.03ᶜ</td>
<td>254 ± 61.22ᶜ</td>
<td>0.69 ± 0.05ᶜ</td>
</tr>
<tr>
<td><em>P. viscida</em> 400mg/kg</td>
<td>522 ± 17.94ᶜ</td>
<td>176.8 ± 28.5ᶜ</td>
<td>222 ± 80.97ᶜ</td>
<td>0.54 ± 0.5ᶜ</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01,(b)p<0.05 as compared to normal. (c)p<0.01,(d)p<0.05 as compared to control.

### Table 6.1.2  Effect of *P. viscida* extract on SOD, GPx, GSH and MDA levels in liver tissues against CCl₄ induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.86 ± 0.20</td>
<td>8.04 ± 0.35</td>
<td>7.35 ± 0.22</td>
<td>0.38 ± 0.20</td>
</tr>
<tr>
<td>CCl₄ Control</td>
<td>0.34 ± 0.10ᵃ</td>
<td>3.14 ± 0.38ᵃ</td>
<td>1.82 ± 0.61ᵃ</td>
<td>1.66 ± 0.10ᵃ</td>
</tr>
<tr>
<td>Silymarin</td>
<td>0.64 ± 0.25ᵈ</td>
<td>6.85 ± 0.26ᶜ</td>
<td>6.28 ± 0.87ᶜ</td>
<td>0.51 ± 0.16ᶜ</td>
</tr>
<tr>
<td><em>P. viscida</em> 200mg/kg</td>
<td>0.56 ± 0.05</td>
<td>6.15 ± 0.31ᶜ</td>
<td>4.56 ± 0.32ᶜ</td>
<td>0.62 ± 0.10ᶜ</td>
</tr>
<tr>
<td><em>P. viscida</em> 400mg/kg</td>
<td>0.71 ± 0.28ᶜ</td>
<td>6.42 ± 0.41ᶜ</td>
<td>5.73 ± 0.55ᶜ</td>
<td>0.05 ± 0.25ᶜ</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01,(b)p<0.05 as compared to normal. (c)p<0.01,(d)p<0.05 as compared to control.
### Table 6.1.3  Effect of *P. viscida* extract on serum GOT, GPT, ALP and bilirubin against paracetamol induce toxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>173.71±10.1</td>
<td>119.52±8.55</td>
<td>121±11.59</td>
<td>0.28±0.04</td>
</tr>
<tr>
<td>Paracetamol Control</td>
<td>536±14.09a</td>
<td>432.21±11.73a</td>
<td>422±17.13a</td>
<td>0.56±0.003a</td>
</tr>
<tr>
<td><em>P. viscida</em> 200mg/kg</td>
<td>242.5±14.5c</td>
<td>378±5.09c</td>
<td>313±24.22c</td>
<td>0.48±0.1c</td>
</tr>
<tr>
<td><em>P. viscida</em> 400mg/kg</td>
<td>225.6±17.23c</td>
<td>265±8.02c</td>
<td>247±20.97c</td>
<td>0.37±0.04c</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01, (b)p<0.05 as compared to normal. (c)p<0.01, (d)p<0.05 as compared to control.

### Table 6.1.4  Effect of *P. viscida* extract on SOD, GPx, GSH and MDA levels in liver tissues against paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.59 ± 0.016</td>
<td>8.05 ± 0.74</td>
<td>7.21 ± 1.82</td>
<td>0.27 ± 0.06</td>
</tr>
<tr>
<td>Control</td>
<td>0.18 ± 0.027a</td>
<td>3.68 ± 0.23a</td>
<td>1.94 ± 1.06a</td>
<td>1.52 ± 0.05a</td>
</tr>
<tr>
<td><em>P. viscida</em> 200mg/kg</td>
<td>0.20 ± 0.063</td>
<td>5.61 ± 0.51c</td>
<td>4.45 ± 1.57d</td>
<td>1.02 ± 0.06c</td>
</tr>
<tr>
<td><em>P. viscida</em> 400mg/kg</td>
<td>0.49 ± 0.075c</td>
<td>6.69 ± 0.23c</td>
<td>6.06 ± 1.52c</td>
<td>0.78 ± 0.07c</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01, (b)p<0.05 as compared to normal. (c)p<0.01, (d)p<0.05 as compared to control.
Fig. 6.1.1 Histopathology of liver in CCl₄ induce toxicity study

Normal

CCl₄ Control

Silymarin

P. viscida 200mg/kg  P. viscida 400mg/kg

Histopathology analysis showed an increased level of tissue necrosis in control group and a lesser level of damage was found in animals treated with P. viscida. This was evident from an increased level of protection to liver parenchyma. There was only a mild level of fatty change in P. viscida treated groups compared to control group.
Fig. 6.1.2 Histopathology of liver in paracetamol induce toxicity study

Normal
Paracetamol Control

*P. viscida* 200mg/kg  *P. viscida* 400mg/kg

Histopathology of liver samples showed, extensive level of liver necrosis along with significant level of fatty changes, in control group. *P. viscida* treatment provided partial protection to the liver tissues compared to control group.
6.2. PROTECTIVE EFFECT OF *PSEUDARTHRIA VISCIDA* AGAINST CISPLATIN INDUCED NEPHROTOXICITY

6.2.1 Introduction

Oxidative stress is found to be one of the important causative factors for nephrotoxicity. More than 20% of the population above 65 years old is found with some kind of renal abnormalities (Nasri, 2013). Cisplatin is a platinum based anticancer drug known to cause nephrotoxicity. It is known to cause cell death due to its ability to cross link DNA strands. It is widely used in the treatment of several types of cancers (Pai and Nahata, 2000). The kidney, the most important organ of the renal system, is retroperitoneal that serve the body as a natural filter of the blood and remove wastes from the body. The kidneys produce and secrete hormones and enzymes that help to regulate red blood cell production, blood pressure, and calcium and phosphate metabolism. Cisplatin is known to cause oxidative stress. This is evident from the increased level of lipid peroxidation and reduced level of endogenous antioxidants, which lead to further deterioration of redox balance in experimental animals treated with cisplatin (Zhang *et al.*, 1992). *P. viscida* is known for its antioxidant property. Hence, in this study, we investigated the protective effect of *P. viscida* against cisplatin induced oxidative stress in Wistar rats.

6.2.2 Materials and Methods

6.2.2.1 Animals

Male Wistar Rats (220-240 g) were purchased from the Small Animal Breeding Station, Agricultural University, Mannuthy, Kerala. The animals were maintained under standardized environmental conditions and fed with standard rat feed (Sai Durga Feeds, Bangalore) and water ad libitum. All the animal experiments conducted in the present study had prior permission from Institutional Animal Ethics Committee (IAEC) and followed guidelines of Animal Ethics Committee, Government of India.
6.2.2.2 Experimental design
Group I animals received five doses of distilled water by gavage and served as normal. Group II served as cisplatin alone treated control. Groups III and IV received \textit{P. viscid.a} extract of doses 200 mg/kg body weight and 400 mg/kg body weight, dissolved in distilled water, for five consecutive days. On day 5, one hour after extract administration all the groups except normal were injected with cisplatin of 25 mg/kg body weight (ip). 72 hours after the administration of cisplatin animals of each group were sacrificed by ether anaesthesia.

6.2.2.3 Determination of serum markers and endogenous antioxidant levels for cisplatin induced nephrotoxicity
At the end of the experiments animals of each group were sacrificed. Blood samples were collected by heart puncture and centrifuged at 2500 RPM for 5 minutes at 4°C to separate the serum. Kidneys were removed immediately after sacrifice and stored at 20°C for further analysis. The homogenization of tissue samples were carried out using Teflon homogeniser (Rotek, India LtD) in 1M tris buffer (pH 7.0) and centrifuged for 30 minutes (4°C) at 10,000 RPM. The supernatants were collected and used for the determination of cellular antioxidants such as superoxide dismutase (2.2. of chapter 2), catalase, reduced glutathione, glutathione peroxidase activities and lipid peroxidation level. The serum samples were used to analyse the level of albumin, creatinine and urea levels. One sample from each group is taken for histopathology.

6.2.2.4 Statistical analysis
The values were expressed in Mean ± SD, for 6 animals in each group. All groups were analysed for one way ANOVA by Dunnetts test using GraphPad InStat software. The groups with 'p' value less than 0.05 were considered statistically significant.
6.2.3 Result

Serum albumin level (Table 6.2.1) was found to be significantly \((p<0.01)\) elevated in control group \((4.18 \pm 0.097)\) compared to normal group \((3.51 \pm 0.032)\) and was found decreasing in \(P. \text{viscida}\) treated groups 200 mg/kg \((4.01 \pm 0.18)\) \((p<0.05)\) and 400 mg/kg \((3.89 \pm 0.039)\) \((p<0.01)\) in a significant manner. Serum creatinine level was also found to be effectively reduced to \(1.98 \pm 0.89\) (200 mg/kg) and \(1.91 \pm 1.64\) (400 mg/kg) in \(P. \text{viscida}\) treated groups in comparison to cisplatin alone control group \((1.79 \pm 0.50)\). Urea level was increased in cisplatin control group \((43.08 \pm 0.52)\) compared to normal group \((21.52 \pm 0.32)\). However significant \((p<0.01)\) reduction in serum urea level was found in \(P. \text{viscida}\) treated groups of 200 mg/kg \((31.30 \pm 1.56)\) and 400 mg/kg \((21.52 \pm 0.32)\).

Kidney tissue samples collected from experimental animals were evaluated for endogenous antioxidants such as SOD, GPX, GSH and for MDA (Table 6.2.2). SOD level in cisplatin alone control group \((0.22 \pm 0.086)\) was significantly lowered \((p<0.01)\) in comparison to normal animals \((0.54 \pm 0.026)\). There was significant enhancement in the level of SOD level in kidney tissues when treated with \(P. \text{viscida}\) of concentrations 200 mg/kg \((0.34 \pm 0.107)\) \((p<0.05)\) and 400 mg/kg \((0.39 \pm 0.060)\) \((p<0.01)\). GPx was found reduced in control group to \(5.21 \pm 0.68\) and was elevated to \(6.65 \pm 0.12\) \((p<0.01)\) and \(7.14 \pm 0.28\) \((p<0.01)\) on treating with \(P. \text{viscida}\) of concentrations of 200 mg/ kg and 400 mg/kg respectively. There was increase in the level of GSH in animals treated with 200 mg/ kg \((5.30 \pm 0.67)\) and 400 mg/kg \((6.74 \pm 1.55)\) of \(P. \text{viscida}\) compared to normal group \((3.15 \pm 1.46)\). MDA level was elevated to \(1.28 \pm 0.40\) nmol/mg protein in control group which was found reduced in animal groups treated with 200 mg/ kg \((0.88 \pm 0.35)\) \((p<0.05)\) and 400 mg/kg \((0.79 \pm 0.19)\) \((p<0.01)\) body weight of \(P. \text{viscida}\).
The kidney weight as percentage of bodyweight (Table 6.2.3) was found to be increased in control group (0.354 ± 0.050) (p<0.01) compared to normal group (0.280 ± 0.022) and was found to be normalized significantly in animals treated with of *P. viscida* of concentrations of 200 mg/kg (0.310 ± 0.015) (p<0.05) and 400 mg/kg (0.297 ± 0.004) (p<0.01).

Histopathology of *P. viscida* (Figure 6.2.1) administered animals was compared with that of cisplatin alone treated groups. There was a decreased level of tissue damage observed in the herbal extract treated groups. Cisplatin had induced severe necrosis of the proximal tubules. Dilation of tubules indicated the increased level of renal tissue damage. Treatment with *P. viscida* reduced the level of necrosis caused by cisplatin in renal tissues.

### 6.2.4 Discussion

Cisplatin is known as an effective anticancer drug to treat and an array of cancers (Rosenberg, 1999). However, it has a bad reputation of adversely affecting the normal functioning of the patient’s kidney. Nephrotoxicity of cisplatin is marked by increased level creatinine (Arany and Safirstein, 1999) as we have observed in the case of the control group. However, there was a significant decrease in the level of creatinine in animals treated with *P. viscida*. Activities of cisplatin in living systems can lead to the formation of free radicals such as superoxides and hydroxyls which consequently leads to oxidative stress and tissue damage. However *P. viscida* treated animals showed increased levels of SOD activity. SOD is known to scavenge superoxide free radicals and thereby reduce the oxidative stress and related damages (Lopez and Luderer, 2004). Nephrotoxicity is also marked by increased level of lipid peroxidation and reduced level of GSH and similar protein thiols. There is a significant decrease in the activity of transport proteins (Nasri, 2013) (Lau, 1999; Kuhlmann *et al.*, 1997). The GSH level showed marked decrease in animals treated with cisplatin alone. Antioxidant
treatments were found to reduce the level of oxidative stress and bring normality to renal functioning. Tocotrienol has shown the ability to reduce proximal tubular injury and lipid peroxidation and thereby enhancing the level of GSH activity (Rafieian-kopaei, 2013). Here in this study elevation of GSH level and reduction in lipid peroxidation could be attributed to the antioxidant activities of *P. viscida*. GPx is known to neutralize hydrogen peroxide and thereby reduce the oxidative stress (Brigelius-Flohe and Maiorino, 2013). GPx level climbed up significantly in *P. viscida* treated animals. The enhancement of endogenous antioxidants in *P. viscida* treated animals can be correlated with reduced levels of both oxidative stress and tissue damage in them. Cisplatin induced tubular damage is marked by loss of tubules to reabsorb water which lead to dehydration and loss of body weight (Mahran et al., 2011; Noroozi et al., 2015). Nephrotoxicity induced by cisplatin is due to the amplified level of tissue damage (Nasri, 2013). However, compared to cisplatin alone control group, kidney weight of *P. viscida* treated animals were lower and almost comparable to that of normal animals. The histopathology result indicated an elevation in the tissue damage level in control animals which was found be reduced in *P. viscida* treated groups.

In conclusion, *P. viscida* enhanced endogenous enzymes in the kidney tissues. There is a marked decrease in serum markers for kidney function in the *P. viscida* treated animals. The protective effect of *P. viscida* against cisplatin could be attributed to the antioxidant properties of the former. This shows the potentiality of *P. viscida* to be utilized in developing any potential ameliorative drug to treat adverse health conditions caused due to exposure to cisplatin treatment regime.
### Table 6.2.1 Effect of *P. viscida* extract on serum parameters such as albumin, creatinine and urea against cisplatin induced nephrotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin (g/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Urea (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.51 ± 0.032</td>
<td>1.79 ± 0.50</td>
<td>19.02 ± 1.76</td>
</tr>
<tr>
<td>Cisplatin Control</td>
<td>4.18 ± 0.097(^a)</td>
<td>2.87 ± 0.53(^a)</td>
<td>43.08 ± 0.52(^a)</td>
</tr>
<tr>
<td><em>P. viscida</em> 200mg/kg</td>
<td>4.01 ± 0.180(^d)</td>
<td>1.98 ± 0.89(^c)</td>
<td>31.30 ± 1.56(^c)</td>
</tr>
<tr>
<td><em>P. viscida</em> 400mg/kg</td>
<td>3.89 ± 0.039(^c)</td>
<td>1.91 ± 1.64(^c)</td>
<td>21.52 ± 0.32(^c)</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01, (b)p<0.05 as compared to normal. (c)p<0.01, (d)p<0.05 as compared to control.

### Table 6.2.2 Effect of *P. viscida* extract on SOD, GPx, GSH and MDA levels in kidney tissues against cisplatin induced nephrotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.54 ± 0.026</td>
<td>8.3 ± 0.95</td>
<td>7.52 ± 2.05</td>
<td>0.57 ± 0.29</td>
</tr>
<tr>
<td>Cisplatin Control</td>
<td>0.22 ± 0.086(^a)</td>
<td>5.21 ± 0.68(^a)</td>
<td>3.15 ± 1.46(^a)</td>
<td>1.28 ± 0.40(^a)</td>
</tr>
<tr>
<td><em>P. viscida</em> 200mg/kg</td>
<td>0.34 ± 0.107(^d)</td>
<td>6.65 ± 0.12(^c)</td>
<td>5.30 ± 0.67(^d)</td>
<td>0.88 ± 0.35(^d)</td>
</tr>
<tr>
<td><em>P. viscida</em> 400mg/kg</td>
<td>0.39 ± 0.060(^c)</td>
<td>7.14 ± 0.28(^c)</td>
<td>6.74 ± 1.55(^c)</td>
<td>0.79 ± 0.19(^c)</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01, (b)p<0.05 as compared to normal. (c)p<0.01, (d)p<0.05 as compared to control.

### Table 6.2.3 Effect of *P. viscida* on kidney weight of Cisplatin treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney weight as percentage of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.280 ± 0.022(^a)</td>
</tr>
<tr>
<td>Cisplatin Control</td>
<td>0.354 ± 0.050(^a)</td>
</tr>
<tr>
<td><em>P. viscida</em> 200 mg/kg + Cisplatin</td>
<td>0.310 ± 0.015(^d)</td>
</tr>
<tr>
<td><em>P. viscida</em> 400 mg/kg + Cisplatin</td>
<td>0.297 ± 0.004(^c)</td>
</tr>
</tbody>
</table>

Values are Mean ± SD for six animals and expressed as the organ weight/ 100g of body weight.
Histopathology of *P. viscida* administered animals was compared with that of cisplatin alone treated groups. There was a decreased level of tissue damage observed in the herbal extract treated groups. Cisplatin had induced severe necrosis of the proximal tubules. Dilation of tubules indicated the increased level of renal tissue damage. Treatment with *P. viscida* reduced the level of necrosis caused by cisplatin in renal tissues.
6.3. PROTECTIVE EFFECT OF PSEUDARTHRIA VISCIDA AGAINST ETHANOL INDUCED GASTRIC ULCER

6.3.1 Introduction

In the living system free radicals are constantly generated and they play an important role in a number of biological processes; some of which are necessary for life (Pacher et al., 2007). In living system reactive oxygen species (ROS) are constantly generated and they can cause extensive damage to tissues and biomolecules which may initiate or propagate many diseases such as inflammation, cancer, liver injury and cardiovascular diseases (Liao and Yin, 2000). Free radicals are closely related with peptic ulcer and gastritis (Salim, 1989). This could further promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and damaging of DNA strands (Schraufstatter et al., 1988).

Although a living system possesses several endogenous defence mechanisms, such as enzymes and antioxidant molecules, which arrest the chain reaction of ROS initiation and production (Halliwell et al., 1995), continuous exposure to ROS for a long time may lead to irreversible oxidative damage. Therefore, antioxidants may have a greater relevance in the prevention and therapeutics of such diseases (Soares et al., 1997). Many natural antioxidants have been isolated from different plant materials. Furthermore, people who consume fruits and vegetables, which are a good source of antioxidants, have a lower risk of heart diseases and many neurological diseases (Stanner et al., 2004). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989). The research into plants with the folkloric use for different aliments should, therefore, be viewed as a fruitful and logical research strategy in the search for new anti-
inflammatory and anti-ulcer drugs. In this study, we examined gastro-protective activity of P. viscida against ethanol induced gastric ulceration.

6.3.2. Materials and methods

6.3.2.1. Animals

Male Wistar rats (150-200 g) were maintained under standardized environmental conditions (22–28°C, 60–70% relative humidity, 12 hr dark/light cycle) and fed with standard rat feed (Sai Durga Feeds, Bangalore, India) and water ad libitum. All the animal experiments conducted during the present study had prior permission from Institutional Animal Ethics Committee (IAEC approved) and strictly followed the guidelines of Animal Ethics Committee, Government of India.

6.3.2.2. Experimental design

Four groups of animals (6 rats/group) were used in this study. Group I was kept as normal without any treatment and all other groups were fasted for 36 hrs and administered with 80% ethanol. Animals of second group received 1ml of 80% ethanol alone. Third and fourth groups received P. viscida extract at a dose of 200 mg/Kg body weight and 400 mg/Kg body weight respectively, one hour prior to the administration of 80% ethanol. The animals were sacrificed after four hours of the administration of ethanol. According to the severity of erosions, the gastric mucosal lesions were graded. Lesion of 1 mm or less is scored as grade 1; 1-2 mm is scored as grade 2 and more than 2 mm are scored as grade 3. The ulcer index was calculated by the formula, 1 × (number of lesions of grade 1) + 2 × (number of lesions in grade 2) + 3 × (number of lesions in grade 3) and then the overall score was divided by a factor 10.

6.3.2.3 Biochemical assays to evaluate the protective effect of P. serratifolia against ethanol induced gastric ulcer in rats

Stomach mucosa was collected by scraping with a blunt knife and its 10% homogenate in tris buffer (pH 7) was prepared. Reduced glutathione (GSH),
in gastric mucosa was determined by the method described by Moron et al. (1979). Glutathione peroxidase (GPx) was measured by the method described by Hafeman et al. (1974). Estimation of Superoxide dismutase (SOD) was done by the method of McCord and Fridovich (1969). Lipid peroxidation was measured by the method of Okhawa et al. (1979). The protein content was determined according to the procedure described by Lowry et al. (1951) (2.2.11. of chapter 2).

6.3.2.4. Statistical analysis
The values were expressed in Mean ± SD, for 6 animals in each group. All groups were analysed for one way anova by Dunnetts test using GraphPad InStat software. The groups with 'p' value less than 0.05 were considered statistically significant.

6.3.3. Results
The effect of *P. viscida* on gastric lesions induced by 80% ethanol is shown in Table. 6.3.1. Ethanol induced an intense gastric mucosal damage in the form of haemorrhagic streaks in control group of rats but not *P. viscida*. Ethanol administration produced gastric damage with an ulcer index of 4.52 ± 0.71 in control group. Compared to the control group *P. viscida* treated groups of rats displayed a marked (p<0.01) and dose dependent protection against ethanol-damage. At the *P. viscida* doses of 200 and 400 mg/kg, the reductions in gastric damage were in the order of 62.39 % and 64.05 % respectively. The biochemical analysis showed that the pre-treatment of the plant extract produced an increase in SOD, GSH and GPx levels (p<0.01), whereas it produced a decrease in lipid peroxidation (Table 6.3.2.). Histopathological analysis of stomach samples showed increased level of damage to gastric mucosal epithelium (Figure 6.3.1). There were severe alterations in the histological architecture. This can be evident from the increased level of haemorrhage, necrosis, erosion and congestion of gastric mucosa. Several instances of gastric lesions in the form of gastric pits were found. The
anomalies were found drastically reduced in animals treated with \textit{P. viscida} extracts.

6.3.4. Discussion
The role of free radical reactions in biology and medicine has become an area of intense interest due to their relationship to chronic diseases. It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events in living organisms. Lipids containing polyunsaturated fatty acids can be oxidized by free radical-mediated reactions. In addition, when oxygen is supplied in excess or their reduction is insufficient, this generates endogenous ROS imbalances with the formation of hydroxyl and superoxide radicals (Rahal \textit{et al.}, 2014). If the endogenous response system, such as antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), is inadequate in scavenging ROS, damage to important macromolecules can be established and generate cellular oxidative stress. The non-enzymatic antioxidants, such as vitamin C, vitamin E and phenolic compounds found in a vegetable-rich diet, play a significant role together with enzymatic defences in the physiological redox balance (Halliwell and Gutteridge, 1998). Several studies have shown that consumption of antioxidant-rich foods decreases levels of oxidative damage in humans. Flavonoids and other polyphenols have powerful antioxidant activities \textit{in vitro}, being able to scavenge a wide range of reactive species, including hydroxyl radicals, peroxyl radicals, hypochlorous acid and (sometimes) superoxide radicals (Rice-Evans, 2000).

At oral doses of 200 and 400 mg/kg, \textit{P. viscida} dose-dependently prevented the ethanol-induced acute haemorrhagic mucosal lesions of the glandular region of the stomach. It has been firmly established that oxidative stress and impaired prostaglandin synthesis contribute to gastric mucosal damage in experimental models of gastric lesions induced by ethanol (Kwiecien \textit{et al.},
In the gastric walls of ethanol administered animals, *P. viscida* significantly replenished SOD, GSH and GPx levels and significantly reduced lipid peroxidation as evident from the decreased MDA level; indicating the capacity of *P. viscida* to prevent oxidative stress. It was reported that, a reduction in gastric glutathione can occur following ethanol consumption and glutathione pre-treatment could subside the gastric damage (Loguercio *et al.*, 1993). However, *P. viscida* significantly replenished the ethanol associated decrease in reduced glutathione. Therefore, it is conceivable that the *P. viscida* extract was effective in reducing the oxidative stress induced by ethanol in rat stomach. In conclusion, the final outcome of this study indicates a cytoprotective role for *P. viscida* affording gastroprotection against gastric mucosal damage induced by ethanol which could be attributed to its antioxidant property.
Table 6.3.1 Effect of *P. viscida* on ulcer index of animals affected with ethanol induced gastric ulceration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer Index</th>
<th>Percentage of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>----</td>
<td>100</td>
</tr>
<tr>
<td>EtOH 80% 1 ml Control</td>
<td>4.52 ± 0.71</td>
<td>100</td>
</tr>
<tr>
<td>EtOH 80% 1ml +200 mg/kg P. viscida</td>
<td>1.61 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.38</td>
</tr>
<tr>
<td>EtOH 80% 1ml +400 mg/kg P. viscida</td>
<td>1.49 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.03</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01,(b)p<0.05 as compared to Control.

Table 6.3.2 Effect of *P. viscida* extract on SOD, GPx, GSH, catalase and MDA levels in liver tissues against ethanol induced gastric ulceration

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
<th>Catalase (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.89 ± 0.02</td>
<td>7.61 ± 0.89</td>
<td>5.48 ± 0.48</td>
<td>0.07 ± 0.002</td>
<td>4.17 ± 0.12</td>
</tr>
<tr>
<td>Control</td>
<td>0.38 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.82 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.02 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.02 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. viscida</em> 200mg/kg</td>
<td>0.59 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.62 ± 0.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.26 ± 0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.77 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. viscida</em> 400mg/kg</td>
<td>0.72 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.27 ± 0.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.83 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.10 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.48 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; for six animals in each group; (a)p<0.01, (b)p<0.05 as compared to normal. (c)p<0.01,(d)p<0.05 as compared to control.
Histopathological analysis of stomach samples showed increased level of damage to gastric mucosal epithelium. There were severe alterations in the histological architecture. This can be evident from the increased level of haemorrhage, necrosis, erosion and congestion of gastric mucosa. Several instances of gastric lesions in the form of gastric pits were found. The anomalies were found significantly reduced in animals treated with *P. viscida* extracts.