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Influence of Beers of Different Alcohol Concentration on Haematological Indices of Male Albino Rats

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Abstract

Background and Objective: This study investigated the influence of beers of different alcohol content on haematological indices of male albino rats. Materials and Methods: Three brands of beers commonly consumed within Nigeria were purchased and administered to the experimental animals for 21 days. Group 1 was the control. Group 2 and group 3 were administered 10 mL/kg bw and 20 mL/kg bw of beer A respectively. Group 4 and group 5 were administered 10 mL/kg bw and 20 mL/kg bw of beer B respectively, while group 6 and group 7 were administered 10 mL/kg bw and 20 mL/kg bw of beer C respectively. The haematological analysis was carried out using Abacus 380. Results: White blood cell (WBC) count increased in all the test groups compared with the normal control. The increase is significant ($P<0.05$) in groups 3, 5, 6 and 7. Lymphocyte (LYM) increased non-significantly ($P>0.05$) in groups 2, 5, 6 and 7, but reduced non-significantly ($P>0.05$) in groups 3 and 4 compared to the control. Mid-size cells (MID) reduced non-significantly ($P>0.05$) in group 6, but increased non-significantly ($P>0.05$) in groups 2, 3, 4, 5 and 7 compared to the control. Granulocyte (GRA) increased non-significantly ($P>0.05$) in group 6, but reduced non-significantly ($P>0.05$) in groups 2, 3, 4, 5 and 7 compared to the control. Red blood cell (RBC), haemoglobin (Hb) and packed cell volume (PCV) showed no significant alteration ($P>0.05$) in all the test groups compared to the control. RBC reduced in all the test groups; Hb reduced in groups 2, 3, 4, 5 and 7, but increased in group 6, while PCV reduced in groups 2, 4 and 7, but increased in groups 3, 5 and 6. Platelet (PLT) and plateletcrit (PCT) increased significantly ($P<0.05$) in groups 2, 3, 4, 5 and 7, but increased non-significantly ($P>0.05$) in group 6 compared to the control. Conclusion: The result showed that regular consumption of these beers of different concentrations as used in this study may induce intoxication and influence certain immune indexes, but may not induce anaemia. It also encourages the production of platelets and may promote the stoppage of bleeding resulting from an injury.

Keywords: Alcohol content; Anaemia; Beer; Haematology; Haemoglobin; Intoxication; White blood cell.

1. Introduction

According to a report, alcoholism is one of the most serious global public health challenges and it is estimated that about two billion people consume alcohol globally, of which 76.3 million people often develop alcohol-related abnormalities [1]. The clinical manifestation of alcoholic effects on the biological system depends on several factors such as the quantity and duration of intake, sex, viral infection, genetic composition, malnutrition, obesity, smoking and iron overload [2]. Severe alcohol intake has been found to increase the risk of bacterial infection, alcohol-induced obstruction of neutrophils and monocytes, decrease leucocytes count in both blood and bone marrow, reduce granulocytes level and increase reticulocytes count [3-6]. Chronic severe alcohol dependence is capable of causing myelosuppression resulting in a slight decrease in all blood cells, blood loss from the gastrointestinal tract, malnutrition and as a result, chronic alcoholic consumers may suffer from moderate anaemia, characterized by enlarged and structurally abnormal RBCs; mildly reduced numbers of WBCs, especially of neutrophils; and moderately to severely reduced numbers of platelets[7]. Griendling and FitzGerald [8], reported that reactive oxygen species generated from alcohol biotransformation was implicated in several physiological aberrations such as rheumatoid arthritis, haemochromatosis, atherosclerosis and cardiovascular disease other than the known alcoholic liver disease.

It has been reported that consumption of alcohol is being used as a psychoactive therapy while alcohol itself has been previously used as an anaesthetic, disinfectant, protein precipitant as well as local irritant [9]. Similarly, light to moderate consumption of alcohol serves as a natural part of the diet, which adds calories to the food and may be effective in reducing stress [10]. Many investigations have suggested that a light alcohol intake results in decreased

insulin resistance and hence, lowers the risk of diabetes [11] and in addition, it increases insulin sensitivity in skeletal muscles, which may serve as a protective mechanism against obesity and diabetes [12].

Currently, there is need for a comparative study on the influence of beers of different alcohol content on haematological indices of male albino rats. This is important because it will provide to researchers and consumers, the results/findings on the implications of consuming these three beers on haematological indices. This study will therefore bridge this gap that exist in literature by providing the findings contained in this study.

2. Materials and Methods

2.1. Beers Used

Three different brands of commonly consumed beers were purchased from a beer joint in Wukari, Nigeria. The beers were of different alcohol concentrations: 2%, 5.2% and 7.5%, and were labelled A, B and C respectively for this study.

2.2. Experimental Animals

Thirty-five healthy male albino rats of 7 weeks of age were used in this study. The animals were bred and kept at the animal house, Department of Biochemistry, Federal University Wukari, Nigeria. They were allowed access to water and feed *ad libitum* throughout the period of the experiment. Standard laboratory protocols for animal studies were followed and methods used were performed following the relevant guidelines and regulations.

2.3. Experimental Design

The method of Imo, *et al.* [13] was used. The animals were randomly placed into seven groups of five animals each. Animals in group 1 served as normal control and were administered a placebo of normal saline, while animals in groups 2, 3, 4, 5, 6 and 7 served as test animals. Groups 2 and 3 animals were administered 10 mL/kg bw and 20 mL/kg bw respectively of the beer labelled A (2% alcohol content). Groups 4 and 5 animals were administered 10 mL/kg bw and 20 mL/kg bw respectively of the beer labelled B (5.2% alcohol content), while groups 6 and 7 animals were administered 10 mL/kg bw and 20 mL/kg bw respectively of the beer labelled C (7.5% alcohol content). The different beers were administered to the corresponding test animals through an oral route. Each test animal received the designated beer twice per day (morning and evening periods) for 21 consecutive days.

2.4. Animal Sacrifice and Blood Collection

After administration of the beers to the animals, they were starved overnight, anaesthetized (using chloroform) and sacrificed by cervical dislocation. Blood samples were collected (by cardiac puncture) using a hypodermic syringe and dispensed into sample tubes containing an anti-coagulant (EDTA) for haematological analysis.

2.5. Haematological Analysis

The levels of WBC (white blood cell) count, LYM (lymphocyte), MID (mid-size cells), GRA (granulocyte), RBC (red blood cell) count, Hb (haemoglobin), PCV (packed cell volume), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), RDWc (red blood cell distribution width count), PLT (platelet), PCT (plateletcrit), MPV (mean platelet volume) and PDWc (platelet distribution width count) were determined using haematological auto-analyzer (Abacus 380).

2.6. Statistical Analysis

Statistical analysis was carried out on the results with the use of One-Way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21. The means were compared for significance at $p \leq 0.05$ and the group results presented as mean ± SD.

3. Results

Results of the haematological analysis are presented in the tables below:

Table-1. Concentrations of white blood cells in rats administered beers of different alcohol content

Parameters	Group 1 (Normal control)	Group 2 (Beer A (2%): 10 mL/kg bw)	Group 3 (Beer A (2%): 20 mL/kg bw)	Group 4 (Beer B (5.2%): 10 mL/kg bw)	Group 5 (Beer B (5.2%): 20 mL/kg bw)	Group 6 (Beer C (7.5%): 10 mL/kg bw)	Group 7 (Beer C (7.5%): 20 mL/kg bw)
WBC ($x10^9$/L)	3.46 ± 0.12^{a}	$4.81 \pm 0.61^{a,b}$	9.13 ± 1.44^{c}	$5.15 \pm 0.69^{a,b}$	$7.39 \pm 2.28^{b,c}$	12.25 ± 2.87^{d}	$6.87 \pm 1.24^{b,c}$
LYM (%)	63.70 ± 4.47^{a}	68.30 ± 5.47^{a}	60.87 ± 3.45^{a}	62.37 ± 6.13^{a}	69.27 ± 3.45^{a}	64.80 ± 7.62^{a}	70.77 ± 5.24^{a}
MID (%)	$11.50 \pm 1.73^{a,b}$	15.67 ± 2.16^{b}	15.50 ± 4.87^{b}	15.93 ± 1.17^{b}	15.77 ± 2.19^{b}	8.03 ± 1.27^{a}	13.07 ± 2.10^{b}
GRA (%)	$24.77 \pm 2.40^{a,b}$	16.07 ± 3.67^{a}	$23.60 \pm 3.04^{a,b}$	$21.73 \pm 6.17^{a,b}$	16.67 ± 6.19^{a}	27.03 ± 6.47^{b}	16.13 ± 4.12^{a}

Results represent mean ± standard deviation of group result obtained (n=5).
Mean in the same row, having different letters of the alphabet are statistically significant ($p<0.05$).

Legend: WBC: white blood cell; LYM: lymphocyte; MID: mid-size cells; GRA: granulocyte.

WBC increased in all the test groups compared with the normal control (table 1). The increase is significant ($p<0.05$) in groups 3, 5, 6 and 7. LYM increased non-significantly ($p>0.05$) in groups 2, 5, 6 and 7, but reduced non-significantly ($p>0.05$) in groups 3 and 4 compared to the control. MID reduced non-significantly ($p>0.05$) in group

6, but increased non-significantly (p>0.05) in groups 2, 3, 4, 5 and 7 compared to the control. GRA increased non-significantly (p>0.05) in group 6, but reduced non-significantly (p>0.05) in groups 2, 3, 4, 5 and 7 compared to the control.

Table-2. Concentrations of selected haematological parameters in rats administered beers of different alcohol content

Parameters	Group 1 (Normal control)	Group 2 (Beer A (2%): 10 mL/kg bw)	Group 3 (Beer A 2%): 20 mL/kg bw)	Group 4 (Beer B (5.2%): 10 mL/kg bw)	Group 5 (Beer B (5.2%): 20 mL/kg bw)	Group 6 (Beer C (7.5%): 10 mL/kg bw)	Group 7 (Beer C (7.5%): 20 mL/kg bw)
RBC (10^{12}/L)	8.36 ± 0.91^{a}	7.12 ± 1.31^{a}	7.30 ± 1.31^{a}	7.19 ± 0.65^{a}	7.90 ± 0.91^{a}	7.89 ± 0.75^{a}	7.00 ± 0.53^{a}
Hb (g/dL)	13.63 ± 1.46^{a}	12.20 ± 2.69^{a}	12.80 ± 2.11^{a}	12.67 ± 1.10^{a}	12.90 ± 1.23^{a}	13.83 ± 1.33^{a}	11.53 ± 1.46^{a}
PCV (%)	42.65 ± 6.06^{a}	40.40 ± 8.74^{a}	43.20 ± 7.25^{a}	41.88 ± 4.13^{a}	44.00 ± 4.83^{a}	43.60 ± 5.18^{a}	37.98 ± 5.20^{a}
MCV (fl)	50.67 ± 1.53^{a}	56.33 ± 2.08^{b,c,d}	59.33 ± 0.58^{d}	58.33 ± 1.53^{c,d}	55.67 ± 0.58^{b,c}	57.00 ± 2.00^{b,c,d}	54.00 ± 3.00^{b}
MCH (pg)	16.37 ± 0.45^{a}	17.13 ± 0.76^{a,b,c}	17.53 ± 0.35^{c}	17.67 ± 0.67^{c}	16.67 ± 0.12^{a,b,c}	17.43 ± 0.20^{b,c}	16.47 ± 0.81^{a,b}
MCHC (g/dL)	32.17 ± 1.46^{b}	30.27 ± 0.21^{a}	29.60 ± 0.10^{a}	30.30 ± 0.52^{a}	29.33 ± 0.81^{a}	30.50 ± 1.14^{a}	30.50 ± 0.44^{a}
RDWc (%)	19.90 ± 0.52^{a}	21.53 ± 0.99^{a,b}	21.40 ± 1.00^{a,b}	22.57 ± 0.31^{b,c}	21.83 ± 1.82^{a,b}	22.90 ± 0.61^{b,c}	24.20 ± 1.25^{c}

Results represent mean ± standard deviation of group result obtained (n=5).
Mean in the same row, having different letters of the alphabet are statistically significant (P<0.05).

Legend: RBC: red blood cell; Hb: haemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDWc: red blood cell distribution width count.

Alcohol intake at doses above did not cause a significant change (P > 0.05) in RBC, Hb, and PCV levels (table 2). RBC was reduced in all the test groups. Compared to the control. Hb reduced in groups 2, 3, 4, 5 and 7, but increased in group 6. PCV reduced in groups 2, 4 and 7, but increased in groups 3, 5 and 6. MCV increased significantly (P<0.05) in all the test groups compared to the control. MCH increased significantly (P<0.05) in groups 3, 4 and 6, but increased non-significantly (P>0.05) in groups 2, 5 and 7 compared to the control. MCHC reduced significantly (P<0.05) in all the test groups compared to the control. Alcohol intake increased RDWc in groups 2, 3 and 5, but the increase was not significant (P>0.05). Alcohol intake however caused a significant increase (P<0.05) in RDWc in groups 4, 6, 7 relative to the control.

Table-3. Concentration of platelets in rats administered beers of different alcohol content

Parameters	Group 1 (Normal control)	Group 2 (Beer A (2%): 10 mL/kg bw)	Group 3 (Beer A (2%): 20 mL/kg bw)	Group 4 (Beer B (5.2%): 10 mL/kg bw)	Group 5 (Beer B (5.2%): 20 mL/kg bw)	Group 6 (Beer C (7.5%): 10 mL/kg bw)	Group 7 (Beer C (7.5%): 20 mL/kg bw)
PLT (10^{9}/L)	3312.67 ± 350.40^{a}	4732.33 ± 739.64^{c}	4462.33 ± 119.25^{c}	4615.33 ± 298.51^{c}	4822.00 ± 153.97^{c}	3821.00 ± 9.54^{a,b}	4393.00 ± 128.71^{b,c}
PCT (%)	1.88 ± 0.31^{a}	2.74 ± 0.47^{c}	2.68 ± 0.10^{c}	2.74 ± 0.23^{c}	2.96 ± 0.20^{c}	2.21 ± 0.29^{a,b}	2.63 ± 0.13^{b,c}
MPV (fl)	5.67 ± 0.42^{a}	5.80 ± 0.10^{a}	6.00 ± 0.10^{a}	5.93 ± 0.21^{a}	5.97 ± 0.12^{a}	5.77 ± 0.06^{a}	5.97 ± 0.15^{a}
PDWc (%)	26.73 ± 1.07^{a}	29.47 ± 0.64^{b}	29.43 ± 0.29^{b}	29.47 ± 0.23^{b}	29.43 ± 0.29^{b}	28.60 ± 0.17^{b}	29.27 ± 0.29^{b}

Results represent mean ± standard deviation of group result obtained (n=5).
Mean in the same row, having different letters of the alphabet are statistically significant (P<0.05).

Legend: PLT: platelet; PCT: plateletcrit; MPV: mean platelet volume; PDWc: platelet distribution width count.

PLT and PCT increased significantly (P<0.05) in groups 2, 3, 4, 5 and 7 compared to the control (table 3). MPV increased non-significantly (P>0.05) in all the test groups compared to the control, while PDWc increased significantly (P<0.05) in all the test groups compared to the control.

4. Discussion

WBC increased numerically in all the groups as compared to the normal rats but only increased significantly in groups 3, 5, 6 and 7 (table 1). The increase of WBC in these groups could be an outcome of alcohol-induced leucocytosis whereas the non-significant increase experienced in group 2 and 4 may be ascribed to mild intoxication that may have been ameliorated by the body protective mechanism such as endogenous antioxidant and immune system. It may also be due to the low volume and percentage alcohol concentration administered. Substantially, this sort of increase may be attributed to lymphocyte infiltration of poisoned cells [14]. On the other hand, this current report is in contrast to the findings of Igboh, *et al.* [15], who ascribed the decline in Hb, PCV, WBC and Lymphocytes in human subjects to the generation of reactive oxygen species, through the microsomal metabolism of alcohol by cytochrome P_{450} whose effects eventually deplete antioxidants and render blood cells very fragile, thus leading to characteristic destruction of the blood cells. There was no significant increase in the lymphocyte counts in all the groups as compared to the normal rats. Akanni, *et al.* [7], deduced that, increase in lymphocyte counts following alcohol exposure could be one of the defensive mechanisms implored to protect the body against the deleterious effect of alcohol. However, in this present study, MID and GRA results did not increase or decrease as compared to the normal rats. GRA helps to attack foreign substances that causes inflammation or infection.

Mild depletion in PCV and Hb content could arise from the toxicity of free radicals metabolized by cytochrome P450 [14, 16]. Xenobiotics can cause haemolytic anaemia when sulphydryl groups of the erythrocyte membrane are oxidized which infects injury to the erythrocyte membrane. Moreover, the depletion in PCV and Hb level may be attributed to a mild obstructed hematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and/or their enhanced removal from circulation [17]. This may be due to the beers administered. Furthermore, xenobiotic oxidants cause elevations in lipid peroxides in red cells accompanying reductions in physiological parameters such as red cell maturation factors, RBCs and Hb [18]. Also, according to Igboh, *et al.* [15], the low Hb,

PCV and RBC experienced in alcoholic subjects are indicative of massive destruction of blood cells, malnutrition due to the ability of alcohol to interfere with availability and utilization of nutrients by the body. Meanwhile, the results obtained in this study showed non-significant variation in these parameters in rats administered the alcohol as compared to the normal rats, hence at variance with the above forgoing findings. The result of this study showed that consumption of the beers may not influence the synthesis of RBC and Hb, hence, the non-significant alteration of the PCV.

Previous studies have shown depletion of RBC, Hb, WBC, PLT and haematocrit counts, while MCV and MCH were reported to be significantly high thus, the picture of the complete blood count from such studies indicated that anaemia, leucopenia and thrombocytopenia are identifiable disorders associated with severe alcohol consumers which are directly proportional to the duration of alcohol use and the quantity consumed [19, 20]. Although, the present study did record a significant decrease in RBC, Hb, WBC, it did agree with the increase in MCV and MCH as previously reported. The result of MCH showed that the duration may not significantly induce macrocytic anaemia, but may do so if the volume or alcohol content is increased. Das and Vasudevan [21], reported an elevated erythrocyte mean cell distribution width count (RDWc) which is an indication of anisocytosis in 40.6% of subjects. The finding on the RDWc was in concordance with this recent report where there was a significant increase in the level of RDWc. This means that consumption of beer may have the ability to induce anisocytosis in an animal.

It was also noted in this investigation that, PLT and PCT increased significantly in all the groups (except group 6) as compared to the normal rats which are not in line with the previous findings as in the forgoing. The result of PLT shows that consumption of beers may stimulate the production of platelets, hence, mat not induce thrombocytopenia and leukaemia. This is because thrombocytopenia has been reported to predict mortality [22]. Also, the present report did not show any significant variation in MPV as compared to the normal rats which imply that the blood platelet volume of the test animals is not lower than that of the normal animals. This correlates with the result of PLT.

5. Conclusion

Regular consumption of the three different beers used in this study for the period as in this study could induce intoxication and may influence certain immune indexes as evident in the increased WBC in the test animals. Administration of the beers may not significantly induce anaemia. Beer consumption encourages the production of platelets and may promote the stoppage of bleeding resulting from an injury.

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