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Effect of Ethanol Extract of *Calotropis procera* Root Bark on Carbon Tetrachloride-Induced Hepatonephrotoxicity in Female Rats

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Abstract

Calotropis procera root is used in traditional medical practice for the treatment of various ailments. The possible hepatoprotective and nephroprotective activities of the ethanolic extract of *C. procera* root in female rats were investigated. Carbon tetrachloride (CCl_4) was used to induce hepatotoxicity and nephrotoxicity with significant ($P < 0.05$) increase in the level of serum enzyme markers of hepatotoxicity and nonenzyme markers of nephrotoxicity. Administration of 150 and 300 mg/kg body weight (bw) of the ethanolic extract of *C. procera* root did not protect the liver and kidney from CCl_4-induced toxicity. Pretreatment with the extract rather potentiated the toxicity induced by CCl_4. This research result did not support the reported hepatoprotective activity of the extract. It is advised strongly that caution should be taken when ingesting alcoholic preparations of *C. procera* root.

Keywords: *Calotropis procera*, Hepatonephroprotective, Nephrotoxicity, Hepatoprotective And CCL_4

1. Introduction

Since ancient times, plants, animals and mineral sources have been used to cure and prevent diseases. Plants are the most researched and have been the major source of drugs that are used in modern medicine today and will continue to provide cure for man (Halilu *et al*., 2012). Numerous medicinal plants justify the medicinal uses of herbs (Dahiru and Obidoa, 2009; Sankaran *et al.,* 2010). Hence, the efficacy of the drugs should be tested by standard experimental methods and there should be adequate data from studies to validate the therapeutic potentials (Girish *et al*., 2009).

Calotropis procera belongs to the family Asclepiadaceae and is a soft wooded, evergreen perennial shrub having few stems, few branches and relatively few leaves concentrated near the growing tip. A copious white sap (referred to as the latex) flows whenever the stems or leaves are cut (Ahmed *et al.,* 2005; Liogier, 1995; Sharma *et al.,* 1997; Howard, 1989). *C. procera* is often found growing in open habitat with little competition and it also grows favourably in dry habitat (Parrotta, 2001). *C. procera* is known by various names like Dead Sea apple, Sodom apple, Swallow wort and Milkweed (Gupta *et al*., 2012).

The capsulated root bark powder is effective in diarrhoea (Ahmad *et al*., 2005) and asthma (Singh *et al.,* 1980). The previous pharmacological studies include report of anticancer (Ahmad *et al.,* 2005), antifungal (Hassan *et al*., 2006) and insecticidal activity (Ahmad *et al*., 2006), antifertility and antiulcer activities (Kamath, 2002), hepatoprotective activity against CCl4-induced hepato-oxidative stress in rats (Patil *et al.,* 2011) and antihelmentic activity (Zaman *et al.,* 2012). The reported hepatoprotective activity of the methanolic root extract prompted us to investigate whether the ethanolic root extract of *C. procera* will also exhibit hepatoprotective and if possible nephroprotective activities in CCl_4-induced hepato-nephrotoxicity.

2. Materials and Methods

2.1. Plant Material

Calotropis procera root was collected from McBride ward Jalingo Taraba State, Nigeria in the month of January, 2011. The plant was identified and authenticated

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by the Department of Plant Science, Modibbo Adama University of Technology, Yola. The root bark was pealed and air dried under room temperature (32 ± 2^0C). The dried root bark was powdered and kept in an air tight container until use.

2.2. Animals

A total number of twenty five female Wister strain albino rats weighing between 120 – 130 g were purchased from the National Veterinary Research Institute Vom, Jos, Plateau State. The primary reason for using the female rats was availability at the time of the study. The secondary reason was the unique effect of the root bark on female as abortifacient. The root is used in formulating local abortifacients taken by women. The animals were housed in stainless steel cages in a well ventilated room and were fed with standard pelleted feed (Vital feed, Grand Cereal and Oil Mills, Ltd Jos) and water *ad-libitum* prior to the experiment.

2.3. Preparation of Extract

The root back powder 50 g was placed in a 1000 ml capacity conical flask and 500 ml of ethanol added to it. The mixture was gently stirred, tightly covered and left for 24 hours. It was then filtered using Whatman number 3 filter paper. The filtrate was then placed in a rotary evaporator at 40°C to remove the solvent. The remaining solvent was finally removed using a decitor. The stock solution of the bark extract was prepared by dissolving 1 g of the extract in small quantity of distilled water and later made up to 100 ml with distilled water.

2.4. Experimental Design

Twenty five female rats were divided into five group of 5 animals each (Groups I – V). Treatment was done as follows:

Group I: Normal control, received food and water only.

Group II: Experimental control, received 0.8 ml/kg bw of CCl_4 (1:1 in olive oil) orally.

Group III: Pretreated with 150 mg/kg bw of *C. procera* bark extract for 7 days and 0.8 ml/kg bw of CCl_4 (1:1 in olive oil) orally on the 7th day.

Group IV: Pretreated with 300 mg/kg of *C. procera* bark extract for 7 days and 0.8 ml/kg bw of CCl_4 (1:1 in olive oil) orally on the 7th day.

Group V: Extract control, received 300 mg/kg bw of *C. procera* extract for 7 days and olive oil only. In all groups, both extracts and CCl_4 were administered to rats orally using gastric tube.

The animals were anaesthetized using ether on the 9th day (48 hours after administering CCl_4). Blood was collected into a sterile centrifuge tube by puncturing the retro-orbital plexus. The blood was allowed to clot for 30 min and later centrifuged at 2500 rpm for 15 min to obtain the serum. The serum was later used to assay for the following biochemical parameters:

Alanine aminotransferase (ALT), aspartate amino-transferase (AST), cholesterol (CHOL), triglyceride (TG), total bilirubin (TB), total protein (TP), creatinine and urea. All parameters were analysed using Randox diagnostic kits (Randox Ransod Ltd, UK).

2.5. Statistical Analysis

The results obtained are presented as mean ± SD. Statistical analysis was performed by one-way Analysis of Variance (ANOVA). Individual comparisons between group means were further determined using t-test and considered significant at $P < 0.05$. Software SPSS version 13 was used for the statistical analysis.

3. Results

Results indicate that administration of CCl_4 significantly ($P < 0.05$) increased the serum levels of ALT, AST, TG and total bilirubin while significantly decreasing the serum protein level when compared to normal control. Pretreatment of Groups III and IV with 150 and 300 mg/kg bw respectively of the extract did not protect the liver against the toxicity of CCl_4. Pretreatment with the extract at both doses (Groups III and IV) significantly ($P<0.05$) increased AST serum level in a dose dependent manner with 300 mg/kg bw of the extract increasing the serum ALT levels when compared with group II (CCl_4 only). Pretreatment of rats with 300 mg/kg bw of the extract prior to administration of CCl_4 produced significant ($P < 0.05$) decrease in total protein concentration compared to normal control (Group I) and experimental control (CCl_4 only). Animals administered 300 mg/kg bw of the extract only (Group V) presented significant ($P < 0.05$) increase in serum ALT, AST and TB levels with significant ($P < 0.05$) decrease in serum TG and TP values when compared with normal control (Table 1).

Table 1. Effect of ethanol root bark extract of *C. procera* on markers of CCl_4-induced hepatotoxicity in female rats. Results are mean ± SD for 5 determinations.

GROUP	ALT (U/L)	AST (U/L)	CHOL (mg/dl)	TG (mg/dl)	TB (mg/dl)	TP (mg/dl)
Group I	11.3 ± 1.7	34.7 ± 4.1	83.2 ± 4.8	75.0 ± 1 2.7	0.19 ± 0.02	8.9 ± 0.8
Group II	30.0 ± 6.7^{*}	130.3 ± 8.7^{*}	89.7 ± 7.7	92.6 ± 7.7^{*}	2.45 ± 0.03^{*}	6.1 ± 0.7^{**}
Group III	31.3 ± 3.6	150.0 ± 8.5^{a}	92.3 ± 16.2	89.7 ± 4.7	2.21 ± 0.17^{b}	5.9 ± 0.8
Group IV	63.7 ± $5.9^{a\&}$	192.7 ± $12.8^{a\&}$	80.2 ± 5.6	94.4 ± 6.5	2.39 ± 0.11	3.1 ± 0.5^{bde}
Group V	31.8 ± 6.3^{*}	94.7 ± 8.2^{*bc}	75.1 ± 7.8	44.2 ± 3.0^{bc}	1.91 ± 0.14	3.0 ± 0.3^{b}

* Significantly ($p < 0.05$) higher when compared to Group I
a Significantly ($p < 0.05$) higher when compared to Group II
b Significantly ($p < 0.05$) lower when compared to Group II
c Significantly ($p < 0.05$) higher when compared to Group III and Group IV
& Significantly ($p < 0.05$) higher when compared to Group III
d Significantly ($p < 0.05$) lower when compared to Group III
e Significantly ($p < 0.05$) lower when compared to Group I

Administration of CCl_4 to rats was seen to significantly ($P < 0.05$) increase the levels of biochemical parameters (creatinine and urea) in serum used to evaluate kidney functions. Pretreatment of animals with both doses of extract did not protect the kidney but rather acted synergistically with CCl_4 to significantly ($P < 0.05$)

increase both levels of serum creatinine and urea. Administration of the extract alone at 300 mg/kg bw also significantly increased the levels of the two parameters (Table 2).

Table 2. Effect of ethanol root bark extract of *C. procera* on markers of CCl_4-induced nephrotoxicity in female rats

Treatment	Creatinine (mg/dl)	Urea (mg/dl)
Group I	48.9 ± 5.2	26.6 ± 0.4
Group II	64.2 ± 4.6*	32.8 ± 5.5*
Group III	83.3 ± 3.0*a	52.0 ± 0.1*a
Group IV	81.2 ± 7.1*a	48.0 ± 4.9*a
Group V	63.7 ± 6.0*	36.8 ± 5.0*

Results are mean ± SD for 5 determinations.

* Significantly ($p < 0.05$) higher when compared to Group I

[a] Significantly ($p < 0.05$) higher when compared to Group II

4. Discussion

Carbon tetrachloride induced hepatic injuries are commonly used animal models for the screening of hepatoprotective plant extracts and the magnitude of hepatic damage is assessed by measuring the level of released cytosolic transaminases including ALT and AST in circulation (Agarwal *et al*., 2006). It is established that hepatotoxicity induced by CCl_4 depends on the cleavage of the carbon-chlorine bond to generate trichloromethyl free radical ($CCl_3^{\cdot}$) that reacts rapidly with oxygen to form a trichloromethyl peroxy radical [$CCl_3O_2^{\cdot}$] (Gutierrez and Solis, 2009). This metabolite possibly attack membrane polyunsaturated fatty acids thereby causing lipid peroxidation leading to impairment of membrane function and liver injury (Gonzalez *et al*., 1995). Increased serum levels of both ALT and AST in this study confirm the destruction of the hepatocytes thereby allowing these enzymes to leak into the circulation. The decreased serum level of total proteins in the serum of carbon tetrachloride-treated rats further suggests the severity of liver toxicity. The liver is a major organ of protein synthesis and any disease in the liver can cause damage of hepatocytes with changes in protein and free amino acid metabolism leading to decreases synthesis and increase wasting via catabolism (Yousef *et al.*, 2006; Wallace, 2007 and El- Shafey *et al.*, 2011).

Administration of the extract at both doses of 150 and 300 mg/kg bw did not protect the liver and kidney of rats in this study rather it increased the severity of the damage. This could possibly be due to the toxic effect of the extract itself on the liver and kidney as evidenced by elevated levels of markers of hepato-nephrotoxicity due to the accumulation of toxic doses of the active principles of the root bark extract of the plant. Findings from this study corroborate the results of Pouokam, *et al.* (2011) who reported marked increases in these parameters after administering aqueous extract of *C. procera*. It also supports the results of Buraimoh *et al*. (2011) who showed that the ethanolic leaf extract of *C. procera* is detrimental to the integrity of the liver tissues as evident in the necrotic nature of the liver. Mohammed *et al*. (2012) also reported the toxicity of the root extract in both female and male rats after long exposure. This study contradicts the reported hepatoprotective activity of the root back extract (Basu *et al*., 1992; and Patil *et al*., 2011). This could possibly be due to different medium of extraction and the sex of the animals used. It is strongly suggested that in-depth toxicological study be carried out to eliminate the ambiguity of reports on the extract of the plant. Caution should be taken while ingesting alcoholic preparations of the root of *C. procera* for any medicinal purpose.

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