Hepatoprotective Effects of African Locust Bean (*Parkia clappertonia*) and Negro Pepper (*Xylopia aethiopica*) in CCl4-Induced Liver Damage in Wistar Albino Rats

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**Abstract:** The aim of this study was to evaluate the protective effects of Negro pepper (*Xylopia aethiopica*) and African locust bean (*Parkia clappertonia*) against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. Carbon tetrachloride (0.5 mL kg⁻¹ b wt.) was administered after 21 days of feeding animals with diets containing Negro pepper (*X. aethiopica*) and African locust bean (*P. clappertonia*). Serum alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) levels 24 h after CCl4 administration decreased significantly (p<0.05) in rats pre-treated with *X. aethiopica* and *P. clappertonia* than in CCl4-treated rats only. Total serum bilirubin also showed a remarkable decrease in rats pre-treated with *X. aethiopica* and *P. clappertonia* when compared to those administered CCl4 alone. Lipid peroxidation expressed by malondialdehyde (MDA) concentration was significantly decreased (p<0.05) in rats pre-treated with *X. aethiopica* and *P. clappertonia* than in rats administered CCl4 alone. Histopathological examinations of rats administered CCl4 alone revealed severe hepatic damage to the liver. However, rats pre-treated with *X. aethiopica* and *P. clappertonia* showed significant improvements in the architecture of rat liver. These findings suggest that *X. aethiopica* and *P. clappertonia* may be protective against CCl4-induced liver damage in rats.

**Key words:** African locust bean, carbon tetrachloride, hepatic injury, lipid peroxidation, negro pepper

**INTRODUCTION**

The liver is known to perform a multitude of essential functions and several disease states, including hepatitis and cirrhosis alter the metabolism of this organ (Attri et al., 2001). The liver is an organ of paramount importance, which plays an essential role in the metabolism of foreign compounds. Human beings are exposed to these compounds through consumption of contaminated foods or exposure to chemical substances in the occupational environment. Oxidative stress resulting from the toxic effects of free radicals on the tissue plays an important role in the pathogenesis of various diseases such as Alzheimer’s disease, Parkinson’s disease and those involving anti-inflammatory processes (Habhi et al., 2008). Free radical induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations (Slater, 1984; Oberley, 1988; Halliwell, 1993).

Carbon tetrachloride is a known hepatotoxin. Its administration in rats enhances hepatic protein oxidation and results in the accumulation of oxidized proteins in the liver (Abraham et al., 1999). A number of recent reports clearly demonstrated that in addition to hepatic problems, CCl4 also causes disorders in kidney, lungs, testis and brain as well as in blood generating free radicals (Chaurbonneau et al., 1986; Ahamad et al., 1987; Ohata et al., 1997; Ozturk et al., 2003). Lipid peroxides produced from unsaturated fatty acids via radicals, cause histotoxicity and promote the formation of additional free radicals in a chain reaction type. The damage or death of tissue usually results in the leakage of the enzymes in the affected tissue(s) into the blood stream (Diegers et al., 1985; Obi et al., 2001). Serum or plasma enzyme levels have been used as markers for monitoring chemically induced tissue damages (Lin and Wang, 1986; Ngaba et al., 1989). The enzymes L-Alanine aminotransferase (L-ALT) [EC. 2.6.1.2] and L-Aspartate (L-AST) [EC. 2.6.1.1] are important enzymes that are often
employed in assessing liver injury (Jaeger et al., 1975; Ngaha et al., 1989; Obi et al., 1998; Patrick-Iwuanyanwu et al., 2007; Patrick-Iwuanyanwu and Wegwu, 2008). The biotransformation of CCl₄ to metabolites is a cytochrome P450-mediated reaction that initiates lipid peroxidation and attendant tissue damage. Sheny et al. (2001) reported that the oxidative damage through free radical generation is among the various mechanisms involved in the hepatotoxic effects of CCl₄. A number of investigators have previously demonstrated that antioxidants prevent CCl₄ toxicity, particularly hepatic toxicity, by inhibiting lipid peroxidation (Teselkin et al., 2000), suppressing alin aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Lin and Huang, 2000) and increasing antioxidant enzyme activity (Kumaravelu et al., 1995).

Herbal treatment of many diseases including hepatopathy is increasing in many countries (Venkukumar and Lattha, 2002; Malaya et al., 2004). Some plants have been shown to have protective antioxidant effects and are therefore hepatoprotective. Such plants include H. rosasinensis (Obi et al., 1998), Ginkgo biloba (Shenoy et al., 2001), Ginger, garlic, a combination of ginger and garlic (Patrick-Iwuanyanwu et al., 2007) and Acanthus montanus (Patrick-Iwuanyanwu and Wegwu, 2008) in CCl₄ hepatotoxicity.

Negro pepper (Xylopia aethiopica) is one of the most pungent spices available. It is found mostly in forest and coastal regions in Nigeria and belongs to the family Annonaceae (Dalziel, 1955; Irvine, 1961). It has an attractive aroma and has been applied in ethnomedicine in the treatment of cough, bronchitis, dysentery and female sterilization. It is believed to aid uterine contraction and is applied as an abortifacient (Iwu, 1986). The extract of the fruit is used to treat malaria in the Hausa land of Nigeria (Etkin, 1997), while oral infusion of the seeds is used as an antitussive agent (Mamoudoukande et al., 1994). Phytochemical evaluation shows that X. aethiopica is rich in alkaloids, tannins, flavonoids, steroids, oligosaccharides and has tolerable levels of cyanogenic glycosides (Ijeh et al., 2004).

The genus Parkia to which the African locust bean belongs is large in the family leguminosraceae. The pods are flat, large, irregular clusters from which the locust bean seeds are obtained. The species of the genus include Parkia filicoides, Parkia biglobosa, Parkia bicolor and Parkia clappertoniana. The locust bean tree is planted mainly because the fruit is rich and provides valuable protein in the dry season (Odunfa and Oyewole 1986). It is also used for medicinal purposes and as a source of mouth wash to relieve toothache.

The aim of this study was however, to evaluate the hepatoprotective effects of Negro pepper (Xylopia aethiopica) and African locust bean (Parkia clappertoniana) against carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Plant material:** Negro pepper and African locust beans were purchased from Choba market, Choba, Rivers State, Nigeria in October, 2008. They were sun dried for 4 days, ground into powdery form using an electric blender (Moulinex) and stored in air-tight containers.

**Animals:** Adult male wistar albino rats (150-170 g) were obtained from the Animal House, Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria in October, 2008. They were housed in standard cages (Griffin and George Modular cage system) and left to acclimatize for 7 days to laboratory conditions before the commencement of experiment. During the acclimatization, the animals were fed with pelleted rat chow and water ad libitum.

In this study, rats were separated into four main groups (6 animals in each group) and given extracts of the drugs 21 days prior to CCl₄ administration. Hepatoprotection was assessed by monitoring the levels of selected biochemical parameters in the blood of rats.

**Experimental protocol:**

**Group 1:** Normal control rats (feed only)

**Group 2:** Received normal feed and 0.5 mL CCl₄ kg⁻¹ b.wt.

**Group 3:** Feed: locust bean (1:1) and 0.5 mL CCl₄ kg⁻¹ b.wt.

**Group 4:** Feed: Negro pepper (1:1) and 0.5 mL CCl₄ kg⁻¹ b.wt.

**Induction of hepatic injury:** Carbon tetrachloride (CCl₄)-induced liver damage was achieved by injecting 0.5 mL CCl₄ kg⁻¹ b.wt. intramuscularly on the 21st day of feeding animals of groups 1 to 4 with commercial feed, African locust beans and Negro pepper as stated above.

**Preparations of samples:** Twenty four hours after the administration of CCl₄, the rats were anaesthetized in chloroform-saturated chamber. The animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture using a 5 mL hypodermic syringe and needle and introduced into an anticoagulant free bottle. Serum was separated by centrifugation at 2500 rpm for 10 min and stored in a refrigerator at 4°C until use. The measurement of different biochemical parameters (L-AST, L-ALT and ALP, LDH and bilirubin) was performed using...
the Humazym MUV - test kit. The liver was excised and fixed in formalin for histological assessment of hepatic damage. Lipid peroxidation was also determined by estimating the malondialdehyde levels, using the method of Hunter et al. (1963) modified by Gutteridge and Wilkins (1980).

Analysis of data: The mean values of the control and test serum activities of a given enzyme were compared using the student's t-test (Zar, 1984). The significant level was set at p≤0.05.

RESULTS AND DISCUSSION

The results of hepatoprotective effects of locust bean (Parkia clappertoniae) and Negro pepper (Xylopia aethiopica) on rats treated with single dose of CCl4 (0.5 mL kg⁻¹ b.wt.) are as shown in Table 1. The results indicate that rats administered with 0.5 mL kg⁻¹ b.wt. CCl4 recorded severe hepatic damage (group 2) when compared to control (group 1) and rats pretreated with African locust bean and Negro pepper (groups 3 and 4, respectively). This was evidenced by a marked elevation in the levels of serum marker enzymes, AST, ALT, ALP and LDH, bilirubin and MDA in rats treated with CCl4 alone. The marker serum enzymes, AST, ALT and LDH recorded the lowest values in the group pre-treated with Negro pepper. However, serum ALP was observed to be lowest in rats pre-treated with African locust bean.

The efficacy of any hepatoprotective drug is indeed dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin (Hukkeri et al., 2003). The remarkable elevation in the liver marker enzymes and bilirubin in CCl4 administered rats in this study is only a confirmation of previous reports on the hepatotoxicity of CCl4. In fact, in most experiments involving the induction of liver injury in experimental animals, administration of CCl4 elicited the elevation in the levels of liver marker enzymes (AST, ALT, ALP and LDH) and Bilirubin resulting in a significant hepatic damage. The elevated levels of these biochemical parameters are direct reflection of alterations in the hepatic structural integrity. The results of marker enzymes levels in rats administered CCl4 alone corroborates those of other markers (Obi et al., 1998; Wegwu et al., 2005; Patrick-Iwuanyanwu et al., 2007; Patrick-Iwuanyanwu and Wegwu, 2008) who reported elevated levels in the serum content of hepatic enzymes in rats administered with CCI4 alone. The elevation of marker enzymes in rats administered CCI4 alone reported in this study is similar to the findings of Prakash et al. (2008) who observed significant hepatic damage in rats treated with single dose of CCl4 from a substantial increase in the activities of SGOT and SGPT. This is indicative of cellular leakage and loss of functional integrity in liver (Sallie et al., 1991). In particular, the increase in the serum level of ALT is indicative of liver damage (Lin and Wang, 1986; Nnaji et al., 1989). These enzymes are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged (Lin and Huang, 2000). However, the reduction in the levels of marker enzymes, ALT and AST in rats pre-treated with Negro pepper (Xylopia aethiopica) and African locust bean (Parkia clappertoniae) as reported in this study is also in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. This is also similar to the findings of Thabrew et al. (1987), who found that serum transaminases returned to normal activities with the healing of hepatic parenchyma and regeneration of hepatocytes.

It is evident that an increase in bilirubin concentration in the serum or tissue is indicative of obstruction in the excretion of bile. Thus, the increased level of bilirubin observed in rats administered with CCI4 alone (group 2 rats) could be attributed to liver damage. However, the decrease in bilirubin levels in pretreated rats is indicative of reversal of liver damage by the beans and spice.

Administration of 0.5 mL CCl4 kg⁻¹ b.wt. has been reported to elevate malondialdehyde (MDA), a product of lipid peroxidation in liver of rats treated with CCI4 only (Shenoy et al., 2001; Patrick-Iwuanyanwu et al., 2007). They attributed the increase in MDA levels to enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation.

<p>| Table 1: Hepatoprotective effects of African locust bean (Parkia clappertoniae) and Negro pepper (Xylopia aethiopica) on ALP, AST, ALT, LDH, Bilirubin and lipid peroxidation (MDA) in CCl4-induced hepatotoxicity in rats |</p>
<table>
<thead>
<tr>
<th>Groups/Treatments</th>
<th>ALP (UL⁻¹)</th>
<th>AST (UL⁻¹)</th>
<th>ALT (UL⁻¹)</th>
<th>LDH (UL⁻¹)</th>
<th>Bilirubin (mg dL⁻¹)</th>
<th>MDA (μmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Feed only</td>
<td>95.4±0.01</td>
<td>145.2±0.02</td>
<td>57.9±0.07</td>
<td>100.1±0.13</td>
<td>0.34±0.04</td>
<td>0.65±0.07</td>
</tr>
<tr>
<td>2 Feed + CCl4</td>
<td>212±0.01</td>
<td>220±0.02</td>
<td>132±0.12</td>
<td>155±0.04</td>
<td>1.0±0.08</td>
<td>2.6±0.08</td>
</tr>
<tr>
<td>3 Feed + Locust bean + CCl4</td>
<td>125±0.01</td>
<td>160±0.10</td>
<td>79±0.11</td>
<td>110±0.12</td>
<td>0.3±0.04</td>
<td>1.3±0.10</td>
</tr>
<tr>
<td>4 Feed + Negro pepper + CCl4</td>
<td>148±0.01</td>
<td>154.4±0.21</td>
<td>61.3±0.21</td>
<td>103.5±0.10</td>
<td>0.32±0.03</td>
<td>1.55±0.40</td>
</tr>
</tbody>
</table>

Values are Mean±SEM, n = 6 rats in each group. Means with different superscript letters (a-d) in the same column are significantly different at the 0.05 level.
of excessive free radicals. This in turn alters the ratio of polyunsaturated to other fatty acids, thus, leading to a decrease in the membrane fluidity, which may be sufficient to cause cell death (Rotruck et al., 1979). The results in this study suggest that pretreatment of rats for 21 days with African locust bean and Negro pepper prior to CCl₄ administration significantly (p≤0.05) reversed these changes. It would be deduced, therefore, that the antioxidant effects of African locust bean and Negro pepper could possibly be its mechanism of hepatoprotection.

Histopathological examinations (Fig. 1a-d) showed defects ranging from massive tissue necrosis, congested central vein, fatty degeneration and infiltration by inflammatory cells in rats treated with CCl₄ alone. However, histological profile of rats pre-treated with locust bean (P. clappertonicana) and Negro pepper (X. aethiopica) exhibited significant liver protection against the toxicant as evidenced by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration (Fig. 1a-d). Histopathological examination of control rats however, revealed normal architecture.

The marked decrease in the levels of serum marker enzymes and MDA in pre-treated rats are indications of the ability of African locust bean and Negro pepper to protect the liver against CCl₄ poisoning. This is corroborated by the architecture of the liver of rats that received African locust bean and Negro pepper prior to CCl₄ administration (Fig. 1a-d).

The findings in this study indicate that pretreatment of rats with African locust bean and Negro pepper 21 days prior to CCl₄ administration caused a marked decrease in the levels of hepato specific serum enzymes. It thus implies that African locust bean and Negro pepper may be protective against CCl₄-induced liver damage in rats.

REFERENCES


