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Biochemical and histological changes associated with Ruzu herbal bitters (RHB) in non-morbid rodents.docx

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ABSTRACT

Background & Aim: In developing countries, the use of herbal preparations has gained much attraction not only for therapeutic purposes but, also for prophylaxis. This study was conducted to determine the effect of a popular Nigeria commercial herbal preparation; Ruzu herbal bitters (RHB) on some biochemical indicators of liver function as well as histology in non-morbid rats.

Experimental: Thirty-five adult Wistar rats divided into seven experimental groups (A-G) of five rats each were used for this investigation. Rats in group A were not treated with RHB and served as control. Rats in groups B-G received 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL and 1.2 mL of RHB twice daily by gavage, respectively. All rats were exposed to experimental conditions for two weeks. Blood was collected and analyzed for Total bilirubin (TB), Direct Bilirubin (DB) as well as activities of alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST). Liver and testicular tissues were processed by standard histological method and stained for microscopy.

Results: Rats treated with experimental doses of RHB exhibited lower levels of TB, DB, AST, ALT and ALP in comparison with rats in the control group which had higher values. Rats in the control group had normal liver and testicular tissue morphology while rats given experimental doses of RHB exhibited hepatocellular degeneration, cytoplasmic degeneration, vacuolation, presence of haemorrhage, inflammatory cell infiltration, condensed nuclei and minimal fibrosis in the periportal area. Testicular tissue appeared normal in RHB treated rats. Thus, administration of RHB on non-morbid rats within experimental conditions appears to significantly improve the biochemical indices of liver function (TB, DB, AST, ALT and ALP) but with residual degenerative effects on liver morphology.

Recommended applications/industries: RHB is therefore recommended for therapeutics and not for prophylaxis.

1. Introduction

Ruzu herbal bitters (RHB) is a aqueously extracted mixture from plant parts consisting of root of *Curculigo pilosa*; stem of *Uvaria chamae* and bark of *Citrullus colocynthis* in a 4:2:4 volume ratio (Kale *et al.*, 2018; Ogunlana *et al.*, 2018; Ogunlana *et al.*, 2020; Obasi *et al.*, 2020; Ogunlana *et al.*, 2021). Phytochemical analysis of the constituent plant parts indicates that they possess antioxidant, antiasthmatic, hepatoprotective and neuroprotective activities (Ogunlana *et al.*, 2018; Ogunlana *et al.*, 2020; Obasi *et*

al., 2020). RHB is claimed to be therapeutic against a wide-ranging disease conditions including obesity, diabetes, erectile dysfunction, typhoid and malaria fevers, arthritis, gastrointestinal disorders, high blood pressure, fibroid, among others (Ogunlana *et al.*, 2020; Obasi *et al.*, 2020; Oyaluna *et al.*, 2021). This so-claimed therapeutic action may not be unconnected with the phytochemicals (alkaloids, flavonoids, tannins, saponins, phenols, glycosides, sterols) as well as bioactive agents (cytotoxic, antibacterial, antifungal, antiviral, anticarcinogenic, antimutagenic, anti-inflammatory, antimalarial) detected in the mixture (Obasi and Ogugua, 2021). A couple of empirical research (kidney and liver function; haematological and lipid profile; glycemic index; anti-inflammatory activity) conducted with the mixture in rodents suggests that it may be antidiabetic, nephroprotective, hepatoprotective, antihyperlipidemic and anti-inflammatory (Obasi and Ogugua, 2020; Ogunlana *et al.*, 2020; Obasi and Ogugua, 2021; Ogunlana *et al.*, 2021). To the best of our knowledge, not much effort has been made to correlate biochemical effect of RHB administration with histological findings. Therefore, this study seeks to not only validate some of the biochemical assertions but to also support this with histological evidence.

2. Materials and Methods

2.1. Research location, experimental materials and study design

This study was conducted at the Animal House, Department of Anatomy, Babcock University, Ilishan-Remo, Ogun State, Nigeria. Thirty-five (35) adult Wistar rats were obtained from the Laboratory Animal facility at Babcock University, housed in well-ventilated plastic cages under diurnal lighting conditions, and fed with standard mouse feed and water ad libitum. RHB were purchased from Sagamu market, Ogun State, Nigeria.

2.2. Ethical consideration

Ethical clearance with reference number NHREC/24/01/2020 was obtained from the Babcock University Health Research Ethics Committee (BUHREC 296/20) before the commencement of the study.

2.3. Study design

Thirty-five (35) adult Wistar rats were divided into seven experimental groups (A-G) with five rats in each group. All rats were fed with pelletized growers mash and water ad libitum. Rats in group A did not receive RHB treatment and served as control. Rats in groups B-G received 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL and 1.2 mL of RHB twice daily by gavage, respectively. All rats were exposed to experimental conditions for 2 weeks. At the end of the exposure period, blood was collected slowly by cardiac puncture through the left ventricle following administration of anesthesia and used for biochemical analysis (Williams *et al.*, 2020). Liver and testicular tissues were thereafter extracted and used for histological analysis.

2.4. Biochemical study

Blood samples from rats in the different groups were collected into lithium heparin bottles, spun with a centrifuge and the sera extracted was used for evaluation of TB, DB as well as activities of AST, ALT and ALP.

2.5. Histological study

Extracted liver and testicular tissues from rats in the different groups were fixed in 10% buffered formalin solution for forty-eight hours and thereafter processed by standard histological method and stained with H&E as well as MT stains. Stained slides were subsequently viewed under the microscope for histopathological observation.

2.6. Statistical analysis

Results of biochemical analysis were computed statistically using SPSS version 23.0. Data are expressed as mean± standard deviation. Analysis of variance (ANOVA) was used to check significance among means of all groups. Probability of ≤ 0.05 was considered significant.

3. Results and discussion

3.1. Biochemical study

Some biochemical indices of liver function (TB, DB, AST, ALT and ALP) were evaluated in Wistar rats exposed to experimental doses of RHB as well as rats

in the control group. Results are expressed as Mean ± S.D. and shown in Table 1; while the graphs of mean plots are shown in Fig. 1. Generally, all rats given experimental doses of RHB exhibited decreased TB, DB, AST, ALT and ALP levels as against rats in the control group. However, statistically significant ($P \leq 0.05$) decrease in value was observed for TB, AST, ALT and ALP; while an insignificant ($P \geq 0.05$) decrease was observed for DB. Rats treated with 0.8 mL and 1.0 mL of RHB had the least TB and DB values, respectively. Rats treated with 0.6 mL of RHB had the least AST and ALT values. Rats treated with 0.4 mL of RHB had the least ALP value. It was also observed that rats treated with 0.8 mL and 1.2 mL of RHB showed slightly higher ALP values than rats in the control group.

Table 1. Biochemical changes (as Mean± S.D) in the liver of Wistar rats exposed to varying quantities of Ruzu herbal bitters by gavage.

Biochemical parameters	Control	0.2 mL	0.4 mL	0.6 mL	0.8 mL	1.0 mL	1.2 mL	P-value
TB	3.13±0.75	1.57±0.56	1.77±0.75	1.66±0.69	0.80±0.46	0.92±0.26	1.46±0.95	0.04
DB	0.91±0.25	0.57±0.19	0.63±0.33	0.42±0.39	0.34±0.46	0.15±0.04	0.62±0.21	0.11
AST	98.60±22.41	63.40±12.50	58.20±38.62	36.80±21.64	71.80±21.45	49.80±28.62	65.40±33.37	0.04
ALT	21.60±3.13	18.20±6.57	11.40±6.15	10.80±6.83	14.60±2.19	21.00±3.67	13.40±6.19	0.01
ALP	173.00±26.87	144.04±36.61	106.80±11.16	120.80±51.72	175.20±88.58	162.80±10.01	216.80±58.11	0.02

Note: Concentration is in mg/dL.
Key: TB (Total bilirubin), DB (Direct bilirubin), AST (Aspartate-aminotransferase), ALT (Alanine-aminotransferase), ALP (Alkaline-phosphatase).

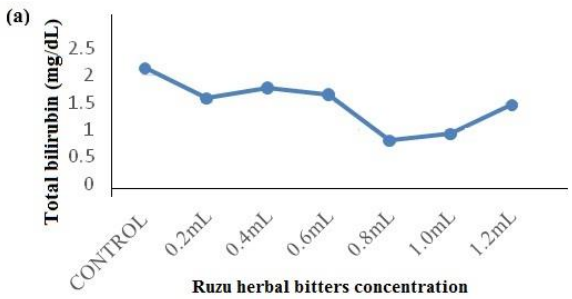
3.1.1. Evaluation of TB and DB

The levels of TB and DB in rats not treated with RHB (control group) as well as rats treated with RHB (experimental dose groups) were evaluated. Numerical data of outcome is shown in Table 1, while graphical plots of numerical data are shown in Fig. 1a-b.

Generally, it was observed that the level of TB and DB in rats treated with experimental doses of RHB was lower than for rats in the control group in a non-dose dependent manner. Rats treated with 0.8mL of RHB had the least TB value of 0.80±0.46 mg/dL as against 3.13±0.75 mg/dL for rats in the control group. Rats treated with 1.0mL of RHB had the least DB value of 0.15±0.04 mg/dL as against 0.91±0.25 mg/dL for rats in the control group. While the difference in value for TB is statistically significant ($P \leq 0.05$); that of DB is not statistically significant ($P \geq 0.05$). Similar reduction in the level of TB and DB were observed by Ogunlana *et al*. (2018); Ogunlana *et al*. (2021) as well as Obasi and Ogugua (2021) in experimental rodents treated with RHB. Higher TB levels were observed by Ogidi *et al*. (2022) in rats treated with experimental doses of RHB than in untreated rats; which may have been due to the concentration of RHB administered to the rats as well as the duration of treatment. Bilirubin levels were noted to be unaltered in rats treated with experimental doses of "Living Bitters" and "Swedish Bitters" as well as rats in the control group (Ojekale *et al.*, 2019). This may be attributed to the composition of individual herbal mixtures. Since the concentration of bilirubin in the serum depends on the rate at which it is excreted following haemoglobin destruction, there seem to be sufficient evidence from our results that RHB probably enhances the process which led to reduction in the levels of TB and DB in RHB treated rats.

3.1.2. Evaluation of AST, ALT and ALP

Amino acid metabolism is accomplished when serum transaminases (AST and ALT) which are normally present in the liver catalyzes the transfer of amine (NH_2) groups from amino acids to keto acids. Destruction of liver cells releases these enzymes with consequent rise in their plasma levels. ALP is also known to be normally present in the liver but, excreted in the bile. In the event that liver function is compromised, serum ALP level is elevated which is a manifestation of retention. In this study, the levels of AST, ALT and ALP in rats not treated with RHB (control group) as well as rats treated with RHB (experimental dose groups) were evaluated. Numerical data of outcome is shown in Table 1, while graphical plots of numerical data are shown in Figs. 1c-e.



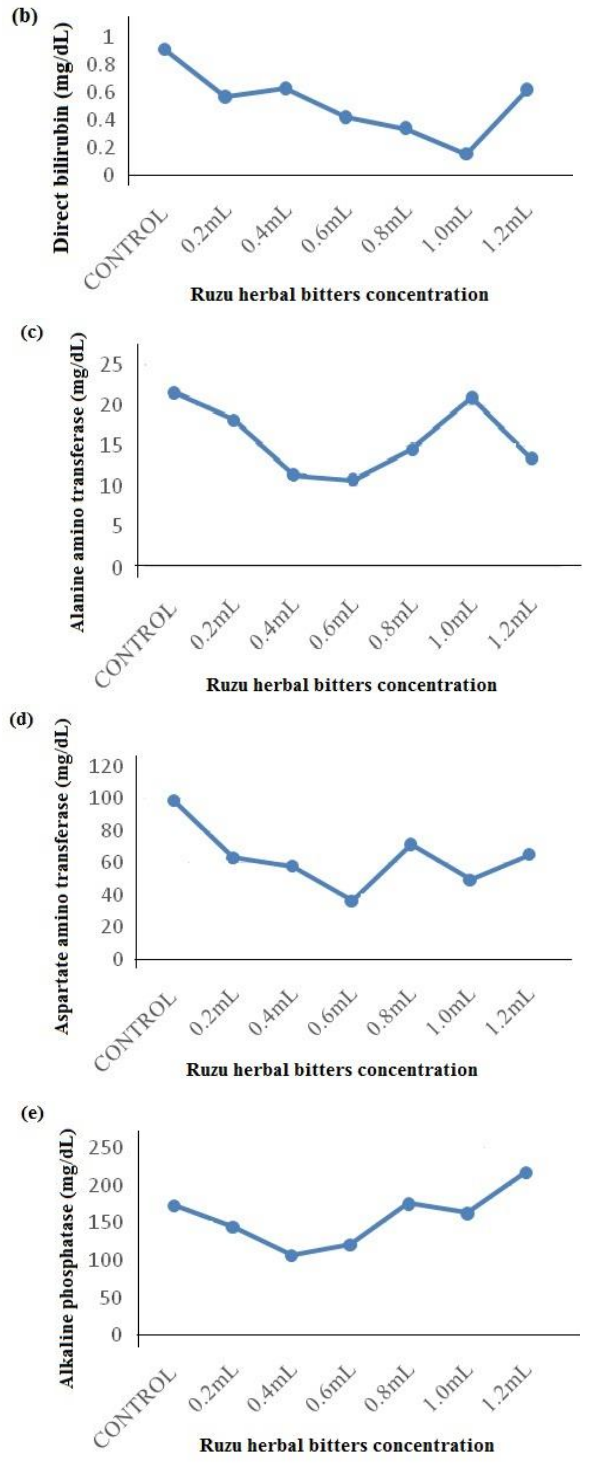


Fig 1. Plots of mean serum levels at control and test doses for TB (a); DB (b); ALT (c); AST (d); ALP (e).

Generally, it was observed that the levels of AST, ALT and ALP in rats treated with experimental doses of RHB were lower than for rats in the control groups in a non-dose dependent manner. Rats treated with 0.6mL of RHB had the least AST value of 36.80±21.64 mg/dL as against 98.60±22.41 mg/dL for rats in the control group. Rats treated with 0.6mL of RHB also had the least ALT value of 10.80±6.83 mg/dL as against 21.60±3.13 mg/dL for rats in the control group. Rats treated with 0.4mL of RHB had the least ALP value of 106.80±11.16 mg/dL as against 173.00±26.87 mg/dL for rats in the control group. Exceptions were observed for rats treated with 0.8mL and 1.2 mL of RHB where ALP values were higher than for rats in the control groups. The differences in value of AST, ALT and ALP between treated and untreated rats were statistically significant ($P \leq 0.05$). Similar reduction in AST, ALT and ALP levels were observed in RHB treated rats by Ogunlana *et al.* (2018); Ogunlana *et al.* (2021) as well as Obasi and Ogugua (2021) when compared to rats in the control groups. This probably means that RHB has a protective effect on the liver. A different picture of increased AST, ALT and ALP levels were observed by Ogidi *et al.*, (2022) in RHB treated rats. This may be due to the concentration of RHB used and the duration of administration. The values of AST and ALT remained unchanged in rats treated with experimental doses of "Living Bitters" and "Swedish Bitters" from the untreated rats (Ojekale *et al.*, 2019). The values of AST, ALT and ALP were also observed not to be significantly altered in rats following administration of "Action Bitters" by Nwachuku and Elekima, (2018). This could be linked to the chemical composition of the different herbal mixtures.

3.2. Histological study

Microscopic observations of the effect of experimental doses of RHB on the liver and testes of rats in the treatment groups as well as the control group are shown in Figs. 2 and 3.

The liver tissues of rats in the control group appears to have normally arranged hepatocytes with no distortions while liver tissues rats treated with experimental doses of RHB exhibited a wide range of non-dose dependent morphological alterations which include haemorrhagic infiltration, presence of inflammatory cells, condensed nuclei, degeneration of hepatocytes, cytoplasmic degeneration and vacuole formation. These changes may have been triggered by the chemical constituents of the herbal mixture resulting in tissue injury. This appears to be consistent

with the observations of Ogunlana *et al*. (2020); Ogunlana *et al*. (2021) and Obasi and Ogugua, (2021) who identified the presence of morphological changes such as mild vascular congestion, moderate diffused steatosis, portal infiltration, inflammatory cells, necrosis and apoptosis in liver tissues of rodents treated with varying doses of RHB.

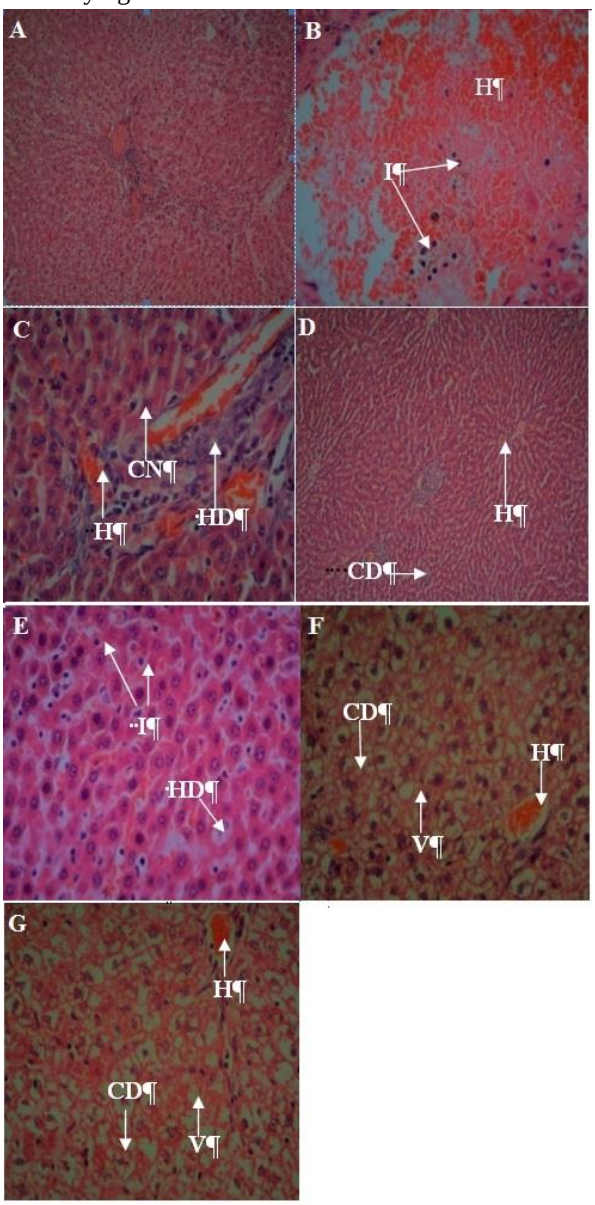


Fig. 2. Selected sections: (A) Liver of rats in the control group showing normally arranged hepatocytes with no distortions (H&E x100); (B) Haemorrhage and inflammatory cells in the liver of rats treated with 0.2mL RHB (H&E x 400); (C) Haemorrhage, condensed nuclei and hepatocyte degeneration in the liver of rats treated with 0.4mL RHB (H&E x 400); (D) Haemorrhage and cytoplasmic degeneration in the liver of rats treated with 0.6mL RHB (H&E x 100); (E) Inflammatory cells and enlarged hepatocyte degeneration in the liver of rats treated with of 0.8mL RHB (H&E x 400); (F) Cytoplasmic degeneration, haemorrhage and vacuolation in the liver of rats treated with 1.0mL RHB (H&E x 400); (G) Cytoplasmic degeneration, haemorrhage and vacuolation in the liver of rats treated with 1.2 mL RHB (H&E x 400). **Key:** H (Haemorrhage); I (Inflammatory cells); CN (Condensed nuclei); HD (Hepatocyte degeneration); CD (Cytoplasmic degeneration); V (Vacuolation).

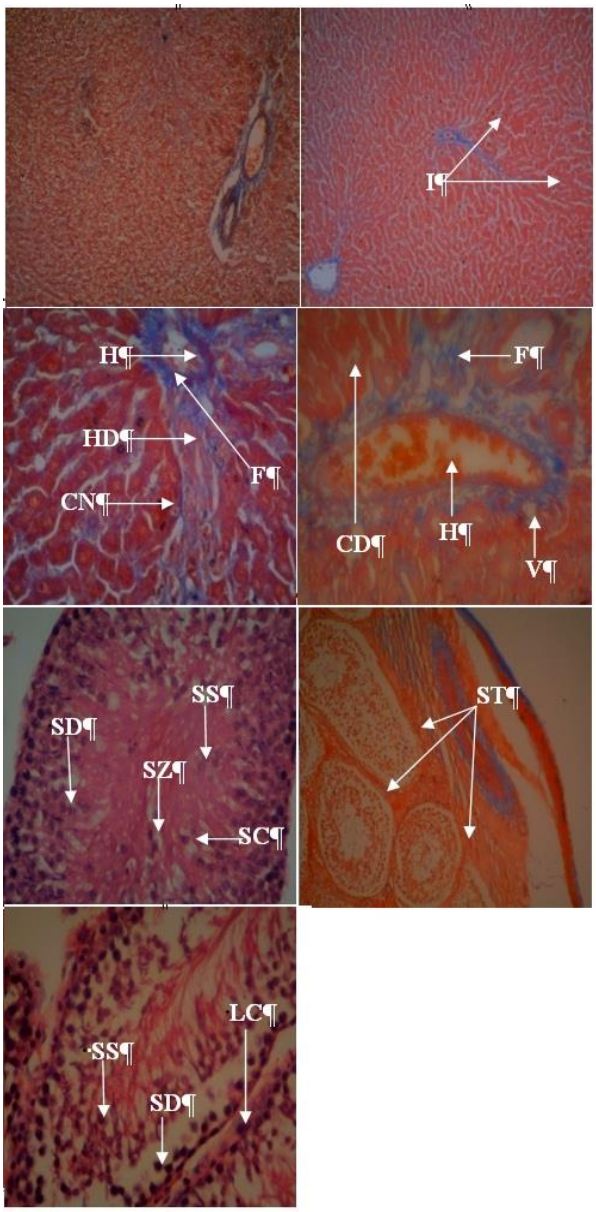


Fig. 3. Selected sections: (A) Liver of rats in the control group with no distortions and collagen fibers stained blue (MT stain x100); (B) Inflammatory cells and collagen fibers stained blue in the liver of rats treated with 0.2mL RHB (MT x 100); (C) Haemorrhage, condensed nuclei and hepatocyte degeneration in the liver of rats treated with 0.4mL RHB (MT x 400); (D) Haemorrhage, vacuolation and cytoplasmic degeneration in the liver of rats treated with 0.6mL RHB (MT x 400); (E) Spermatids, sertoli cells, spermatozoa and spermatocytes in a seminiferous tubule from testis of rats in

the control group (H&E x 400); (F) Seminiferous tubules from testis of rats in the control group with collagen fibers stained blue (MT x 100); (G) A seminiferous tubule from testis of treatment group with normal leydig cells, spermatids and spermatocytes (H&E x 400). **Key:** I (Inflammatory cells); H (Haemorrhage); CN (Condensed nuclei); HD (Hepatocyte degeneration); V (Vacuolation); CD (Cytoplasmic degeneration); F (Fibrosis); LC (Leydig cells); ST (Seminiferous tubules); SD (Spermatids); SC (Sertoli cells); SS (Spermatocytes); SZ (Spermatozoa).

Obasi and Ogugua (2020) also identified eosinophilic tubular casts in the renal tubules of tissues from the kidney of rats treated with RHB. The morphology of testicular tissues from rats in the control group as well as RHB treated groups appears to have normal seminiferous tubules with features such as spermatids, sertoli cells, leydig cells, spermatozoa and spermatocytes. The retention of normal testicular architecture in RHB treated rats may be as a result of metabolism of the chemical constituents of RHB before reaching tissues distant to the liver such as the testes. This is consistent with the observation of Obasi and Ogugua (2020) who reported normal splenic tissue morphology in rats treated with different doses of RHB as well as untreated rats.

4. Conclusion

Liver function tests are most often employed to determine the presence of liver disease, the type of liver disease as well as the extent and progression of liver disease. Tests of excretion by the liver, evaluation of synthesis by the liver and evaluation of enzyme activity in the liver are the common laboratory investigations performed to determine liver function. With the lower levels of TB, DB, AST, ALT and ALP observed in RHB treated rats in this study; we conclude that RHB is an effective formulation that improves the processes of excretion as well as enzyme activity by the liver. However, with the residual morphological distortions observed in sections of RHB treated rats, it is suggested that further studies be done to ascertain the concentration of RHB to be administered and duration of administration that would prevent such developments.

5. References

Kale, O.E., Akinpelu, O.B., Bakare, A.A., Yusuf, F.O., Gomba, R., Araka, O.C., Ogundare, T.O., Okolie, A.C., Adebawo, O. and Odutola, O. 2018. Five traditional Nigerian polyherbal remedies protect against high fructose fed, streptozotocin-induced type 2 diabetes in male Wistar rats. *BMC Complementary and Alternative Medicine*, 18(1): 160.

Nwachuku, E.O. and Elekima, I. 2018. Evaluation of the effect of Action bitters (herbal mixture) on some biochemical indices of Albino rats. *Asian Journal of Research in Biochemistry*, 3(4): 1-8.

Obasi, D.C. and Ogugua, V.N. 2020. Effect of Ruzu herbal bitters on the kidney function and haematological parameters of Alloxan-induced diabetic rats. *International Journal of Scientific and Engineering Research*, 11(5): 63-86.

Obasi, D.C., Ogugua, V.N., Obasi, J.N. and Okagu, I.U. 2020. Phytochemical, nutritional and anti-nutritional analyses of Ruzu herbal bitters. *Journal of Pharmacy and Biological Sciences*, 15(1): 4-17.

Obasi, D.C. and Ogugua, V.N. 2021. Effect of Ruzu herbal bitters on the liver function and lipid profile parameters of Alloxan-induced diabetic rats. *Journal of Clinical and Experimental Hepatology*, In press.

Ogidi, O.I., Ayibaene, F., Shonubi, O.O. and Anani, R.O. 2022. Biochemical study on the effects of Ruzu herbal bitters formulation on Wistar Albino rats. *Biomedical Journal of Scientific and Technical Research*, 41(1): 32434-32439.

Ogunlana, O.O., Ogunlana, O.E., Ugochukwu, S.E. and Adeyemi, A.O. 2018. Assessment of the ameliorative effect of Ruzu herbal bitters on the biochemical and antioxidant abnormalities induced by high fat diet in Wistar rats. *International Journal of Pharmacology*, 14(3): 329-341.

Ogunlana, O.O., Ogunlana, E.O., Adekunbi, T.S., Adetuyi, O.B., Adegboye, B.E. and Iheagwam, F.N. 2020. Anti-inflammatory mechanism of Ruzu bitters on diet-induced non- alcoholic fatty liver disease in male Wistar rats. *Evidence-Based Complementary and Alternative Medicine*, 2020, Article ID 5246725, 8 pages.

Ogunlana, O.O., Adetuyi, O.B., Adekunbi, T.S., Adegboye, B.E., Iheagwam, F.N. and Ogunlana, O.E. 2021. Ameliorative effect of Ruzu herbal bitters on high-fat diet induced non-alcoholic fatty disease in Wistar rats, *Journal of Pharmacy and Pharmacognosy Research*, 9(3): 251- 260.

Ojekale, A.B., Lawal, O., Lasisi, M.O., Bamigbose, C.A. and Dosu, F.A. 2019. Bioactive compounds in herbal bitter drinks and effects on selected hepatic

biomarkers in Albino Wistar rats in Lagos, Nigeria. *Journal of Applied Environmental Management*, 23(12): 2139-2142.

Oyaluna, Z., Abolaji, A.O. and Babalola, C.P. 2021. Effects of Ruzu herbal bitters, atraditional Nigerian polyherbal drug on longevity and selected toxicological indices in *Drosophila melanogaster*. *Biointerface Research in Applied Chemistry*, 11(2): 9638-9645.

Williams, S.C., Linske, M.A. and Stafford III, K.C. 2020. Humane use of cardiac puncture for non-terminal phlebotomy of wild-caught and released *peromyscus* spp. *Animals (Basel)*, 10(5): 829.