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herba polonica
From Botanical to Medical Research
Vol. 63 No. 3 2017

DOI: 10.1515/hepo-2017-0017

EXPERIMENTAL PAPER

Acute and sub-chronic toxicity evaluations of aqueous extract from stem bark of *Grewia mollis* (*Malvaceae*) in rats

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Summary

Introduction: Different parts of *Grewia mollis* Juss. (*Malvaceae*) are commonly used in folk medicine to treat several ailments, including diarrhea, ulcers, rickets, cough and fever. Although several studies have proved its therapeutic effectiveness, there are very few toxicological studies on the plant.

Objectives: This study was carried out to evaluate the acute and sub-chronic toxicity of the aqueous extract of *G. mollis* stem bark (GM) in animals.

Methods: In the acute study, rats were orally administrated with GM at doses of 150, 300, 600, 1200, 2400, 4800 and 9600 mg/kg to determine the oral medial lethal dose (LD_{50}). In the chronic study, rats received three doses of GM (150, 300 and 600 mg/kg) for 28 days. After the treatments, food intake, body weights, biochemical, hematological and histopathological parameters were analyzed.

Results: The LD_{50} was estimated to be >9600 mg/kg. No significant alterations in the animal's body weight gain, relative organs weight, serum biochemical analysis, hematological or histopathological analyses of liver, kidneys, lungs, heart and spleen were observed.

Conclusions: The results of this study provided evidence that oral administration of GM at dose of 600 mg/kg is relatively safe in rats and may not exert severe toxic effects.

Keywords: ***Grewia mollis, LD_{50}, subchronic, haematology, histopathology***

INTRODUCTION

Herbal preparations and natural remedies are commonly used in developing countries for the treatment of various diseases as an alternative for most conventional medicines [1]. Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities.

However, there is little information or evidence available concerning the possible toxicity that medicinal plants may cause to the consumers [2]. Medicinal plants are used for the treatment of several diseases based on the belief that they present low toxicity due their natural origin and their long-standing use in various cultures. However, the treatment with medicinal plants, as the use of orthodox medicines, may cause adverse effects and drug interactions [3]. Therefore, the imperative becomes to evaluate the pharmacological and toxicological properties of these plants, especially those commonly used by people to identify possible toxic effects and their real therapeutic effects.

Grewia mollis Juss. (*Malvaceae*) is a slow-growing shrub or small tree, often multi-stemmed and can grow up to 10.5 m tall [4]. It occurs widely in tropical Africa, from Senegal and Gambia eastward to Somalia and southward to Angola, Zambia and Zimbabwe. In Nigeria, the mucilaginous bark and leaves are commonly used in soups; dried and ground they are mixed with bean-meal to make cakes [5]. The mucilage from the bark and leaves are applied to ulcers, cuts, sores rickets, rheumatism, anal prolapse, cough and snakebites [5, 6]. In Togo, a decoction of stem bark and leave are drunk to treat diarrhoea, and a maceration is taken to ease childbirth [6, 7]. In Central Africa, sap from root-shavings is used to treat sore eyes, whereas a liquid obtained by kneading the root bark in water is drunk to treat stomach-ache, colic and poisoning by certain plants [6, 7]. Antimicrobial [8], antioxidant and hepatoprotective activity [9] has been reported from the ethanol and methanol extracts of the leaves, roots and stem bark of *G. mollis*. Phytochemical screening of *G. mollis* revealed the presence of tannins, saponins, flavonoids, glycosides, phenols, steroids and the absence of alkaloids in the leaves and stem bark [10].

Although several studies have proved its therapeutic efficacy, only two toxicological studies with *G. mollis* stem bark can be found in literature. A histopathological study [10] with only 500 mg/kg of *G. mollis* intraperitoneally for two weeks was carried out revealed that the organs did not show any significant morphological alteration. In another study [11], powdered stem bark of *G. mollis* mixed with the normal diet at 0, 1, 5 and 10% and fed to only male Wistar rats for 28 days. Significant increases in serum transaminases activities, accompanied by decreased food intake were observed in rats fed the stem bark at 10% dietary level.

Based on *G. mollis* use in traditional practices and the literature references, the present study was undertaken to evaluate the comprehensive acute and sub-chronic toxicity studies in both male and female animal models by administering aqueous extract of *G. mollis* stem bark (GM) orally.

MATERIAL AND METHODS

Plant material

Leaves and stem bark of *G. mollis* collected from Suleja, Niger state, Nigeria in August, 2015 were identified and authenticated by Mallam Ibrahim Muazzam of the Department of Traditional Medicine and Medicinal Plant Research, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The voucher specimen (NIPRD/H/6785) was deposited at the NIPRD herbarium for future reference. The stem bark was sun dried then powdered using a mechanical grinder. The powdered material (200 g) was boiled in 2.2 l of distilled water for 30 minutes, and allowed to cool and then filtered. The filtrate was then concentrated in a rotar vapor to give a total yield of 23.38% (*w/w*). The dried extract was preserved in an air tight clean glass container and kept in a refrigerator maintained at -4°C until use. The extract was dissolved in distilled water and various concentrations of stock solutions (50 mg/ml to 1000 mg/ml) were used for the acute and subchronic studies.

Animals

Albino Wistar rats (150–170 g) of either sexes were used for the experiment. The animals were obtained from the Animal House, School of Basic Medical Science, Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were acclimatized for two weeks and fed with standard feed and tap water *ad libitum*. Animals were exposed to natural lighting conditions and were handled in accordance with international principles guiding the use and handling of experimental animals [12] approved by the Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria (EC/FP/016/11).

Acute oral toxicity study

The acute toxicity study of GM was carried out according to a modified method described by Miller and Tainter [13]. Overnight fasted male rats were randomly distributed into eight groups of five rats

per group. Group (1–7) orally received 150, 300, 600, 1200, 2400, 4800 and 9600 mg/kg of aqueous extract of *G. mollis* stem bark respectively while group 8 received distilled water (5 ml/kg). Observation was made for changes in behavior of the rats, mortality and morbidity over a period of 24 h and any signs of delayed toxicity for two weeks.

Sub-chronic oral toxicity

A sub-chronic repeated daily dose (28 days) study was conducted in both male and female rats. Forty rats were randomly distributed into four groups of 10 rats per group. Each group contained five male and five female rats. Groups I–III orally received 150, 300 and 600 mg/kg of GM, respectively, while group IV received distilled water (5 ml/kg). All animals were weighed at start of treatment and once a week. Measurements of food consumption were made weekly. Any signs of toxicity and mortality were also recorded daily throughout the study period.

At the end of the experiment, all rats were anesthetized by chloroform inhalation, and blood samples were collected via cardiac puncture into non-heparinized and EDTA-containing tubes for biochemical and haematological analyses. After blood collection, the animals were sacrificed by cervical dislocation, and their organs were isolated. The heart, liver, spleen, kidneys and lungs were excised, weighed and examined macroscopically. Relative organ weights of the excised organs were determined. The heart, liver, spleen, kidneys and lungs were then fixed in formosaline for histopathological examination.

Haematological and biochemical analysis

The following haematological parameters were analyzed using Human Automated Hematology System Analyzer (ERMA PCE 210, ERMA, Japan): hemoglobin (Hb), packed cell volume (PCV), white cell count (WBC) and differential count (granulocyte, lymphocyte and monocyte), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and random blood glucose.

The following serum biochemical parameters were measured to investigate major toxic effects in tissues and, specifically, effects on kidney and liver using a using standard diagnostic test kits (Randox laboratories, UK) on Automated Clinical System (VIS-7220G, Biotech Engineering Management Company Limited, UK; Analyzer ISE 4000 SFRI, France): alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TB) conjugated bilirubin (CB), globulin, albumin, total protein, electrolytes, creatinine (Cr), urea and lipid profile.

Histopathological analysis

Histopathological analysis was carried out on the preserved tissues and organs. The organs listed above were harvested and fixed in 10% formo-saline for a period of at least 24 h, dehydrated with alcohol, embedded in paraffin, cut into 4-5 μm thick sections, and stained with haematoxylin-eosin dye. Stained were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) at 400 × magnification.

Statistical analysis

All values are expressed as mean ± SEM. Comparisons between groups were performed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the Graph prism 6. A p value of <0.05 was considered significant.

RESULTS

Acute oral toxicity (LD_{50})

Aqueous extract of *G. mollis* stem bark at a dose of 9600 mg/kg had no adverse effect on the behavioral responses of the tested rats up to 14 days of observation. Physical observations indicated no signs of changes in the skin, fur, eyes mucous membrane, tremors, salivation, and diarrhoea of the rats. Neither mortality at the tested dose nor weight loss was observed in the rats affected (tab. 1).

Sub-chronic toxicity of *G. mollis* stem bark

No clinical signs of toxicity (as diarrhea, piloerection, and convulsions) and death were observed during the 28 days of treatment with *G. mollis* stem bark at 150, 300 and 600 mg/kg, or vehicle. The bodyweight gain of the animals during the period of treatment was similar among the animals treated with GM or vehicle as shown in figures 1 and 2.

Table 1.

Acute oral toxicity study of *Grewia mollis* in rats

	Log dose	Sedation	Diarrhea	Tremor	Mortality
Control	-	0/5	0/5	0/5	0/5
GM 150 mg/kg	2.17	0/5	0/5	0/5	0/5
GM 300 mg/kg	2.47	0/5	0/5	0/5	0/5
GM 600 mg/kg	2.77	1/5	0/5	0/5	0/5
GM 1200 mg/kg	3.07	2/5	0/5	0/5	0/5
GM 2400 mg/kg	3.38	2/5	0/5	0/5	0/5
GM 4800 mg/kg	3.68	2/5	0/5	0/5	0/5
GM 9600 mg/kg	3.98	4/5	0/5	0/5	0/5

GM: *Grewia mollis.*

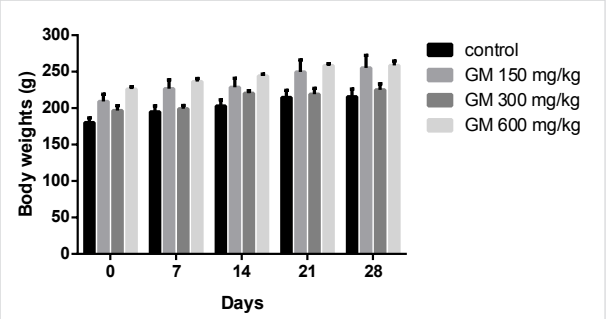

Figure 1.

Effect of 28 days oral treatment with *Grewia mollis* stem bark (GM) on body weights in male Wistar rats. Each bar represents mean±SEM

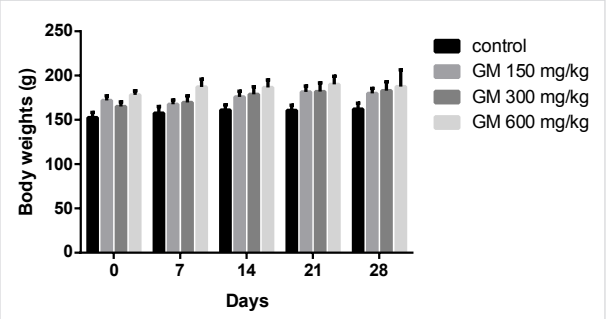

Figure 2.

Effect of 28 days oral treatment with *Grewia mollis* stem bark (GM) on body weights in female Wistar rats. Each bar represents mean±SEM

Relative organs weight

The values of relative weight (%) of heart, lungs, liver, kidney and spleen from animals treated for 28 days with GM or vehicle are presented in table 2 (male and female). There were no significant differences in the relative weight of these organs among groups.

Table 2.

Effects of the subchronic oral administration of *Grewia mollis* on relative organs weights in rats.

	Control	*G. mollis* [mg/kg/day]		
		150	300	600
Male				
Liver [%]	3.47±0.12	3.05±0.06	3.30±0.05	2.89±0.09
Kidney [%]	0.33±0.01	0.31±0.02	0.34±0.02	0.36±0.02
Heart [%]	0.35±0.01	0.31±0.01	0.36±0.01	0.32±0.01
Spleen [%]	0.46±0.03	0.47±0.03	0.44±0.03	0.51±0.06
Lungs [%]	0.69±0.03	0.63± 0.07	0.62±0.03	0.60±0.02
Female				
Liver [%]	3.32±0.11	3.28±0.10	3.35±0.09	3.28±0.40
Kidney [%]	0.36±0.02	0.34±0.02	0.35±0.02	0.33±0.02
Heart [%]	0.39±0.02	0.39±0.04	0.39±0.01	0.37±0.04
Spleen [%]	0.46±0.04	0.40±0.04	0.36±0.01	0.40±0.03
Lungs [%]	0.83±0.03	0.77±0.03	0.75±0.04	0.72±0.02

Values are mean ± SEM.

Food intake

There were no significant differences in food intake during treatment among the animals treated with GM or with vehicle, as shown in figures 3 and 4.

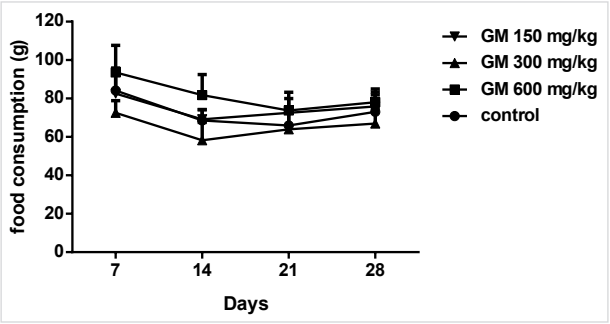

Figure 3.

Effect of 28 days oral treatment with *Grewia mollis mollis* stem bark (GM) on food consumption in male Wistar rats. Each point represents mean±SEM

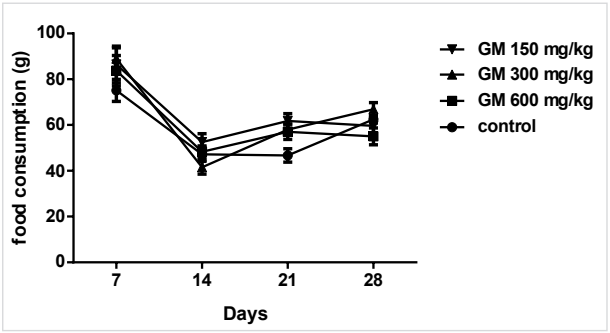

Figure 4.

Effect of 28 days oral treatment with *Grewia mollis mollis* stem bark (GM) on food consumption in female Wistar rats. Each point represents mean±SEM

Haematological parameters

The effects of sub-chronic treatment on the haematological parameters are presented in table 3. Most haematology measures (haemoglobin, hematocrit, red blood cells distribution width, total white blood cells, neutrophils, lymphocytes and platelet count) in treated rats were not significantly different from the controls, with the exception of granulocytes and monocytes in the male rats. The changes in lymphocytes and monocytes were not dose dependent because they were only observed in the group treated with 150 and 300 mg/kg (p<0.05) of GM, not in the group treated with the highest dose (600 mg/kg).

Biochemical parameters

The biochemical profiles of treated and control groups are shown in table 4. *G. mollis* extract had no effect on the serum electrolytes, such as bicarbonate, sodium, potassium, and chloride. The kidney function parameters (urea and creatinine) did not reveal any relevant changes following 28 day oral administration of GM. No statistically significant differences in glucose, total protein, globulin, albumin, liver function parameters (ALT and ALP) were noted with the exception of marginal variations both in male and female rats. However, there was a significant (p<0.05) increase in AST at 150 mg/kg of GM in the female rats.

Lipid profile

In table 5, there were no significant changes in total cholesterol, high density lipoprotein and low density lipoproteins. However, there was a significant (p<0.05) dose-dependent decrease in total triglycerides in male rats after 28 day of oral administration of GM.

Table 3.

Effects of the sub-chronic oral administration of *G. mollis* on hematological parameters in rats

	Control	*G. mollis* [mg/kg/day]		
		150	300	600
Male				
WBC [×10³/μl]	7.58±0.46	6.28±0.46	7.12±0.33	6.26±0.27
LY [%]	68.38±3.06	76.78±5.67	81.94±3.69	75.38±3.82
MO [%]	12.26±0.72	6.20±0.67*	5.32±0.25*	10.18±0.12
GR [%]	18.96±1.80	19.02±0.74	10.74±0.33*	16.84±0.89
RBC [× 10⁶/μl]	8.22±0.44	7.84±0.41	8.26±0.11	8.87±0.33

	Control	*G. mollis* [mg/kg/day]		
		150	300	600
Male				
HGB [g/dl]	14.32±0.44	13.44±0.81	14.40±0.18	15.16±0.38
HCT [%]	42.68±2.05	39.02±1.86	48.94±2.24	43.50±2.50
MCV [fl]	51.76±1.68	49.86±0.41	50.74±1.22	48.82±0.95
MCH [pg]	17.38±0.40	17.10±0.22	17.36±0.14	17.06±0.26
MCHC [g/dl]	33.64±0.74	34.32±0.52	34.32±0.69	35.06±1.11
PLT [$\times 10^3/\mu l$]	222.60±5.10	185.80±8.99	299.62±11.89	196.60±6.12
Female				
WBC [$\times 10^3/\mu l$]	10.93±0.27	9.15±0.58	9.08±0.36	8.97±0.12
LY [%]	73.74±3.25	56.30±3.53	65.94±3.9	73.42±6.21
MO [%]	11.06±1.17	12.22±1.48	14.20±0.55	7.93±0.66
GR [%]	14.22±1.12	15.04±1.12	19.06±0.84	16.88±0.43
RBC [$\times 10^6/\mu l$]	7.50±0.17	7.25±0.08	8.60±1.30	8.01±0.41
HGB [g/dl]	13.18±0.43	12.90±0.12	13.33±0.67	12.94±0.47
HCT [%]	37.22±0.95	37.04±0.44	42.66±2.31	38.54±3.11
MCV [fl]	49.68±1.40	51.04±0.61	49.18±1.28	47.80±1.39
MCH [pg]	17.52±0.33	17.72±0.11	16.06±1.14	16.16±0.39
MCHC [g/dl]	35.36±0.75	34.80±0.54	33.04±2.83	33.92±1.28
PLT [$\times 10^3/\mu l$]	228.00±7.76	266.25±8.78	239.50±7.10	250.00±8.72

Control group received 5 ml/kg distilled water. Values are mean ± SEM (n = 10], $p<0.05$ compared with control. WBC – white blood cells; LY – lymphocytes; MO – monocytes; GR – granulocytes (mainly neutrophils); RBC – red blood cells; HGB – haemoglobin; HCT – haematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelets.

Table 4.

Effects of the subchronic oral administration of *Grewia mollis* on some biochemical parameters in rats

	Control	*G. mollis* [mg/kg/day]		
		150	300	600
Male				
Urea [mg/dl]	42.20±2.41	36.10±2.21	37.21±1.67	36.20±1.77
Cr [mg/dl]	0.68±0.05	0.64±0.02	0.62±0.02	0.64±0.02
Na^+[μmol/l]	146.22±0.73	143.60±0.67	142.40±1.53	143.62±1.77
K^+ [μmol/l]	5.20±0.12	5.44±0.27	5.10±0.13	5.50±0.21
HCO_3^- [μmol/l]	27.40±1.72	27.44±1.50	26.82±0.8	24.80±1.24
Cl^- [μmol/l]	104.40±0.87	102.60±0.5	104.60±1.50	105.62±1.24
ALT [IU/l]	35.22±2.23	32.30±1.22	35.10±1.34	25.80±2.57
AST [IU/l]	77.20±2.31	92.40±1.51*	83.40±3.92	71.22±3.35
ALP [IU/l]	26.60±3.12	21.20±1.46	24.82±0.86	19.64±2.13
TP [mg/dl]	6.60±0.23	6.94±0.14	6.34±0.08	7.04±0.38
Albumin [mg/dl]	3.74±0.06	3.76±0.07	3.62±0.02	3.9±0.08
Globulin [mg/dl]	2.86±0.24	3.18±0.12	2.74±0.08	3.18±0.31
TB [mg/dl]	0.56±0.05	0.62±0.05	0.56±0.04	0.58±0.03

CB [mg/dl]	0.26±0.02	0.24±0.02	0.26±0.02	0.33±0.03
Glucose [mg/dl]	65±5.19	80.2±4.22	75±4.59	70.4±4.47
Female				
Urea [mg/dl]	42.44±1.94	38.40±2.71	37.80±3.39	32.20±1.06
Cr [mg/dl]	0.66±0.07	0.66±0.02	0.60±0.04	0.58±0.03
Na^+[μmol/l]	145.32±1.14	140.40±1.61	144.22±1.14	144.80±1.02
K^+ [μmol/l]	5.18±0.27	4.72±0.19	5.48±0.16	5.86±0.35
HCO_3^- [μmol/l]	24.60±2.01	25.34±1.78	26.80±1.77	25.50±1.58
Cl^- [μmol/l]	106.20±0.94	103.80±1.35	104.40±1.32	106.44±1.41
ALT [IU/l]	30.40± 1.83	33.60±0.60	34.20±1.28	27.21±1.22
AST [IU/l]	86.80±3.82	91.88±1.35	92.22±3.30	66.50±3.31
ALP [IU/l]	20.60±1.16	26.20±1.26	24.24±1.8	19.40±1.72
TP [mg/dl]	6.92±0.18	7.04±0.25	6.96±0.22	6.86±0.43
Albumin [mg/dl]	4.06±0.14	3.98±0.09	4.02±0.10	4.02±0.22
Globulin [mg/dl]	2.86±0.16	3.06±0.18	2.94±0.18	2.86±0.20
TB [mg/dl]	0.58±0.06	0.48±0.06	0.64±0.05	0.62± 0.05
CB [mg/dl]	0.24±0.05	0.26±0.05	0.24±0.05	0.26±0.02
Glucose [mg/dl]	62.80±3.56	80.80±2.19	71.2 0± 5.8	79.12±5.74

Control group received 5 ml/kg distilled water. Values are mean ± SEM (n=10), *p<0.05 compared with control. Cr – creatinine, ALP – alkaline phosphatase, AST – aspartate transaminase, ALT – alanine transaminase, ALP – alkaline phosphatase, TP – total protein, TB – total bilirubin, CB – conjugated bilirubin.

Table 5.

Effects of the subchronic oral administration of *Grewia mollis* on lipid profile in rats

	Control	*G. mollis* [mg/kg/day]		
		150	300	600
Male				
TC [mg/dl]	68.4±2.90	61.8±2.27	53±2.58	62±2.44
TG [mg/dl]	91.8±2.83	76.8±1.53*	63.2±3.56*	71.2±3.78*
HDL [mg/dl]	37±2.64	37.6±1.28	29.4±1.91	38±1.92
LDL [mg/dl]	13.04±2.59	11.24±1.82	10.96±1.40	10.96±1.64
Female				
TC [mg/dl]	61.4± 3.6	58.6±1.56	61.8± 2.35	59.8±4.16
TG [mg/dl]	71±0.89	70.4±4.69	63.4±2.97	64.6±3.20
HDL [mg/dl]	33.4±2.87	33.6±0.97	39.6±2.20	36.8±2.67
LDL [mg/dl]	13±1.79	10.92±2.29	9.72±1.33	13.48±1.78

Control group received 5 ml/kg distilled water. Values are mean ± SEM (n=10), *p<0.05 compared with control. TC – total cholesterol, HDL – high density lipoproteins, LDL – low density lipoproteins, TG – total triglycerides.

Histopathology

Histopathological sections of lungs, heart, liver, spleen and kidneys are shown in figures 5 and 6. There were no lesions or pathological changes related to treatment with GM were observed in the organs of the animals from the treatment groups as compared to the untreated group.

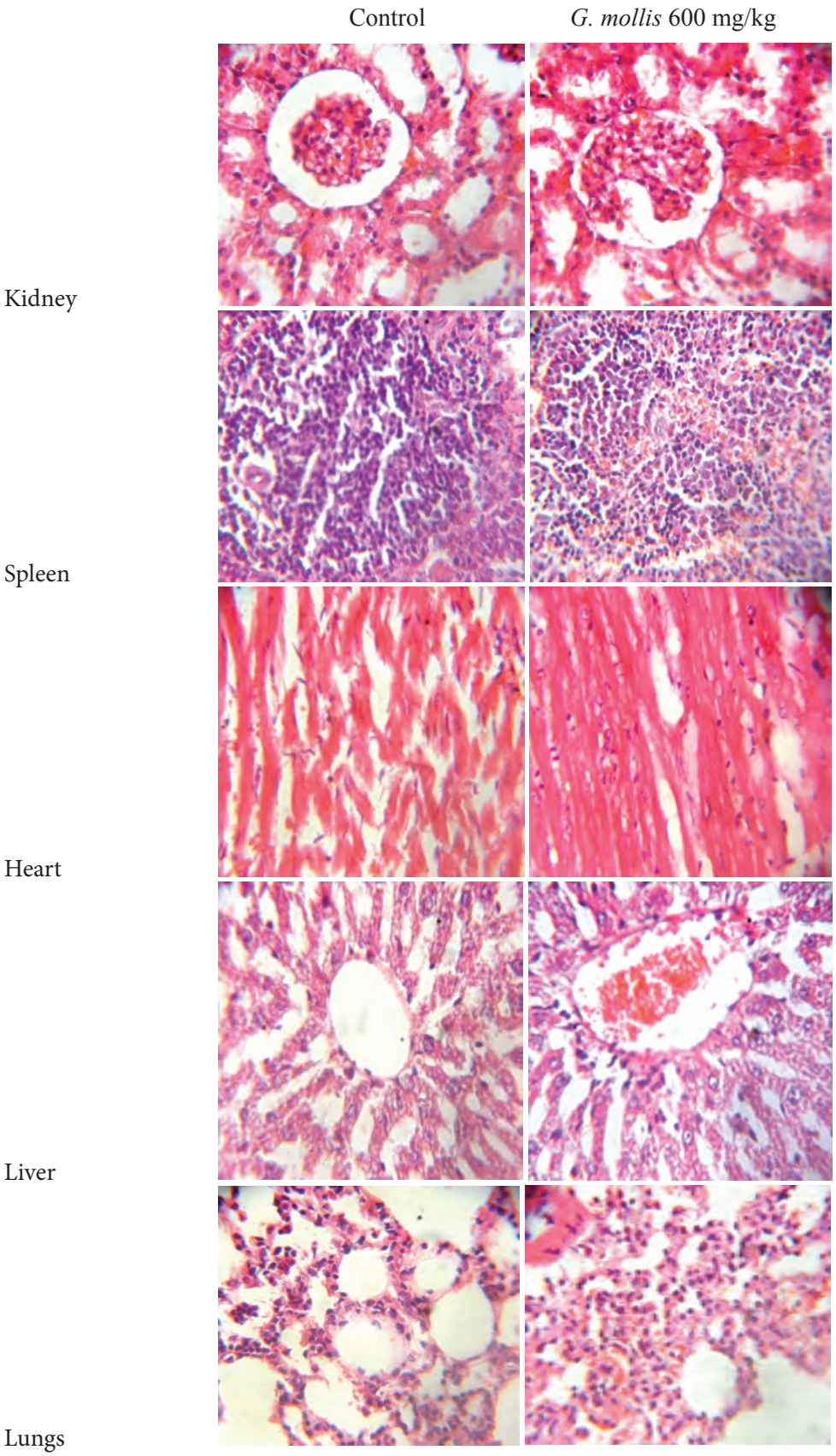

Figure 5.

Representative photomicrographs for the heart, kidneys, liver and spleen of male Wistar rats treated orally with the control (water) or highest dose of *Grewia mollis* (600 mg/kg) for 28 days

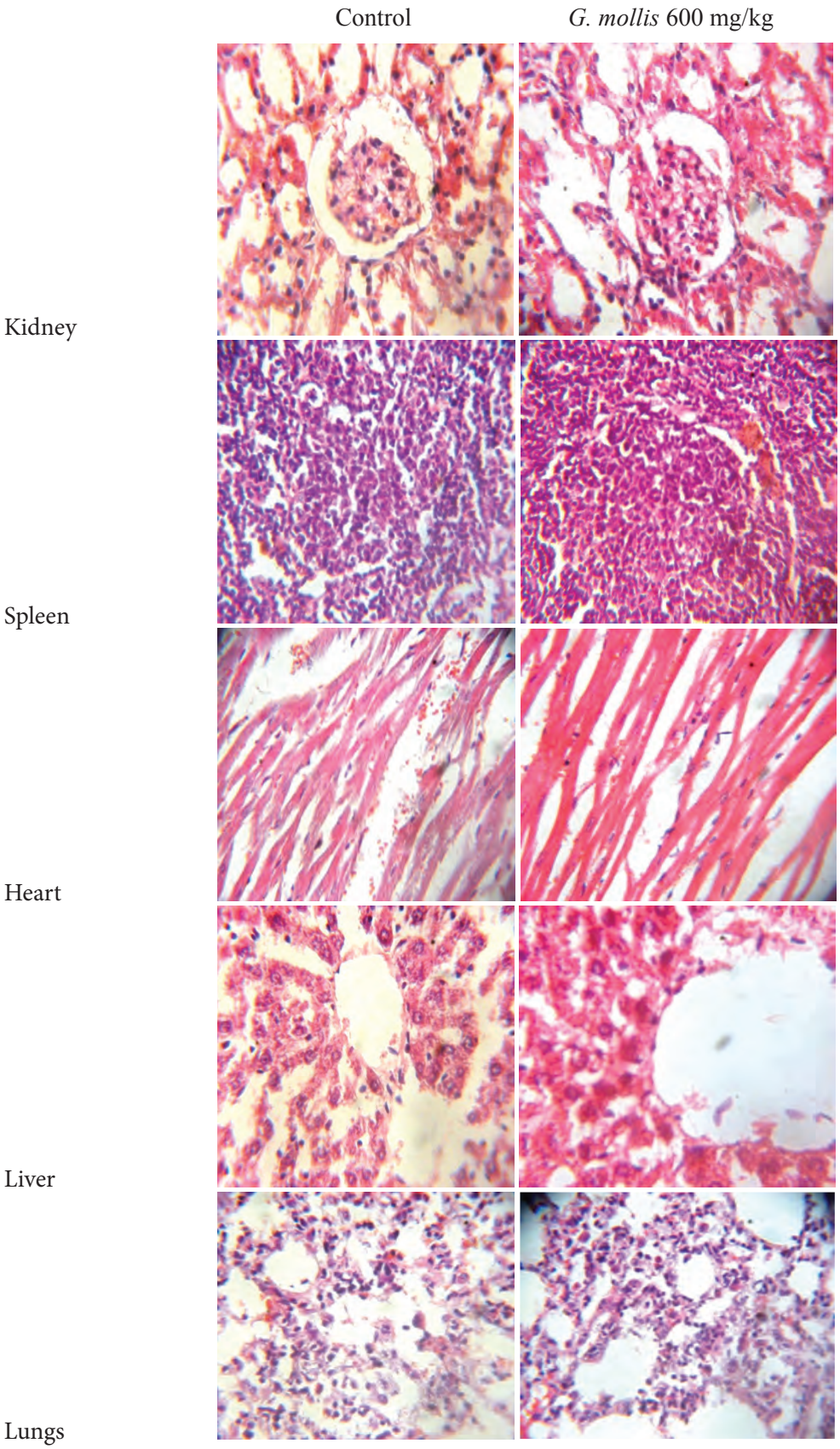

Figure 6.

Representative photomicrographs for the heart, kidneys, liver and spleen of female Wistar rats treated orally with the control (water) or highest dose of *Grewia mollis* (600 mg/kg) for 28 days

DISCUSSION

Based on the long-term use of medicinal plants by humans, the information on the acute and sub-chronic toxicity studies on medicinal plants or preparations derived from them should be obtained in order to enhance the confidence in their safety to humans, and set criteria for the selection of a safe dose in humans [14]. Despite widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. Few scientific data are available to confirm the safety profile of repeated exposure of *G. mollis* extracts [10, 11]. However, there is no safety study undertaken to evaluate and focus on the oral acute and sub-chronic toxicity of aqueous extract of *G. mollis* stem bark in both sexes of rodents in an attempt to replicate its chronic use as food or medicine in humans.

In the evaluation of the toxic properties of medicinal plants, the first step is usually LD_{50} determination. Results from the acute toxicity study can help determine the potential types of drug activity from the LD_{50} values obtained [13]. In the present study, the oral acute toxicity assay at a dose of 9600 mg/kg did not cause signs of toxicity, changes in behavior or mortality up to 14 days of observation and LD_{50} value was shown to be greater than 9600 mg/kg. Hence, the extract was classified as relatively nontoxic when administered orally [15]. However, study [10] has previously reported an LD_{50} of 1500 mg/kg when the extract was administered intraperitoneally.

Toxicological evaluation involving sub-chronic studies is used to determine the undesirable effects of continuous or repeated exposure of medicinal plants over a period of the life span of experimental animals, such as rodents [16]. These studies provide information on target organ toxicity and they can be used to determine appropriate dose regimens for longer term studies in humans [17]. In the present study the sub-chronic toxicity of *G. mollis* stem bark was evaluated in rats at doses of 150, 300 and 600 mg/kg/day for 28 days. There were no significant changes in animal behavior, food consumption, body weight gain as well relative organ weights in either sex of the animals when compared with the control. Significant alterations in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to a toxic substance [18-20].

Analysis of blood parameters is relevant to risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the data are translated from animal studies [21]. Sub-chronic administration of *G. mollis* did not significantly affect the haematological parameters with the exception of granulocytes and monocytes in the male rats. The changes in lymphocytes and monocytes were not dose dependent because they were only observed in the group treated with 150 and 300 mg/kg, not in the group treated with the highest dose (600 mg/kg). Since there were no corresponding changes observed in the other parameters, the significant changes in granulocytes and monocytes are not related to treatment with *G. mollis* stem bark extract [22].

Biochemical parameters evaluation is usually done to determine the possible alterations in hepatic and renal functions influenced by the plant extracts or compounds. The liver and kidneys have demonstrated to play crucial roles in various metabolic processes and are, therefore, particularly exposed to the toxic effects of exogenous compounds [23]. There were no significant changes in the liver function parameters among the treated groups when compared with the control; however the AST enzyme was significantly by 150 mg/kg dose of the extract. This effect was not dose dependent and the elevated values were within reference values, thus cannot be attributed to the plant extract [22]. This result is in contrast to previous studies [11] which report a significant increase in AST enzymes suggesting liver damage by the extract. The reason may be a result of the effect of the stem bark powder in the diet of rats on AST which is different from the aqueous extract used in this study. The kidney function parameters such as urea and creatinine did not reveal any significant changes following 28 day oral administration of extract. The lack of significant alterations in the levels of ALT, AST, creatinine, and BUN are good indicators of liver and kidney functions [24], which suggests that sub-chronic administration of *G. mollis* stem bark extract did not alter the hepatocytes and kidneys of the animals.

Hyperlipidemia, which is the elevation of lipids in the blood, is a major risk factor in cardiovascular diseases [25]. In this study, the extract significantly decreased total triglycerides in male rats suggesting that it may be useful in preventing atherosclerosis in cardiovascular diseases. In the present study, histopathological evaluation of the sub-chronic oral ingestion of *G. mollis* stem bark extract show that no pathological changes were observed in the photomicrographs of the liver, spleen, lung, kidneys and heart after 28 days oral administration. Therefore, these data confirmed that the extract has no toxic effect on organs in rats.

CONCLUSION

In summary, oral administration of *G. mollis* extract did not cause any death or treatment-related toxicity on organs of rats in the acute or sub-chronic toxicity studies at dose of 150, 300 and 600 mg/kg of extract. In addition, no observed adverse effect level (NOAEL) was detected for dose of up to 600 mg/kg/day for rats. Based on the formula for dose translation [26, 27], the human equivalent dose (HED) of the *G. mollis* extract is 97.8 mg/kg/day. For 70 kg adults, the HED is 6.8 g/day of *G. mollis* extract or 29.1 g/day of powdered stem bark. However, the safety of *G. mollis* extract in humans needs further investigation.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Gerald Eze of the Department of Anatomy, School of Basic Medical Sciences, University of Benin for his professional assistance in interpretation of the photomicrographs in this study.

AUTHOR CONTRIBUTIONS

The study was designed by II, carried out by II and AP. The manuscript was prepared by II and AP. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflict of interest: Authors declare no conflict of interest.

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Ocena ostrej i subchronicznej toksyczności wodnego ekstraktu z kory *Grewia mollis* (*Malvaceae*) u szczurów

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Streszczenie

Wstęp: Poszczególne części *Grewia mollis* Juss. (*Malvaceae*) powszechnie są stosowane w medycynie ludowej w leczeniu wielu schorzeń, w tym biegunek, krzywicy, kaszlu, gorączki. Chociaż wiele badań udowodniło skuteczność terapeutyczną, niewiele jest badań toksykologicznych tej rośliny.

Cele: Badanie to zostało przeprowadzone w celu oceny ostrej i subchronicznej toksyczności wodnego ekstraktu z kory *G. mollis* (GM) u zwierząt.

Metody: W badaniu toksyczności ostrej szczurom podano dożołądkowo GM w dawkach 150, 300, 600, 1200, 2400, 4800 i 9600 mg/kg w celu wyznaczenia średniej dawki śmiertelnej (LD_{50}). W badaniu nad oceną toksyczności subchronicznej szczury otrzymywały GM w trzech dawkach (150, 300 i 600 mg/kg) przez 28 dni. Zwierzęta analizowano oceniając je pod względem: spożycia pokarmu, zmian masy ciała, oznaczania parametrów biochemicznych, hematologicznych i histopatologicznych.

Wyniki: Wartość LD_{50} oznaczono na poziomie >9600 mg/kg. Podawanie wyciągu nie wpłynęło istotnie na: zmiany przyrostu masy ciała zwierząt, średniej masy narządów, wartości parametrów biochemicznych surowicy, parametrów hematologicznych lub obrazu histopatologicznego wątroby, nerek, płuc, serca i śledziony.

Wnioski: Wyniki tego badania dostarczyły dowodów, że subchroniczne podawanie dożołądkowe GM w dawce 600 mg/kg jest stosunkowo bezpieczne u szczurów i może nie wywierać poważnych objawów toksycznych.

Słowa kluczowe: ***Grewia mollis, LD50, podawanie subchroniczne, hematologia, histopatologia***