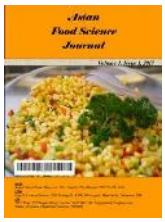

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# Reproductive Toxicity & Biomarker Response of Male Albino Rats *(Rattus norvegicus)* to a Daily Dose of Beer

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author OEO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author LKSB managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.*

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## ABSTRACT

The effect of Beer was evaluated on Albino rats. Sperm count, kidney function test, liver test, red blood cell, pack cell volume, haemoglobin, white blood cell, platelets, lymphocytes were evaluated. The results revealed the mean serum Na, K and Cl reduced from 155.33, 6.13 and 99.33 in week 1 to 146.33, 4.80 and 87.3 in week 4 with a significant difference ($P<0.05$) across the group when compared to the average control for Na and K. HCO3 had a mean of 19.0 in week 1 and a mean of 21 in week 4 in the treated group and the control group had 23.33 in week 1 and 24.33 in week 3. AST had a mean of 47.33 in week 1 which reduced to 23.33 in week 4 while ALT had a mean of 14.00 in week 1 and 15.00 in week 4 with a significant difference ($P<0.05$) across the group. The mean serum protein had a mean of 61.77 in week 1 and a mean of 68.11 in week 4 but the treated group was generally lower than the control group. Mean PCV reduced from 33.33 in week 1 to

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25.13 in week 4, Hb from 11.20 in week 1 to 12.60 in week 3 then up to 13.35 in week 4 with a significant difference (P<0.05) when comparing the test with the average control, WBC had a mean of 8.60 in week 1 and 9.03 in week 4 in the treated group, Platelet count was 300 in week 1 and 514 in week 4 with significant difference (P<0.05) when compared to the average control. RBC had a mean of 5.0 in week 1 and 7.23 in week 4. Lymphocyte for week 1 had a mean value of 65 and decreased to 62.60 in week 4 with a significant difference (P<0.05) when compared to the average control. While the mean sperm count was 62.60, 115 and 125 in week 1, 2 and 3 with the control group having 84, 475 and 575 in week 1, 2 and 3 respectively. This showed that Beer had a negative effect with a significant difference (P<0.05) on the sperm count in the test when compared with the control. These findings demonstrate that Beer had a detrimental effect on the sperm count, liver kidney and on the haematological parameter.

*Keywords: Beer; biomarker response; reproductive toxicity; rats; liver; kidney; hematology.*

## 1. INTRODUCTION

It has been reported that over 2 billion people consume alcohol worldwide and this may result in health implications which affect work, family life productivity etc. [1,2]. In industrialised countries, heavy intake of alcohol is a leading cause of preventable mortality and morbidity, second only to cigarette smoking [2]. The production and consumption of alcohol occur in most cultures of the world, from hunter-gatherer people to nation-states [3]. Beer is an alcoholic beverage made from barley (malt), hops, water, yeast and other ingredients. Globally, alcohol consumption has increased in recent decades, with all or most of that increase in developing countries [4,5]. According to a research carried out, beer is one of the oldest and most widely consumed alcoholic drinks in the world. It is also believed that beer is the third most consumed liquid after water and tea [6]. Reasons for drinking alcoholic beverages such as beer vary and include: being part of a standard diet, medical purposes, relaxant effects, euphoric effects, recreational purposes, artistic inspiration, putative aphrodisiac effects and happiness [7]. It has been shown in epidemiological studies that moderate consumption of alcoholic beverages has a protective effect against the clinical complications of coronary heart disease [8]. A study in developing monkey has demonstrated the detrimental effect of alcohol on the activation of hormone secretion that accompanies female puberty [9]. Research with adult rats has shown that alcohol increases opioid activity in the brain [10]. Leucocytes, erythrocyte and thrombocyte production and functions are affected directly by alcohol intake. Liver damage secondary to alcohol abuse also impacts red blood cells and the homeostatic mechanisms [11]. Nutritional deficiencies are caused not only by poor dietary habits practiced by alcohol abusers but by the effect of alcohol on the absorption, storage and utilisation of several vitamins [12]. Besides cardiovascular disease, many of the pathophysiological effects of alcohol ingestion are related to the pathway of ethanol metabolism [13]. Alcohol suppresses platelet production and causes thrombocytopenia which results in platelet abnormalities; inhibition of platelet aggregation [14]. Cell counts reflects the kinetics of entry and loss of cells from circulation and cell morphology reflects the status of individual cells which is a direct reflection of the health of the bone marrow, the circulation and the tissues [11]. In human, high alcohol consumption is associated with serious disorders of spermatogenesis and a reduction in testosterone and the reproductive process generally [15,16, 17]. This work is carried out to determine the effect(s) of beer on liver function, renal function, haematological parameters and sperm count on male albino Wister rat (*Rattus norvegicus*).

## 2. MATERIALS AND METHODS

*Experimental Setup:* 24 adult male rats were used, weighing from 170 to 220 g. The animals were divided into two (2) groups. Group 1 was the treatment group containing 12 male albino Wister rats. Group 2 was the control group of 12 male rats. The animals were housed in a well-constructed cage at a temperature ranging from 24°c to 26°c. They were fed with a standard diet and water from the period of acclimatization till the end of the experiment. Depending on the body weight of rats, 1.3 to 1.9 ml of Beer (S. brand) was administered daily through the oral route using a syringe. The average adult human weighs about 60 kg and they drink about 33cl of beer daily (330 ml). This weight was used to determine what 1 g body weight will be exposed to and multiplied by the weight in gram of the rat to obtain its final exposure concentrations.

## 2.1 Biochemical Analysis

Standard procedures were ensured during the collection of the blood, sperm and liver samples prior to biochemical analysis. The epididymal sperm count was done with Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and light microscope at 40× magnifications [18]. The plasma activity of Alkaline Phosphatase (ALP) was determined using Radox kit (colorimetric method) of [19]. Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen [20]. The plasma activity of aspartate transaminase AST and alanine transaminase ALT was determined using Reitman and Frankel method [21]. The serum electrolytes were determined using ISO 4000 Automated electrolyte analyser. SFRI, France.

## 2.2 Method of Data Analysis

Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

## 3. RESULTS

The results for Hepato-renal analysis Table 1 indicate that for Na, a mean value of 155.33 in week 1, 144.33 in week 2, 131.35 in week 3 and 146.33 in week 4 was recorded with a control of 133.33 in week 1, 157.33 in week 2, 136.33 in week 3 and 149.33 in week 4. The average control was 137.66. There was a significant difference ($P<0.05$) when comparing the Na level across the week. The mean potassium in the treated group was 6.13 in week 1, 4.40 in week 2, 3.90 in week 3 and week 4 was 4.80, for the control group, a mean value of 4.36 in week 1, 7.23 in week 2, 5.0 in week 3 and 5.10 in week 4. The average control was 5.42. There was a significant difference ($P<0.05$) across the group when compared to the average control. Cl had a mean value of 99.33 in week 1, 99.33 in week 2, 96.33 in week 3, and 87.33 in week 4 in the treated group, and the control group had a mean of 100.33 in week 1, 100.33 in week 2, 109.33 in week 3 and 86.33 in week 4 having an average control of 98.66. There was no significant difference ($P>0.05$) across the week. The mean value of Bicarbonate in the treated group was 19.00 in week 1, 26.00 in week 2, 21.00 in week 3 and 26.00 in week 4. The control group had a mean value of 23.33 in week 1, 23.33 in week 2, 24.33 in week 3 and 23.00 in week 4 with an average control of 23.66. There was also significant differences ($P<0.05$) across the week. The AST and ALT had a mean value of 47.33 and 14.00 in week 1, 30 and 7 in week 2, 24.0 and 12.0 in week 3, 23.33 and 15.0 in week 4 in the treated group with the control group having a mean of 17.33 and 10.33 in week 1, 34.33 and 10.0 in week 2, 23.33 and 11.00 in week 3, 23.00 and 13.00 in week 4 with an average control of 24.99 and 10.44 respectively. There was a significant difference ($P<0.05$) across the week in only ALT levels. A mean value of 61.77, 57.05, 52.33 and 68.11 in week 1, 2, 3 and 4 respectively was recorded for serum protein in the treated group. While the control group was 65.70, 72.30, 69.23 and 73.27 in weeks 1, 2, 3 and 4 respectively with an average control of 69.07. There was a significant difference ($P<0.05$) when comparing the results across the week.

The result of Haematological Analysis is shown in Table 2; Mean PCV for the treated group was 33.33, 34.50, 32.50 and 25.13 in weeks 1, 2, 3 and 4, the control group had 26.33, 32.53, 32.83 and 39.03 in weeks 1, 2, 3 and 4 with an average control of 30.56. Although there was significant difference ($P<0.05$) across the week. The mean Hb level in the control group was 9.0, 9.90, 10.33 and 13.83 in weeks 1, 2, 3 and 4 while the treated group had 11.20, 10.70, 10.43 and 12.60 in weeks 1, 2, 3 and 4 with an average control of 9.74. There was no significant difference ($P>0.05$) across the week. The RBC and WBC in the treated group was 5.0 and 8.60 in week 1, 6.54 and 9.53 in week 2, 5.88 and 8.43 in week 3, 7.23 and 9.03 in week 4, the control group had a mean of 4.33 and 9.0 in week 1, 5.56 and 9.85 in week 2, 6.04 and 7.43 in week 3, 6.90 and 6.20 in week 4 with an average control of 5.31 and 8.76. There was a significant difference ($P<0.05$) across the week in RBC alone. The blood platelet and lymphocyte had a mean value of 300 and 65 in week 1, 513 and 81.5 in week 2, 356 and 75.20 in week 3, 514.33 and 62.60 in week 4 in the treated group, while the control group had a mean value of 270 and 70 in week 1, 335.33 and 84.40 in week 2, 423 and 78.2 in week 3, 416.33 and 84 in week 4. The average control was 345 and 77.53 for the blood platelets and lymphocytes respectively with a significant difference ($P<0.05$) across the week. The sperm count Table 3 had a mean of 62.6,115,125 and 675 in week 1, 2, 3 and 4 respectively in the treated group while the control group had a mean of 84, 475, 575 and 475 in week 1, 2, 3 and 4 respectively with a significant difference across ($P<0.05$) the week.

**Table 1. Results of hepato-renal analysis**

| | | Na(mmol/l) | K(mmol/l) | Cl(mmol/l) | HCO3(mmol/l) | AST(mmol/l) | ALT(mmol/l) | Protein |
|---|---|---|---|---|---|---|---|---|
| Week 1 | control | 133.33±2.52$^{b}$ | 4.36±0.26$^{b}$ | 100.33±4.51$^{a}$ | 23.33±0.58$^{a}$ | 17.33±3.51$^{b}$ | 10.33±1.53$^{b}$ | 65.70±12.19$^{a}$ |
| | test | 155.33±2.52$^{a,A}$ | 6.13±0.06$^{a,A}$ | 99.33±1.53$^{a,A}$ | 19.00±0.00b,C | 47.33±11.50$^{a,A}$ | 14.00±2.00$^{a,A}$ | 61.77±3.54a,AB |
| Week 2 | control | 157.33±22.50$^{a}$ | 7.23±2.55$^{a}$ | 100.33± 18.50$^{a}$ | 23.33±1.53$^{a}$ | 34.33±3.51$^{b}$ | 10.00±2.00$^{a}$ | 72.30±3.36$^{a}$ |
| | test | 144.33±0.58$^{a,B}$ | 4.40±0.10$^{a,AB}$ | 99.33±0.58$^{a,A}$ | 26.00±0.00$^{b,A}$ | 30.00±2.00$^{a,A}$ | 7.00±1.00$^{a,C}$ | 57.05±4.57$^{a,AB}$ |
| Week 3 | control | 136.33±10.50$^{a}$ | 5.00±0.60$^{a}$ | 10 9.33 ±4.51$^{a}$ | 24.33±3.51$^{b}$ | 23.33±5.51$^{a}$ | 11.00±4.00$^{a}$ | 69.23±2.15$^{a}$ |
| | test | 131.33±5.51$^{a,C}$ | 3.90±0.30$^{b,B}$ | 96.33± 3.51$^{a,A}$ | 21.00±2.00$^{a,BC}$ | 24.00±1.00$^{a,A}$ | 12.00±0.00$^{a,AB}$ | 52.33±0.35$^{b,B}$ |
| Week 4 | control | 149.33±0.58$^{a}$ | 5.10±0.10$^{a}$ | 86.33±1.00$^{a}$ | 23.00a±1.00$^{a}$ | 23.00±1.00$^{a}$ | 13.00±1.00$^{a}$ | 73.27±2.15$^{a}$ |
| | test | 146.33±1.53$^{b,AB}$ | 4.80± 0.10$^{b,AB}$ | 87.33±3.51$^{a,A}$ | 26.00a±1.00$^{a,A}$ | 23.33±0.58$^{a,A}$ | 15.00±1.00$^{a,A}$ | 68.11±10.80$^{a,A}$ |
| | Average control | 137.66±9.02$^{BC}$ | 5.42±0.88$^{AB}$ | 98.66±7.12$^{A}$ | 23.66±1.65$^{AB}$ | 24.99±3.38$^{A}$ | 10.44±2.13$^{B}$ | 69.07±4.96$^{A}$ |

*$^{a-b}$ Different letters in the same column indicate significance difference (p<0.05) within the week.*
*$^{A-B}$Diffferent letters in the same column indicate significance difference (p<0.05) across the week*

**Table 2. Result of haematological analysis**

| | | PCV(%) | HB(g/dl) | RBC(x10$^{12}$) | WBC(x10$^{9}$) | Platelet(x10$^{9}$) | Lymphocyte(x10$^{9}$) |
|---|---|---|---|---|---|---|---|
| Week 1 | control | 26.33±1.52$^{b}$ | 9.00±0.30$^{b}$ | 4.33±0.15$^{a}$ | 9.00±2.50$^{a}$ | 270.00±0.00$^{b}$ | 70.00±5.00$^{b}$ |
| | test | 33.33±1.52$^{a,A}$ | 11.20±0.50$^{a,A}$ | 5.00±0.20$^{a,c}$ | 8.60±1.40$^{a,A}$ | 300.00±10.00$^{a,B}$ | 65.00±3.00$^{a,BC}$ |
| Week 2 | control | 32.53±2.95$^{a}$ | 9.90±0.90$^{a}$ | 5.56±0.70$^{a}$ | 9.85±5.65$^{a}$ | 335.33±106.00$^{b}$ | 84.40±1.40$^{a}$ |
| | test | 34.50±0.50$^{a,A}$ | 10.70±0.20$^{a,B}$ | 6.54±0.38$^{a,AB}$ | 9.53±4.95$^{a,A}$ | 513.00±15.00$^{a,A}$ | 81.50±0.00$^{b,A}$ |
| Week 3 | control | 32.83±3.95$^{a}$ | 10.33±1.15$^{a}$ | 6.04±0.64$^{a}$ | 7.43±2.85$^{a}$ | 423.00±108.00$^{a}$ | 78.20±1.40$^{a}$ |
| | test | 32.50$^{a}$±0.40$^{a,A}$ | 10.43±0.05$^{a,B}$ | 5.88±0.27$^{a,BC}$ | 8.43±3.65$^{a,A}$ | 356.00±35.00$^{a,B}$ | 75.20±4.10$^{a,AB}$ |
| Week 4 | control | 39.03±2.35$^{a}$ | 13.83±0.45$^{a}$ | 6.90±1.60$^{a}$ | 6.2±0.05$^{b}$ | 416.33±3.51$^{b}$ | 84.00±0.70$^{a}$ |
| | test | 25.13±0.95$^{b,B}$ | 12.60±1.00$^{a,A}$ | 7.23±0.15$^{a,A}$ | 9.03±0.25$^{a,A}$ | 514.33±2.51$^{a,A}$ | 62.60±3.70$^{b,C}$ |
| | Average control | 30.56b±2.69$^{A}$ | 9.74±0.70$^{B}$ | 5.31b±2.75$^{C}$ | 8.76±2.75$^{A}$ | 345.00±54.40$^{B}$ | 77.53±2.12$^{A}$ |

*$^{a-b}$ Different letters in the same column indicate significance difference (p<0.05) within the week.*
*$^{A-B}$Different letters in the same column indicate significance difference (p<0.05) across the week*

**Table 3. Results of sperm count**

| | | Sperm Count ($x10^6$) |
|---|---|---|
| Week 1 | Control | $84.00\pm125.00^{a}$ |
| | Test | $62.60\pm35.00^{b,C}$ |
| Week 2 | Control | $475.00\pm25.00^{a}$ |
| | Test | $115.00\pm75.00^{b,C}$ |
| Week 3 | Control | $575.00\pm175.00^{a}$ |
| | Test | $125.00\pm75.00^{b,A}$ |
| Week 4 | Control | $475.00\pm50.00^{a}$ |
| | Test | $675.00\pm25.00^{a,A}$ |
| | Average control | $508.33\pm402.22^{B}$ |

*$^{a-b}$ Different letters in the same column indicate significance difference ($p<0.05$) within the week.*
*$^{A-B}$Different letters in the same column indicate significance difference ($p<0.05$) across the week*

## 4. DISCUSSION

The increase in Sodium levels in the first week and then a decrease in the second week, third week and fourth week when compared to the respective control for the week indicates that beer might have affected the ability of the kidney to balance the blood electrolyte either through affecting the Antidiuretic hormone or causing damage to the kidney. This might be due to the alcoholic content of the beer. This result agrees with [22] who reported adverse effect of alcohol on the kidney of rats. This might still be the reason why the blood potassium level and Choride level reduced from a mean of 6.13 and 99.33 in week1 to 4.80 and 87.33 in week 4, indicating that prolonged consumption of beer (alcohol) has negative effect on the kidney. This finding is consistent with [23] that stated that alcohol is one of the numerous factors that can compromise kidney health and interfere with kidney function through acute or chronic consumption. The level of serum bicarbonate was significantly ($P<0.05$) low in the first week and third week of treatment, this is in agreement with [24]. The results for AST and ALT indicates a high level of AST and ALT in week 1 which was significantly higher in the treated group compared to the control group for that week. The AST and ALT levels later decreased from 47 and 14 in week 1 to 24 and 12 in week 3, indicating liver damage. The level of AST and ALT was fairly similar to the control on the fourth week showing that after 7 days of withdrawal, the liver might have started recovering from the damage. The considerably lower mean value of serum protein for week 1, 2, 3 and 4 in the treated group compared to the control of the various weeks shows that the continuous consumption of Beer (Alcohol) reduces the protein level and may be as a result of protein synthesis inhibition by alcohol intake [25]. The results from haematological parameters of this study obtained indicate that RBC, WBC and Platelets were abnormally high across the week, while there was a reduction in the blood PCV and Hb in the treated group from 33.33, and 5.0 in week 1 to 25.13 and 12.60 in week 4 compared to the control of 26.33 for PCV and 9.0 for Hb in week 1, 39.03 for PCV and 13.83 for Hb in week 4. Lymphocyte also reduced significantly across the group when compared to the average control. This finding is in conformity with the report of [22], that treatment of rats with alcohol may have adverse effect on the bone marrow, kidney and haemoglobin metabolism [26]. In his findings showed that heavy drinking (alcoholism) affects haematological parameters. The observed Significant decrease ($P<0.05$) in the level of Sperm count recorded across the week when compared to the control during the period of treatment from week I to week 3 can be attributed to heavy consumption of alcohol (in this case Beer) [27,28]. The last week (week 4) which is 7 days after withdrawal revealed a positive effect on the sperm count showing that the rat recovered from the adverse effect of beer (Alcohol).

## 5. CONCLUSION

The result clearly indicates that a daily consumption of Beer (Alcohol) has a huge detrimental effect on the sperm count and fertility at large; it also has detrimental effect on the liver and kidney. Therefore, moderate consumption of Beer and other alcoholic beverages will help avoid these adverse health conditions.

## COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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