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Research Article

Hepatoprotective Fractions from Methanol Extract of *Tetracarpidium conophorum* (African walnut) seeds

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Abstract

Tetracarpidium conophorum (African walnut) is an African plant with a lot of indigenous claims. The present study was to investigate the hepatoprotective effect of fractions [Hexane (HEX-F), Dichloromethane (DCM-F) and, ethyl acetate (EA-F)] from crude methanol extract of *T. conophorum* (African Walnut) seeds in rats intoxicated with carbon tetrachloride after 24 hr and 48 hr of induction respectively. Sixty male Wistar rats were distributed equally into fifteen groups. Group IA (control) received distilled water and olive oil (i.p), group IIA rats were intoxicated with CCl_4 in olive oil (600 mg/kg, i.p.) only on the 8_{th} day, groups IIIA was given 100 mg/kg of sylimarin, while groups IVA-IXA were administered the different fractions at 250 mg/kg and 500 mg/kg doses respectively for 7 days, thereafter intoxicated with CCl_4 on the 8_{th} day. Groups XA-XVA was intoxicated with CCl_4 on the 8_{th} day and administered 250 mg/kg and 500 mg/kg of the fractions at 1hr, 6hr, 12hr, 18 hr and 24 hr. This procedure was repeated for a different set of sixty (groups IB-VXB) male rats after 48 hrs of induction. . Results show that there were significant increases ($p<0.001$) in alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl trans peptidase (γ GT) and alkaline phosphatase (ALP) activities in rats administered CCl_4 after 24 hr and 48 hr respectively, but pretreatment and post treatment with the hexane and ethyl acetate fractions possess potent hepatoprotective action against CCl_4-induced hepatic damage by lowering liver marker enzymes. Therefore methanol extract of *T. conophorum* exhibited hepatoprotective activity against CCl_4 induced liver damage.

Key Words: *African walnut, liver damage, Hepatoprotection and Carbon tetrachloride*

INTRODUCTION

The liver is the core organ involved in xenobiotic reactions and has the ability to transform hydrophobic compounds into water-soluble ones that are more easily removed by the body (Lehman-McKeeman, 2008). The activity of the liver in the uptake of xenobiotics is mediated primarily by cytochrome P_{450} (CYP_{450}). Although biotransformation reactions have a lot to do with the detoxification process that takes place in the liver, but in some cases, the metabolism of xenobiotics is injurious to cells due to the synthesis of highly reactive metabolites (free radical, reactive oxygen species) that are more toxic than the parent compound. These metabolites can react directly with cellular macromolecules or start chain reactions (Cui *et al*., 2009). Carbon tetrachloride (CCl_4) is a small, lipophilic molecule that disperses easily in the lipid compartments of the body and is metabolized in the hepatocytes. Its mechanism of toxicity needs cytochrome P_{450} that play a role in the conversion of CCl_4 to trichloromethyl (CCl_3.)(Weber *et al*., 2003) which initiate lipid peroxidation. These oxidized lipids then ravage the membranes of liver cells, causing swelling and necrosis of hepatocytes, resulting in the release of cytosolic hepatic enzymes.

T. conophorum also known as African walnut is usually called okhue in Bini, Igbo as ukpa (Igbo), and Yoruba as awusa or asala (Oke, 1995). Phytochemicals such as betulinic acid, α-amyrin, ricinine, gallic acid and vanillic acid from *T. conophorum* seed have been isolated and structurally elucidated by our research team. "This plant has several medicinal properties such as antioxidant and immunostimulatory properties (Uadia *et al*., 2012); improve fertility, antimicrobial (Ajaiyeoba and Fadare, 2006) and anticancer activities (Herbert *et al*., 1998). It is of importance to note that the methanol extract of *T. conophorum* seeds from which the fractions were obtained has hepatoprotective potentials (Oriakhi *et al*., 2018). Consequently the objective of this research is to assess the hepatoprotective effect of fractions obtained from methanol extract of *T. conophorum* seeds.

MATERIALS AND METHODS

Collection of Plant materials and Extract Preparation: *T. conophorum* seeds and extract preparation was done according to the method described by Oriakhi *et al*. (2018)

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Fractionation by Vacuum Liquid Chromatography: Precisely 300 g of methanol extract was mixed with certain amount of silica and was placed on top of a column (75 cm height, and 10cm in diameter) packed with silica gel(60–200 mesh, Merck), which was connected to a vacuum pump. Stepwise elution of column was carried out with 100 % hexane (HEX-F), dichloromethane (DCM-F), ethyl acetate (EA-F) to obtain the respective fractions. Fractions collected were evaporated to dryness and yield determined.

Hepatoprotective status of Fractions from *T. conophorum* seeds in rats induced with carbon tetrachloride after 24 h induction: Sixty (60) albino rats (Wistar strain) of average weight 150 ±10 g were used in this study, with 4 rats per group. Experimental scheme was carried out as described by Oriakhi *et al*. (2018)

- **Group I (Control):** Distilled Water only
- **Group II**: CCl_4 only (600 mg/kg in olive oil intraperitoneally)
- **Group III**: Silymarin (100 mg/kg body weight) daily for 7 days + CCl_4 on the 8th day
- **Groups IV&V**: HEX-F of *T. conophorum* seeds (250 mg/kg and 500 mg/kg body weight orally) daily for 7 days + CCl_4 on the 8th day
- **Groups VI&VII**: DCM-F of *T. conophorum* seeds (250 mg/kg and 500 mg/kg body weight orally respectively) daily for 7 days + CCl_4 on the 8th day
- **Groups VIII&IX**: EA-F of *T. conophorum* seeds (250 mg/kg and 500 mg/kg body weight orally respectively) daily for 7 days + CCl_4 on the 8th day
- **Groups X&XI**: CCl_4 + HEX-F of *T. conophorum* seeds (250 mg/kg and 500 mg/kg body weight orally respectively), at 1h, 6 h, 12 h, 18 h and 24 h.
- **Groups XII&XIII**: CCl_4 + DCM-F of *T. conophorum* seeds (250 mg/kg and 500 mg/kg body weight orally respectively), at 1h, 6 h, 12 h, 18 h and 24 h
- **Groups XIV&XV**: CCl_4 + EA-F of *T. conophorum* seeds (250 mg/kg and 500 mg/kg body weight orally respectively), at 1h, 6 h, 12 h, 18 h and 24 h

Hepatoprotective status of fractions from *T. conophorum* seeds in rats induced with carbon tetrachloride after 48 h induction: Sixty (60) albino rats (Wistar strain) of average weight 150 ±10 g were used in this study, with 4 rats per group (labeled group IB-XVB). The same design was repeated for rats intoxicated with carbon tetrachloride after 48 h of induction.

Blood Sample Collection: At the end of each experiment, blood sample was collected according to the method described by Oriakhi *et al*. (2018)

Ethics and Guidelines: Guide and care of the rats was in agreement with the principles of laboratory animal care (NIH, 1985)

Laboratory Investigation: ALT and AST activities were revealed using Radox Kit (Reitman and Frankel, 1957), while γ-GT activity was determination by Teitz (1987). Alkaline phosphatase (ALP) activity was determined using Teco kit and method described by Kochmar and Moss (1976)"

Statistical Evaluation: Statistics were expressed as the mean ± S.E.M using SPSS software. Differences between means were assessed by Duncan's multiple range tests (***p<0.001; **p<0.01; *p <0.05).

RESULTS

Hepatoprotective status of fractions from methanol extract of *T. conophorum* seeds in CCl_4 induced hepatic damage in rats after 24h of induction: The effect of Hexane, dichloromethane and ethyl acetate fractions of *T. conophorum* seeds on serum AST, ALT, ALP, and γ-GT in CCl_4 induced liver damage in rats after 24 hr is shown in Table 1.

The fractions revealed concentration dependent protection in contrast to CCl_4-induced liver injury as detected from the increased activities (P<0.001) of AST, ALT, ALP and γ-GT. The elevated concentration of these biomarkers found in the serum of rats administered CCl_4 only after 24 hr, were virtually return to normal by the fractions, except DCM-F at 250 mg/kg given to the rats, and was insignificant when compared to rats given CCl_4 only. Hex-F demonstrated the highest hepatoprotective activity, tailed by EA-F. Hex-F displayed the same therapeutic effect to that of silymarin. Post-treatment of both Hex-F and EA-F at a dose of 500 mg/kg given to the rats for every 6 h for 24 h showed notable decrease (P<0.001, P< 0.05) in AST and ALT activities when compared to group II (CCl_4) but showed no significant difference when given 250 mg/kg of the fractions.

Table 1:
Hepatoprotective status of fractions from methanol extract of *T. conophorum* seeds in CCl_4 induced hepatic damage in rats after 24 hr of induction

(n =4)	(mg/kg Body wt.)	AST(U/l)	ALT(U/l)	ALP(U/l)	γ-GT(U/l)
Group I (control)		52.50±0.50	57.90 ± 0.06	20.54±0.43	2.04±0.01
Group II (CCl_4 only)	600	***209.15±0.79$_a$	***83.37 ±0.55$_a$	***73.22±0.36$_a$	***7.55±0.15$_a$
CCl_4 + Silymarin	100	***70.40±0.50$_b$	***40.20± 0.50$_b$	***23.30±0.20$_b$	***2.20±0.02$_b$
HEX-F + CCl_4	250	***180.00±5.20$_b$	**70.12±0.42$_b$	***50.20±1.20$_b$	***5.90±0.20$_b$
HEX-F + CCl_4	500	***100.52±0.80$_b$	***41.34±0.90$_b$	***27.22±0.07$_b$	***3.50±0.05$_b$
DCM-F+ CCl_4	250	208.50±6.50$_b$	82.11±2.51$_b$	64.40±2.50$_b$	*6.80±0.25$_b$
DCM-F + CCl_4	500	***171.03±0.86$_b$	***64.49±1.19$_b$	***56.00±0.25$_b$	***5.50±0.11$_b$
EA-F + CCl_4	250	*190.00±4.80$_b$	74.00±3.50$_b$	***48.20±2.20$_b$	***4.20±0.10$_b$
EA-F + CCl_4	500	***164.56±1.65$_b$	***61.96±0.50$_b$	***33.43±0.24$_b$	***3.00±0.01$_b$
CCl_4 + HEX-F	250	198.20±8.00$_b$	82.00±1.50$_b$	**62.00±4.20$_b$	***6.00±0.02$_b$
CCl_4+ HEX-F	500	***149.03±1.36$_b$	***68.05±2.85$_b$	***37.48±0.21$_b$	***4.80±0.05$_b$
CCl_4+ DCM-F	250	208.00±3.80$_b$	83.00±5.50$_b$	70.00±3.00$_a$	***7.00±0.31$_b$
CCl_4+ DCM-F	500	204.03±0.88$_b$	75.50±1.20$_b$	**61.14±0.22$_b$	***6.20±0.22$_b$
CCl_4+EA-F	250	196.53±0.27$_b$	82.50±2.00$_b$	64.00±2.00$_b$	***5.60±0.06$_b$
CCl_4+ EA-F	500	***169.00±2.50$_b$	*71.41±0.57$_b$	***46.66±0.29$_b$	***3.20±0.02$_b$

Data were expressed as Mean ± S.E.M, n= 4 rats in each group, figures with letter a is compared with control group; while b show significant difference with the CCl_4 only group

Table 2:
Hepatoprotective status of fractions from methanol extract of *T. conophorum* seeds in CCl_4 induced hepatic damage in rats after 48 hr of induction

(n =4)	(mg/kg Body wt.)	AST(U/l)	ALT(U/l)	ALP(U/l)	γ-GT(U/l)
Normal(control)		51.80±0.35	20.12±0.22	30.54±0.43	2.60±0.25
CCl_4 only	600	***218.31±1.57$_a$	***77.15±3.02$_a$	***83.40±0.41$_a$	***6.80±0.15$_a$
CCl_4 + Silymarin	100	***63.50±0.02$_b$	***18.40±0.05$_b$	***31.90±1.50$_b$	***2.70±0.30$_b$
HEX-F + CCl_4	250	***140.65±2.88$_b$	*62.00±2.50$_b$	***46.00±2.20$_b$	5.50±0.25$_b$
HEX-F + CCl_4	500	***102.61±2.62$_b$	***34.27±0.39$_b$	***33.85±3.19$_b$	**2.90±0.20$_b$
DCM-F+ CCl_4	250	198.5±6.50$_b$	76.03±0.16$_b$	70.00±3.20$_b$	6.00±1.20$_b$
DCM-F + CCl_4	500	***159.90±6.10$_b$	74.00±0.50$_b$	*64.12±0.29$_b$	5.02±0.22$_b$
EA-F + CCl_4	250	***164.40±4.50$_b$	64.20±1.20$_b$	***50.20±2.00$_b$	4.80±0.20$_b$
EA-F + CCl_4	500	***113.96±0.76$_b$	**58.61±2.07$_b$	***31.34±4.15$_b$	**3.30±0.30$_b$
CCl_4 + HEX-F	250	***175.60±2.64$_b$	70.00±6.20$_b$	74.00±4.20$_b$	6.20±0.52$_b$
CCl_4+ HEX-F	500	***161.80±5.44$_b$	***49.52±0.45$_b$	***36.73±0.50$_b$	*3.60±0.10$_b$
CCl_4+ DCM-F	250	210.00±8.50$_b$	70.20±5.50$_b$	80.60±6.80$_b$	6.51±0.50$_b$
CCl_4+ DCM-F	500	***181.92±0.27$_b$	68.90±2.20$_b$	***54.96±5.81$_b$	5.10±0.38$_b$
CCl_4+EA-F	250	***184.40±4.80$_b$	74.80±4.00$_b$	68.00±5.20$_b$	5.80±1.50$_b$
CCl_4+ EA-F	500	***170.00±5.50$_b$	***47.57±0.27$_b$	***49.51±0.22$_b$	*3.80±0.20$_b$

Data were expressed as Mean ± S.E.M, n= 4 rats in each group, figures with letter a is compared with control group; while b show significant difference with the CCl_4 only group

There was similarly insignificant difference in AST and ALT activities when given 250 and 500 mg/kg of DCM-F, but exhibited significant decrease (P<0.01) in ALP activity when given 500 mg/kg of the fraction. However, there was a significant rise in γ-GT activity for all fractions in a dose dependent fashion, when compared to group II.

Hepatoprotective status of fractions from methanol extract of *T. conophorum* seeds in CCl_4 induced hepatic damage in rats after 48 h of induction

Hepatoprotective status of fractions (HEX-F, DCM-F, and EA-F) obtained from the methanol extract of *T. conophorum* seeds on serum AST, ALT, ALP, and γ-GT in CCl_4 induced hepatic damage in rats after 48 h is shown in table II. CCl_4 elicited substantial liver damage after 48 h, as indicated by observable increases (p<0.001) in AST, ALT, ALP and γ-GT values when compared to rats in control group. Pre-treatment with hexane fraction and ethyl acetate fraction at 250 and 500 mg/kg for 7 days bring about significant decrease (P< 0.001) in AST, ALT, ALP and γ-GT activities when compared to group II. Conversely treatment with dichloromethane fraction produced insignificant reductions in all parameters under consideration when compared to group II. Post-treatment with hexane fraction and ethyl acetate fraction at 250mg/kg and 500 mg/kg for every 6 h for 48 h caused significant reduction (P < 0.001) in AST activity when compared to rats given CCl_4 only, while there was reduction in ALT and ALP activities in rats administered 250 mg/kg and 500 mg/kg, but showed insignificant reduction (P>0.05) at a dose of 250 mg/kg. Dichloromethane fraction displayed significant reduction in AST and ALP activities at a dose of 500 mg/kg, but no significant change (P >0.05) was observed in ALT and γ-GT activities at both doses of the fraction

DISCUSSION

The current study validates the hepatoprotective effect of fractions from methanol extract of *T. conophorum* seed against CCl_4 induced hepatic injury in rats. Hepatobillary enzymes (AST, ALT and ALP) are predominantly present in the liver (Zeashan *et al*, 2008). Upon pathological damage of the liver, the enzymes leaks out into the blood stream, raising serum concentrations of these enzymes (Dolai *et al*., 2012). Thus elevated serum ALT, AST and ALP levels in CCl_4 administered rats is suggestive of cellular leakage and loss of functional integrity of cell membrane (Eidi *et al*., 2014)

In this study administration of CCl_4 at a dose of 600 mg/kg after 24 and 48 h of induction respectively causes hepatic injury in rats. Serum activities of AST, ALT, ALP and γ-GT activities were significantly elevated (P< 0.05) in the rats administered with CCl_4 only. This was congruent with the findings of Oriakhi *et al*. (2018). Pre-treatment of the fractions (250 mg/kg and 500 mg/kg) of *T. conophorum* seed revealed maximum protection against CCl_4 induced alterations in the serum enzyme levels (Table 1). The hexane fraction at both doses demonstrated greatest hepatoprotection, followed by ethyl acetate fraction. The activity of 500 mg/kg body weight of hexane fraction was analogous to that of silymarin (100 mg/kg body weight). Post-treatment of both HEX-F and EA-F at a dose of 500 mg/kg gave remarkable decrease (P<0.001, P< 0.05) in AST and ALT activities when compared to group II (CCl_4) but no significant difference when treated with 250 mg/kg of the fractions. These findings correlate with other researcher which state that walnut extract diminishes the increased enzyme activities in the plasma caused by CCl_4. Nevertheless, DCM-F was unable to ameliorate the injury of the liver brought about by CCl_4 at both concentrations of 250 and 500 mg/kg, but displayed significant decrease (P<0.01) in ALP activity when given 500 mg/kg of the fraction. There was a significant reduction in γ-GT activity for all fractions in a dose dependent manner, when compared to group II. The ability of DCM-F to lower the activities of ALP and γ-GT suggest that it could impede the liver from obstructive hepatic disease. It is of interest to note that n-hexane and ethyl acetate fractions were able to safeguard the liver from hepatic injury and this restorative property is similar to silymarin. One of the mechanisms of action of silymarin is its inhibitory effect on cytochrome P_{450} detoxification system. Therefore the hepatoprotective properties of n-hexane and ethyl acetate fractions may have resulted from their abilities to inhibit cytochrome P_{450}, and may be ascribed to the presence of the antioxidative activities of secondary metabolites in the fractions.

In conclusion, this study validated the protective activity of fractions from methanol extract of *T conophorum* seed in CCl_4

induced hepatic injury. So we recommended that the active principles present in the fraction(s) should be isolated and characterized.

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