

Effects of various alcoholic bitters on the haematological parameters of albino wistar rats.

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Abstract

Due to easy accessibility of alcohol, people readily get intoxicated which results in various devastating health consequences. This study evaluated the effects of various alcoholic bitters on the haematological parameters of albino wistar rats. Forty two (42) male albino rats of wistar strain were used for this study and grouped into 7 groups of 6 animals. Group 1 served as normal control and were given distilled water. Group 2 were treated with Action Bitters (AB), group 3 were treated with Alomo Bitters (ALB), group 4 were treated with Origin Bitters (OB), group 5 were treated with 1960 bitters, group 6 which was the alcoholic control were treated with Local Gin (LG) and group 7 were treated with Local gin+B complex. The alcoholic bitters were administered to the rats at a dosage of 2.68 ml/kg body weight which is equivalent to 75 kg body weight of man consuming a bottle of these drinks (75 cl) at a time per day. The administration was done twice daily for a period of thirty (30) days using orogastric tube. Twelve hours after the last administration, the rats were sacrificed and blood was obtained via cardiac puncture into a well labeled EDTA bottle. Results of haematological indices estimation indicated a significant ($p < 0.05$) decrease in RBC count of groups II (3.75 ± 0.26), IV (3.89 ± 0.35), V (3.71 ± 0.23), VI (1.96 ± 0.29) and VII (3.74 ± 0.31) when compared with the normal control (4.82 ± 0.21) while group III (4.03 ± 0.34) showed no significant ($p > 0.05$) changes compared with normal control (4.82 ± 0.21). However, Hb levels of animals in groups II (13.46 ± 0.69), III (13.85 ± 0.47), IV (13.44 ± 0.99), V (13.44 ± 0.93) and VII (13.48 ± 0.81) shows no significant ($p > 0.05$) changes compared with those of normal control (14.85 ± 0.94) while animals in group VI (8.49 ± 0.61) recorded a significant ($p < 0.05$) decrease compared with the normal control (14.85 ± 0.94) and other treated groups. The PCV levels of animals in group II (24.12 ± 1.14), III (24.53 ± 1.02), IV (25.04 ± 1.37), V (24.78 ± 1.28) and VII (24.56 ± 1.82) recorded no significant ($p > 0.05$) changes compared with the control (25.18 ± 5.09). Moreover, the platelet count of animals in group II (303.47 ± 31.97), III (278.66 ± 18), IV (226.05 ± 15.16), V (255.81 ± 20.01), VI (151.19 ± 12.15) and VII (242.89 ± 18.47) shows a significant ($p < 0.05$) decrease when compared with the normal control (314.09 ± 9.68). However, the WBC count of all the animals in group II (3.22 ± 0.30), III (3.21 ± 0.25), IV (3.22 ± 0.21), V (3.50 ± 0.20), VI (1.52 ± 0.17) and VII (3.09 ± 0.15) were all significantly ($p < 0.05$) lower when compared to the normal control (4.62 ± 0.15). The results also showed significant ($p < 0.05$) decrease in the differential count of all the treated groups compared to the normal control. The findings of this study suggest that chronic ingestion/consumption of these alcoholic bitters may induce haematotoxicity. Hence, there is need for public enlightenment on the dangers of these bitters.

Keywords: Alcoholic bitters, Haematology, Albino rats, Haemolysis.

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Introduction

The earliest origins of alcoholic bitters can be traced back as far as the ancient Egyptians, who may have infused medicinal herbs in jars of wine [1]. This practice was further developed during the middle ages, where the availability of distilled alcohol coincided with a renaissance in pharmacognosy, which made possible far more concentrated herbal bitters and tonic preparations. Many of the various brands and styles of digestive bitters made today reflect herbal stomachic and tonic preparations whose roots are claimed to be traceable back to Renaissance era pharmacopeia and traditions [1].

Bitters are traditionally an alcoholic preparation flavored with botanical herb so that the end result is characterized by a bitter, sour, or bittersweet flavor. Bitters are produced from root extracts and herb, from the therapeutic content of (primarily) tropical and subtropical plant and spices [2]. Bitters are usually dark in color and valued for their ability to promote appetite and digestion hence they are use as patent medicine, digestion aids and as flavoring in cocktails [3]. Unorthodox traditional medicine practice which employ the use of herbs (medicinal plants) in treatment of ailment have gained much publicity and recognition, seemingly elusive to the system of orthodox medical practice. Medicinal plants have been defined as those plants which contain in one or more of their organs, substances that can be used for the synthesis of useful drugs [4]. Scope of

modern science may have widened the differences in terms of medication between orthodox and unorthodox/traditional medicine, this gap seems to be closing fast as the current trend is that they are both adopting practices from each other [4]. Numerous longstanding brands of bitters were originally developed as patent medicines, but are now sold as digestives bitters, sometimes with herbal properties, and cocktail flavorings.

Alcoholic beverages such as Alomo bitters, Action bitters, Origin bitters, Pasa bitters, 1960 bitters, Osomo bitters etc. makes an impact in the alcoholic beverages market despite the initial fears over the hygiene level of their product and their composition [5,6]. The claim that it is restorative and sex energy boosting, continue to lure customers to patronize them in mass. The botanical ingredients used in preparing bitters have historically consisted of aromatic herbs, bark, roots, and/or fruit for their flavour and medicinal properties. Some of the more common ingredients are cascarilla, cassia, gentian, orange peel, and cinchona bark etc. Most bitters contain water and alcohol, the latter of which functions as a solvent for botanical extracts as well as a preservative. Bitters have been claimed to help heal piles/haemorrhoids and improve sexual function, enhance blood circulation, purification of blood by the kidneys, blood pressure regulation through arterial dilatation and prevent formation of kidney stones, cleanse the colon of impurities and have also been said to possess anti-tumor properties [7,8].

They are also said to have anti-inflammatory, antibiotic and antifungal properties. Bitters have also been said to ensure good digestion of fats and oils, and proper functioning of the excretory functions of the liver, reduce accumulated fat (triglycerides) and cholesterol levels thereby conferring on it hypolipidaemic properties [8].

The focus of this study however, is to evaluate the effects of alcohol intoxication on some haematological indices in albino Wistar rats (Table 1).

Groups	Number of animals	Administration
1 (Normal control)	6	Normal saline
2	6	Action bitters (2.68 ml/kg bw)
3	6	Alomo bitters (2.68 ml/kg bw)
4	6	Origin bitters (2.68 ml/kg bw)
5	6	1960 bitters (2.68 ml/kg bw)
6 (Alcoholic control)	6	Local gin (2.68 ml/kg bw)
7	6	Local gin (2.68 ml/kg bw) +vit. B-complex

Table 1. Experimental design and administration schedule for the effect of various alcoholic bitters on the haematological indices of albino wistar rats.

Alcohol consumption abuse has been blamed on various social, economic as well as health challenges. It has also been considered as one factor responsible for high violent death rates in most populations and a common cause of hospital admissions throughout the western world. Acute or chronic alcohol consumption causes degeneration in different internal organs and systems of the body as well as weight loss. Consumption of alcoholic bitters is increasingly high in Nigeria because consumers believe that it contains body purifiers, it is anti-malaria, sex energy boosting, anti-diabetic, hypo-lipidemic etc.

Alcohol which is a major constituent of these bitters has been proven capable of causing disease conditions depends on different factors which includes malnutrition, contaminants of viral infectious of the liver, gastric predisposition etc. The medicinal use of these extract from plants are well documented but there still little or no information as regard this. This study is justified by the fact that it will scientifically establish the effect of alcoholic bitters on haematological indices.

Materials and Methods

Chemicals and reagents

Biochemical assay kits used in this analysis were obtained from three separate laboratories: Dialab, Randox and Teco diagnostics. Others include Action bitters, Alomo bitters, Origin bitters and 1960 bitters obtained from supermarket Otuoke, Nigeria, while the Local gin was obtained from a local drinking joint in Otuoke, and the vitamin B-complex was obtained from Otuoke pharmacy, Otuoke, all in Bayelsa State. Chloroform, Normal saline and distilled water of analytical grades were also used.

Experimental animals

Forty-two (42) male rats of albino wistar strain weighing 80-120 g were used for this study. The rats were obtained from the pharmacology Department, faculty of basic medical sciences of the University of Port Harcourt, and were used for the study. The animals were allowed one week acclimatization, after which they were reweighed and housed in plastic cages with plastic bottom and wire-mesh top, under controlled environmental conditions of temperature ($28 \pm 2^\circ\text{C}$), relative humidity ($50 \pm 5\%$) and a twelve-hour light/dark cycle. The animal facility was adequately ventilated and the animals maintained regularly on the commercial rat chow. Tap water and food were provided ad libitum throughout the experimental period.

Experimental design and treatment of animals

The experimental design employed consisted of 42 wistar rats of albino strain divided into 7 groups of 6 animals each. Group 1 is the normal control, and received distilled water, group 2 received Action bitters, group 3 received Alomo bitters, group 4 received Origin bitters, group 5 received 1960 bitters, while group 6 is the alcoholic control and received local gin while group 7 received local gin plus vitamin B-complex twice daily

at a dose equivalent to a man (75 kg body weight), consuming a bottle of these drinks (75 cl) at a time per day. The experimentation lasted for thirty days (1 month).

Blood sample collection and preparation

At the end of the experiment, the animals were sacrificed and blood (5 ml) obtained from each of them via cardiac puncture into non-heparinized plain test tubes. Another blood (1 ml) was obtained into heparinized tubes for haematological analysis.

The blood (5 ml) in non-heparinized plain test tubes were allowed to clot after which were centrifuged and the serum collected with a pasture pipette, from which the haematological parameters were analyzed.

Haematological assay

Hematological analysis of Total Red blood cell count, Total Platelet Count, Total White Blood Cell Count, Packed cell volume, Haemoglobin, Differential white cell count were carried out using auto haematological analyzer.

Statistical analysis

Data obtained was expressed as mean \pm SEM and analysis was done Statistical Package for Social Scientists (SPSS version 21.0). Values at $p < 0.05$ were considered significant in comparison with appropriate control.

Results

Effect of treatment on hematological parameters

The effects of alcoholic bitters on the RBC, HB, PCV, Platelet and WBC of albino wistar rat are presented in Table 2.

Group	RBC ($\times 10^6$ cells/ μ l)	HB (g/dl)	PCV (%)	Platelet ($\times 10^6$ cells/ μ l)	WBC ($\times 10^6$ cells/ μ l)
NC	4.82 \pm 0.21	14.85 \pm 0.94	25.18 \pm 5.09	314.09 \pm 9.68	4.62 \pm 0.15
AB	3.75 \pm 0.26*	13.46 \pm 0.69	24.12 \pm 1.14	303.47 \pm 31.97	3.22 \pm 0.30*
ALB	4.03 \pm 0.34	13.85 \pm 0.47	24.53 \pm 1.02	278.66 \pm 18.72	3.21 \pm 0.25*
OB	3.89 \pm 0.35*	13.44 \pm 0.99	25.04 \pm 1.37	226.05 \pm 15.16*a	3.22 \pm 0.21*
1960	3.71 \pm 0.23*	13.44 \pm 0.93	24.78 \pm 1.28	255.81 \pm 20.01*	3.50 \pm 0.20*
LG	1.96 \pm 0.29*abcd	8.49 \pm 0.61*abcd	16.97 \pm 1.43*abcd	151.19 \pm 12.15*abcd	1.52 \pm 0.17*abc
LGB	3.74 \pm 0.31*e	13.48 \pm 0.81e	24.56 \pm 1.82e	242.89 \pm 18.47*ae	3.09 \pm 0.15*e

Note: Mean \pm Standard Error of Mean (SEM), n=6, *significant at $p < 0.05$ compared with group 1: (NC), a=significant at $p < 0.05$ compared with group 2: (AB), b=significant at $p < 0.05$ compared with group 3: (ALB), c=significant at $p < 0.05$ compared with group 4: (OB), d=significant at $p < 0.05$ compared with group 5: (1960), e=significant at $p < 0.05$ compared with group 6.

Abbreviations: AB: Action Bitters; ALB: Alomo Bitters; OB: Orign Bitters; LG=Local Gin; LGB: Local Gin+B complex; RBC: Red Blood Cell; HB: Haemoglobin; PCV: Pack Cell Volume; WBC: White Blood Cell.

Table 2. Effect of various alcoholic bitters on the haematological parameters of albino wistar rats (RBC, HB, PCV, Platelet, WBC).

The result reveals that the RBC count ($\times 10^6$ cells/ μ l) of animals in group II (3.75 \pm 0.26), III (4.03 \pm 0.34), IV (3.89 \pm 0.35), V (3.71 \pm 0.23), VI (1.96 \pm 0.29) and VII (3.74 \pm 0.31) were all significantly ($p < 0.05$) lower compared with the normal control (4.82 \pm 0.21), while group VI (1.96 \pm 0.29) shows a significant ($p < 0.05$) decrease compared to other treated groups.

Results shows that the hemoglobin count of animals in group II (13.46 \pm 0.69), III (13.85 \pm 0.47), IV (13.44 \pm 0.93), V (13.44 \pm 0.99) and VII (13.48 \pm 0.81) shows no significant ($p > 0.05$) changes when compared with the normal control (14.85 \pm 0.94).

However, the hemoglobin count of animals in group VI (8.49 \pm 0.61) showed a significant ($p < 0.05$) difference and decrease when compared with the normal control (14.85 \pm 0.94) and other treated groups.

The PCV results obtained showed that the PCV count of animals in group II (24.12 \pm 1.14), III (24.53 \pm 1.02), IV (25.04 \pm 1.37), V (24.78 \pm 1.28) and VII (24.56 \pm 1.82) have no significant ($p > 0.05$) changes compared with the control (25.18 \pm 5.09).

However, the PCV count of animals in group VI (16.97 \pm 1.43) showed a significant ($p < 0.05$) decrease when compared to group II, III, IV, V and VII and the normal control.

The results obtained reveals that the platelet count of all the animals in group II (303.47 \pm 31.97), III (278.66 \pm 18), IV (226.05 \pm 15.16), V (255.81 \pm 20.01), VI (151.19 \pm 12.15) and VII (242.89 \pm 18.47) shows a significant ($p < 0.05$) decrease when compared with the normal control (314.09 \pm 9.68).

However, the platelet count of animals in group IV (226.05 \pm 15.16), V (255.81 \pm 20.01), VI (151.19 \pm 12.15) and VII (242.89 \pm 18.47) showed a significant difference when compared with the normal control (314.09 \pm 9.68).

The result obtained showed that the WBC count ($\times 10^6$ cells/ μ l) of all the animals in group II (3.22 \pm 0.30), III (3.21 \pm 0.25), IV (3.22 \pm 0.21), V (3.50 \pm 0.20), VI (1.52 \pm 0.17) and VII (3.09 \pm 0.15) were all significantly ($p < 0.05$) lower when compared to the normal control (4.62 \pm 0.15).

The comparative analysis of the normal control group and the treated group indicated that there are significant ($p < 0.05$) differences at in all of the differential counts considered in this experiment.

The result shows that there is a significant decrease ($p < 0.05$) in the differential count of all the treated groups compared to the normal control (Table 3).

Group	Monocyte (%)	Lymphocyte (%)	Neutrophil (%)	Basophil (%)	Eosinophil (%)
NC	8.05 \pm 0.49	66.52 \pm 1.45	22.21 \pm 1.69	1.82 \pm 0.15	6.09 \pm 0.27
AB	3.86 \pm 0.36*	43.52 \pm 3.93*	18.41 \pm 1.70*	1.16 \pm 0.13*	3.28 \pm 0.30*
ALB	3.71 \pm 0.42*	44.98 \pm 5.30*	16.69 \pm 1.17*	1.06 \pm 0.10*	3.67 \pm 0.20*

OB	3.68 0.37 [*]	±	50.90 3.68 [*]	±	18.74 1.25 [*]	±	1.19 0.16 [*]	±	3.47 0.37 [*]	±
1960	3.86 0.34 [*]	±	45.39 3.38 [*]	±	16.71 1.44 [*]	±	1.14 0.07 [*]	±	3.64 0.39 [*]	±
LG	2.39 0.33 ^{abcd}	±	28.09 2.44 ^{abcd}	±	11.37 0.70 ^{abcd}	±	0.93 0.08 [*]	±	2.43 0.27 ^{bcd}	±
LGB	4.16 0.46 ^e	±	35.29 2.94 [*]	±	17.39 0.87 ^e	±	1.22 0.08 [*]	±	3.52 0.54 ^e	±
Note: Mean ± Standard Error of Mean (SEM), n=6, *significant at p<0.05 compared with group 1: (NC), a is significant at p<0.05 compared with group 2: (AB), b is significant at p<0.05 compared with group 3: (ALB), c is significant at p<0.05 compared with group 4: (OB), d is significant at p<0.05 compared with group 5: (1960), e is significant at p<0.05 compared with group 6.										
Abbreviations: AB: Action Bitters; ALB: Alomo Bitters; OB: Orign Bitters; LG: Local Gin; LGB: Local Gin+B complex; NC: Normal control										

Table 3. Effect of various alcoholic bitters on the differential white blood cell count (Monocyte, Leukocyte, Neutrophil, Basophil, Eosinophil).

Discussion

Blood is a bodily fluid in animals which delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from same cells. Blood component includes plasma (the liquid portion, which contains water, proteins, salts, lipids, and glucose), red blood cells, white blood cells, and cell fragments called platelets. [9].

Blood plays an important role in regulating the body's systems and maintaining homeostasis. It performs many functions within the body, including; supplying oxygen to tissues (bound to hemoglobin, which is carried in red cells), supplying nutrients such as glucose, amino acids, and fatty acids either dissolved in the blood or bound to plasma proteins (e.g., blood lipids), removing waste such as carbon dioxide, urea, and lactic acid, immunological functions, including circulation of white blood cells and detection of foreign material by antibodies, coagulation, which is one part of the body's self-repair mechanism (blood clotting by the platelets after an open wound in order to stop bleeding), messenger functions, including the transport of hormones and the signaling of tissue damage, regulating body pH, regulating core body temperature, hydraulic functions, including the regulation of the colloidal osmotic pressure of blood [10].

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extract on blood related functions [11]. In this study, prolonged administration of the alcoholic bitters significantly decreased haemoglobin concentration, red blood cell count, packed cell volume, platelet count, white blood cell count as well as other differential counts were also affected compared to their respective control groups.

The decline in haematological parameters is probably due to generation of free radicals (reactive oxygen species, ROS) through the microsomal metabolism of alcohol via cytochrome P450. Reactive Oxygen Species (ROS) is known to deplete antioxidants and depletion of antioxidants renders blood cells very fragile, thus leading to accelerated destruction of the blood cells [12].

The Red Blood Cell counts of all treated groups were generally reduced. Although, there was no significant (p>0.05) difference observed with the administration of Alomo bitters compared with the normal control but there was a significant (p<0.05) changes observed with the administration of AB, OB, 1960, LG and LGB when compared with the control. Alterations in the red blood cell structure can result in impaired blood flow, chronic anaemia, endothelial dysfunction, ischemia, hypertension, risk for cardiovascular diseases and lysis of red blood cells at physiological conditions. [13,14].

However, haemoglobin concentrations were generally reduced in all the treated groups. There was no significant (p>0.05) difference observed with the administration of AB, ALB, OB, 1960 and LGB when compared with the normal control but a significant (p<0.05) difference was observed with LG when compared to the control and other treated groups.

The PCV count were also reduced in all the treated groups and there was no significant (p>0.05) difference observed with the administration of AB, ALB, OB, 1960 and LGB when compared with the control but LG shows a significant (p<0.05) difference when compared with the control and other treated groups. The low PCV count could be as a result of malnutrition [15]. Alcohol suppresses appetite and causes ulceration of the intestine. These factors could interfere with nutrient availability and utilization by the body which can lead to malnutrition.

Moreover, the platelet counts were also generally reduced in all the treated groups. However, there was no significant (p>0.05) difference observed with the administration of AB and ALB when compared with the control but a significant (p<0.05) difference was observed with the administration of OB, 1960, LG and LGB when compared with the control. The Red blood cell decreases may be associated with fewer platelets in the body, so experiencing alcoholic anemia can reduce the body's ability to form clots.

The WBC count also shows a significant (p<0.05) decrease in all the treated groups and there was a significant (p<0.05) difference observed with the administration of AB, ALB, OB, 1960, LG and LGB when compared with the control. The low white blood cells are likely due to the effect of massive destruction of blood cells which can lead to anaemia and weaken immune system [15].

Furthermore, decreases were also observed in the differential count in all the treated groups. There were significant (p<0.05) difference observed with the administration of AB, ALB, OB, 1960, LG and LGB when compared with the control.

Generally, the findings of this study suggest that alcohol abuse can induces a wide array of adverse effects as evident in the

observed indicators for erythrocytopenia, thrombocytopenia and leucopenia. We opine therefore, that excessive alcohol ingestion should be avoided. Moreover, it is rather unnecessary to ingest an excessive amount of a substance that has capacity to induce hematological toxicity.

Conclusion

In conclusion, the low haematological parameters are indicative of massive haemolysis, which can lead to anaemia and weaken immune system. Again, low haematological parameters could be as a result of malnutrition. Alcohol suppresses appetite and causes ulceration of the intestine. These factors could interfere with nutrient availability and utilization by the body, which can lead to malnutrition. Hence, it may be concluded that from this study that the consumption of alcoholic bitters appears to be toxic and is capable of inducing severe haemolysis/hematological alterations.

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