NJBMB (2022) 37(3) 181-187

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

Biochemical and Haematological Responses to Chronic Consumption of some Herbalized Nigerian Alcoholic Drinks

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OPEN ACCESS

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ARTICLE HISTORY
Received: 16/03/2022
Reviewed: 13/05/2022
Revised: 30/08/2022
Accepted: 30/08/2022
Published: 30/09/2022

CITATION
Odey M.O., Mboso E.O., Ogar I., Iwara I.A., Adindu E.A., Godfrey C.O., Ibor M.E., Osim M.E., Emuru E.O. (2022). Biochemical and Haematological Responses to Chronic Consumption of some Herbalized Nigerian Alcoholic Drinks, *Nigerian Journal of Biochemistry and Molecular Biology*. *37*(3), 181-187

ABSTRACT

The unconfirmed perceptions of herbal-based alcoholic beverages having therapeutic, anti-malarial, and aphrodisiac properties have led to a surge in use of these products in many poor nations. Herein, we investigated biochemical and haematological effects of some commonly consumed herbalized alcoholic beverages in Cross River State, Nigeria using rat. Male wistar rats were treated with Action bitters, Alomo bitters, Origin bitters, 1960 bitters, Local gin, and Local gin plus vitamin B-complex, daily at a dose of 2.68 mL/kg body weight. A control group was given distilled water. After 90 days exposure, rats were anaesthetized in chloroform vapour and blood collected via cardiac puncture. Serum biochemical parameters; total protein, albumin, creatinine, and haematological parameters; haemoglobin, red blood cell, packed cell volume, platelet, white blood cell, basophils, neutrophils, eosinophils, lymphocyte and monoctes were measured. There were significant decreases ($P < 0.05$) in total protein and albumin of groups that received local gin, and local gin + vitamin B-complex. Also, the creatinine in all the alcohol-treated groups were significantly decreased ($P < 0.05$), compared with control. These suggest hepato and nephro-toxicities were significantly decreased ($P < 0.05$) in alcohol-treated groups compared with control group, suggesting haemato-toxicity. The results further showed that Local Gin induced more pronounced deleterious biological effects compared to other tested alcoholic beverages. Overall, the decrease in biochemical and haematological parameters in treatment groups compared with control suggests a probable mode of action and provide a mechanistic insight by which alcoholic beverages induce biochemical and haematological toxicity.

Keywords: *Biochemical changes, Alcoholic beverages, Haematological parameters, Wistar rat*

INTRODUCTION

Chronic consumption of locally made herbal-based alcoholic beverages, as well as their possible downstream physiological and biochemical effects, continues to be on the rise in many developing nations including Nigeria (Adeloye *et al.*, 2019). The reasons for drinking alcoholic beverages vary, and they include being part of a standard diet, medical objectives, relaxing effects, euphoric effects, recreational purposes, artistic inspiration, alleged aphrodisiac benefits, and happiness (Harter *et al.*, 2013). Alcohol is a psychoactive substance (Odey *et al.*, 2019). It has anesthetic and antiseptic qualities (Horai *et al.*, 2018), and it can be employed as a protein precipitant (fixative) (O'Shea *et al.*, 2010), and a local irritant (Harter *et al.*, 2013). The metabolic effect could be positive or negative (O'Keefe *et al.*, 2007).

It has been widely reported that prolonged alcohol intake can lead to a variety of ailments, including coronary heart

disease (CHD) (Mehlig *et al.*, 2014), liver disease (Liamis *et al.*, 2000; Lewis, 2006), and is responsible for approximately 5.9% of global deaths (Clarke *et al.*, 2017). Alcoholic beverages provide food energy. Although each gram of alcohol has about 7.1Kcal, excessive consumption might raise the risk of weight gain and the development of obesity or malnutrition (Vasanthi *et al.*, 2012; Nestel, 1965). Many biological effects of excessive alcohol use have been linked. These include those of the central nervous system, liver, kidney, lipid metabolism, and cardiovascular disease (Ruffle, 2014; Nahar *et al.*, 2007). The alcohol in alcoholic beverages contains ethanol (grain alcohol), whose metabolic product (acetaldehyde) is a poison in high doses and can lead to liver cirrhosis (Ginsberg *et al.*, 2010; Horai *et al.*, 2018). Alcoholic beverages contain primarily water, ethanol, and sugar. They are frequently regarded as empty caloric beverages because they contain none of the essential nutrients required for cellular respiration. Alcohol causes toxicity to the blood-forming organs (viz. bone marrow); the blood cell precursors; and the mature red blood cells (RBC), white blood cells (WBC), and platelets resulting in fewer than-normal or non-functional mature blood cells. This may lead to serious medical complications among alcohol abusers, such as anaemia, fatigue, shortness of breath, light headedness, and even reduced mental capacity, abnormal heartbeats, risk of serious infection and interference with blood clotting (Yakubu *et al.*, 2017).

Despite the data on the negative effects of chronic alcoholism, alcohol consumption continues to rise and is a source of concern for human health in Africa and its sub-region (Vasanthi *et al.*, 2012; Gonzalez-Reimers *et al.*, 2015; Adeloye *et al.*, 2019). Recently, several alcoholic beverage products fortified with various types of herbs and plant products, such as Alomo Bitters (AlB), Action Bitters (AcB), Origin Bitters (OrB), 1960 bitters, and Local Gin (LG), have gained widespread acceptance in Nigerian markets and are widely consumed, especially in Cross River State. These products lack scientific screening, with vague product descriptions, and unknown active components (Odey *et al*., 2019). They have unconfirmed claims implying their nutritional and medical importance because they are thought to cure and/or alleviate a variety of known ailments such as waist pain, menstrual cramps, cardiovascular disorders, digestive difficulties, anti-malarial and aphrodisiac properties, including the production of spermatocytes in males (Harter *et al*., 2013; Horai *et al.*, 2018). Despite these claims, there is little or no evidence on the potential biochemical and toxicological effects of their chronic use. As a result, the purpose of this study was to look at the physiological, biochemical, and toxicological effects of chronic ingestion of these products in a mammalian model.

MATERIALS AND METHODS

Chemicals and reagents: Total protein, albumin and creatinine kits were purchased from Randox Laboratories Limited, United Kingdom (UK). Alcoholic drinks; Action bitters (AcB), Alomo bitters (ALB), Origin bitters (OrB), 1960 bitters, Local gin (LG) and vitamin B-complex were all purchased from a commercial store in Calabar, Nigeria. All other chemicals were of the highest commercially available analytical grade.

Experimental animal's acclimation and handing procedure: All experimental procedures were reviewed, approved and performed in accordance with the Cross River University of Technology's Ethics Committee guidelines. A total of forty-two (42) male Wistar rats weighing 100 –120 g were obtained from the animal house of the Department of Medical Biochemistry, Faculty of Basic Medical Sciences of Cross River University of Technology. Wistar rats were maintained in the laboratory at 28.0 ± 2.0 °C, Relative humidity of 50.0 ± 5.0 % under a 12:12 h light and dark photoperiod. The animals were fed *ad libitum* with vital feed and tap water, and maintained in the laboratory for two weeks. After fourteen (14) days acclimatization period, rats were divided into seven experimental (7) groups of six (6) animals each. The experimental control group received distilled water, while other groups received oral intubation of Action bitters (AcB), Alomo bitters (ALB), Origin bitters (OrB), 1960 bitters, Local gin (LG) and Local gin plus vitamin B-complex (LG+VtB) daily at a dose of 2.68 mL/kg for ninety days. The dose was determined based on the assumption of four (4) adults with a total body weight of 280 kg (70 kg each) consuming 75 cl of the administered alcoholic beverage.

Sample collection: At the end of the 90-day experimental period, rats were anaesthetized in chloroform vapour and blood collected via cardiac puncture of the left ventricle using a 5 mL syringe. Part of the blood was transferred into an EDTA-tube, and used for haematological analysis, while the other part emptied into plain sample tube and allowed to clot. Thereafter, it was centrifuged at 3000 rpm for 15 min using table top centrifuge (0412-1 Cole Medical Instrument Co. Ltd, England). Serum was collected and preserved for biochemical assays.

Biochemical and haematological assays: Total protein, albumin and creatinine levels were measured in serum using kits purchased from Randox Laboratories Limited, United

Kingdom (UK) following the manufacturer's protocols. The full blood count and differential count were measured using auto heamatolo gyanalyzer (Mindray Beston India).

Statistical analysis

Data obtained was tested by One-way ANOVA followed by post-hoc analysis (Duncan's multiple range test) between exposure concentrations and control group, values were considered significantly different at $p<0.05$. Statistical analysis was performed using the Prism GraphPad 5 (GraphPad software, La Jolla, USA).

RESULTS AND DISCUSSION

Changes in Total protein, Albumin and Creatinine Levels: Total protein, albumin and creatinine levels were measured in male Wistar rats following treatment with alcoholic beverages over a 90-day exposure period. The results showed a significant decrease in total protein in the group that received the Local gin and Local gin + Vit. B-complex, compared to the control (Figure 1). Albumin level was significantly decreased ($p<0.05$) in groups that received Local gin, Local gin + vit. B-complex, 1960 bitters and Alomo bitters, compared to the normal control (Figure 2). Creatinine level in all exposed groups was significantly decreased compared to control (Figure 3).

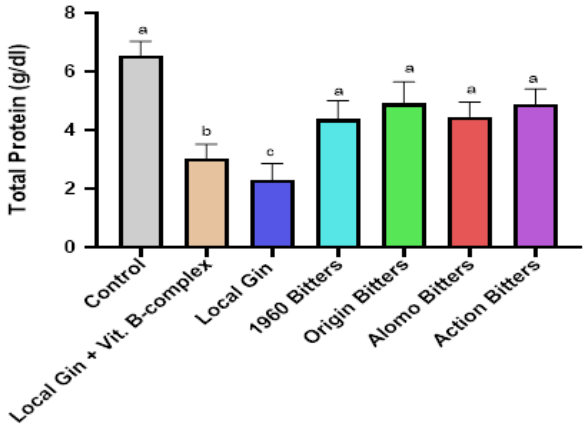

Figure 1. Changes in serum total protein of animals exposed to chronic consumption of herbalized alcoholic beverages

a = not significantly different from control P>0.05
b = significantly different from control P<0.05
c = significantly different from control and Origin bitters

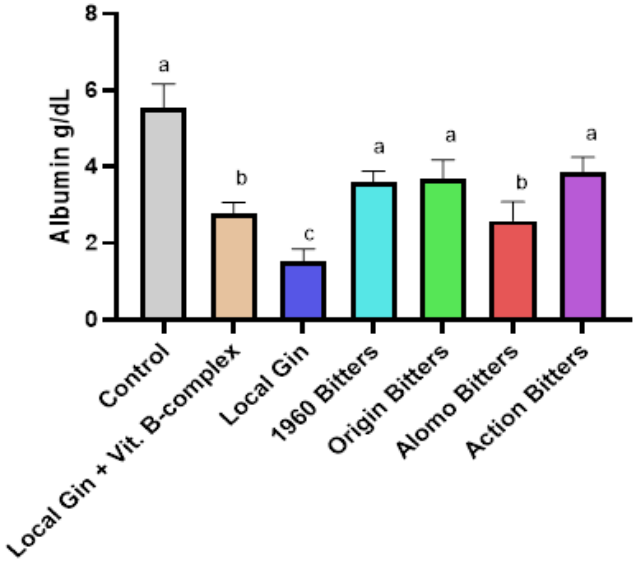

Figure 2: Changes in serum albumin of animals exposed to chronic consumption of herbalized alcoholic beverages

a = not significantly different from control P>0.05
b = significantly different from control P<0.05
c = significantly different from control and Action bitters

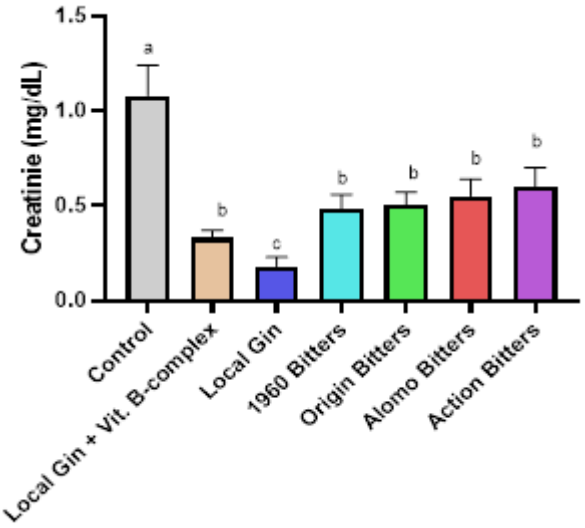

Figure 3. Changes in serum creatinine of animals exposed to chronic consumption of herbalized alcoholic beverages

a = not significantly different from control P>0.05
b = significantly different from control P<0.05
c = significantly different from control and Action bitters

The Alterations in total protein, albumin and creatinine levels following 90-day administration of bitter alcoholic beverages in male Wistar rats are presented in figures 1-3 above; (1) Total protein (2) Albumin (3) Creatinine. Data is presented as mean values ± standard error of mean (SEM). Different alphabets indicate significant difference ($p<0.05$) between exposure concentration and control (n= 6).

Effect on haematological parameters

Changes in haematological parameters were assessed by measuring haemoglobin (Hb) level, red blood cell (RBC), packed cell volume, platelet, white blood cell (WBC), basophils, neutrophils, eosinophils, lymphocyte and monoctes levels in male Wistar rats following treatment with alcoholic beverages for over a 90-day exposure. There was a significant decrease ($p<0.05$) in the group that received Local gin, compared to the normal control and all the treated groups, in the full blood parameters (Table 1).

Table 1. Full Blood Count of Animals Exposed to Chronic Consumption of Nigerian Herbalized Alcoholic Beverages

Alcoholic beverages	Red blood cell (x 10^6cell/µL)	Haemglobin (g/dL)	Packed cell volume (%)	Platelet (x 103 cells/µL)	White blood cell (x 10^3 cells/µL)
Control	4.82±0.21[a]	14.85±0.94[a]	31.02±1.07[a]	314.10±9.68[a]	4.62±0.15[a]
Local gin	1.96±0.30[b]	8.49±0.61[b]	16.97±1.43[b]	151.20±12.15[b]	1.52±0.17[b]
Local gin + Vit. B-complex	3.74±0.31[a]	13.48±0.81[a]	24.56±1.82[c]	242.90±18.47[a]	3.42±0.36[c]
1960 Bitters	3.71±0.23[a]	13.44±0.93[a]	24.78±1.28[c]	255.80±20.01[a]	3.50±0.20[c]
Origin Bitters	3.89±0.35[a]	13.44±0.99[a]	25.04±1.37[c]	226.10±15.16[c]	3.22±0.21[c]
Alomo Bitters	4.03±0.34[a]	13.85±0.47[a]	24.53±1.02[a]	278.70±18.72[a]	3.21±0.25[c]
Action Bitters	3.75±0.26[a]	13.46±0.69[a]	24.53±1.02[c]	228.20±27.86[c]	3.22±0.30[c]

Values are expressed as mean ±SD, n = 6; a = not significantly different from Control $p>0.05$; b = significantly different from Control and test groups at $p<0.05$; c = significantly different from Control at $p<0.05$

The differential blood count (Table 2), shows the percentage level of the different types of white blood cells.

The neutrophil levels in groups that received Local gin, and Local gin + vit. B-complex was significantly reduced ($P<0.05$) compared to the control. The percentage eosinophil level showed a significant decrease in all the treated groups, compared to the control. The percentage basophils levels were also significantly decreased in all the treated groups, compared to the control. The percentage lymphocyte levels were significantly decreased in the Local gin and Local gin + vit. B-complex treated groups, compared to the control. The monocyte levels were all significantly decreased in the treated groups, compared to the control.

Table 2. Differential Blood Count of Animals Exposed to Chronic Consumption of Nigerian Herbalized Alcoholic Beverages

Alcoholic beverages	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Lymphocytes (%)	Monocytes (%)
Control	22.22±1.67[a]	6.09±0.27[a]	1.82±0.15[a]	61.52±4.85[a]	8.05±0.49[a]
Local Gin	11.37±0.70[b]	2.43±0.27[c]	0.93±0.80[c]	28.09±2.44[b]	2.39±0.32[c]
Local Gin + Vit. B-complex	15.90±0.76[c]	3.52±0.54[c]	1.22±0.08[c]	35.28±2.94[c]	4.16±0.46[c]
1960 Bitters	16.71±1.45[a]	3.64±0.39[c]	1.14±0.07[c]	45.40±3.38[a]	3.86±0.34[c]
Origin Bitters	18.74±1.25[a]	3.47±0.39[c]	1.18±0.15[c]	50.90±3.68[a]	3.68±0.37[c]
Alomo Bitters	16.69±1.27[a]	3.67±0.21[c]	0.06±0.10[c]	43.98±5.64[a]	3.71±0.42[c]
Action Bitters	18.41±1.70[a]	3.28±0.30[c]	1.16±0.13[c]	44.02±4.24[a]	3.86±0.36[c]

Values are expressed as mean ±SD, n = 6; a = not significantly different compared to the Control at $p>0.05$; b = significantly different compared to the control and Origin Bitters at $p<0.05$; c = significantly different compared to the Control at $p<0.05$

DISCUSSION

In Nigeria and other developing countries, there is increasing consumption of herbal-based alcoholic beverages due to general speculations suggesting that these products are medicinal, and/or therapeutic to most ailments (including waist pain and menstrual cramps), anti-malarial and aphrodisiac (Harter *et al.*, 2013). However, this is without

adequate information on their negative physiological and biochemical effects. Herein, we investigated the possible toxicological, biochemical and physiological effects in rats, of some commonly consumed herbalized alcoholic beverages in Nigeria. The results demonstrated that experimental exposure of rats to these alcoholic beverages for 90days led to decrease in levels of total protein, albumin, creatinine, haemoglonin, red blood cell, packed cell volume, platelet, white blood cell, basophils, neutrophils, eosinophils, lymphocyte and monocyte. Some of these changes were significant.

A decrease in total protein and albumin levels are indicators of compromised cellular functions and integrity including hepatocellular damage, necrosis, altered membrane permeability and cholestasis from the toxicological standpoint (Venukumar *et al.*, 2004; Tasduq *et al.,* 2006; Odey *et al.*, 2022). The effect of the chronic consumption of herbalized alcoholic beverages on serum lipid profile, electrolytes, liver enzymes and total body weight changes has been reported by (Odey *et al.*, 2019), and it corroborated this finding. Albumin functions in transportation and maintenance of interstitial fluid homeostasis throughout the body (Kerner *et al.,* 2005; Odey *et al.*, 2022). The result of this study showed decreased levels of total protein and albumin in alcohol exposed groups compared to control. These decreases may be due to hepatocellular damage via hepatotoxicity, occasioned by the chronic alcohol consumption. Decrease total protein and albumin levels as observed in this study is consistent with the previous report of low levels of these proteins in prolonged alcohol consumption (Kyoko *et al.*, 2008).

Creatinine is produced from the breakdown of creatine and phosphocreatine and can also serve as an indicator of renal function (Bellocco *et al.,* 2012). Creatine is synthesized in the liver, pancreas, and kidneys from the transamination of the amino acids arginine, glycine, and methionine. Creatine then circulates throughout the body and is converted to phosphocreatine by the process of phosphorylation in the skeletal muscle and brain (Schaeffner *et al.,* 2005). Herein we observed low creatinine concentration in alcohol-treated groups compared with control. Such decrease may suggest nephrotoxicity. Decreased creatinine concentrations as observed in this study is consistent with the previous report suggesting decrease creatinine concentration in chronic alcoholism (White *et al.*, 2009). Consistently, we observed a lower level of all measured biochemical parameters (total protein, albumin, creatinine) in the LG treated group compared with other treatments and control and this may suggest that LG induced a higher biochemical and toxicological effects in rats compared to the herbalized alcoholic beverages.

Furthermore, we observed low concentration of haematological parameters such as haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), platelet, white blood cell (WBC), basophils, neutrophils, eosinophils, lymphocyte and monocytes in alcohol-treated groups compared with control. Alcohol exerts a direct toxic effect to the bone marrow resulting in vacuolization of the bone marrow precursor cells, anaemia, leukemia and thrombocytopenia (Akanni *et al.*, 2010). It also affects the functions of the leucocytes and platelets leading to impaired cellular immunity. Haematological functions are affected indirectly from nutritional deficiency, chronic liver disease and other metabolic derangement (Chu, 2000). This has also been supported by (Jaana, 2004) who reported that alcohol has a wide spread direct and indirect effects on the haematological system which mimics and obscures other disorders. Leucocytes, Erythrocytes and Thrombocytes production and functions are affected directly. Liver damage secondary to alcohol abuse also impacts red blood cells and haemostatic mechanism (Akanni *et al.,* 2010). The decrease concentration of haematological parameters such as observed in this study is consistent with the report of (Eyong *et al,* 2008).

CONCLUSION

The Biochemical and haematological evaluation of the effects of chronic consumption of some herbalized Nigerian alcoholic beverages shows negative impacts on the parameters assessed. The significant reduction in serum biochemical and haematological parameters due to chronic consumption of these beverages shows that the consumers of these products are predisposed to changes that may be deleterious to biochemical and physiological processes. Public health awareness, outreaches and enlightenment campaigns are necessary to inform vulnerable individuals with predisposed health conditions on the short, mid and long-term health risks associated with the chronic intake of these and other similar herbal-based alcoholic beverages.

AUTHORS' CONTRIBUTIONS

All authors participated in the study conception, design, experimentation and the interpretation of data. The manuscript was written by MOO, MEO and IAI. All authors commented and approved the revised manuscript for publication.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ACKNOWLEDGEMENT

The authors are grateful to the staff at Primary Health Centers in Calabar, Cross River State, Nigeria for their technical assistance throughout the study duration.

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