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Research Article

Evaluation of acute and sub-chronic toxicities of ulcer fast®: a bi-herbal formula in male Wistar albino rats

Kingsley C. Patrick-Iwuanyanwu*, Jane A. Emerue

Department of Biochemistry (Toxicology Unit), Faculty of Chemical Sciences, College of Natural and Applied Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria

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***Correspondence to:**
Kingsley C. Patrick-Iwuanyanwu,
Email: kctrendy@yahoo.co.uk

ABSTRACT

The acute and sub-chronic toxicities of ulcer fast® (UF) - A commercial bi-herbal formula prepared with *Alstonia boonei* and *Xylopia aethiopica* in male Wistar albino rats was evaluated. There was no mortality in rats administered 2000 mg/kg body weight (BW) of UF in an acute toxicity study. A significant ($p \leq 0.05$) increase in daily consumption of feed and fluid intake in experimental rats after 28 days was recorded followed by a progressive increase in BW of rats administered 50, 100 and 200 mg/kg BW of UF in a dose-dependent manner. Alanine amino transferase, aspartate amino transferase, alkaline phosphatase, lactate dehydrogenase, triacylglycerides and creatinine increased significantly ($p \leq 0.05$) in rats treated with UF, whereas urea and fasting blood sugar decreased significantly ($p \leq 0.05$) in a dose-dependent manner when compared with control. There was a marginal decrease in serum calcium ion and phosphate ion following the administration of UF when compared with control. Packed cell volume and hemoglobin decreased significantly ($p \leq 0.05$) in rats treated with UF, whereas white blood cell increased significantly ($p \leq 0.05$) in a dose-dependent manner when compared with control. Histological examination of the liver, kidney, heart and lungs showed normal architecture in control group, whereas hepatocytes of rats treated with 50, 100 and 200 mg/kg BW of UF were characterized by slight periportal fatty change, marked change and ballooning degeneration. Heart muscle of rats treated with 200mg/kg BW of UF showed slight inflammation while histological examination of the lungs showed areas of interstitium damage and diffuse alveolar damage in rats treated with UF. In conclusion, indiscriminate administration of UF could be of public health concern and long-term exposure may cause a significant potential health risk.

Keywords: Ulcer fast, Bi-herbal formula, Sub-chronic toxicity, Acute toxicity, Histological examination, Risk assessment

INTRODUCTION

Over the years, the use of herbs in the treatment of illnesses has been very successful, and sufficient use of its historic usage has been useful in drug discovery and development. Herbal drugs are prescribed widely due to their effectiveness, less side-effects and relatively low cost.[1] Medicinal herbs and herbal formulas are generally considered to be safer than conventional drugs. However, there is no evidence for the quality, safety, and efficacy of most commonly used herbal formulas. According to World Health Organization about 80% of the world population relies on traditional medicine for primary health care and more than 30% of the plant species have been used medicinally. The usage of herbal medicines has its advantage as they do not produce many side-effects commonly seen after long-term administration of synthetic drugs.[2] There are limited clinical trials to determine efficacy and safety of traditional herbal medicines. As such, one of the major drawbacks of these medicines is limited bioavailability and poor absorption when taken orally.[3,4] At present, herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers, and adaptogens.[5] In spite of such wide acceptability, the number of standardized herbal drugs is less due to lack of regulatory standards and implementation protocols. Standardization requires a natural plant product to be authenticated at origin itself by adoption of good agricultural and manufacturing practices.[6-10] There is an apparent trend of adding drugs or analogues to herbs to make them more effective, especially for weight loss and enhanced sexual function.[11] Furthermore, the adverse effects of some herbs have been reported.[12-14]

Ulcer fast® (UF) is a commercial bi-herbal preparation commonly used as an ulcer remedy. It contains a combination

of *Alstonia boonei* and *Xylopia aethiopica*. *A. boonei* (*Apocyanaceae*) is a large ever-green and medicinal plant used extensively in West, East and Central Africa for the treatment of malaria, fever, intestinal helminthes, rheumatism micturition, insomnia, chronic diarrhea, rheumatic pains, anti-inflammatory and hypertension and also for the prevention of diseases.[15-19] The stem bark of *A. boonei* has been listed in the African Pharmacopoeia as anti-venom for snake bites and in the treatment of arrow poisoning.[17,19,20]

X. aethiopica, commonly called Negro pepper is one of the most pungent spices used in various traditional dishes of Western and Central Africa. It is found mostly in forest and coastal regions in Nigeria and belongs to the family Annonaceae.[21,22] The plant is used in decoction to treat dysentery, cough, bronchitis, ulceration, skin infection and female sterility. Several studies have shown that *X. aethiopica* extracts possess antibacterial,[23-26] antifungal[27] and anti-plasmodial[28] activities. *X. aethiopica* has been reported to increase antioxidant defense and protects rats from the adverse effects of irradiation.[29,30] The present study was, therefore, carried out to evaluate the acute and sub-chronic toxicity profile of UF® in male Wistar albino rats.

METHODS

Source of materials

Four bottles of UF®, a commercial herbal preparation, produced by Alive Herbals in Accra, Ghana with NAFDAC Reg: 020-815-1645 was purchased from Trado-Medical Centre at the Isaac Boro Park in Port Harcourt, Rivers State, Nigeria.

Experimental animals

Healthy male Wistar albino rats weighing between 120 and 150 g, obtained from the Animal House of the Department of Physiology, University of Nigeria, Enugu Campus, Enugu State, Nigeria were used for this study. They were divided into four groups of 10 rats per group. The animals were housed in a cross-ventilated room and kept under standard environmental condition of 12/12 hrs light/dark cycle. They were housed in polypropylene cages (five animals per cage) and were fed with standard rat pellet and water *ad libitum*. They were allowed to acclimatize for 7 days to the laboratory conditions before the experiment. The experiment was performed in accordance with the guidelines established by the European Community for the Care and Use of Laboratory Animals and approved by Departmental Animal Ethical Committee.

Experimental procedure

Acute toxicity test

Healthy male Wistar albino rats weighing between 140 and 160 g maintained under standard laboratory conditions were used for acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guidelines 425.[31] A total of 10 animals were used, which received a single oral dose of 2000 mg/kg body weight (BW) of UF. Animals were kept overnight fasting prior to drug administration of crude extracts by oral gavage. After administration of UF, food was withheld for further 3-4 hrs. Animals were observed individually at least once during first 30 mins after dosing, periodically during first 24 hrs (with special attention during the first 4 hrs) and daily thereafter for a period of 14 days. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, lacrimination, perspiration, pilo-erection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes were noted.[31]

Feed and water intake

The weight of feed and volume of water consumed by rats in each group were measured daily as the difference between the quantity of feed and water supplied and the amount remaining after 24 hrs respectively.

BW change

Rats in all the groups were weighed twice every week during the period of treatment and on the last day of study. Doses of the extract administered were adjusted accordingly.

Sub-chronic toxicity study

Subchronic toxicity study was carried out in accordance with OECD 407 guidelines.[32] 40 healthy male Wistar albino rats were used for the sub-chronic study. They were divided into four groups of 10 rats per group. Group I, which served as the control received normal saline, standard diet and water only while Groups 2, 3 and 4 were, administered 50, 100 and 200 mg/kg BW of UF respectively. The drugs were administered using a curved, ball-tipped stainless steel feeding needle for a period of 28 days. All the rats had free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality (Table 1).

Sample collection

At 24 hrs after the 28 days of oral administration of UF, the rats were anaesthetized in a chloroform-saturated chamber after which the animals were sacrificed by cervical dislocation method. Blood samples were obtained by cardiac puncture from each rat by means of a 2 ml hypodermic syringe and needle. One portion was collected into clean dry ethylenediaminetetraacetic acid bottles for the estimation of hematological parameters namely: packed cell volume

Table 1: Treatments administered to different groups.

Group	Treatment	Duration	Number of rats
Control	Normal saline+feed+water	28 days	10
50 mg/kg BW	Normal feed+water+50 mg/kg BW of UF	28 days	10
100 mg/kg BW	Normal feed+water+100 mg/kg BW of UF	28 days	10
200 mg/kg BW	Normal feed+water+200 mg/kg BW of UF	28 days	10

BW: Body weight, UF: Ulcer fast

(PCV), white blood cells (WBC) and hemoglobin (Hb) using an automated hematological machine (Cell-DynTM Abbot, US). Another portion was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 mins. The sera were separated, stored at −4°C used for evaluation of biochemical parameters namely: alanine transaminase (ALT), aspartate transaminase (AST) levels and alkaline phosphatase (ALP) levels, total cholesterol, total and conjugated bilirubin, serum urea nitrogen and creatinine using commercial kits obtained from Randox Laboratories, UK. Electrolyte levels were estimated using the method of analysis as prescribed by Trinder.[33,34]

Hematological analysis

The WBC and the differentials were estimated using the improved Neubauer counting chambers as described by Dacie and Lewis.[35] The Hb concentration was determined by the Cyameth-Hb method while the PCV was determined by the micro method also as described by Dacie and Lewis.[35]

Histopathological study

A portion of the liver, kidney, heart and lung of all the rat groups was fixed separately in 10% buffered neutral formalin for 48 hrs followed by bovine solution for 6 hrs and then processed for paraffin embedding. By using a microtome, sections of 5 µm thickness were taken, processed in alcohol-xylene series and were stained with alum-hematoxylin and eosin[36] and subjected to histopathological examination.

Statistical analyses

The results are expressed as mean±standard error of the mean. One-way analysis of variance was employed for between and within group comparison while Student's t-test was used for paired comparison. 95% level of significance ($p \leq 0.05$) was used for the statistical analysis.

RESULTS

The result of the acute toxicity study showed no mortality or physical changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, pilo-erection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000 mg/kg BW of UF. Since none of the mentioned toxic signs and symptoms or mortality was observed in the animals at the above-mentioned dose, 50, 100 and 200 mg/kg BW of test drug were selected for the study. The results of daily feed and fluid intakes are shown in Table 2. Data from the study showed a significant ($p \leq 0.05$) increase in the daily feed and fluid intake in rats administered UF in a dose-dependent manner when compared with control with rats administered 200 mg/kg BW recording the highest (Table 2). In addition, there was also a progressive increase in BWs of rats administered 50, 100 and 200 mg/kg BW UF when compared with control. The result of the effect of oral administration of UF on serum marker enzymes and biochemical parameters are as shown in Tables 3 and 4 respectively. There was a significant ($p \leq 0.05$) increase in the levels of liver marker enzymes (ALT, AST, ALP), lactate dehydrogenase (LDH) and biochemical parameters (triacylglyceride and creatinine), whereas urea and fasting blood sugar (FBS) decreased significantly ($p \leq 0.05$) in rats administered UF when compared with control. There was a marginal increase in the levels of sodium ion and potassium ion when compared with control. However, rats administered UF showed a marginal decrease in the levels of calcium ion, chloride ion and phosphate ion when compared with control (Table 5). Result from the study on the effect of administration of UF on hematological parameters showed significant ($p \leq 0.05$) decrease in PCV and Hb in the groups administered UF in a dose-dependent manner when compared with control whereas WBC increased significantly ($p \leq 0.05$) in experimental rats in a dose-dependent manner when compared with control (Figure 1).

Table 2: Daily feed and fluid intake of UF treated rats.

UF (mg/kg)	Feed intake (g/day)	Fluid intake (mL/day)
Control	24.45±1.10	35.00±1.45
50 mg/kg	30.35±0.92	40.50±1.33
100 mg/kg	38.48±0.87	47.10±1.10
200 mg/kg	45.00±0.76	52.55±0.90

n=10 values (mean±SEM). SEM: Standard error of the mean, UF: Ulcer fast

Results of the effect of administration of UF on histological examination of the liver showed normal architecture in control group, whereas rats administered 50, 100 and 200 mg/kg BW were characterized by slight periportal fatty change, marked change, and ballooning degeneration (Figure 2a-d).

Table 3: Effect of UF® on serum biochemical parameters of male Wistar albino rats after 28 days.

Parameters	Control	50 mg/kg	100 mg/kg	200 mg/kg
ALT (U/L)	63.31±38.21	86.72±11.55[a]	124.71±7.56[b]	190.70±9.24[c]
AST (U/L)	343.60±10.82	550.70±80.43[a]	662.60±34.70[b]	686.68±32.56[c]
ALP (U/L)	20.33±121	25.42±10.17[a]	44.10±8.22[b]	61.67±0.21[c]
LDH (U/L)	406.70±15.27	466.71±41.63[a]	480.30±96.44[b]	493.35±47.26[c]
Triacylglyceride	1.59±0.57	1.76±0.15[a]	1.97±0.09[b]	2.14±0.31[c]
Creatinine	69.00±1.00	77.07±8.96[a]	82.08±5.00[b]	96.01±4.36[c]
Urea (µmol/L)	2.27±0.35	1.73±0.55[a]	1.53±0.06[b]	0.93±0.15[c]
FBS (µmol/L)	11.17±5.25	8.43±1.23[a]	6.70±0.7[b]	6.37±1.01[c]

n=10 values (mean±SEM). Superscripts (a-c) is significantly different at p≤0.05 when compared with control. ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, FBS: Fasting blood sugar, UF: Ulcer fast

Table 4: Effect of UF® on serum electrolyte levels of male Wistar albino rats.

Group	Na^+ (mg/dl)	K^+ (mg/dl)	Ca^{2+} (mg/dl)	Cl^- (mg/dl)	HPO_4 (mg/dl)
Control	135.67±2.52	8.50±0.10	2.61±0.32	90.06±0.70	1.37±0.15
50 mg/kg	145.67±2.08	6.87±0.40	1.77±0.15	90.06±1.75	1.27±0.31
100 mg/kg	138.33±4.93	7.70±0.55	1.67±0.12	85.00±0.40	0.92±0.75
200 mg/kg	145.67±3.22	6.53±0.31	1.77±0.15	86.10±1.21	1.7±0.10

n=10 values (mean±SEM), SEM: Standard error of the mean, UF: Ulcer fast

Table 5: Effect of UF® on selected hematological parameters in male Wistar albino rats.

Group	PCV	Hb	WBC
Control	41.50±2.12	13.67±1.53	5.9±1.27
50 mg/kg	32.67±5.78[a]	11.32±1.73[a]	10.2±4.86[a]
100 mg/kg	29.00±3.00[b]	8.87±0.90[b]	9.4±5.13[a]
200 mg/kg	22.67±14.57[c]	6.93±4.08[c]	15.87±1.30[b]

n=10 values (mean±SEM). Superscripts (a-c) is significantly different at p≤0.05 when compared with control,
SEM: Standard error of the mean, UF: Ulcer fast, PCV: Packed cell volume, WBC: White blood cells, Hb: Hemoglobin

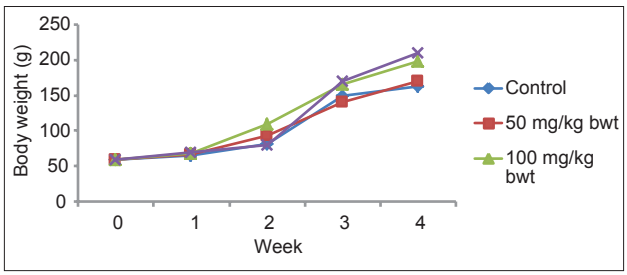

Figure 1: Effect of ulcer fast® on body weight changes in control and treated rats.

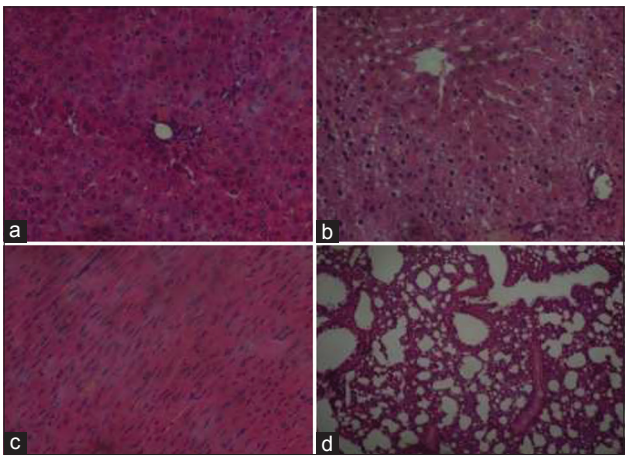

Figure 2: A cross section of control rat liver (a) normal hepatocytes; and groups treated with 50 mg/kg body weight (BW), (b) slight periportal fatty change; 100 mg/kg BW, (c) slight periportal change 200 mg/kg BW, (d) a marked change and ballooning degeneration (magnification ×200).

Histological examination of rat kidney of control rats showed normal architecture, whereas rats administered 50, 100 and 200 mg/kg BW UF were characterized by normal glomeruli, interstitium and vascular system (Figure 3a-d); on the other hand, histological examination of heart of control rats showed normal heart muscles. Similarly, rats administered 50 and 100 mg/kg BW UF also showed normal heart muscle whereas rats in the group administered 200 mg/kg BW UF were characterized by slight inflammation of the heart muscle (Figure 4a-d). Finally, histological examination of lungs of control rats showed normal alveolar and interstitium, whereas rats in group administered 50, 100 and 200 mg/kg BW UF were characterized by diffuse interstitium damage and diffuse alveolar damage (Figure 5a-d).

DISCUSSION

The use of herbal products is increasing, and over-the-counter herbal supplements are perceived by the public

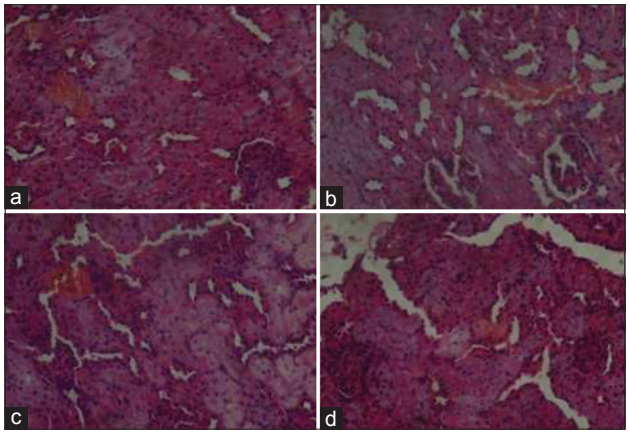

Figure 3: Photomicrographs of rat kidney administered with ulcer fast showing (a) Control with normal architecture, (b) 50 mg/kg body weight (BW) showing normal glomeruli, tubules, interstitium and vascular system, (c) 100 mg/kg dose showing normal glomeruli, tubules, inters ilium and vascular system, (d) 200 mg/kg dose showing normal glomeruli, tubules, inters ilium and vascular system (magnification ×200).

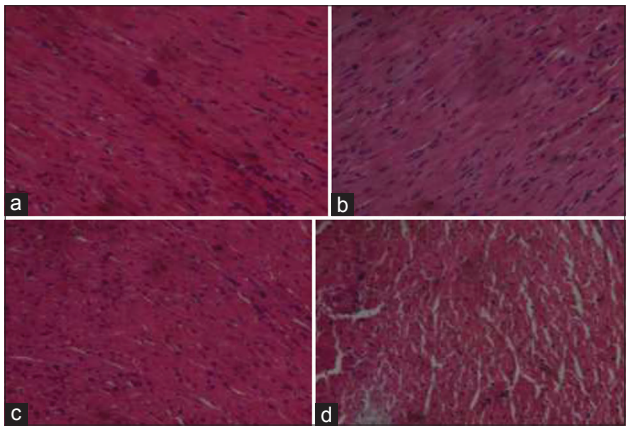

Figure 4: Photomicrograph of rat heart treated with ulcer fast and stained with H and E, (a) Control showing normal heart muscles, (b) 50 mg/kg dose, showing normal heart muscle, (c) 100 mg/kg dose showing normal heart muscle, (d) 200 mg/kg dose showing slight inflammation of the heart muscle (magnification ×200).

as "safe" and "harmless." Although the majority of them are safe, some herbal medicines carry risks.[37] Heavy metal contamination, adulteration with western pharmaceuticals and inclusion of prohibited animal and plant ingredients are regularly reported in ethnic medicines.[37] The present study investigated the toxicity effects of UF - A commercial herbal preparation in male Wistar albino rats. Data obtained from acute toxicity of UF showed no mortality or physical changes in skin and fur; eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerction, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors, and convulsion) among rats administered up to 2000 mg/kg

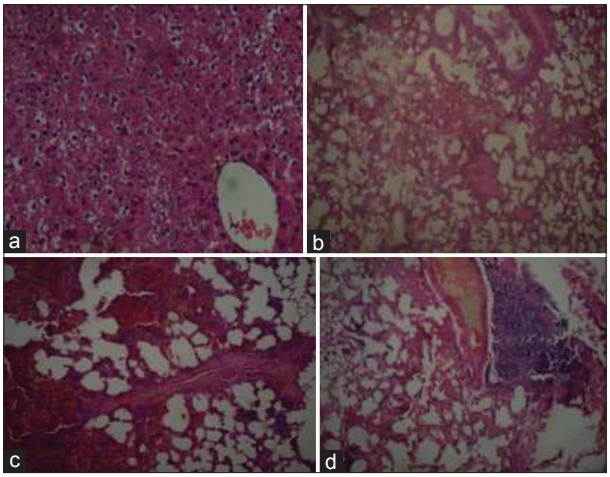

Figure 5: Photomicrograph of lung of control rat, (a) normal alveolar and interstitium and rats treated with 50 mg/kg ulcer fast (UF), (b) diffuse interstitium damage; 100 mg/kg body weight (BW) UF, (c) diffuse alveolar damage and 200 mg/kg BW UF, (d) diffuse alveolar damaged lung (magnification ×200).

BW of UF. This observation suggests that the drug could be considered relatively safe especially when administered orally where absorption may not be complete due to inherent factors limiting absorption in the gastrointestinal tract.[38,39] In a similar manner, sub-chronic administration of UF had no negative effect on the normal growth of rats suggesting that it did not cause any alterations in carbohydrates, protein or fat metabolism in the experimental animals. This finding suggests that UF did not adversely interfere with nutritional benefits (e.g. weight gain, stability of appetite) expected of animals that are continuously supplied with food and water *ad libitum*.[40] Changes in BW have been used as an indicator of adverse effects of drugs and chemicals.[41]

The significant ($p \leq 0.05$) elevation of liver marker enzymes ALT, AST, ALP may be an indication of liver damage.[42,43] Transaminases such as ALT and AST are well-known indicators of liver function and used as biomarkers to conclude the probable toxicity of drugs and xenobiotics.[44] A mild or higher activity of AST indicates liver injury or myocardial infarction.[45,46] ALT on the other hand catalyzes the conversion of alanine to pyruvate and glutamate. Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury.[47-49] Alkaline phosphatase is more specific to detect obstruction in the biliary system. It is found in both the liver and the bile and it leaks into the bloodstream in a manner similar to that of the ALT and AST.[50] The increased level of LDH observed in the present study is suggestive of damage to cardiac muscle of the rats.

The elevation of creatinine and urea in this present study is an indication that UF may adversely affect the concentration of creatinine and urea in experimental rats. The elevation of urea and creatinine levels in the serum is taken as index of drug-induced nephrotoxicity.[51-53] Urea, a waste product of

protein catabolism may rise and accumulate when the kidney is defective indicating that the rate of serum urea production exceeds the rate of its clearance.[54] The increased level of serum triacylglycerides in experimental rats may be attributed to increased availability of free fatty acid, glycerophosphate, decreased very-low-density lipoprotein in the serum and decreased removal of triglyceride and cholesterol from serum due to diminished lipoprotein activity.[55,56] The reduction in the level of blood glucose (FBS) may be due to the ability of UF to prevent the absorption of glucose in the gut and/or increased insulin secretion by pancreatic stimulation.[57]

Hematological parameters are important health indices and are of diagnostic significance in routine clinical evaluation of the state of health. The reduction in the level of PCV in this study indicates that UF may have adversely affected erythrocyte concentration. Fairbarks[58] showed that xenobiotic causes hemolytic anemia when sulfhydryl groups of the erythrocyte membrane are oxidized, which inflicts injury to the erythrocytes membrane. The observed reduction in the levels of PCV and Hb may have occurred due to lysis of blood cells leading to the suppression of blood cell synthesis by any of the active ingredient found in this drug. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell (RBC) membrane to oxidative damage probably as a result of depletion of reduced glutathione on the surface of the RBC.[59-61] The elevation of WBC may be attributed to a combination of physiological and biochemical factors in the metabolic system of the animal resulting to the defensive mechanism in the immune system of the rats. The observation in this study is in agreement with the report of Adisa et al.,[62] who reported increased WBC count and a corresponding decrease in PCV values in albino rats infected with *Trypanosoma brucei*. The increase in the WBC count may be attributed to the induction of systemic or localized inflammatory responses by the ingestion of UF.

The histopathological changes in the liver, kidney, lungs and a mild change in the heart corroborates results of biochemical parameters and may be explained by the fact that UF induced oxidative stress, which may account for the various degenerative changes in these organs. In conclusion, the result of this study indicated an elevation in liver marker enzymes, biochemical parameters, induction of anemia and increased WBC counts. Therefore, long-term administration of UF may be toxic to vital organs in the body.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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