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Original Research Article

Acute Oral Toxicity of *Grewia mollis* Stem Bark Extract in Wistar Rats

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ABSTRACT

Grewia mollis is a medicinal plant and natural food additive and belongs to the family Malvaceae. The utilisation of the plant in traditional folk medicine is employed for the treatment of various diseases. Furthermore, it functions as a binding agent in numerous locally produced food products. Nevertheless, the available data about its safety is inadequate. The acute oral toxicity of *G. mollis* stem bark extract was determined in female Wistar rats using a fixed-dose procedure**.** Ten (10) female Wistar rats were allocated into two groups. *G. mollis* stem bark extract was administered orally to one group at 2000 mg/kg of body weight, while the other group (the control) received distilled water. There was no death in either the extract-treated or control groups. Therefore, the median lethal dose (LD50) of the *G. mollis* stem bark extract was considered greater than 2000 mg/kg of body weight.*G. mollis* stem bark elevated alkaline phosphatase activity and reduced serum cholesterol levels.It also increased serum total protein and sodium levels. However, no gross or histopathologic lesions were found in any of the examined organs. These results indicate that oral administration of *G. mollis* stem bark extract had no toxicological effects in rats and support the potential use of the stem bark extract as a safe natural food additive and therapeutic option.

Keywords: Acute toxicity, *Grewia mollis*, Stem bark, Oral, Rat.

Introduction

The plant *Grewia mollis* (Malvaceae) is widely used in food and traditional medicine in many African countries. For instance, *G. mollis* is used as a thickening agent in some locally produced foods, such as *punkasau*, made from corn flour, and *kosai* or *akara*, made from bean flour.[1,2] The extract also serves as a binder in *dawadawa*, a locally produced spice in Nigeria and other West African countries.[3]In Chad and certain communities in North-East Nigeria, the decoction of *G. mollis* stem bark is added to the malt flour-water mixture to enhance the sedimentation of suspended particles and produce clear beer.[4] Aside from its use in food processing, the *G. mollis* plant has numerous therapeutic uses, such as treating snake bite, constipation, liver disease, arthritis, inflammation, malaria, typhoid, and wound healing.[5-8] The extracts derived from the bark and leaves of the *G. mollis* plant are used to treat fever, and the fruit is also consumed for the same therapeutic purpose.[9]*G. mollis* stem bark has also been found to have potential antibacterial properties.[10] The leaves contain potent antioxidant compounds that protect against carbon tetrachloride-induced hepatotoxicity.[11]The leaves were also reported to have high antibacterial activity.[12]This suggests that the plant could be a valuable natural remedy for combating bacterial infections. Previous toxicological studies on G. mollis stem bark have concentrated on the short-term toxicity of both the extract[13] and the stem bark powder[1] in rodents, as well as the acute (LD_{50}) toxicity associated with its ethanol extract.[14]

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However, there is insufficient information on the acute oral toxicity of *G. mollis* stem bark. This study assesses the acute oral toxicity of an aqueous extract derived from the stem bark of *G. mollis* in female Wistar rats. Additionally, understanding the acute oral toxicity of the stem bark extract will help in determining appropriate dosage regimens for potential food and therapeutic applications.

Materials and Methods

Chemicals

The chemicals used in this study were of analytical grade and were obtained from Sigma-Aldrich Chemical Corporation USA (St. Louis, Missouri, USA) and BDH Chemicals Limited (Poole, Dorset, England). The test kits for serum biochemical tests were products of Randox Laboratories Limited (Antrim, UK) and Agape Diagnostics, Switzerland.

Animals

Female Wistar rats, aged 7-8 weeks, were obtained from the National Veterinary Research Institute in Vom, Plateau State, Nigeria. The rats were housed in polyethylene cages with stainless steel tops and were maintained at a temperature of 24-25 °C under a 12-hour light-dark cycle. Tap water and a standard rodent pellet diet (Vital Feed, Jos, Nigeria) were administered *ad libitum*. Before starting the treatment, the animals were acclimatised for seven days.The Institutional Animal Care and Use Committee (IACUC) of Modibbo Adama University, Yola, approved the experiment with approval number MAU/FLS/2021/028. Animals were cared for using standard procedures.[15]

Plant material

Fresh inner stem bark of *G. mollis* was obtained from Bati village, Fufore local government area of Adamawa State. The plant sample was collected in the month of February 2021 and identified by a taxonomist in the Department of Plant Sciences at Modibbo Adama University Yola, and the specimen was deposited in the herbarium of MAU Yola with voucher number MAUH/GM/2021/04-162. To prevent the loss of active ingredients, the inner stem bark was shredded and air-dried in the

laboratory at room temperature (25-28 ˚C) for 14 days. The dried plant sample was stored in a clean, sealed glass container.

Extraction of the stem bark
The dried stem bark of *G. mollis* was extracted with distilled water at room temperature. A 4 L graduated beaker was filled with 1 L of distilled water. Then, while stirring with a glass rod, 100 g of the shredded inner stem bark was continuously added. The infusion was increased to 3 litres by the addition of distilled water and maintained at room temperature (25-28 °C). After about 24 hours, the mixture was stirred for 5 minutes, and the viscous solution was filtered through a double-layered muslin cloth to remove extraneous matter**.** The filtrate was lyophilised, and it was subsequently ground into a fine powder using an electrical blender. The dried powdered stem bark extract was stored in a dry, clean, airtight container until the time of administration. For the study, a solution (100 mg/ml) of *G. mollis* stem bark extract (GME) was prepared immediately prior to dosing.

Experimental design
Acute oral toxicity was determined in healthy adult female nulliparous Wistar rats using the fixed-dose procedure described in the Organisation for Economic Co-operation and Development (No. 420)**.**[16]Two female rats were sequentially administered 300 mg/kg and 2000 mg/kg of GME orally (sighting study)**.** In the main study, ten female Wistar rats were randomly divided into two groups of five rats each. Before dosing, food was withheld for duration of one night, while water was made available *ad libitum*. One group was orally administered GME at a dose of 2000 mg/kg. The control group was administered distilled water at a dose of 2 ml/kg. After dosing, the rats were deprived of food for another three hours. The animals in the extract-treated groups and control groups were closely monitored for mortality and clinical signs, including changes in behaviour patterns and alterations in physiological systems, including the skin, mucous membranes, and central nervous system.

Determination of body weight
Rats were weighed (g) immediately prior to GME administration. Animal body weights were determined weekly, and at the end of the study,before sacrifice**.**

Determination of food consumption
The amount of food consumed by animals in each cage was recorded daily by measuring the amounts of food provided in a cage (g) and the amount of food remaining after 24 hours:

Food consumption = Amount of food provided in a cage (g) – Amount of food remaining (g)

Determination of water consumption
Water consumption was recorded daily by measuring the amounts of water provided in a cage (ml) and the remaining after 24 hours:

Water consumption = Amount of water provided in a cage (ml) – Amount of water remaining (ml)

Determination of absolute and relative organ weights
The organs were weighed with a bench scale. The relative organ weight [organ to body weight ratio (%)] of each rat was calculated using the formula:

Relative organ weight (%)

$$= \frac{\text{Absolute weight of organ (g)}}{\text{Final body weight (g)}} \times 100$$

Collection of blood sample
At the end of the study, the rats were fasted for 12 hours; animals were deprived of food but had free access to water. The animals were euthenised by administering ketamine (90 mg/kg) and xylazine (90 mg/kg) via the intraperitoneal route. Blood was collected via the retro-orbital plexus.[17]A portion of the whole blood sample was collected in ethylenediaminetetraacetic acid (EDTA)-coated tubes for the assessment of haematological parameters. The remaining blood sample was collected in plain tubes without anticoagulant, allowed to clot, and subsequently centrifuged at 3000 g for 15 minutes to obtain serum for the biochemical assay.

Determination of serum biochemical parameters
Serum transaminase (alanine aminotransferase and aspartate aminotransferase) and alkaline phosphatase activities, along with concentrations of urea and creatinine, total protein, albumin, and bilirubin, were measured using test kits obtained from Randox Laboratories Limited. The test kits for determining serum cholesterol and triglycerides were products of Agape Diagnostics, Switzerland. These parameters were assayed following the instructions described by the manufacturer. A semi-automated electrolyte analyser (RD-171, China) was used to measure serum electrolytes (chloride, potassium, and sodium).

Determination of haematological indices
Blood samples from animals in both the treated and control groups were used to measure major haematological parameters such as leucocyte counts, platelets, erythrocyte counts, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. Measurements were performed with an automated haematology analyser.

Histopathological examination
After animal sacrifice, the livers, kidneys, hearts, lungs, and spleens of necropsied rats were harvested and kept in 10% neutral buffered formalin prior to undergoing dehydration by successive alcohol exchanges.They were then cleared with xylene, embedded in paraffin blocks, and cut using the rotary microtome at a thickness of 5 µm. They were then stained with haematoxylin and eosin dyes to demonstrate general tissue structure.[18] The sections were examined using a light microscope, and photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd., 1999–2004).

Statistical analysis
The results are presented as the mean ± standard error (SE). Data were analysed using an independent samples t-test with the Statistical Package for the Social Sciences (SPSS, version 26). The mean values of the GME group and the control were compared and $p < 0.05$ was chosen as the statistical level of significance.

Results and Discussion

Clinical observations
The animals in the GME-treated group showed weakness and decreased mobility for the first four hours, but they rapidly recovered. All animals administered GME survived to the end of the study **(Table 1)**. The LD_{50} of the GME is therefore considered to exceed 2000 mg/kg. The result is consistent with the findings of[13], who found that oral treatment of GME at 150-9600 mg/kg body weight in Wistar rats did not result in death. The observed high LD_{50} value (more than 2000 mg/kg) indicates that GME has low toxicity.[19]

Table 1: Behavioural patterns of rats given an acute oral dose of *G. mollis* stem bark extract

Parameter	Clinical observations									
	Control					GME 2000 mg/kg bw				
	30min	4hrs	24hrs	7days	14days	30min	4hrs	24hrs	7days	14days
Skin colour	N	N	N	N	N	N	N	N	N	N
Piloerection	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Eyes	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N
Urine colour	N	N	N	N	N	N	N	N	N	N
Faeces	N	N	N	N	N	N	N	N	N	N
Motor activities	N	N	N	N	N	↓	↓	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Convulsion and tremor	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Itching	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Coma	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

N, normal; NF, not found; ↓, decrease.; bw, body weight

Effect on body weight, food and water consumption

Changes in body weight are a clear indicator of the adverse effects caused by substance testing (Table 2).[20,21]. However, there was no significant difference ($p > 0.05$) in body weight between the groups administered GME and the control group. This indicates that the extract did not affect the overall health status of the rats. In both weeks 1 and 2, the mean daily consumption of food and water by the GME-treated group was similar to that of the control group. Food and water consumption are often used as indicators of toxicity and can affect animal body weight and blood and cellular concentration of biochemical indices. The lack of significant change in food and water consumption suggests sufficient nutrient intake and hydration in the GME-treated rats compared to the control group.

Table 2: Effect of acute oral administration of GME on the body weight of rats

Dose	Initial Body Weight (g)	Final Body Weight (g)	Weight Gain (%)
Control	134.86 ± 5.02	165.28 ± 8.56	22.56
GME 2000 mg/kg bw	134.30 ± 4.45	163.30 ± 5.41	21.64

Tabulated values are Mean ± SE, n= 5 ;bw, body weight.

Effect on serum biochemical parameters

The serum alkaline phosphatase activity increases significantly in the GME-treated group (Table 3**).** Cholestasis, a disease condition where bile flow is reduced, leading to elevated bile in the liver and blood, is usually accompanied by increased alkaline phosphatase activity and bilirubin concentration. [22-25] However, the concentration of serum total bilirubin in the GME-group was comparable to the control group, suggesting that the observed increase in ALP activity could be due to mild cholestasis or sources outside of the liver. The group administered GME showed an increase in total protein concentration. The increase in serum total protein may be due to increased hepatic synthesis elicited by some phytochemicals present in GME. Similarly, the administration of GME evoked an increase in serum sodium concentrations in the GME group.An increase in blood sodium concentration may occur as a consequence of reduced renal excretion[26-28]or dehydration.[29,30]

Table 3: Effect of acute oral administration of GME on serum biochemical parameters of rats

Parameter	Control	GME 2000 mg/kg bw
AST (U/L)	88.40 ± 2.54	90.80 ± 1.39
ALT (U/L)	89.20 ± 6.89	91.60 ± 6.00
ALP (U/L)	113.40 ± 6.84	134.80 ± 3.71*****
TBIL (µmol/L)	1.26 ± 0.27	1.84 ± 0.10
Total protein (g/dL)	7.34 ± 0.26	8.36 ± 0.31*
Albumin (g/dL)	3.88 ± 0.16	3.86 ± 0.11
Triglycerides (mg/dL)	94.20 ± 5.05	81.20 ± 5.86
Total cholesterol (mg/dL)	78.40 ± 1.50	66.60 ± 2.84*
Creatinine (mg/dL)	1.18 ± 0.12	0.85 ± 0.12
Urea (mg/dL)	62.00 ± 2.26	63.96 ± 2.94
Chloride (mmol/L)	96.00 ± 6.35	99.80 ± 4.75
Potassium (mmol/L)	19.40 ± 1.91	16.60 ± 1.03
Sodium (mmol/L)	122.20 ± 4.83	147.20 ± 5.36*

Values are Mean ± S.E.M, n= 5; *significantly different compared to control ($p< 0.05$). AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin.

However, the concentrations of comparable renal fun-ction indices did not differ significantly in relation to renal impairment. Specifically, the blood levels of creatinine, urea, potassium, and chloride were not significantly different between the GME-treated group and the control group, indicating that there was little effect on kidney function.Dehydration is unlikely, as the mean daily water consumption of GME-treated rats for each week was similar to that of the control group.According to the serum lipid profile, the animals treated with GME had significantly lower serum total cholesterol concentrations.

Studies have shown that a high concentration of cholesterol in the blood increases the risk of cardiovascular disease.[31-34] Therefore, a reduction in total serum cholesterol would be beneficial, as it will reduce the risk of cardiovascular disease.

Effect on haematological parameters

The GME-treated group exhibited no significant change in haematological parameters (Table 4). Haematological indicators are important in assessing the health status of animals and humans. Alterations in specific blood parameters, such as erythrocyte count, haemoglobin levels, and packed cell volumes, are associated with the condition of anaemia. White blood cells function as indicators of immune system, whereas platelet count is related to bleeding and clotting disorders. The absence of notable changes in these parameters in the GME-treated group suggests that the extract did not affect the haematopoietic tissues.

Table 4: Effect of acute oral administration of GME on haematological parameters of rats

Parameter	Control	GME 2000 mg/kg
Leukocyte counts (10^9/L)	8.54 ± 1.82	9.48 ± 1.30
Erythrocyte counts (10^{12}/L)	6.33 ± 0.79	7.07 ± 0.24
Packed cell volume (%)	39.06 ± 4.91	46.18 ± 1.92
Hemoglobin (g/dL)	11.42 ± 1.05	12.68 ± 0.47
MCV (fL)	61.78 ± 1.05	65.26 ± 1.31
MCH (pg)	18.56 ± 10.61	17.90 ± 0.13
MCHC (g/L)	30.04 ± 1.08	27.50 ± 0.49
Platelet counts (10^9/L)	173.60 ± 22.62	228.60 ± 19.80

Values are mean ± standard error of mean (S.E.M), n= 5; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Effect on relative organ weight and histopathological features of organs

Generally, changes in organ weight are considered sensitive indicators of toxicity.[35-37] Administration of GME did not cause adverse changes in the organs, as evidenced by the comparable absolute and relative organ weights between the GME-treated groups compared to the control (Figure 3 and Figure 4). Furthermore, the microscopic examination of selected organ sections from the control and GME-treated groups revealed no abnormalities (Figure 5). The liver, kidney, heart, lungs, and spleen sections had normal histological features with no observable lesions.

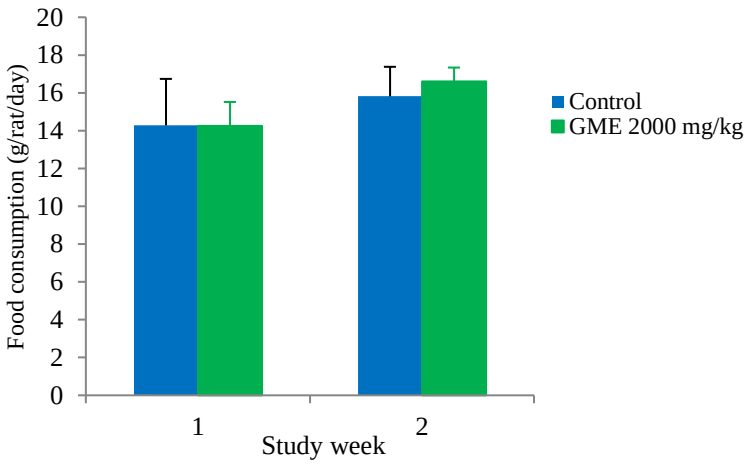

Figure 1: Effect of acute oral administration of GME on food consumption of rats. The GME-treated group and the control group show no significant difference ($p > 0.05$) in mean daily food consumption during weeks 1 and 2.

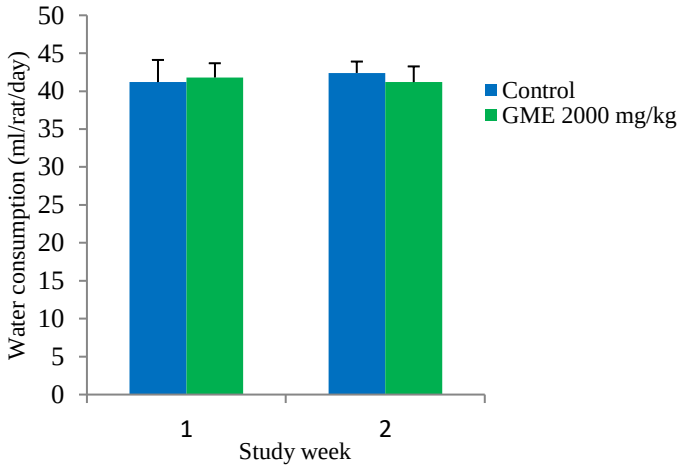

Figure 2: Effect of acute oral administration of GME on food consumption of rats. The GME-treated group and the control group show no significant difference ($p > 0.05$) in mean daily water consumption during weeks 1 and 2.

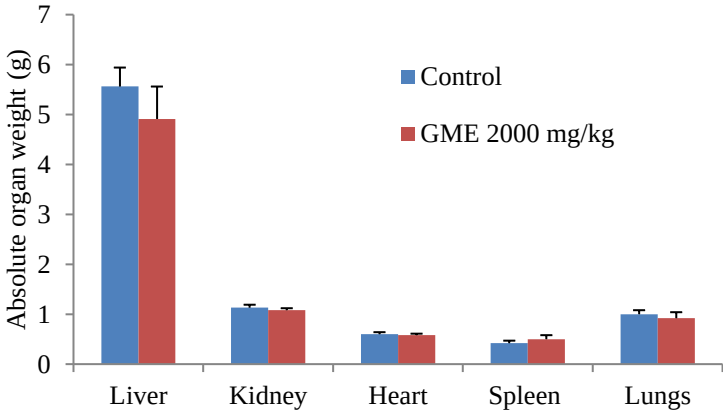

Figure 3: Effect of acute oral administration of GME on absolute organ weight of rats. There is no significant ($p > 0.05$) difference between organ weight of GME-treated rats and the control.

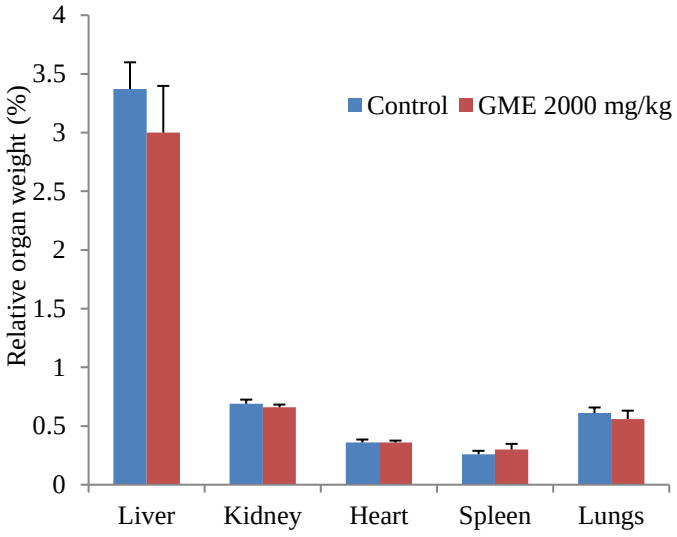

Figure 4: Effect of acute oral administration of GME on relative organ weight of rats. There is no significant ($p > 0.05$) difference between relative organ weight of GME-treated rats and the control.

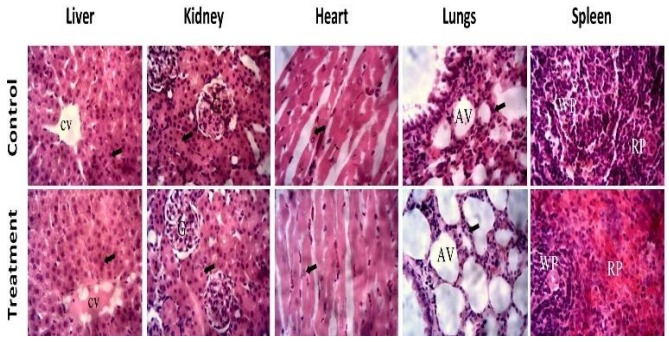

Figure 5: Photomicrographs of tissue sections from the control and treatment (GME 2000 mg/kg) groups: central vein (CV), hepatocytes (arrows), glomerulus (G), renal tubules (arrows), cardiac muscle fibres (arrows), alveoli (AV), and interstitium (arrows), white pulp (WP), and red pulp (RP) with no observable histological changes. H & E, × 400

Conclusion

Oral administration of GME to rats caused significant increases in serum alkaline phosphatase activity, total protein and sodium concentrations accompanied by a reduction in serum total cholesterol concentrations with no observable alterations in organ histology. Furthermore, the LD50 value exceeded 2000 mg/kg body weight. Therefore, the GME was deemed safe for short-term consumption. However, the minor changes observed in biochemical markers raise concerns regarding the potential long-term effects of similar exposure.

Conflict of Interest

The authors declare no conflict of interest.

Authors Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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