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Effects of Chronic Alcohol Ingestion on Hematological Parameters in Albino Mice Experimentally Challenged with *Escherichia coli* Strain 0157:H7

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Abstract The aim of this study was to investigate the effect of chronic alcohol consumption on hematology when challenged with *E. coli* strain 0157:H7 using albino mice as experimental model. Eight weeks old mice (26.6 – 35.3 g) of both sexes were used in the study and were divided into 6 groups of 12 mice each using stratified random selection method. Group 1 was given 10 % ethanol (V/V) in their drinking water. Group 2 received 20 % of ethanol. The third group received 30 % of ethanol while Group 4 and 5 received 40 % of alcohol *ad-libitum* respectively. Group 6 served as control and received only distilled water. The alcohol-treated groups received ethanol for 3 weeks to establish a chronic state of alcoholism and Groups 1-4 were then challenged with *E. coli* strain 0157:H7 for 7 days. Blood samples were collected via the median canthus of the eyes from the retrobublar plexus. The blood samples were allowed to clot and the sera was obtained by aspiration into Bijou bottles for hematology analysis like packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), neutrophil, lymphocytes, monocytes, eosinophil, and basophil. There was a significance difference (P< 0.05) in the mean WBC, RBC, PCV and Hb values between various groups. The means of WBC of the groups exposed to 10 % alcohol with *E. coli* (9.2±0.1) had a significantly higher (P<0.05) value than all the other alcohol-treated groups. The mean PCV of groups exposed to 40 % alcohol with *E. coli* (36.67±0.88), 30 % alcohol with *E. coli* (37±1.08) and 20 % alcohol with *E. coli* (37.50±1.19) were significantly (P<0.05) less than that of groups exposed to 10 % alcohol with *E. coli* (39.60±1.40). Groups that consumed 10% alcohol with *E. coli*, water with *E. coli* (41.67±0.88), and water without *E. coli* (42.25±1.31) were significantly (P<0.05) lower than the group that consumed 40 % alcohol without *E. coli* challenge (46.25±0.75), but higher than the groups treated with alcohol and challenged with *E. coli*. There was significant difference (P< 0.05) in the RBC mean values between the various groups. The mean values of groups exposed to 40% alcohol with *E. coli* (899.2±116.58) and 30 % alcohol with *E. coli* (923.3±38.37) were significantly (P<0.05) lower than 20 % alcohol with *E. coli* (978.3±46.39), water with *E. coli* (985±31.75), water without (998.8±85.81) and 40 % alcohol without *E. coli* (1068.2±22.58). Neutrophil, lymphocyte and monocyte values across the various groups revealed significant differences (P< 0.05) among the different groups. The results showed that chronic alcohol (ethanol) consumption has adverse pathologic effects on the packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), neutrophil, lymphocytes, monocytes, eosinophil, and basophil. Although alcohol is generally obtained from the fermentation of starch-containing food, its abuse and daily consumption causes damage hematological parameters in the body. Thus, when such a body is challenged with a pathogenic organism, there is less resistance to systemic entry of the cells by the organism, faster access to body cell due to the dehydration effect, and a quick necrotic time due to the toxins produced by such pathogenic organisms.

Keywords: *alcohol, ethanol, albino mice, Escherichia coli strain 0157:H7, packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), neutrophil, lymphocytes, monocytes, eosinophil, and basophil*

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1. Introduction

Alcohol primarily is in the form of ethyl alcohol or ethanol. It has occupied an important place in the history of humankind for at least 8000 years [1]. Sugar fermentation into alcohol occurs regularly in nature through contact with air borne yeast. It is presently known that alcohol in moderate amounts relieves anxiety and fosters a feeling of euphoria [2].

Alcohol is known to have a dose related toxic effect on both skeletal and cardiac muscles. Numerous studies have shown that alcohol can depress cardiac contractibility and thus leading to cardiomyopathy [3]. Fatty acid ethyl esters (formed from the enzymatic reaction of ethanol with free fatty acid appear to play a role in the development of this disorder [4]. Females are at a higher risk of alcohol induced cardiomyopathy than are the males [5]. Clinical studies indicate an increased incidence of hemorrhagic and ischemic stroke in persons who drink more than 40 g - 60 g of alcohol per day [6].

Escherichia coli is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some, such as serotype O157:H7 can cause serious food poisoning in humans, and are occasionally responsible for product recalls [7]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 [8], and by preventing the establishment of pathogenic bacteria within the intestine [9,10].

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination [11,12]. The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of metagenics, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology and microbiology.

Differences in *E. coli* strains are often detectable only at the molecular level. However, they may result to changes in the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of fecal contamination in environmental samples [11,12]. New strains of *E. coli* evolve through the natural biological process of mutation and through horizontal gene transfer [13]. Some strains develop traits that can be harmful to the host animal. These virulent strains typically cause a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world [14]. More virulent strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immuno-compromised [9,14].

The aim of this study was to investigate the effect of chronic alcohol consumption on hematology when challenged with *E. coli* strain 0157:H7 using albino mice as experimental model.

2. Materials and Methods

2.1. Preparation and Harvest of *E. coli* Strain 0157:H7

The bacteria were collected from an already identified and characterized stock (ECO 6) which has previously being maintained for research purposes in the Microbiology laboratory of the Department of Veterinary Microbiology and Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. With a sterile wire-loop, some bacteria were streaked into a petri-dish containing Mac Conkey agar. A colony from here was harvested and sub-cultured on an Eosin Methylene blue agar to confirm that the colony was *Escherichia coli*. Nutrient broth (100 ml) was prepared and 10 ml of the broth was put into 10 test-tubes. These were clogged with cotton-wool and autoclaved at 121 °C for 15 min. A colony of *E. coli* was harvested from the growth on the Eosin methylene blue agar growth and was introduced into the first test-tube. This was properly mixed and a 1 ml of the mixed solution was collected and transferred into the next test-tube. This process was used to achieve a serial dilution in all the 10 test-tubes. 20 ml of Mac Conkey's agar was poured into 10 universal bottles and these were autoclaved. After allowing to cool, 0.1 ml of the mixture in each of the above serially diluted test-tubes was introduced into each of the universal bottles, after a mild, yet thorough mixing, the mixture was poured into already sterilized Petri-dishes for growth of colonies. Twenty-four (24) h later, the plates were examined and colony growth was counted. The amount per dilution was used to ascertain the amount in the parent stock, and this was used to determine the volume for infecting the mice.

2.2. Animals and Experimental Design

Eight weeks old albino mice (26.6 – 35.3 g) of both sexes were used in this study. The mice were sourced from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized for 14 days before the commencement of the experiment and were fed with commercial feed (Vital feed®, Grand Cereal Oil Mill Ltd., Nigeria) and provided with clean water *ad libitum*. The albino mice were divided into 6 groups of 12 mice each using stratified random selection method, and were kept in clean cages in the Animal House of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Group 1 was given 10 % ethanol (V/V) in their drinking water. Group 2 received 20 % of ethanol solution. The third group received 30 % of ethanol while group 4 and 5 received 40 % of alcohol *ad-libitum* respectively. Group 6 served as normal control and received only water. The alcoholic groups received ethanol for 3 weeks to establish a chronic state of alcoholism before groups 1-4 were then challenged with *E. coli* strain 0157:H7. Group 5 was not challenged with *E. coli*.

From the result obtained after the colony count, it was deduced that the parent stock contained about $2.3X10^8$ CFU/ml. 0.5 ml of the parent stock, which contains about 1.15×10^4 CFU and was used to challenge the mice intraperitoneally. All challenged animals were weighed 2 days after to evaluate the effect of the challenge on their

weight. Seven days after the challenge, the mice in each group were humanely euthanized and dissected. Ethical conditions governing the conduct of experiments with life animals were strictly observed [15,16,17].

2.3. Serum and Blood Harvesting

Blood samples were collected from the above challenged mice via the median canthus of the eyes from the retrobublar plexus. The blood samples were collected into Bijou bottles for hematology analysis which included packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), neutrophil, lymphocytes, monocytes, eosinophil, and basophil.

2.4. Body Weight

The rats were weighed with a manual weighing balance. They were weighed by putting the rats into a plastic container that had been balanced to zero (0). The weight were taken and recorded in grams (g). This was done on a weekly basis.

2.5. Packed Cell Volume (PCV)

Microhaematocrit method was used for the estimation of the packed cell volume. A heparinized capillary tube was used to collect blood from the sample bottles by capillary action up to three quarter (¾) full. The filled ends of the capillary tubes were sealed up with plasticine by holding and passing it gentle through the plasticine to avoid breakage. The capillary tube were then centrifuged using a microhaematocrit centrifuge, after placing the capillary tubes into the grooves of the centrifuge with the sealed end facing outward, in order to avoid spillage. This was centrifuged at 10,000 revolutions per minute (rpm) for 5 min. Thereafter, Hawksely microhaematocrit reader was used to read the PCV as a percentage (%).

2.6. Haemoglobin Concentration (HbC)

The cyanomethaemoglobin method was used in the estimation of haemoglobin concentration. 4mls of Drabkins solution was added to clean transparent bottles and then 20 µl of blood was added into each bottle. Afterwards, the diluents and blood was mixed adequately and allowed to stand for about 10 min to enable maximum conversion of haemoglobin to cyanomethaemoglobin.

A standard solution (blank solution) in a clean cuvette was placed into the electronic spectrophotometer to zero it. Then, the content of each transparent bottle was poured into the wiped cleaned cuvette, placed into the spectrophotometer and the reading was taken in optical density. These readings were later converted to absolute haemoglobin values in grams per deciliter (g/dl).

2.7. Total Leukocyte Count

20 µl of blood was pipetted into a small test tube containing 380 µl of white blood cell diluting fluid to make a 1:20 dilution of the blood sample. The diluted sample was loaded onto the improved Naubauer counting chamber and all cells in the four corner square (primary squares) were counted using a light microscope at x10 objective. The values gotten was multipled by fifty (50) to obtain the total white blood cell per microliter of blood [18].

2.8. Differential Leukocyte Count

The blood sample was shaken gently and a drop of blood was placed on a clean grease free microscope slide. The drop of blood was carefully smeared on the slide using a cover slip to make a thin smear. The smear was air dried and then stained by Leishman technique. The stained slides were later examined with oil immersion objective using light microscope. Hundred (100) cells were counted by the longitudinal counting method and each cell type was identified and scored using the differential cell counter. Results for each type of white blood cell were expressed as a percentage of the total count and converted to the absolute value for microliter of blood [18].

2.9. Statistical Analysis

The values obtained were computed into means and analysed using Analysis of variance (ANOVA). The means were separated using Duncan's multiple range tests at 95% confidence.

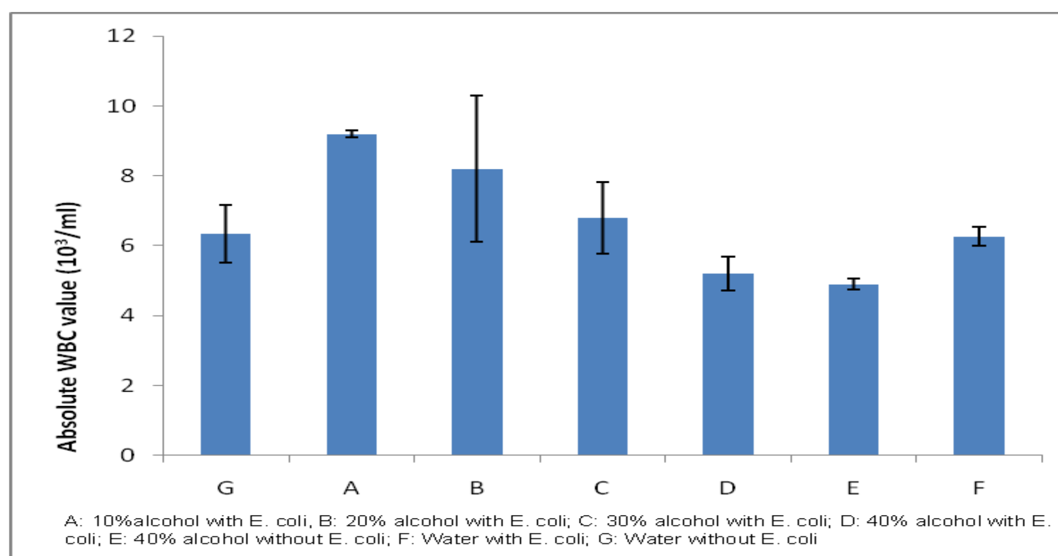

Figure 1. Comparison of mean WBC count of the different groups

3. Results

3.1. Effect of Alcohol and *E coli* Treatments on WBC Count of the Different Groups

There is a statistical significance (P< 0.05) in the mean WBC values between various groups. The means of WBC of the groups exposed to 40 % alcohol with *E. coli* (5.2±0.49) and 40 % alcohol without *E. coli* (4.9±0.15) were significantly P<0.05) less than the groups that took only water with *E. coli* (6.3±0.26), water without *E. coli* (6.35±0.83), 30 % alcohol with *E. coli* (6.8±1.03) and 20 % alcohol with *E. coli* (8.2±2.09). It was also observed that the groups exposed to 10% alcohol with *E. coli* (9.2±0.1) had a significantly higher (P<0.05) value than all the above mentioned groups (Figure 1).

3.2. Effect of Alcohol and *E coli* Treatments on Mean RBC Count of the Different Groups

There was significant difference (P< 0.05) in the RBC mean values between the various groups. The mean values of groups exposed to 40 % alcohol with *E. coli* (899.2±116.58) and 30% alcohol with *E. coli* (923.3±38.37) were significantly (P<0.05) lower than 20 % alcohol with *E. coli* (978.3±46.39), water with *E. coli* (985±31.75), water without *E. coli* (998.8±85.81) and 40 % alcohol without *E. coli* (1068.2±22.58). However, group of 10 % alcohol with *E. coli* (1.135.0±38.79) is significantly (P<0.05) higher than all above mentioned groups (Figure 2).

3.3. Effect of Alcohol and *E coli* Treatments on Mean PCV of the Different Groups

There was significant difference (P<0.05) in the PCV mean values between the different groups. The mean PCV of groups exposed to 40 % alcohol with *E. coli* (36.67±0.88), 30 % alcohol with *E. coli* (37±1.08) and 20 % alcohol with *E. coli* (37.50±1.19) were significantly (P<0.05) less than that of groups exposed to 10 % alcohol with *E. coli* (39.60±1.40). Groups that consumed 10 % alcohol with *E. coli*, water with *E. coli* (41.67±0.88), and water without *E. coli* (42.25±1.31) were significantly (P<0.05) lower than the group that consumed 40 % alcohol without *E. coli* challenge (46.25±0.75), but higher than those of other mentioned groups (Figure 3).

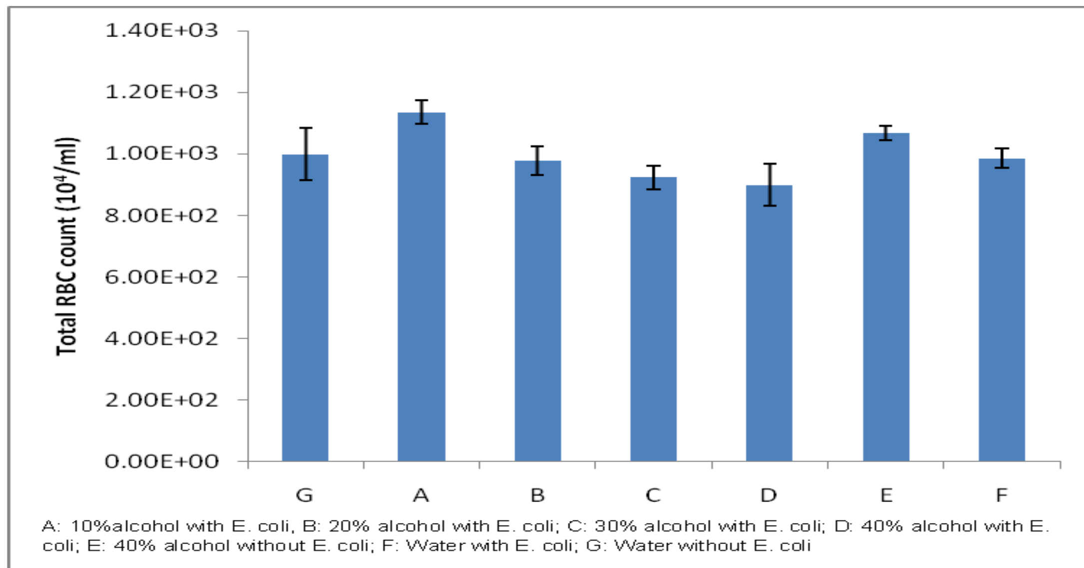

Figure 2. Comparison of mean RBC count of the different groups

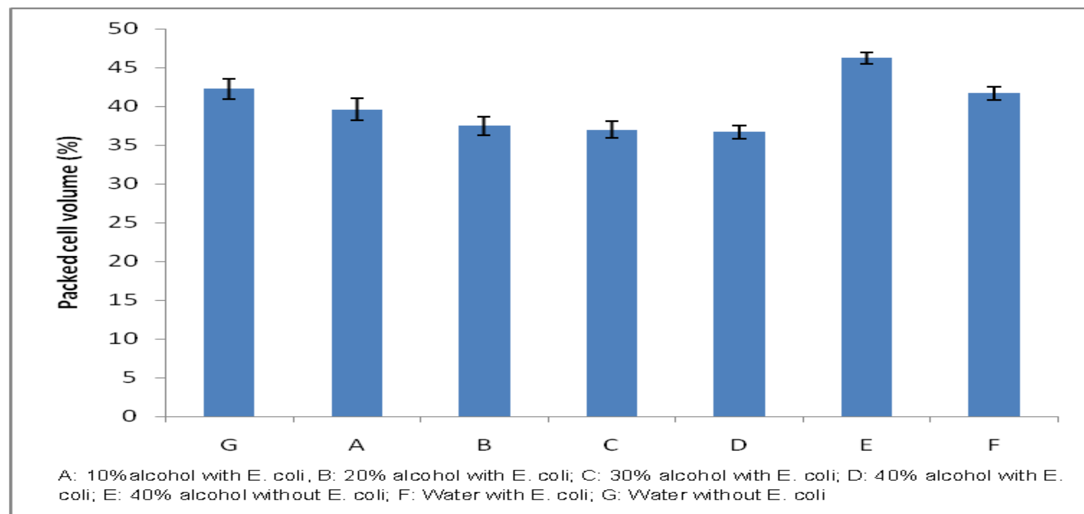

Figure 3. Comparison of mean PCV of the different groups

3.4. Effect of Alcohol and *E coli* Treatments on Mean Hemoglobin Concentration of the Different Groups

There was a significance (P<0.05) in the mean Hb concentration values between the groups. The groups exposed to 40 % alcohol with *E. coli* (10.20±0.97), 30 % alcohol with *E. coli* (10.40±.61) and 20 % alcohol with *E. coli* challenge (10.63±0.56) were significantly (P<0.05) less than the groups that consumed 10 % alcohol with *E. coli* (11.00±0.32). This group was significantly lower than groups that received water with *E. coli* (12.50±0.00) and water without *E. coli* challenge (12.68±0.48). Between water alone and 40 % alcohol without *E. coli* (12.80±0.31), there was an increase which was not statistically significant (P>0.05) (Figure 4).

3.5. Effect of Alcohol and *E coli* Treatments on Mean Neutrophil Counts

Neutrophil value across the various groups as seen in Figure 5 reveals that water without *E. coli* challenge (832.25±171.32) and 40 % alcohol with *E. coli* (889.33±27.65) are significantly (P<0.05) lower than the mean value from 40 % alcohol without *E. coli* (2006.0±181.07). However, 40 % alcohol without *E. coli* is significantly (P<0.05) lower than water with *E. coli* (2437.7±101.33) and 30 % alcohol with *E. coli* (2538.0±687.65). 20 % alcohol with *E. coli* (3535.0±9.00.26) and 10 % alcohol with *E. coli* (4787.0±151.38) had mean values significantly higher than all other groups (Figure 5)

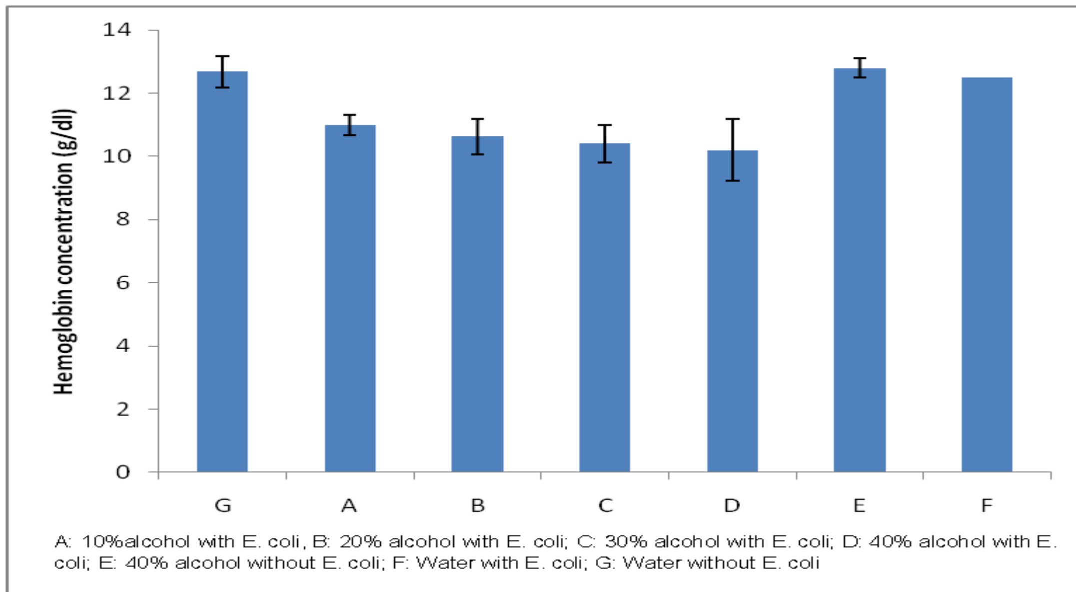

Figure 4. Comparison of mean Hemoglobin concentration of the different groups

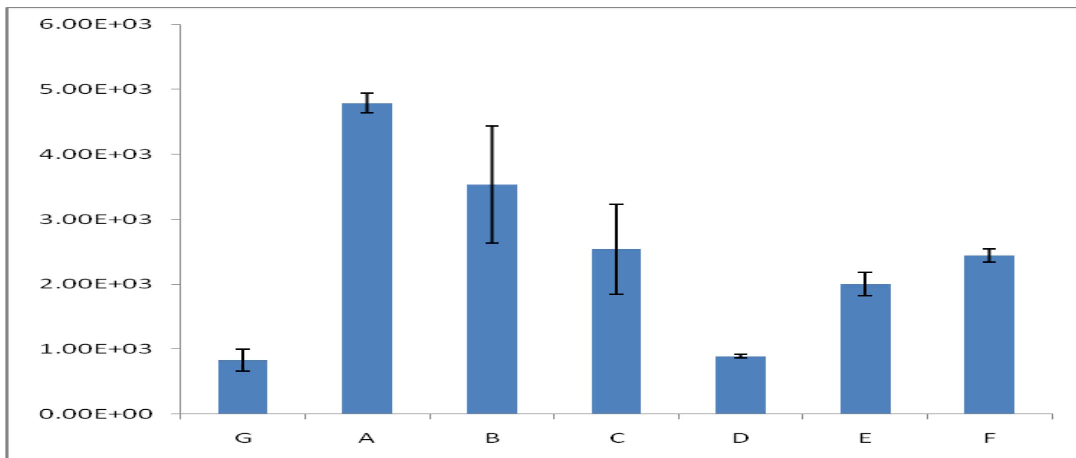

Figure 5. Comparison of mean Neutrophil counts of the different groups

Key for graph: A: 10 % alcohol with *E. coli*, B: 20 % alcohol with *E. coli*; C: 30 % alcohol with *E. coli*; D: 40 % alcohol with *E. coli*; E: 40 % alcohol without *E. coli*; F: Water with *E. coli*; G: Water without *E. coli*

3.6. Effect of Alcohol and *E coli* treatments on mean Lymphocyte counts

Lymphocyte analysis revealed that 40 % alcohol with *E. coli* (1669.3±40.34) was significantly (P<0.05) lower than 40 % alcohol without *E. coli* (2827.2±139.28). Water with *E. coli* (3750.0±155.89), 30% alcohol with *E. coli* (3904.5±396.31), 20 % alcohol with *E. coli* (4206.83±1049.92) and 10 % alcohol with *E. coli* (4275.80±29.95) had means that were significantly (P<0.05) greater than the group with 40 % alcohol without *E. coli* (1669.30±40.34). The positive control group that took water without any *E. coli* challenge (5303.80±609.76)

had the greatest lymphocyte value and this was significantly different from all other groups (Figure 6).

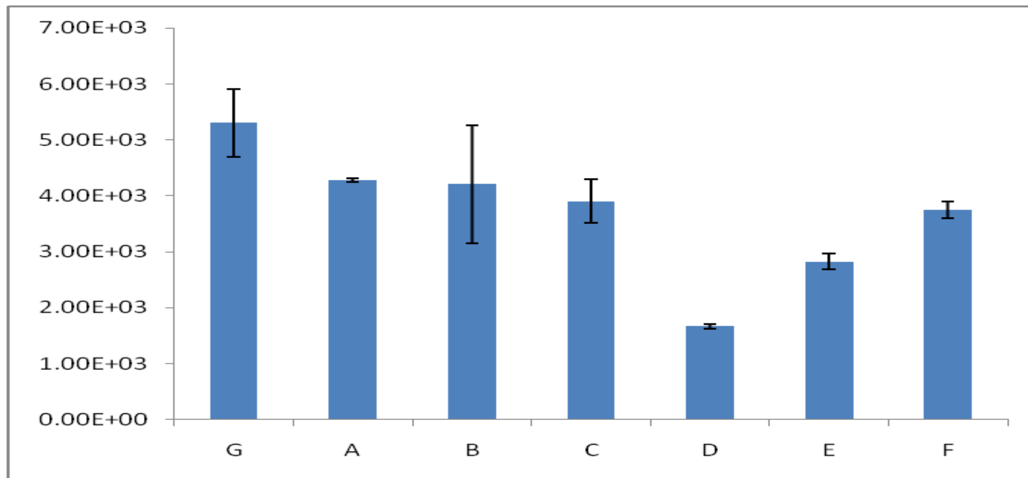

Figure 6. Comparison of mean Lymphocyte counts of the different groups

Key for all graphs: A: 10 % alcohol with *E. coli*, B: 20 % alcohol with *E. coli*; C: 30 % alcohol with *E. coli*; D: 40 % alcohol with *E. coli*; E: 40 % alcohol without *E. coli*; F: Water with *E. coli*; G: Water without *E. coli.*

3.7. Effect of Alcohol and *E coli* Treatments on Mean Monocytes Counts

The monocyte analysis revealed that 40 % alcohol without *E. coli* (17.00±12.02), 10 % alcohol with *E. coli* (46.80±29.57), 40 % alcohol with E. coli (69.00±1.15), water without *E. coli* (75.75±10.00) and 30 % alcohol with *E. coli* (112.25±11.89) were all significantly (P<0.05) lower than group exposed to 20 % alcohol with *E. coli* (318.25±146.24). There was no defined progression in the mean values in this analysis (Figure 7).

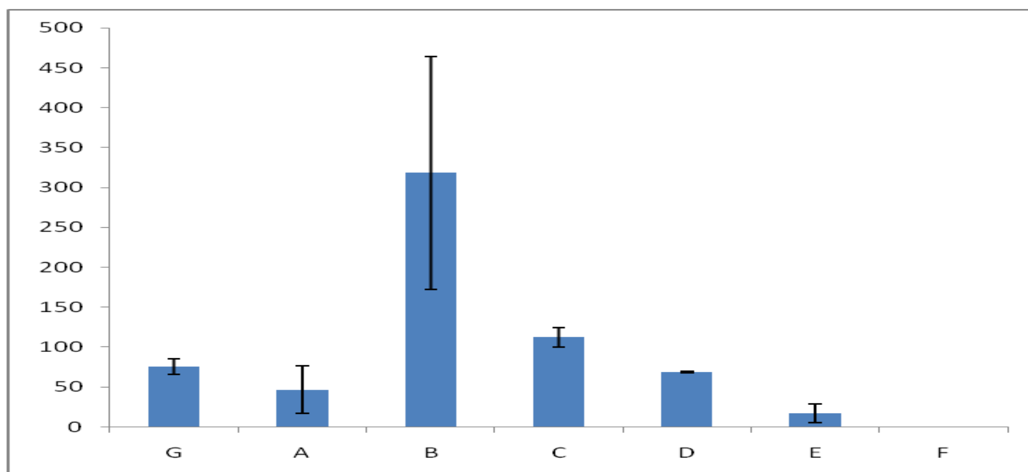

Figure 7. Comparison of mean Monocytes counts of the different groups

Key for all graphs: A: 10 % alcohol with *E. coli*, B: 20 % alcohol with *E. coli*; C: 30 % alcohol with *E. coli*; D: 40 % alcohol with *E. coli*; E: 40 % alcohol without *E. coli*; F: Water with *E.* coli; G: Water without *E. coli*

4. Discussion

From the results obtained from the blood analysis, it was noticed that alcohol consumption in the presence of *E. coli* infection generally causes significant changes in the blood parameters of animals. This may be caused by the effect of the alcohol on the various organs and the toxins produced by the *E. coli* in the blood [19]. It was also observed that the highest level of significance is seen within the PCV means. Alcohol caused a decrease in the total WBC value and in the presence of E. coli, this decrease was more noticeable. The higher the alcohol concentration, there is a corresponding decrease in the total WBC value. This decrease in the WBC mean value agrees with Szabo [20], who noticed a 5 fold depression of immunity between a 10 % and 30 % alcohol consuming group. We showed that alcohol alone causes an increase in the level of RBC in the body. But when challenged with *E. coli*, there was a concentration-dependent decrease in the RBC mean value. The reason for this cannot be fully explained, but it is thought to be due to dehydration of the body. Alcohol has been known to cause dehydration of tissues increasing the water content of the blood which is eventually eliminated by the kidneys [21]. The atria arrhythmia could cause a relative increase in the blood volume in circulation as seen in this work. From this work, we saw that alcohol causes an increase in the PCV (Packed Cell Volume) and Hemoglobin concentration values, but when challenged with *E. coli*, there is a significant drop. This may be due to the reasons enumerated in the point above. These values are directly proportional to the RBC values of the animal. Alcohol

caused a significant increase in the neutrophil level of the mice, and in the presence of *E. coli* challenge, the neutrophil level dropped significantly. The pseudo increase appears to be as a result of haemo-concentration of the blood, which when challenged with an infection like *E. coli*, caused a fall in the number of the neutrophils.

Alcohol consumption causes a decrease in the lymphocyte level of the blood, and when challenged with *E. coli*, this level is further lowered significantly. The studies revealed that areas of necrosis and neutrophilic infiltration which progressed further to cirrhosis was due to an increase in the concentration of ethanol consumed. This agrees with other findings [22]. The primary effects are fatty infiltration of the liver, hepatitis, and cirrhosis. Because of its intrinsic toxicity, alcohol can injure the liver in the absence of dietary deficiencies [23].

Certain strains of *E. coli*, such as O157:H7, O121 and O104:H21, produce potentially lethal toxins. Food poisoning caused by *E. coli* is usually caused by eating unwashed vegetables or undercooked meat. O157:H7 is also notorious for causing serious and even life-threatening complications such as haemolytic-uremic syndrome. Severity of the illness varies considerably. It can be fatal, particularly to young children, the elderly or the immunocompromised, but is more often mild. If *E. coli* bacteria escape the intestinal tract through a perforation (for example from an ulcer, a ruptured appendix, or due to a surgical error) and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. Recent research suggests that treatment with antibiotics does not improve the outcome of the disease, and may in fact significantly increase the chance of developing haemolytic-uremic syndrome [24].

Intestinal mucosa-associated *E. coli* are observed in increased numbers in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis [25]. Invasive strains of *E. coli* exist in high numbers in the inflamed tissue, and the number of bacteria in the inflamed regions correlates to the severity of the bowel inflammation enterotoxigenic *Escherichia coli* (ETEC).

Alcohol consumption elevates the level of tissue plasminogen activator, a clot dissolving enzyme [26], decreasing the likelihood of clot formation. Decreased fibrinogen concentrations seen following alcohol consumption also could be cardio-protective [27], and epidemiological studies have linked the moderate consumption of alcohol to platelet formation inhibition [28]. Cardiac conditions, including a prolongation of the QT interval, prolongation of ventricular re-polarization and sympathetic stimulation are seen in alcohol consumption [29,30].

5. Conclusion

In this work, it was noticed that alcohol causes a haemo-concentration. This concentration was not observed in the groups that took only water. When challenged with *E. coli*, these secrete toxins which cause faster damage (death) to the blood cells.

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