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Tissue repair response of *Tetracarpidium conophorum* seed extract against paracetamol - induced hepatotoxicity in Wistar rats

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Abstract
This study evaluated the liver and kidney integrity of ethanolic seed extract of Walnut (*Tetracarpidium conophorum)* on paracetamol (PCM) induced hepatotoxicity via its reactive intermediate metabolite N-acetyl-1,4-benzoquinone imine (NAPQI) in albino rats. PCM is an over the counter drug and commonly abused. Overdose of PCM causes toxic effect to the liver and other tissues as endogenous enzymes will leak out to show its toxicity. The rats were divided into five groups of five rats each. (group one received n-saline, group 2: PCM alone, group 3: PCM + 250mg/kg extract, group 4: PCM + 500mg/kg extract and group 5: PCM + 750mg/kg extract. All the animals were starved for 24hrs before the administration of PCM to groups 2 - 5. After 48hrs, Gps 3 - 5 were given 250, 500 and 750mg/kg of TC extract respectively for 7 days and then the animals were sacrificed. The levels of the serum transaminase enzymes were used to assess the integrity of the liver. The oral treatment of rats with different doses of the extract showed significant ($P \leq 0.05$) hepatoprotective activity against NAPQI induced hepatotoxicity by decreasing the activities of transaminases. The HDL levels of the TC extract revealed an increase from the PCM induced level of 43.46±0.97 - 49.44±0.68mg/dl. The TC extracts also reduced the serum levels (mg/dl) of Urea and Cr^{-} from 25.66±0.31 - 20.32±0.37 and 0.68±0.02 - 0.59±0.01 respectively. These findings suggest that the walnut seed may contain cytoprotective and antihepatotoxic properties.

Keywords: Paracetamol, hepatotoxicty, cytoprotective, walnut and NAPQI

Introduction
Tetracarpidium conophorum (Juglandaceae) is also known as the Nigerian walnut, black walnut or conophor [1]. It is an important crop because it has diverse medicinal uses. The plant is cultivated principally for the nuts which are cooked and consumed as snacks [2]. The nut is rich in protein and yields high food energy value [3, 4]. It is believed traditionally in Gabon that when the seeds are eaten by the husbands of pregnant women it mitigates the risk of miscarriage [5]. Also the fresh nuts are believed to be an antidote for snake bite antidote [6]. Walnuts have been shown to decrease endothelial dysfunction associated with high fat diets [7]. Walnut is useful in treating Rheumatism, gout, cold, kidney pain, heavy menstrual bleeding, as a blood cleanser and to expel worms [8]. Different parts of the plant have been reported to show antioxidant properties [9]. Phytochemical study shows the presence of alkaloids, flavonoids, saponins, phenols and its compounds and tannins [10].
Acetaminophen or paracetamol, also known by the brand name Tylenol and Panadol, is usually well tolerated in prescribed dose, but overdose is the most common cause of drug-induced hepatotoxicity and acute liver failure worldwide [11]. The liver damage is not due to the drug itself but to its metabolite (N-acetyl-p-benzoquinone imine (NAPQI)) which is toxic and produced by cytochrome P-450 enzymes in the liver [12]. Under normal circumstances, this metabolite is detoxified by conjugating with a phase 2 enzyme, glutathione. In an overdose, a large amount of NAPQI is generated, which overwhelms the detoxification process and leads to liver cell damage. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants that in turn, injure hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also lead to oxidative stress [13]. Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside the liver. This promotes further liver damage [14]. Different parts of the plants have been reported to have polyphenolic compounds and antioxidant properties [9, 20] and based on these therefore, the study investigated the cytoprotective effect of *Tetracarpidium conophorum* seed extract on NAPQI induced liver damage.

Materials and Method

Plant Collection and Extraction

Fresh walnut seeds were collected from Eziome village in Mbieri town of Imo State, Nigeria in the month of June. The walnuts were identified and authenticated in the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, Abia State University, Uturu by the Institutions herbarium, Chikodi Okechukwu. A voucher specimen (ABSU/FB50) has been deposited in the Institutional herbarium for reference purpose.

Plant Extraction

The seeds were separated from their husks and other inner particles, after which the seeds were sliced into smaller fractions, oven-dried and powdered mechanically. 130g of the sample was extracted in 95% ethanol, with the aid of the soxhlet extraction unit. The extract was obtained after extraction, taken to the rotary evaporator, where the ethanol was fully evaporated leaving the crude extract which was kept in an air tight container ready for use.

Animals

This study was carried out using albino rats of both sexes weighing between 121 - 198g. They were obtained from the animal house of the department of zoology, University of Nigeria, Nsukka. The rats were randomly divided into 5(five) groups of 5 animals each and housed in plastic cages and allowed free access to feed and water *ad libitum*. They were acclimatized to laboratory condition for 2 weeks before commencement of the experiment and maintained under standard laboratory conditions of temperature (20-25 ^{0}C, 12 h/12 h light/ dark cycles). The conduct of this study was guided by the provisions of the experimental ethics committee on Animal use of the Faculty of Biological Sciences, Abia State University, Uturu, Abia State, Nigeria.

Experimental Protocol

A total of 25 rats were randomly divided into 5 groups of 5 rats each comprising of Group 1 serving as normal saline (control), Group II serving as negative control PCM (3g/kg), group III -PCM (3g/kg) + 250mg/kg TC extract, Group IV received PCM (3g/kg) + 500mg/kg TC extract and Group V received PCM (3g/kg) + 750mg/kg TC extract

Paracetamol induced toxicity model

This model is used to produce experimental liver damage. Paracetamol was administered orally as a single dose of 3g/kg. After 48 h, they were treated with the test drugs for 7 days. At the end of the experiment blood was withdrawn by cardiac puncture for biochemical profile studies to determine the levels of ALT, AST, ALP, Serum Cholesterol, albumin, total proteins, bilirubin, and creatinine. The value of the test is compared with the control using suitable statistical analysis

Sample Collection

The rats were sacrificed anesthetically with chloroform and blood withdrawn by cardiac puncture for biochemical analysis in sterilized centrifuge tubes. They were allowed to clot at room temperature before being spun in the centrifuge at 3000pm for 10 minutes for estimation of biochemical parameters, including triglycerides (TG), high- density lipoprotein (HDL), creatinine and urea using established procedures using kits from Randox Laboratories Ltd UK.

Statistical Analysis

The results were expressed as multiple comparisons of mean ± standard error of mean (SEM). One-way Analysis of Variance (ANOVA) was used to determine the significance and the probability level of less than 5% was considered as significant.

Results

Table 1: Effects of *Tetracarpidium conophorum* on liver function indices of paracetamol-induced hepatotoxicity in wistar rats.

Groups	Treatment	ALT (U/L)	ALP(U/L)	ALP(U/L)	T. Bilirubin(g/dl)
1	Normal control	26.40±1.57^{d}	40.20±0.66^{d}	66.20±0.80a	0.75±0.03^{c}
2	PCM (3g/kg)	53.00±2.10^{a}	127.20±1.16^{a}	67.80±0.86a	1.50±0.05^{a}
3	PCM + TC (250mg/kg)	36.60±0.75^{b}	126.60±0.81^{a}	61.80±0.80b	1.32±0.04^{b}
4	PCM + TC (500mg/kg)	29.40±1.03^{cd}	110.40±0.93^{b}	62.00±0.71^{b}	1.16±0.05^{b}
5	PCM + TC (750mg/kg)	31.40±1.33^{c}	106.00±2.21^{c}	57.60±0.93^{c}	1.24±0.08^{b}

Results are expressed as means±SEM. n=5 Means accompanied by similar lowercase letters in the same column are not significantly different at $p< 0.05$ by ANOVA.

Table 2: Effects of *Tetracarpidium conophorum* on biochemical indices of paracetamol- induced toxicity test.

Groups	Treatment	Urea(mg/dl)	Creatinine(mg/dl)
1	Normal control	15.54±0.45^{d}	0.56±0.01^{c}
2	PCM	25.66±0.31^{a}	0.68±0.02^{a}
3	PCM + TC (250mg/kg)	22.56±0.67^{b}	0.61±0.01^{b}
4	PCM + TC (500mg/kg)	19.10±0.82^{c}	0.60±0.01^{bc}
5	PCM + TC (750mg/kg)	20.32±0.37^{c}	0.59±0.01^{bc}

Results are expressed as Means±SEM. n=5. Means accompanied by similar lower case letters in the same column are not significantly different at $p< 0.05$ by ANOVA.

Table 3: Effects of *T. conophorum* on lipid profile of Paracetamol induced wistar rats.

Groups	Treatment	T. Cholesterol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)
1	Normal control	82.10±0.94^{b}	77.70±0.79^{a}	50.06±0.51^{a}
2	PCM	87.40±0.61^{a}	79.88±1.67^{a}	43.46±0.97^{b}
3	PCM + (250mg/kg)	59.96±1.36^{d}	73.98±0.46^{b}	44.98±1.45^{b}
4	PCM + (500mg/kg)	65.94±1.85^{c}	77.74±0.61^{a}	49.24±0.53^{a}
5	PCM + (750mg/kg)	67.68±1.27^{c}	77.28±0.54^{a}	49.44±0.68^{a}

Results are expressed as means±SEM. Means accompanied by similar lowercase letters in the same column are not significantly different at $p< 0.05$ by ANOVA.

The result of the effects of *Tetracarpidium conophorum* seed extract on biochemical indices of paracetamol-induced wistar rats is presented in table 1. The result revealed an increase in the serum levels of ALT, AST, ALP, and Total bilirubin, following the administration of PCM (3000mg/kg) per body weight of the animals. This increase was statistically significant ($p< 0.05$) between the control for ALT, AST and Total Bilirubin. In the same vein, *T. conophorum* seed extract also caused a reversal of the serum levels of ALT, AST, ALP and Total Bilirubin. when compared with negative control group. This decrease following the administration of the plant extract was statistically significant ($p< 0.05$) in a dose dependent manner. However, a non-significant difference ($p>0.05$) was observed in the level of ALP between the control and the acetaminophen-induced group.

The result of the effects of *T. conophorum* on kidney function indices of paracetamol induced wistar rats is presented in table 2. The result revealed a significant increase ($p< 0.05$) in the level of urea and creatinine following the administration of paracetamol to the experimental animals, when compared to the normal control, that received n-saline. However, a significant ($p< 0.05$) reduction was observed following the administration of the seed extract of the plant at different doses of 250, 500 and 750mg/kg per body weight when compared with the PCM induced group.

The result of the effects of *T. conophorum* on liver profile of Paracetamol-induced wistar rats is presented in table 3. The results showed a significant variation ($p< 0.05$) in the serum levels of cholesterol, Triacylglycerol, and high density lipoprotein (HDL) following the administration of paracetamol to the animals *viz*-a-*viz* the normal control. The extract of the plant elicited a decrease in the level of total cholesterol and triacylglycerol when compared to the toxicant group. PCM showed a significant reduction ($P< 0.05$) in HDL when compared with the control though has a comparable level with TC (250mg/kg) hence, the level of HDL was significantly increased ($p< 0.05$) after the administration of the intervention group (500 and 750mg/kg).

Discussion

Paracetamol is commonly abused as an Over the Counter (OTC) drug. Its overdose causes hepatic damage [15, 16] by its reactive metabolite NAPQI via oxidative processes and free radical production. In this study, PCM caused significant increases in the levels of Creatinine and Urea compared to the control group. Elevations in these serum levels are indicative of renal toxicity [17]. The effect of TC administration reversed these increases caused by PCM showing its ameliorative effect on the kidney. This could be attributed to its Flavonoid content eliciting its antioxidant properties. In the same trend, PCM alone elicited a statistical increase in serum TG and Total Cholesterol with a reduction in HDL but were reversed by intervention group (250, 500 and 750mg/kg) which is in line with another work being reported [18]. This could also be attributed to its saponin content which has been reported to cause a reduction in TGs and T.cholesterol [19, 20] indicating that the extract possesses cytoprotective activity.

On the liver biomarkers, the statistical significant increase elicited by PCM on serum levels of ALT, AST and Total Bilurubin reversed by intervention group of all doses revealed that TC extract also possesses a hepatoprotective activity. This suggests that the biochemical restoration could be due to the ability of the extract to inhibit cytochrome P450 or ability to promote PCM glucuronidation [21].

The phytochemical constituents of *Tetracarpidium conophorum* revealed the presence of Alkaloids, Flavonoids, Steroids, Phenols, Tannins and Saponins and Vitamin content with appreciable amount of vitamins A, C and E [10]. Flavonoids have been reported to exhibit antioxidant [22, 23] and hence hepatoprotective activities. Also saponins possess hepatoprotective activity through modulation of its antioxidant properties [24]. Tannins equally exerts free radical scavenging and hepatoprotective activities [25]. Based on these reports from its phytochemical constituents, it shows or suggests that T.C induced-hepatoprotective activities could have been possible from the synergistic involvement of flavonoids, saponins and tannins. The mechanism of protection may have been via activation of liver regeneration, by the enhancement of protein and Glycoprotein synthesis or rapid detoxification and excretion [26] or prevention of lipid peroxidation process and stabilization of hepatocyte membranes [27]. Also the contribution of powerful antioxidants, Vitamin C and E contained in walnut seed may have aided in the cytoprotective activities.

Conclusion

The results obtained in this study suggest that the seed extract of Walnut (*Tetracarpidium conophorum*) possesses hepatoprotective and cytoprotective activities against Paracetamol- induced hepatotoxicity. These positive effects may have been elicited through the synergistic involvement of the Phytochemical compounds present.

Authors' Contributions

NC designed and managed the research work, AC analyzed the data and prepared the draft of the manuscript, EO was involved in the collection of plants and animals / plant extraction. NC and SO performed the experiment. All authors participated in the reading and confirmation of the final version of the manuscript for publication.

Conflict of Interests: The authors declare no conflict of interest

Ethical consideration

All the experimental procedures involving the use of animals were conducted in accordance to the guiding principles for research as recommended by the Organization for Economic Co-operation and Development and approved by the experimental ethics committee on animal use of the Faculty of Biological Sciences, Abia State University, Uturu Abia State, Nigeria.

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