Anti-toxicity and antioxidant effects of methanolic extract of *Paullina pinnata* on carbon tetrachloride (CCl₄) induced tissue damage in wistar rats

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ABSTRACT

The present study was carried out to evaluate the anti-toxicity and antioxidant effects of methanolic extract of *Paullina pinnata* on carbon tetrachloride (CCl₄) induced tissue damage in wistar rats. Experimental rats were randomly divided into five groups of five rats each. Group 1: served as the control rats (olive oil + DMSO), Group 2: were induced with CCl₄ and olive oil in ratio 1:1; 2ml/kg bw only by intraperitoneal administration. Group 3: were administered with Silymarin at 50 mg/kg bw, Group 4 and 5: were induced with CCl₄ and treated with extract of *P. pinnata* 500 and 1000 mg/kg b.wt respectively). Biochemical, hematological parameters and histo architecture of the liver and kidney as well as antioxidants activities were assayed for using standard procedure. The group treated with the methanolic extract of *P. pinnata* were able to reduced elevated level of hematological values and bio markers enzymes when compared to CCl₄ untreated group in the liver, kidney and serum. It also significantly increase the antioxidant activities. The histopathological studies in the liver and kidney of rats also supported that *P. pinnata* extract markedly reduced the toxicity of CCl₄ and preserved the histo architecture of the kidney and liver tissue to normal and near normal respectively. The results suggested that methanolic extract of *P. pinnata* could palliate the liver injuries perhaps by its antioxidative effect, hence eliminating the deleterious effect of toxic metabolites from the CCl₄.

Keywords: Antioxidant activities, Biochemical and Hematological parameters, Methanolic extract, *Paullinna pinnata*, Toxicity

1. INTRODUCTION

Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. Large number of medicinal plants has been investigated for their antioxidant properties. [1] hypothesized that natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress. Reactive oxygen species (ROS) exert oxidative damaging effects by reacting with nearly every molecules found in living cells.
including protein, lipid, amino acids and DNA, if excess ROS are not eliminated by antioxidant system. They play important roles in aging and in the pathogenesis of age related disorders such as cancer, hypertension, atherogenesis, Alzheimers disease and Parkinsons disease.

Previous study have been suggested that carbon tetrachloride (CCl₄) has been used extensively to study hepatotoxicity in animal models by initiating lipid peroxidation, thereby causing injuries to kidney, heart, testis and brain [2] in addition to liver pathogenesis. Effects such as fatty degeneration, fibrosis, hepatocellular apoptosis and carcinogenicity have been associated with CCl₄ toxicity. To increase antioxidant activity in our body and that could be achieved by consumption of vegetables, fruits or edible plants is the most practical way to fight degenerative diseases. There is an increasing interest in natural antioxidants e.g. polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases. Previous studies suggested that different parts such as seeds, leaves and bark of stem and root known to contain substantial amounts of phytoconstituents such as phenolics, flavonoids, tannins having the ability to inhibit the free radicals that are excessively produced, hence can act as antioxidants [4,5].

*Paullinia pinnata* (Sapindaceae) is a woody or sub-woody climber at dump site and stream banks of the forest and jungle regrowth in the savannah zone. The common vernacular names in Nigeria include *goron dorina*; *yatsa biyar* (Hausa) *edefina*; *aliligo* (Igbo) and *ogbe-okiye* (Yoruba). In traditional medicine, various parts of *P. pinnata* are used for treating various diseases. In South West Nigeria, Fred-Jaiyesimi and Anthony, 2011 [6] reported the use leaf juice of *P. pinnata* for the treatment of sore throat. An infusion is used for fever while the roots are used for the treatment of leprosy, jaundice, snake bites, nausea and vomiting [7]. The whole plant is used in Ghana to treat dysentery. The Previous phytochemical investigations of *P. pinnata* have shown the presence of triterpene, saponins, cardiotonic catechol and tannins [8]. Two flavone glycosides, diosmetin-7-O-(2''-O-ß-D-apiofurasosyl-6''-acetyl-ß-D-glucopyranoside) and tricetin-4''-O-methyl-7-O-(2''-O-ß-D-apiofurasosyl 6''-acetylß-D-glucopyranoside), have also been isolated from the leaves [9]. Miemanang et al., [10] also reported the isolation of paullinoside A, ß-sitosterol and ß-amyrin from the leaves, while Annan and Houghton, Isolated paullilupeone and paullilupeol from the roots.

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant material

Fresh leaf of *Pauliana pinnata* was collected from Ikere Ekiti in Ekiti State. The plant were identified and authenticated at Ekiti state University Ado Ekiti in the department of botany by Mr Omotayo F.O with UHAE NUMBER 2015/26.

### 2.2. Extracts preparation

The leaves were air dried and grounded to fine powdered form and used for solvent extraction. For sample preparation, 500g of dried sample were extracted with 95% methanol at 25°C for 48h and concentrated using a rotary evaporator under reduced pressure at 40°C to yield 29.6% (w/w) of the initial raw material.

### 2.3. Experimental groupings

Animals and treatment studies were carried out using male Sprague Dawley rats weighing 180±10g. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions. They had free access to standard diet and fresh water ad libitum. For acute toxicity studies, 30 rats were divided randomly into 5 groups, each comprising 6 animals. Group (I) the controls received only vehicles; olive oil (0.5 ml/kg bwt) and DMSO (0.5 ml/kg bwt) and fed with a normal diet for 7 days. Group II (induction controls) received single dose of CCl₄ (CCl₂ + Olive oil in 1:1 ratio; 2 ml/kg bwt; i.p). Group III received silymarin (50mg/kg, oral), (Will and Asha., 2006) as the standard reference drug, once in a day for 7 days respectively along with the intraperitoneal administration of CCl₄ on day 1. Group IV and V received only PPM (500 and 1000 mg/kg; oral administration) once in a day for 7 days. At the end of 7 days, 24h of the last treatment, all the animals were anesthetized in an ether chamber and sacrificed. The liver and kidney was removed and placed at 4°C after perfusion with ice cold saline.

### 2.4. Blood Sample Collection and Analysis

Blood samples for haematological analysis were collected from all the rats through the retro-orbital venous plexus under ether-induced anaesthesia, into heparinized tubes while the sample for serum biochemistry was collected into plain tubes. From the blood samples collected, packed cell volume (PCV) was determined by micro haematocrit method, haemoglobin concentration (Hb) by...
cyanmethaemoglobin method while the red blood cells (RBC) and white blood cells (WBC) were counted using haemocytometer. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of PCV, Hb and RBC count. Blood smear were then stained with Giemsa to obtain the differential leucocyte counts. Blood was collected from the jugular vein of the animals under deep anaesthesia with chloroform for biochemical analysis. The liver and kidney tissues were harvested for histological examination. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, uric acid, albumin, and total protein were determined using Randox kit.

2.5. Determination of in vivo antioxidant activity

10% homogenate of liver tissue was prepared in 100 mM KH2PO4 buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the following experiments as described below.

2.6. Measurement of lipid peroxidation (LPO)

The assay for membrane lipid peroxidation was done with some modification. The reaction mixture in a total volume of 3.0ml contained 1.0ml tissue homogenate, 1.0ml of TCA 10%, and 1.0ml TBA0.6%. The test tube was placed in boiling water bath for about 45min. The test tube was shifted to ice bath and then centrifuged at 2500× g for 10min. The amount of malondialdehyde form in each of the samples was observed by measuring the optical density of the supernatant at 532nm. The result were expressed as the nmol MDA formed/gram tissue by using a molar extinction coefficient of 1.56×10^5 M^-1 cm^-1. With the help of formula

$$LPO = \frac{\text{vol. of assay} \times \Delta O.D \times 10^9}{1.5 \times 10^5 \times \text{vol. of conjugate enzyme} \times \text{protein mg}}$$

2.7. Measurement of reduced glutathione (GSH) level

The GSH content in colon was determined by the method of Jollow, et al., 1974 [11] in which 1.0ml of PMS fraction (10%) was mixed with 1.0ml of sulphosalicylic acid (4%). The sample were incubated at 4°C for at least 1h and then subjected to centrifugation at 1200g for 15min at 4°C. The assay mixture contained 0.4ml filtered aliquot, 2.2ml phosphate buffer (0.1M, pH7.8) and 0.4ml DTNB (10Mm) in total volume of 3.0ml. The yellow colour developed was read immediately at 412nm on spectrophotometer. The GSH content was calculated as μmol DTNB conjugate formed/gram tissue using molar extinction coefficient of 13.6×10^3 M^-1 cm^-1. With the help of formula

$$\text{GSH} = \frac{\Delta O.D \times \text{vol. of assay} \times 100}{1.36 \times \text{vol. of conjugate enzyme} \times \text{protein mg}}$$

2.8. Measurement of glutathione-S-transferase (GST) activity

The reaction mixture consisted of 2.4ml phosphate buffer (0.1M, pH 6.5), 0.2ml reduced glutathione (1.0mM), 0.2ml CDNB (1.0Mm) and 0.2ml of cytosolic fraction in a total volume of 3.0ml. The changes in absorbance were recorded at 340nm and the enzyme activity was calculated as μmol CDNB conjugate formed/minute/mg protein using a molar extinction coefficient of 9.6×10^3 M^-1 cm^-1. With formula

$$\text{GST} = \frac{\Delta O.D \times \text{vol. of assay} \times 100}{0.081 \times \text{vol. of conjugate enzyme} \times \text{protein (mg)}}$$

2.9. Measurement of glutathione reductase (GR) activity

The assay system consisted of 1.6ml phosphate buffer (0.1M, pH 7.6), 0.1ml EDTA (0.5mM), 0.05ml oxidized glutathione, 0.1ml NADPH (0.1mM) and 0.1ml of 10% PMS in a total volume of 2.0ml. The enzyme activity was assessed at 25°C by measuring the disappearance of NADPH at 340nm and was calculated as nmol NADPH oxidized/minute/mg protein using molar extinction coefficient of 6.22×10^3 M^-1 cm^-1. With the help of formula

$$\text{GR} = \frac{\Delta O.D \times \text{vol. of assay} \times 100}{6.22 \times \text{vol. of enzyme} \times \text{protein mg}}$$

2.10. Measurement of catalase (CAT) activity

In brief, the assay mixture consist of 2.0ml phosphate buffer (0.1M, pH 7.4), 0.95ml hydrogen peroxide (0.019M) and 0.05ml of PMS (10%) in a final volume of 3.0ml. Changes in absorbance were recorded at 240nm. The catalase activity was calculated in terms of nmol H2O2 consumed/minute/mg protein. With the help of formula

$$\text{Catalase} = \frac{\Delta O.D \times \text{vol. of assay} \times 0.081}{0.081 \times \text{vol. of conjugate enzyme} \times \text{protein mg}}$$
2.11. Histological analysis

Small liver specimens were extracted, placed in formalin solution, and processed routinely by embedding in paraffin. Tissue sections (4-5 µm) were stained with haematoxylin-eosin for general features and examined under light microscope (Celestron).

2.12. Statistical analysis

Data are presented as mean ± standard deviation. For establishing significant differences between groups, data were analyzed by the One-way ANOVA of variance followed by the Tukey post hoc test. Values were considered statistically significant if P value is less than 0.05 (p < 0.05), using sigmastat 2.0 for Window (soft stat, jan Raff, CA).

3. RESULTS

3.1. Biochemical analysis

Phase-II detoxifying enzymes present in liver cell endoplasmic reticulum is the one accountable for the activation of CCl4 to form trichloromethyl and peroxytrichloromethyl free radicals. These can react covalently with several biomolecules such as nucleic acid, lipid and protein resulting in cellular membrane deterioration, increased absorbity and trickle out of cytoplasmic ALT, ALP and AST. Serum Levels of ALT, AST and ALP should serve as hepatotoxicity indicator. Indeed, CCl4 dispensation produced significant elevations of serum ALT, AST and ALP compared to the normal control group. However, treatment of rats with 500 and 1000mg/kg b.w. P. pinnata extract significantly decreased these serum biochemical indices as compared with the CCl4 treatment group in liver, serum and kidney homogenate. Also rats treated with 500 and 1000mg/kg b.w. P. pinnata extract significantly decreased albumin, uric acid and creatinine level as compared with the CCl4 untreated group. There is increase levels of TRIG in the CCl4 untreated animals compared to the control. This consequence was retained in the animal groups that were given P. pinnata extract when compared to both the control and CCl4 untreated groups. On the other hand, animals treated with P. pinnata show a significant decline in the level of total protein when compared to both the control and CCl4 untreated groups (Table 1)

3.2. Haematology

Modulatory activities of P. pinnata and the consequence of CCl4 administration on the haematological parameters of the wistar rats are shown in Table 2. There was a significant reduction (P<0.01) in the PCV, Hb concentration, and RBC values when compared with those of the CCl4 untreated group. MCV, MCH and MCHC shows no significant difference in the rats that were exposed to CCl4 untreated group, treated with P. pinnata extract and those of the normal control. The total WBC count reduced significantly (P<0.05) in all the groups exposed to CCl4 when compared to those of the extract treated group (500 and 1000mg/kg bw). No significant changes was observed neutrophil, eisonephil and monocyte counts in normal control, CCl4 induced, Silymarin treated and the groups exposed to 500 and 1000mg/kg of the extract while the lymphocyte counts was significantly higher (P<0.05) in the groups exposed to 500 and 1000mg/kg of the extract than those of CCl4 untreated group.

Antioxidant status in the blood serum, liver and kidney homogenate was estimated by determining the activities of catalase (CAT), thiobarbituric acid reactive substances (TBARS), glutathione reductase (GR), glutathione peroxidase (GPx) and reduced glutathione (GSH) levels. Increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems were one of characteristics of oxidative stress. The antioxidant activity of CAT, GR, GPx and GSH significantly decreased, while TBARS significantly increased in the CCl4 untreated group of rats. Administration of P. pinnata significantly decreased the elevated TBARS, and also significantly increased the reduced antioxidant enzyme activities. (Table 3).

4. DISCUSSION

A generalized reduction in the cellular elements in the blood (pancytopenia) was observed, that CCl4 administration produced as shown by microcytic hypochromic anaemia, thrombocytopenia and lymphopenia in the blood as proven by the reduction in the PCV and RBC with the exception of total WBC counts, although, there was lymphopenia. We believe that this reduction in the formed elements in the blood is stress induced because of the leucocytosis observed in these rats might not have been a result of significant increase in WBC production, but by the release of marginated neutrophils and other neutrophil pool into the circulation. Toxic chemicals and drugs are detoxified by one of the crucial organs in our body called the liver.
Table 1. Effect of Methanolic Extract of *Paullina pinnata* on Maker Enzyme Activities

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>UNTREATED</th>
<th>STANDARD</th>
<th>500mg/kg</th>
<th>1000mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Kidney</td>
<td>Serum</td>
<td>Liver</td>
<td>Kidney</td>
<td>Serum</td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
| TG     | 93.6±7.44 | 94.3±4.62 | 178±3.63 | -        | 190±22.8  | 65.4±20.5  | -        | 92.0±3.12 | 33.9±3.34 | -        | 59.5±3.26 | 54.4±2.18 | -        | 94.8±0.10  |<|>0.05 compared with control group of rats. *represent P <0.05 compared with control group of rats. "represent P <0.05 compared with standard treated group of rats.

Table 2. Effect of Methanolic Extract of *Paullina pinnata* on Hematological parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCV</th>
<th>HB</th>
<th>RBC</th>
<th>MCH</th>
<th>MCHC</th>
<th>MCV</th>
<th>N</th>
<th>L</th>
<th>E</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.0±1.73</td>
<td>15.0±0.577</td>
<td>7.50±0.289</td>
<td>7.43±1.16</td>
<td>20.0±0.001</td>
<td>20.0±0.010</td>
<td>60.0±0.0002</td>
<td>3.3±5.61</td>
<td>58.0±4.36</td>
<td>0.333±0.333</td>
</tr>
<tr>
<td>Untreated</td>
<td>24.0±11.6acdk</td>
<td>8.01±3.388d</td>
<td>4.00±1.93acdk</td>
<td>15.3±3.46de</td>
<td>20.0±0.0176</td>
<td>33.3±0.0433</td>
<td>40.0±10.0</td>
<td>13.3±4.41</td>
<td>79.0±3.21acde</td>
<td>--</td>
</tr>
<tr>
<td>Standard</td>
<td>41.3±1.86b</td>
<td>13.8±0.623</td>
<td>6.89±0.309b</td>
<td>7.50±1.22</td>
<td>20.0±0.0167</td>
<td>33.3±0.2777</td>
<td>60.0±0.0009</td>
<td>26.0±4.36</td>
<td>31.3±2.67b</td>
<td>0.333±0.333</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>44.3±2.33b</td>
<td>14.7±0.882b</td>
<td>7.39±0.389b</td>
<td>5.23±0.176b</td>
<td>19.8±0.167</td>
<td>33.1±0.278</td>
<td>60.0±0.0009</td>
<td>28.0±6.00</td>
<td>25.0±2.89ab</td>
<td>3.67±1.45</td>
</tr>
<tr>
<td>1000mg/kg</td>
<td>51.7±3.84b</td>
<td>17.2±1.30</td>
<td>8.44±0.484b</td>
<td>4.27±0.561b</td>
<td>20.0±0.0252</td>
<td>33.4±0.0524</td>
<td>60.0±0.0009</td>
<td>26.0±4.16</td>
<td>27.0±4.36ab</td>
<td>1.00±0.577</td>
</tr>
</tbody>
</table>

Results were expressed in Mean±S.E.M (n=5). *represent P <0.05 compared with control group of rats. "represent P <0.05 compared with CCl4 induced group of rat. "represent P <0.05 compared with standard treated group of rats.

Protective effect of *Paullina pinnata* against carbon tetrachloride (CCl4) induced tissue damage in wistar rats.
Protective effect of Paullina pinnata against carbon tetrachloride (CCl₄) induced tissue damage in wistar rats

Table 2. Effect of Methanolic Extract of Paullina pinnata on antioxidant parameters

<table>
<thead>
<tr>
<th>Control</th>
<th>Untreated</th>
<th>Standard 500mg/kg</th>
<th>Standard 1000mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Kidney</td>
<td>Serum</td>
<td>Liver</td>
</tr>
<tr>
<td>GPx</td>
<td>24.6±2.5</td>
<td>14.8±0.7</td>
<td>11.3±3.9</td>
</tr>
<tr>
<td>GR</td>
<td>108±3.65</td>
<td>100±2.96</td>
<td>60.9±1.35</td>
</tr>
<tr>
<td>LPO</td>
<td>8.31±0.7</td>
<td>11.8±2.2°</td>
<td>9.96±2.12°</td>
</tr>
<tr>
<td>CAT</td>
<td>2.27±0.1°</td>
<td>1.35±0.2°</td>
<td>1.29±0.10</td>
</tr>
<tr>
<td>GSH</td>
<td>128±9.05</td>
<td>108±7.87</td>
<td>72.4±3.64</td>
</tr>
</tbody>
</table>

Results were expressed in Mean± S.E.M (n=5). *represent P <0.05 compared with control group of rats. †represent P <0.05 compared with CCl₄ induced group of rat. ‡represent P <0.05 compared with standard treated group of rats.
It was reported that CCl₄ is suitable to cause lipid peroxidation in experimental animals within a few minutes after administration and it’s prolong use results in liver cirrhosis and fibrosis by lipid peroxidation pathway. It is generally accepted that reactive free radical (CCl₇⁺), generated by reductive metabolism of CCl₄ by hepatic cytochrome P₄₅₀ which is believed to induce lipid peroxidation and degradation or deterioration of cellular membranes is responsible for its toxicity. Lipid peroxidation is not only reaction initiated by CCl₄ but also reduction in tissue antioxidant activities, such as CAT and SOD which may result from oxidative modification of these proteins [12]. Our results showed that administration of *P. pinnata* extract effectively protect the cell against the loss of these antioxidant activities after CCl₄ administration. It may does this either by protecting the cells from oxidative damage by ROS and free radicals or its phytochemical constituents which reveal the presence of terpenoids, tannins, saponins and flavonoids, in *P. pinnata* may explain its role in hepatoprotection by inhibiting the free radicals mediated damage. Takeoka and Dao [13] claimed that tannin, triterpens and flavonoids were antioxidant agent and may interfere with reactive oxygen species formation, also Babu et al., [14] stated that hepatoprotective activities of certain flavonoids are known. The synthesis of anti-oxidant enzymes have been shown to be stimulated by phytochemicals and detoxification systems at the transcriptional level, through antioxidant response elements [15], and to increase δ-glutamylcysteine synthesis[16]. The increased level in LPO in liver caused by CCl₄ suggests enhanced lipid peroxidation leading to liver damage and failure of antioxidant defense mechanism to prevent formation of excessive reactive oxygen species. Treatment with *P. pinnata* significantly reverses these changes. Hence it is may be that the mechanism of hepatoprotection of *P. pinnata* is due to its antioxidant effect.

Our data confirmed previous observations on the hepatocellular damage in CCl₄ toxicity, because serum ALT, ALP and AST which are markers of hepatocellular damage increased significantly in the group exposed to CCl₄ untreated group. Whereas the extract markedly decreased the activities of these liver function enzyme. The increased activities of liver marker enzymes such as ALP, ALT and AST in the serum of CCl₄ induced rats indicate damage to hepatic cells. Damage to the cell integrity of the liver by CCl₄ is resulted by an increase in the activity of AST, which is flowed into circulation after cellular damage since ALP is an ectoenzyme of the hepatocytes’ plasma membrane. CCl₄-mediated acute toxicity increased absorptivity of the hepatocytes membrane and cellular leakage. In the group treated with CCl₄ only, the ALT, ALP and AST activities were significantly increased compared with the control group, indicating severe hepatocellular damage. In contrast, *Pauliana pinnata* extract markedly decreased the release of ALT, ALP and AST in serum, liver and kidney homogenates. The increase in total serum protein (TSP) and significantly lower serum albumin observed in those groups that received the plant extract when compared with control and CCl₄ untreated group may be due to short course duration of the experiment. Significant decrease in serum albumin had been associated with active cirrhosis and biliary liver damages. Elevated serum levels of creatinine and uric acid in the CCl₄ untreated group in this study was found to reduce significantly sequel to administration of *Pauliana pinnata* extract. This suggests that the plant extract may prevent kidney damage (nephroprotective) which produced the observed neutrophilia in those rats that induced with CCl₄ untreated group, under the influence of the stress hormone cortisol and catecholamine. It has been shown that acute CCl₄ toxicity on haematological parameters led to transient reduction in the Hb concentration and reticulocyte count as well as PCV and RBC counts by extension which is similar to our observation in the present study. Treatment with *Pauliana pinnata* extract in this study was observed to increase the PCV, RBC, Hb and lymphocyte to values that were higher than values in those rats that did not receive the extract. This shows that *Pauliana pinnata* extract significantly reduced the damaging effects of CCl₄ in the treated rats. Although the mechanism of the protection cannot be ascertained at the moment, it may be due to the antioxidant properties of the extract.

5. CONCLUSION

The CCl₄ Induced hepatotoxicity produced in rats leading to hepatic injury triggers the generation of toxic radicals which can be masked by using a correct antioxidant in adequate amount. The use of methanolic leaf extracts of *Pauliana pinnata* protects the liver from damage by CCl₄ as evident by improved histologic result (table 4) and biochemical markers of liver damage. The hemorrhage caused by CCl₄ in the liver was minimized by use of plant extract as flavonoids are known to be vascular protectors. The mechanism of the hepatoprotective action of the plant is uncertain but may be related to the ability of the plant to inhibit lipid peroxidation in the liver. On the basis of results obtained it can be concluded that the methanolic leaf extract of
**Paullina pinnata** seems to possess hepatoprotective, nephroprotective and hematoprotective activity in *albino* rats. Further studies are needed to evaluate mechanism of action and the potential usefulness of this extract in clinical conditions associated with liver damage.

**Conflict of Interest**

The authors declare that they have no conflicts of interest.

**References**


*Protective effect of Paullina pinnata against carbon tetrachloride (CCl₄) induced tissue damage in wistar rats*