ACUTE AND SUB CHRONIC TOXICITY PROFILE OF METHANOL EXTRACT OF *TETRACARPIDIUM CONOPHORUM* SEEDS ON WISTAR ALBINO RATS

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ABSTRACT

Tetracarpidium conophorum has been reported to possess hepatoprotective, antioxidant, and anti-diabetic properties. This study aimed to determine the toxicity profile of methanol extracts of T. *conophorum* seed, both acutely and sub chronically. The test for acute toxicity was performed using a modified Lorke method. There were four groups of five rats each for the examination of sub-chronic toxicity. Over the course of 30 days, groups two, three, and four received 500, 1000, and 2000 mg/kg of the extract, respectively, with group one serving as the control. The extract did not cause any toxicity symptoms or rat mortality during the acute toxicity test, while the median lethal dose (LD50) of the methanol extract of T. *conophorum* was determined to be ≥ 5000 mg/ kg body weight. Results also show that sub-chronic administration of the extract did not significantly (p > 0.05) influence body weight, hematological parameters, and food intake. Relative organs weights were not significant (p > 0.05) compared to the control, while the serum triglyceride levels were significantly (p < 0.05) elevated in 500 and 1000 mg/kg extract-treated groups without significant alterations of other biochemical parameters (liver enzymes, renal function, antioxidant enzymes). This study shows that T. *conophorum* seed extract is safe, as demonstrated by the acute and sub-chronic toxicity.

Keywords: *Tetracarpidium conophorum,* toxicity, extracts, acute, sub chronic

INTRODUCTION

Medicinal plants and products play an essential role in our daily lives. Since the dawn, people have used plants or parts of them as food and medicine. According to reports, over 70% of Africans and Asians depend on natural product remedies (WHO, 2015). This is due to the fact that they are usually affordable and readily available. Several current and established objectives exist for employing plants as sources of medicinal compounds (Awodele *et al*., 2013). These comprise the isolation of bioactive components, the structural elucidation of lead compounds for the synthesis of pharmacological molecules that would function as pharmacologic instruments, and the use of the entire plant or a portion of it as herbal medicine (Atanasov *et al*.,2015; Oreagba *et al*., 2011). Metabolites from plants combines to create complex substances that can be beneficial or detrimental. The lack of a specific dose is one of the major drawbacks of using ethnomedicinal plants (Adeosun *et al.,*2022). However, most advanced countries impose certain degrees of laws and regulations for the safety monitoring, product standardization, and quality assurance of any such natural substance (Aliakbarzadeh *et al*., 2016; Stickel *et al.,* 2015). However, some practitioners of alternative and complementary medicine routinely criticize the WHO certification program's attempts to regulate the quality of natural goods (WHO, 2015). This explains why there are divergent opinions regarding the various medicinal benefits of plants (Awodele *et al*., 2013).

Scientists have developed three concepts to guarantee safety. Any substance or product that benefits a living creature must first undergo a study to demonstrate its safety characteristics. The second is to evaluate the chemical makeup of the conventional therapeutic ingredient. Toxicological reports are therefore required for every medical plant or product and for verifying their public acceptance (Kale *et al*., 2016). The last phase establishes the rules for looking into the suggested folkloric application, a step toward developing and discovering new drugs. Numerous government organizations

have continued to publish data on herbs, including usage patterns, toxicity information, results of clinical trials, and analysis of side effects associated with herbal medicines. Studies have connected several therapeutic effects to an antioxidant system that aids in combating the numerous types of free radicals continuously produced by the body to meet its unique metabolic needs. For regularly used herbal medications, including ginger, ginseng, milk thistle, and turmeric, among others, reports from animal research about their economic significance, toxicological effects, and herb-drug interactions have been published (El-Seedi *et al*., 2013; Wang *et al*., 2011). Nevertheless, despite initiatives to advance drug discovery and development, only a small subset of medicinal plants has been studied and assessed for its potential to be poisonous.

The aforementioned facts necessitate assessing the toxicological profile of *Tetracarpidium conophorum,* commonly called the African walnut. *T. conophorum* is a perennial climber that grows in sub-Saharan Africa's moist woodland zone (Oke, 1995). It is cultivated mainly for its nuts which can be boiled, roasted, or fried (Edem et al., 2009; Oke, 1995). Compared to other nuts, African walnuts have an adequate amount of Omega-3 fatty acids and negligible Omega-6 fatty acids (Bassey *et al*., 2009). According to Uadia *et al*. (2012), this plant has various therapeutic benefits, including immunostimulatory and antioxidant qualities. Based on this background, we sought to assess the acute and sub-chronic toxicological effects *of* methanol extract of *Tetracarpidium conophorum* seeds in female Wistar rats.

MATERIALS AND METHODS

Collection of Plant materials and Preparation of Plant Extract

T. conophorum seeds were gathered in November 2021 from an open forest in Edo State, Nigeria. The fresh walnut seeds' authenticity was confirmed by the Department of Plant Biology and Biotechnology at the University of Benin,

Nigeria and herbarium specimen was assigned voucher number UBHe0153. The seeds were cleaned, shelled, chopped into bits, and dried. After being dried, the samples were pulverized and kept in an airtight container. Thereafter, 1 kg of *T. conophorum* seed powder was extracted for 72 h in 5000 mL of 100% methanol. The samples were filtered and the filtrate was then evaporated using a rotary evaporator, yielding 32%. The resulting yield was kept in an airtight bottle and refrigerated at 4ºC.

Animals

Female Wistar rats (140 ± 10 g) were utilized for this study. The animals were obtained from the University of Benin's animal house, where they were kept. They were accustomed to laboratory circumstances for 7 days prior to the study. The animals were provided with regular rodent food and free access to fresh water. All animal testing was done in accordance with NIH criteria (National Research Council, 1985).

Acute Toxicity Testing

Twelve female Wistar albino rats, were acclimatized for a week in clean cages before being randomly separated into 4 groups of 3 animals each. According to Lorke (1983) method, groups 1, 2, and 3 received 10, 100, and 1000 mg/kg body weight *T. conophorum* methanol extract daily for 14 days. Water was given orally to the control group's fourth group every day for 14 days. The animals were watched for any changes in behavior over the course of 14 days, including excitement, exhaustion, diarrhea, itching, curled tails, shivering, hair loss, and mortality.

Determination of median lethal dose (LD$_{50}$)

Based on the acute toxicity test findings, three groups of Wistar female albino rats weighing an average of 150 ± 10 g each was given 1600, 2900, and 5000 mg/kg body weight of the methanol extract of   *T. conophorum* orally. Over the course of 24 h, death was monitored. Then, the Lorke (1983) method was used to calculate the LD$_{50}$. The geometric mean of the dose that resulted in 100% death and the dose that produced no mortality was used to compute the acute toxicity LD$_{50}$. After two weeks of watching the animals, the investigations ended. Recovery and weight increase following each examination were seen as indicators of test survival.

Sub-chronic Toxicity Study

For this investigation, twenty adult female albino rats were employed, which were split into four groups of five each. The methanol extract of *T. conophorum* seeds was administered orally to three of the groups at doses of 500, 1000, and 2000 mg/kg body weight each day for 30 days, whereas the control group got just distilled water during that time. Rats were given an overnight fast after being exposed for 30 days, and then their blood was drawn via heart puncture for hematological and biochemical tests. Then, the animals were sacrificed, and the kidney, liver, heart, lungs, and spleen were removed and weighed. The wet weight of each organ and the animal's total weight were used to compute the relative weight of the various organs.

(i) Weekly body weight

The rats body weight was recorded using a sensitive balance during the acclimatization period, once before dosing began, once every week throughout the dosing period, and once on the day of sacrifice. The weekly dose of administration was determined based on weekly variations in body weight.

(ii) Relative organ weight

The animals were all put to death by cervical dislocation on the 30th day. Different organs, including the heart, liver, lungs, spleen, kidneys, and testes, were removed and weighed (absolute organ weight). Using the procedure described in (Aniagu *et al.,* 2005), the relative organ weight of each animal was determined.

$$Re\,l\,ativeorganweight = \frac{Absoluteorganweight(g)}{Bodyweightofrat(g)}\,x100$$

Blood Sample Collection

After the administration of the methanol extract of *T. conophorum* for 24 h in the acute toxicity and 30 days in sub chronic toxicity, the female Wistar albino rats were made unconscious by cervical dislocation. The fasting blood was collected from a prominent artery and directly from the heart after dissecting the female Wistar albino rats into 3 different sample test tubes. A portion was immediately transferred into tubes containing EDTA for the automated hematology analyzer (Sysmex America Inc., USA) to measure the hematological parameters. A second quantity of blood was placed in plain tubes for serum biochemical analysis. With the aid of standard Randox kits, the blood was centrifuged, and the serum examined for levels of creatinine, urea, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total and direct bilirubin, high-density lipoproteins (HDL), albumin (ALB), total and direct bilirubin, and cholesterol. Using an Ilyte auto-analyzer, sodium, potassium, chloride, and bicarbonate ions were measured.

Statistical analysis

Where appropriate, the data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range tests using Graph Pad Prism Version 7.p values $\leq$ 0.05 were regarded as significant. The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates.

RESULTS

Acute Toxicity

In those receiving 1000 mg/kg body weight within 24 hours, neither physical signs of intoxication nor mortality were noticed. However, in the second phase, rats that received 1600, 2900, and 5000 mg/kg body weight initially displayed symptoms of hyperactivity but afterward appeared calm. Throughout the 24-hour monitoring period and the two weeks that followed, no deaths were noted in these groups. The methanol extract of *T. conophorum* has an estimated median lethal dose (LD50) of 5000 mg/kg body weight.
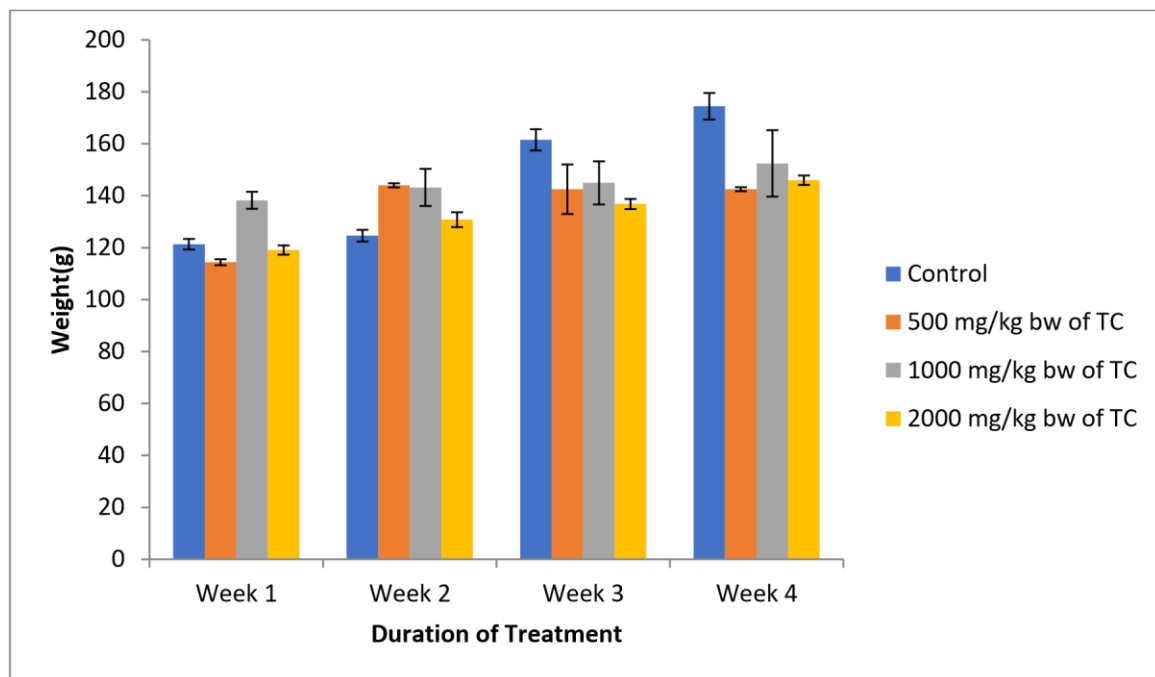
Figure 1: Sub chronic toxicity of methanol extract of *T. conophorum* seeds on body weight.

Table 1: Effect of various doses of methanol extract of *Tetracarpidium conophorum* seeds on relative % weight of organ

Organs	Treatment (mg/kg body weight)			
	Control	**500**	**1000**	**2000**
Liver	2.70±0.65[a]	3.37±0.38[a]	3.50±0.19[a]	3.31±0.32[a]
Lungs	0.98±0.03[a]	1.00±0.08[a]	1.08±0.06[a]	1.15±0.08[a]
Kidney	0.73±0.08[a]	0.69±0.01[a]	0.84±0.06[a]	0.78±0.06[a]
Spleen	0.36±0.01[a]	0.44±0.03[a]	0.46±0.03[a]	0.45±0.003[a]
Heart	0.44±0.01[a]	0.45±0.03[a]	0.42±0.03[a]	0.45±0.07[a]

Values are expressed as mean± SEM(n=5). Different lower-case letters represent significant difference between means at $P < 0.05$.

Haematological parameters

Table 2 shows that there was a significant increase ($P < 0.05$) in the white blood cell counts (WBC) in rats administered 1000 and 2000 mg/kg of methanol extract of *T. conophorum* seeds. Also, there was a significant increase ($P < 0.05$) in lymphocytes when the rats were given methanol extract of *T. conophorum* seeds at all doses investigated when compared with the control. In contrast, the extract significantly reduced ($P < 0.05$) platelets at all doses investigated when compared with the control.

Table 2: Sub chronic toxicity of methanol extract of *T. conophorum* seeds on haematological parameters

Parameters	Treatment (mg/kg body weight)			
	Control	**500**	**1000**	**2000**
WBC (10^3/µl)	[a]7.15 ± 0.55	[a]9.40 ± 0.40	[b]10.00 ± 0.08	[b]10.05 ± 0.05
RBC (10^6/ µl)	[a]8.75 ± 0.28	[a]8.52 ± 0.06	a9.52 ± 1.36	[a]8.33 ± 0.16
HB (%)	0.73 ± 0.08	0.69 ± 0.01	0.84 ± 0.06	0.78±0.06
HCT (g/dl)	36.00 ± 0.01	44.00 ± 0.03	46.00 ± 0.03	45.00±0.003
MCV (fl)	0.44 ± 0.01	0.45 ± 0.03	0.42 ± 0.03	0.45±0.07
MCH (pg)	[a]19.15 ± 0.15	18.95 ± 0.15	19.50 ± 0.50	19.05±0,15
MCHC (%)	35.15 ± 0.35	35.50 ± 0.50	33.05 ±0.05	34.40±0.10
LMY (%)	63.55 ± 2.05	81.05 ±7.65	90.05±2.95	92.10±2.70
PLT (10^3/ µl)	[a]573.00 ± 63.00	[b]484.00 ± 26.00	[c]272.50 ± 6.50	[d]119.50 ±1.50

Data are expressed as mean ± SEM (n=5). Different lowercase letters represent significant difference between means at $P < 0.05$. WBC; white blood cell count, RBC; red blood cell count, HCT; haematocrit, HB; haemoglobin, MCH; mean cell haemoglobin; MCV; mean cell volume, MCHC; mean cell haemoglobin concentration, PLT; platelet count, LYM; lymphocyte, Mo; monocytes

Effect of methanol extract of *T. conophorum* seeds on liver enzymes

Figure 2 reveals the effect of methanol extract of *T. conophorum* seeds on serum ALT, AST and γ-GT activities in both control and test rats. The effect of methanol extract of *T. conophorum* seeds on serum ALT, AST and γ-GT activities were not significantly different ($P > 0.05$) in the test groups when compared to control.

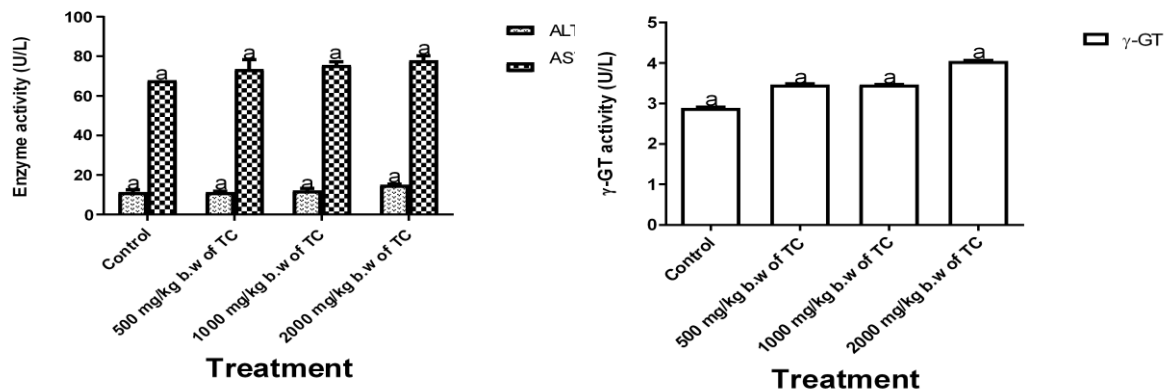Figure 2: Sub chronic toxicity of methanol extract of *T. conophorum* seed on ALT, AST and γ-GT activities

Effect of methanol extract of *T. conophorum* seeds on serum liver metabolite

The effect of methanol extract of *T. conophorum* seeds on both serum albumin, total protein, total and direct levels were not significantly different ($P > 0.05$) in the test groups when compared to control.
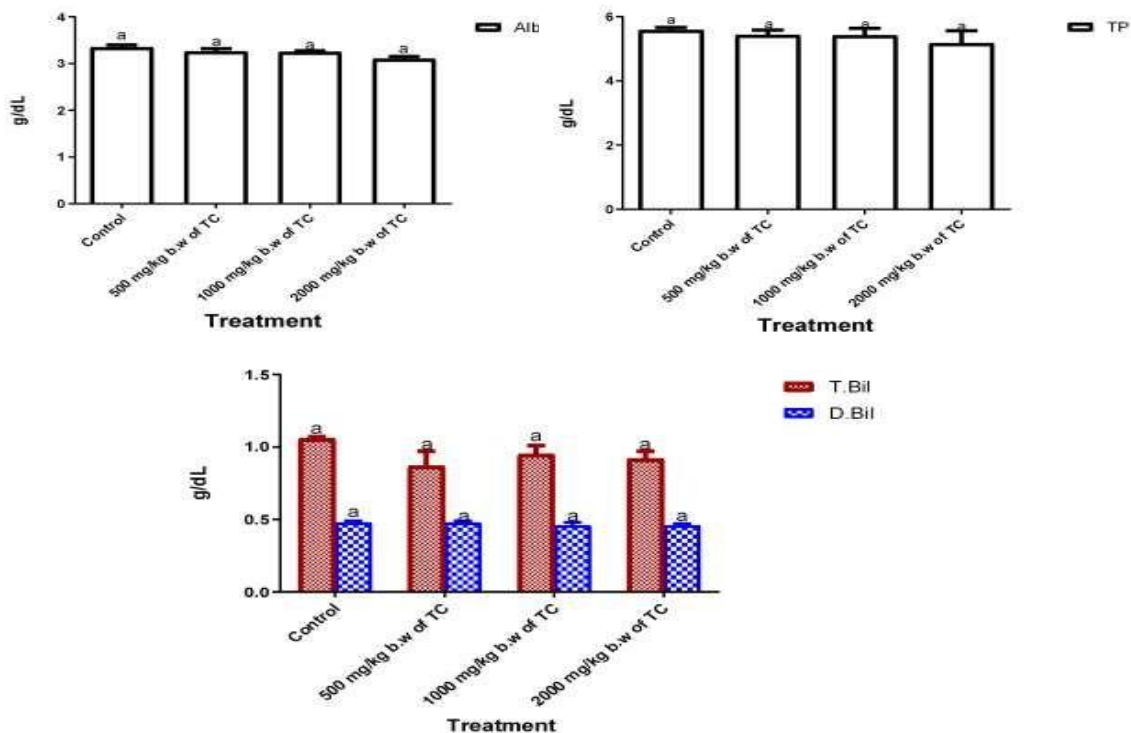

Figure 3: Sub chronic toxicity of methanol extract of *T. conophorum* seeds on serum albumin, total protein, total and direct bilirubin levels

Effect of methanol extract of *T. conophorum* seed on Kidney function

The effect of a methanol extract of *T. conophorum* seeds on the blood levels of urea and creatinine in test and control rats is shown in Figure 4. Rats given 500, 1000, and 2000 mg/kg body weight of the methanol extract of *T. conophorum* did not differ significantly from controls in terms of serum creatinine levels ($P > 0.05$). However, it was noted that rats given 1000 and 2000 mg/kg body weight of the extract had slightly higher urea levels than the control group.
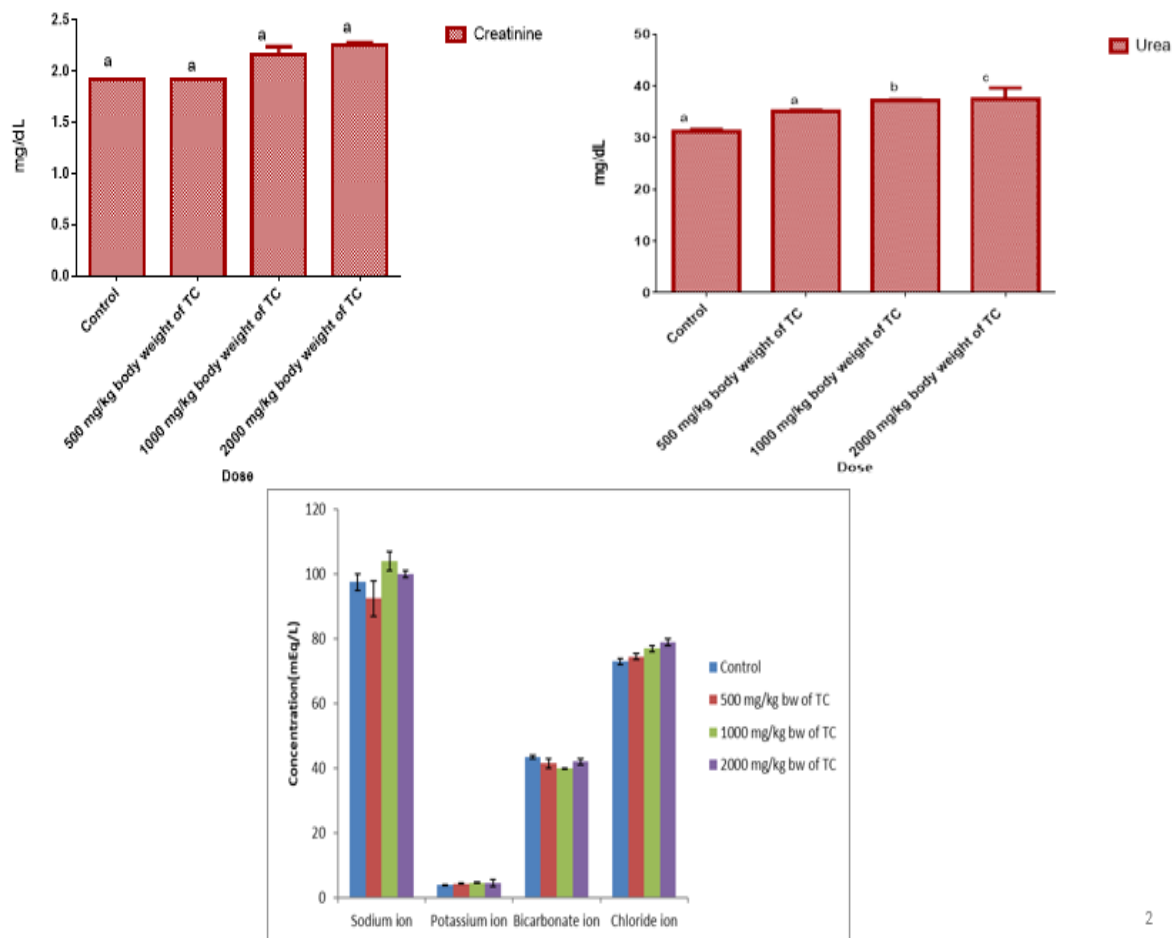
Figure 4: Sub-chronic toxicity of methanol extract of *T. conophorum* on serum creatinine and urea levels

Effect of methanol extract of *T. conophorum* seeds on Lipid Profile

Figure 5 shows the effect of methanol extract of *T. conophorum* seeds on serum Total cholesterol, HDL-C, LDL-C and Triglycerides levels in both control and test rats. The effect of methanol extract of *T. conophorum* seeds on serum Total cholesterol, HDL-C and LDL-C levels was not significantly different ($P > 0.05$) in the test groups when compared to control. However, there is a significant increase ($P < 0.05$) in serum triglycerides in rats administered with 2000 mg/kg body weight of methanol extract of *T. conophorum* seeds when compared to control.
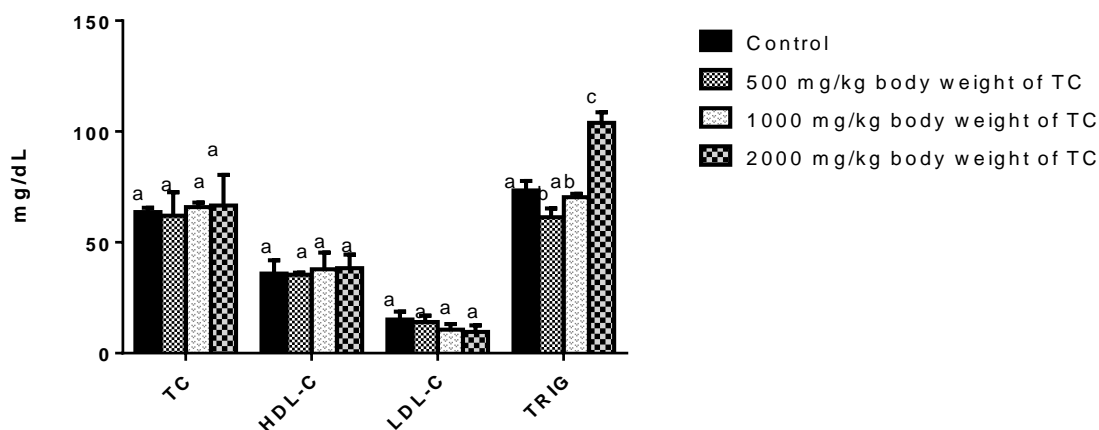

Figure 5: Sub-chronic toxicity of methanol extract of *T. conophorum* seeds on serum lipid profile levels

Effect of methanol extract of *T. conophorum* seeds on Creatine kinase activities

The effect of methanol extract of *T. conophorum* seeds on creatine kinase (CK) in rats is shown in Figure 6. The result show that there were no significant differences in the creatine kinase activities in rats administered 500 and 1000 mg/kg body weight of the methanol extract of *T. conophorum* seeds respectively compared with control. However, rats administered 2000 mg/kg of methanol extract of *T. conophorum* significantly decrease CK activity when compared to the control.
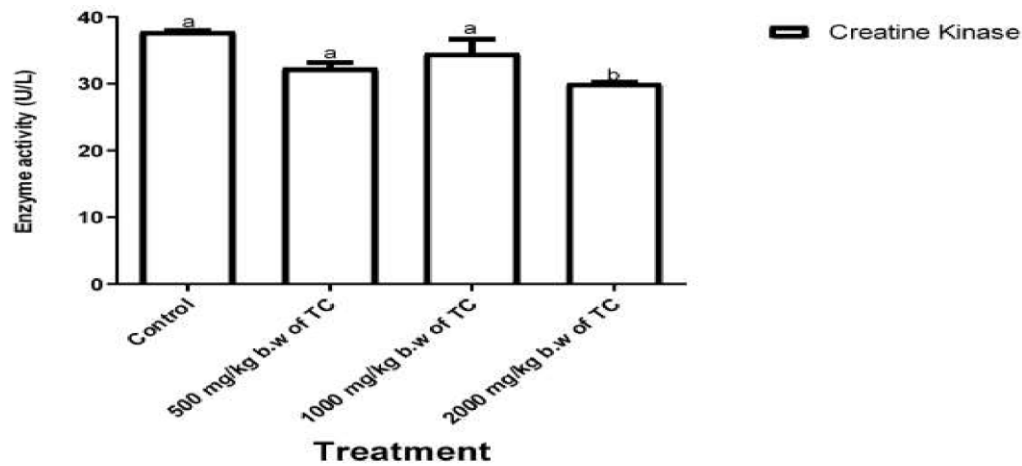
Figure 6: Sub-chronic toxicity of methanol extract of *T. conophorum* on serum creatine kinase activities

Effect of methanol extract of *T. conophorum* seeds on Antioxidants enzymes

The effect of methanol extract on antioxidant enzymes is shown in Figure 7. The results show that there were no significant differences in superoxide dismutase (SOD), catalase (CAT) activities in rats administered 500, 1000 and 2000 mg/kg body weight of the methanol extract of *T.* *conophorum* seeds respectively compared with control. However, there was a gradual increase in superoxide dismutase activities in a dose dependent manner. Similarly, a dose dependent increase in glutathione peroxidase and glutathione S transferase activities in rats administered 500, 1000 and 2000 mg/kg body weight of *T. conophorum* seeds when compared to control
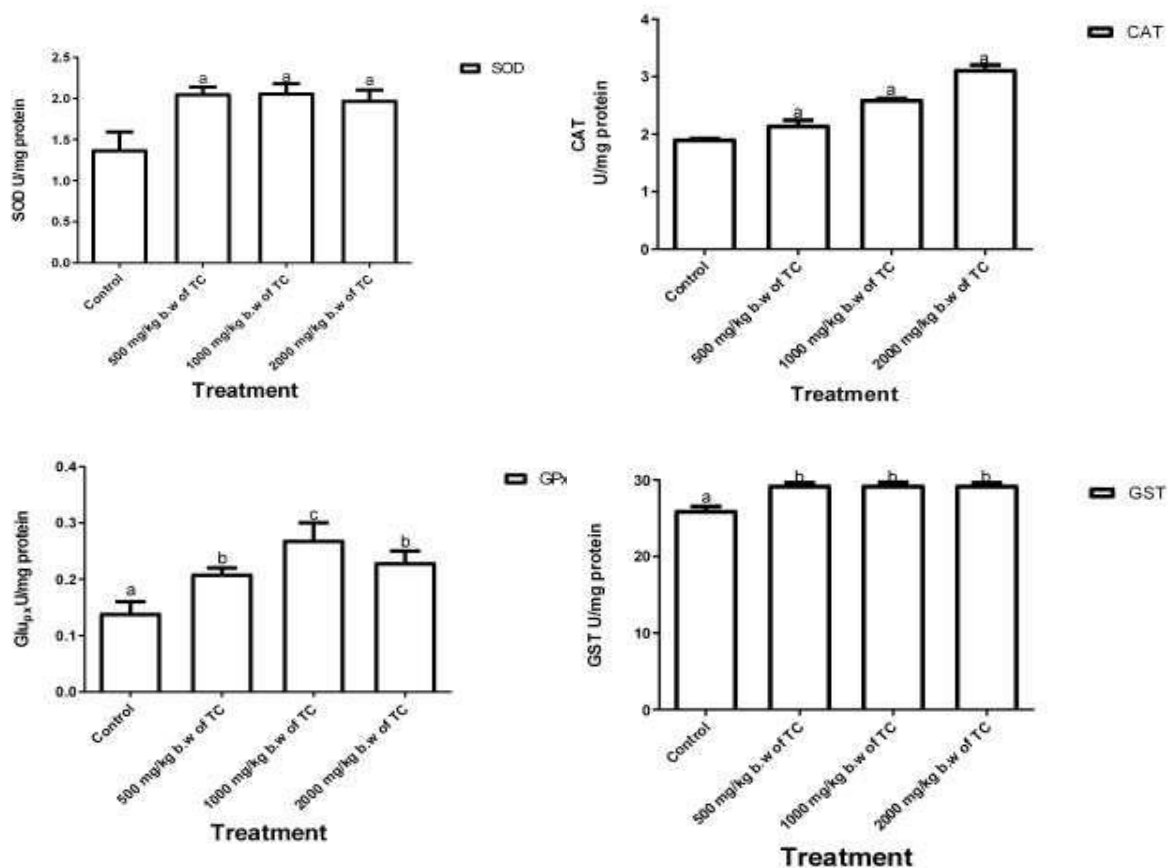

Figure 7: Sub-chronic toxicity of methanol extract of *T. conophorum* seeds on superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase and glutathione S transferase activities

Effect of methanol extract of *T. conophorum* seeds on glutathione and lipid peroxidation levels

The effect of methanol extract of *T. conophorum* seeds on glutathione and lipid peroxidation levels is shown in Figure 8. It was observed that there was a dose dependent increase in reduced glutathione levels in rats administered 500, 1000 and 2000 mg/kg body weight of *T. conophorum* seeds when compared to control. Also, there was no significant differences in lipid peroxidation levels in rats administered 500, 1000 and 2000 mg/kg body weight of the methanol extract of *T. conophorum* seeds respectively compared with control.
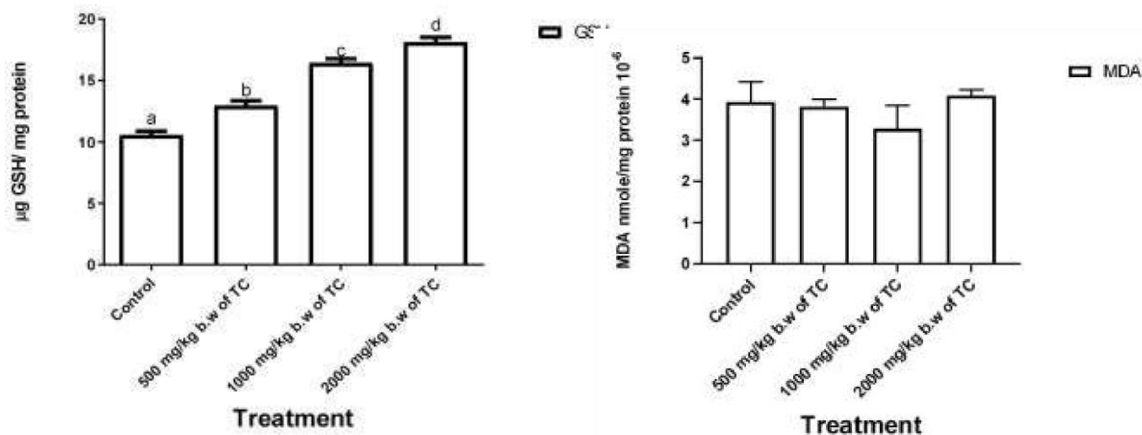

Figure 8: Sub-chronic toxicity of methanol extract of *T. conophorum* seeds on glutathione and lipid peroxidation levels

DISCUSSION

Herbal products have been employed in traditional medicine because they include chemical compounds that may have therapeutic effects for treating various human ailments (Hosseinzadeh *et al*., 2015). However, experimental studies have revealed that some of these herbal compounds are hazardous, making it necessary to investigate the toxicological impact of plants with therapeutic properties (Ndhlala *et al.,* 2013). International regulatory organizations like the Food and Drug Administration (FDA) have advocated effective preventative measures against using herbal products without adequate scientific and toxicological data (Kale *et al*., 2019; De Smet, 2004). Based on this context, this study assesses the acute and subchronic toxicity of *Tetracarpidium conophorum* (African walnut) seeds.

The investigation of the acute toxicity of medicinal plants aids in identifying potential adverse effects following a brief period of time and a single dose of administration. Additionally, it is used in the first stages of research to examine the pharmacological effects of novel medicinal drugs, notably LD50 determination (Kpemissi *et al*., 2020; Musila *et al*., 2017; Ugwah-Oguejiofor *et al*., 2019). In this acute toxicity study, the experimental animals tolerated the extract of *T. conophorum* seeds, even at higher dosages. No mortality was detected at the extract's highest dose of 5000 mg/kg body weight. Substances with LD_{50} values greater than or equal to 5000 mg/kg are considered relatively safe (Abraham and Ahmad, 2021). Therefore, the LD_{50} value obtained indicates that the extract can be regarded as non-toxic on acute exposure to rats.

A second investigation was conducted to investigate the sub chronic toxicity because there were no harmful effects discovered in the acute trial. In the sub chronic toxicity research, all rat groups had normal appearances before, during, and after the therapy. As shown in Table 1, the food intake patterns of the groups administered the methanol extract of *T. conophorum* seeds (the tested groups) did not substantially differ from the control group the next week, and no group experienced any mortality. According to Unuofin *et al*. (2018), relative organ weight is a sensitive indicator in toxicity studies. No detectable difference was found between the treated rats' overall weight (Table 2) and relative organ weight in the current investigation. No significant difference was observed between the treated rats' overall weight (Table 2) and relative organ weight (Table 3) compared to the control. These results may then confirm the low toxicity of the extract.

Haematopoietic toxicity may show up as a reduction in the number of circulating cells, structural and functional abnormalities, and, less frequently, morphological changes (El Kabbaoui *et al.,* 2017). In order to determine how plant extracts, affect an animal's blood system, it is crucial to evaluate haematological parameters. Analysing blood parameters is important since it offers a wealth of data. It can indicate haematopoietic function (myeloid lineage cells), allergy occurrence (white blood cells), or intravascular consequences such hemolysis (Okokon *et al*., 2010). When compared to the control group, the extract had no effects on lymphocytes, monocytes, mean cell volume, mean cell haemoglobin concentration, red blood cell count, haematocrit, or hemoglobin. However, the levels of WBC were significantly $P < 0.05$ higher in the extract-treated group than in the control group. Additionally, a dose-dependent significant reduction in platelet count was seen in the extract-treated groups compared to the control groups. Leukocytes, also known as white blood cells (WBC), are immune system cells that help the body fight off foreign substances and infectious diseases. An increase in the number of white blood cells (leukocytosis) in the blood is a sign of infection or a response to a toxic environment (Okokon *et al*., 2010). The administration of plant extract may alter phagocytes' primary defense against invaders by consuming and eliminating them, hence promoting cellular inflammatory processes (Esteban *et al*., 2015).

The liver is in charge of the body's detoxification, whilst the kidneys aid in the cleansing of the blood and the disposal of waste. Evaluations of the liver and kidney's health and functionality are essential for determining the toxicity profile of medications and plant extracts. Therefore, the biochemical analysis conducted in the current study may reveal potential liver and kidney damage brought on by ingesting the extract. The hepatic function enzymes and their metabolites were unaltered in Wistar rats that received methanol extract of *T. conophorum* (500, 1000, and 2000 mg/kg) at all doses tested.

Similarly, the extract had no effect on the antioxidant enzymes compared to the control. The increase in TC in female rats treated with the methanol extract of the plant mixture (200 and 400 mg/kg) correlated to the increase in HDL-C can therefore be taken as an improvement in lipid profile. This corroborates the results of Akomolafe *et al.* (2017), where they found no significant changes in serum markers of lipid profile in the subchronic toxicity of *T. conophorum.*

CONCLUSION
The outcome of the study has shown that the methanol extract of *T. conophorum* seeds is relatively safe on acute and sub chronic administration.

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