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Toxicological study of "*Mendim me zõn*": A traditional tea made with *Solanum aethiopicum* Shum berries

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Abstract

Mendim me zõn is a traditional tea made with *Solanum aethiopicum* Shum berries; a plant belonging to the Solanaceae family. It is a Bantu beverage consumed for its revitalizing properties. Powdered berries of this plant are now distributed and consumed in many countries around the world. Bioactive substances such as glycoalkaloids, galactosamine and fucose, among others which are potentially toxic, can be found in Mendim me zõn. However, very few studies have looked in the safety of this increasingly popular beverage. The present work aimed therefore to carry out a toxicological study of this drink, focusing on acute and sub-chronic toxicity. To do this, 50 g of powdered berries obtained in the locality of Ngaoundere, Adamawa Region of Cameroon. They were infused in 10 L of boiling water for 10 min. The extract was used to study acute and subchronic toxicity using rats as animal model, during 14 and 42 days respectively. Clinical, biochemical and histopathological analyses were carried out on male and female rats given progressive doses of the extract. The results showed that there was no death at any dose. Signs of toxicity including vomiting, diarrhoea, abdominal pain, self-mutilation, apathy, agitation, continuous grooming, drowsiness, stupor, tremors, hair erection, difficulty in breathing and anorexia were not observed in rats. Repeated intake of Mendim me zõn increased appetite and proved to be laxative. Microscopic sections of the liver and kidney showed normal architecture. Blood parameters remained within normal ranges. However, at high doses (1000 mg/kg/day), extract increased erythrocytes while white blood cells decreased in males. In female platelets and mean corpuscular volume increased. With the exception of bilirubin, consumption of extract statistically reduced the parameters linked to renal and hepatic function. These results might suggest that Mendim me zõn is safe beverage.

Keywords: Toxicological study; Mendim me zõn; tea*; Solanum aethiopicum* Shum berries; Safety

1. Introduction

Solanum aethiopicum L. Shum is an annual and herbaceous plant that can reach 1.5 to 1.8 m in height, belonging to the Solanaceae family [1]. This plant is widespread in Central, West, East and Southern Africa; and widely cultivated in Uganda [2]. It has strong stems and deep roots, and is drought resistant. The berries are grouped in clusters on one side of the stem, their colour change from green to red during ripening. The diameter of berry varies between 1 and 2 cm. The seeds are flat and scattered in the pulp [3]. The berries of *S. aethiopicum* Shum are eaten raw or in the form of traditional beverage known to the *Bantu* peoples, as "*Mendim me zõn*" [4,5].

Some consumers claim that "*Mendim me zõn*" possesses detoxifying and revitalizing properties. It is traditionally used to prevent cancer and diabetes [6]. Globally, many bioactivities are associated with species of *Solanum* gender, including anticancer, anti-inflammatory, antihyperlipidemic, antihyperglycemic, antipyretic, antimicrobial, antihypertensive, neuro and hepatoprotective [7]. Numerous scientific evidences have been established functional and therapeutic virtues of *S. aethiopicum* Shum berries. They are also used to treat parasitical [8] and metabolic syndrome diseases [5,9]. The

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antiviral properties of these berries have highlighted one of the types of HIV [10]. These virtues have been attributed to their bioactive substances such as amins, carbohydrates, minerals, vitamins and secondary metabolites including calystegin alkaloids, phenolic compound, lectins and glycoalkaloids [7, 11]. Our previous works have shown that these berries possess proteolytic activity, ergogenic effect; and prevent the development of metabolic syndrome [5,12,13].

However, chemical analysis of *S. aethiopicum* Shum berries showed substances with toxic potential including glycoalkaloids, fucose, galactosamine [11,14]. The safety of *S. aethiopicum* Shum extract remains to be proven. the Organisation for Economic Co-operation and Development [15, 16] suggests studying acute and subchronic toxicity. Therefore, the objective of this study is to evaluate the safety of "*Mendim me zõn*"**,** focused on the acute and subchronic toxicity using the rat as animal model.

2. Material and methods

2.1. Experimental conditions

2.1.1. Preparation of extract

The berries of *S. aethiopicum* Shum were obtained in the locality of Ngaoundéré, Adamawa Region of Cameroon. They were dried, ground and stored in the plastic sachets. Powder (50 g) were infused in 10 L of boiling water for 10 min, and filtered using Whatman paper n°4. After 24 hours passed to the rotavapor at 40°C, the obtained filtrate was dried in oven at 40°C during 72 hours. Dried extract was stored at 4°C until rat treatment.

2.1.2. Animal acclimatisation

The animal experiments were conducted according to the OECD guidelines (2008). The toxicological evaluation of extract of *S. aethiopicum* Shum was carried out on healthy albino Wistar rats obtained from the animal house of the Faculty of Science of the University of Ngaoundere in Cameroon. The animals were acclimatised for the last ten (10) days prior to the test under laboratory conditions. These were: temperature 24 ± 2°C, relative humidity 70%, daily exposure to a cycle of about 12 h of darkness and 12 h of light. In addition, they were provided with tap water and food to satiation, as adapted to the AIN-93 formula [17]. The composition of the food consumed by the animals during the experiment was: corn starch (50%), casein (20%), sucrose (12%), soja oil (8%), cellulose (5%), minerals (3.5%) and multivitamin (1.5). The food and water were changed every 24 hours.

The administered doses were prepared by dissolving the corresponding amounts of extract of *S. aethiopicum* Shum in distilled water [as vehicle]. A constant volume of 1 mL of the extract per 100 g of rat body weight was administered by gavage through an orogastric tube [15,16]. The rats were fasted for 12 hours before the start and end of the experiment, without interruption of the water diet.

2.2. Acute toxicity

2.2.1. Preparation of animals

Acute toxicity was the median lethal oral dose (LD50), expressed as mass of extract of *S. aethiopicum* Shum per unit weight of rat. The experiment was conducted on 9 nulliparous, non-pregnant female rats [15] aged 8-10 weeks and weighing 95-113 g. The animals were randomly selected, marked for identification and placed individually in the plastic cages.

2.2.2. Doses

The weight of the animals was determined immediately prior to the administration of extract of *S. aethiopicum* Shum. The dose to be administered was calculated according to the fasting body weight of each animal. Thus, the quantities 18, 31, 56, 100, 177, 313 and 505 mg corresponding to doses 175, 310, 550, 990, 1750, 3100 and 5000 mg/kg were administered as a single dose [15].

2.2.3. Treatment of rats

The animals were treated one by one as follows: the starting dose (175 mg/kg) was given to one rat, depending on the survival after 48 hours, the following doses were given, so on until the limit dose (5000 mg/kg) where three animals were treated. To proceed to the next dose, it was necessary to ensure that the previous dose did not cause death after 48 hours. After administration, food deprivation was continued for the first 4 hours to maximise the effect of the extract. The median lethal dose (LD50) was estimated.

2.2.4. Observations

The animals were observed individually, at least once during the first 30 minutes after administration of the extract and regularly during the first 24 hours [with particular attention to the first 4 hours], and daily thereafter for 14 days. All signs of toxicity (vomiting, diarrhoea, abdominal pain, self-mutilation, apathy, agitation, continuous grooming, drowsiness, stupor, tremors, hair erection, difficulty in breathing and anorexia) were observed and recorded, as well as weight changes.

2.2.5. Autopsy

At the end of the test period, the animals were weighed and then immolated under anaesthesia (by placing the animals in a jar containing chloroform-soaked cotton wool) by cutting the jugular vein. The animals underwent a complete and detailed macroscopic autopsy, including an examination of the external body surface and all orifices as well as the cranial, thoracic and abdominal cavities. They were then dissected and the organs (eyes, liver, kidneys, digestive tract, sexual organs, brain and lungs) were examined macroscopically and weighed.

2.3. Subchronic toxicity of extract of S. aethiopicum Shum

2.3.1. Treatment of animals

Extract of *S. aethiopicum* Shum was administered by gavage at three dose levels: 0, 250, 500 and 1000 mg/kg/day rat body weight. These were calculated on the basis of the body mass of the animals and readjusted weekly. Sixty Wistar albino rats of both sexes aged 6-7 weeks and weighing 53-71 g were randomly assigned to the different groups (control and treated). After randomisation, the mean body weights of the four groups of 12 rats each (6 females [nulliparous and non-pregnant] and 6 males) were measured. Individually labelled rats were housed in colony cages of 6 rats according to sex. The extract was administered orally and daily [1 mL/100 g body weight]. Group I corresponded to the control (did not receive the extract). Groups II, III, IV and IV' (satellite group) correspond to the experimental groups that received 250, 500, 1000 mg and satellite of extract of *S. aethiopicum* Shum per kg of rat body weight, respectively. Signs of toxicity and mortality were observed for 28 days [16]. The satellite group was observed for an additional two weeks [without treatment].

2.3.2. Clinical study of animals

Signs of toxicity

All animals were observed daily from the beginning to the end of treatment. These clinical observations are made outside the cage where the animals are housed and at fixed times (7 a.m. and 5 p.m.). They included the following symptoms: changes in the condition of the skin, hair, eyes and mucous membranes, the appearance of secretions and excretions, neuro-vegetative reactions (secretion of tears, horripilation, variation in pupillary diameter, abnormal breathing), incoordination of locomotion, posture and reactions to handling, as well as the presence of clonic or tonic movements and stereotyped (excessive grooming, repetitive circular runs) or bizarre behaviour (self-mutilation, walking backwards).

Body weight, feed intake and food efficiency

The animals were weighed at the start of treatment and at least once a week for 4 weeks. Food intake, water intake and food efficiency were also measured. The body mass of the animals was expressed in g of the average of 6 rats of each sex. Body mass gain was the difference between final and initial body mass. Food intake was the difference between the amount of food offered and the amount remaining, expressed in g per day per rat for 6 rats per sex. Food efficiency (FE) was expressed as g of mass gained per amount of food ingested per rat per day.

2.3.3. Macroscopic examination

The animals were sacrificed as described above. The following vital organs: brain, heart, epididymis, liver, kidneys, spleen, testes, ovaries, pancreas and lungs were removed immediately and weighed; the organ coefficients were deducted by calculating the ratio between the mass of organ and final mass of animal.

2.3.4. Histopathological analysis

Histopathological evaluation was conducted according to the standard guide [18]. This analysis was performed on the liver and kidney of male and female control animals, which had daily received 1000 mg/kg Extract of *S. aethiopicum* Shum for 28 days. The organs were pre-fixed in 10% neutral formalin solution and histopathological analysis was

performed according to conventional methods. Briefly, liver and kidney tissues were placed in histological cassettes, dehydrated in a 100% ethanol bath for 45 min twice, cleared in a xylene bath for 01 h twice, and impregnated in two successive baths of melted paraffin each for 01 h. The slides obtained are analysed using a photonic microscope coupled to a photomicrographic system.

2.3.5. Haematological analysis

Blood samples are collected from the jugular vein of the animals under anaesthesia in tubes containing an anticoagulant (EDTA, K+). It was immediately mixed with the blood samples. The analyses were performed using an automated haematology analyser (Auto Haematology Analyzer, BC. 3000 Plus, SHENZHEN MINDRAY, Hamburg, Germany). The haematological parameters determined are those defined by the Harmonised Guide for Testing of Animals in Toxicological Studies [19]. Thus, the following blood parameters are determined: total and differential leukocyte count, erythrocyte and platelet count, haemoglobin concentration, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean platelet volume [16].

2.3.6. Biochemical analyses

The serum was prepared as follows: blood collected from the jugular vein in dry tubes was centrifuged at 3500 x g for 10 min at 5°C, the supernatant (serum) obtained was placed in Eppendorf's tube and stored at -20°C. The following parameters were determined according to standard guidelines [19], glucose (Glu), urea (Ur), creatinine (Cr), total protein, albumin (Alb), globulin (Glo), calcium (Ca), sodium (Na), potassium (K), total cholesterol (Cho), appropriate evaluation of hepatocellular functions: glutamopuryvate transferase serum (GPTS), glutamo-oxalo-acetate transferase serum (GOTS) and hepatobiliary: alkaline phosphatase (ALP), gamma glutamyl transferase (γ-GT), total bilirubin (Bil). In addition, the lipid profile in serum is determined: cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG). Commercial Biolabo kits (Maizy, France) were used for the determination of these biochemical parameters. The measurements were performed with a spectrophotometer (Spectrophotometry 3000 Plus, Evolution, Hamburg, Germany).

2.3.7. Statistical data analysis

For each parameter, the results obtained were expressed as the mean ± standard deviation of 06 animals. The differences between the test groups and the control group were determined using the analysis of variance (ANOVA) and Duncan's test at the 5% level. Statgraphics Version 5.0 software was used for these analyses.

3. Result and discussion

3.1. Acute toxicity of Extract of *S. aethiopicum* Shum

Signs of toxicity related to the ingestion of extracts containing glycoalkaloids of the Solanaceae, saponins or tannins such as vomiting, diarrhoea, abdominal pain, self-mutilation, apathy, agitation, continuous grooming, drowsiness, stupor, tremors, hair erection, difficulty in breathing and anorexia [20] were not observed in rats at any dose. The presence of anthraquinones in the extract could participate in the control of gastrointestinal disorders [21]. In addition, it was possible that ripening of the fruits has resulted in the modification of the glycosylated fraction of glycoalkaloids and saponins, thus reducing the harmfulness of *S. aethiopicum* Shum fruits [22]. Single administration of doses ranging from 175 to 5000 mg/kg did not produce toxic manifestations and death. This would be due to the pharmacological compounds contained in the extract such as flavonoids, saponins, alkaloids, N-acetyl neuraminic acid and mannitol [14] which play an important role in health optimisation.

This observation suggests that the median lethal dose (LD50) is above the limit dose (5000 mg/kg). According to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS), extract of *S. aethiopicum* Shum is therefore not classified as acutely toxic [23]. In addition, it is known that any substance or mixture of substances with an LD50 greater than 5000 mg/kg, has no acute oral toxicity [24].

3.2. Repeated dose oral toxicity study of extract of *S. aethiopicum* Shum

3.2.1. Growth parameters of rats

Table 1 shows the effect of repeated-dose oral administration of extract of *S. aethiopicum* Shum for 28 days on thirst, appetite, body mass and food efficiency. The body mass of the rats did not change; with the exception of Group III rats in males and Group IV rats in females, which had lower body masses than the control group ($P < 0.05$). However, the gain in body mass showed no difference between the groups of animals. The pharmacological substances contained in

extract of *S. aethiopicum* Shum would be involved in all phases of metabolism, from food ingestion to excretion. Other plant extracts containing saponins, alkaloids, flavonoids, tannins and polyphenol have been shown to promote body weight gain in rats [25].

The water intake did not change compared to the control group. However, food intake increased with extract of *S. aethiopicum* Shum administration compared to control animals. Other studies have suggested that the administration of plant extracts rich in pharmacological substances such as saponins increases appetite [26,27]. This is probably the case for the saponins contained in extract of *S. aethiopicum* Shum.

However, an opposite effect observed in terms of food efficiency in females. This observation could be explained by the fact that extract of *S. aethiopicum* Shum limited the bioavailability of nutrients. The interactions between the substances in the crude extract and the constituents of the diet offered to the rats would certainly have reduced its utilisation. These results suggest that extract of *S. aethiopicum* Shum increased appetite, but was also laxative due to the presence of anthraquinones [21].

Table 1 Changes in body weight, water and food intake, and food efficiency after 28 days of repeated-dose extract of *S. aethiopicum* Shum administration to male and female rats

Doses (mg/kg)	**Body weight (g)**			**Water intake (mL/rat/day)**	**Food intake (g/rat/day)**	**Food efficiency**
	Initial	**Final**				
		28 days	**42 days**			
Males n = 06						
0	64.5 ± 7.1	178.1 ± 11.5	-	24.1 ± 2.4	20.3 ± 2.9	5.58 ± 0.9
250	62.2 ± 7.3	183.4 ± 15.6	-	25.8 ± 3.3	28.5 ± 2.6*	4.3 ± 0.6*
500	60.5 ± 6.5	167.6 ± 8.3*	-	23.5 ± 3.7	30.8 ± 5.6*	3.5 ± 0.4*
1000	62.1 ± 7.7	178.9 ± 10.0	-	24.3 ± 3.3	35.5 ± 2.8**	3.3 ± 0.3*
Satellite	64.0 ± 4.9	180.1 ± 12.5	206.4 ± 16.7	23.6 ± 4.0	36.5 ± 4.2**	3.2 ± 0.7*
Females n = 06						
0	61.5 ± 4.1	152.5 ± 13.0	-	26.3 ± 3.1	19.2 ± 2.3	4.7 ± 0.3
250	60.2 ± 5,8	145.9 ± 17.7	-	26.0 ± 4.1	24.0 ± 1.7*	3.6 ± 0.4*
500	57.6 ± 3.3	143.5 ± 14.1	-	25.8 ± 2.2	28.0 ± 4.1*	3.1 ± 0.6*
1000	58.3 ± 5.1	142.6 ± 13.3*	-	26.1 ± 3.5	30.8 ± 1.9*	2.7 ± 0.3*
Satellite	60.6 ± 4.8	147.1 ± 13.4	179.6 ± 14.5	26.5 ± 4.2	31.0 ± 3.6*	2.8 ± 0.4*

* significantly different compared to the control group ($P < 0.05$).; ** value significantly different compared to the control group ($P < 0.01$).

3.2.2. Effect of repeated doses-oral administration on organ coefficient

Macroscopic observation of the organs revealed no abnormalities compared to control rats. Oral administration of extract of *S. aethiopicum* Shum at doses ranging from 250 to 1000 mg/kg did not affect ($p > 0.05$) absolute and relative organ weights after 28 days of experimentation. Previous work suggested that there is a balance between body and organ weights when organ coefficients are similar between treated and control animals [28].

3.2.3. Effect of repeated doses-oral administration on architecture of kidney and liver

The kidney and liver architectures of treated animals with 1000 mg/kg did not change after 28 days of experimentation. Lu [29] reported that even when hepatocytes are necrotic, they are replaced by new hepatocytes, except for irreversible malignancies. The kidney has a strong compensatory capacity that allows it to return to normal function after significant morphological or metabolic disturbances [30]. Toxic manifestations in the liver such as hepatocyte necrosis or hypertrophy, steatosis, dilatation of sinusoidal capillaries, obstruction of the bile duct or portal vein were not observed as much in treated animals as in controls. In the kidney, renal collecting tubule obstruction, loss of brush border, glomerular capsule changes and tubular necrosis were not observed.

3.2.4. Effect of repeated doses-oral administration on haematological parameters

Table 2 Effect of repeated oral administration of extract of *S. aethiopicum* Shum for 28 days on haematological parameters in male and female rats

Parameters	Doses (mg/kg/day)				
	0	250	500 mmmg/kg	1000	Satellite
Male n= 06					
Erythrocytes (10^{12}/L)	7.25 ± 0.48	7.90 ± 0.34	7.72 ± 0.45	8.73 ± 0.51*	8.59 ± 0.52*
Lymphocytes (10^9/L)	4.21 ± 0.58	4.24 ± 0.75	4.08 ± 0.52	3.91 ± 0.56	4.38 ± 0.34
Granulocytes (10^9/L)	5.31 ± 0.97	4.95 ± 0.71	4.25 ± 0.49*	4.64 ± 0.68	4.79 ± 0.43
White blood cells (10^9/L)	8.25 ± 0.30	7.95 ± 0.17	7.79 ± 0.36	7.28 ± 0.49*	7.40 ± 0.41
Haemoglobin (g/dL)	15.81 ± 1.69	16.19 ± 0.88	16.03 ± 1.29	15.31 ± 0.21	15.97 ± 0.90
Haematocrits (%)	40.60 ± 2.26	43.73 ± 3.06	40.90 ± 1.70	45.46 ± 2.25	43.38 ± 4.46
Platelets (10^9/L)	684 ± 18.43	682 ± 20.86	693 ± 19.67	714 ± 12.79	708 ± 22.21
VGM (fL) [1]	50.45 ± 2.27	50.95 ± 1.87	50.24 ± 2.58	50.30 ± 2.81	50.47 ± 2.03
HCM (pg) [2]	19.18 ± 1.94	18.45 ± 0.96	18.60 ± 1.02	18.70 ± 1.11	18.53 ± 1.33
CCMH (g/dL) [3]	38.28 ± 1.24	39.46 ± 1.95	40.50 ± 1.56*	37.73 ± 1.78	37.31 ± 1.86
VPM (fL) [4]	8.01 ± 0.54	8.15 ± 0.86	8.36 ± 0.33	7.86 ± 0.46	7.67 ± 0.49
Female n= 06					
Erythrocytes (10^{12}/L)	6.39 ± 0.42	6.45 ± 0.61	6.17 ± 0.13	6.77 ± 0.67	6.51 ± 0.73
Lymphocytes (10^9/L)	4.73 ± 0.34	4.94 ± 0.43	4.07 ± 0.52	4.78 ± 0.32	4.63 ± 0.37
Granulocytes (10^9/L)	4.73 ± 0.60	4.23 ± 0.15	3.94 ± 0.29	4.16 ± 0.30	4.30 ± 0.31
White blood cells (10^9/L)	8.32 ± 0.39	7.99 ± 0.50	8.19 ± 0.37	8.29 ± 0.32	7.79 ± 0.40
Haemoglobin (g/dL)	16.90 ± 0.92	17.22 ± 0.67	17.13 ± 0.33	16.88 ± 0.61	16.83 ± 0.77
Haematocrits (%)	41.25 ± 2.17	40.63 ± 2.07	38.81 ± 1.90*	39.87 ± 2.51	39.05 ± 1.63
Platelets (10^9/L)	715 ± 24.46	718 ± 44.14	716 ± 26.27	750 ± 33.70*	776 ± 17.38*
VCM (fL)[1]	51.63 ± 1.93	52.28 ± 1.12	51.95 ± 1.60	53.07 ± 2.07*	53.80 ± 1.53*
HCM (pg)[2]	19.70 ± 1.12	19.51 ± 1.24	20.31 ± 0.92	19.78 ± 0.48	19.60 ± 0.75
CCMH (g/dL)[3]	39.39 ± 1.34	39.48 ± 1.19	40.16 ± 1.01	40.98 ± 1.33	41.71 ± 0.40*
VPM (fL)[4]	7.19 ± 0.50	7.01 ± 0.63	7.27 ± 0.91	7.26 ± 0.34	7.39 ± 0.42

* significantly different compared to the control group ($P < 0.05$).; ** value significantly different compared to the control group ($P < 0.01$).; [1]mean corpuscular volume; [2]mean corpuscular haemoglobin; [3]mean corpuscular haemoglobin concentration; [4]mean platelet volume.

The influence of repeated administration of the extract of *S. aethiopicum* Shum for 28 days on the haematological parameters of male and female rats was evaluated and the results are shown in Table 2. The analysis of variance shows that the only differences between the treated and control groups observed for the following parameters: erythrocytes (dose 1000 mg/kg and satellite), granulocytes (dose 500 mg/kg), white blood cells (dose 1000 mg/kg) and mean corpuscular haemoglobin concentration (dose 500 mg/kg) for males. On the other hand, the values for haematocrit (dose 500 mg/kg), platelets (dose 1000 mg/kg and satellite group), mean corpuscular volume (dose 1000 mg/kg and satellite group) and mean corpuscular haemoglobin concentration (satellite group) were different from the control group in female rats. The haematological profile of the test animals was not similar to that of the control animals.

However, the values of the haematological parameters were within the range of normal values for the age and strain of animals used [31]. This can be explained by the fact that the flavonoids contained in extract of *S. aethiopicum* Shum are

protected red blood cells from haemolysis and at the same time promote their production [32]. The good condition of the kidney would have favoured the secretion of erythropoietin which stimulates the production of red blood cells in the bone marrow.

3.2.5. Effect of repeated doses-oral administration on biochemical parameters

Renal function parameters

Nephrotic functions are assessed by the following serum parameters, such as creatinine, urea and electrolytes [33]. Repeated doses-oral administration of the extract of *S. aethiopicum* Shum for 28 days decreased creatinine, urea, calcium, sodium and potassium levels (Table 3). This trend was significant for creatinine, urea and calcium. The reduction in serum creatinine could be explained by the fact that extract of *S. aethiopicum* Shum would have harmonised creatinine biosynthesis with the capacity of the kidney to clean up. Adeyemi et al [34] reported that high concentrations of sodium, potassium, urea and creatinine are associated with renal dysfunction.

The decrease in creatinine, urea and electrolytes in serum can be explained by excretion of these compounds in the urine or faeces. This reduction maintains the levels at normal values, due to the action of pharmacological substances in extract that could be involved in all stages of metabolism [27]. Furthermore, extract of *S. aethiopicum* Shum could act by decreasing the bioavailability of calcium and other constituents such as proteins that are eliminated in the faeces. It is clear that the concentration of serum urea depends on the amount of protein taken in. Thus, the mannitol in extract of *S. aethiopicum* Shum would have inhibited sodium reabsorption and promoted water and sodium elimination [35].

Table 3 Biochemical parameters of renal function in male and female rats after 28 days of extract of *S. aethiopicum* Shum administration

Parameters	**Doses (mg/kg/day)**				
	0	**250**	**500**	**1000**	**Satellite**
Males n = 06					
1Cr (mg/dL)	0.56 ± 0.04	0.44 ± 0.05*	0.41 ± 0.05*	0.39 ± 0.02*	0.38 ± 0.04*
2Ur (mg/dL)	24.50 ± 1.87	21.66 ± 1.21*	20.33 ± 1.96*	20.50 ± 1.64*	21.00 ± 2.19*
Ca (mg/dL)	98.0 ± 10.09	88.33 ± 9.99*	82.83 ± 8.01*	81.00 ± 8.36*	83.16 ± 7.57*
Na (mM)	139.16 ± 8.61	129.66 ± 14.82	120.16 ± 9.72*	118.0 ± 13.85*	117.50 ± 13.36*
K (mM)	4.30 ± 0.23	3.98 ± 0.21	3.91 ± 0.19	3.86 ± 0.22	3.88 ± 0.24
Females n= 06					
1Cr (mg/dL)	0.62 ± 0.07	0.54 ± 0.05	0.48 ± 0.04*	0.45 ± 0.04*	0.46 ± 0.05*
2Ur (mg/dL)	25.43 ± 2.25	23.66 ± 1.63	22.33 ± 2.65	22.16 ± 2.13*	21.83 ± 2.31*
Ca (mg/dL)	9.13 ± 0.74	7.72 ± 0.46*	7.51 ± 0.44*	7.85 ± 0.77*	8.33 ± 0.91
Na (mM)	133.16 ± 6.24	126.33 ± 8.93	122.50 ±9.26*	122.66 ± 12.86*	111.83 ± 6.30**
K (mM)	4.46 ± 0.47	4.36 ±0.42	4.06 ± 0.31	3.96 ± 0.17	3.86 ± 0.13

[1] Creatinine; [2] Urea; * significantly different compared to the control group ($P < 0.05$).; ** value significantly different compared to the control group ($P < 0.01$).

3.2.6. Liver function parameters

Serum hepatobiliary and pancreatic function parameters following repeated doses-oral administration of extract of *S. aethiopicum* Shum for 28 days are presented in Table 4 A (male) & B (female). There was no significant difference between the experimental and control groups of rats in terms of albumin, total protein, globulin and low-density lipoprotein (LDL) levels (males and females). This trend towards a decrease in blood protein compounds, although not significant, suggests that subchronic administration of extract of *S. aethiopicum* Shum maintains liver function at a normal level. The decrease in serum glucose can be due to the effect of saponins, glycoalkaloids and mannitol contained in extract of *S. aethiopicum* Shum. The anti-diabetic virtues of *S. aethiopicum* were mentioned by Gbolade [6]. Repeated administration of extract of *S. aethiopicum* Shum affected the lipid profile. Thus, the significant decrease in triglyceride

and cholesterol levels can be explained by the action of substances such as saponins in extract. Saponins extracted from the fruits of a related species (*S. anguivi*) have been shown to lower lipid parameters [36]. These substances act by inhibiting pancreatic lipase and limiting intestinal absorption of lipids [37] and/or by promoting lipid catabolism in the liver [38]. The bioactive substances of the extract inhibited pancreatic lipase and amylase and promoted the excretion of lipids and carbohydrates through the faeces [39].

With regard to bilirubin, repeated administration for 28 days lead to a significant increase in its serum level regardless of the sex of the animal. It is known that the increase in bilirubin is associated with increased haemolysis. This could be attributed to the saponins in extract of *S. aethiopicum* Shum. On the contrary, considering the erythropoietic power of this extract observed above, the red blood cells became more numerous and haemolysis. Furthermore, the elevation of serum bilirubin may be due to damage to the heart and liver [34]. However, no abnormalities were observed in these two organs.

The decrease of the activity of the liver-related enzymes can be explained by the hepatoprotective effect of glucosamine and glucuronic acid contained in extract of *S. aethiopicum* Shum. Increased activity of these enzymes is always related to liver tissue damage. The reduction in liver enzyme activity may suggest that extract of *S. aethiopicum* Shum has a hepatoprotective effect [40]. These observations confirm the results of the histopathological analysis of the liver and kidney.

Table 4 A Biochemical parameters of hepatobiliary and pancreatic functions of males after 28 days of extract of *S. aethiopicum* Shum administration

Parameters	**Doses (mg/kg/day)**				
	0	**250**	**500**	**1000**	**Satellite**
Glu (mg/dL)	104.50 ± 5.13	100.83 ± 4.83	93.16 ± 4.07*	88.0 ± 4.19*	85.16 ± 3.06*
Alb (g/dL)	3.96 ± 0.08	3.71 ± 0.17	3.65 ± 0.25	3.66 ± 0.16	3.58 ± 0.16
Prot (g/dL)	6.95 ± 0.28	6.75 ± 0.19	6.61 ± 0.42	6.26 ± 0.21	6.15 ± 0.16
Glo (g/dL)	2.98 ± 0.33	3.03 ± 0.19	2.96 ± 0.59	2.60 ± 0.12	2.56 ± 0.20
Bil (mg/dL)	0.42 ± 0.02	0.51 ± 0.03	0.54 ± 0.05*	0.59 ± 0.04*	0.58 ± 0.03*
ALP (U/L)	77.16 ± 13.73	77.33 ± 9.15	74.15 ± 7.35	66.50 ± 3.08*	61.33 ± 12.01*
GOTS (U/L)	98.17 ± 7.31	83.43 ± 37.22*	88.50 ± 4.27*	85.66 ± 14.66*	85.16 ± 8.37*
GPTS (U/L)	44.83 ± 8.15	40.66 ± 5.12	35.33 ± 6.62	34.85 ± 7.93*	35.17 ± 5.42
γ-GT (U/L)	21.33 ± 2.80	17.83 ± 2.32	17.50 ± 2.42	15.0 ± 2.60*	17.20 ± 2.78
Chlo (mg/dL)	69.66 ± 4.54	68.50 ± 2.51	62.66 ± 2.65*	64.33 ± 3.55*	63.83 ± 2.31*
HDL (mg/dL)	47.17 ± 1.94	42.0 ± 1.78	40.0 ± 2.28*	37.50 ± 2.66*	37.0 ± 1.80*
LDL (mg/dL)	24.83 ± 4.45	26.66 ± 5.64	27.50 ± 2.42	25.83 ± 2.78	24.33 ± 2.16
TG (mg/dL)	107.0 ± 8.19	94.0 ± 10.77	86.83 ± 4.57*	85.66 ± 7.42*	88.50 ± 7.12*

Glu (Glucose), TP (total protein), Alb (albumin), Glo (globulin), Chlo (cholesterol), GPTS (glutamopuryvate transferase serum), GOTS (glutamo-oxalo-acetate transferase serum), ALP (alkaline phosphatase), gamma glutamyltransferase (γ-GT), Bil (bilirubin), Chlo (cholesterol), HDL (High density lipotrpotein), LDL (Low density lipoprotein) and TG (triglycerides).;* significantly different compared to the control group ($P < 0.05$).; ** *value significantly different compared to the control group ($P < 0.01$).*

Table 4 B Biochemical parameters of hepatobiliary and pancreatic functions of females after 28 days of extract of *S. aethiopicum* Shum administration

Parameters	Doses (mg/kg/day)				
	0	**250**	**500**	**1000**	**Satellite**
Glu (mg/dL)	112.66 ± 7.25	103.50 ± 5.24*	96.33 ± 4.71**	89.83 ± 2.56**	89.50 ± 7.01**
Alb (g/dL)	4.15 ± 0.19	3.93 ± 0.16	3.70 ± 0.18	3.75 ± 0.21	3.82 ± 0.14
Prot (g/dL)	7.03 ± 0.36	6.75 ± 0.15	6.60 ± 0.25	6.35 ± 0.18	6.31 ± 0.20
Glo (g/dL)	2.88 ± 0.42	2.81 ± 0.31	2.91 ± 0.16	2.60 ± 0.08	2.48 ± 0.26
Bil (mg/dL)	0.44 ± 0.02	0.51 ± 0.03	0.54 ± 0.05*	0.56 ± 0.04*	0.56 ± 0.06*
ALP (U/L)	71.83 ± 6.11	66.67 ± 3.39	51.84 ± 7.73*	51.50 ± 4.23*	41.66 ± 3.01**
GOTS (U/L)	104.82 ± 18.14	95.33 ± 8.73	92.17 ± 7.22*	85.67 ± 8.43*	98.0 ± 11.33
GPTS (U/L)	41.50 ± 3.45	36.50 ± 5.01	32.30 ± 14.78*	39.81 ± 4.79	36.80 ± 3.60
γ-GT (U/L)	19.33 ± 2.34	16.51 ± 1.87	14.50 ± 2.43	13.0 ± 2.28*	11.66 ± 3.21*
Chlo (mg/dL)	71.66 ± 4.92	70.67 ± 4.41	65.67 ± 6.21*	64.83 ± 3.92*	66.50 ± 3.27
HDL (mg/dL)	50.84 ± 3.31	48.85 ± 2.48	42.50 ± 4.41*	39.33 ± 2.87*	41.67 ± 3.66*
LDL (mg/dL)	29.21 ± 2.13	32.66 ± 1.63	30.50 ± 5.68	31.16 ± 2.93	28.67 ± 3.21
TG (mg/dL)	119.17 ± 10.41	91.50 ± 9.87*	75.66 ± 4.17**	74.0 ± 6.69**	78.83 ± 6.11**

Glu (Glucose), TP (total protein), Alb (albumin), Glo (globulin), Chlo (cholesterol), GPTS (glutamopuryvate transferase serum), GOTS (glutamo-oxalo-acetate transferase serum), ALP (alkaline phosphatase), γ-GT (gamma glutamyltransferase), Bil (bilirubin), Chlo (cholesterol), HDL (High density lipotrpotein), LDL (Low density lipoprotein) and TG (triglycerides).; * significantly different compared to the control group ($P < 0.05$).; ** value significantly different compared to the control group ($P < 0.01$).

4. Conclusion

At the end of our work on the toxicity of *Mendim me zõn*, traditional tea made with *Solanum aethiopicum* Shum berries; we found the following:

- the median lethal dose of extract of S. aethiopicum Shum was greater than 5000 mg/kg;
- After repeated doses-oral administration of Mendim me zõn for 28 days, food efficiency reduced; histological analysis of the liver and kidney revealed normal architecture. The haematological profile of the rats was normal, suggesting an erythropoietic potential of this beverage. This tea also showed hepato-protective, nephroprotective, hypoglycaemic and lipid-lowering effects. In summary, Mendim me zõn was free of acute toxicity at 5000 mg/kg, subchronic toxicity at 1000 mg/kg body weight per day.

Compliance with ethical standards

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Disclosure of conflict of interest

No potential conflicts of interest relevant to this work were reported.

Statement of ethical approval

All procedures involving animals in this work were conducted in accordance with Organization for Economic Co-operation and Development (OECD) for the toxicological study of chemical products.

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