JOPAT Vol 21(2), 992 - 1002, December. 2022 Edition
ISSN2636 – 5448 *https*://dx.doi.org/10.4314/jopat.v21i2.18

HEPATORENAL FUNCTION OF WISTAR RATS TREATED WITH ALOMO AND JEKOMO, AN ALCOHOLIC HERBAL BITTERS IN NIGERIA

*Maria Modupe Adeyemi[1], Olayinka M. Afolabi[1] and Nifemi Balogun[1]
Department of Chemistry and Biochemistry, Caleb University Lagos Nigeria

ABSTRACT

Increased demand for herbal remedies and natural quest for alcohol consumption has positioned alcoholic herbal bitters an acclaimed blood detoxifying and liver cleansing potential as an ideal drink without scientific validation. This study assessed the hepatorenal effect of Alomo and Jekomo, commonly consumed alcoholic bitters in male Wistar rat. Thirty male Wistar rats weighing 110 to 130 g were divided into six groups of five animals each and treated with distilled water, ethanol, Alomo and Jekomo Alcoholic Bitters at 2.68 mL/kg body weight respectively for 28 days. The serum and kidney homogenate were used to determine biochemical parameters such as total protein, albumin, creatinine, urea and bilirubin following standard methods. There was a significant increase ($p < 0.05$) in serum and kidney levels of total protein and albumin in alcohol treated groups. A significant increase ($p < 0.05$) in serum creatinine levels of ethanol and kidney homogenate of Jekomo bitters treated group when compared with control. A significant increase ($p < 0.05$) in serum and kidney urea and bilirubin concentration was also observed in all alcohol treated groups when compared with control. A combined elevation in urea and creatinine and bilirubin suggest a moderate to severe form of kidney and liver damage induced by the alcoholic herbal bitters. As essential markers of kidney function, the recorded elevation of urea and creatinine shows a potential exposure to renal dysfunction. Prolonged and increased consumption of these alcoholic herbal bitters should be discouraged to prevent hepatocellular injury or damage.

Keywords: Alcoholic herbal bitters, Alomo bitters, Jekomo bitters, Kidney, Liver. ***Corresponding author contact:** maria.adeyemi@calebuniversity.edu.ng

INTRODUCTION

Herbal bitters are made up of numerous groups of chemical compounds extracted from the herbs and roots of medicinal plants with common characteristics of a bitter taste. They act to increase the vital energy centers in the body [1]. The use of herbs (medicinal plants) in treatment of ailment has gained much

publicity and recognition which has led to the repackaging of herbal bitters, blend of herbs, spices, roots and seeds as tonic and medicinal products. Herbal bitters have been reported to possess anti-inflammatory [2], anti-tumour [3], antibiotic [4], anti-fungal [5] and hypolipidemic properties [6]. Herbal bitters act on the pancreas thereby helping to normalize blood sugar levels, promote the production and release of pancreatic enzymes [7]. Alcoholic herbal bitters have been claimed by sellers and consumers to help heal piles and or hemorrhoids, improve sexual function, enhance blood circulation, purification of blood by the kidneys, cleanse the colon of impurities, blood pressure regulation through arterial dilatation and prevent formation of kidney stones. Alcohol is a psychoactive drug that provides energy (7.1 kcal/g), however, excessive intake can increase the risk of weight-gain and the development of obesity or malnutrition [8]. The National Agency for Food and Drug Administration Control (NAFDAC) stated in Spirit Drink Regulations 2019 [9] that alcoholic herbal drinks must not contain more than 15% of absolute alcohol by volume and also warned against the deleterious and potential harmful effects of some herbal bitters in Nigeria. Certain biological effects associated with alcohol consumption includes, changes in lipid profile and other hepatocellular markers which may result in liver dysfunction and hepatocellular damage [10, 11]. Interestingly, the alcohol in alcoholic beverages is ethanol (grain ethanol) which is considered as a toxin in cases of excessive consumption and can result in liver cirrhosis [12]. The metabolism of alcohol in the liver by alcohol dehydrogenase and microsomal ethanol-oxidizing system (MEOS) generates the production of toxic metabolites which interferes with the metabolism of the body's essential nutrients, and the accumulation of these toxins processed in the liver may result in alcohol liver disease or hepatic damage [11, 13].

Many of these drinks are illicitly distilled in obscure locations and some manufacturers claim the bitter ingredient is a secret recipe [14]. Due to consumers' belief about its therapeutic effects, there is tendency towards excessive consumption and resultant increase in health risk. Several products from different manufacturers of alcoholic beverages, fortified with diverse kinds of herbs and plant constituents such as Alomo bitters and Jekomo has gained wide acceptance in Nigeria with no insight as to the effect of these alcoholic herbal bitters on the kidney and liver. Therefore this study investigated the hepatorenal effects of sub-acute consumption of Alomo and Jekomo herbal bitters in a rat model.

MATERIALS AND METHODS

Chemicals and Reagents

Total protein, Albumin, Creatinine, Urea and Bilirubin kits were purchased from Fortress Diagnostics, United Kingdom. Alcoholic bitter drinks; Alomo and Jekomo herbal bitters were purchased from a commercial store at Imota in

Ikorodu local government area of Lagos state Nigeria. All chemicals used were of pure analytical grade.

Experimental Animals and Grouping

Thirty healthy, male Wistar rats weighing 110 - 130 g were divided into six groups of five animals each. The animals were purchased from Babcock University animal facility Ilishan-Remo, Ogun state, Nigeria. The animals were allowed to acclimatize for two weeks at Caleb University, Imota Lagos Nigeria animal house. They were kept in aerated cages in a well-ventilated room, under controlled environmental conditions (photo-period: 12hrs light and 12hrs dark cycle; temperature: 25–27°C; humidity: 45–55%). Tap water and feed; standard rat pellet were provided ad libitum throughout the experimental period. After 14days of acclimatization period, rats were divided into six groups, the experimental control received distilled water, while other groups received oral intubation of Alomo bitters, Jekomo bitters and Ethanol at a dose of 2.68 mL/kg for 28days. The dosing was determined according to Odey et al., [11]. Ethical approval protocols as given by Caleb University research ethics committee were strictly adhered to.

Sample collection

Body weight of rats were measured weekly and at the end of the 28 days treatment, the rats were anaesthetized on chloroform vapor and blood collected by cardiac puncture at the left ventricle using a 5mL syringe into plane bottles and centrifuged at 3000rpm for 15min. The serum collected with a pasture pipette was stored below 0°C. The Kidney was excised using forceps and scissors and homogenized in ice-cold 0.25M sucrose solution. The homogenates were diluted using a dilution factor 1:9 w/v ratio. The diluted homogenates was further centrifuged at 5000 rpm for 10 minutes and the supernatants collected with a pasture pipette and stored at below 0°c for biochemical assays.

Serum and Kidney Homogenate Biochemical Assays

Serum and Kidney homogenate levels of total protein, albumin, creatinine, urea, and bilirubin were measured using kits purchased from Fortress Diagnostics, United Kingdom following manufacturer's protocol.

Statistical Analysis

Data obtained was presented as Mean ± Standard deviation and tested by One-way ANOVA followed by post-hoc analysis (Duncan's multiple range test) between alcoholic herbal bitters and control group, values were considered significantly different at $p < 0.05$ using GraphPad Prism 9.0 (GraphPad software, USA)

RESULTS

A decrease in body weights of rats was observed in all alcohol treated groups after 28 days of treatment exposure (Table 1). A significant decrease ($p < 0.05$) was observed in rats treated with Jekomo (141.2 ± 4.2 g) and ($p < 0.01$) in rats treated with Ethanol (120.5 ± 5.3 g) when compared with control (150.2 ± 7.8 g).

Table 1: Changes in weight of Wistar rats after treatment with Alcoholic herbal drinks for 28days (n = 5 animals per treatment group).

Treatment group	Weight (g)
Control	150.2 ± 7.8
Ethanol	120.5 ± 5.3*
Alomo bitters	145 ± 3.0
Jekomo bitters	141.2 ± 4.2*

Values are represented as Mean ± Standard deviation.

*indicates significant difference from control at $p < 0.05$

Changes in biochemical characteristics such as total protein, albumin, creatinine, Urea and Bilirubin in serum and kidney homogenates of rats treated with alcoholic herbal bitters for 28days shows that alcohol significantly modulated the levels of hepatorenal indices in male Wistar rats (Figures 1-10). There was a significant increase ($p < 0.05$) in serum and kidney homogenate levels of total protein and albumin in alcohol treated groups (Figures 1 – 4). A significant increase ($p < 0.05$) in serum creatinine levels was observed in ethanol treated group and a significant decrease in Jekomo when compared with control (Figure 5). There was no significant difference ($p > 0.05$) in creatinine levels of Action bitters treated groups. However, a significant increase ($p < 0.05$) in kidney homogenate creatinine levels was observed in Jekomo bitters when compared with control. (Figure 6). A significant increase ($p < 0.05$) in serum and kidney homogenate Urea concentration was observed in all alcohol treated groups when compared with control (Figures 7 and 8). There was a significant increase ($p < 0.05$) in Bilirubin levels of rats treated with Alcohol bitters (figure 9 - 10).

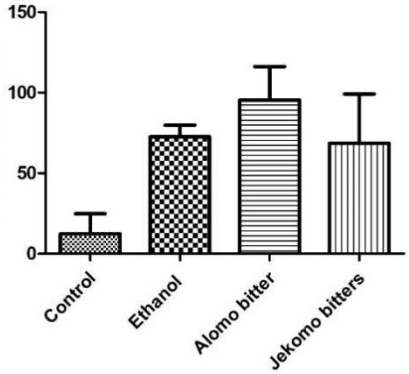

Figure 1: Serum total protein (g/L) levels of rats administered with Alomo and Jekomo bitters

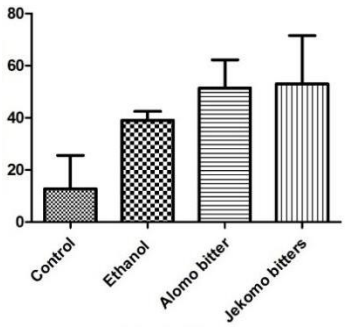

Figure 2: kidney homogenate levels of total protein (g/L) levels of rats administered with Alomo and Jekomo bitters

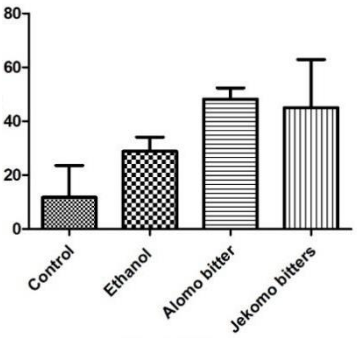

Figure 3: Serum albumin (g/L) levels of rats administered with Alomo and Jekomo bitters

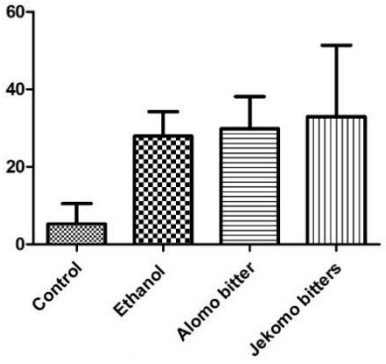

Figure 4: kidney homogenate albumin (g/L) levels of rats administered with Alomo and Jekomo bitters

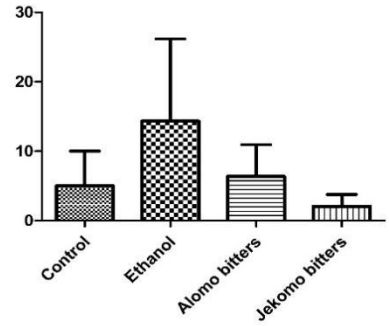

Figure 5: Serum creatinine (mg/dL) levels of rats administered with Alomo and Jekomo bitters

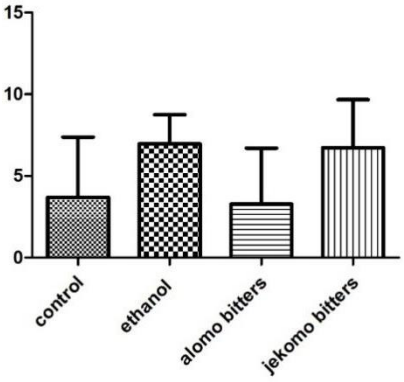

Figure 6: kidney homogenate creatinine (mg/dL) levels of rats administered with Alomo and Jekomo bitters

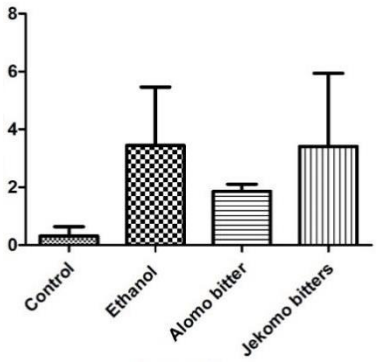

Figure 7: Serum Urea (mg/dL) levels of rats administered with Alomo and Jekomo bitters

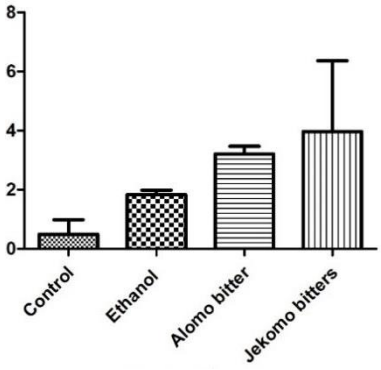

Figure 8: kidney homogenate Urea (mg/dL) levels of rats administered with Alomo and Jekomo bitters

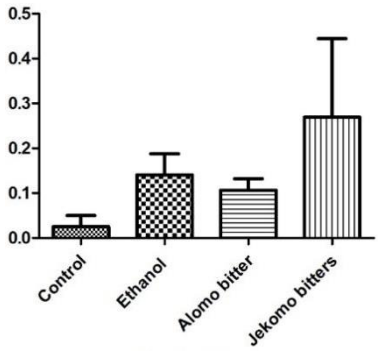

Figure 9: Serum bilirubin (mg/dL) levels of rats administered with Alomo and Jekomo bitters

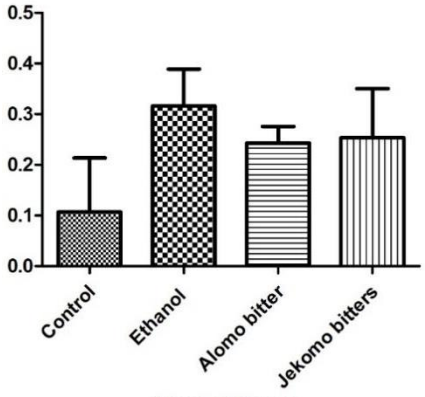

Figure 10: kidney homogenate bilirubin (mg/dL) levels of rats administered with Alomo and Jekomo bitters

DISCUSSION

The smooth endoplasmic reticulum of liver is a metabolic clearing house for endogenous (e.g. proteins) and exogenous (e.g. drugs and alcohol) substances. The liver as a clearance and transformation center for chemicals exposes it to toxic injury [15]. The liver, kidney and blood are seen to be the sites of accumulation of chemicals; hence urea, total protein, albumin, bilirubin and creatinine are sensitive and reliable biochemical indices for evaluation of renal function [4]. Determining markers of liver and kidney biosynthetic capacity such as albumin and total protein concentration evaluated showed a significant increase in alcoholic herbal treated group for evaluation of total protein and albumin which highlights the hepatoprotective effect of the herbal constituents. This protective effect observed from the alcoholic herbal groups may be as a results of the bioactive compounds present in the herbs. Albumin is a blood protein synthesized in hepatocytes and transports various substances, including bilirubin, fatty acids, metals, ions, hormones, drugs and xenobiotics. Since the half-life of albumin is approximately 21 days, the increased albumin concentration of the exposed rats is suggestive of decreased degradation rate which is approximately 4% per day [16]. Serum total protein concentration indicates the functional capacity of the liver to synthesize albumins which its composition can either prevent or initiate hepatocellular injury [11, 14, 17].

The significant increase in creatinine level observed in Jekomo herbal bitters is similar to the report of Oyewo *et al*., [18], Anyasor and Ogunbiyi, [2] and Elechi-Amadi *et al.* [19], but different from the findings of Anionye *et al.,* [7] where the creatinine value of the rats treated with alcoholic bitters showed no significant difference. Anyasor and Ogunbiyi [2] reported increased oxidative stress on the liver and kidney when anti-oxidant and anti-inflammatory properties of polyherbal preparations such as Swedish bitters, Yoyo bitters, Action bitters were evaluated. There might be a possibility that the significant increase in creatinine value as observed in the ethanol and Jekomo treated groups interfere with the renal functions. However, the creatinine levels of animals treated with the alcoholic herbal were significantly lower ($p < 0.05$) when compared with ethanol group.

The significant increase in serum and homogenate urea and bilirubin concentration is similar to the findings of Oyewo *et al.* [18] and Anyasor & Ogunbiyi [2] but different from the report of Oforibika and Uzor [20] and Anionye *et al*. [7] who reported no significant difference. Urea and Creatinine are waste products of protein metabolism that are excreted through the kidney. Elevated Creatinine in the serum reflects decreased glomerular filtration rate, while increased urea indicates dysfunctional reabsorption [21, 22]. Urea levels can also be increased by other factors, such as dehydration; which is commonly associated with alcohol

intake, antidiuretic drugs [22] and diet. Short administration of Alomo and Jekomo herbal drinks showed no negative effect on kidney however, a misuse or overdose might complicate the renal functions if intake is not properly guided because urea and creatinine which are not excreted through the kidney alters protein metabolism which will eventually lead to kidney and liver dysfunction and or damage.

Conclusion

A slight elevation in urea, creatinine and bilirubin levels observed in Jekomo alcoholic herbal bitters could suggest a potential risk for kidney and liver damage. Prolonged and increased consumption of these alcoholic herbal bitters should be discouraged to prevent hepatorenal injury or damage.

Acknowledgement

The authors will like to appreciate the efforts of Mr Akindele Adigun, a technologist at the Department of Chemistry and Biochemistry, Caleb University Lagos Nigeria for technical assistance.

Conflict of Interest

There is no conflict of interest whatsoever.

References

1. Nwachuku, E.O, and Elekima, I. (2019). Evaluation of the effect of action bitters (herbal mixture) on some biochemical indices of albino rats. *Asian J of Res in Biochem*, 1-8
2. Anyasor, H.G, and Ogunbiyi, A.I. (2017). Antioxidant and anti-inflammatory properties of selected polyherbal preparations: Oroki herbal; Swedish bitters and Yoyo bitters. *Oxid Antioxid Med Sci*, 6(2):25- 29.
3. Oforibika, G.A, and Oforibika, D.A. (2020). Estimation of Toxicity Level of Three Different Herbal Bitters (Confam, G. Winco and 1960 Roots) on some Serum Lipid Profile Parameters and the Heart of Wister Albino Rats. *J Health Sci Res*, 15-20.
4. Waribo, H.A., Edamisan, E., Elekima, I., and Bartimaeus, E.A.S. (2017). Effect of Oral Consumption of Action Bitters on Renal Indices of Apparently Healthy Subjects in Port Harcourt Metropolis. *Eur J Pharm and Med Res*, 4(10): 62-67
5. Al-Aboody, M.S., and Mickymaray, S. (2020). Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics*, *9*(2), 45.
6. Oreagba, I.A., Oshikoya, K.A., & Amachree, M. (2011). Herbal medicine use among urban residents in Lagos, Nigeria. *BMC Complemt Altern Med*, *11*(1), 1-8.
7. Anionye, J.C., Onyeneke, E.C., Eze, G.I., Edosa, R.O., Agu, K.C., Omorowa, E.F., Oghagbon, E.S. (2017). Evaluation of the effects of super bitters on albino rats. *J Sci Res*, *2*(1):1-24.

8. Vasanthi, H.R., Parameswari, R.P., DeLeiris, J., Das, D.R. (2012). Health benefits of wine and alcohol from neuroprotection to heart health. *Frontiers in Biosciences*, 4:1505 – 1512.
9. Spirit Drink Regulation (2019). National Food and Drug Agency Control, 1-16
10. Gupta, S., Ahirwar, D., Jhade, D., Kumar, S.K., Bharti, A. (2010). Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Aerial parts of Mentha arvensis Linn. *Inter J. Pharma Sci Drug Res*, 2: 261 – 264
11. Odey, M.O., Ibor, O.R., Ujong, U.P., Chukwuka, A.V., Anden, A.B. (2019). Modulation of Biochemical Responses in Rats following Consumption of some herbalized Nigerian Alcoholic drinks. *Afri. J. Biomed Res*, 22(3):353 – 362.
12. Ginsberg, H., Olesfsky, J., Farquhar, J.W., Reaven, G.M. (2010). Moderate ethanol ingestion and plasma triglyceride levels. A study in normal and hypertriglyceridemic persons. *Annual inter Med*, 80:143 – 149
13. Strogner, J.M., Eassey, J.M., Baldwin, J.M., Miller, B.I. (2014). Innovative alcohol use assessing the prevalence of alcohol without liquid and other non-oral routes of alcohol and administration. *Drug and Alcohol Dependence*, 142:74 - 78.
14. Ibrahim, O., & Adigun, R.O. (2018). A review of alcoholic bitters proliferation in Nigeria: A friendly multifaceted killer looming across the nation. *J Med Biomed Res, 2022*; 17 (1-2): 63 -79.
15. Saukkonen, J.J., Cohn, D.L., Jasmer, R.M., Schenker, S., Jereb, J.A., Nolan, C.M., Sterling, T.R. An official ATS statement: hepatotoxicity of antituberculosis therapy. A.merican journal of respiratory and critical care Med, 2006; *174*(8): 935-952.
16. Peralta, R., and Pinsky, M.R. (2016) Hypoalbuminemia. Drugs and Diseases. http://emedicine.medscape.com/article/166724-overview Updated: Aug 16, 2016
17. Singal, A.K., Jampana, S.C., & Weinman, S.A. (2011). Antioxidants as therapeutic agents for liver disease. *Liver Inter, 31*(10):1432-1448.
18. Oyewo, E.B., Adetutu, A., and Adebisi, J.A. (2013). Immunomodulatory activities of Yoyo bitters: recommended dose precipitated inflammatory responses in male Wistar rats. PJBS, *16*(24): 1904-1912.
19. Elechi-Amadikn, K.N., Briggs, O.N., Opusunju, B.H., Batubo, N.P., Ubulom, R.R., Solomon, Q.E. (2020). Toxicological Effects of Overdose of Some Herbal Bitters Commonly Consumed in South Southern

Nigeria. *Asian J Med Prin and Clin Pract, 3*(2):35-39.

20. Oforibika, G.A., and Uzor, C.A. (2020). Study of the renal function of Wistar albino rat treated with three different herbal bitters (Confam, G. Winco and 1960 Roots). Direct Research *J Public Health Envi Tech, 5*(1), 1-5.
21. Owumi, S.E., and Dim, U.J. (2019). Manganese suppresses oxidative stress inflammation and Caspase-3 activation in rats exposed to Chlorpyrifos. T*oxicol Rep*, 6: 202 – 209
22. Yassin, M.M., Adas, T.O., and Yassin, M.M. (2021). Serum Glucose, Bilirubin, Liver Enzymes, Renal Parameters, Protein Profile and some electrolytes in adult male domestic rabbits intoxicated with Chlorpyrifos. *J Toxicol Risk Assess*, 7(1):037