Original Article

JHSMR Journal of Health Science and Medical Research

Long-Term Exposure to Bitters (A&Y) Exacerbates Inflammation in Wistar Rats: A Study on Male Renal Function

Tony Ezike, M.Sc., Adams Omoaghe O, Ph.D., Nnamdi Ejieh, M.Sc., Samuel Olaniyi K, Ph.D.

Department of Physiology, Faculty of Basic Medical Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State P.M.B.5454, Nigeria.

Received 6 August 2024 ● Revised 18 December 2024 ● Accepted 12 January 2025 ● Published online 13 May 2025

Abstract:

Objective: This study aims to evaluate the effects of long-term exposure to bitters (A&Y) on inflammation and renal functions in male Wistar rats. Specifically, the research focuses on assessing weight changes, lipid profiles, pro-inflammatory and anti-inflammatory markers, oxidative and anti-oxidative markers, renal function markers, electrolytes, and kidney histology.

Material and Methods: Forty-nine male Wistar rats weighing between 160-180g were used for this study and were evenly divided into 7 groups (n=7): control (normal saline, 1ml), Y bitter low dose [YBL, 0.22 ml/kg], Y bitter average dose [YBA, 0.43 ml/kg], Y bitter high dose [YBH, 0.65 ml/kg], A bitter low dose [ABL, 0.22 ml/kg], A bitter average dose [ABA, 0.43 ml/kg] and A bitter high dose [ABH, 0.65 ml/kg] administered for 8 weeks. Biochemicals were assayed using enzyme-linked immunosorbent assays kits. Hematoxylin and eosin stains were used for kidney histology. Statistically significant values (p-value<0.05) were accepted.

Results: The results of malondialdehyde, glutathione transferase, superoxide dismutase, catalase, creatinine, urea, and gamma glutamyl transferase significantly increased. The inflammatory cytokines like tumor necrotic factor, C-reactive protein, and interleukin-6 significantly increased, whereas the anti-inflammatory cytokine interleukin-1 significantly decreased in the bitters-treated groups. The histology results showed a progressive decrease in the glomerular count of the bitters treated groups.

Conclusion: The findings of this study suggest that the arbitrary use of bitters has a significant negative impact on kidney health, leading to the development of glomerular nephritis and architectural changes in the renal tissue at a dose differential severity.

Keywords: anti-inflammatory marker, bitters, pro-inflammatory cytokines, renal function, renal morphology

Contact: Adams Omoaghe O, Ph.D.
Department of Physiology, Faculty of Basic Medical Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State P.M.B.5454, Nigeria.
E-mail: omoagheolalekan@gmail.com

J Health Sci Med Res
doi: 10.31584/jhsmr.20251204
www.jhsmr.org

Introduction

Bitters are extracts from the herbs and roots of tropical and subtropical plants and spices used for various medicinal purposes, such as improving digestion and reducing inflammation[1]. The majority of the bitters are either aqueous or liquor blends, the latter of which serves as a preservative and solvent for extractions, depending on the brands and types[2]. Among other therapeutic claims is purification of blood, prevention of kidney stone formation, expression of anti-inflammatory, antibacterial, and antifungal potentials[3,4]. In addition, bitters are polyherbal preparations and many of their constituents are unknown. However, some extracts have been revealed, such as *Mangifera indica leaves, Sorghum bicolor stem, Cassia sieberiana root, Ocimum basilicum leaves, Aloe vera, Acinos arvensis, Citrus aurantifolia, and Cinamomum aromticum*[5]. Therefore, with the surge in the number of people having kidney problems, coupled with the increased consumption of different blends of bitters owing to their claims of curing everything, except death, without empirical evidence. To investigate the chronic consumption of these selected bitters, a questionnaire was developed in order to select the 2 bitters with the highest respondents, code names Y and A bitters. Therefore, this study aimed to investigate the harmful effects of bitter drinks on the kidneys of Wistar rats.

Material and Methods

Bitters

Bitters A and Y were purchased from First Oasis Pharmacy shop, Ado-Ekiti, Ekiti State, Nigeria.

Animals grouping and treatment

Forty-nine male Wistar rats weighing between 160-180 g were bought and used for this study. They were kept in the animal house of ABUAD and housed in plastic cages under standard laboratory conditions with access to water and standard diet ad libitum. Forty-nine male Wistar rats were obtained from Afe Babalola University and allowed to acclimatize for one week. The Wistar rats were evenly divided into 7 groups (n=7): control (normal saline, 1 ml), Y bitter low dose [YBL, 0.22 ml/kg], Y bitter average dose [YBA, 0.43 ml/kg], Y bitter high dose [YBH, 0.65 ml/kg], A bitter low dose [ABL, 0.22 ml/kg], A bitter average dose[ABA, 0.43 ml/kg] and A bitter high dose [ABH, 0.65 ml/kg] administered for 8 weeks and approved by the Institutional Research Ethics Committee (ABUAD/HREC/08/03/2024/255A).

Sacrifice and collection of samples

After administration with bitters, the animals were sacrificed by cervical dislocation and dissected. The rats were cardiac punctured to collect blood in a heparinized tube; centrifugation at 3000 rpm for 15 min at room temperature was used to collect blood samples. Frozen plasma was stored until the biochemical assay.

Preparation of tissue homogenates

Kidneys (500 mg each) were carefully separated from the attached adipose tissue and homogenized with phosphate buffer solution (PBS). The mixtures were centrifuged at 3000 rpm for 15 min at room temperature, and the supernatants were collected and frozen for biochemical assay.

Biochemical analysis

The activities of malondialdehyde (MDA), catalase (CAT), glutathiones s-transferase (GST), super oxide dismutase (SOD), urea (BXC0123), creatinine (BXC0111) and Kidney Gamma-glutamyl-transferase (GGT) (BXC0361) were determined with kits from Foortress Diagnostics Limited, United Kingdom. The interleukin 6 (IL-6) (E-EL-R0015), Interleukin 10 (IL-10) (E-EL-R0016), tumor necrosis factor alpha (TNF-α) (BXC0361) and C-reactive

protein (CRP) (E-EL-R0506) were analyzed by enzyme-linked immunosorbent assays (ELISA) using rat ELISA kits (Foortress Diagnostics Limited, United Kingdom). Lipid profiles, namely total cholesterol (TC), triglyceride (TG) (BXC0271), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were assayed. Below is a description.

Determination of lipid peroxidation (LPO)

MDA was used to measure LPO, using the method reported by Farombi et al.[24] with Foortress Diagnostics kits and the clear solution's absorbance at 540 nm. The equation, ∑=1.56×105 L/mol/cm, was used to calculate the amount of MDA, where ∑ represents the extinction coefficient.

Determination of IL-6

An ELISA sandwich format was used with interleukin-6, using the method reported by Deuster and Moy[25]. The reagents were carefully handled in such a way as not to produce foam inside the vials. To test the specified number of samples, we calculated the number of microplate strips and wells needed for controls and standards. A sufficient number of microplate strips were taken out from the pouch. To the relevant wells, 100L of the sample and 1 control solution were introduced. They were then shielded at room temperature (18°C–25°C) for 1 hour before the complete removal of the cover and cleaning of the plates, according to the protocol. After that, each well received chromogen Tetramethylbenzidine substrate (TMB substrate) solution and was left to incubate for 12 to 15 minutes at room temperature in the dark. Finally, each well had 100L of Stop Reagent applied to it. The addition of the Stop Reagent quickly produced the desired effects. Spectrophotometers were used to measure the absorbance of each well using a main wavelength of 450 nm and 620 nm (approximate range: 610 to 650 nm).

Determination of IL-10

An ELISA sandwich format was used with interleukin-10, using the method reported by Baeck and Svensen[26]. These wells were pipetted with a sample. A biotinylated antibody directed against human IL-10 was added after washing away the non-bound IL-10 and other sample elements. Streptavidin and horseradish peroxidase were used to quantitatively measure IL-10 concentration in the samples. In each well of the microplate, a conjugate was added. A TMB-substrate solution was applied to each well following an additional wash phase. A stop solution containing sulfuric acid was then added, and the resultant yellow product was detected at 450 nm. By directly comparing the sample's IL-10 concentration to the assay's standard curve, the sample's IL-10 concentration could be identified.

Blood electrolyte analysis

The blood electrolytes (Na^+, K^+, Cl^-, Ca^{2+}) were also analyzed after using an automated blood electrolyte analyzer, following the manufacturer's instructions.

Hematoxylin and eosin (H&E) staining

H&E staining was performed[6]; 100 g of ammonium alum, 50ml of absolute alcohol, 100ml of distilled water, 2.5 g of mercuric oxide, and 40 ml of glacial acetic acid. Method: Boiling water was used to dissolve ammonium alum, whereas 100% alcohol was used to dissolve the hematoxylin. After combining the 2 ingredients, they were heated to a boiling temperature. The mixtures were taken off the heat source and combined with mercuric oxide, and then allowed to cool. Next, glacial acetic acid was added to facilitate quick nuclear staining. The eosin was dissolved in water and then mixed with 95% alcohol (1 part alcohol to 4 parts eosin solution). A few drops (0.4 ml) of acetic

acid were added to the finished mixture in order to make the stain more intense. Sections were deparaffinized in xylene for 10–20 minutes and rehydrated using 100% and 95% alcohol, rinsed in both distilled and tap water. Hematoxylin-stained sections were then washed in tap water for 3–5 minutes. Eosin was used to stain the slides for 1–4 minutes. Slides were divided and dehydrated into 2 groups: 5–6 dips of 95% alcohol and 6 dips of 100% alcohol. In xylene, slides were cleared twice. Lastly, Permount was used to mount the slides on an OPTO-Edu Industrial Light Microscope (China).

Histological cell counts were obtained from the photomicrographs using Image J software for Windows.

Data analysis and statistics

All data are expressed as the mean and standard error of the mean. Statistical group analysis was carried out using GraphPad Prism software version 8.5. We compared the mean values of the variables among the groups using one way analysis of variance. A post hoc analysis was performed using Tukey's test. Differences were considered statistically significant at p-value<0.05.

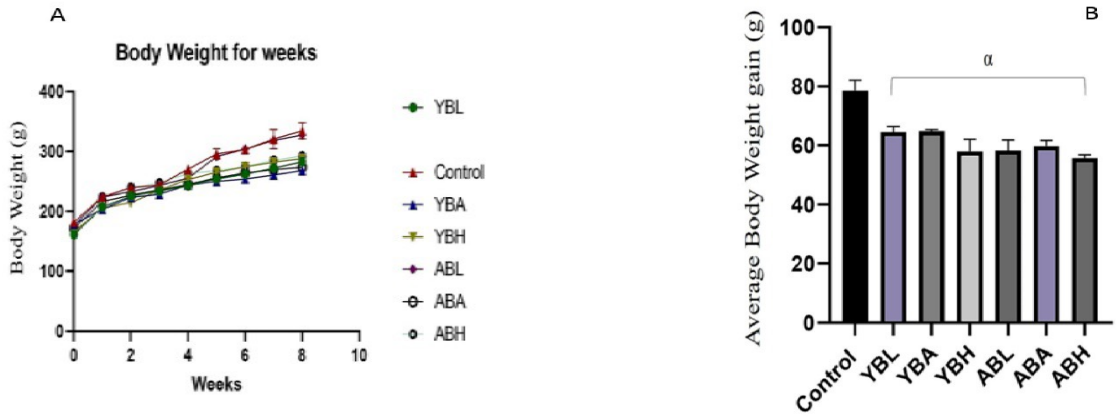
Results

Progressive weight gain was observed on a weekly basis, as depicted in Figure 1A, mostly in the control group. The average body weight gain (Figure 1B) in the control group was significantly increased compared with the other groups (p-value<0.05). The average body weight was reduced in the low-dose Y bitters group compared to the average and high-dose groups. The results of plasma TC (Figure 2A) in the control group showed no significant difference compared to the Y bitters low and average dose and the A bitters low dose groups. However, there was a significant reduction (p-value<0.05) in plasma TG levels (Figure 2B) in the control group compared to the other groups (p-value<0.05). Reduced plasma TG levels in bitters-treated groups follow a staircase pattern. A significant decrease in LDL (Figure 2C) was observed in the Y bitters and A bitters high-dose treatment groups compared to the control group (p-value<0.05). Plasma HDL levels (Figure 2D) were significantly higher in the average and high-dose groups treated with A bitters compared to the other groups (p-value<0.05). The results (Figure 3A and B) showed that MDA was significantly increased in the bitters-treated groups in a dose-dependent pattern compared to the control group (p-value<0.05). Plasma SOD levels (Figure 3C) were significantly increased in the high-dose Y bitters and A bitters groups compared to the control group. Kidney SOD levels (Figure 3D) showed a significant dose-dependent increase in bitters-treated rats compared with the control rats (p-value<0.05). The results of plasma CAT (Figure 3E) increased significantly in high-dose Y bitters, average bitters, and high-dose A bitters compared to the control, whereas the results of kidney CAT (Figure 3F) increased significantly in the bitters-treated groups compared to the control group (p-value<0.05). The GST results followed a similar pattern.

The kidney pro-inflammatory markers (TNF-a, IL-6, and CRP) (Figure 4 A, B, and C) significantly increased in the bitters-treated groups compared to the control group in a dose-dependent manner (p-value<0.0.5), whereas the kidney anti-inflammatory markers (IL-10) significantly decreased in the bitters-treated groups compared to the control group in a dose-dependent manner (Figure 4D) (p-value<0.05).

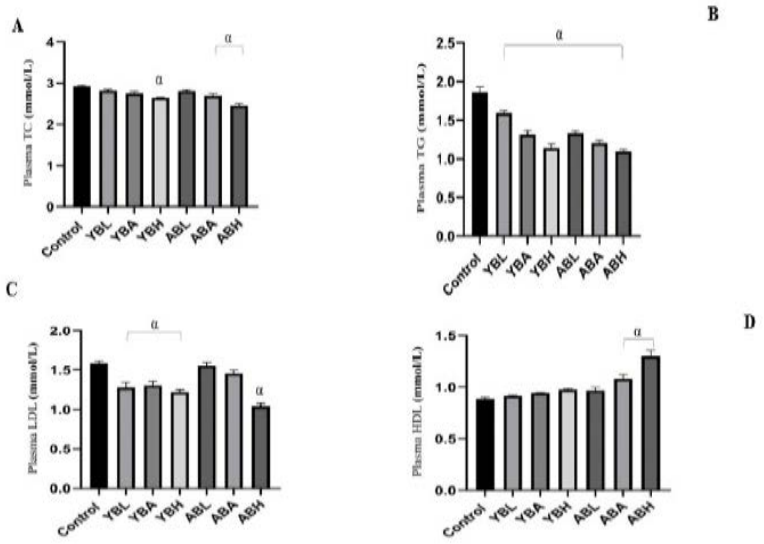
Kidney creatinine levels (Figure 5A) significantly increased in the bitters-treated groups compared to the control group. Kidney urea levels (Figure 5B) increased significantly in the bitters-treated groups in a dose-dependent pattern compared with the control group, whereas kidney GGT levels (Figure 5C) significantly increased in the bitters-treated groups compared with the control group (p-value<0.0.5). Plasma electrolytes (Figure 6A–D) showed significant decreases in chloride, calcium,

potassium, and sodium ions in the bitters-treated groups compared with the control group (p-value<0.05). The results of kidney histology (Figure 7: G1-G7) showed the control group's (G1) histologic architecture of glomeruli with a normal glomerular count compared to the significant progressive decrease in the bitters-treated groups.



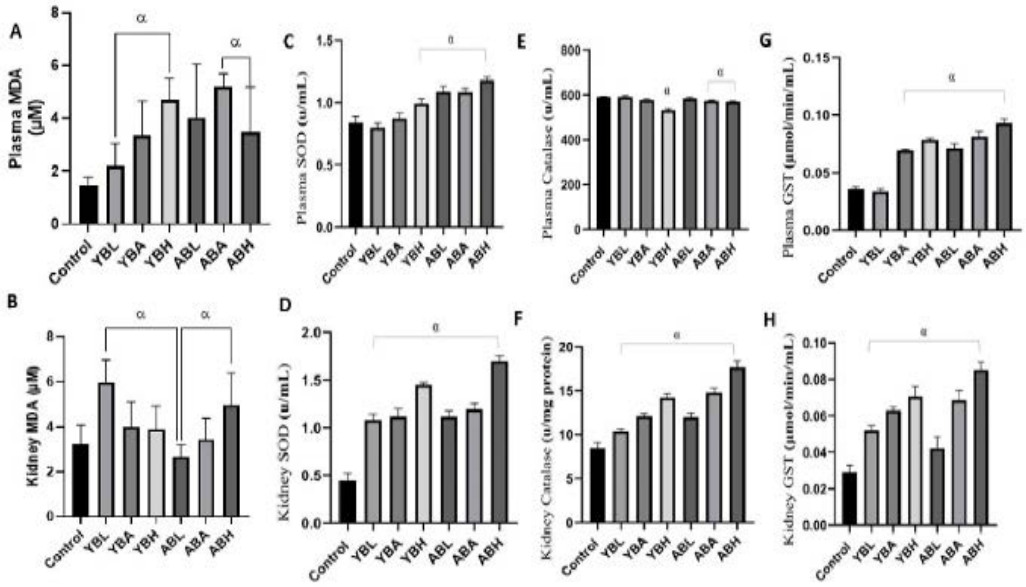
YBL=Y Bitters Low-dose, YBA=Y Bitters Average-dose, YBH=Y Bitters High-dose, ABL=A Bitters Low-dose, ABA=A Bitters Average-dose, ABH=A Bitters Low-dose

Figure 1 Average Body weight gain (g) of each group of animals over 8 weeks at (p-value<0.05)



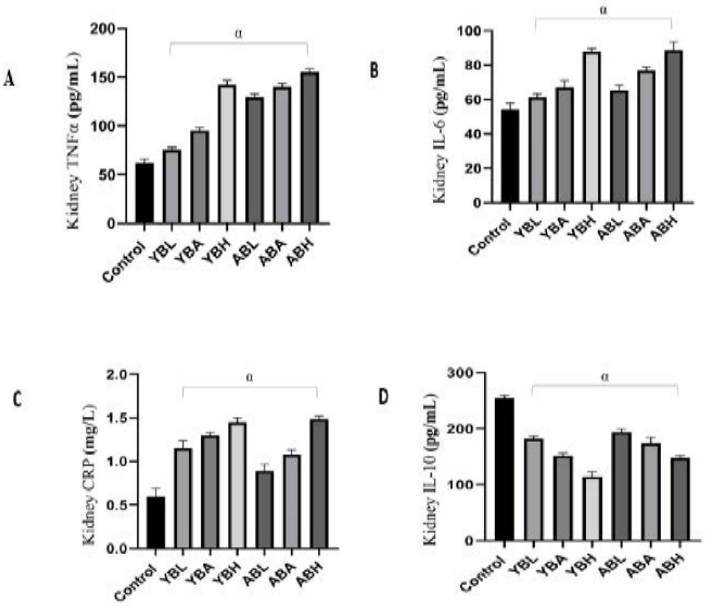
YBL=Y Bitters Low-dose, YBA=Y Bitters Average-dose, YBH=Y Bitters High-dose, ABL=A Bitters Low-dose, ABA=A Bitters Average-dose, ABH=A Bitters Low-dose

Figure 2 Effects of Bitters Y and A on bitters on lipid profile (p-value<0.05)



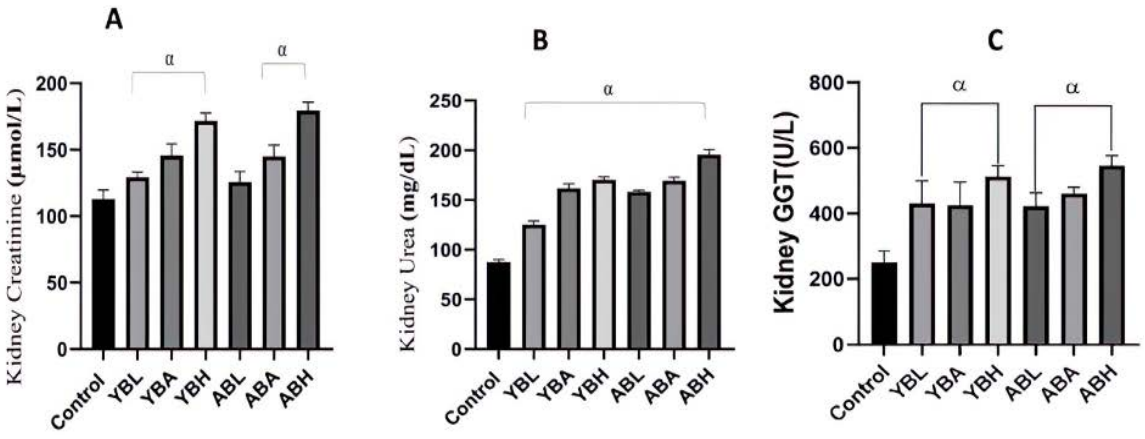
YBL=Y Bitters Low-dose, YBA=Y Bitters Average-dose, YBH=Y Bitters High-dose, ABL=A Bitters Low-dose, ABA=A Bitters Average-dose, ABH=A Bitters Low-dose

Figure 3 Effects of Bitters Y and A on bitters on lipid profile (p-value<0.05)



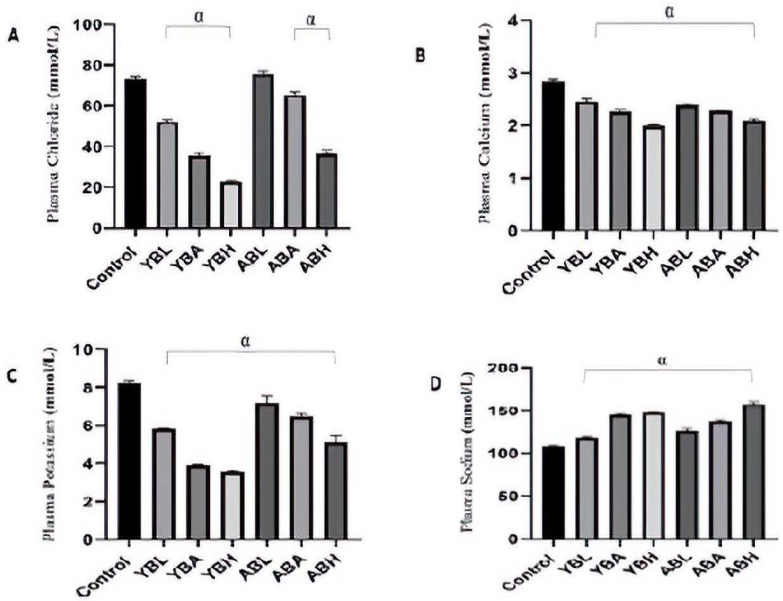
YBL=Y Bitters Low-dose, YBA=Y Bitters Average-dose, YBH=Y Bitters High-dose, ABL=A Bitters Low-dose, ABA=A Bitters Average-dose, ABH=A Bitters Low-dose

Figure 4 Effect of Bitters Y and A on pro and anti-inflammatory markers at (p-value<0.05)



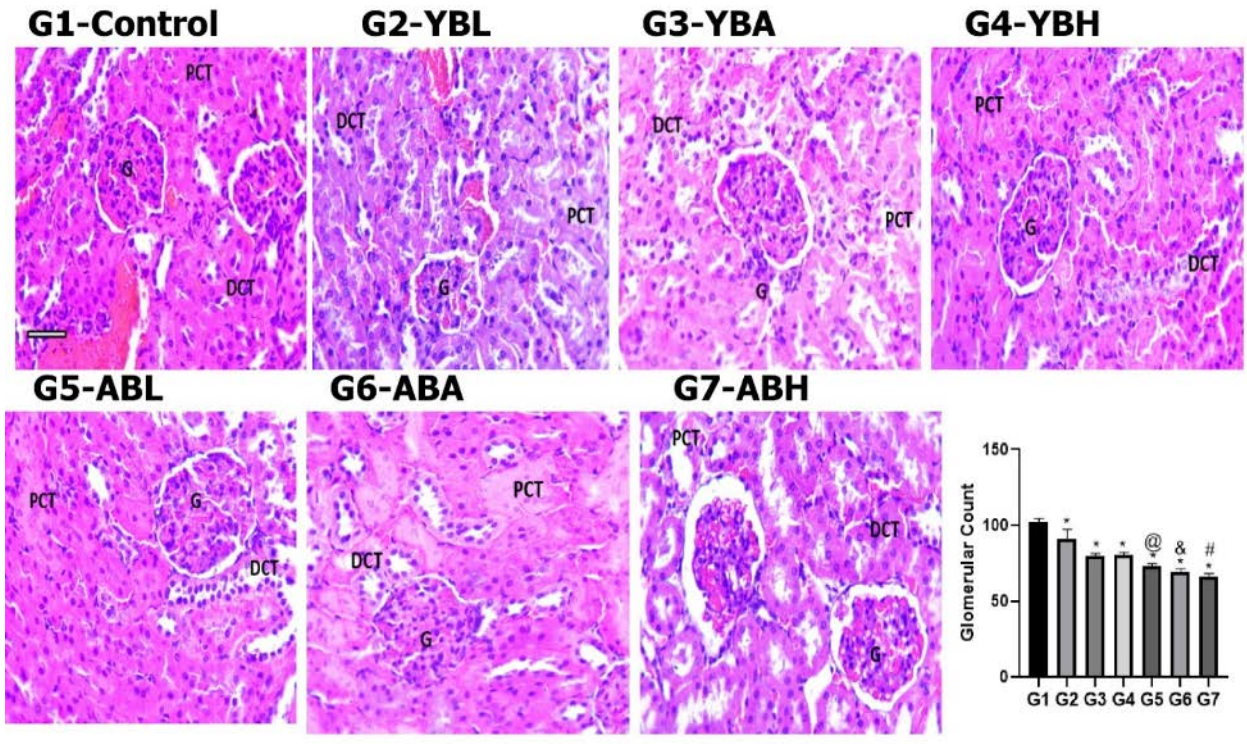
YBL=Y Bitters Low-dose, YBA=Y Bitters Average-dose, YBH=Y Bitters High-dose, ABL=A Bitters Low-dose, ABA=A Bitters Average-dose, ABH=A Bitters Low-dose

Figure 5 Effect of Y and A bitters on the renal markers at (p-value<0.05)



YBL=Y Bitters Low-dose, YBA=Y Bitters Average-dose, YBH=Y Bitters High-dose, ABL=A Bitters Low-dose, ABA=A Bitters Average-dose, ABH=A Bitters Low-dose

Figure 6 Effect of Y and A bitters on the blood electrolyte levels at (p-value<0.05)



YBL=Y Bitters Low-dose, YBA=Y Bitters Average-dose, YBH=Y Bitters High-dose, ABL=A Bitters Low-dose, ABA=A Bitters Average-dose, ABH=A Bitters Low-dose

Figure 7 Histology (H&E) of the kidney (Mag. X800, Scale bar: 42 UM). Showing the glomerular cells count and peritubular cells.

Discussion

Our findings suggest that the chronic consumption of the bitter tonic (Y and A) is associated with increased oxidative stress, inflammation, and renal damage, likely due to ROS. All the groups progressively gained weight throughout the period of the study, although the control group had a significant average weight gain compared to the bitter administered groups and this finding was supported by[7], while another reported differently[3] on the weight change, probably as a result of a longer duration of treatment. The lipid profile results revealed that TG, cholesterol, LDL, and HDL showed a dose-dependent reduction and increase in the bitters-treated groups compared to the control group, as established in previous studies[8,9]. Conversely, these findings were not evident in certain studies[3].

LPO was a prominent indicator of oxidative stress in the bitter-treated groups, as evidenced by increased MDA levels both in the plasma and kidney tissues[10,11] with very significant alterations in the conformation, normal architecture, and integrity of the membrane as a result of oxidation and any major shift. Inflammation is a way the body protects itself against a variety of insults, mainly from ROS, a subset of free radicals that disrupts cellular functions through the alteration of tissues due to

increasing inflammation[12]. Reactive oxygen species strongly stimulate the expression of inflammation and increase the production of inflammatory markers like TNF-α, interleukin (IL)-6, and systemic inflammatory markers like CRP[12,13]. Dose-dependent amplification of TNF-α and IL-6 has been established[14,15,16]. IL-10 maintains tissue homeostasis by inhibiting disproportionate inflammatory responses, suppressing immune reactions, delaying tissue fibrosis, and promoting tissue repair. A decrease in IL-10 may be due to the long-term use of bitters.

Superoxide dismutase, CAT, and glutathione are the first-line defense antioxidants protecting the body against hazardous radicals, such as superoxide anion, peroxisomal, and mitochondrial hydrogen peroxide, respectively. A significant increase in the levels of these antioxidants, particularly SOD, could be linked to the high amount of free radicals generated from the chronic use of bitters, which leads to cellular and tissue damage[3]. High SOD and MDA levels indicate the presence of generated free radicals. Glutathione eliminates and protects against reactive nitrogen and oxygen species that help antioxidant enzymes as co-substrates; hence, its substantial expression in kidney tissues supports detoxification and the lowering of oxidative stress from the chronic use of bitters, as customary to[15] and contrary to the report of[17]. Urea and creatinine are regular markers of renal function test. Urea is a significant metabolic waste from amino acids, while creatinine is a nitrogenous waste produced during the metabolism of skeletal muscles. Renal function markers increased significantly in a dose-dependent pattern in the bitter administered groups as established[18,19] and deviated from other reports[20], possibly due to differences in the dosage and duration of treatments. GGT, a secretory enzyme localized in the liver, pancreas, and kidneys, is a cytochrome P450 secretase. It has been demonstrated that GGT may be a potential therapeutic agent. Our results revealed a significant increase in the GGT in the kidney in the bitters-treated groups, indicative of tubular damage, which agreed with the previous report[21]. Our plasma electrolyte analyses showed that more calcium, chloride, and potassium ions were excreted in the bitters administered groups compared with those in the control group, accompanied by the retention of plasma sodium[10,22] and also disagreed with others[23]. These electrolyte imbalances may be associated with impaired kidney function. The photomicrographs showed a progressive decrease in dose-dependent patterns in the glomerular counts of the bitters administered group's histoarchitecture of the glomeruli against the earlier position reported[15]. However, the long-term effects of bitters on the kidneys can be attributed to this progressive loss of the glomerulus.

Finally, while our study provides valuable insights into the effects of bitters on kidney function, there are several limitations that should be considered when interpreting our results as the study was conducted in male Wistar rats and may not be generalizable to other animal models or humans.

Conclusion

The findings of this study suggest that the arbitrary use of bitters has a significant negative impact on kidney health, leading to the development of glomerular nephritis and architectural changes in the renal tissue at a dose-dependent severity.

Authors' contributions

For the conceptualization of the project, ET, OAO, and OSK played significant roles. Data curation was managed by OAO, ET, and EN, while formal analysis was carried out by OAO, ET, and EN as well. The funding acquisition

responsibilities were handled by OAO, ET, and EN. In terms of investigation, ET, OAO, and EN were actively involved. Methodology development was a collaborative effort by OAO, ET, and OSK. Project administration duties were managed by OAO, ET, and EN, who also took care of resource allocation and software development. Supervision was overseen by OAO, ET, and OSK, ensuring that all aspects of the project adhered to the required standards. Validation tasks were performed by ET, OAO, and OSK. Visualization contributions were made by ET, OAO, EN, and OSK, enhancing the project's clarity and presentation. The writing of the original draft was done by OAO and ET, while the reviewing and editing process was a collective effort by ET, OAO, EN, and OSK.

Availability of data and material

The authors declare that data supporting the findings of this study will be made available on request.

Acknowledgement

We acknowledge the support of our families and colleagues throughout the period of this research.

Conflict of interest

The authors declare no conflict of interest.

References

1. Ogwu MC, Ikhajiagbe B. Vernonia amygdalina Delile (Asteraceae): An overview of the phytochemical constituents, nutritional characteristics, and ethnomedicinal values for sustainability. In: Herbal Medicine Phytochemistry: Applications and Trends. Cham (CH): Springer International Publishing; 2024. p. 1–29.
2. Ogechi N, Nwachuku E, Ibioku E. Evaluation of the effect of action bitters (herbal mixture) on some biochemical indices of albino rats. Asian J Res Biochem 2019;3:1–8.
3. Anionye JC, Onyeneke EC, Eze GI, Edosa RO, Agu KC, Omorowa EF, et al. Evaluation of the effects of super bitters on albino rats. J Sci Res 2017;2:1–24.
4. Kamal A, Zafar S, Abbas M. "Ethnomedicinal use, phytochemistry, and pharmacological potential of action bitters: a review." Arabian J Chem 2022:15;33–47.
5. Anyasor GN, Ogunbiyi B, Akinlusi IA. Antioxidant and anti-inflammatory properties of selected polyherbal preparations: Oroki Herbal, Swedish Bitters, and Yoyo Bitters. Oxid Antioxid Med Sci 2017;6:25–9.
6. Oyekunle AO, Ige AO, Olaoye MO, Adewoye EO. Unripe Musa paradisiaca fruit diet ameliorates impaired glucose regulation caused by iron-induced oxidative stress. African J of Biomed Res 2017;20:301–8.
7. Alabi MA, Fagbohunka BS, Sunday RM, Ojo FA. Safety potential of Yoyo Bitter on lipid indices, hematology, and renal function in experimental animals. J Int Res Med Pharm Sci 2015;4:89–93.
8. John OK, Adejumo SA, Uduak EE, Isibor A. Effects of action bitters on serum lipid profile of wistar albino rats. Niger J Physiol Sci 2017;29:155–61.
9. Omobolanle AF, Olaoye IM, Oluwatosin A, Kalejaiye NO. Anti-obesity activity of Rida Herbal Bitters in high fat diet-induced obese rats. J Med Res 2022;8:36–41.
10. Adeyemi OS, Fambegbe M, Daniyan OR, Nwajei I. Yoyo Bitters, a polyherbal formulation, influenced some biochemical parameters in Wistar rats. J Basic Clin Physiol Pharmacol 2012;23:135–8.
11. Ebuehi OAT, Ayobami LO, Ajagun-Ogunleye OM. Biochemical impact of Carica papaya (Pawpaw) leaves extract and Ruzu Bitters on hematology and brain histology in Sprague Dawley rats. Recent Prog Nutr 2023;3:1–26.
12. Grigore A, Vulturescu V, Neagu G, Ungureanu P, Panteli M, Rasit I. Antioxidant anti-inflammatory evaluation of a polyherbal formula. Pharmaceuticals 2022;15:114.
13. Ratheesh M, Jose SP, Sheethal S, Sindhu A, Sandya S, Asish A. Ameliorating effect of polyherbal formulation (Febrojith) on acute and chronic inflammatory model via inhibiting inflammation and oxidative stress. J Ethnopharmacol 2023;116824.
14. Oyewo EB, Adetutu A, Adebisi JA. Immunomodulatory activities of Yoyo Bitters: recommended dose precipitated inflammatory response in male Wistar rats. Pak J Biol Sci 2013;16:1904–12.
15. Omotosho OI, Olayinka T, Oyewole O. Biochemical studies on toxicity of Yoyo Bitters (a polyherbal preparation) on liver and

kidney functions of Wistar rats. Afr J Tradit Complement Altern Med 2018;15:318–24.

16. Ogoun TR, Ogidi OI, Aye T. Toxicity studies of Yoyo Cleanser Bitters poly–herbal formulation in albino rats. World J Pharm Res 2022;11:1–11.
17. Waribo HE, Edamisan IE, Ebirien–AS. Effect of oral consumption of Action Bitters on renal indices of apparently healthy subjects in Port Harcourt metropolis. Asian J Biochem Genet Mol Biol 2021;9:14–9.
18. Adeyemi MM, Afolabi OM, Balogun N. Hepatorenal function of Wistar rats treated with Alamo and Jekomo, an alcoholic herbal bitter in Nigeria. J Phytomed Ther 2022;21:992–1002.
19. Aderonke SO, Collins O. Toxicological evaluation of Hb Cleanser® Bitters on biochemical parameters in Wistar rats. GSC Biol Pharm Sci 2022;20:16–21.
20. Oforibika GA, Uzor CA. Study of the renal function of Wistar albino rats treated with three different herbal bitters. Direct Res J Public Health Environ Technol 2020;5:1–5.
21. Famurewa AC, Showunmi F, Folawiyo AM, Epete M, Okike PI, Onuoha MC, et al. Biochemical alterations in the liver and kidney of rats following sub–acute administration of aqueous extract of stem–bark of Anacardium occidentale (cashew tree). Asian J Res Biochem 2018;3:1–8.
22. Patrick–Iwuanyanwu KC, Nkpaa KW. Toxicity effect of sub–chronic oral administration of Class Bitters®—a polyherbal formula on serum electrolytes and hematological indices in male Wistar albino rats. J Xenobiotics 2015;5:5369.
23. Ogechi N, Ibioku E. Evaluation of the effect of Action Bitters (herbal mixture) on some biochemical indices of albino rats. Asian J Res Biochem 2019;3:1–8.
24. Farombi E, Tahnteng J, Agboola A, Nwankwo J, Emerole G. Chemoprevention of 2–acetylaminofluorene–induced hepatotoxicity and lipid peroxidation in rats by kolaviron—A Garcinia kola seed extract. Food Chem Toxicol 2000;38:535–41.
25. Deuster PA, Moy EM. Interleukin 6: review of its role in exercise and inflammation. Mil Med Res 2014;1:66–75.
26. Baeck SS, Svendsen SJ. IL–10: a mediator of tolerance or inflammation. Int J Immunopathol Pharmacol 2019;32:141–50.