RESEARCH ARTICLE

Evaluation of the toxicological effect of methanol extract of unfermented *theobroma cacao* in wistar albino rats

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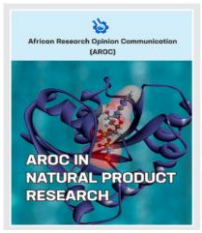

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Abstract

Background: The pod of Theobroma cacao is usually processed into a variety of beverages for human consumption; hence this study investigated its safety to vital organs at different doses. **Method**: Phytochemical, nutrient and antinutrient composition of unfermented *theobroma cacao* were determined using standard procedures. A total number of twenty-four (24) adult male Wistar albino rats (140 - 200g) were randomly divided into four groups of six rats each and were administered thus: Group I (normal control) rats were intraperitoneally administered with normal saline (1ml/kg b.w./day) for twenty-one days while group II, III and IV were intraperitoneally administered 200mg/kg, 400mg/kg and 600mg/kg b.w/day of Theobroma cacao for twenty-one days. During the course of the treatments, blood was extracted on days 0, 14, and 21 to determine the biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), while the liver and kidneys of the experimental animals were harvested for histopathological examinations at the end of the treatment. **Results:** The acute toxicity studies showed no toxicity up to 5000mg/kg of the extract. Treatment with the extract showed no significant difference ($p>0.05$) in the weight of the rats when compared with the control. A Significant decrease ($p\leq0.05$) was observed in total bilirubin concentration and alanine aminotransferase (ALT) activity of the treated groups when compared with the control group while Aspartate aminotransferase (AST) activity showed a significant increase ($p\leq0.05$) in some of the treated groups. The microscopic examination of the liver and kidney tissues showed no histopathological changes in the extract-treated groups. **Conclusion:** The observed results showed that unfermented Theobroma cacao is rich in antioxidants and has little or no toxicological effect on albino rats.

Keyword: *Theobroma cacao*; liver enzymes; toxicity; phytochemicals; antinutrients

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1.0 Introduction

Cocoa (*Theobroma cacao*) belongs to the genus *Theobroma*, a group of small trees which grow in the Amazon basin and other tropical areas of South and Central Africa. They are classified under the subfamily Sterculioidea of the mallow family Malvaceae. The medicinal value of cocoa plants have assumed a more important dimension in the past few decades owing largely to the discovery that, extracts from cocoa plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potentials [1]. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, heart diseases, cancer, Alzheimer's diseases, Parkinson's disease and the aging process [2].

Cocoa bean and its product (cocoa liquor, cocoa powder, and dark chocolate) are food sources rich in phenolic compounds [3]. The pharmacologically active ingredients of cocoa seeds include amines, alkaloids, fatty acids, polyphenols, magnesium, phenylethylamine, theobromine, caffeine and N-acylethanolamines [4]. The health promoting properties of cocoa beans are attributed to their phenolic compounds, catechins and flavonols [5], which are potent antioxidants that can attenuate inflammatory processes and other disease states. Cocoa products also contain high levels of biologically active polyphenols that exert both acute and chronic antioxidant-associated health

benefits [6] Cocoa and cocoa products have also been shown to suppress the development of atherosclerotic lesions [7], decrease platelet function [8] increase dermal blood flow [9], inhibits the proliferation of human breast cancer cells [10] possess hypoglycaemic properties and decrease oxidation of LDL cholesterol [11].

Due to the fact that cocoa is processed into many food products such as chocolates and confectionery coating and as flavours in cakes, ice cream etc; this study was aimed at evaluating, phytochemical, nutritive and antinutritive composition of methanol extract of unfermented *Theobroma cacao as well as* its toxicological effect on liver function enzymes and histology of the liver and kidney of albino rats.

2 Materials and methods

2.1 Collection and Preparation of plant extract

Unfermented seeds of *Theobroma cacao* were obtained from a cocoa farm in Cross River state, Nigeria and were authenticated by a botanist, at the Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State. The seeds were de-coated, sun-dried for fourteen (14) days, ground and sieved into fine powder (cocoa powder). A methanol extract of *Theobroma Cacao* was obtained by macerating 1500 g of the cocoa powder in 2000 ml of methanol for 24 h after which the extract was filtered through whatman No.1 filter paper. The filtrate was concentrated under reduced pressure.

2.2 Phytochemical analysis

Qualitative and quantitative phytochemical screening of the extract was carried out using the Standard methods described by **[12, 13].** The procedure for the determination of each phytochemical is elaborated below.

Alkaloids: 1mL of Methanol extract of unfermented cacao seeds was mixed with 1mL of Wagner's reagent (iodine in potassium iodine solution). The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Terpenoids: A 2mL of chloroform was mixed with 1mL methanol extract of unfermented cacao seeds for reaction to take place, a few drops of concentrated sulphuric acid was then slowly added to the reaction mixture. The appearance of a reddish-brown coloration signified the presence of terpenoids.

Tannins: A 2mL of 5% ferric chloride was added to 1mL methanol extract of unfermented cacao seeds. The presence of tannin was indicated by the appearance of a dark-blue or greenish-black colour.

Flavonoids: A 1mL of 2N sodium hydroxide was added to 2mL methanol extract of unfermented cacao seeds. The appearance of a concentrated yellow colour indicated the presence of flavonoids.

Saponins: A 2mL of distilled water was added to 2mL methanol extract of unfermented cacao seeds and shaken for 15 min in a graduated cylinder. The appearance of a foam layer signified the presence of saponins

Steroids: A 10mL of chloroform was added to 1mL methanol extract of unfermented cacao seeds after which 10mL concentrated sulphuric acid was intermittently added. The appearance of red colour in the upper layer and yellowish-green in the sulphuric acid layer signified the presence of steroids.

Glycosides: This was carried out using the Liebermann's test. 2.0 ml of acetic acid and 2 ml of chloroform were added to methanol extract of unfermented cacao seeds. The mixture was then cooled after which H_2SO_4 was added concentrated. The appearance of a green colour showed the presence of glycosides

2.3 Analysis of anti-nutrient compositions

Antinutrients analysis was carried out for the determination of the following: phytates by the method of Markkar *et al*., [14]; oxalates by the method of Underwood [15] and haemaglutinin by spectrophotometric method, hydrogen cyanides by the method outlined in AOAC [16]

2.4 Analysis of proximate compositions

Proximate analysis was carried out on the dried samples of the seeds according to the procedure of Association of Official Analytical Chemist (AOAC (1998) and on the previously described method by Nguyen [17]. The determination of moisture content was carried out using a hot-air oven set at 100 °C overnight. Kjeldahl method was used in the determination of nitrogen

content after which the crude protein content was calculated by multiplying nitrogen content by a factor of 6.25. Crude lipids in the cocoa seed were extracted using the Soxhlet method, the determination of the crude lipid was then determined after oven-drying the seed powder at 100 °C for 30 min. Ash content was measured by heating the cacao seed powder in a furnace at 600 °C for 5 h. Carbohydrates (include crude fiber) was calculated from Equations (1) and (2) [Nguyen) as follows:

Carbohydrate (% by fresh weight) = 100 − moisture − crude protein − crude lipid − ash (1)

Carbohydrate (% by dry weight) = 100 − crude protein − crude lipid − ash (2)

2.5 Experimental animals

Eighteen (18) albino mice of both sexes (17 - 25g) and twenty-four (24) adult male wistar albino rats (140 - 200g) were used for the acute toxicity study (LD_{50}) and the toxicological studies respectively. All the animals were obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. They were acclimatized for seven days in the animal house of the Department of Home Science, under adequate ventilation at room temperature, with 12-hour light/dark cycle before the experiment. Water and grower feeds were provided for them *ad libitum* throughout the experiment.

2.6 Ethical approval

Ethical approval was obtained from the faculty animal research ethics committee of the Faculty of Basic Medical Sciences (FAREC-FBMS) University of Calabar with the approval number: 022-TBCM-0521

2.7 Acute toxicity study

The median lethal dose (LD50) of the methanol extract of unfermented *Theobroma cacao* was determined by administering the extract to six groups of albino mice at doses of 10, 100, 1000, 1600, 2900 and 5000mg/kg bw respectively according to the method of Lorke (1983), as illustrated by Amos *et al*., [18]

2.8 Experimental design

The wistar rats were randomly divided into four study groups (1-4) of six animals each. The groups and doses administered were: group I (normal control): rats administered p.o with normal saline (1ml/kg b.w./day), group II rats were administered p.o with 200mg/kg, group III rats received p.o 400mg/kg b.w/day of *Theobroma cacao* extract while group IV rats were administered p.o with 600mg/kg b.w/day of *Theobroma cacao*. Rats in the treatment groups received extracts once daily for 21 days which was the duration of the study.

2.9 Collection of blood sample

Blood samples of the rats were collected into sterilized centrifuge tubes on day zero, 14 and 21 of the experiment and were immediately spurned at 4000 rpm for 15 mins. The plasma was collected into clean labelled sample tubes and used for analysis [19]

2.10 Analysis of biochemical parameters

Serum liver enzymes; alanine amino-transferase (ALT) and aspartate amino-transferase (AST) were measured according to the method of Reitman and Frankel [20], alkaline phosphatase (ALP) was determined according by colorimetric method as described by Kind and King [21] and total bilirubin was determined according to the method of Jendrassik [22] using Randox kits.

2.11 Histopathological examination

Twenty-four (24) hours after the last administration, the animals were anaesthetised using chloroform and dissected, tissue samples (kidney and liver) were removed, blotted dry, and immediately fixed in 10% formalin, dehydrated in a graded ethanol (50–100%) series, cleared in xylene, and embedded in paraffin for histological examination according to the method of Drury et al. [23]. Five-microthick sections were stained with haematoxylin and eosin, the changes observed using a microscope were recorded and photographs of the pathological lesions taken [24].

2.12 Statistical analysis

The results obtained were expressed as mean ± SEM. Tests of statistical significance were carried out using two-way analysis of variance (ANOVA). The statistical product and service solution (SPSS), version 18 was used. ($p \leq 0.05$) was considered statistically significant.

3 Results

3.1 Phytochemical, proximate and anti-nutrient composition of methanol extract of unfermented *Theobroma cacao*

The qualitative and quantitative phytochemical composition of methanol extract of unfermented *T. cacao* showed relatively high concentration of bioactive compounds such as reducing sugars, tannins and flavonoids (Tables 1 and 2). The glycosides and alkaloids were obtained at moderate concentration, while the soluble carbohydrates, terpenoids, saponins and steroids detected at low concentrations. Result of proximate analysis showed *T. cacao* was high in carbohydrate content (46.12 ± 5.71), crude protein (14.09 ± 2.35), ash (5.23 ± 1.01), moisture (12.80 ± 2.05), crude fat (11.40 ± 3.01) and crude fibre (7.37 ± 2.30) (Table 3). The antinutrients discovered in the methanol extract of unfermented *T. cacao* include phytates, hydrogen cyanides, oxalates, tannins and haemaglutinins (Table 4).

Table 1: Qualitative phytochemical composition of methanol extract of unfermented *T. cacao*

Phytochemicals	***Presence***
Reducing sugar	+++
Soluble carbohydrates	+
Alkaloids	++
Terpenoids	+
Tannins	+++
Flavonoids	+++
Saponins	+
Steroids	+
Glycosides	++

key; + = present in low concentration, **++** = present in moderately high concentration, **+++ =** present in very high concentration

Table 2: Quantitative phytochemical composition of methanol extract of unfermented *T. cacao*

Phytochemical	Concentration (mg/g)
Reducing sugars	572.50 ±10.00
carbohydrates	1.91 ± 0.05
Alkaloids	3.57 ± 0.17
Terpenoids	0.35 ± 0.01
Tannins	5.94 ± 0.30
Flavonoids	5.46 ± 0.58
Saponins	1.43 ± 0.08
Steroids	1.18 ± 0.04
Glycosides	2.84 ± 0.11

Values are mean ±SEM of triplicate determinations

Table 3: proximate composition of methanol extract of unfermented *T. cacao*

Parameters	Concentration (%)
Crude Protein	14.09 ± 2.35
Carbohydrates	46.12 ± 5.71
Moisture	12.80 ± 2.05
Crude fat	11.40 ± 3.01
Crude fibre	7.37 ± 2.30
Ash	5.23 ± 1.01

Values are mean ±SEM of triplicate determinations

Table 4: Antinutrient composition of methanol extract of unfermented *T. cacao*

Constituent	Conc. (mg/g)
Tannins	2.80 ± 0.02
Phytates	629.00 ± 12.70
Oxalates	123.95 ± 3.18
Haemaglutinins	755.50 ± 6.36
Hydrogen Cyanide	0.55 ± 0.00

Values are mean ±SEM of triplicate determinations

3.2 Acute toxicity profile of methanol extract of unfermented *T. cacao*

The acute toxicity test of methanol extract of *Theobroma cacao* showed neither toxicity nor behavioural changes in animals up to 5000mg/kg body weight (Table 5)

Table 5: Acute toxicity profile of methanol extract of unfermented *Theobroma cacao*

Doses	Mortality	Toxicity
10	0/3	None observed
100	0/3	None observed
1000	0/3	None observed
1600	0/3	None observed
2900	0/3	None observed
5000	0/3	None observed

3.3 Effect of methanol extract of unfermented *T. cacao* on liver enzymes and bilirubin of rats

The administration of unfermented *Theobroma cacao* did not cause any significant difference ($p \geq 0.05$) in the alkaline phosphate (ALP) and aspartate aminotransferase (AST) activities of all the treated groups after 21 days when compared with the control group (tables 6-7). There were however significant decreases ($p \leq 0.05$) in alanine aminotransferase (ALT) activity and total bilirubin concentration in all the treated groups after 14 and 21 days when compared with the control group (tables 8-9).

Table 6 Effect of methanol extract of unfermented *Theobroma cacao* on ALP activity of Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	600mg/kg b.w
Day 0	29.33 ± 3.48[a]	30.67 ± 0.33 [a]	30.33 ± 0.33 [a]	27.67 ± 0.88 [a]
Day 14	32.67 ± 1.45 [a]	28.67 ± 0.67 [a]	29.33 ± 1.20 [a]	29.33 ± 0.67 [a]
Day 21	30.00 ± 1.15 [a]	32.67 ± 1.33 [a]	32.00 ± 1.15 [a]	30.67 ± 0.67 [a]

Means with different lower case subscripts ($_{a, b, c}$) across the row i.e. between groups are significantly different at p≤0.05. ALP: Alkaline phosphate

Table 7: Effect of methanol extract of unfermented *Theobroma cacao* on AST activity of Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	600mg/kg b.w
Day 0	51.33 ±1.33 [a]	51.33 ± 6.36 [a]	56.67 ± 2.73 [a]	52.67 ± 0.88 [a]
Day 14	58.00 ± 2.00 [a]	46.67 ± 2.90 [b]	57.00 ± 3.61 [a]	50.67 ± 3.53 [a]
Day 21	58.00 ± 2.00 [a]	58.67 ± 1.33 [a]	59.33 ± 1.76 [a]	58.67 ± 4.37 [a]

Means with different lower case subscripts ($_{a, b, c}$) across the row i.e. between groups are significantly different at p≤0.05. AST: Aspartate transaminase

Table 8: Effect of methanol extract of unfermented *Theobroma cacao* on ALT activity of Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	600mg/kg b.w
Day 0	42.00 ± 2.30 [a]	40.33 ± 0.88[a]	42.67 ± 0.88 [a]	43.67 ± 0.67 [a]
Day 14	42.33 ± 1.67 [a]	36.67 ± 1.76[b]	33.67 ± 0.88[b]	33.33 ± 1.45[b]
Day 21	44.67 ± 0.89 [a]	35.33 ± 2.60 [b]	31.67 ± 0.67[b]	28.33 ± 1.20[b]

Means with different lower case subscripts ($_{a, b, c}$) across the row i.e. between groups are significantly different at p≤0.05. ALT: Alanine transaminase

Table 9: Effect of methanol extract of unfermented *Theobroma cacao* on Total Bilirubin in Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	600mg/kg b.w
Day 0	1.50 ± 0.06 [a]	1.47 ± 0.03[b]	1.33 ± 0.03[b]	1.40 ± 0.06[c]
Day 14	1.40 ± 0.06 [a]	1.16 ± 0.24[ab]	1.00 ± 0.10[a]	0.97 ± 0.12[b]
Day 21	1.33 ± 0.14 [a]	1.03 ± 0.09[a]	0.90 ± 0.10[a]	0.63 ± 0.09[a]

Means with different lower case subscripts ($_{a, b, c}$) across the row i.e. between groups are significantly different at p≤0.05

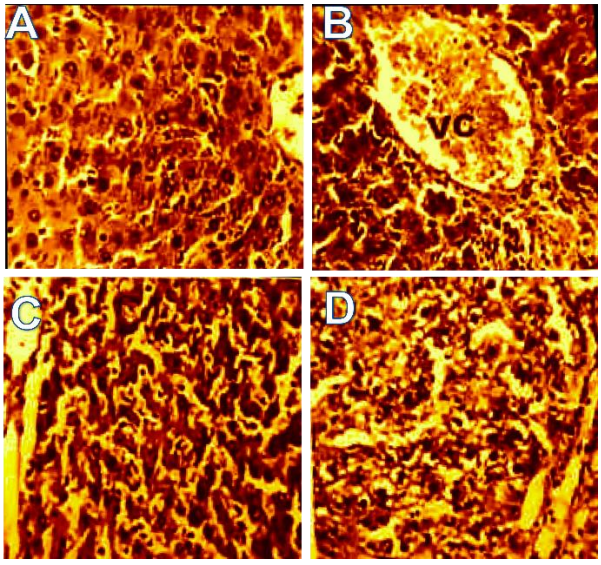

Figure 1: Photomicrographs haematoxylin and eosin-stained liver tissues of (A) control showing normal liver architecture, (B) 200mg/kg bw T. *cacao* showing venous congestion. (C), 400mg/kg bw, and (D) 600 mg/kg T. *cacao* with no remarkable histopathological changes. H&E x 400

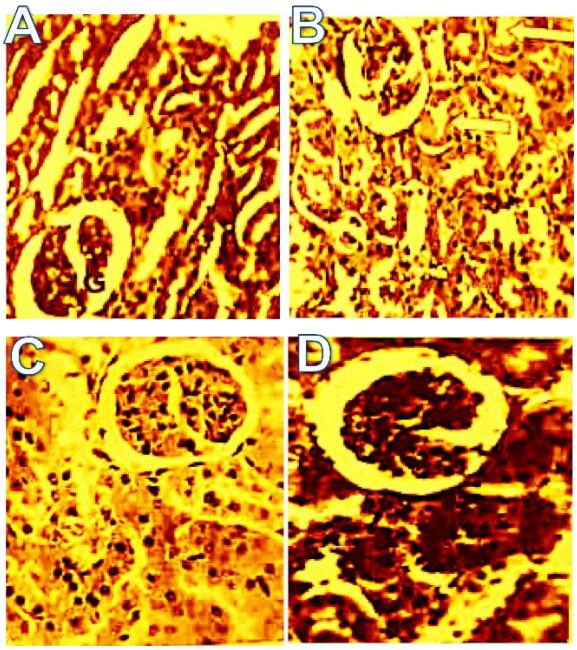

Figure 2: Photomicrographs of haematoxylin and eosin-stained kidney tissues of (A) control showing normal glomerulus, (B) 200mg/kg bw T. *cacao* showing dilated tubules. (C), 400mg/kg bw, and (D) 600 mg/kg T. *cacao* with no remarkable histopathological changes. H&E x 400

3.3 Effect of methanol extract of unfermented *T. cacao* on the histology of liver tissues of wistar albino rats.

Compared with the control group (Figure IA), the administration of 400mg/kg (Figure 1C) and 600mg/kg (Figure 1D) of methanol extract of unfermented *T. cacao* caused no significant changes to the histology of the liver tissues of experimental animals. However, animals administered with 200mg/kg methanol extract of T. cacao, showed venous congestion but no liver cell necrosis (Figure 1B).

3.4 Effect of methanol extract of unfermented T. cacao on the histology of Kidney tissues of wistar albino rats

Photomicrograph of kidney sections from control rats (Figure 2) shows normal glomerulus (G) and renal tubules. Rats administered 200mg/kg of *T. cacao* extract (Figure 2B) showed dilated tubules while kidney sections from rats given 400mg/kg (Figure 2C) and 600mg/kg (Figure 2D) showed no remarkable histological changes.

4.0 Discussion

Studies have shown that plant extract possesses constituents which are known to exhibit medicinal as well as physiological activities [25]. Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms [26]. They have diverse functions which include determination of colours, strength to plants, defence against predators, and attraction of insects for pollination and feeding etc, while some are waste products [27].

The qualitative phytochemical evaluation of unfermented *Theobroma cacao* showed a very high presence of flavonoids, reducing sugar, and Tannins in the methanol extract, also this study revealed a moderate presence of alkaloids and glycosides in the extract. Soluble carbohydrates, terpenoids, saponins and steroids were detected at low concentrations. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 [28] and this property may

explain the mechanisms of antioxidative action of *Theobroma cacao*. Their important antioxidant activity helps in the removal of oxidant free radicals [29] which may be the reason for protection against cardiovascular and degenerative diseases. Reducing sugar help to reduce certain oxidizing agents [30], through the aldehyde functional group which allows the sugar to act as a reducing agent. The aldehyde can be oxidized via a redox reaction in which another compound is reduced during the oxidation of aldoses. Tannins play a role in protection from predation, perhaps also as pesticides and in plant growth regulation [31].

Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention [32]. Theobroma cacao containing these compounds, may serve as a potential source of bioactive compounds useful in the treatment of cancer, also effective in protecting the kidneys and may have also shown potential antibacterial and antiviral effects [33]. Alkaloids inhibit certain mammalian enzyme activities such as phosphodiesterase, thus prolonging the action of cyclic AMP (cAMP) [34]. Glycosides are known to lower blood pressure and improve heart performance [35]. The presence of glycosides in this plant could, thus, make it useful in the treatment of cardiac failure. Saponins have some characteristics which include the formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness [36]. they are major ingredients in traditional Chinese medicine and thus are responsible for most of the observed biological effects [37]. Steroids are very important compounds due to their metabolic relationship with compounds such as sex hormones [38]. They also have antibacterial properties [39]. This plant is thus proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

The proximate analysis of unfermented Theobroma cacao in this study revealed crude protein, ash, moisture, fats, carbohydrates and crude fibre. The crude protein value in this study shows that the plant is a good source of protein. The moisture content obtained is an indication that it is not liable to microbial spoilage. The crude fat content observed shows that unfermented Theobroma cacao is a good source of oil. The Crude fibre was reported to have hypocholesterolemic properties [40]. It is known that a diet low in fibre is undesirable and could cause constipation [41]. Food fibre aids the absorption of trace elements in the gut and reduces the absorption of cholesterol. The ash content is the measure of the mineral content; the ash content result thus shows the presence of minerals in Theobroma cacao extract.

The presence of antinutrients is equally a major factor limiting the proper utilization of the nutrients in plant seeds. Values obtained for phytates, oxalates and haemagglutinin in this study are higher than those reported for fermented cocoa powder [42] and it also agrees with the recommendation of researchers that cocoa should be fermented prior to utilization as food [42]. Phytates inhibit the functions of some digestive enzymes and oxalates cause irritation and swelling in the mouth and throat [43]. Tannins are a group of polyphenolic compound chelate metals such as iron and zinc and thus reduce the absorption of these nutrients, inhibit digestive enzymes and may precipitate proteins [44]. Haemaglutinins are proteins which agglutinate red blood cells. The biological effects of haemagglutinin probably result from their affinity for sugars; they may bind to carbohydrate moieties of the cells of the intestinal wall and cause a non-specific interference with nutrient absorption [45, 46]. Hydrogen cyanides (HCN) is known to inhibit metal-containing enzymes such as cytochrome oxidase which is responsible for the energy-providing processes in the cell where oxygen is utilized, HCN is present in this extract at a very low concentration which will likely pose no toxic effect. Therefore, because of the presence of these antinutrients, unfermented Theobroma cacao should be consumed in limited amounts. This is because the toxic substances may accumulate in the body and may lead to a decline in certain aspects of health, such as the function of the nervous system and inhibition of oxidative phosphorylation.

The liver is a target organ that plays a key role in the detoxification of toxic substances ingested into the body [47] hence toxicity could cause hepatic injury which is an interplay of different metabolic process dysfunctions including DNA damage [48] and lipid peroxidation [49] amongst others. Numerous authors have reported that Symptoms of Liver damage due to toxicity include elevated aspartate transaminase (AST), alanine transaminase (ALT) and total bilirubin and these

elevations could possibly be a result of leakage of these enzymes from the liver cystole [50]. It is for this reason that the authors decided to investigate the acute toxicity of methanol extract of unfermented Theobroma cacao in order to ascertain whether the extracts may have any adverse effects on the liver and kidneys of albino rats models. Preliminary toxicity was carried out to know the "No Observed Adverse Effect Level" (NOAEL). [50]. The present study showed that the extract concentrations up to 5000mg/kg had no weight and behavioural changes in the animals tested.

Lowering of enzyme level is an indication of hepatoprotective action of Theobroma cacao extract. Administration of Theobroma cacao at all doses significantly decreased alanine transaminase (ALT) and total bilirubin compared with the control showing that Theobroma cacao has the ability to preserve normal liver function. Alkaline phosphatase (ALP) showed no significant difference between the treated groups and the control group and aspartate transaminase (AST) showed a significant increase in some of the treated animals, this may be due to some secondary metabolite that may be found in the plant. This result agrees with the finding of Feudjo et al. [51].

Histopathological examination is increasingly being recognized as a valuable tool for assessing the level of toxicity of plant-based food in rats. The kidney serves as a major route of excretion of metabolites of xenobiotic and receives the largest proportion of postbranchial blood and therefore, it is more likely to undergo histopathological alterations under chemical stress [52]. Although liver marker enzyme levels are not a direct measure of hepatic injury, they show the functional status of the liver. The liver appears normal, as there is no major damage. This would allow it to carry out de novo biosynthesis of cholesterol required to combine with such substrates as glycerol and free fatty acids released from lipolysis to yield VLDL. It is a common reason that normal architecture and integrity of the liver is required for this to occur. The kidney tissue also appears normal as there is no observed damage at a higher dose.

5.0 Conclusion

In conclusion, *Theobroma cacao* contain many bioactive compounds which may be an important sources of minerals, vitamins and energy, all of which are associated with decreased risk in development of several diseases. Also, the results presented above showed that the administration of extract of unfermented *Theobroma cacao* to rats has no or little toxicological effect on the studied index. However, some antinutrients were observed in high concentration, which may make the unfermented cocoa less suitable than the fermented one.

Conflicts of interest: The authors declared no conflict of interest exist

Author's contributions: All authors participated in research design. Author EIA, ACA & LUSE conducted the research work, Author EIA, and ARA wrote the manuscript. While Authors EIA, RUU and ARA revised the manuscript and the research design. All authors read and approved the final manuscript.

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