Aqueous Extract of Combretum dolichopentalum Leaf - a Potent Inhibitor of Carbon Tetrachloride Induced Hepatotoxicity in Rats

Kalu F.N., Ogugua V.N., Ujowundu C.O., and Nwaoguikpe R.N.

ABSTRACT

The aqueous extract of Combretum dolichopentalum leaves were evaluated for its protective activity against CCl₄-induced liver damage. The concentration of 250 and 500 mg/kg b.w of C. dolichopentalum leaf extract were administered to different group of rats prior to CCl₄ administration. Both 250 and 500 mg/kg of the extract significantly (P<0.05) reduced the activity of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase when compared to rats administered CCl₄ only. Also the concentration of non-enzyme markers of hepatic dysfunction such as total bilirubin and lipid peroxidation product-malonyldialdehyde was reduced by C. dolichopentalum. But the concentration of total protein and total cholesterol was increased when compared to rats administered CCl₄ only. This finding suggests that C. dolichopentalum leaves possessed rich hepatoprotective principles against CCl₄ induced toxicity of the liver.

Keywords: Combretum dolichopentalum, hepatotoxicity, protection, inhibitor.

INTRODUCTION

Carbon tetrachloride (CCl₄), although having narcotic and anaesthetic properties, is a hepatotoxic agent which induces formation of reactive oxygen specie. It may cause depletion of antioxidant enzymes and antioxidant substrates to induce oxidative stress. CCl₄ requires bioactivation by Cytochrome P₄₅₀ System of phase 1 reaction in liver and yield the reactive metabolic trichloromethyl radical (CCl₃) and proxy trichloromethyl radical (00CCl₃) (Chatterjea and shinde, 2007a). These free radicals can bind with polyunsaturated fatty acid (PUFA), forming alkoxy (R.) and peroxy radicals (ROO.), that can initiate lipid peroxidation, cause damage in cell membrane, change enzyme activity and finally induce hepatic injury or necrosis (Weber et al., 2003). Lipid peroxidation is considered to be of fundamental importance in cell ageing and damage (Popovic et al., 2006). At present, in spite of an increasing need for agents to protect the liver from damage, modern medicine lacks a reliable liver protective drug. Therefore a number of natural substances have been studied to evaluate the effect of their protective activities (Willett, 1994). Combretum dolichopentalum belongs to the family of combretaceae. The leaf of C. dolichopentalum is rich in flavonoids and good macronutrient content (Kalu et al., 2011). This study was designed to evaluate the hepatoprotective potency of administering the aqueous extract of C. dolichopentalum leave in carbon tetrachloride induced liver damage.
MATERIALS AND METHODS

Plants and Animal Preparation

The leaves of *C. dolichopentalum* were collected from a farm within the University of Nigeria Nsukka, Enugu State in October, 2010. The leaves were air dried and made into powder using a grinding mill. In this study, 20 albino rat weighing between 120-200 g were purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State. The animals were housed and kept at room temperature in a cage under 12/12 hour light/dark and were fed and maintained ad libitum on water and growsers mash for seven days.

Experimental design:

The rats were divided into four groups of five rats each. Each group of five (5) animals was treated as follows;

**Group I:** Rats were administered olive oil (0.04 ml/animal) only.

**Group II:** were administered single dose of CCl₄ in olive oil

**Group III:** were administered with 250 mg/kg b.w aqueous extract of *C. dolichopentalum* for seven days prior to CCl₄ administration.

**Group IV:** Pre-treated with 500 mg/kg b.w aqueous extract of *C. dolichopentalum* for seven days prior to CCl₄ administration.

CCl₄ was administered in olive oil (2:1) 1.5 ml/kg b.w to induce liver damage. The Protocol used in this research observed the guidelines on the care and well being of research animals (NIH, 1985) and was approved by the Departmental Ethics Committee.

Preparation of aqueous extract

The dried leave of the plant were pulverized into fine powder with a grinding mill. 50 g of the powder was mixed with 500 ml of distilled water, and allowed to stand for 48 hours and filtered with a mash, followed by a whatman filter paper. The filtrate was then evaporated using rotary evaporator at reduced temperature >50°C to obtain 13 % yield of the extract.

Blood collection and serum preparation

Rats from all groups were sacrificed 24 hours after CCl₄ administration. Blood was collected via the ocular vein and allowed to stand for 10 min before centrifuging at 3000 rpm for 15 min to separate serum. Serum separated was used for the estimation of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP), Lipid peroxidation product (MDA), Total Bilirubin (T.B), Total Protein (T.P) and Total Cholesterol (T.C).

Biochemical Studies

Lipid peroxidation product (MDA) was estimated by the method of Wallin *et al.*, (1993). The effect of aqueous extract of *C. dolichopentalum* (AECD) on liver function enzymes was estimated by the method of Reitman-frankel (1975); Total cholesterol was estimated by the method of Allain *et al.*, (1976); Total bilirubin was estimated by the method of Jendrassik and Grof, (1938). Total protein was estimated by the method described by Tietz, (1995) using Randox laboratory test Kits (Antrim, UK).

Statistical Analysis

Statistical analysis was carried out using statistical package for the Social Sciences (SPSS). One way ANOVA was used to determine statistical difference between two means when P<0.05.

RESULTS

Table 1 represents the values obtained from the effect of pretreatment of rats with *C. dolichopentalum* aqueous leaf extract on Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP). The activities of AST, ALP and ALT Significantly (*p*<0.05) increased in group II (CCl₄ alone) compared to group I. The pretreatment of the rats with 250 and 500 mg/kg of the aqueous extract of *C. dolichopentalum* for seven days prior to CCl₄ administration significantly (*p*<0.05) decreased the activities of serum ALT, AST and ALP when group III and IV is compared to group II.

**Table 1:** Effect of CCl₄ toxicity and aqueous *C. dolichopentalum* leaves on Enzyme markers of liver damage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CCl₄</th>
<th>250mg/kg AECD + CCl₄</th>
<th>500mg/kg AECD + CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (IU/L)</td>
<td>69.20±2.06</td>
<td>80.05±1.15*</td>
<td>75.26±2.69**</td>
<td>71.95±1.21*</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>148.75±13.77</td>
<td>208.75±28.39*</td>
<td>183.75±11.09*</td>
<td>171.25±6.29*</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>53.70±15.18</td>
<td>95.05±2.83*</td>
<td>87.15±5.91*</td>
<td>78.10±6.26**</td>
</tr>
</tbody>
</table>

Values represent means ± SEM; n=4, *p* ≤ 0.05 as compared to normal, *p* ≤ 0.05 when compared to CCl₄.

**Table 2:** Effect of CCl₄ toxicity and aqueous extract of *C. dolichopentalum* leaves, on non-enzyme markers of liver damage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>CCl₄</th>
<th>250mg/kg AECD + CCl₄</th>
<th>500mg/kg AECD + CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (%TBARS)</td>
<td>46.01±12.71</td>
<td>78.26±6.26*</td>
<td>72.09±8.63*</td>
<td>67.35±7.82*</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>7.32±0.30</td>
<td>5.89±0.43*</td>
<td>6.34±0.13*</td>
<td>6.46±0.25*</td>
</tr>
<tr>
<td>T.B (mg/dl)</td>
<td>1.30±0.81</td>
<td>2.22±0.21*</td>
<td>1.84±0.12</td>
<td>1.57±0.32</td>
</tr>
<tr>
<td>T.C (Mmol/L)</td>
<td>3.73±0.15</td>
<td>2.72±0.24*</td>
<td>2.95±0.32*</td>
<td>3.05±0.23*</td>
</tr>
</tbody>
</table>

Values represent means ± SEM; n=4, *p* ≤ 0.05 as compared to normal, *p* ≤ 0.05 when compared to CCl₄.

Table 2 shows the effect of pretreatment with aqueous extract of *C. dolichopentalum* leaves on non-enzyme markers in CCl₄ induced hepatotoxicity. The concentration of total cholesterol and total protein were decreased when treated with CCl₄ alone (group II) compared to group I. Pretreatment with *C. dolichopentalum* extract caused an increased in the concentration of total cholesterol and total protein in group III and IV. The concentration of total bilirubin was significantly (*P*<0.05) increased in group II compared with group I. Pretreatment with
250 and 500 mg/kg of *C. dolichopentalum* leaf extract prior to CCl\textsubscript{4} administration resulted in significant (P<0.05) decrease in the concentration of total bilirubin compared to the group administered CCl\textsubscript{4} alone.

**DISCUSSION**

The significant increase in the activities of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase is an indication of hepatic damage following CCl\textsubscript{4} administration. Inducement of hepatic injury with CCl\textsubscript{4} is an experimental model widely used for hepatoprotective drug screening in which CCl\textsubscript{4} is biotransformed by hepatic microsomal cytochrome P\textsubscript{450} to produce trichloromethyl free radicals. These hepatotoxic metabolites can react with biomolecules such as proteins and lipids in the membrane of cells or organelles leading to necrosis of the hepatocytes and release cytosolic transaminases (Sil et al., 2006; Chatterjea and shinde, 2007; Gutierrez and Solis, 2009). Other researchers have reported increased activities of AST, ALT, ALP and bilirubin due to CCl\textsubscript{4} hepatotoxicity (Dahiru et al., 2005; Galati et al., 2005; Gutierrez and Solis, 2009). Increases in both transaminases are found in liver damage, with ALT much higher than AST (Chatterjea and Shinde, 2007). In this study, the AST was found to be much higher than ALT. This may be due to atrophy of the liver and kidney caused by induction with CCl\textsubscript{4}. The higher concentration of AST could also suggest necrosis of the liver and kidney. The decrease in liver enzymes as a result of the treatments of the rats with *C. dolichopentalum* extract indicates a stabilization of plasma membranes as well as repair of hepatic tissue damage caused by CCl\textsubscript{4} (Szymonik-Lesinik et al., 2003; Tirkey et al., 2005). This effect may be as a result of the tannin content which is known to “tar” the outermost layer of the mucosa and thereby render it less permeable and more resistant to chemical and mechanical injury or irritation (Asuzu and Omu, 1988). The decrease in the activities of serum transaminases is in agreement with the commonly accepted view that serum activities of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thrabrew et al., 1987).

The concentration of malonyldialdehyde a lipid peroxidation product which increased on administration of CCl\textsubscript{4} suggests increased oxidative stress on the tissues of the rats. The significant (P<0.05) decrease in the concentration of MDA in rats pretreated with extracts of *C. dolichopentalum*. The decrease in MDA concentration could be attributed to the antioxidant and free radical scavenging properties of *C. dolichopentalum* extracts. Therefore, the reduction of CCl\textsubscript{4} induced- oxidative stress by anti-lipid peroxidative activity might be a mechanism of antioxidant action of *C. dolichopentalum*.

The increase in the concentration of serum bilirubin is an index of the degree of jaundice. This could possibly be the result of increased production, decreased uptake by the liver, decreased conjugation, decreased secretion from the liver or blockage of bile ducts (Bun, 2006). Decrease in the concentration of total cholesterol, could be as a result of inhibition of enzymes necessary for the synthesis, esterification, oxidation and excretion of cholesterol. Pretreatment with *C-dolichopentalum* extract was observed to elevate the total cholesterol level close to normal; Cholesterol concentration within the recommended range is important for the synthesis of steroids hormones (Chatterjea and Shinde, 2007).

**CONCLUSION**

The results of this study indicates that the aqueous extract of *Combretum dolichopentalum* leaves possessed potent hepatoprotective activity against CCl\textsubscript{4} induced liver damage in rats.

**REFERENCES**


