Journal of Biosciences and Medicines, 2023, 11, 63-75
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Synergistic Mixture of *Cyperus esculentus*, *Phoenix dactylifera* and *Cocos nucifera* Aqueous Extract: Its Liver and Kidney Benefits in Male Albino Rat Model

Tamuno-Boma Odinga[1*], Cletus Barizoge Lemii[2], Iyaeneomi Ransome Daka[2], Christine U. Gabriel-Brisibe[3], Sarah K. Enebeli[2], Iyingiala Austin-Asomeji[4], Felicia Ucheawaji Edward[2]

[1]Department of Biochemistry, Faculty of Science, Rivers State University, Port Harcourt, Nigeria
[2]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Basic Medical Science, Rivers State University, Port Harcourt, Nigeria
[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Science, Rivers State University, Port Harcourt, Nigeria
[4]Department of Community Medicine, Faculty of Clinical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria
Email: *Bomaodinga@gmail.com

How to cite this paper: Odinga, T., Lemii, C.B., Daka, I.R., Gabriel-Brisibe, C.U., Enebeli, S.K., Austin-Asomeji, I. and Edward, F.U. (2023) Synergistic Mixture of *Cyperus esculentus*, *Phoenix dactylifera* and *Cocos nucifera* Aqueous Extract: Its Liver and Kidney Benefits in Male Albino Rat Model. *Journal of Biosciences and Medicines*, **11**, 63-75.
https://doi.org/10.4236/jbm.2023.1110007

Received: July 7, 2023
Accepted: October 9, 2023
Published: October 12, 2023

Abstract

Background: The synergistic mixture of *Cyperus esculentus*, *Phoenix dactylifera* and *Cocos nucifera* (STCD) aqueous extract is a common drink in Port Harcourt, Nigeria. It is assumed to have various health benefits. However, the synergistic mixture of the content has not been studied scientifically, hence the need to evaluate its effect on the liver and kidney being part of the body's metabolic organs. **Aim:** This study evaluated the synergistic mixture of *Cyperus esculentus*, *Phoenix dactylifera* and *Cocos nucifera* (STCD) aqueous extract in male albino rats. **Methods:** Acute toxicity LD_{50} of STCD was carried out, afterwards, fifteen male albino rats were grouped into three groups with 5 rats in each group; Control, 200 mg/kg, 400 mg/kg STCD. The rats were administered STCD orally 24 hourly, for 21 days, with feed and water *ad libitum*. At the end of the experiment, blood samples were collected for biochemical analysis of the liver and kidney biomarkers, while the liver and kidney tissues were harvested for histopathological examination using standard laboratory methods. Descriptive statistics were computed and expressed as Mean ± SD. One-way ANOVA and Turkeys test was performed. P value ≤0.05 was considered statistically significant. **Results:** Acute toxicity LD_{50} of STCD was observed to be ≥2404.2 mg/kg body weight. An increase in the percentage body weight difference of 8.39% and 2.86% was observed for 200

and 400 mg/kg STCD groups. Also, the liver weight was observed to increase in 400 mg/kg (3.92 ± 1.42) in comparison to the control group (3.48 ± 1.61), a decrease in the kidney weight was observed in all groups administered STCD in comparison to the control group. Administration of STCD at both 200 and 400 mg/kg revealed a decrease in the concentration of the hepatic biomarkers AST, ALT, ALP, TP, Albumin, Total and conjugated bilirubin. The kidney biomarker Urea was observed to decrease in concentration for 200 mg/kg STCD (4.60 ± 1.83) and 400 mg/kg STCD (4.76 ± 0.74) when compared to the control group (6.32 ± 2.74). A decrease in Creatinine was observed in 200 mg/kg (91.80 ± 34.69) and 400 mg/kg (98.60 ± 15.53) in comparison to the control group (117.60 ± 42.88). The histological examination of the liver of rats administered STCD revealed structural normal central vein, hepatocytes and portal tract. The kidney examination revealed normal glomeruli and normal tubule. **Conclusion:** The findings of this study opine that STCD improved the health of both the liver and kidney as evidenced via the biomarkers and histological examinations of the liver and kidney. This study therefore recommends the intake of STCD at moderate doses for improved liver and kidney function due to its bioactive compounds and nutritional content.

Keywords

Cyperus esculentus (*Tiger nuts*), *Phoenix dactylifera* (*Dates*) and *Cocos nucifera* (*Coconuts*), Liver, Kidney, Male Rats

1. Introduction

Cyperus esculentus commonly known as Tiger nut, and by several other names in Nigeria, including Akiausa, Aya, and Ofio by the Igbo, Yoruba, and Hausa tribes respectively [1]. It is also called yellow nut sedge, "Souchet" in French, "ermandeln" in German, and "chufa" in Spanish is a weed plant of the tropical and Mediterranean regions [2]. The sweet almond-like tubers of tiger nuts are known for their high nutrient content and health benefits; Oleic acid (64.2% - 68.8%), glucose, phosphorus, potassium, Calcium, Zinc, Copper, Sodium, Manganese, vitamins C and E [2] [3] [4]. The high starch (20% - 30% DW), fat (20% - 28% DW), and protein (nearly twice that of cassava) of tiger nuts [5].

Phoenix dectylifera also known as Date palm fruits are tropical fruits grown on a date palm tree in small clusters. It is regarded in most Arabian countries as a staple food, as an essential part of the diet, and generally consumed around the world [6]. Dates are consumed naturally and in processed forms such as paste, syrup, pickles, jams, jellies, and are used in various baking or confectionery items alongside chocolate, coconut, honey, vinegar, and other ingredients [7]. It serves as a quick source of energy due to its high Carbohydrate content (mostly sucrose, glucose and fructose) (70% - 80%) [8], it's also rich in nutrients such as high in fiber and contain a variety of vitamins and minerals, including large levels of calcium, iron, fluorine, and selenium they are also low in salt, its antioxidant, promotes brain health, promote labour induction naturally and hypoglycemic potency

[9]. The biological activities could be attributed to its phytochemical constituents, which including carotenoids, phenolics, flavonoids, and anthocyanins, all of which have antioxidant, antimutagenic, and immune-modulatory activities [10]. In addition to their nutritional value, dates are said to offer several medical benefits such as aphrodisiac, immune booster, strength, fitness, and pain relief, as well as protection against many diseases such as cancer and heart disease [11].

Coconut (*Cocos nucifera*) belonging to the Arecaceae family holds quite an importance in the Indian traditional medicinal system. Coconut milk and coconut meat contain dietary phosphorous that prevents oxidative damage and increases the activity of antioxidant enzymes in the intestine and hepatic pancreas of young [12] [13]. Coconut milk contains coconut oil, which may boost HDL (good) cholesterol and reduce LDL (bad) cholesterol. Improvements in these markers may reduce your risk of heart disease [14].

Phoenix dactylifera fruits, *Cocos nucifera* nuts and *Cyperus esculentus* nuts are commonly sold in many markets and also widely consumed as food in many parts of the world. These three plant materials are commonly used in synergism for the preparation of different types of drinks. In Nigeria, they are used in preparing the drink known as "Kayan Mata and Kayan Maza in the Hausa language of Nigeria". This drink is widely consumed in Nigeria for its "assumed health benefits" including aphrodisiacs activity in male. Although the individual plants have been explored for their compositions nutritionally and therapeutically, the synergistic mixture of the content has not been studied scientifically, hence this study to evaluate the effect of the drink on the hepatic and renal biomarkers of the drink in male albino rats [15].

In this study, we carried out the LD_{50} of Synergistic Tiger nut, Coconut and Date (STCD) seed aqueous extract and evaluated its effect on the hepatic and renal biomarkers in male albino rats.

2. Materials and Methods

2.1. Acute Toxicity Studies (LD_{50}) of STCD

The acute toxicity of STCD was determined by the OECD [16] procedure outlined by Lorke [17] as reported by Adefisayo *et al.*, [18]. The rats were fasted overnight prior to experiment. Rats of either sex were divided into 2 phases. In the first phase of the study, 9 rats were divided into 3 groups of 3 rats each and they were treated with STCD by gavage at the doses of 10, 100, and 1000 mg/kg. In the second phase, 8 rats were divided into 4 groups of 2 rats each and they were treated with STCD by gavage at the doses of 850, 1700, 3400, and 6800 mg/kg. The general behavior of the animals was observed continuously for 1 hour after treatment, then intermittently for 4 hours, and finally hourly for the next 24 hours. The LD_{50} was determined using the formula:

$$LD_{50} = \sqrt{a \times b}$$

where a = least dose that killed any rat;

b = highest dose that did not kill any rat.

2.2. Measurement of Body Weight Change

The animals were weighed weekly for all groups using a digital weighing balance to access weekly weight gain or weight loss

2.3. Experimental Design

A total numbers of fifteen healthy male albino rats with a weight of 250 - 300 g were obtained from the animal house of the Department of Pharmacology, Rivers State University, Port Harcourt, Nigeria. The animals were housed in clean plastic cages, placed in well ventilated house conditions (temperature: (22 ± 3)˚C; photoperiod: 12 h natural light and 12 h dark; humidity: 45% - 50%). They were allowed unrestricted access to rat pellets (Feed Company) and tap water. The animals were acclimatized for 7 days.

2.4. Plant Material and Preparation of Extract

Fresh seeds of tiger nuts, dates and coconut were purchased from mile 1 market in Port Harcourt and authenticated at the herbarium of the Plant Science and Biotechnology department of Rivers State University. Tiger nuts (400 g) were cleaned to remove impurities, washed and put in a bowl. The dates (120 g) were cut into two so as to remove the seeds, then washed and put in a bowl. The coconut (200 g) was removed from the shell, diced and washed. All were pulverized with a manual blender, adding 150 ml of water until a uniformed mixture was obtained. The mixture was filtered using a sieve and the resulting filtrate was put into a bottle until use. This synergistic mixture was prepared daily for the 21 days of administration.

2.5. Experimental Protocol

After acclimatization of the animals, the animals were properly grouped, Group 1) animals acting as the control group were administered with normal rat feed and distilled water, group 2) animals acting as the low dose, were administered 200 mg/kg of the synergistic aqueous mixture (tiger nut, dates and coconut), and group 3) animals which was the high dose were administered 400 mg/kg of the synergistic mixture (tiger nut, dates and coconut). The administration in both group 2 and 3 was done orally using an oral gavage tube. The administration lasted for 21 days and animals were sacrificed.

2.6. Collection of Samples (Blood, Liver and Kidney)

Sacrifice was done in three batches the first was carried out after 7 days, the second after 14 days and the third after 21 days. The animals were sacrificed under chloroform anesthesia, followed by decapitation and dissection. Blood samples were collected through cardiac puncture and put in plain sample bottles, and testes were carefully harvested from each animal for histological examination. Testes samples meant for histological analysis were immediately fixed 10% normal saline.

2.7. Histological Analysis

The liver and kidney tissue biopsies of the rats were fixed in 10% formalin, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. The tissues were then cut into 2- to 3-mm-thick sections by a microtome, fixed on the slides, and stained with hematoxylin-eosin. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan), and photomicrographs were taken with a Leica DM 750 camera at ×100 magnification.

2.8. Statistical Analysis

The results obtained were expressed as mean ± standard deviation. Data were analyzed using one-way analysis of variance followed by post hoc test using Turkeys test, and p-value less than 0.05 was considered statistically significant. The statistical analysis was performed with the aid of IBM SPSS, Version 25.

3. Results

3.1. Acute Oral Toxicity Test (LD50) of STCD

The oral administration of STCD at all concentrations up to 1700 mg/kg had no symptom of toxicity in the experimental rats, however, a difference in the body weights of the rats was observed. Symptoms of diarrhea and fatigue were observed in the rats administered 3400 and 6800 mg/kg, this resulted to 50% mortality of the rats. The other 50% were administered 100 mg/kg of lime fruit juice, which resuscitated them. The oral LD_{50} of STCD was determined to be ≥2404.2 mg/kg body weight in adult male albino rats as shown in Table 1 below.

Table 2 shows the body weight difference of the experimental rats used for the study. From the table a 30.95% increase in the body weight of the control group rats was observed, while 8.39% and 2.86% increase in the body weight of rats administered 200 mg/kg and 400 mg/kg body weight extract were observed respectively.

Table 3 shows the effect of the mixed extract on the biomarkers of kidney

Table 1. Acute oral toxicity test (LD50) of STCD.

Number of Rats	Dose (mg/kg)	Sex	Mortality
Phase 1			
3/3	10	Male	0/3
3/3	100	Male	0/3
3/3	1000	Male	0/3
Phase 2			
2/2	850	Male	0/2
2/2	1700	Male	0/2
2/2	3400	Male	1/2
2/2	6800	Male	1/2

LD_{50} = Lethal dose; LD_{50} $\sqrt{a \times b}$ of STCD = $\sqrt{3400 \times 1700}$ mg/kg = 2404.2 mg/kg body weight. Therefore, LD_{50} of STCD is ≥2404.2 mg/kg body weight in adult male albino rats.

function in male albino rats. Blood Urea was observed to decrease in concentration in the groups administered 200 mg/kg and 400 mg/kg STCD in comparison to the control group, albeit not significant at 0.05 level. Also, a decrease in Creatinine, potassium and sodium was observed in 200 and 400 mg/kg STCD when compared to control group. The observable increases were not significantly decreased ($p \leq 0.05$). Chloride and Bicarbonate had a dose dependent non-significant ($p \leq 0.05$) increases in groups administered 200 and 400 mg/kg STCD when compared to the control group.

Table 4: Effect of Synergistic mixture of *Cyperus esculentus, Phoenix dactylifera* and *Cocos nucifera* on the hepatic biomarkers. A decrease in the enzymatic biomarkers AST, ALT, and ALP. A decrease also in Total protein, Albumin, Total bilirubin and Conjugated bilirubin were observable in the 200 and 400 mg/kg STCD in comparison to the control group. The observed decreases were not statistically significant at $p \leq 0.05$.

3.2. Histological Observations

The photomicrographs of the liver and kidney sections of the experimental rats are shown in Figures 1-6. The liver of the control group rats was structurally

Table 2. Body weight difference in experimental rats exposed to Synergistic mixture of *Cyperus esculentus, Phoenix dactylifera and Cocos nucifera.*

Groups	Initial body weight (g)	Final body weight (g)	% body weight difference	Liver weight (g)	Kidney weight (g)
Group 1 (control)	69.80 ± 7.50	121.00 ± 30.83	30.95	3.48 ± 1.61	1.04 ± 0.09
Group 2 (200 mg/kg STCD)	88.20 ± 5.12	121.20 ± 29.20	8.39	3.36 ± 0.68	0.78 ± 0.38
Group 3 (400 mg/kg STCD)	111.80 ± 6.72	123.00 ± 11.68	2.86	3.92 ± 1.42	0.98 ± 0.04

Values are expressed as mean ± standard deviation.

Table 3. Effect of Synergistic mixture of *Cyperus esculentus, Phoenix dactylifera and Cocos nucifera* on Some Kidney biomarkers.

Groups	Urea (mg/dl)	Creatinine (μmol/L)	Potassium (mEq/L)	Sodium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)
Group 1 (Control)	6.32 ± 2.74[a]	117.60 ± 42.88[a]	5.22 ± 0.44[a]	158.00 ± 8.89[a]	74.00 ± 6.96[a]	24.80 ± 3.03[a]
Group 2 (200 mg/kg STCD)	4.60 ± 1.83[a]	91.80 ± 34.69[a]	4.98 ± 1.54[a]	153.60 ± 29.09[a]	71.60 ± 17.59[a]	27.60 ± 1.67[a]
Group 3 (400 mg/kg STCD)	4.76 ± 0.74[a]	98.60 ± 15.53[a]	3.54 ± 0.74[a]	123.00 ± 16.72[c]	76.00 ± 18.41[a]	26.80 ± 3.63[a]

Table 4. Effect of Synergistic mixture of *Cyperus esculentus, Phoenix dactylifera and Cocos nucifera* on selected hepatic biomarkers.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dL)	Albumin (g/dL)	Total Bilirubin (mEq/L)	Conjugated Bilirubin (mEq/L)
Group 1 (Control)	31.80 ± 5.81[a]	13.28 ± 4.65[a]	61.00 ± 39.69[a]	76.80 ± 6.38[a]	41.40 ± 5.68[a]	7.22 ± 1.54[a]	3.98 ± 0.97[a]
Group 2 (200 mg/kg STCD)	33.60 ± 7.09[a]	13.24 ± 6.07[a]	58.60 ± 15.45[a]	66.20 ± 7.59[a]	38.40 ± 6.50[a]	6.90 ± 1.47[a]	3.84 ± 1.36[a]
Group 3 (400 mg/kg STCD)	31.80 ± 5.81[a]	9.84 ± 2.80[a]	47.80 ± 18.91[a]	66.20 ± 9.39[a]	39.00 ± 7.31[a]	6.64 ± 1.09[a]	3.66 ± 0.67[a]

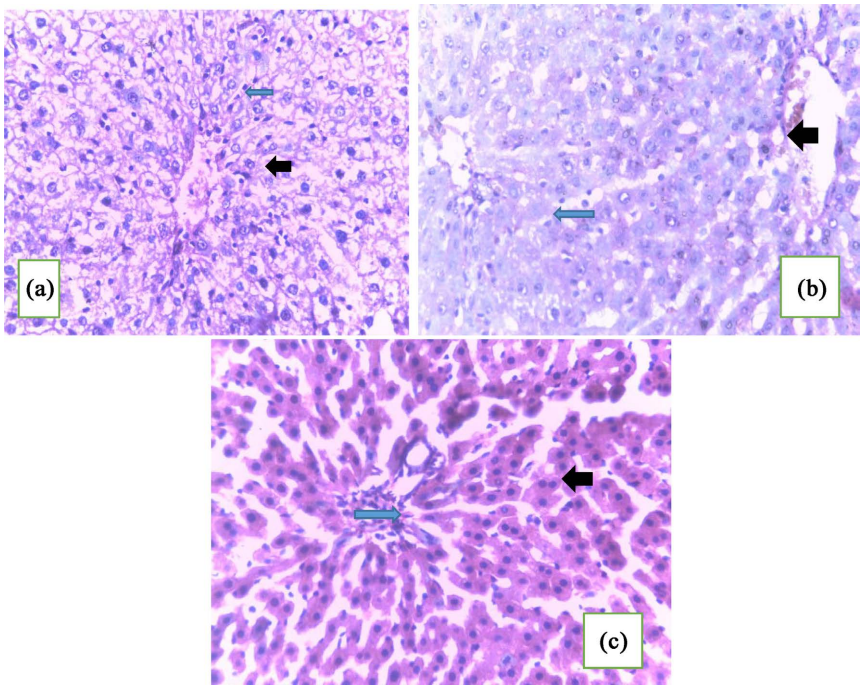

Figure 1. Photomicrograph of the Liver for the Control group. The photomicrographs for the liver of the control group reveal thus; (a) normal central vein (black) and mild infiltration of the hepatocytes (blue), (b) show normal central vein (black) and normal hepatocytes (blue), (c) normal portal tract (blue) and normal hepatocytes (black).

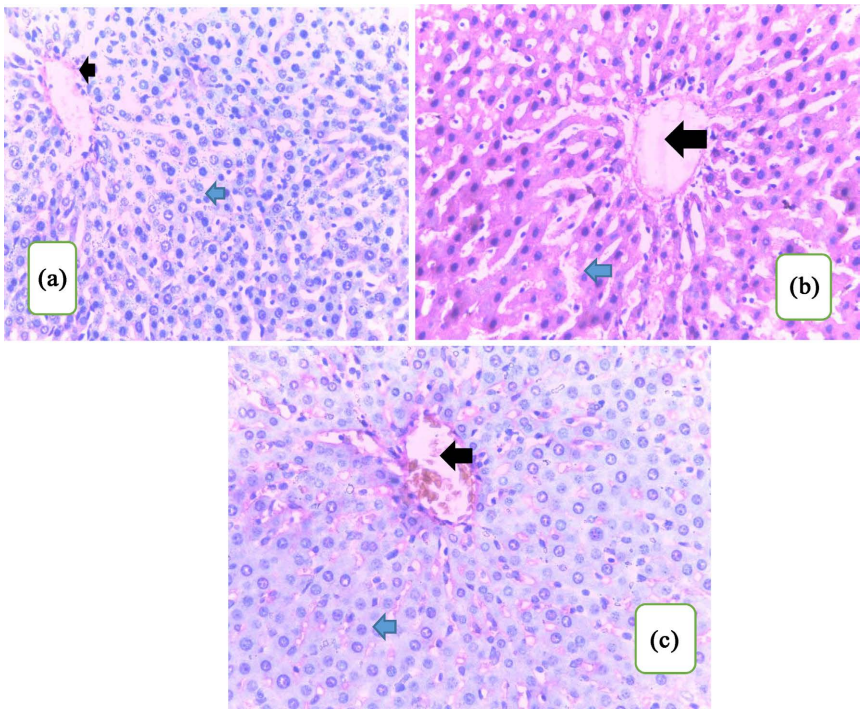

Figure 2. Photomicrograph of the Liver for the 200 mg/kg STCD rats. The photomicrographs revealed that the liver of the rats administered 200 mg/kg STCD as follows: (a) Section of the liver show normal central vein (black) and normal hepatocytes (blue) (b) Section of the liver show normal central vein (black) and normal hepatocytes (blue) (c) Section of the liver show normal central vein (black) and normal hepatocytes (blue).

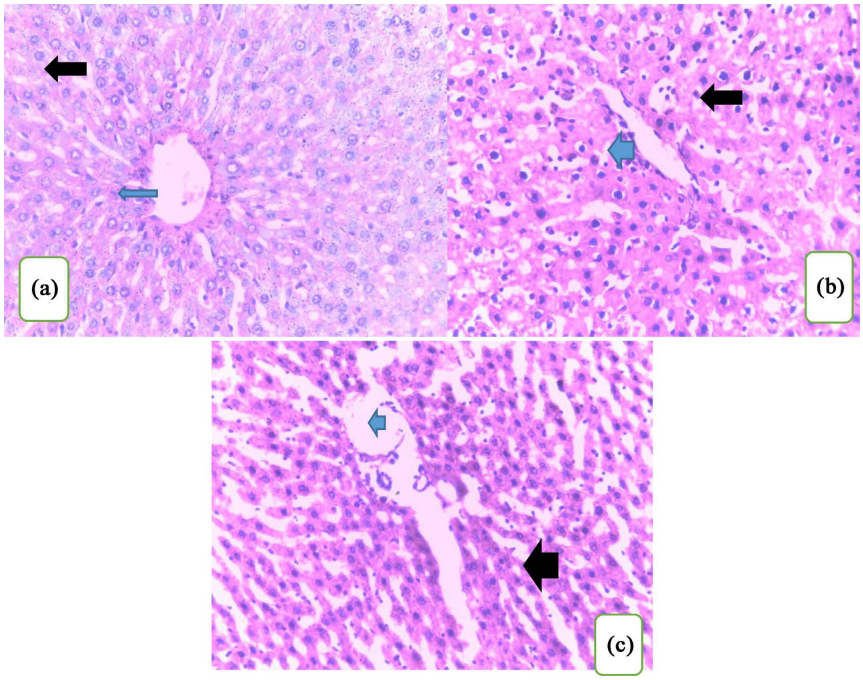

Figure 3. Photomicrograph of the Liver for the 400 mg/kg STCD rats. The photomicrographs revealed that the liver of the rats administered 200 mg/kg STCD as follows: (a) Section shows normal portal tract (blue) and normal hepatocytes (black), (b) section shows normal central vein (blue) and normal hepatocytes (black), (c)section shows normal central vein (blue) and normal hepatocytes (black).

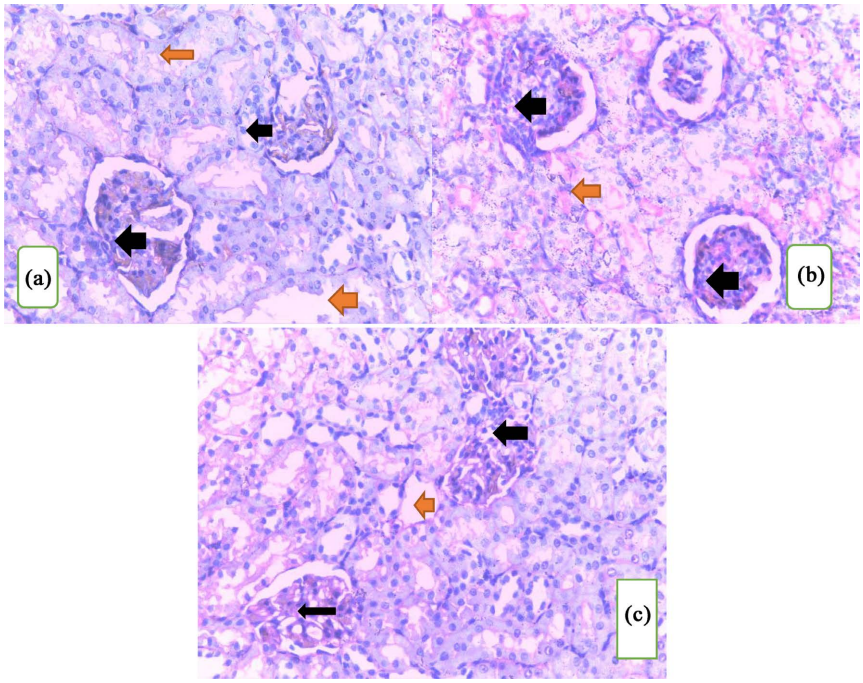

Figure 4. Photomicrograph of the Kidney for the control group rats. The photomicrographs revealed that the kidney of the rats in the control group are as follows: Section of kidney shows normal glomeruli (black) and normal tubule (red) (b) Section of kidney shows normal glomeruli (black) and normal tubule (red) (c) Section of kidney shows normal glomeruli (black) and mild fatty infiltration of the tubules (red).

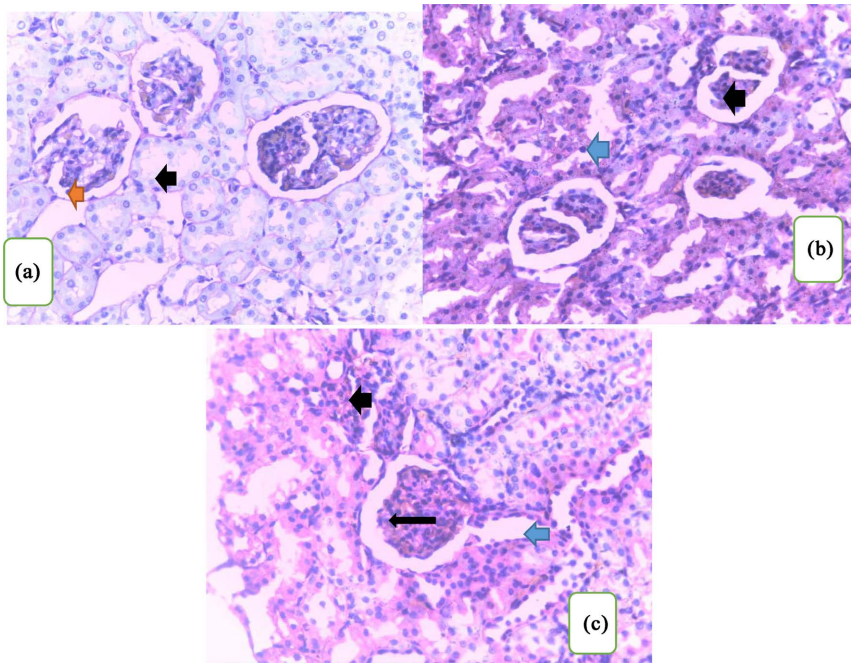

Figure 5. Photomicrograph of the Kidney for the 200 mg/kg STCD rats. The photomicrographs revealed that the kidney of the rats in the 200 mg/kg STCD group are as follows: (a) Section of kidney shows normal glomeruli (black) and normal tubule (red) (b) section of kidney shows normal glomerulus (blue) and normal tubules (black) (c) section of kidney shows normal glomerulus (black) and normal tubules (blue).

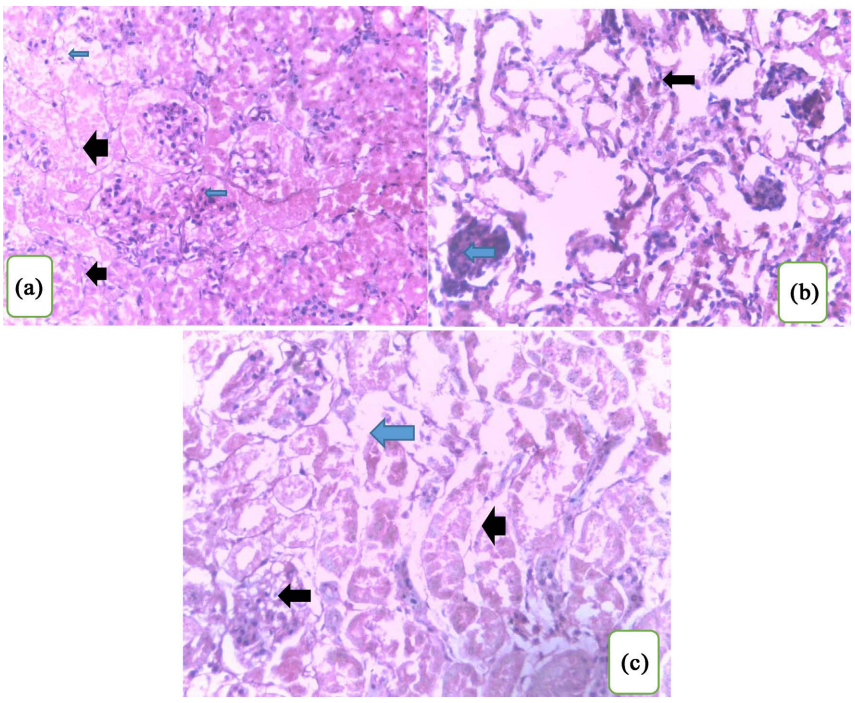

Figure 6. Photomicrograph of the Kidney for the 400 mg/kg STCD rats. The photomicrographs revealed that the kidney of the rats in the 400 mg/kg STCD group are as follows (a) Section of kidney shows normal glomeruli (black) and normal tubule (red) (b) section of kidney shows normal glomerulus (blue) and normal tubules (black) (c) section of kidney shows normal glomerulus (black) and normal tubules (blue).

normal, having normal central vein, normal hepatocytes, normal portal tract and mild infiltration of the hepatocytes. The rats administered 200 and 400 mg/kg STCD orally also showed normal central veins and normal hepatocytes.

The kidneys of the rats in the control groups were structurally normal with normal glomeruli, normal tubule and mild infiltration of the tubules. Also, rats administered 200 and 40 mg/kg STCD showed Section of kidney with normal glomeruli and normal tubule.

4. Discussion

The synergistic aqueous mixture of *Phoenix dactylifera* fruits, *Cocos nucifera* nuts and *Cyperus esculentus* (*STCD*) from this study showed no mortality of the male albino rats at all doses administered. However, 3400 and 6800 mg/kg STCD led to diarrhea of the rats and 50% mortality was observed thereafter. Acute toxicity LD_{50} of STCD was observed from this study to be ≥2404.2 mg/kg body weight in adult male albino rats.

The administration of STCD led to an increase in the body weight of the rats at all administered dose. However, an increase in the % mean body weight difference was observed in the order; control group > 200 mg/kg STCD > 400 mg/kg STCD. The increase in the % mean body weight difference of the control group rats could be attributed to the feed and water allowed to the rats' *ad libitum* without any administration. Rat feed is composed of the nutrient content; 18.50% protein, 3% fats, 6% crude fibers, 7% crude ash and 12% moisture [19]. Animals allowed to unhampered exposure to high nutritional content food, water and standard conditions has recorded an immense body weight increase in experimental rats [19]. Albeit the increase in the body weight of the rats administered 200 and 400 mg/kg STCD, a decrease in the % mean body weight difference was observed in the groups administered 200 mg/kg and 400 mg/kg extract when compared to the control group. The oral gavage method of administration of extract to experimental rats has been reported [20] by Patricia *et al.*, (2011) to have undulate effects on experimental rats, of which could lead to stress, result in passive reflux if the stomach is overfilled, aspiration pneumonia, pharyngeal, esophageal and gastric irritation or injury with structure formation. From the above literature, the decrease in the percentage body difference in the extract administered group could be attributed to the accompanying stress in the administration of the extracts.

The synergistic aqueous mixture of *Cyperus esculentus, Phoenix dactylifera and Cocos nucifera* (STCD) on the liver biomarkers revealed that STCD led to a decrease in the blood AST, ALT, Total Bilirubin and conjugated bilirubin in comparison to the control group. An elevation in the enzymatic hepatic biomarkers has been associated with various disease conditions, including liver dysfunction [21]. The regular intake of bioactive compounds is associated with beneficial effects on chronic diseases, including liver diseases [22]. The inherent bioactive constituents of the seeds could be attributed to the potency of STCD to

improve the status of the liver as evidenced in **Table 4** which revealed a decrease in the concentration of AST, ALT and ALP in 200 and 400 mg/kg STCD rats in comparison to the control group. **Figures 1-3** also showed the histology of the liver having normal structural integrity of the veins, portal tracts and hepatocytes. The non-enzymatic biomarkers of liver function; Total protein, Albumin, Total bilirubin and conjugated bilirubin also had an insignificant decrease in concentration for all administered group when compared to the control group. An irregular or significant increase or decrease in blood Total protein and Albumin may be associated with various disease conditions including liver and disease diseases, cancer or infections such as hepatitis [23].

A decrease in Urea has been associated with malnutrition, low-protein diet, impaired metabolic activity in the liver due to parenchymal liver disease or rarely, to congenital deficiency of urea cycle enzymes [24]. Creatinine is excreted primarily by the kidney. The rich nutritional content of Tiger nuts in Vitamins and minerals such as Phosphorus, potassium, vitamins B1, E and C [2] can be attributed to the contribution of the extract to improve the functions of the kidney. Tiger nuts, Dates and Coconuts has also been reported to reduce the risk of colon cancer, prevent constipation, balancing of the nervous system and improve the antioxidant status of the body [25] [26].

5. Conclusion

The findings of this study opines that the synergistic mixture of *Cyperus esculentus, Phoenix dactylifera and Cocos nucifera* (STCD) had no toxicity in all doses administered, improved the health of both the liver and kidney as evidenced via the biomarkers and histological examinations of the liver and kidney. This study therefore recommends the intake of STCD at moderate doses for improved liver and kidney function due to its bioactive compounds and nutritional content.

Authors' Contributions

The study was jointly designed and conducted by all authors. All authors read and approved the final version of this manuscript.

Ethics Approval

All animals were treated in strict accordance with the National Institutes of Health Guide for the care and Use of Laboratory Animals. Experimental protocols were approved by the Ethics Committee of the Rivers State University, Nigeria.

Data Availability

The data generated and analyzed in the present study are available from the corresponding author upon reasonable request

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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