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Sub-acute toxicity assessment of *Sapium ellipticum* (Hochst) Pax ethanol leaf extract in Wistar rats

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ABSTRACT

Objective: To determine the toxicity of *Sapium ellipticum* (*S. ellipticum*) ethanol leaf extract in animal model.**Methods:** Three groups (I, II and III, $n = 10$) of Wistar rats were respectively given 0.5 mL corn oil, 400 and 800 mg/kg BW of *S. ellipticum* extract, twice daily, for a period of 14 days. Lethal dose (LD_{50}) was determined and morbidity was assessed via repeated dose administration of the extract.**Results:** *S. ellipticum* at the doses employed did not cause any significant changes in the serum concentrations of ALT, AST and ALP compared to the control group. The level of total serum cholesterol was significantly ($P < 0.05$) and dose-dependently reduced in the extract-treated animals by 13.5% and 16.0% compared to the control animals. Total plasma protein concentration and body weight were respectively increased by 8.7% and 13.7% at 800 mg dosage of the extract. The intraperitoneal (*i.p.*) and intramuscular (*i.m.*) LD_{50} values of *S. ellipticum* leaf extract were determined as 979.80 and 1341.60 mg/kg BW respectively while the oral (*p.o.*) LD_{50} was estimated to be greater than 45000 mg/kg BW.**Conclusions:** The observations noted in this study apparently validate the use of *S. ellipticum* ethanol leaf extract in folklore medicine in terms of herbal safety.

1. Introduction

Several studies have affirmed the efficacy of a number of plants in the treatments of diseases compared to some modern or conventional drugs which have failed to yield positive results[1]. However, the problem of herbal toxicity remains a great concern in the use of plants or herbs for complementary or alternative treatments of diseases. The reason is not far-fetched; some plants which have worked like a miracle have been unfortunately found to contain extremely toxic substances capable of causing more damage to consumers[2-5]. It is therefore essential to assess the toxicity of plants which are commonly employed in folk medicine.

Sapium ellipticum (Hochst) Pax (*S. ellipticum*) is widely used in folk medicine[6,7]. It belongs to the family Euphorbiaceae and is commonly referred to as jumping seed tree. *S. ellipticum* is common in eastern and tropical Africa. In southwest part of Nigeria, particularly among the Ilorin indigenes, the plant is popularly referred to as *aloko-agbo*.

A few scientific investigations have been conducted on *S. ellipticum*. Adesegun *et al.*[8] in their *in vitro* study reported

antioxidant properties of the stem bark extract of the plant. Cytotoxicity screening of selected Nigerian plants used in traditional cancer treatment on HT29 (colon cancer) and MCF-7 (breast cancer) cell lines (HeLa cervix adenocarcinoma cells) indicated that *S. ellipticum* leaf extract showed the highest cytotoxic activity among other plants with anticancer potential[9]. The phytochemical constituents, *in vitro* antioxidant capacities and antiparasitodal activities of *S. ellipticum* stem bark extracts were documented by Nana *et al.*[10]. Edimealem *et al.*[11] in their study demonstrated the presence of lupeol, lupeol acetate and stigmasterol in the stem bark extract of *S. ellipticum*. This present study therefore aimed to assess the toxicity of the leaf extract of the plant.

2. Materials and methods

2.1. Collection of *S. ellipticum* leaves

Fresh *S. ellipticum* leaves were collected in the month of December, 2012 from a forest in a suburb of Ibadan, southwest of Nigeria. The harvested leaves were taxonomically authenticated by a botanist (Mr. TK Odewo) at the Lagos University Herbarium (LUH), Nigeria, where a specimen was deposited and assigned a voucher number (LUH 5423).

2.2. Preparation of *S. ellipticum* leaf extracts

The plant material was freed of extraneous materials, air dried at room temperature (25–28 °C) and milled to fine powder using a

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All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health. The experiments were approved by the Ethical Committee of the Faculty of Sciences, Lead City University.

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Waring blender. Then 300 g of the powdered sample was macerated in 2.5 L of the extracting solvent (absolute ethanol) at room temperature. The mixture was allowed to stand for 72 h and stirred intermittently with a glass rod to facilitate extraction. Filtration of the mixture was achieved with a muslin cloth (maximum pore size 2 mm). The resulting filtrate was further filtered through Whatman filter paper (No. 42) and subsequently reduced in volume with a rotary evaporator at 40 °C. Final elimination of solvent and drying were done using a regulated water bath at 40 °C.

2.3. Management of animals

All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health[12]. The experiments were approved by the Ethical Committee of the Faculty of Sciences, Lead City University.

The animals were handled humanely, kept in plastic suspended cages, placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided with rat pellets (Ladokun feeds), and water *ad libitum* and subjected to natural photoperiod of 12 h light and 12 h dark cycle. The animals were allowed to acclimatize for two weeks prior to the commencement of the study.

2.4. Determination of lethal dose of *S. ellipticum* ethanol leaf extract

Lethality studies to determine the LD₅₀ of the extract was performed according to the combined procedures described previously[13,14] with slight modification. The LD₅₀ was assessed for three routes of administration: intraperitoneal (*i.p.*), intramuscular (*i.m.*) and oral (*p.o.*). For intraperitoneal (*i.p.*) and intramuscular (*i.m.*) administration, forty male rats were randomly assigned to 10 groups, with each group having 4 animals. They were respectively treated with 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 mg/kg BW of the extract. The animals were then returned to their respective cages, allowed to have free access to pellets and drinking water 3 h later. They were thereafter monitored for clinical signs, symptoms, behavioral change, feeding pattern and mortality within 24 h of the experiment. Animals were observed individually once during the first 30 min after administration, periodically during the first 24 h (with more attention during the first 4 h), and daily for a period of 14 days.

For the oral LD₅₀ determination, three different sets of animals were used. The first set of animals were randomly divided into five groups, each containing 4 rats. They were treated with 1000, 2000, 3000, 4000 and 5000 mg/kg BW respectively with no mortality recorded after 24 h. In the second phase, doses of 6000, 8000, 10000, 12000 and 14000 mg/kg BW were respectively administered to another set of animals. When no mortality was recorded, a third set of animals equally assigned to 5 groups were respectively treated with doses of 15000, 20000, 30000, 40000 and 45000 mg/kg BW of the extract. They were closely observed for negative behavioral changes and mortality within 24 h of the experiment. The same pattern of observation used for the intraperitoneal and intramuscular studies as described above was followed. The lethal dose of the extract for the different routes was calculated using the formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

where, D₀ = maximum dose that produces 0% mortality; D₁₀₀ = minimum dose that produces 100% mortality.

2.5. Repeated dose toxicity assessment of ethanol leaf extract of *S. ellipticum*

Repeated dose toxicity study was carried out to determine the

effects of *S. ellipticum* ethanol leaf extract on blood chemical parameters. Thirty rats were assigned to three groups (*n* = 10) and labeled Groups I, II and III. The groups were respectively given corn oil (0.5 mL), 400 and 800 mg/kg BW of *S. ellipticum* extract twice daily for 14 days. After the last administration, the animals were fasted overnight and sacrificed by cervical dislocation and blood samples were collected through cardiac puncture into heparinized bottles. The collected blood samples were centrifuged at 3000 r/min for 10 min and the resultant supernatant in each case was used for blood chemistry analyses.

2.5.1. Determination of liver enzyme activity

The activities of alanine amino transferase (ALT, EC. 2.6.1.2.) formerly known as glutamate pyruvate transaminase (SGPT) and aspartate amino transferase (AST, EC. 2.6.1.1) formerly known as glutamate-oxaloacetate transaminase (SGOT) were estimated by the use of endpoint colorimetric diagnostic kits (Randox Laboratories Limited, England) according to the method of Reitman and Frankel[15]. Alkaline phosphatase (ALP) activity was determined by the use of Sigma diagnostic kits (Sigma Diagnostic, USA) according to the method of Englehardt[16].

2.5.2. Estimation of total protein, globulin and albumin concentrations

The concentrations of total plasma protein and albumin were estimated using Sigma diagnostic kits (Sigma Diagnostic, USA). The level of globulin was obtained by difference.

2.5.3. Lipid profile analysis

Total cholesterol (T-chol) and total triglycerides (TAG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined using test kits (Linear Chemicals).

2.6. Histopathological effects of *S. ellipticum* leaf extract

The liver and kidneys were removed from each rat and preserved in buffered 10% formalin solution for histopathological processing and examination. These organs were first gross examined for any observable lesions or tissue derangements before they were processed using the automatic tissue processor. The technique involved dehydrating the fixed tissues placed in tissue baskets with their respective labels and passing them through graded alcohol (70%, 90%, 95% and 100%) solutions. The tissues were removed after dehydration and moved into xylene solution baths to clear the alcohol and facilitate molten wax impregnation. The tissues were finally sectioned using rotary microtome (at 5 µm thickness), stained with haematoxylin and eosin (H&E) and then examined microscopically using standard techniques[17].

2.7. Statistical analysis

Data analysis was performed using statistical software, Prism graphpad, version 6.4. The statistically significant difference between groups was analyzed using One-way ANOVA followed by independent-sample *t* test. The level of significance was set at *P* < 0.05. The results were presented as mean ± SEM.

3. Results

3.1. LD₅₀ determination of *S. ellipticum* ethanol leaf extract

The LD₅₀ values of the *S. ellipticum* leaf extract through intraperitoneal (*i.p.*) and intramuscular (*i.m.*) routes were determined as 979.80 and 1341.60 mg/kg BW respectively. Oral administration

of the extract (to a dose of 45000 mg/kg BW) did not cause any negative behavioral changes in the animals, and no mortality was recorded within and after 24 h of the experiment; increased appetite was observed in the animals.

3.2. Repeated dose effects of *S. ellipticum* ethanol leaf extract on Wistar rats

The effects of repeated administration of *S. ellipticum* extract twice daily for a period of 14 days to physiologically normal rats are summarized in Figures 1–4. Figure 1 shows that *S. ellipticum* extract caused significant ($P < 0.05$) and dose dependent gain in body weight of rats by 8.9% and 13.7% compared to the 5.1% gain observed in the control animals. Treatment of rats with *S. ellipticum* at the doses employed (400 and 800 mg/kg BW) relatively to the control group did not cause any significant changes in the serum concentrations of ALT, AST and ALP in rats. Nonetheless, administration of *S. ellipticum* at a dose of 400 mg/kg BW resulted in a negligible and non-significant increase in both ALT and ALP, as well as a slight decrease in AST (Figure 2).

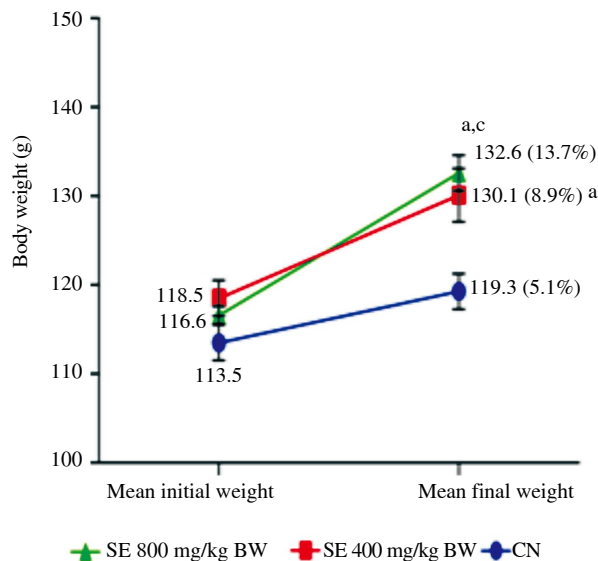


Figure 1. Repeated dose effects of *S. ellipticum* on the body weights of rats.

Values are expressed as mean of six rats ($n = 10$). CN: Control rats treated with corn oil; SE: *S. ellipticum*. ^a: $P < 0.05$ when compared to control; ^c: $P < 0.05$ when compared to 400 mg/kg.

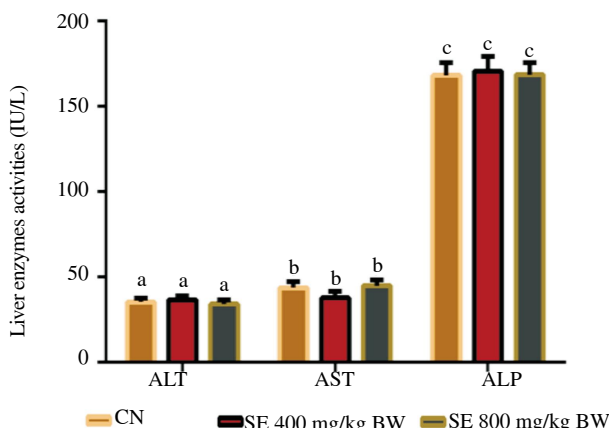


Figure 2. Repeated dose effect of *S. ellipticum* on selected liver enzymes (ALT, AST and ALP) in Wistar rats.

Values are expressed as mean \pm SEM ($n = 10$). CN: Control rats treated with corn oil; SE: *S. ellipticum*. Bars with the same letter are not significantly different.

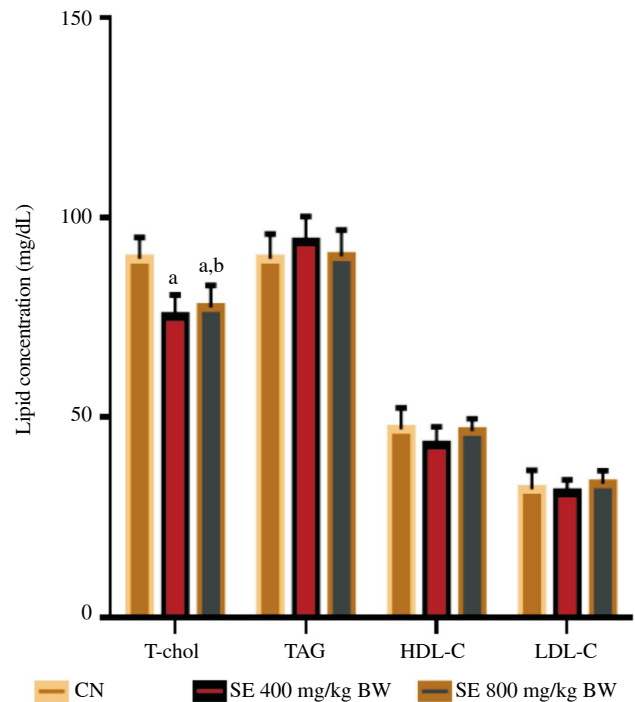


Figure 3. Repeated dose effect of *S. ellipticum* on lipid metabolism in Wistar rats.

Values are expressed as mean \pm SEM ($n = 10$). CN: Control rats treated with corn oil; SE: *S. ellipticum*. ^a: $P < 0.05$ when compared to control; ^b: $P < 0.05$ when compared to 400 mg/kg BW.

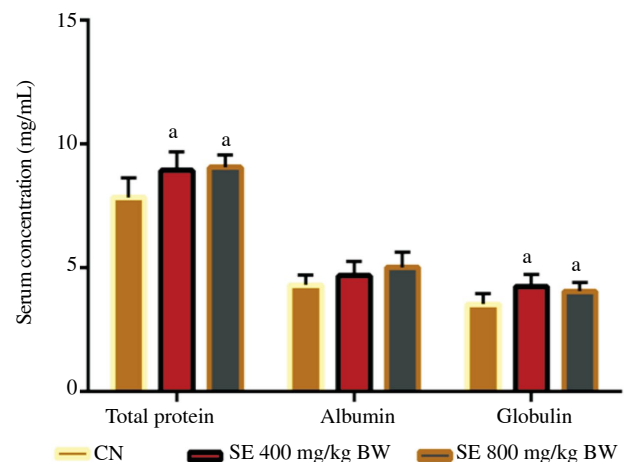


Figure 4. Repeated dose effect of *S. ellipticum* on serum total protein, albumin and globulin.

Values are expressed as mean \pm SEM ($n = 10$). CN: Control rats treated with corn oil; SE: *S. ellipticum*. ^a: $P < 0.05$ when compared to control.

Administration of *S. ellipticum* at doses of 400 and 800 mg/kg BW caused significant ($P < 0.05$) reduction in serum T-chol level in rats by 13.5% and 16.0% respectively compared to the control animals. Conversely, the extract at both doses failed to cause any significant alteration in the levels of other parameters (TAG, HDL-C and LDL-C) in the animals. However, slight increase and decrease were noted in TAG and HDL-C respectively following *S. ellipticum* administration at 400 mg/kg BW (Figure 3). Total plasma protein concentration was also significantly increased by 7.4% and 8.7% respectively relative to control (Figure 4).

3.3. Histopathological effects of *S. ellipticum* leaf extract

Figures 5 and 6 show the histological structure of the liver and kidney of control rats and those administered with *S. ellipticum*

extract (800 mg/kg BW). No visible lesion or tissue derangement was observed in the liver tissues. The kidney of both control and extract-treated animals showed mild intratubular and interglomerular infiltrations.

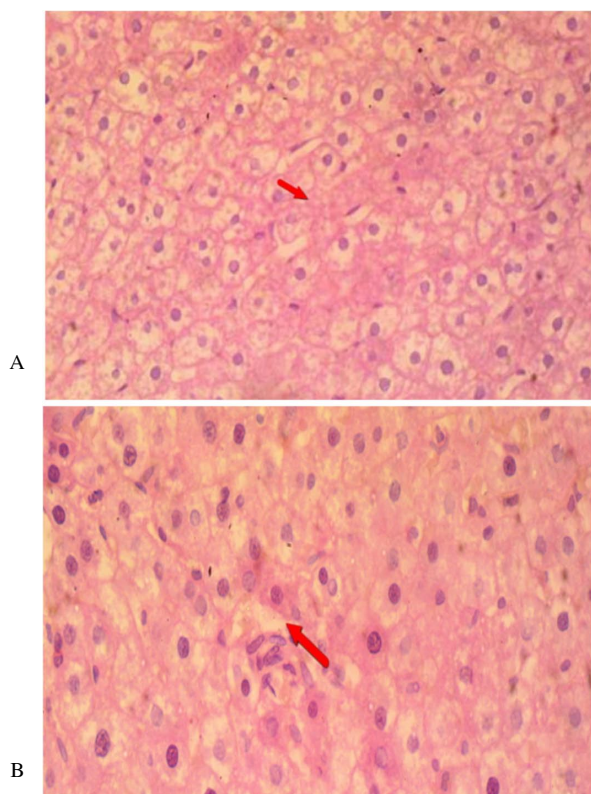


Figure 5. Photomicrograph of thin sections (5 μ m) of liver tissues of *S. ellipticum*-treated rats (800 mg/kg) (A) and control (B) (400 \times), showing normal liver histology (red arrow).

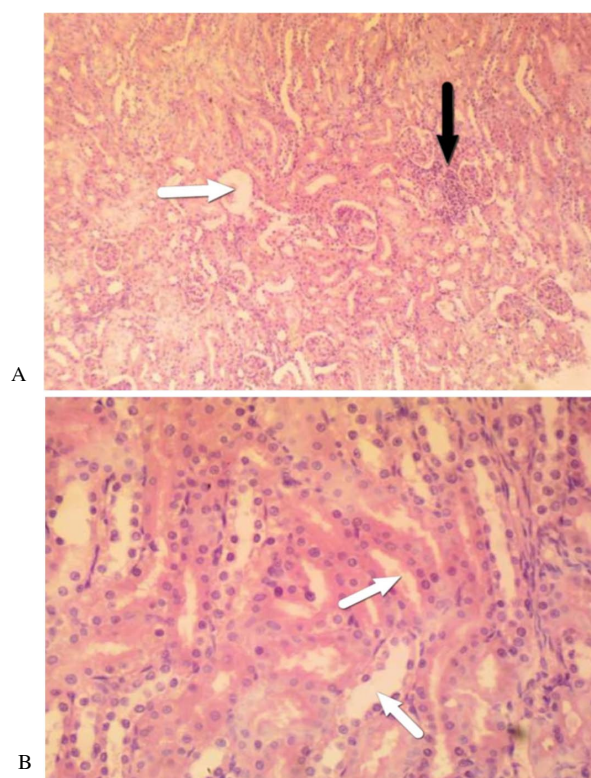


Figure 6. Photomicrographs of thin sections (5 μ m) of kidney tissues of control (A) and *S. ellipticum*-treated rats (B) (100 \times) showing mild intratubular cellular infiltration (white arrow) and interglomerular cellular infiltration (black arrow).

4. Discussion

Herbal toxicity is a major cause for concern in the therapeutic application of plant materials; hence, the knowledge of the toxicity profile of *S. ellipticum* is important, particularly in terms of its lethal dose (LD₅₀) and repeated dose toxicity. LD₅₀ is generally described as the dose or concentration of a test material (plant, chemical, drug, etc.) that causes mortality in 50% of the animals (rats, mice, etc.) in a dose group. Its values are useful in comparing the relative acute hazards of substances, especially when no other toxicology data are available. More importantly, the LD₅₀ value of a material indicates its safe dose range through a particular administration route. Nonetheless, many important observations of toxicity are not represented by LD₅₀ values or by slopes of dose-response curves for lethality. For example, information about morbidity and pathogenesis may have more toxicological significance than mortality and these are not revealed by the LD₅₀ of a substance. Acute toxicity in the form of repeated dose toxicity (evaluation of toxicological indices) is therefore necessary to provide more useful preliminary information on the toxic nature of a new material for which no other toxicology information is available[18].

ALT, AST and ALP are the major serum marker enzymes of hepatic injury and represent markers of tissue derangement[19]. These enzymes play significant role in diagnosis of diseases, assessment of drugs and plant extracts for safety and toxicity[20]. Their concentrations in the plasma are collectively used as an indicator of the liver functional and structural integrity. High serum levels (beyond defined limits for normal liver physiology) of these enzymes occur as a result of leakage from the liver cytosol into the blood stream following hepatic structural damage or injury[21]. The liver is the primary and major site for metabolism of most foreign substances (xenobiotics) in the body and is arguably the most susceptible organ[22]. Chemical agents, such as those used in laboratories and industries, biological agents (such as microcystins) as well as herbal remedies can inflict hepatic injury. More than 900 drugs have been implicated in causing liver injury[23].

In this study, the hepatotoxic effect of *S. ellipticum* extract was investigated by comparing the serum levels of ALT, AST and ALP in rats treated with different doses (400 and 800 mg/kg BW) with those in a control group. The results observed in the treated groups suggest that the structural and functional integrity of the liver was not compromised by the *S. ellipticum* extract at the administered doses.

ALT is specific for the liver[24] but AST is found also in other tissues including the red blood cells, the cardiac and the skeletal muscle[25]. ALP is located in all tissues of the body but particularly in large amounts in the biliary duct of the liver[26], and obstruction of this duct increases the level of the enzyme in the plasma. The enzyme is commonly used to assess the integrity of plasma membrane and endoplasmic reticulum of tissues[27]. The maintenance of serum ALP concentration in this study suggests that the integrity of the various membrane systems was not compromised by the administration of the extract. The slight decrease noted in AST level suggests a possible improvement in cellular integrity and functionality of the liver and other tissues where AST is primarily located. It also implies that the extract has the potential to enhance tissue regeneration following previous damage. These findings agree with the report of Rao *et al.*[28] which stated that no significant change was observed in AST and ALT activity following treatment of rats with *Commiphora molmol* for 24, 48 and 72 h. Overall, lack of significant changes in the levels of serum aminotransferases and ALP in the present study suggests that *S. ellipticum* is safe, and exhibits no deleterious effect on the liver as well as other body tissues.

The effect of *S. ellipticum* extract on the lipid profile of rats was also studied in the current investigation. Lipid profile is the term

that collectively describes the amounts of T-chol, TAG, LDL-C and HDL-C in milligram per deciliter (it may also include the measurement of phospholipids and other lipids). This profile is used to assess the risk of cardiovascular disorders (CVDs) and is altered in the serum in various disease states such as diabetes[29]. Except for HDL-C, high level of all lipids in the blood is arguably a risk factor in the etiology and progression of cardiovascular disorders[30]. LDL is one of the lipoprotein components of the blood. It transports cholesterol mainly to the arterial wall. This results in the build-up of insoluble lipid on the wall of the arteries thereby reducing blood flow and increasing the pressure on the arterial wall as well as the heart.

The deposition of cholesterol on the arterial wall results in a condition known as arteriosclerosis which is the major cause of cardiovascular disorders. In contrast, HDL binds to arterial cholesterol and transports it to the liver for metabolism. People with high levels of HDL-C seem to have fewer problems with CVDs, while those with low HDL-C have increased rate of CVDs. Thus, substances that increase the plasma HDL-C and decrease LDL-C will play an important role in reducing the risk of CVDs.

In this study, *S. ellipticum* leaf extract at the administered doses (400 and 800 mg/kg BW) was observed to cause significant reduction in the serum levels of T-chol in physiologically normal rats, suggesting that the extract possesses anti-hypercholesterolemic effect. It, however, failed to show any significant alteration in the serum levels of other lipid molecules.

Surprisingly, slight increase and decrease were noted in TAG and HDL-C respectively. This observation is a bit disturbing as cholesterol level is strongly connected to the levels of other lipid molecules. Cholesterol is synthesized from long chain fatty acids which are attached to the glycerol side chain of TAG. Thus, increase in the TAG level often increases the synthesis of cholesterol from the liver. Also the serum levels of HDL-C, LDL-C, and VLDL-C collectively constitute the total cholesterol level in the serum. It will then be reasonable to suggest that the reduction in total cholesterol following *S. ellipticum* administration may have probably occurred through the induction or suppression of certain enzymes critical to the metabolism of cholesterol.

It also implies that the extract may have facilitated the mobilization of cholesterol from the blood into peripheral tissues which may be responsible for the slight decrease in HDL-C. Besides, the decline in the HDL-C may have been influenced by the action of the extracts on the activities of lecithin cholesterol acyl transferase (LCAT), which plays a key role in the maturation of HDL-C particles[31]. The anti-hypercholesterolaemic effect observed with *S. ellipticum* extract in the current study is similar to that in previous report by Adebayo et al.[32].

Proteins are essential and indispensable to the functional and structural integrity of body cells and tissues. They are primarily synthesized in the liver and a compromise in protein system is the basis for several disease conditions or pathologies. Albumin and globulin constitute the total plasma proteins and are usually in ratio of 2:1. Thus, albumin constitutes the major component of the total plasma protein. It is lowered in chronic liver damage, malnutrition as well as during impaired protein metabolism. Decline in serum

albumin level has severe negative impact on the body system.

The present study showed that *S. ellipticum* extract at both doses produced reasonable increase in total protein concentration in rats. Particularly, the level of serum globulin was increased, indicating facilitated absorption of protein in the intestine as well as efficient functionality of the liver[33]. Enhanced intestinal absorption of protein may provide the liver with sufficient supply of amino acids to synthesize serum proteins, leading to a rise in serum proteins (e.g. globulin). Conversely, impaired intestinal absorption of protein or liver damage may impair the synthesis of serum proteins in the liver and results in decreased serum proteins levels.

Chan et al.[34] demonstrated that serum albumin level is usually reduced in chronic liver disease and congestive heart failure. The effect of *S. ellipticum* on serum proteins observed in this study reflects a positive role of the extract in protein absorption and synthesis. More importantly it reveals extremely low toxicity for the investigated extract.

In light of the prescriptions of Gad and Chengelis[35], the LD₅₀ values and the outcome of the various toxicological evaluations obtained in this study suggest that *S. ellipticum* leaf extract has extremely low toxicity. The extract at the administered doses during the administration period did not cause significant negative alterations in the evaluated indices, suggesting that the structural and functional integrity of vital tissues was not compromised in the experimental animals. This view is substantiated by the fact that histological architectures of the liver and kidney of *S. ellipticum* treated animals were comparable to those of the control animals. Besides, the significantly high oral LD₅₀ (45 000 mg/kg BW) further demonstrates that the extract is non toxic and safe for oral consumption. This result is consistent with the oral LD₅₀ value greater than 25 g/kg BW reported by Wansi et al.[36] for the root bark extract of *S. ellipticum* in mice.

The high oral LD₅₀ value associated with *S. ellipticum* in the present study could possibly be due to biotransformation of the components of the extract into non-toxic metabolites in the gastrointestinal tract of the animals by the action of certain modifying or detoxifying enzymes. It may also be attributed to the fact that the metabolites of the extract are easily eliminated from the body system, consequently preventing bioaccumulation to a toxic level.

The increased food intake in the extract-treated animals as observed in this study is suggestive of the presence of digestive or appetizing agent(s) in *S. ellipticum* leaf extract.

In conclusion, the results of the lethality and morbidity studies apparently validate the use of *S. ellipticum* leaf extract in folklore medicine in terms of herbal safety.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Ernst E. Herbal remedies. In: Stoleran IP, editor. *Encyclopedia of psychopharmacology*. Berlin: Springer Berlin Heidelberg; 2015, p. 733-8.
- [2] Aziato L, Antwi HO. Facilitators and barriers of herbal medicine use in Accra, Ghana: an inductive exploratory study. *BMC Complement Alternat Med* 2016; **16**(1): 142.
- [3] Ardalan MR, Rafieian-Kopaei M. Is the safety of herbal medicines for kidneys under question? *J Nephropharmacol* 2013; **2**(2): 11.
- [4] Efferth T, Kaina B. Toxicities by herbal medicines with emphasis to traditional Chinese medicine. *Curr Drug Metab* 2011; **12**(10): 989-96.
- [5] Asif M. A brief study of toxic effects of some medicinal herbs on kidney. *Adv Biomed Res* 2012; **1**: 44.
- [6] Gove AD, Hylander K, Nemomissa S, Shimelis A, Enkossa W. Structurally complex farms support high avian functional diversity in tropical montane Ethiopia. *J Trop Ecol* 2013; **29**(2): 87-97.
- [7] Burkill HM. *The useful plants of West Tropical Africa*. Vol. 1-3. 2nd ed. London: Royal Botanic Gardens, Kew; 1995.
- [8] Adesegun SA, Elechi NA, Coker HA. Antioxidant activities of methanolic extract of *Sapium ellipticum*. *Pak J Biol Sci* 2008; **11**: 453-7.
- [9] Sowemimo A, Van de Venter M, Baatjies L, Koekemoer T. Cytotoxicity evaluation of selected Nigerian plants used in traditional cancer treatment. *J Med Plants Res* 2011; **5**(11): 2442-4.
- [10] Nana O, Momeni J, Nzangué Tepongning R, Ngassoum MB. Phytochemical screening, antioxydant and antiplasmodial activities of extracts from *Trichilia roka* and *Sapium ellipticum*. *J Phytopharmacol* 2013; **2**(4): 22-9.
- [11] Edimeale A, Reneela P, Tsegaye J. Phytochemical investigation of *Sapium ellipticum*. *Nat Prod Plant Resour* 2013; **3**(5): 1-6.
- [12] National Institutes of Health. *Strategic plan for NIH obesity research*. Bethesda: National Institutes of Health; 2004.
- [13] Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol* 1983; **54**(4): 275-87.
- [14] Botham PA. Acute systemic toxicity-prospects for tiered testing strategies. *Toxicol In Vitro* 2004; **18**(2): 227-30.
- [15] Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; **28**: 56-63.
- [16] Englehardt A. Measurement of alkaline phosphatase. *Aerztl Lab* 1970; **16**: 42-3.
- [17] Arthur SJ, John B. *A colour Atlas of histopathological staining techniques*. London: Wolfe Medical Publishers, Ltd.; 1978, p. 14-20.
- [18] Gad SC, Smith AC, Cramp AL, Gavigan FA, Derelanko MJ. Innovative designs and practices for acute systemic toxicity studies. *Drug Chem Toxicol* 1984; **7**(5): 423-34.
- [19] Sandler NG, Koh C, Roque A, Eccleston JL, Siegel RB, Demino M, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. *Gastroenterology* 2011; **141**(4): 1220-30.
- [20] El-Kader MA, Mohamed NZ. Evaluation of protective and antioxidant activity of thyme (*Thymus vulgaris*) extract on paracetamol-induced toxicity in rats. *Aust J Basic Appl Sci* 2012; **6**: 467-74.
- [21] Sun Y, Yang J, Wang LZ, Sun LR, Dong Q. Crocin attenuates cisplatin-induced liver injury in the mice. *Hum Exp Toxicol* 2014; **33**(8): 855-62.
- [22] Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug Metab Rev* 2012; **44**(1): 88-106.
- [23] Grant LM, Rockey DC. Drug-induced liver injury. *Curr Opin Gastroenterol* 2012; **28**(3): 198-202.
- [24] Philip R, Mathias M, Kumari SN, Gowda DK, Shetty JK. Evaluation of relationship between markers of liver function and the onset of type 2 diabetes. *Nitte Univ J Health Sci* 2014; **4**(2): 90.
- [25] Gatsing D, Aliyu R, Kuate JR, Garba IH, Jaryum KH, Tedongmo N, et al. Toxicological evaluation of the aqueous extract of *Allium sativum* bulbs on laboratory mice and rats. *Cameroon J Exp Biol* 2005; **1**(1): 39-45.
- [26] Karim SM, Rahman MR, Shermin S, Sultana R. Correlation between aminotransferase ratio (ast/alt) and other biochemical parameters in chronic liver disease of viral origin. *Delta Med Coll J* 2015; **3**(1): 13-17.
- [27] Olajide JE, Akanji AM, Sanni M, Omale J. Acetylsalicylic acid and cellular damage in kidney of metabisulphite treated rats. *Anim Res Int* 2008; doi: 10.4314/ari.v2i3.40875.
- [28] Rao RM, Khan ZA, Shah AH. Toxicity studies in mice of *Commiphora molmol* oleo-gum-resin. *J Ethnopharmacol* 2001; **76**(2): 151-4.
- [29] Panel ED, Grundy SM. An international atherosclerosis society position paper: global recommendations for the management of dyslipidemia. *J Clin Lipidol* 2013; **7**(6): 561-5.
- [30] Ray KK, Kastelein JJ, Matthijs Boekholdt S, Nicholls SJ, Khaw KT, Ballantyne CM, et al. The ACC/AHA 2013 guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular disease risk in adults: the good the bad and the uncertain: a comparison with ESC/EAS guidelines for the management of dyslipidaemias 2011. *Eur Heart J* 2014; **35**(15): 960-8.
- [31] Glomset JA. The plasma lecithin: cholesterol acyl transferase reaction. *J Lipid Res* 1968; **9**: 155-67.
- [32] Adebayo HA, Aliyu R, Gatsing D, Garba HI. The effects of ethanolic leaf extract of *Commiphora africana* (Burseraceae) on lipid profile in rats. *Int J Pharmacol* 2006; **2**(6): 618-22.
- [33] Grant GH, Kachmar JF. *Fundamental of clinical chemistry*. 3rd ed. Tietz NW, editor. Philadelphia: W.B. Saunders Company; 1987, p. 298-320.
- [34] Chan S, McCowen KC, Bistran BR, Thibault A, Keane-Ellison M, Forse RA, et al. Incidence, prognosis, and etiology of end-stage liver disease in patients receiving home total parenteral nutrition. *Surgery* 1999; **126**(1): 28-34.
- [35] Gad SC, Chengelis CP. *Acute toxicology testing: perspectives and horizons*. Caldwell: The Telford Press; 1988.
- [36] Wansi SL, Nguelefack-Mbuyo EP, Nchouwet ML, Miaffo D, Nyadjieu P, Wabo JP, et al. Antidiarrheal activity of aqueous extract of the stem bark of *Sapium ellipticum* (Euphorbiaceae). *Trop J Pharm Res* 2014; **13**(6): 929-35.