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Sub-chronic toxicity of ethanol leaf extract of *Syzygium guineense* on the biochemical parameters and histopathology of liver and kidney in the rats

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ABSTRACT

Background: Syzygium guineense Wall. leaf is being used as a traditional medicine against hypertension and diabetes mellitus. Unlike its efficacy, the safety profile of this plant upon long-term administration has not been investigated yet. Therefore, this study investigated the sub-chronic toxicity of *S. guineense* leaves in rats.
Methods: Wistar albino rats, 10/sex/group were randomly assigned into four groups. Group I-III respectively received 250, 500, and 1000 mg/kg of body weight of 70 % ethanol extract of*S. guineense* leaves for 90 consecutive days. Group IV (control) received distilled water. Throughout the experiment, clinical observations were carried out, food intake and weight of the rats also were measured. Finally, different biochemical parameters, organ weight, and histopathology of liver and kidneys were evaluated.
Results: Administration of 70 % ethanol extract of*S. guineense* leaves decreased food intake and body weight gain of the test animals. Rats treated with 1000 mg/kg of *S. guineense* extract showed significantly increased serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels. Serum urea levels also increased in female rats treated with 500 and 1000 mg/kg body weight of *S. guineense*. Moreover, the blood glucose level of rats treated with 1000 mg/kg body weight was significantly decreased compared to the control group. However, the histology of the liver and kidneys were not significantly altered by any of the doses administered.
Conclusion: Administration of*S. guineense* in rats at a dose of 1000 mg/kg body weight affected the food consumption, weight gain, and serum levels of liver and kidney enzymes suggesting that *S. guineense* intake at high doses may be toxic. Therefore, liberal consumption of *S. guineense* leaves should be taken curiously and cautiously.

1. Introduction

Usage of plant in therapy dates back to antiquity, for all civilizations, not being an actual “discovery” [1]. Many millions of the world’s populations rely on herbal medicines. Herbal medicine contributes a lot to all people to get access to health care. In developing countries including Africans, 65–80 % of the population depends largely on medicinal plants for basic healthcare [2]. Similarly, a great number of Ethiopian populations use traditional medicine for their health care. The low cost, easy accessibility, and community trust are some of the reasons mentioned by

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; DPX, Dibutylphthalate Polystyrene Xylene; EPHI, Ethiopian Public Health Institute; H & E, hematoxylin and eosin; IRB, institutional review board; OECD, Organization for Economic Co-operation and Development; SDM, standard deviation of mean SPSS: statistical package for social science; TMMRD, Traditional and Modern Medicine Research Directorate.

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the users to choose herbal therapies [3].

Syzygium guineense Wall. is a medium to large evergreen water-loving dicotyledon tree distributed in many African countries [4]. The leaves of *S. guineense* are traditionally used for the treatment of diabetes mellitus [5], malaria [6], wound [7], herpes zoster infection [8], and many others. *S. guineense* leaves have many scientifically proven pharmacologic effects. It showed potential antivenom [9], antidiabetic [10], antihypertensive [11], antimalarial [12], and anti-inflammatory [13] properties. It is also reported as a good analgesic agent [13].

The phytochemical constituents of *S. guineense* leaves, as identified by various investigators, are alkaloids, terpenoids, anthraquinones, flavonoids, tannins, saponins, glycosides, and triterpenes. It also contains sugars, proteins, lipids, polyphenols, and essential oils. These constituents are responsible for its wide range of pharmacologic activities [12–14]. Currently, various phytochemicals or secondary metabolites of plants are tested and effective against different illnesses. Flavonoids are reported to have anti-inflammatory, antioxidation, antimicrobial, antiallergic, and immunomodulatory properties [15] and effective against atherosclerosis, diabetes, and Alzheimer's disease [16]. Saponins also possess immunomodulatory and anti-inflammatory activity and inhibit cancer cell proliferation [17]. Moreover, polyphenols and carotenoids are effective in preventing ophthalmic diseases induced by oxidative stress and inflammation [18,19].

Plant products have potent protective effects against toxic insults [20–22]. However, several researchers have shown that many medicinal plants have potential toxicity on cellular and biochemical parameters of blood [23,24] and histopathology of various internal organs like the liver and kidney [25–29]. Thus, validating the efficacy and assessing the safety of herbal therapies is mandatory [30]. Prior researches conducted to assess the safety of *S. guineense* leaves are of short duration (acute or sub-acute) [10,25,31,32]. Their findings also were inconsistent with each other. Moreover, the toxic effect of *S. guineense* on long term administration has not been determined yet. It is recommended to investigate the toxic effect of this plant based on long-term use since it would be the future source of antihypertensive/antidiabetic drugs [10, 11]. Therefore, the aim of this study was to evaluate the toxic effect of 70 % ethanol extract of *S. guineense* leaves on the histology of liver and kidneys and biochemical parameters in rats, following long term administration. This study is going to address a known gap in knowledge as it is the first of its kind investigating the long-term effects of *S. guineense* and hence, advances the knowledge in the field. The results of this investigation could also be used as baseline data for further investigation.

2. Materials and methods

2.1. Plant collection and extraction

Fresh leaves of *S. guineense* were collected from the suburb of Woliso town, Oromia region, Ethiopia. The leaves were identified by a taxonomist in the National Herbarium, Department of Plant Biology and Biodiversity Management, Addis Ababa University, Ethiopia, where a voucher specimen (MS 001) was deposited. The cleaned and shade dried leaves were coarsely powdered by an electric mill. The powder was macerated with 70 % ethanol and frequently rotated by an orbital shaker for 24 h. The mixture was then filtered by Whatman paper (No 1, 18 cm diameter). Then the filtrate was concentrated with a rotatory evaporator (Büchi Rota Vapor R-205, Switzerland) at 40℃ and the concentrate was further dried by a hot water bath at 45℃. The dried, solvent-free extract was packed in a sealed glass and kept in a refrigerator at -4℃ until used for the experiment [33].

2.2. Experimental animals

The experimental animals were young male and female (nulliparous) Wistar albino rats, 220−260 g weight, and age between 10–12 weeks. The source of experimental rats was the Ethiopian Public Health Institute (EPHI) animal breeding unit. Rats were acclimatized for a week and accommodated for the experiment in the laboratory of the Traditional and Modern Medicine Research Directorate (TMMRD) of EPHI. The rats were maintained in a stainless-steel cage at room temperature (23 ± 3℃) with a relative humidity of 50 ± 10 % under a controlled alternating 12 -h light-dark cycle. Throughout the experiment, a conventional laboratory diet and an unlimited supply of drinking water were supplied.

Eighty (forty male and forty female) rats were randomly assigned into four groups, each consisted of ten rats/sex/group. Rats were randomized using a computer-based random order generator. Groups one to three received 250, 500, and 1000 mg/kg of body weight of the plant extract, respectively, based on a previous efficacy study [10]. The fourth group (negative control) received distilled water (1 mL/100 g of body weight). Rats were administered for 90 days via an intragastric tube. The overall experimental procedures were conducted in compliance with Organization for Economic Co-operation and Development (OECD) guideline test number 408 [34]. Outcomes were blindly measured by the investigator who is unaware of the treated and control rats.

2.3. Clinical observation, body weight and food intake measurement

Throughout the treatment period, a general clinical observation was made every morning. Any signs of toxicity: changes in the skin, motor and sensory function, unusual respiratory pattern, and self-mutilation were recorded before and after dosing. All rats were also observed for severe toxicity, morbidity, and mortality daily. The weight of rats was measured on the first day of administration, weekly thereafter, and at necropsy, then the weight gain was calculated and the weight gain difference among groups was compared. Additionally, food intake was measured daily until the end of the experiment.

2.4. Liver and kidneys weight

At the end of the treatment period, rats were subjected to overnight fasting and then anesthetized with an intraperitoneal injection of pentobarbital (150 mg/kg of body weight) [35]. A blood sample was taken before dissecting the rats. A longitudinal incision was made through the anterior abdominal wall, thereupon, the liver and kidneys were carefully dissected free of fat and examined for any gross pathological alterations. The weight of the liver and kidneys was measured by an electric balance sensitive to 0.0001 g. The relative organ weight was calculated by dividing the absolute organ weight by the final rat weight and multiplying by a hundred.

2.5. Clinical chemistry analysis

At termination, a fasted blood sample was taken for clinical chemistry assessment. Five-six mL of blood was collected via cardiac puncture. The blood was placed in a plane test tube for an hour. To obtain serum, the blood was centrifuged for 10 min with an electrical centrifuge at 3500 rpm. Then serum was separately collected by micropipette and kept in a vial. Finally, the serum was immediately analyzed by an automated clinical chemistry analyzer and the following clinical chemistry investigations indicating renal and hepatic functions were recorded: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, total protein, albumin, glucose, and total cholesterol.

2.6. Histopathology of the liver and kidneys

Samples from the liver and kidneys were obtained to assess treatment-related histopathologic changes. The samples were fixed in 10 % formalin overnight. Tissue processing and staining protocols were based on the basic steps mentioned in Bancroft's theory and practice of

histological techniques [36]. Briefly, the tissues were dehydrated by ascending series of alcohol (40 %, 50 %, 70 %, 80 %, 90 %, 100). Tissues were cleared by xylene. Then the tissues were impregnated and embedded with melted paraffin wax. Finally, to stain the tissues, a five μm section was made. To remove excess wax and facilitate tissue adherence on the slides, the slides with the ribbons were placed in a hot oven (40−45 °C) for 20−30 min.

The staining procedure was conducted as follows: the slides were dewaxed with xylene (I, II, and III) for five minutes in each, dehydrated with descending series of alcohol (absolute alcohol I, absolute alcohol II, 90 % alcohol, 80 % alcohol, and 70 % alcohol) for two minutes in each, washed with running tap water for two minutes. Then the slides were stained with Harris hematoxylin for 6−10 min, cleaned with running tap water for 10 min, immersed in acid alcohol for 2–3 seconds, and counterstained by eosin Y for 1−2 min. The stained slides were dehydrated by ascending series of alcohol (80 %, 95 %, absolute alcohol I, and II) for two minutes in each and cleared with xylene I, II, and III, two minutes in each. Finally, the cleared slides were mounted with Dibutylphthalate Polystyrene Xylene (DPX) and covered with a cover-slip [36].

Once the sides were dried, a detailed microscopic examination for any treatment-related changes was performed by a pathologist using a binocular light microscope. Histology of liver and kidney in the treatment groups was compared with the control group. After microscopic examination, representative photomicrographs were captured by an automated built-in digital microscope camera (Leica EC4, Germany) under 10x and 40x objective lens magnification, total magnification 100x and 400x, respectively.

2.7. Statistical analysis

Data analysis was performed by a statistical package for social science (SPSS) version 24 and results were expressed as mean ± standard deviation of mean (SDM). To check the difference between groups, a one-way analysis of variance (ANOVA) followed by Turkey (to test any difference among the four groups) and Dunnett (for the difference between control and treated groups) post Hoc tests were conducted. P-value < 0.05 was considered statistically significant.

2.8. Ethical approval

A letter of ethical approval was obtained from the Department of Anatomy graduate committee and institutional review board (IRB) of the College of Health Sciences, Addis Ababa University (protocol number AAUMF03−008) in compliance with OECD guideline, test number 408 [34] for the care and use of experimental animals. Rats used in this study were kept in the highest standards for the humane use of animals in the biomedical research laboratory of EPHI. They were not subjected to any unnecessary painful and terrifying situations. Administration of the test substance was carried out by experts and maximum effort was applied to prevent them from the pathogen. Before rats were sacrificed, to avoid pain and suffering, they were anesthetized with an intraperitoneal injection of pentobarbital. Finally, sacrificed rats were disposed of humanely by the laboratory standards of EPHI.

3. Results

3.1. Clinical observations

Cage side clinical observations were conducted daily before and after dosing periods. The result of these records did not exhibit any changes in skin, hair, and mucus membranes. Moreover, no change in respiratory pattern, motor activity, self-mutilation, or any other signs of toxicity were observed. There was no death recorded throughout the experiment, which indicated that the rats well tolerated the 90 days oral administration of the test plant.

3.2. Food intake and weight gain

The levels of food intake and weight gain of the test animals are presented in Table 1. Male rats treated with a high dose of the plant extract showed a significant reduction in food intake compared to the control group. However, there was no significant reduction in food intake in the low and middle dose groups. On the other hand, female rats treated with 1000 mg/kg of the test plant showed decreased food intake (148.4 ± 5.3 g) compared to the control (162.6 ± 12.2 g) and the other treatment groups. Male and female rats treated with 1000 mg/kg of *S. guineense* extract showed a significant reduction in weight gain compared to that in the control and low dose treated groups (Table 1).

3.3. Weight and gross examination of the liver and kidneys

At necropsy, a macroscopic examination of the liver and kidneys was performed. No gross abnormalities in the color, texture, size, and shape of the liver and kidneys were observed across the experimental groups (Fig. 1 a & b). The relative weight of the liver and kidneys presented in Table 2 did not show significant variation between the control and treated groups.

3.4. Effects on the biochemical profile of rats

The biochemical profile of the rats (clinical chemistry) is displayed in Tables 3 & 4 . Male rats treated with a high dose of *S. guineense* extract (1000 mg/kg) had significantly elevated levels of ALT (66.0 ± 9.7) and AST (179.5 ± 20.2) compared to those in the control group (ALT, 44.3 ± 7.5 and AST, 132.8 ± 22.7). AST was also significantly elevated compared to those in the rest of the groups. Serum levels of ALP in rats treated with 500 and 1000 mg/kg of the extract were significantly higher compared to those in low dose treated as well as the control groups. In addition, the serum glucose level was reduced in rats treated with 1000 mg/kg of the plant extract compared to that in the control group. However, there was no significant variation between the treatment and control groups in terms of the other liver and kidney function tests (Table 3).

In female rats, there was a significantly higher level of ALT (82.9 ± 5.9) in rats treated with 1000 mg/kg of the plant extract compared to that in the control group (58.5 ± 16.8) as well as in the low dose (250 mg/kg) treated group (Table 4). In addition, a significantly higher levels of serum AST, ALP, and urea were detected in the middle and high dose treated groups compared to those in the control and low dose treated groups. The blood glucose level was significantly lower (87.0 ± 29) in rats that received 1000 mg/kg of the test substance compared to the

Table 1
Weight gain and food intake of rats treated with 70 % ethanol leaf extract of *S. guineense*.

Gender of the rats	Food intake and weight gain	Group			
		Group I 250 mg/kg	Group II 500 mg/kg	Group III 1000 mg/kg	Group IV Control
Male	Food intake (g) n=10	224.7 ± 16.4	219.3 ± 2	206.3 ± 23.2*	231.2 ± 14.4
	Weight gain/rat (g)	91.2 ± 6.1	85.0 ± 12.8	70.0 ± 10.0**	93.8 ± 6.6
Female	Food intake (g) n=10	162.5 ± 8.2	160.5 ± 8.3	148.4 ± 5.3***	162.6 ± 12.2
	Weight gain/rat (g)	39.8 ± 5.0	34.4 ± 4.6	25.8 ± 5.6 **	39.0 ± 4.2

Results are expressed as mean ± SMD.
* Significantly different from group IV.
** Significant difference with group one and four.
*** Significant difference with all the other groups (p-value<0.05), One-Way ANOVA.

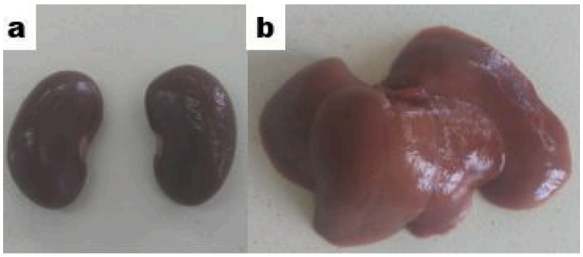

Fig. 1. Photograph of rat kidneys (a) and liver (b) showing normal gross structures following administration of 70 % ethanol leaf extract of *S. guineense* (1000 mg/kg).

Table 2
Relative organ weight of rats treated with 70 % ethanol leaf extract of *S. guineense.*

Gender of the rats	Organ weight (g)	Group			
		Group I (250 mg/kg)	Group II (500 mg/kg)	Group III (1000 mg/kg)	Group IV (Control)
Male	Liver	3.7 ± 1.3	2.7 ± 0.1	2.8 ± 0.2	3.3 ± 0.8
	Kidney	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Female	Liver	3.4 ± 0.5	3.5 ± 0.4	3.6 ± 0.5	3.7 ± 0.4
	Kidney	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1

Results are expressed as mean ± SMD, One-Way ANOVA.

Table 3
Biochemical profile of male rats treated with 70 % ethanol leaf extract of *S. guineense.*

Tests	Group			
	Group I (250 mg/kg)	Group II (500 mg/kg)	Group III (1000 mg/kg)	Group IV (Control)
ALT (U/L)	44.9 ± 13.3	44.8 ± 5.0	66.0 ± 9.7*	44.3 ± 7.5
AST (U/L)	124.3 ± 12.9	113.5 ± 9.4	179.5 ± 20.2**	132.8 ± 22.7
ALP (U/L)	70.3 ± 1.5	103.0 ± 7.0*!	100.3 ± 6.5*!	72.3 ± 2.1
Urea (mg/dL)	52.7 ± 6.2	40.1 ± 3.6	41.6 ± 8.5	47.8 ± 4.1
Creatinine (mg/dL)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.0
Albumin (g/dL)	3.9 ± 0.5	4.1 ± 0.1	4.1 ± 0.2	4.2 ± 0.1
Total protein (g/dL)	6.1 ± 0.6	6.0 ± 0.2	6.3 ± 0.2	6.1 ± 0.4
Total cholesterol (mg/dL)	42.8 ± 2.4	36.6 ± 2.1	42.9 ± 2.6	36.8 ± 1.7
Glucose (mg/dL)	101.8 ± 10.5	91.5 ± 14.8	88.8 ± 12.6*	125.6 ± 12.8

Results are expressed as mean ± SMD.
For all p-value was <0.05, One-Way ANOVA.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase.
* Significantly different from control group (Dunnett test).
** Significant difference with all the other groups.
*! Significant difference with groups I and IV.

control group (129.0 ± 25.0) (Table 4).

3.5. Effects on the histology of the liver and kidneys

Administration of ethanol leaf extract of *S. guineense* for 90 days did not produce a significant change in hematoxylin and eosin (H & E) stained histology of the liver. Only some fatty changes were observed in the liver of one rat treated with 1000 mg/kg of the plant extract (Fig. 2 a & b). In all the groups, there were no structural alterations observed. Its microscopic structures: the portal triad, bile duct system, the hepatocytes, and the sinusoids appeared normal (Fig. 2 c & d). In addition, any dose of the plant extract did not affect the histology of the kidney. The glomerular capillaries, the Bowman's capsule, the afferent and efferent arterioles, and the renal tubes did not show any structural alterations across all the groups (Fig. 3).

Table 4
Biochemical profile of female rats treated with 70 % ethanol leaf extract of *S. guineense.*

Tests	Group			
	Group I (250 mg/kg)	Group II (500 mg/kg)	Group III (1000 mg/kg)	Group IV (Control)
ALT (U/L)	59.4 ± 9.5	67.0 ± 6.0	82.9 ± 5.9**	58.5 ± 16.8
AST (U/L)	164.1 ± 9.9	190.1 ± 2.2**	219.0 ± 5.1*!	162.9 ± 11.4
ALP (U/L)	114.0 ± 3.5	130.0 ± 8.5*	171.0 ± 6.9*!	105.0 ± 5.0
Urea (mg/dL)	55.1 ± 3.3	80.3 ± 7.8**	72.2 ± 13.0*	53.9 ± 7.6
Creatinine (mg/dL)	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.1
Albumin (g/dL)	3.9 ± 0.2	3.5 ± 0.2	3.6 ± 0.2	4.0 ± 0.2
Total protein (g/dL)	5.9 ± 0.5	5.4 ± 0.1	5.4 ± 0.07	5.9 ± 0.6
Total cholesterol (mg/dL)	66.8 ± 4.5	67.4 ± 6.8	67.5 ± 3.7	85.8 ± 27.4
Glucose (mg/dL)	107.5 ± 8.8	98.2 ± 10.2	87.0 ± 29.0*	129.0 ± 25.0

Results are expressed as mean ± SMD.
For all p-value was <0.05, One-Way ANOVA.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase.
* Significantly different from group four (Dunnett test).
** Significant difference with groups one and four.
*! Significant difference with all the other groups.

4. Discussion

The best-registered organ toxicity because of herbal therapy is hepatic toxicity [37,38]. Abnormal liver function tests are the primary manifestation of hepatic toxicity. The liver is highly vulnerable to drug-induced damage due to its drug concentration and metabolic function [37,39]. Another organ that is affected by the toxic insults is the kidney. The high blood flow to the kidney, its function in urine concentration, and metabolic activation of xenobiotics predispose it to the blood-born toxicant to a greater extent than the other organs [40]. The current study was aimed at investigating any toxic effect of *S. guineense* on the structure and function of the liver and kidneys following 90 days of administration in rats.

The results of this study indicated that administration of 70 % ethanol extract of *S. guineense* leaves reduced food intake and weight gain in the test animals. It also resulted in significant reduction in serum glucose level. Serum levels of ALT, AST, ALP, and urea were increased. These effects were noticeable especially in the rats treated with the high dose of *S. guineense.*

Body weight change is an important indicator of toxicity, disease progression, and response to treatment [41]. In our study decreased food intake and weight gain were observed in male and female rats treated with 1000 mg/kg of the plant extract. It is known that *S. guineense* is a tannin-rich plant [42,43]. Tannins damage the epithelial lining of the gastrointestinal tract and reduce food intake [44]. This may be the reason for the reduced food intake and weight gain observed in the high dose treated rats.

In the present study, upon clinical observation, all experimental rats did not show treatment-related behavioral changes and other signs of toxicity. The weight of the liver and kidneys was not significantly altered by treatment with the test substance. This observation is supported by the findings of other researchers [32]. However, increased liver and kidney weight have been reported in mice treated for 42 days with 200

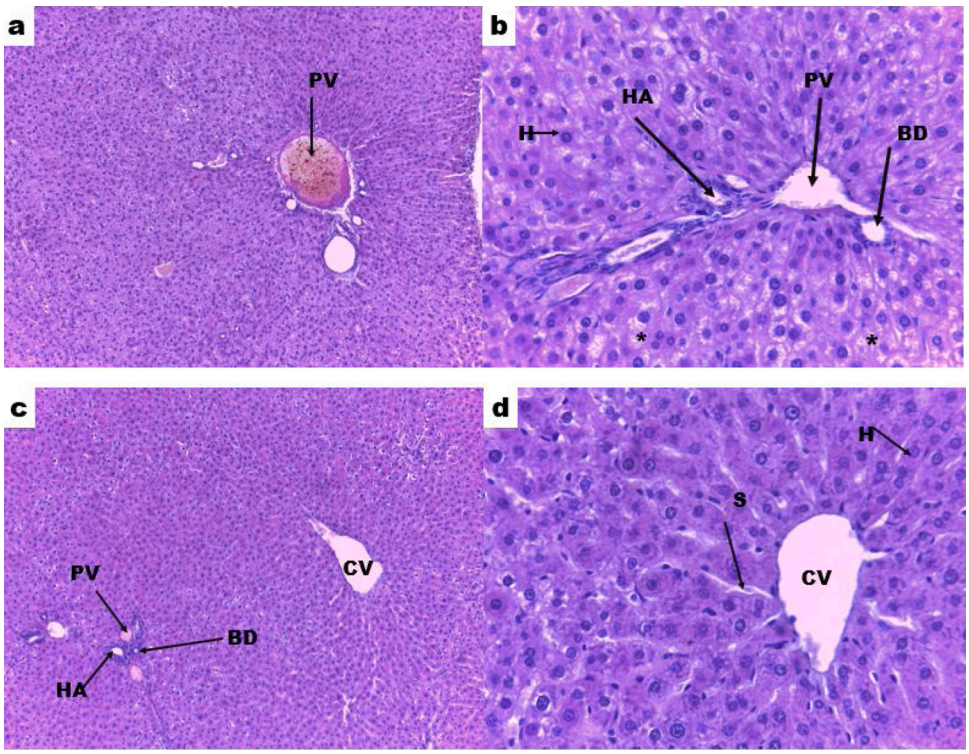

Fig. 2. Photomicrograph of rat liver, a & b sections taken from a rat treated with 1000 mg/kg of 70 % ethanol extract of *S. guineense* leaves showing a fatty change (*), c & d sections taken from control group showing normal liver. PV: portal vein, HA: hepatic artery, BD: bile duct, CV: central vein, H: hepatocyte, S: sinusoid; H and E stain, 100x (a &c) and 400x (b & d) total magnification.

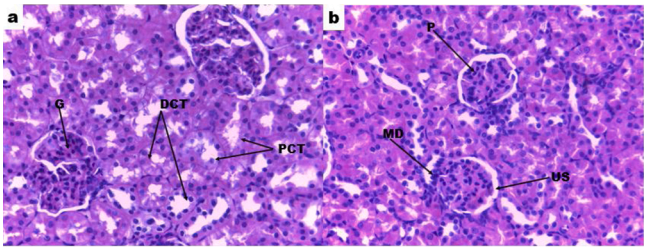

Fig. 3. Photomicrograph of rat kidney showing normal microscopic structures, (a) section taken from a rat treated with 1000 mg/kg of 70 % ethanol extract of *S. guineense* leaves, (b) section taken from control group. G: glomerulus, PCT: proximal convoluted tubule, DCT: distal convoluted tubule, MD: macula densa cells, P: podocytes, US: urinary space; H and E stain, 400x total magnification.

and 600 mg/kg, respectively, of aqueous leaf extract of *S. guineense* [31]. Another six weeks study on mice also reported increased liver weight at doses of 200 and 400 mg/kg of aqueous leaf extracts of *S. guineense* [25]. This discrepancy may be due to the differences in the test animals and the type of extraction methods used in the respective studies.

The presence of tissue damage in the liver can be evaluated by measuring serum level of enzymes like AST, ALT, and ALP. Tissue damage results in the release of extra AST and ALT to the blood circulation and thus, the blood level of such enzymes is increased. Therefore, these enzymes are important biomarkers of liver damage. ALT is the most sensitive liver enzyme that indicates liver cell damage [45].

In the current study, the serum level of ALT was significantly increased in male and female rats treated with 1000 mg/kg of the plant extract. Although increased AST level may not be specific for liver damage, our study revealed an increased serum AST level in the high dose treated group (1000 mg/kg). The serum level of ALP can be increased due to bile duct obstruction, liver damage, or bone disease [46]. Our finding indicated an increased serum level of ALP in rats treated with 500 and 1000 mg/kg of *S. guineense* extract. *S. guineense*'s alkaloids may have been responsible for the observed increase in liver enzymes since other research findings also have reportedly indicated that the plant's alkaloids were toxic [47,48]. Nevertheless, these increments in serum levels of AST, ALT, and ALP were not followed by tissue damage in the liver. Administration of *S. guineense*, even at the high dose, did not produce a significant inflammation or cellular alteration in the liver. This indicates that consumption of *S. guineense* leaves at a high dose only affected some functions of the liver or maybe it takes a chronic administration to observe cellular alteration in the liver. Moreover, short-term (4 weeks) administration of *S. guineense* in rats did not result in a significant change in the liver enzymes [32]. This difference may have been due to the longer duration of the treatment period employed in our study.

Serum urea and creatinine levels are the commonly employed renal function tests. The creatinine level indicates the glomerular filtration rate and the serum urea level indicates the renal excretion capacity [49]. In the present study, serum level of creatinine was not altered by treatment with the test substance. There was no significant difference in the serum level of creatinine in both male and female rats of treatment and control groups. This is consistent with the histological finding of the kidney in which no structural abnormalities were observed in any of the treatment groups. Similar findings were also reported by other researchers [32,41].

The serum urea level in male rats did not vary significantly between the treatment and control groups. However, elevated serum urea levels were recorded in female rats treated with 500 mg/kg (80.3 ± 7.8) and 1000 mg/kg (72.2 ± 13.0) of the plant extract.

In the current study, serum levels of total protein, albumin, and cholesterol did not change significantly in all the treated and control rats. Similar findings have been reported in short-term studies [10,32].

Reduction of blood glucose level was observed in male and female rats, treated with 1000 mg/kg of *S. guineense* extract. This finding supported the hypothesis that *S. guineense* had hypoglycemic effect. The

anti-diabetic effect of *S. guineense* leaves has been reported by many researchers [9,10,32]. This may be due to secondary metabolites of *S. guineense* such as flavonoids and terpenoids [43]. It has been reported that flavonoids lower the blood glucose level by modulating a glucose transporter protein [50].

In our investigation, treatment of animals with *S. guineense* leaves extract did not show significant microscopic changes on the normal architecture of the liver and kidney. Our finding is in line with Loha et al. [32] and Amare [31]. However, a study conducted by Abba et al. [25] reported the presence of hemorrhagic necrosis and cytoplasmic vacuolations in the mice liver following six weeks administration of 600 mg/kg of body weight of aqueous extract of *S. guineense*. In our study, some fatty changes were observed in one male rat treated with 1000 mg/kg of the extract. Steatosis (fatty change) is an abnormal accumulation of fat in the liver cells. This accumulation can be due to an imbalance among fat intake, synthesis, breakdown, and secretion [51].

5. Conclusion

Administration of 70 % ethanol leaf extract of *S. guineense* at a high dose reduced the food intake and weight gain of rats. In addition, treatment of rats with *S. guineense* resulted in increased serum levels of ALT, AST, ALP, and urea. Treatment of rats with *S. guineense* significantly reduced serum glucose level. These effects were noticeable especially in the rats treated with the high dose of *S. guineense*. Administration of *S. guineense* extract did not result in alteration in the histology of the liver and kidneys. Further studies involving chronic administration of the plant extract should be conducted to clarify the inconsistencies between the functional and histopathological findings. In general, the consumption of *S. guineense* leaves at a high dose should be taken curiously and cautiously.

Author contributions

Melese Shenkut Abebe: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, and writing-original draft.

Girma Seyoum: Conceptualization, supervision, methodology, validation, review and editing.

Kaleab Asres & **Yonas Bekuretsion**: Supervision, methodology, resources, validation, review and editing.

Abiy Abebe & **Demiraw Bikila**: Investigation, methodology, resources, validation, review and editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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