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

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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

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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.

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 14 days 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 28 days. 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).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Weight (g)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>150.2 ± 7.8</td>
</tr>
<tr>
<td>Ethanol</td>
<td>120.5 ± 5.3*</td>
</tr>
<tr>
<td>Alomo bitters</td>
<td>145 ± 3.0</td>
</tr>
<tr>
<td>Jekomo bitters</td>
<td>141.2 ± 4.2*</td>
</tr>
</tbody>
</table>

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

![Creatinine Levels](image)

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

![Serum Urea Levels](image)

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

![Urea Levels](image)

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

![Bilirubin Levels](image)
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.

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Conflict of Interest
There is no conflict of interest whatsoever.

References


