Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 13, Issue 2, 2024, 66
https://doi.org/10.33263/LIANBS132.066

Hepatic and Nephro-Modulatory Activity of Oral and Repeated Exposure to Ruzu Bitters (a Nigerian Polyherbal Formulation) in Wistar Rats

Abolanle A. A Kayode [1,3,*], Omowumi T. Kayode [2,3], Oyebolanle O. Adesoye [3]

[1] Phytomedicine Research, Drug Discovery & Development Laboratory, Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ilishan-Remo, Ogun State, Nigeria
[2] Department of Biochemistry, College of Basic and Applied Sciences, Mountain Top University, Ogun State, Nigeria;
[3] Department of Chemical & Food Sciences, Bells University of Technology, Ogun State, Nigeria
* Correspondence: kayodeab@babcock.edu.ng;

Scopus Author ID 35224908600
Received: 30.04.2023; Accepted: 7.01.2024; Published: 30.06.2024

Abstract: This study aimed to evaluate the toxic effect of Ruzu Herbal Bitters (RHB). Thirty male Wistar rats were allocated into five groups of 6 animals each. In the space of 28 days, distilled water and RHB were administered orally at concentrations of 0.04, 0.08, 0.13, and 0.20 mL/kg body weight, respectively. After this treatment, the animals were sacrificed, and the plasma, liver, and kidney obtained were used for the determination of Protein concentration, Creatinine, Urea, Bilirubin, Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate Dehydrogenase Enzyme (LDH) activities. All experiments were done using standard methods. Significant ($p < 0.05$) alterations were observed in (ALT, AST, ALP, LDH, Albumin, and Total bilirubin). All doses of RHB significantly ($p < 0.05$) increased the serum AST and ALT levels. Furthermore, similar significant ($p < 0.05$) alterations were observed for the kidney function parameter creatinine. All doses of RHB caused insignificant ($p < 0.05$) elevations in the level of urea. There was a dose-dependent fluctuation in albumin levels in the blood. Significant decreases in the lipid profile and total protein concentrations were observed. These findings revealed that moderate consumption of doses of RHB might be safe.

Keywords: kidney; liver; polyherbal; Ruzu herbal bitters; safety; toxicity.

1. Introduction

The traditional utilization of herbs for treating ailments and diseases is the oldest type of medical practice known to humankind, and it has been utilized in all societies for ages. Herbal medicines are made up of plant portions or crude extracts containing several unpurified metabolites that synergistically act [1-3]. It has become a common traditional practice in some cases, that a combination of plants or their extracts is used in the treatment of certain ailments with the belief by the herbalists that the individual plants contain different therapeutic agents which, when combined, will give a better therapeutic efficacy for a particular disease or multiple diseases than that of a single plant [4,5]. Therefore, most herbal preparations work in synergy, resulting in polyherbal mixtures.

Over the last 10 years, people in developing and developed countries have shown an overwhelming interest in herbal medicine, and its acceptance is rising fast. Poverty and limited

access to modern medicine have made about 80% of the people in developing countries rely on the use of herbal medicine as their major source of health care. Due to this dependence and increased use of herbal products, misuse and adulteration of available herbal products have led to dissatisfaction and deadly cases for consumers and manufacturers [5-7].

Ruzu Herbal Bitters (RHB) is a polyherbal mixture that is an aqueous preparation of three different plants: *Curculigo pilosa* (squirrel groundnut) root (40%), *Uvaria chamae* (bush baby) stem (20%), and *Citrullus colocynthis* (bitter apple) bark (40%). It is produced by Ruzu Natural Health Product and Services, Nigeria, with a NAFDAC Registration Number: A7-1102L [8]. RHB is commercially available, and the manufacturers infer that the product has the following medicinal functions, as indicated in the leaflet of the product: management/treatment of erectile dysfunction, high blood sugar, cardiovascular diseases, irregular menstruation, fertility issues, pains, overweight issues, gastrointestinal disorders, and organ detoxifications amongst others [6-9]. RHB's anti-inflammatory activity in preventing nonalcoholic fatty liver disease in rats was confirmed by [8,10]. Evaluating RHB's safety is essential in knowing its potential adverse effects, as it has become a vastly treasured polyherbal medicine that is now widely consumed for its biological and medicinal benefits. There are no reports on its toxic effect. Therefore, this study aimed to evaluate RHB's safety and potential toxic effects.

2. Materials and Methods

2.1. Animal grouping and administration.

Thirty rats (Wistar strains) between the weight of 150 and 200 g were housed in cages under well-ventilated conditions and were fed with standard commercial feed and clean water. Ruzu herbal bitters were orally administered daily for 28 days using the dosage on the prescription label as a guide. Animals were distributed into five groups containing six rats each:
Group A: Oral administration of 0.2 mL/kg body weight distilled water (control group);
Group B: Oral administration of 0.04 mL/kg body weight Ruzu bitters;
Group C: Oral administration of 0.08 mL/kg body weight Ruzu bitters;
Group D: Oral administration of 0.13 mL/kg body weight Ruzu bitters;
Group E: Oral administration of 0.20 mL/kg body weight Ruzu bitters;

Determination of creatinine: this was carried out according to the method described by Tietz *et al*. [11]

Determination of urea: the method of Veniamin andVakirtzi–Lemonias [12] was used to determine urea concentration.

Determination of bilirubin: the dimethyl sulphoxide method described by Tietz *et al*. [11] was used for bilirubin determination.

Lipid profile: total cholesterol, triglyceride, HDL, and LDL cholesterol. Trinder's CHOD-PAD enzymatic colorimetric method [13] was used.

Determination of alkaline phosphatase (ALP) The method of Wright *et al*. [14] was employed.

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Activities of AST and ALT were determined following the principle described by Reitman & Frankel [15].

2.2. Histopathological analysis.

The tissues were registered according to the nature of the tissue, laboratory number, and research topic. The sample areas are cut and placed inside a well-labeled tissue embedding cassette. The tissue is then processed using a 24-hour automatic tissue processor ranging from 17 to 19 hours. The tissue processor contains 12 beakers, 10 glass beakers, and 2 thermostatically controlled electric metal beakers containing paraffin wax. Beaker 1 contains 10% of normal saline for complete fixation. Beakers 2-8 contain alcohol ranging from 70% alcohol to absolute alcohol to remove water from the tissue samples. Beakers 9-10 contain clearing agents (xylene L and H), which completely clear the dehydrating agent off the tissue sample. Beaker 11-12 contains embedding agents. After being processed, the tissues are embedded using an automatic embedding center. The paraffin wax becomes solidified when it is cold. This forms a support medium for the tissue during sectioning. The tissues are then cut using a microtome and placed on a hot plate for 30 minutes using the stain H&E staining method. It was stained in Haematoxylim for 10 minutes, then rinsed in water. 1% acid alcohol was used to differentiate. It was rinsed in water for 5 minutes. It was stained in 1% Eosin for 2-5 minutes and rinsed in tap water. 70% alcohol, 95% alcohol, and absolute alcohol were used to dehydrate. D.P.X was used to clear in xylene and then viewed under the microscope.

The study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies [16].

2.3. Statistical analysis.

Data generated from this study were expressed as means ± standard error of means (SEM) and analyzed using the GraphPad Prism 6 software. The significance difference was calculated using one-way ANOVA with the significance level set at $P \leq 0.05$.

3. Results and Discussion

The effect of the RHB on the liver function parameters, which include AST, ALT, ALP, and LDH are shown in Figure 1. The result revealed that the group treated with the highest dose of RHB (0.20 mL/kg *b. w*) had a significant ($p < 0.05$) reduction in the LDH and AST activity in the liver tissues when compared with the control. There was a significant ($p \leq 0.05$) increase in the ALT levels in all the treatment groups compared to the control. The ALP level was significantly decreased in all the groups compared to the control.

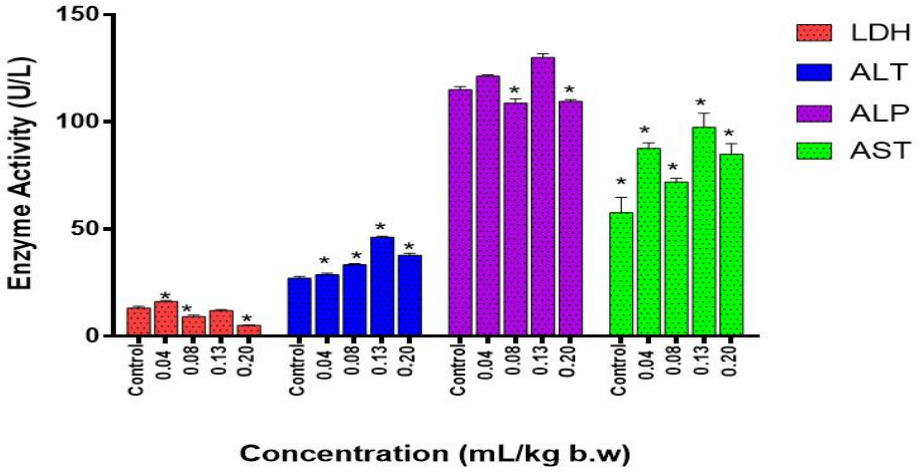

Figure 1. Effect on enzyme activities in the liver.

The result showed a significant ($p \leq 0.05$) decrease in the cholesterol level in all the groups (Figure 2). Also, there was a significant ($p \leq 0.05$) decrease in triglyceride in the groups given 0.04, 0.08, 0.20 (mL/kg *b. w*) of Ruzu bitters. In contrast, the triglyceride level was significantly elevated in the group given 0.13 (mL/kg b. w) compared to the control. The high-density lipoprotein (HDL) levels were significantly ($p \leq 0.05$) depleted in the entire treatment group. Ruzu bitters led to a significant decrease in the LDL level compared to the control.

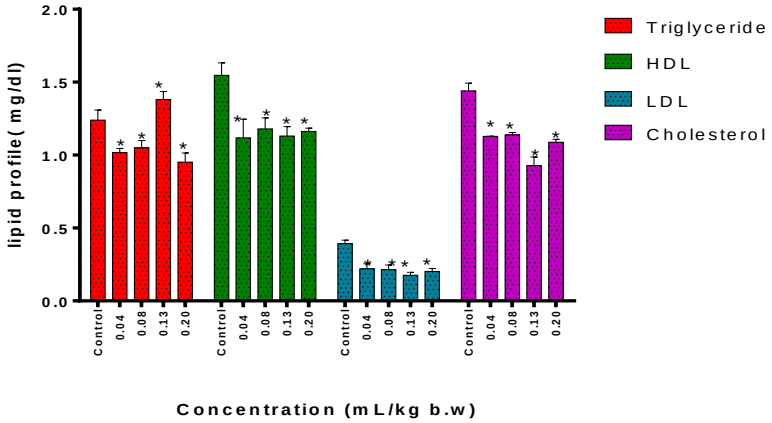

Figure 2. Ruzu herbal bitter lowered the lipid profile.

There was no significant ($p \leq 0.05$) change in the kidney urea level when all the treatment groups were compared to the control. In contrast, the kidney creatinine level was significantly reduced ($p \leq 0.05$) when compared to the control in all the treatment groups (Figure 3).

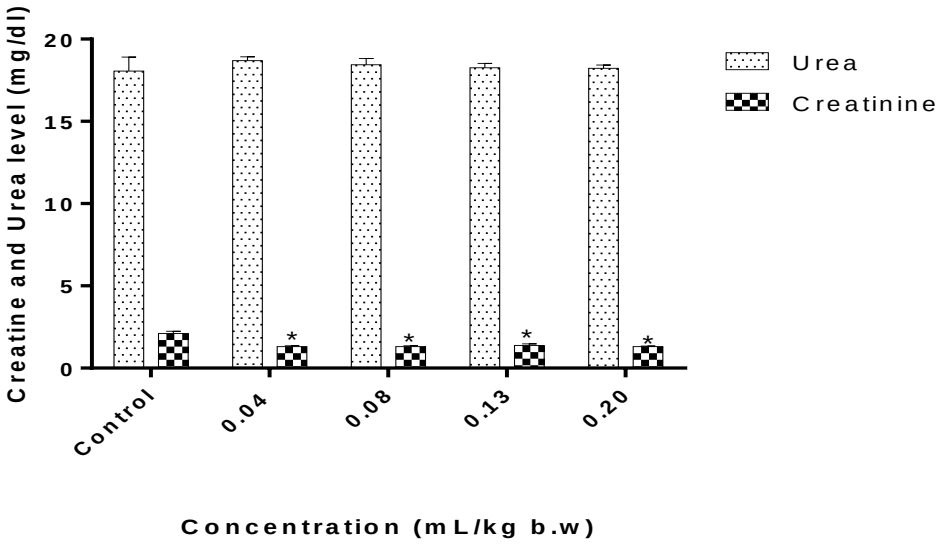

Figure 3. Effect on the kidney urea and creatinine levels.

The albumin levels of the group treated with 0.04 (mL/Kg *b. w*) and 0.08 (mL/Kg *b. w*) were significantly ($p \leq 0.05$) reduced, while there was no significant change in the group treated with 0.13 (mL/Kg *b. w*) and 0.20 (mL/Kg *b. w*). Creatinine level was significantly reduced ($p \leq 0.05$) in all the treatment groups compared to the control. (Figure 4)

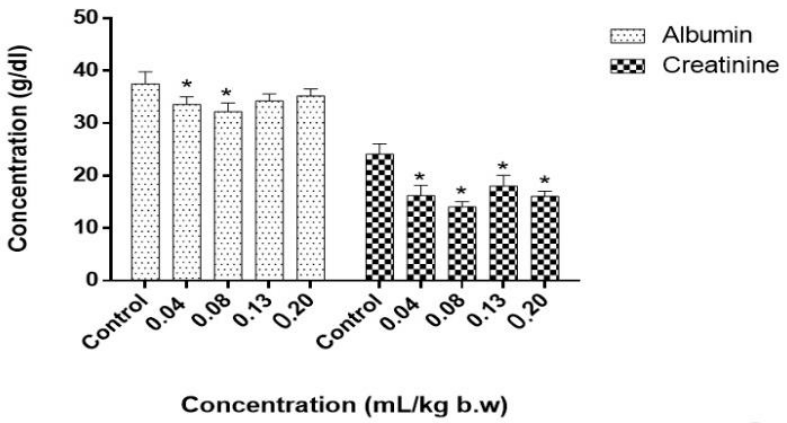

Figure 4. Effect on plasma creatinine and albumin levels.

In the liver, Ruzu bitter caused varying degrees of alterations in the albumin levels compared to the control (Figure 5). Albumin levels were significantly ($p \leq 0.05$) higher in the group given 0.04 (mL/Kg *b. w*) and 0.013 (mL/Kg *b. w*) and lower in the group given 0.08 mL/Kg and 0.020 (mL/Kg *b. w*). Meanwhile, the bilirubin level was significantly reduced ($p \leq 0.05$) compared to the control in all the treatment groups.

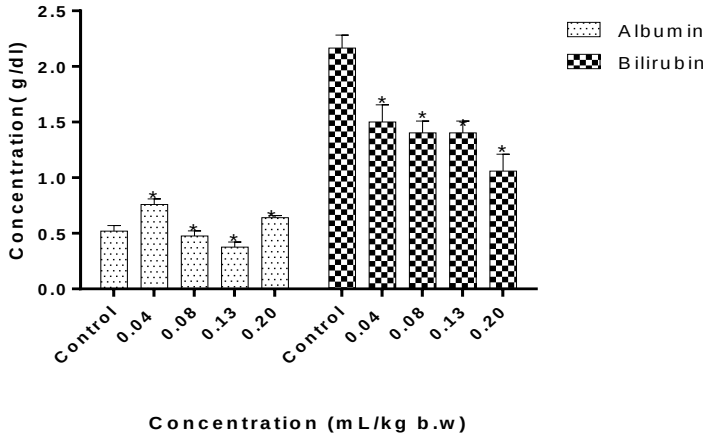

Figure 5. Effect on liver albumin and bilirubin levels.

There was a varying degree of change in the blood's total protein concentration compared to the control (Figure 6). The protein level in the blood was significantly ($p \leq 0.05$) higher in the group given 0.04 (mL/Kg *b. w)*; there was no significant difference in the other groups. Meanwhile, the total protein concentration in the liver was significantly reduced ($p \leq 0.05$) compared to the control in all the treatment groups.

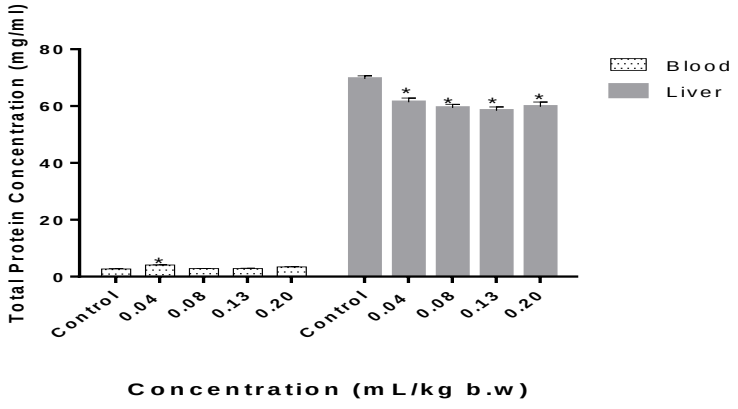

Figure 6. Effect on total protein concentration.

There was a significant ($p \leq 0.05$) increase in the ALT and AST levels in all the treatment groups compared to the control. Ruzu bitter caused varying degrees of alterations in the levels of LDH and ALP compared to the control.

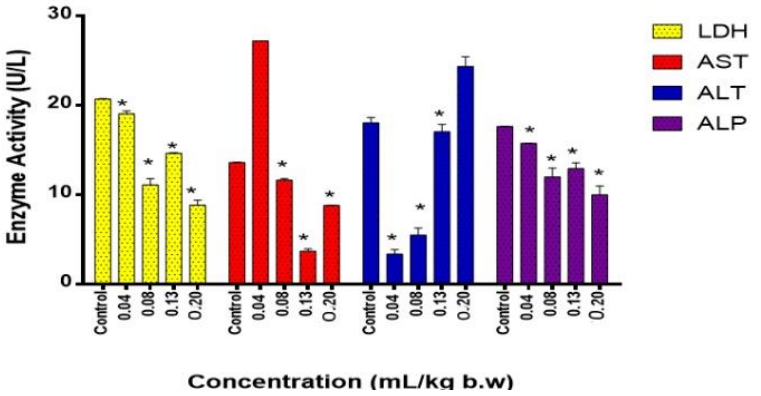

Figure 7. Effect on enzyme activities in the blood.

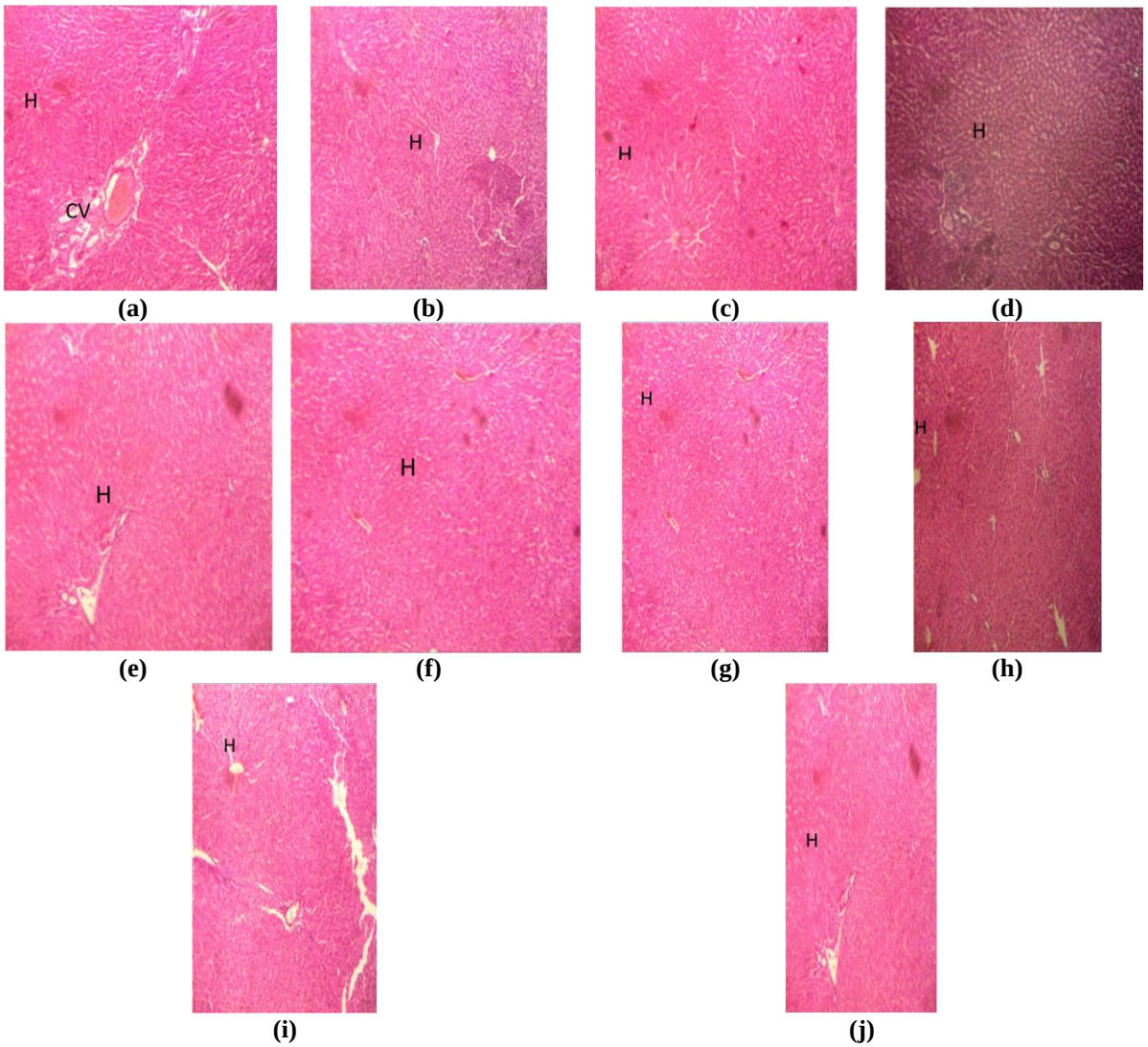

Figure 8. (a) and **(b)** Photomicrographs showing the liver of rats administered 0.13 mL/kg body weight of distilled water (Control group). Normal liver architecture with Central vein (CV) and hepatocyte (H); **(c)** and **(d)** Photomicrograph showing the liver of rat administered 0.04 mL/kg body weight of Ruzu Herbal Bitters. Normal liver architecture with hepatocyte (H); **(e)** and **(f)** Photomicrographs showing the liver of rats administered 0.2 mL/kg body weight of Ruzu Herbal Bitters. Normal liver architecture with hepatocyte (H); **(g)** and **(h)** Photomicrographs showing the liver of rats administered 0.08 mL/kg body weight of Ruzu Herbal Bitters. Normal liver architecture with hepatocyte (H); **(i)** and **(j)** Photomicrograph showing the liver of rat administered 0.13 mL/kg body weight of Ruzu Herbal Bitters. Normal liver architecture with hepatocyte (H).

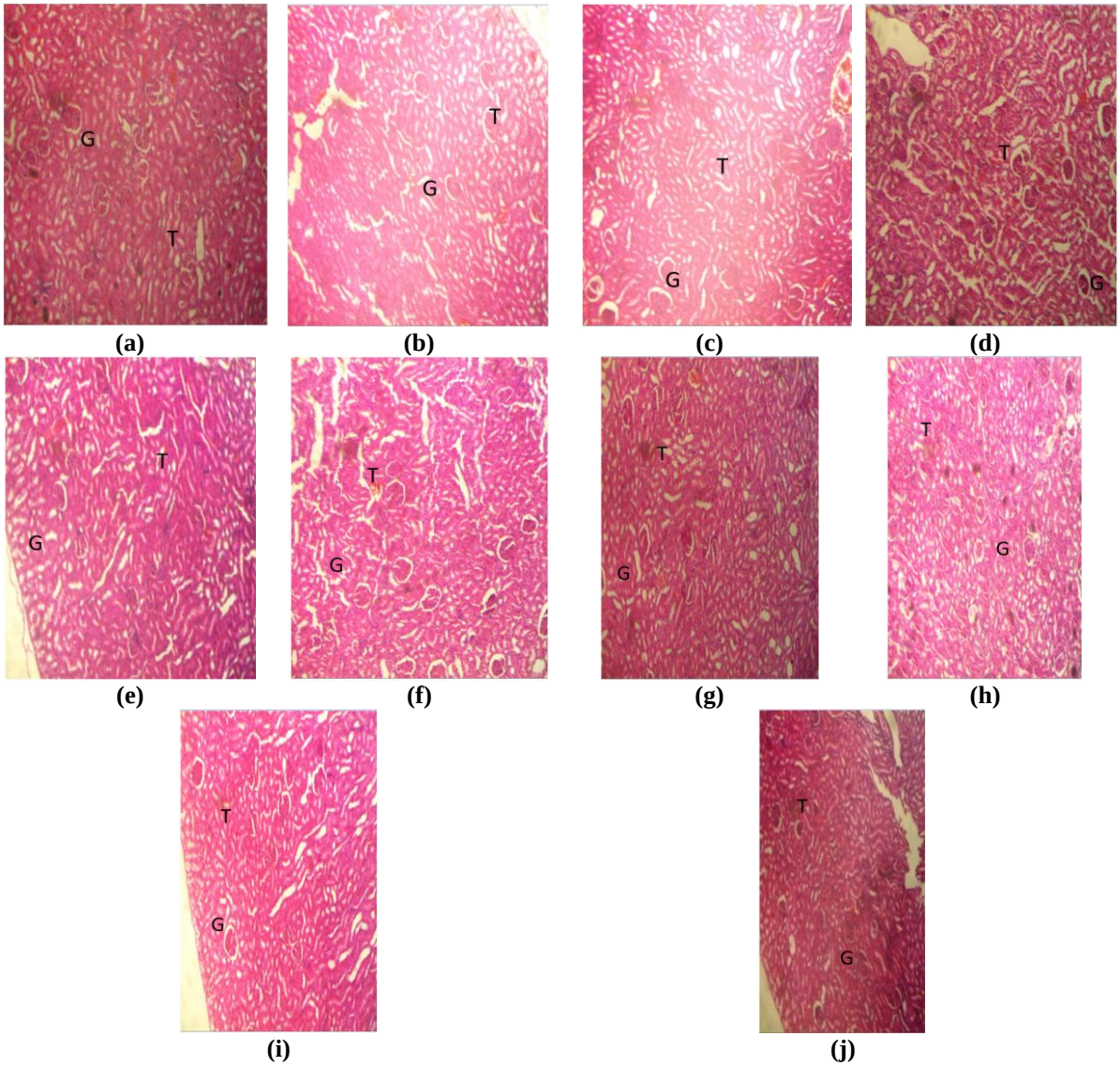

Figure 9. (a) and **(b)** Photomicrograph showing the control group with normal kidney with tubule (T) and glomeruli (G).; **(c)** and **(d)** Histology of the 0.04 mL/kg treated Ruzu Herbal Bitters showing normal kidney with tubule (T) and glomeruli (G).; **(e)** and **(f)** Histology of the 0.08 mL/kg treated Ruzu Herbal Bitters showing normal kidney with tubule(T) and glomeruli (G).; **(g)** and **(h)** Histology of the 0.013 mL/kg treated Ruzu Herbal Bitters showing normal kidney with tubule (T) and glomerulii (G); **(i)** and **(j)** show the histology of the 0.2 mL/kg treated RB showing normal kidney with tubule (T) and glomeruli (G).

LDH levels were higher in the groups given 0.04 (mL/Kg *b. w*) and 0.013 (mL/Kg *b. w*) and significantly ($p \leq 0.05$) lower in the groups given 0.08 (mL/Kg *b. w*) and 0.020 (mL/Kg *b. w*) while the ALP level significantly reduced in the groups treated with 0.08 (mL/Kg *b. w*) and 0.20 (mL/Kg *b. w*) and increased in the groups treated with 0.04 (mL/Kg *b. w*) and 0.13 (mL/Kg *b. w*) (Figure 7).

Poisonous or unfavorable effects of pharmacological agents are inclined by the degree to which they go into the body and how they relate to different body tissues and vital organs like the liver and kidney [17,18]. In this study, the toxic effect of Ruzu herbal bitters was evaluated. It is crucial to assess the function of vital organs like the liver and kidney in the body because these organs have diverse mechanisms to eliminate toxic substances and decrease their toxic effects. Although the kidney and liver are excellent toxin eliminators, they can be damaged during toxin elimination [17-21]. The potential toxic and lethal effects of RHB on hepatic and renal functions can be revealed by assessing biochemical factors.

The ALT, AST, LDH, and ALP are conventional enzymes used to assess liver damage or impairment [19,22-24]. A great increase in the concentration of these enzymes in the blood is key to gaining information about the healthy state of the liver or any changes that might have occurred in the hepatocyte membrane permeability [20,25,26]. Cell impairment or cell death can be detected by the presence of LDH in the extracellular space; increased levels of LDH are found in diseases that affect the heart, liver, and skeletal muscle, as in hematological disorders and diseases relating to abnormal cell growth [21,27], a significant decrease in the LDH level was observed in this study. Ailments such as viral hepatitis, liver damage, bile duct problems, infectious mononucleosis, or myopathy are usually suggested when there is an elevated level of ALT and AST in the blood, so these enzymes are used for screening liver impairments.

In the high-dose treatment, ALT displayed a noticeable increase in the liver and also in the blood, while AST and LDH were significantly raised in the blood; the observed increase raises a concern that RBH may have hepatotoxic properties. The plasma alkaline phosphate ALP level also decreased gradually. Blocking of the bile flow is usually known to be followed by an increase in the alkaline level [22,23,28-30]. Therefore, it is obvious that the RHB drug did not upset the bile flow. At the lowest dose of RHB, there was an increase in the blood's total protein concentration, while in the other groups, the total protein did not increase significantly ($p \geq 0.05$) with the dose. The decline in total protein level often infers protein loss, which can be caused by malabsorption resulting from liver damage [24-26,31-33]. There was a dose-dependent fluctuation in albumin levels in the blood. The synthesis level of albumin in the liver is usually determined by its plasma level [15,17,27,34]; RHB seems to have an effect on the level of synthesis in the liver. Bilirubin is known as the regular end-product of heme metabolism in the body, and it can cause damage to the brain of infants. At the same time, elevated levels have been implicated in many chronic diseases that affect adults [28-30,35-38]. In this study, the bilirubin level was significantly reduced. The creatinine level in the kidney exhibited a significant decrease ($p \leq 0.05$), while there was no considerable change in the Urea level in all the treatment groups. Among the non-protein nitrogenous substances known are creatinine and urea; both are end products of protein metabolism that must be constantly removed [31,39]. Urea and creatinine are analytes used to evaluate renal function because the body depends on the renal system to excrete these compounds [32,33,40-42]. This implies that RBH does not possess a nephrotoxic property. RHB led to a decrease in the lipid profile, and the presence of hypolipidemic agents in RHB might be responsible for the observed decrease in the plasma total cholesterol and triglyceride levels. The reduced LDL-cholesterol levels observed in all the treated groups are confirmatory to the fact that the RHB can reduce the risk of cardiovascular diseases, and this supports the traditional use of the polyherbal drug, although a decrease in the HDL-cholesterol level was observed when compared to the control. The light microscopic examinations of rat liver and kidney sections showed intact and normal cellular architecture in the control group as well as in the groups treated with Ruzu Herbal Bitters. This is consistent with the results obtained for the liver and kidney function markers discussed previously.

4. Conclusions

Oral and repeated exposure to Ruzu Herbal Bitters may have no adverse effects on the functions of the liver, kidney, and atherogenic indices in rats. These findings showed that moderate consumption of doses of Ruzu Herbal Bitters might be safe.

Funding

This research received no external funding

Acknowledgments

This research has no acknowledgement.

Conflicts of Interest

The authors declare no conflict of interest.

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