The assessment of alcohol effects on red blood cell indices in rats

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INTRODUCTION

Alcohol can cause a significant alteration of cells, tissues, and organs. In particular, ethanol exposure induces cell membrane remodeling in different cells and lipid vesicles including membrane fluidization.[1] Moreover, erythrocyte’s stability based on various factors and it has been reported that increased levels of erythrocyte hemolysis and chronic anemia is related to alcoholism.[2,3] These phenomena may be related to the effects of alcohol on the red blood cells (RBCs) membrane because unmetabolized ethanol can have a direct effect on membrane properties, which is generally causing an increase in fluidity.[4,5] Interestingly, alcohol metabolites can induce oxidative stress in cell membranes and as RBCs do not express alcohol dehydrogenase enzyme or ethanol-inducible cytochrome P-450 (CYP2E1) so catalase is considered as the only erythrocyte enzyme capable of oxidative metabolizing ethanol to acetaldehyde.[6]

ABSTRACT

Introduction: Alcohol has many serious effects on cells and tissues and consequently causes major health diseases. Erythrocyte functions are depending on many factors which are shown increased in red blood cells (RBCs) dysfunction is related to alcoholism. Objective: The aim of the present study is to evaluate the effects of alcohol on erythrocyte indices. Materials and Methods: The experiment includes 20 rats divided into four groups under the same environment and same weight which are continuously fed with different alcohol concentrations (15%, 20%, and 25%), and one control that feeds with water only for 21 days. Results: The findings are that RBC’s indices increased significantly (*P < 0.05) with the alcohol concentrations such as mean corpuscular volume, mean corpuscular hemoglobin (Hb) (MCH), MCH concentration, Hb, and hematocrit except the RBC counts. Conclusion: In conclusion, alcoholism can induce different effects on RBC indices particularly size and mass of Hb and its concentration in RBCs which can cause anemia and may return normal after alcohol abstinence.

KEY WORDS: Alcohol, Anemia, Erythrocyte, Macrocyte, Red blood cell indices
structure), lowered resistance to infection, and a decrease in the ability to fight off infections.[13]

The aim of the study is to assess the effect of different alcohol concentrations consumption on RBC indices such as erythrocyte (RBC) counts, hemoglobin (Hb) concentration, hematocrit (HCT) percentage (percentage of RBCs in blood), mean corpuscular volume (MCV) (RBCs size), mean corpuscular Hb (MCH) (mass of Hb in RBCs), and mean corpuscular Hb concentration (MCHC) in rats.

MATERIALS AND METHODS

Materials
Ethylene diaminetetraacetic acid (EDTA) tubes, plain tubes, microhematology tubes, diethyl ether as esthetic agent for rats, 20 rats, diluted ethanol (15%, 20%, and 25%), different syringe size, Genex count 60 devices to analyze the blood samples, microscope, hematoxylin and eosin stain, cages, thermometer, gastric lavage tube, and stainless steel cages were used.

Methods

Experiment protocol and animal care
A total of 20 rats with same weight (200 g/rat) were divided into four groups which include five rats in each cage. Group I was the control while Group II was 15% alcohol feeding rats, Groups III and IV were fed with 20% and 25% alcohol, respectively. The rats were housed into stainless steel cages in an animal house with a 25°C to keep the adaptation of the rats in the same environment. The humidity was between 50% and 60% and all the animals care procedures were guided according to the committee of animal house in the University of Baghdad.

Alcohol administration for 21 days
Alcohol of different concentrations was prepared (15%, 20%, and 25%). Moreover, alcohol of various concentrations was used to feed the rats which were fed for 21 days as continuous feeding by gastric lavage with daily observation to ensure normal and proper feeding. Moreover, one of the five groups was fed with water (control group).

Blood sampling
Before feeding, blood samples were drawn from all rats to check the normal values of different erythrocyte properties (RBCs count, Hb, HCT, MCH, MCHC, and MCV), then blood samples were collected from the tail vein of rats after using diethyl ether as esthetic. After 21 days, blood samples were collected again to evaluate the changes in RBC’s indices.

RBC’s indices measurement (RBCs count, Hb, HCT, MCH, MCHC, and MCV)
Samples were analyzed in laboratory using Genex count 60 device to count the values of RBC indices (RBCs count, Hb, HCT, MCH, MCHC, and MCV). Furthermore, all samples were collected in EDTA tubes to prevent clotting until the required tests were done.

Kidney and liver histological analysis
Paraffin blocks procedure was used to analyze the tissue of kidney and liver after alcohol feeding to check if there is relation between RBC’s indices and the histology of these organs. First of all, rats were sacrificed by overdose of anesthesia and their kidneys and livers were removed and sacked in formalin bottles. Tissues were inserted in paraffin blocks in very precisely spaced and sections from each block were cut using microtome then placed in microscope slide. These slides were stained with hematoxylin and eosin to analyze the tissues under the microscope.

Statistical analyses
The experimental data were presented by mean ± S.E. Moreover, Minitab software was used to analyze the data and t-test was used to evaluate the data before and after ethanol administration. The differences were considered when \( P > 0.05 \).

RESULTS
In this study, the RBC indices and histology of kidney and liver are analyzed to assess the effect of alcohol on these cells. The results are shown in Table 1 which represents the control group and the other groups after 21 days of alcohol feeding with 15%, 20%, and 25% concentrations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I control</th>
<th>Group II 15%</th>
<th>Group III 20%</th>
<th>Group IV 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count</td>
<td>5.172±2.28</td>
<td>5.84±0.48</td>
<td>5.92±0.34</td>
<td>5.2±0.71</td>
</tr>
<tr>
<td>HGB</td>
<td>10.58±4.86</td>
<td>12.24±1.21</td>
<td>12.3±0.95</td>
<td>10.76±1.6*</td>
</tr>
<tr>
<td>HCT</td>
<td>29.3±13.06</td>
<td>33.44±3.11</td>
<td>34.02±2.16</td>
<td>30.14±3.96*</td>
</tr>
<tr>
<td>MCV</td>
<td>56.5±1.286</td>
<td>57.28±0.73</td>
<td>57.52±1.68*</td>
<td>58.04±1.52*</td>
</tr>
<tr>
<td>MCH</td>
<td>19.8±1.54</td>
<td>20.86±0.46*</td>
<td>20.7±0.612*</td>
<td>20.58±0.759*</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.32±2.08</td>
<td>36.6±0.339</td>
<td>36.06±0.66*</td>
<td>35.58±1.056*</td>
</tr>
</tbody>
</table>

Suffix (*) indicates significant change, RBCs: Red blood cells, HCT: Hematocrit, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, SD: Standard deviation
RBC Indices Assessment
Alcohol has different impacts on various RBC’s parameters depending on concentration. MCH of RBCs in rats was fed with 15% alcohol shows a significant change ($P^* < 0.05$) in compare with the control ones, but the others RBC’s indices are not shown any significant changes [Figure 1].

Moreover, rats were fed with 20% alcohol show more significant values of different RBC’s parameters such as MCH, MCV, and MCHC ($P^* < 0.05$) in compare to the control samples [Figure 2].

Furthermore, alcohol concentration of 25% induces a significant change ($P^* < 0.05$) in all RBC’s indices (MCH, MCV, MCHC, HB, and HCT) except RBCs count [Figure 3].

Histological assessment of kidney and livers tissue of the rats
The histological findings are shown no major changes or injury in renal tissues, that there is no major change or injury in renal tissues, particularly in interstitial fibroblasts which is responsible for erythropoietin secretion which is responsible on stimulation of bone marrow to produce RBCs [Figure 4].

However, liver tissue demonstrates certain changes in the groups exposed to ethanol when compared to control Group I such as dilated and congested central veins, vacuolar degeneration of cytoplasm of hepatocytes with foci of necrosis and bile leakage [Figure 5].

DISCUSSION
Alcohol has long been listed in textbook as a cause of macrocytosis and anisocytosis with or without folate deficiency. In the present study, it is a trial to investigate the acute effect of alcohol consumption on RBC’s indices. The observations are shown that alcohol concentration is directly proportional with the RBC’s properties, particularly MCV, MCH, MCHC, and HCT [Table 1]. Acute alcohol toxicity has a direct effect on folate metabolism which is important for hematopoietic system. Moreover, short exposure for alcohol can interfere with several folate aspects such as folate intake, intestinal absorption, and mainly

Figure 1: The significant change ($P^* < 0.05$) of the mean corpuscular of hemoglobin after 21 days of alcohol feeding with 15% concentration

Figure 2: The effect of 20% alcohol concentration on different red blood cells parameters with a significant change in mean corpuscular hemoglobin, mean corpuscular volume, and MCH concentration ($P^* < 0.05$) after 21 days of rats feeding
storage and release of folate from the hepatocyte. This is clinically manifested as macrocytosis, megaloblastosis, anisocytosis, and a point to anima.\cite{14} Therefore, this can be explained the observation of significant ($P^*$ < 0.05) increase in MCV of the RBCs after 21 days of all alcohol concentrations in rats [Figures 1-3]. Further, in this point, histological study of liver [Figure 4] appears that even in acute alcohol intake, liver damage can happen and may affect folate storage and release as a result macrocytosis observed in all rats [Table 1].

The adverse effects of alcohol on the blood synthesis or hematopoietic system are both direct and indirect. The direct sequels of excessive alcohol consumption include toxic effects on the bone marrow, the blood cell precursors, mature RBCs, white blood cells, and platelets. The indirect effects include nutritional deficiencies that compromise the production and function of various blood cells. These direct and indirect effects of alcohol collectively can result in serious medical problems for alcoholism.\cite{15,16}

In this study, the effect of acute exposure of three different concentrations of alcohol (15%, 20%, and 25%, respectively) on the various red blood cell indices is assessed in rats. Since alcohol’s toxic effects are dose dependent, anemia which is represented by change in RBCs capacity of dealing with oxygen is also related with increasing the concentration of administered alcohol.\cite{17,18} Moreover, the MCV and MCH were significantly more in alcohol administered groups when compared to control group, an increased MCV can be found in patients with folic acid or Vitamin B12 deficiency (as in the case of megaloblastic anemia) because alcohol can cause malnutrition.\cite{12} Liver diseases cause characteristic structural abnormalities in these cells, resulting in fewer than normal or non-functional mature blood cells and particularly increase in RBC’s size (MCV) due to elevation of lipid membrane as shown in Figure 5.\cite{12,17,18}

Elevated MCV and MCH appear to be characteristic of alcohol abusers in the presence or absence of anemia. Interestingly, this can be supported that MCV
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has frequently been used as screening procedure for detecting alcohol abuse because it has relatively low sensitivity for this purpose.[13]

Moreover, the presence of enlarged RBC’s in the blood can be indicative of a variety of disorders in addition to alcoholism, including different kinds of anemia and a dysfunction of the thyroid gland, the enlarged RBCs (increase in MCV) in patients with macrocytosis generally are uniformly round, in contrast to the more oval cells characteristic of megaloblastic anemia.[16,20]

Thus, people who drink excessive amounts of alcohol can develop macrocytosis even in the absence of other factors associated with RBC enlargement such as alcoholic liver disease or folic acid deficiency; precise mechanism underlying macrocytosis still is unknown. However, alcohol is suggested to be the main cause of macrocytosis and strongly related to folate metabolism disorder and liver damage as shown in Figure 4. Hence, alcohol appears to interfere directly with RBC development because the macrocytes disappear within 2–4 months of abstinence.[21,22]

In addition, the findings of the study report that MCH and MCHC are significantly increased (P* < 0.05) with alcohol concentrations [Figures 2 and 3]. The explanation of this phenomenon can be attributed to either RBCs hemolysis or folate deficiency.[23] According to the histological study of the hepatocytes [Figure 4] which is demonstrated a complicated damage in the liver of the rats after 21 days of alcohol intake which may lead to decrease folate release from the liver as a result RBC’s mean corpuscular Hb and Hb concentration (MCH and MCHC) are increased [Figures 2 and 3]. Moreover, alcohol can induce hemolysis for RBCs which can lead to increase the Hb and consequently the MCH and MCHC.[2] Therefore, 25% concentration of alcohol significantly increases (P* < 0.05) the Hb [Figure 3] in addition to MCH and MCHC.[2]

Furthermore, iron is essential to RBC functioning and iron deficiency which is commonly caused by excessive blood loss can result in anemia. In many alcoholic patients, blood loss and subsequent iron deficiency are caused by gastrointestinal bleeding. Iron deficiency in alcoholics often is difficult to diagnose; however, it may be masked by symptoms of other nutritional deficiencies (e.g., folic acid deficiency) or by coexisting liver disease and other alcohol-related inflammatory conditions.[24-27]

Importantly, RBCs production and maturation are stimulated by erythropoietin secretion from peritubular fibroblast in renal cortex.[28,29] The tissue assessment of the kidney of the rats after different concentrations of alcohol intake for 21 days is shown that there is no significant damage in the peritubular site which is responsible for erythropoietin secretion as a result no significant change in RBCs count (P > 0.05); therefore, short period of alcohol exposure does not appear to affect renal tissue function and particularly erythropoietin secretion [Figure 5].[30]

CONCLUSION

Acute alcohol exposure can cause increase in RBCs size (MCV) and macrocytosis anima due to lipid membrane damage as a result of liver damage. Moreover, Hb which is incorporated in RBCs also elevated significantly after alcohol administration and that leads to MCH, MCHC, and HCT increase. Finally, alcohol may induce folate deficiencies and liver damage that can lead to RBC’s indices abnormalities.

REFERENCES


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