HEPATOPROTECTIVE EFFECTS OF PHYLLANTHUS VASUKII
PARTHIPAN ET AL. (PHYLLANTHACEAE) AGAINST CCL\textsubscript{4}
INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT
The aim of the present study is to investigate the hepatoprotective activity of Phyllanthus vasukii ethanol root extract against Carbon tetrachloride (CCL\textsubscript{4}) intoxicated rats, which showed significant elevation in serum enzymes, bilirubin and lipid peroxidation of the liver tissues, SGOT, SGPT and ALP and reduction in body weight, serum total protein, SOD, CAT, GRD, GSH and GPx activity. Treatment with ethanol extract of P. vasukii plant altered the above parameters to the levels of near normal. All the above results were comparable with the standard drug silymarin (100 mg kg\textsuperscript{-1}) treated group. Thus the present study ascertains that the ethanol root extract of P. vasukii possesses significant hepatoprotective activity.

KEYWORDS: Hepatoprotective activity, Carbon tetrachloride (CCL\textsubscript{4}), serum enzymes, silymarin.

INTRODUCTION
The plant Phyllanthus vasukii belongs to the family Phyllanthaceae. The genus Phyllanthus is large and distributed widely in tropical and subtropical countries of the world. It has been in use as herbal medicine for a long time in China, India, Brazil and South-East Asian nations. In China herbal decoctions are applied for the treatment of hepatitis B, hypertension, dropsy and throat sore (Xia, 1997). The Phyllanthus species are employed by the local people of Thailand, Latin America and Africa to cure jaundice, renal calculi and malaria etc.
(Poompache and Chudapongse, 2011; Moreira et al., 2013; Omulolokoli et al., 1997). The fruits of *P. emblica* was used as a tonic in Indian Ayurveda and often used to cure liver disease (Adil et al., 2010; Nain et al., 2012). *P. emblica* is one of the constituents of Triphala in India. The fruits of bhuiamiki, a combination of five *Phyllanthus* species are a therapy in urinary diseases. The leaves of *P. polyphyllus*, also called as sirunelli are used as remedies for diabetes, jaundice, wound, fever and inflammation. In Chinese Traditional Medicine (CTM), *P. urinaria* is employed to treat jaundice, enteritis, diarrhoea, dropsy and to cure hepatitis B. More than several hundreds of phytoconstituents were reported from different species of *Phyllanthus*, which mainly constitute lignins, triterpenoids, flavonoids and tannins.

**MATERIALS AND METHODS**

**Plant material**

Roots of plant *Phyllanthus vasukii* was collected from *P. vellore*, Namakkal District, Eastern Ghats, Tamilnadu, India. The plant was described by Parthiban et al. (2017). A voucher specimen was deposited at the Herbarium of Botany Department, Bharathiar University, Coimbatore, India.

**Acute toxicity study**

Normal healthy wistar male albino rats weighing 180-240 g were purchased from the animal house of Kongunadu Arts and Science College, Coimbatore, India. They were kept in well ventilated room of the animal house at 25 ± 2°C and relative humidity of 42-55%, light and dark cycles of 12:12 h respectively for one week before and during the experiments. All the rats were provided with standard pellet diet from Gulmohur brand, M/s Hindustan Lever Ltd., Mumbai, India and water ad libitum and food was withdrawn 18-24 h before the experiment. All the experiments were performed according to OECD - 423 guidelines. The Institutional animal ethical clearance was obtained for this experiment (01/2016/IAEC/KASC).

The hepatoprotective effect of the ethanol extract and antioxidant activity of the root of *P. vasukii* was studied using the CCl₄-induced hepatotoxicity test. The acute toxicity test in rats showed that the root extract could be classified as non-toxic since, a dose of 2000 mg kg⁻¹ b.wt. did not cause mortality.

**Experimental Design**

Rats were divided into six groups of six rats in each group. Group I (control) rats were administered a single daily dose of saline 2.5 mL kg⁻¹ body weight, p.o. Group II received
carbon tetra chloride (CCl₄) 2.5 mL kg⁻¹ body weight i.p. Test groups from group III to V were administered orally 100, 200 and 400 mg kg⁻¹ body weight of ethanol extract of *Phllanthus vasukii* respectively, in the form of aqueous suspension once daily. Group VI received Silymarin, the hepatoprotective drug (Sigma grade), at a dose of 100 mg kg⁻¹, p.o., along with CCl₄. The experimental duration was 14 days. CCl₄ was administered as 30% solution for every half an hour. Rats were sacrificed 48 h after the last injection. Blood was collected, allowed to clot and serum separated. Liver was dissected out and further used for biochemical studies.

**Biochemical Determinations**

The biochemical parameters like serum total protein (Lowry *et al*., 1951), albumin and globulin (Wolfson, 1948), serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), Serum alkaline phosphatase (ALP) (King and Armstrong, 1934), Serum bilirubin (Mulloy and Evelyn, 1937) were determined. The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. γ-Glutamyl transferase (γ-GT) was estimated by the method of Szasz (1969). Liver homogenates (10% w/v) were prepared in ice cold 10 mM tris buffer (pH 7.4). Enzymatic antioxidants superoxide dismutase (SOD) (Das *et al*., 2000), Catalase (Sinha, 1972) and non-enzymatic antioxidant glutathione peroxidase (GPx) (Beutler *et al*., 1984), glutathione reductase (GRD) (Goldberg and Spooner, 1983) and reduced glutathione (GSH) (Moran *et al*., 1979) were also assayed in liver homogenates.

**Statistical Analysis**

The data were expressed as the mean ± S.E.M. the difference among the mean has been analysed by one way ANOVA. *p*<0.001, *p*<0.01 and *p*<0.05 were considered as statistically significant using SPSS software.

**RESULTS**

The acute toxicity test showed that the ethanol extract of *P. vasukii* was non-toxic since a dose higher than the limit did not show toxic symptoms or mortality upto 2000 mg kg⁻¹ b.wt. The results of hepatoprotective effects of ethanol root extract of *P. vasukii* on CCl₄ intoxicated rats are presented in Table 1. The differences between the initial body weight of the liver damaged control Group I showed loss of body weight by 9.55%, whereas the liver damaged Group II treated with extracts at the 100, 200 and 400 mg kg⁻¹ b.wt. showed loss of weight by 3.18 and 3.34% respectively. Meanwhile, at a dose of 400 mg kg⁻¹ b.wt. showed
increase in the body weight. Ethanol root extract of *P. vasukii* at the doses of 100, 200 and 400 mg kg\(^{-1}\) b.wt. significantly reduced the total protein and albumin to almost normal level after CCl\(_4\) induced hepatotoxicity. Thus the *P. vasukii* extract restored liver weight. The total protein and albumin levels were significantly (*p* < 0.01) decreased to 6.31 g dL\(^{-1}\) and 3.68 g dL\(^{-1}\) in CCl\(_4\) intoxicated rats from the levels of 8.24 g dL\(^{-1}\) and 4.5 g dL\(^{-1}\) respectively in the normal Group I.

In CCl\(_4\) induced liver damage model, the levels of the liver marker enzymes SGOT, SGPT and ALP were significantly increased (Table 2). After that the intoxicated rats were treated with various doses of *P. vasukii* root ethanol extract such as 100, 200 and 400 mg kg\(^{-1}\) b.wt. Only the higher dose of extract has improved the altered serum enzyme levels of SGOT, SGPT and ALP in all three hepatotoxicant - treated groups as compared to the respective hepatotoxin treated concurrent control.

The levels of total bilirubin, conjugated, unconjugated and γ–glutamyl transferase were also found to be significantly elevated in CCl\(_4\) induced hepatotoxicity (Table 3). Treatment of intoxicated rats with ethanol root extract of *P. vasukii* improved altered all three bilirubin levels and γ–glutamyl transferase as compared to the respective CCl\(_4\) (or) hepatotoxicant - treated Group II and control Group III which was treated with a standard drug, Silymarin.

Table 4 shows effect of root *P. vasukii* extract on the levels of mitochondrial lipid peroxidation, cellular antioxidant enzymes such as SOD, CAT and GPx and non-enzymatic antioxidant GSH activities in CCl\(_4\) intoxicated rats and compared with control. A significant reduction in the activity of scavenging mitochondrial enzymes and non-enzymatic antioxidants with an increase in the extent of lipid peroxidation in the liver damaged control rats were observed. These adverse changes were reversed to near normal values in liver damaged rats treated with root extracts of *P. vasukii* at different doses viz. 100, 200 and 400 mg kg\(^{-1}\) b.wt. respectively. The results were well compared with Silymarin, a standard drug.

The root extract at the dose of 100, 200 and 400 mg kg\(^{-1}\) was administered orally once daily for 14 days. The substantially elevated serum enzymatic activities of serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), conjugated and unconjugated bilirubin due to carbon tetrachloride (CCl\(_4\)) treatment were dose dependently restored towards normalization. Meanwhile the decreased activities of antioxidant enzymes LPO, GPx, SOD, CAT and GSH
were also restored towards normalization. In addition, the root extract also significantly prevented the elevation of marker enzymes and depletion of reduced glutathione content in the liver of CCl₄ intoxicated rats in a dose dependent manner.

Table 1. Effect of root ethanolic extract on the body weight of in the normal, liver damaged and drug treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Initial Body weight (Gm)</th>
<th>Final Body weight (Gm)</th>
<th>Mean weight Gain (G↑)/ loss (G↓) (Gm)</th>
<th>% of Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.9% Saline</td>
<td>204.16±6.54</td>
<td>216.50±7.30</td>
<td>12.34</td>
<td>6.04</td>
</tr>
<tr>
<td>Liver damaged Control</td>
<td>0.9% Saline</td>
<td>216.80±7.60</td>
<td>196.16±5.18*</td>
<td>20.64</td>
<td>9.52</td>
</tr>
<tr>
<td>Liver Damaged Animal + ROOT Extract</td>
<td>100(mg kg⁻¹)</td>
<td>211.50±5.80</td>
<td>204.80±4.50ns</td>
<td>6.70</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td>200(mg kg⁻¹)</td>
<td>208.16±4.18</td>
<td>201.20±3.60ns</td>
<td>6.96</td>
<td>3.34</td>
</tr>
<tr>
<td></td>
<td>400(mg kg⁻¹)</td>
<td>215.60±7.30</td>
<td>221.60±5.75ns</td>
<td>6.00</td>
<td>2.78</td>
</tr>
<tr>
<td>Standard Drug (Silymarin)</td>
<td>100(mg kg⁻¹)</td>
<td>204.80±6.70</td>
<td>219.50±4.90*</td>
<td>14.70</td>
<td>7.18</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05 as compared with Normal Control to liver damaged control.

**Group I**: Rats received normal saline was served as a normal control.

**Group II**: CCl₄ hepatic toxicity induced control: Rats received 2.5 mg kg⁻¹ body weight of CCl₄ for 14 days.

**Group III**: Liver injured rats received ethanol extract of root of *P. vasukii* at the dose of 100 mg kg⁻¹ body weight for 14 days.

**Group IV**: Liver injured rats received ethanol extract of root of *P. vasukii* at the dose of 200 mg kg⁻¹ body weight for 14 days.

**Group V**: Liver injured rats received ethanol extract of root of *P. vasukii* at the dose of 400 mg kg⁻¹ body weight for 14 days.

**Group VI**: Liver injured rats received standard drug silymarin at the dose of 100 mg kg⁻¹ bodyweight for 14 days.
Table 2. Effect of root ethanolic extracts of *P. vasukii* 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>Parameters</th>
<th>Dose</th>
<th>T Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G Ratio</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control I</td>
<td></td>
<td>0.9% Saline</td>
<td>8.24±1.12</td>
<td>4.51±0.98</td>
<td>3.73±0.24</td>
<td>1.2:1</td>
<td>13.46±0.18</td>
<td>16.31±0.27</td>
<td>163.16±3.18</td>
</tr>
<tr>
<td>Liver damaged Control</td>
<td></td>
<td>0.9% Saline</td>
<td>6.31±0.65*</td>
<td>3.68±0.13*</td>
<td>2.63±0.13*</td>
<td>1.4:1</td>
<td>56.27±1.68**</td>
<td>64.16±2.18**</td>
<td>231.80±6.56**</td>
</tr>
<tr>
<td>Liver Damaged Animal+ root</td>
<td></td>
<td>100(mg kg⁻¹)</td>
<td>7.54±1.31</td>
<td>4.14±0.26</td>
<td>3.40±0.22</td>
<td>1.2:1</td>
<td>31.60±1.13*</td>
<td>28.26±1.31*</td>
<td>193.10±7.31*</td>
</tr>
<tr>
<td>Extract</td>
<td></td>
<td>200(mg kg⁻¹)</td>
<td>8.11±1.56a</td>
<td>4.36±0.35</td>
<td>3.75±0.16</td>
<td>1.2:1</td>
<td>18.21±1.56a</td>
<td>21.15±1.3a</td>
<td>181.56±3.16a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400(mg kg⁻¹)</td>
<td>8.46±1.37a</td>
<td>4.51±0.73a</td>
<td>3.95±0.11</td>
<td>1.1:1</td>
<td>14.96±1.27aa</td>
<td>18.13±1.02aa</td>
<td>174.16±4.27a</td>
</tr>
<tr>
<td>Standard Drug (Silymarin)</td>
<td></td>
<td>100(mg kg⁻¹)</td>
<td>8.21±1.16a</td>
<td>4.38±0.67ns</td>
<td>3.83±0.35a</td>
<td>1.1:1</td>
<td>12.96±1.13aa</td>
<td>14.16±1.36aa</td>
<td>169.22±3.86aa</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05; **P <0.01 as compared with Normal Control to liver damaged control :a P<0.05 ;aa P<0.01 as compared with liver damaged control to drug treated animal NS: not significant.
Table 3. Effect of root extracts of *P. vasukii* on serum Total, conjugated, unconjugated bilirubin and GGTP levels in the normal control, liver injured and drug treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Dose</th>
<th>Total Bilirubin (Mg/dl)</th>
<th>Conjugated (Mg/dl)</th>
<th>Unconjugated (Mg/dl)</th>
<th>GGTP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control I</td>
<td></td>
<td>0.9% Saline</td>
<td>0.81±0.05</td>
<td>0.21±0.03</td>
<td>0.60±0.021</td>
<td>8.16±0.65</td>
</tr>
<tr>
<td>Liver damaged Control</td>
<td></td>
<td>0.9% Saline</td>
<td>2.65±0.16**</td>
<td>1.14±0.31**</td>
<td>1.51±0.076**</td>
<td>31.65±1.31**</td>
</tr>
<tr>
<td>Liver Damaged Animal+ root Extract</td>
<td></td>
<td>100(mg kg⁻¹)</td>
<td>1.31±0.21*</td>
<td>0.41±0.05ns</td>
<td>0.90±0.034ns</td>
<td>24.16±1.82**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200(mg kg⁻¹)</td>
<td>1.08±0.14a</td>
<td>0.32±0.07a</td>
<td>0.76±0.012</td>
<td>16.26±0.75a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400(mg kg⁻¹)</td>
<td>0.92±0.07aa</td>
<td>0.26±0.05aa</td>
<td>0.66±0.035a</td>
<td>11.22±0.56aa</td>
</tr>
<tr>
<td>Standard Drug</td>
<td></td>
<td>Silymarin</td>
<td>100(mg kg⁻¹)</td>
<td>0.87±0.04aa</td>
<td>0.27±0.04aa</td>
<td>0.60±0.018a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Standard Drug</td>
<td></td>
<td></td>
<td></td>
<td>9.86±0.75aa</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05; **P <0.01; ***P <0.001 as compared with Normal Control to liver damaged control: a P<0.05; aa P<0.01 - as compared with liver damaged control to drug treated animal NS: not significant.
Table 4. Effect of root ethanolic extracts of *P. vasukii* 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</th>
<th>LPO (n mole of MDA/mg protien)</th>
<th>GPX (u/mgProtien)</th>
<th>GRD (u/mg)</th>
<th>SOD (u/mg)</th>
<th>CAT (u/mg)</th>
<th>GSH (u/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control I</td>
<td>0.9% Saline</td>
<td>2.523±0.016</td>
<td>4.211±0.031</td>
<td>0.431±0.026</td>
<td>0.316±0.015</td>
<td>3.72±0.036</td>
<td>33.16±0.16</td>
</tr>
<tr>
<td>Liver damaged Control</td>
<td>0.9% Saline</td>
<td>6.316±0.062**</td>
<td>2.081±0.036**</td>
<td>0.254±0.021**</td>
<td>0.154±0.026**</td>
<td>2.08±0.013**</td>
<td>16.16±0.21**</td>
</tr>
<tr>
<td>Liver Damaged Animal</td>
<td>100(mg kg⁻¹)</td>
<td>4.115±0.016*</td>
<td>3.116±0.017ns</td>
<td>0.296±0.036*</td>
<td>0.193±0.0188</td>
<td>2.76±0.026ns</td>
<td>19.31±0.26*</td>
</tr>
<tr>
<td></td>
<td>200(mg kg⁻¹)</td>
<td>3.541±0.026ns</td>
<td>3.618±0.075a</td>
<td>0.351±0.073a</td>
<td>0.271±0.013ns</td>
<td>3.18±0.018a</td>
<td>23.18±0.35ns</td>
</tr>
<tr>
<td></td>
<td>400( mg kg⁻³)</td>
<td>2.864±0.018a</td>
<td>4.136±0.084aa</td>
<td>0.418±0.054aa</td>
<td>0.326±0.015a</td>
<td>3.65±0.036a</td>
<td>29.36±0.76aa</td>
</tr>
<tr>
<td>Standard Drug</td>
<td>100(mgkg⁻¹)</td>
<td>2.656±0.036a</td>
<td>4.118±0.076aa</td>
<td>0.403±0.018aa</td>
<td>0.318±0.054a</td>
<td>3.51±0.027aa</td>
<td>31.84±0.54aa</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05;**P <0.01 as compared with Normal Control to liver damaged control: a P<0.05 ;aa P<0.01 as compared with liver damaged control to drug treated animal NS: not significant.
DISCUSSION

The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxic agent is the index of its protective effect. The present study indicated that a significant decrease in serum protein concentration in liver injured rats while *P. vasukii* extract treated groups showed a significant increase in the protein levels in the serum which indicates the control over the breakdown of body protein by *P. vasukii* extract and Silymarin at the concentration of 100 mg kg\(^{-1}\) b.wt. This is in accordance with the observation of Andallu and Varadacharyulu (2002).

The hepatoprotective effects of *P. vasukii* on CCl\(_4\) in toxicated rats are represented in the tables. The CCl\(_4\) treated control the serum, SGOT, SGPT and ALP levels were increased with contrast the groups treated with 100 and 200 mg kg\(^{-1}\) b.wt. of *P. vasukii* extract decrease significantly the elevated levels of SGOT, SGPT and ALP towards normalization. The treatment with *P. vasukii* extract 400 mg kg\(^{-1}\) b.wt. showed highly significant activity which is almost comparable to the group treated with silymarin, a potent Hepatoprotective drug ended as reference standard standard. The present study showed, the *P. vasukii* extract possess hepatoprotective activity as evidence by a significant inhibition in the elevated levels of serum enzyme activities induced by CCl\(_4\). Hepatotoxic compounds are known to cause marked elevation in enzyme activities. In the present study, the treatment with *P. vasukii* extract attenuated the increases the activities of SGOT, SGPT and ALP produced by CCl\(_4\) indicating that *P. vasukii* extract protects liver injury induced by CCl\(_4\) towards normalization.

Serum ALP and bilirubin are also associated with liver cell damage. The ALP activity and serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury (Girish *et al.*, 2009). CCl\(_4\) induction caused a significant elevation of enzyme ALP and the bilirubin level has been attributed to the damage. Structural integrity of liver, because they are cytoplasmic in location and related into circulation after cellular damages indicating development of hepatotoxicity (Gutierrezl and Solis, 2009). The administration of *P. vasukii* extract have prevented the increased serum marker enzyme ALP and bilirubin level. This is in agreement with the commonly accepted view that serum levels of ALP return to normal with the healing of hepatic parenchyma and the regeneration of hepatocysts (Thabrew and Joice, 1987).
An increase in LPO indicates serious damage to cell membranes, inhibition of several enzymes and cellular function (Thirunavukarasu et al., 2001). In the present study, an increase in the levels of mitochondrial LPO was found in the CCl₄ induced rats. It is well known that SOD, CAT, GPx and GRD play an important role as protective enzyme against free radical formation in tissues (Oberly and Buettner, 1974). Several investigations reported that the reduced activities of SOD, CAT, GPx and GRD in hepatoprotective rats due to down regulation of the SOD and CAT gene induced by free radical and also by certain humoral factors (Anderson et al., 1994 and Slaga, 1995). The present study indicates a reduction in the activities of mitochondrial SOD, CAT, GPx and GRD. Reduced glutathione, which is a substrate for glutathione peroxidase neutralize hydroxyl radicals and singlet oxygen. Since it is present in high concentration in the cells, it protects cells from free radical attack (Knotsky, 1990).

SOD, catalase, GPx and GRD activities were significantly decreased in CCl₄ treated rats compared to normal controls. However activities of these enzymes were a near normal in CCl₄ treated rats with P. vasukii treatment. Rats treated with CCl₄ showed control double necrosis with lymphocytic and fatty infiltration most of the mammals have an effective mechanism to prevent and neutralize the free radical induced damage, which is accomplished by a set of endogenous enzymes, such as SOD, catalase, GPx and GRD. As the balance between reactive oxygen species production and antioxidant defenses is lost, the ‘Oxidative stress’ results which through a series of events deregulates the cellular function, leading to various pathological conditions. An antioxidant compound might contribute partial, total elevations of such damage. SOD, CAT, GPx and GRD constitute a mutually supportive team of defense against reactive oxygen species. In our study the decline in the activities of these enzymes in CCl₄ administrated rats and their reversal to near normal in CCl₄ intoxicated rats treated with P. vasukii extract indicates that lipid peroxidative and oxidative stress elicited by CCl₄ in toxication is nullified due to the effect of P. vasukii treatment. This observation is in agreement with, hepatoprotective and antioxidant activities reported in Boehmeria ravea (Lin et al., 1995).

In the present study, intraperitonial administration of CCl₄ plus oral treatment has markedly elevated the ALP, GPT and GOT activities in the serum and liver tissues of the control group of animals. In the present study P. vasukii treatment enhanced mitochondrial enzymatic and
non-enzymatic antioxidants and suppressed lipid peroxidation in during and post inhibition supplemented animals.

CONCLUSION
The present study has demonstrated that ethanol root extract of *P. vasukii* has hepatoprotective activity against CCl₄ induced hepatotoxicity in rats. It is suggested that, phytochemical constituents in *P. vasukii* plant play on important role an antioxidant for prevention of oxidative hepatic damage. The enhanced levels of antioxidant enzymes and reduced amount of lipid peroxides are suggested to be the major mechanism of *P. vasukii* ethanol extract in prevents the development of liver damage induced by CCl₄.

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REFERENCES
Analysis, VCH Weinhem, Germany, 1983; 258-265.
10. King EJ and Armstrong AR. Determination of serum and bile phosphatase activity, 
Cannadian Medical Association Journal, 1934; 31: 56-63.
51: 299-304.
12. Lin CC, Lin WC, Chang CH, Namba T. Anti-inflammatory and hepatoprotective effects 
13. Lowry OH, Rosenbrough NJ, Farr AL. and Randall RJ. Protein measurement with the 
Reitman S. and Frankel SA. Colorimetric method for the determination of serum glutamic 
14. Malloy HT and Evelyn KA. The determination of bilirubin with the photometric 
15. Moran MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and 
glutathione Transferase activities in rat lung and liver. Biochemicaet Biophysica ACTA, 
1979; 582-67.
16. Moreira J, Klein-Júnior LC, Cechinel Filho V and De Camposuzzi F. “Anti-
hyperalgesic activity of corilagin, a tannin isolated from Phyllanthusniruri L. 
17. Nain P, Saini V, Sharma S and Nain J. “Antidiabetic and antioxidant potential of 
35: 1141-149.
19. Okhawa H, Qohishi N, Yagi K. Assay of lipid peroxides in animal tissues by 
20. Omulokoli E, Khan B and. Chhabra SC. “Antiplasmodial activity of four Kenyan 
medicinal plants,” Journal of Ethnopharmacology, 1997; 56(2): 133–137.
21. Poompachee K and Chudapongse N. “Comparison of the antioxidant and cytotoxic 
activities of Phyllanthusvirgatus and Phyllanthusamarusextracts,” Medical Principles 


