Hepatoprotective Potentials Of Hibiscus Rosasinensis Petal anthocyanin 
Extracts Against Carbon tetrachloride-Induced Acute Liver Damage in Wistar 
Rats. 
Onyesom I*. Mordi J. Opajobi AO. and #Esume CO.

ABSTRACT
Carbon tetrachloride (CCL₄) is haloalkane that possesses a hepatotoxic effect. 
Material and Methods: The impact of anthocyanin fractions obtained from 
Hibiscus rosasinensis petal on carbon tetrachloride (CCL₄) – induced acute liver 
damage in wistar rats was studied using a combination of alanine transferase 
(ALT) activity value and liver: body weight gain ratio as indices.

Results: CCL₄ treatment significantly increased both ALT value and the liver: 
body weight gain ratio at the 1% probability level when compared with the 
control values.

Conclusion: Pre-treatment with the anthocyanin fractions reduced the levels of these markers and hence, the 
degree of liver damage, though with varying potentials. The lead precipitated, non – slimy red fraction 
possessed the greatest protective property on the rat liver when compared with the other anthocyanin 
fractions so tested.

KEY WORDS: Anthocyanin, carbon tetrachloride, alanine transferase, Hibiscus rosasinensis, liver damage.

Carbon tetrachloride (CCL₄) is 
haloalkane that possesses a 
hepatotoxic effect. The hepatotoxicity 
has been extensively studied and its proposed 
mechanism involves initial reductive 
dechlorination of carbon tetrachloride (CCL₄) 
to trichloromethyl radical (CCL₃) which 
subsequently precipitate membrane lipid 
peroxidation and hence liver damage¹. 
Investigations have demonstrated that the 
liver injury produced by CCL₄ could be 
prevented or greatly modified by pre-treating 
animals with various antioxidants².

Anthocyanin, a water-soluble 
glycoside of anthocyanidins, is a plant 
pigment. They are part of the C15 phenolics 
known collectively as flavonoids with the 
typical A-ring benzoyl and B-ring 
hydroxycinnamoyl system.

Anthocyanins are used in industries mostly 
as colourants and flavour enhancers. 
However, they serve protective functions in 
plants, hence speculated to have antioxidant 
properties³. This tendency may be due to 
their phenolic structure which shows 
antioxidant activity towards a variety of easily 
oxidizable substances and might be part of 
anthocyanin defense mechanisms against free 
radical mediated damage⁴.

This study reports the protective capacities of 
partially purified extracts of H. rosasinensis 
petal anthocyanin against carbon 
tetrachloride-induced liver damage.

Materials and Methods

Animals: Forty-nine (49) adult albino rats 
(Wistar strain) with an initial mean weight of 
154±16g (135-170g) were used for the study. 
The rats were obtained from the Animal Unit, 
Faculty of Pharmacy, University of Benin, 
Benin City, Edo State, Nigeria.

Collection of Hibiscus Flowers: Hibiscus 
flowers (Hibiscus rosasinensis) were 
collected from the environment in University of 
Benin, Ugbowo, Benin City.

* . Department of Medical Biochemistry, 
a . Department of Pharmacology 
Delta State University, Abraka, Nigeria.

© Sudan JMS Vol. 3, No. 1, Mar 2008 33
Extraction and Purification of Anthocyanins: Anthocyanins were extracted from flower petals and purified as previously described\(^5\). The extracts obtained from the stepwise purification techniques were respectively labelled: AN\(_1\), AN\(_2\), AN\(_3\), AN\(_4\) and AN\(_5\). AN\(_1\) is the crude anthocyanin extract and AN\(_2\), the lead precipitated extract, while AN\(_3\) is the 12 x 3.5cm sephadex G-50 column fraction. AN\(_4\) and AN\(_5\) are 50 x 10cm sephadex G-50 column fractions 1 and 2, respectively. AN\(_2\) was obtained from crude, while the column fractions were obtained from AN\(_2\).

Treatment of animals and anthocyanin administration:
The rats were allowed to acclimatize to the feed and laboratory condition for 7 days. Thereafter, they were divided into seven experimental groups with seven rats each. They were provided with feed (rat pellets) and clean water ad libitum.

Rats in groups 1 and 2 were given 0.25ml (5%) ethanol/100g body weight, 5 days a week for four (4) weeks. Rats in groups 3,4,5,6 and 7 received a solution of AN\(_1\), AN\(_2\), AN\(_3\), AN\(_4\) and AN\(_5\) extracts respectively, in 5% ethanol (1:1v/v) at a dose of 0.25ml/100g body weight. All administrations were given orally by intubation.

Administration of Carbon Tetrachloloide \((\text{CCL}_4)\):
After the 4 – week anthocyanin treatment period, the rats in groups 2, 3, 4, 5, 6 and 7 received 0.6ml \text{CCL}_4 (in corn oil, 1:1v/v) per 100g body weight by subcutaneous injection after a 12-hour fast. The animals were then sacrificed after 18 hours of the \text{CCL}_4 treatment. The dosing regimen and the administration method were based on previous experience\(^5\).

Collection of Blood and Liver Samples:
Each rat was anaesthetised in chloroform saturated chamber and the abdominal and thoracic regions were surgically opened to expose the liver and the heart. Whole blood sample was then collected from the heart using 5ml hypodermic syringe and needle into lithium heparinised sample bottle. After leaving to stand on ice for few minutes, the sample was centrifuged at 1200 x g for about 5 min at room temperature in order to separate the plasma which was collected and stored frozen in bijou bottle. Analysis was done within 48 hours of collection. The liver was also excised, washed and weighed fresh.

Analysis of specimens:
Plasma Alanine transferase (ALT) activity value was determined by a colorimetric method\(^7\), using commercially available reagent test kit supplied by Quimica Clinica Aplicada, S.A., Spain.

Statistics:
Analysis of variance (ANOVA) was used to compare group values, followed by Newman-Keuls post-hoc test\(^8\) to determine statistical significance between the groups. Differences were considered significant when P< 0.05.

Results:
The results obtained from the study were shown on Tables 1 and 2. Table 1 presents the changes in plasma alanine transferase (ALT) activity values for the various experimental groups. From Table 1, it can be observed that carbontetrachloride \((\text{CCL}_4)\) treatment significantly (P<0.01) increased ALT activity value by over 700% when compared with the control value.
Table 1: Changes in mean plasma alanine transferase (ALT) activity values for the different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALT Activity (IU/L)</th>
<th>Percentage mean increase compared with G1</th>
<th>Percentage mean decrease compared with G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>61.89±1.51*</td>
<td>-</td>
<td>88.08</td>
</tr>
<tr>
<td>G2</td>
<td>CCl4</td>
<td>519.23±54.34*</td>
<td>738.95</td>
<td>-</td>
</tr>
<tr>
<td>G3</td>
<td>AN1 + CCl4</td>
<td>190.00±15.97*</td>
<td>207.00</td>
<td>63.41</td>
</tr>
<tr>
<td>G4</td>
<td>AN2 + CCl4</td>
<td>161.30±59.52*</td>
<td>160.62</td>
<td>68.93</td>
</tr>
<tr>
<td>G5</td>
<td>AN3 + CCl4</td>
<td>225.40±50.07</td>
<td>264.19</td>
<td>56.96</td>
</tr>
<tr>
<td>G6</td>
<td>AN4 + CCl4</td>
<td>226.40±51.01*</td>
<td>265.81</td>
<td>56.91</td>
</tr>
<tr>
<td>G7</td>
<td>AN5 + CCl4</td>
<td>327.54±65.60*</td>
<td>425.23</td>
<td>36.92</td>
</tr>
</tbody>
</table>

ALT activity values are expressed as mean±SD of seven rats per group.
*Significantly different from the control value (P<0.01)

\(^+\) Significantly different from the CCl4 – treated (G2) value (P<0.01)

AN1 – AN3 represents the various fractions of the anthocyanin extracts:
- AN1: Crude (slimmy red) extract
- AN2: Lead precipitated (non – slimmy red) extract
- AN3: 12 x 3.5cm Sephadex G-50 column fraction.
- AN4: 50 x 10cm Sephadex G – 50 column (single red pigment) first fraction.
- AN5: 50 x 10cm Sephadex G-50 column (blue-violet pigment) second fraction.

Table 2: Mean Liver: Body weight gain Ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Liver weight (g)</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Weight gained (g)</th>
<th>Liver: Body weight gain ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>3.44±0.99</td>
<td>156.00±21.92</td>
<td>175.00±12.91</td>
<td>19</td>
<td>0.1811</td>
</tr>
<tr>
<td>G2</td>
<td>CCl4</td>
<td>4.42±0.34</td>
<td>165.50±1.59</td>
<td>165.50±1.41</td>
<td>0.5*</td>
<td>8.8400*</td>
</tr>
<tr>
<td>G3</td>
<td>AN1 + CCl4</td>
<td>3.76±0.99</td>
<td>149.30±10.73</td>
<td>173.10±9.04*</td>
<td>23.8</td>
<td>0.1580</td>
</tr>
<tr>
<td>G4</td>
<td>AN2 + CCl4</td>
<td>3.59±0.14</td>
<td>162.50±18.65</td>
<td>189.00±13.25</td>
<td>20.5</td>
<td>0.1751</td>
</tr>
<tr>
<td>G5</td>
<td>AN3 + CCl4</td>
<td>3.77±0.23</td>
<td>136.00±47.71</td>
<td>176.20±26.65</td>
<td>40.2*</td>
<td>0.0938*</td>
</tr>
<tr>
<td>G6</td>
<td>AN4 + CCl4</td>
<td>4.03±0.22</td>
<td>154.00±4.42</td>
<td>181.70±5.13</td>
<td>27.7</td>
<td>0.1455</td>
</tr>
<tr>
<td>G7</td>
<td>AN5 + CCl4</td>
<td>3.82±0.91</td>
<td>155.80±7.79</td>
<td>188.90±5.44*</td>
<td>33.1</td>
<td>0.0202*</td>
</tr>
</tbody>
</table>

Weight values are expressed as mean±SD of seven rats per group.
* Significantly (P<0.01) higher than the control value.
+ Significantly (P<0.01) lower than the control value.

This large increase suggests hepatic injury. However, rats that were challenged by the anthocyanin extracts had between 160-425% increments in ALT activity value, an evidence of a measure of hepatoprotection. The lead precipitated extract (AN2) possessed the highest degree of protection and the 50x10cm Sephadex G-50 column second extract (AN3) the lowest, as judged by their ALT values. Table 2 shows the liver weight: body weight gain ratio. The group of rats challenged with 12x3.5cm Sephadex G-50 column fraction (AN3) gained the highest weight followed by the group given the 50x10cm Sephadex G-50 column second fraction (AN3). The liver weight: body weight gain ratio for the CCl4 treated group had the highest value, indicating that CCl4 increased liver weight – an evidence of inflammation, with very minimal gain in body weight. The liver weight: body weight gain ratio for the rats that received...
AN₂ compares well with the control value. AN₂ extract appears to have the highest potential of protecting the liver against CCl₄-induced toxicity.

**DISCUSSION**

Measurement of plasma ALT activity value has been used as an index of acute liver damage induced by CCl₄ exposure. Koji, *et al.*⁶ observed a significant (P<0.01) increase in serum ALT activity value (from 37 IU/L to 553 IU/L), 24 hours after administration (subcutaneous injection) of 0.6ml/kg body weight of a mixture of carbon-tetrachloride (CCl₄) in olive oil (1:1 v/v) to 12–hours fasted rats. They attributed such outrageous increase to acute liver damage occasioned by CCl₄ toxicity. In this present study, the significant (P<0.01) increase (738.95%) in ALT activity value (519.23 IU/L) for the CCl₄-treated rats (Table 1) further confirms the hepatotoxic potential of CCl₄. But, pre-treatment with anthocyanin extracts reduced the significant increase in plasma ALT activity induced by CCl₄ administration. However, the various fractions of the anthocyanin extract differ in their ability to protect the liver from CCl₄ acute damage as evidenced by the ALT activity values (Table 1). The AN₂-lead precipitated, non-slimmy red extract possessed the greatest protective property, while AN₅ – the sephadex second fraction, showed the least protecting ability. Similarly, Ficus carica leaf extract has been observed to possess a measure of hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity in rats⁹.

The liver: body weight gain ratio for the CCl₄-treated group was statistically demonstrated to be the highest (P<0.01), suggesting that CCl₄ toxicity increased liver weight (size) but significantly reduced the gain in body weight by mechanism(s) that have not been clearly established.

Carbon tetrachloride could damage the liver via injuries caused by free radical (CCl₃) - mediated lipid peroxidation.¹⁰ From the available data, it appears that the lead precipitated, non-slimmy red extract of anthocyanin, AN₂ possesses the highest antioxidant potential. The AN₂ fraction should be characterized and studied for its potent antioxidant property against acute liver damage caused especially by CCl₄ and allied substance(s).

**REFERENCES**


