HEPATOPROTECTIVE ACTIVITY OF *CHRYSOHYLLUM ALBIDUM* AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

The leaf extract of *Chrysophyllum albidum* was studied for hepatoprotective activity against rats with induced liver damage by carbon tetrachloride (CCl4). The rats were divided into five groups of eight rats per group. Animals of group A served as normal and were given only vehicle (distilled water) for 7 days. Animals of group B (positive control) were administered with vehicle on the first four days, and with the vehicle and CCl4 on the fifth, sixth and seventh day. The animals of groups C, D and E were respectively administered with 500, 1000 and 1500 mg/kg of extract & distilled water for the first four days, and with distilled water, extract and CCl4 on the last three days. Animals were subsequently anaesthetized and blood samples were collected for alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein and albumin assays; liver organ was isolated and processed for histopathological studies. The results showed that the levels of AST, ALT, ALP and total bilirubin were significantly higher in rats treated with CCl4 indicating liver injury, while these parameters were reduced significantly (p < 0.05) after treatment of rats with the extract. The hepatoprotective activity of *C. albidum* was also supported by histopathological studies of liver tissue. The liver tissue of rats in the group treated with CCl4 showed marked centrilobular fatty degeneration and necrosis while the groups treated with plant extract showed signs of protection against this toxicant as evidenced by the absence of necrosis.

Keywords: *Chrysophyllum albidum*, Sapotaceae, carbon tetrachloride, hepatoprotective property, histopathological studies.

INTRODUCTION

The liver is the central organ in the metabolism and detoxification of drugs and toxins. Consequently, drugs affect the liver more frequently than any other organ and place the liver at great risk for toxic damage (Bussieres and Habra, 1995). After absorption by the intestines, drugs reach the liver via the portal system. In the hepatocytes, these chemicals undergo complex metabolic processes to be converted into hydrophilic substances, readily soluble in the blood stream and easily eliminated thereafter (Lee, 2003). Drugs or their metabolites can cause toxic effect on the liver. Many of the intermediate metabolites have a short half-life, some estimated to be less than a minute, which makes detecting them a challenging task (Park et al., 2005). This chemical-driven liver damage is referred to as hepatotoxicity. The use of herbal medicine can be traced back to 2100 BC in ancient China at the time of the Xia dynasty and during the Vedic period in India. The first written reports are timed to 600 BC with Charaka Samhita in India and to 400 BC with the early notes of the Eastern Zhou dynasty in China (Dhiman and Chawla, 2005). The study of African medicinal plants has not in the past been taken as seriously, or documented as fully, as Indian and Chinese traditional medicines (Adebayo, 2010). Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied (Adebayo et al., 2010). *Chrysophyllum albidum* belongs to the Sapotaceae family and native to the Central, Eastern and Western Africa (Amusa et al., 2003). The plant is specifically distributed in Nigeria, Uganda, Niger, Cameroon and Cote d’ Ivoire (Adewusi, 1997). It is often called the white star apple and distributed throughout the southern part of Nigeria (Idowu et al., 2006). The fruit is popularly called “agbalumo” and “udara” in South Western and Eastern Nigeria respectively. From our previous investigation, we observed that the leaf extract of *C. albidum* significantly reduced the levels of liver function parameters (Adebayo et al., 2010). Many folk remedies from plant origin are tested for their potential hepatoprotective effect on liver damage in experimental animal model. Carbon tetrachloride (CCl4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts (Rubinstein, 1962; Suja et al., 2002). CCl4 is biotransformed by the cytochrome P450.
system to produce the trichloromethyl free radicals, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation (Recknagel et al., 1989). The study is therefore aimed at investigating and validating the hepatoprotective properties of the leaf extract of *C. albidum* in CCl4 induced liver cell damaged rats.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *C. albidum* were obtained from the campus of Covenant University, Canaan land, Ota, Ogun State, Nigeria in November, 2009. The plant was authenticated at the Department of Pharmacognosy, University of Lagos, Lagos, Nigeria and a voucher specimen (PCGH 435) was deposited in the herbarium for reference purpose.

**Preparation of extracts**

The procedure described by Adebayo et al. (2010) was adopted. The leaves of *C. albidum* were collected and air-dried in the laboratory for two weeks after which they were blended into fine powder. 400g were extracted with 95% ethanol. Evaporation of the extract in a rotatory evaporator (Buchi 461, Switzerland) at 40°C gave a yield of 98g.

**Experimental animals**

Male albino rats (40) of Wistar strain obtained from the University of Agriculture, Abeokuta, Ogun State, Nigeria weighing between 200-230g were used for the experiment. Animals were maintained in 12-h light: 12-h dark at a controlled temperature (25 ± 3°C), humidity (60 ± 5%) and kept in the animal house of the Department of Biological Sciences, Covenant University, Ogun State, Nigeria. The animals were allowed to acclimatize for six weeks. Feed and water were given *ad libitum*. All animals were treated in accordance with the recommendations of National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH, 1985).

**Experimental Design**

The model described by Chakraborti and Handa (1989) was employed with some modifications. The rats were divided into five groups of eight rats per group. The animals of group A served as normal control group and were given only vehicle (distilled water, 1 ml/kg b.w.) for 7 days. The animals of group B (positive control) were administered with vehicle on the first four days, and with the vehicle and CCl4 (50% solution of CCl4 in liquid paraffin, 2 ml/kg b.w.) on the fifth, sixth and seventh day. The animals of groups C, D and E were respectively administered with 500, 1000 and 1500mg/kg b.w. of ethanolic extract and distilled water for the first four days, and with distilled water, ethanolic extract and CCl4 on the last three days. Animals were subsequently anaesthetized and blood samples were collected for aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and total bilirubin, assays.

**Blood collection and preparation of sample**

At the end of the treatment period, the rats were anaesthetized in diethylether prior to dissection. The blood was then collected by cardiac puncture into lithium heparinized bottles. Plasma was obtained by centrifuging the blood at 10, 000 revolution per minute for 15 minutes into clean bottles and stored at –20°C until required for biochemical assays (Adebayo et al., 2006). The liver was also collected and fixed with 10% formaldehyde for histopathological examination.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GROUP A (Negative Control)</th>
<th>GROUP B (Positive Control)</th>
<th>GROUP C (500 mg/kg)</th>
<th>GROUP D (1000 mg/kg)</th>
<th>GROUP E (1500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>28.00 ± 4.36</td>
<td>62.80 ± 11.04</td>
<td>41.75 ± 5.27 (75.2%)</td>
<td>28.70 ± 2.22 (121.8%)</td>
<td>26.75 ± 4.21 (128.8%)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>107.86 ± 4.79</td>
<td>123.25 ± 3.75</td>
<td>104.83 ± 5.88 (17.1%)</td>
<td>91.71 ± 3.10 (16.2%)</td>
<td>88.33 ± 3.36 (17.6%)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>228.19 ± 11.09</td>
<td>487.57 ± 19.00</td>
<td>386.27 ± 28.60 (44.4%)</td>
<td>354.90 ± 11.87 (58.1%)</td>
<td>317.86 ± 31.93 (74.4%)</td>
</tr>
<tr>
<td>Total Bilirubin (µmol/L)</td>
<td>14.31 ± 3.18</td>
<td>26.41 ± 3.13</td>
<td>26.18 ± 3.87 (1.6%)</td>
<td>16.51 ± 3.43 (69.2%)</td>
<td>11.15 ± 1.68 (106.6%)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.65 ± 0.21</td>
<td>3.55 ± 0.07</td>
<td>3.98 ± 0.22</td>
<td>3.62 ± 0.34</td>
<td>3.60 ± 0.25</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.41 ± 0.56</td>
<td>6.96 ± 0.20</td>
<td>8.25 ± 1.39</td>
<td>7.43 ± 0.50</td>
<td>7.51 ± 0.81</td>
</tr>
</tbody>
</table>

Values represent mean ± SE of 8 replicates. *a* *p* < 0.05 versus positive control; *b* *p* < 0.05 versus negative control; *c* *p* < 0.05 positive control versus negative control. Values in parenthesis are percentage decrease of parameters analyzed (*p* < 0.05) after pretreatment with the extract and CCl4 with respect to the control groups.
Analysis of biochemical parameters
Commercial test kits obtained from Randox Laboratories, United Kingdom were used for all biochemical parameters measured. Standard methods were used to estimate aspartate amino transferase (AST), alanine aminotransferase (ALT) (Reitman and Frankel (1957)), alkaline phosphatase (ALP) (Tietz et al., 1983), total protein (Weichselbaum, 1946), albumin (Doumas et al., 1971) and total bilirubin (Doumas et al., 1973).

Histopathological analysis
Small pieces of liver fixed in 10% buffered neutral formalin were processed for embedding in paraffin (Aliyu et al., 2007). Sections of 5-6µm thickness were stained with hematoxylin and eosin, examined for histopathological changes under a compound microscope.

STATISTICAL ANALYSIS
All values were expressed as mean ± S.E. and Tukey’s post hoc test was done to analyze significant difference between different groups using the statistical analysis software package SPSS (version 13). Values with $p < 0.05$ were considered as significant.

Fig. 1. Photomicrograph of (a): liver of rat treated with CCl4 showing sinusoidal dilation and focal centrilobular necrosis, H&E x160 (b): liver of rat treated with CCl4 and 500 mg/kg bw of extract of C. albidum showing mild centrilobular fatty degeneration, H&E x160 (c): liver of rat treated with CCl4 and 1000 mg/kg bw of C. albidum extract showing moderate sinusoidal dilation and fatty degeneration, H&E x160. (d): liver of rat treated with CCl4 and 1500 mg/kg bw of C. albidum extract showing reduced dilation of the sinusoids and centrilobular fatty degeneration, H&E x 640 (e): liver architecture showing normal features of control group H&E 400×.
RESULTS

Effect of ethanolic leaf extract of *C. albidum* on CCl4 induced liver injury in rats with reference to biochemical changes in plasma is shown in Table 1. The CCl4 treated (positive control) group showed a significant (*p* < 0.05) increase in the activity of aspartate aminotransferase (AST) (62.80 ± 11.04), alanine aminotransferase (ALT) (123.25 ± 3.75), alkaline phosphatase (ALP) (487.57 ± 19.00) and plasma total bilirubin (26.41 ± 3.13), indicating the liver injury caused by CCl4. Animals treated with the extract of *C. albidum* showed a significant (*p* < 0.05) decrease in the activity of AST (representing 75.2%, 121.8% and 128.8% reduction for groups C, D & E respectively); while the level of ALT was significantly (*p* < 0.05) reduced by 17.1%, 16.2% and 17.6% in Groups C, D & E respectively. Similarly, the activity of ALP and total bilirubin were significantly (*p* < 0.05) lowered across the groups when compared with the CCl4 treated group. However, total protein and albumin were not significantly (*p* >0.05) different in the treated groups when compared with the control groups. Histologically, rats induced with CCl4 showed sinusoidal dilation and focal centrilobular necrosis while rats treated with the extract of *C. albidum* showed significant protection against liver injury from CCl4 as evidenced in mild centrilobular fatty degeneration and reduced sinusoidal dilation across the all the treatment groups (Fig. 1).

DISCUSSION

The potency of any hepatoprotective agent is dependent on its ability to either reduce the harmful effects or maintain the normal hepatic physiological mechanism, which have been caused by a hepatotoxin (Hukkeri *et al.*, 2003). The results of biochemical parameters revealed the elevation of enzyme level in CCl4-treated group, indicating that CCl4 induces damage to the liver (Table 1). Most experiments involving the induction of liver injury by CCl4 is usually accompanied by the elevation in the levels of liver enzyme markers (AST, ALT and ALP). The elevated levels of these biochemical parameters are direct reflection of alterations in the hepatic structural integrity (Patrick-Iwuanyanwu *et al.*, 2010). Liver injury by toxicants causes cellular leakage and loss of functional integrity (Sallie *et al.*, 1991). ALT is a cytoplasmic enzyme found in very high concentration in the liver and an increase of this specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function (Adebayo *et al.*, 2010a). The elevated ALT and AST in CCl4-treated group was significantly reduced upon treatment with the extract of *C. albidum* indicating hepatoprotection from the toxicant. It was observed that the extract significantly normalized the elevated ALT and AST across the treatment groups. CCl4 treated rats showed elevated ALP activity which was significantly lowered by the extract. High level of ALP is an indicator of obstructive jaundice and intra-hepatic cholestasis (Adebayo *et al.*, 2010b). Rats treated with higher doses of the extract exhibited appreciable reduction in plasma total bilirubin suggesting the absence of jaundice and the effectiveness of the extract in activating a normal functional status of the liver. Histological findings also corroborate the biochemical investigations; the liver cells of CCl4 treated group revealed marked sinusoidal dilation and centrilobular fatty degeneration. The incidence of liver damage was reduced after with the plant extract. The result obtained is in an agreement with the findings of Sethuraman *et al.* (2003) where the ethylacetate extract of *S. brevistigma* was found to significantly reduce elevated level of AST, ALT, ALP and total bilirubin in CCl4 liver induced rats. In a similar study, these parameters were significantly lowered when rats were treated with ethanol and aqueous extracts of *P. santalinus* in CCl4 induced hepatocellular injury (Manjunatha, 2006). Some phytochemicals have been linked with hepatic protection. Active compounds like flavonoids, triterpenoids, saponins and alkaloids are known to possess hepatoprotective property (Baek *et al.*, 1996; Tran *et al.*, 2001; Vijyan *et al.*, 2003; Xiong *et al.*, 2003). Preliminary phytochemical investigation shows the presence of flavonoids, triterpenoids and tannins in *C. albidum* (Adebayo *et al.*, 2010b). Our laboratory has isolated and characterized some bioactive flavonoids and chromenes from this plant. The hepatoprotective property may be due to individual or combined effects of these phytochemicals. The exact phytochemical responsible for the hepatoprotective property needs further investigation.

CONCLUSION

The study has shown that the administration of graded doses of ethanolic extract of *C. albidum* could protect the liver from CCl4 induced liver damaged in rats. The present finding has provided information on the possible use of the plant for the treatment of hepatic dysfunction.

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