Protective Effects of *Bixa orellana* Seed Oil on Carbon tetrachloride Induced Liver Damage in Rats

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Abstract: The effects of pretreatment with *Bixa orellana* seed oil on CCl₄-induced liver damage were determined in male Wister rats (150±10g). Rats were pretreated with *Bixa orellana* seed oil at 0, 1, 5 or 10% (w/w) through dietary exposure for 4 weeks before a single intraperitoneal injection of CCl₄. Serum biochemical parameters, liver lipid peroxidation and relative organ weights were determined. Pretreatment with *Bixa orellana* seed oil (10%) resulted in significant (p<0.05) reduction in serum liver marker enzymes activities, total bilirubin concentration, lipid peroxidation and relative liver weights induced by CCl₄ administration. These results show that dietary exposure to *Bixa orellana* seed oil exhibited moderate protection against CCl₄-induced hepatotoxicity in rats.

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Key words: *Bixa orellana*; seed oil; hepatoprotection; CCl₄; rat

1.0 Introduction

Hepatotoxicity resulting from exposure to environmental chemicals is a global major public health problem. A number of hepatotoxicants of different types have been documented (Timbrell, 1991). Carbon tetrachloride (CCl₄) is used commonly as a chemical model for the induction of hepatic injury in experimental animals. CCl₄ is activated in the liver by liver cytochrome P₄₅₀ enzymes to form reactive toxic metabolites which can cause liver injury in animals and humans (Uchleke and Werner, 1975; Gonzalez, 1988). Liver damage is associated with membrane lipid peroxidation and cell necrosis (Rush *et al.*, 1986; Recknagel *et al.*, 1989; Williams and Burkh, 1990).

*Bixa orellana* is a plant of the family Bixaceae. The colored seeds coat yields a carotenoid dye used in coloring foods, drugs and cosmetics. The dye has been shown to have antimicrobial activity against Gram positive bacteria including, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis* and reduced activity against *Escherichia coli*, *Serratia marcesens*, *Candida utilis* and *Aspergillus niger* (Irobi *et al.*, 1996). The oil obtained from *Bixa orellana* seeds has been shown to contain fatty acids such as capric, palmate, stearic, oleic and linoleic acid; the unsaturated oleic and linoleic fatty acids being the most abundant (Silva *et al.*, 2008).

Although the seed coat dye is the most widely used in foods, in some countries including Nigeria, the whole seed is crushed and the powder used in food preparations to enhance food color. Though several studies have been carried out on the dye (Preston and Richard, 1980; Irobi *et al.*, 1996; Paumgartten *et al.*, 2002), little is known about the seed oil especially concerning its effects against chemically induced cell injury. In this study, we report data on the effects of *Bixa orellana* seed oil on CCl₄-induced hepatotoxicity in rats.

2.0 Materials and Methods

2.1 Sample collection and Extraction of *Bixa orellana* seed oil.

The *Bixa orellana* seeds were purchased from Jimeta main market Yola, Adamawa state, Nigeria. The seeds were transferred into a lidded plastic container, moistened and shaken vigorously to remove the seed coat. The mixture was diluted with tap water and the seeds were sieved out and further washed with tap water. The decoated seeds were sun dried and later dried at constant weight in the oven at 60°C. The dried samples were first crushed into coarse particles with a pestle before being ground to fine powder using an electric blender. Oil in the powdered sample was extracted using petroleum ether (60% fraction) using soxhlet apparatus with a yield of about 4.2%. The oil obtained was stored in a brown bottle at room temperature until the commencement of the study.

2.2 Animals

Male Wister rats were purchased from the animal house of University of Jos, Jos, Nigeria. The animals were acclimatized for one week before the start of the study. The animals were housed in plastic
cages at room temperature and were fed pelleted diet (Grand cereals Ltd., Jos, Nigeria).

### 2.3 Chemicals
The kits for the determination of serum alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities were products of Randox Laboratories Co. (Atrium, UK). Thiobarbituric acid (TBA) and carbon tetrachloride (CCl4) were products of Aldrich chemical company, Milwaukee, USA. Other chemicals and reagents utilized were of highest purity and were purchased from local firms, Nigeria.

### 2.4 Experimental Design
Twenty five male Wister rats weighing 150 ± 10g were allocated to 5 groups of 5 rats each (Group I – V). Group I (control) and Group II were fed the normal diet only. Group III, Group IV and Group V, were fed diet mixed with *Bixa orellana* seed oil at 1, 5, and 10% (w/w) respectively, for four weeks. On the last day of the pretreatment, hepatotoxicity was induced using a single dose of CCl4 dissolved in olive oil(1:1), administered intraperitoneally at 2ml/kg body weight (Vanitha et al., 2007) to animals of Group II , III, IV and V. After 24 hours of CCl4 administration, the rats were sacrificed under ether anaesthesia and blood collected by cardiac puncture. Serum was prepared and used for the determination of serum biochemical parameters. Serum AST, ALT, ALP and total bilirubin levels were assayed using commercial kits (Randox laboratories Co.Atrium, UK). Liver from rats treated with CCl4 seed oil on CCl4-induced lipid peroxidation was determined as thiobarbituric acid reactive substance (TBARS) and expressed as the amount of malondialdehyde (MDA) (Uchiyama and Mihara, 1978).

### 2.5 Statistical Analysis
The data are expressed as mean ± standard error (S.E.M) for five replicates and evaluated by one way ANOVA followed by Dunnett test (Dunnett, 1955). All results were compared with respect to control and as well as the group treated with CCl4 only (Group II). The level of significance was set at p< 0.05.

### 3.0 Results
Rats treated with CCl4 showed a significant (p< 0.05) increase in serum liver enzymes activities (*Table 1*). These included ALT, AST and ALP. The elevated levels of the liver enzymes were also accompanied with significant (p< 0.05) increases in serum total bilirubin concentrations. Pretreatment with *Bixa orellana* seed oil caused significant (p< 0.05) dose dependent decreases in the CCl4-induced elevation of serum liver enzymes and total bilirubin concentrations. Administration of the *Bixa orellana* seed oil at 10% dietary level restored the plasma ALP and total bilirubin levels to normal levels.

The effect of pretreatment with *Bixa orellana* seed oil on CCl4-induced lipid peroxidation is shown in *Table 2*. The levels of malondialdehyde (MDA) in the group treated with CCl4 alone increased significantly (p< 0.05) when compared with the control group. Conversely, pretreatment with *Bixa orellana* seed oil (10%) significantly (p< 0.05) decreased CCl4-induced hepatic lipid peroxidation in a dose dependent manner.

Treatment with CCl4 resulted in significant (p<0.05) increase in rat relative liver weights when compared with the control group (*Table 3*). Mean final body weights were not affected by either CCl4 or *Bixa orellana* seed oil administration. Pretreatment with *Bixa orellana* seed oil (10%) resulted in significant (p < 0.05) reduction in the mean relative liver weight of rats. Relative liver weights in the 10% group were comparable to those of the control.

### Table 1: Effects of pretreatment with *Bixa orellana* seed oil on CCl4-induced increases in serum ALT, AST, ALP and Total bilirubin levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Total Bil. (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: control</td>
<td>66.25±4.51</td>
<td>45.41±3.82</td>
<td>42.56±3.19</td>
<td>0.61±0.12</td>
</tr>
<tr>
<td>Group II: CCl4</td>
<td>192.63±4.76</td>
<td>88.34±4.05</td>
<td>58.27±2.37</td>
<td>0.93±0.12</td>
</tr>
<tr>
<td>Group III: 1% oil + CCl4</td>
<td>188.38±5.98</td>
<td>80.62±3.68</td>
<td>54.66±2.36</td>
<td>0.91±0.11</td>
</tr>
<tr>
<td>Group IV: 5% oil + CCl4</td>
<td>173.67±4.02</td>
<td>76.86±5.77</td>
<td>46.97±3.88</td>
<td>0.83±0.13</td>
</tr>
<tr>
<td>Group V: 10% oil + CCl4</td>
<td>156.50±5.58</td>
<td>68.84±5.62</td>
<td>43.63±3.26</td>
<td>0.75±0.14</td>
</tr>
</tbody>
</table>

Tabulated values are mean±S.E.M, n=6
ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphatase; Total Bil., Total bilirubin
a:Significantly different from control group (p<0.05)
b:Significantly different from the group treated with CCl4 only (p<0.05)
Table 2: Effects of pretreatment with *Bixa orellana* seed oil on CCl₄-induced increases in liver lipid peroxidation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (mmoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: control</td>
<td>33.55 ± 4.22</td>
</tr>
<tr>
<td>Group II: CCl₄</td>
<td>58.61 ± 3.68*</td>
</tr>
<tr>
<td>Group III: 1%</td>
<td>56.22 ± 3.72*</td>
</tr>
<tr>
<td>Group IV: 5% + CCl₄</td>
<td>57.53 ± 4.15*</td>
</tr>
<tr>
<td>Group V: 10% + CCl₄</td>
<td>44.63 ± 4.39*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M, n=5; MDA, malondialdehyde
a: Significantly different from control group (p<0.05)
b: Significantly different from the group treated with CCl₄ only (p<0.05)

Table 3: Effects of pretreatment with *Bixa orellana* seed oil on CCl₄-induced changes in relative liver weight.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean final body weight (g)</th>
<th>Relative liver weight (g/100g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: control</td>
<td>264.47±5.71</td>
<td>1.55±0.12</td>
</tr>
<tr>
<td>Group II: CCl₄</td>
<td>259.24±6.11</td>
<td>2.25±0.11*</td>
</tr>
<tr>
<td>Group III: 1% oil + CCl₄</td>
<td>268.65±4.98</td>
<td>2.27±0.14*</td>
</tr>
<tr>
<td>Group IV: 5% oil + CCl₄</td>
<td>260.53±5.92</td>
<td>2.18±0.12*</td>
</tr>
<tr>
<td>Group V: 10% oil + CCl₄</td>
<td>262.16±6.34</td>
<td>1.81±0.13*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M, n=5.
a: Significantly different from control group (p<0.05)
b: Significantly different from the group treated with CCl₄ only (p<0.05)

4.0 Discussion

Hepatotoxicity was observed in rats treated with CCl₄ alone as shown by increased serum ALT and AST which may suggest liver damage (Chawla, 1999) following CCl₄ administration. The observed significant increases in serum ALP activity and total bilirubin concentration further demonstrate hepatotoxicity in rats arising from cholestatic liver injury (Chawla, 1999; Somchit et al., 2004). Pretreatment with *Bixa orellana* seed oil protected against CCl₄-induced hepatotoxicity as demonstrated by significant decreases in serum liver marker enzyme levels and other biochemical indices.

The biochemical marker of lipid peroxidation, malondialdehyde (MDA) increased significantly in all animals treated with CCl₄ when compared with the control. CCl₄ is known to increase lipid peroxidation (Recknagel et al., 1989; Mansour, 2000; Glei et al., 2002). The elevated levels of MDA in the CCl₄-treated groups indicate lipid peroxidation elicited by administration of CCl₄. Pretreatment with *Bixa orellana* seed oil protected against CCl₄-induced liver lipid peroxidation, as shown by reduction in the level of MDA. Consumption of *Bixa orellana* seed oil may decrease liver organ susceptibility to lipid peroxidation and invariably oxidative stress. Pretreatment with *Bixa orellana* seed oil also protected the liver against any change in relative organ weight which normally reflects the pathological state of the organ. The relative liver weights were consistent with serum ALT and AST activities, and liver tissue MDA levels which further demonstrate the hepatoprotective potentials of *Bixa orellana* seed oil. Earlier reports suggested that natural compounds of plant origin can protect against CCl₄-induced toxicity by quenching free radicals and inhibiting lipid peroxidation caused by CCl₄ metabolism (Biasi et al., 1991; Chidambaram et al., 2002). Such antioxidant activities are usually attributed to carotenoids and allied substances which could be important in the observed hepatoprotective effects of *Bixa orellana* seed oil. Interestingly, the seed coat has been shown to contain carotenoids (Trimanna, 1981; Mercadante et al., 1997). It is therefore possible that the seed oil also contain carotenoids or other substances with antioxidant potential. The administration of *Bixa orellana* seed oil to rats provided moderate dose dependent protection against CCl₄-induced hepatotoxicity since meaningful antihepatotoxic effects were observed in the high dose (10%) group only.

5.0 Conclusion

Inclusion of *Bixa orellana* seed oil in the animal diet protected against CCl₄-induced liver injury. Thus, consumption of the seed oil may protect against hepatotoxicity caused by environmental chemicals and other prooxidants by inhibiting liver tissue lipid peroxidation.
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