Effects of Maalox Plus® Antacid and PureCal® Calcium Supplements on Haematological and Biochemical Parameters of Rats Subjected to Alcohol Intoxication

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Abstract

Habitual intake of alcohol is associated with health complications including depletion of the body’s stores of magnesium and calcium. In chronic alcoholism, treatment with magnesium and calcium has shown to normalize elevated enzyme activities and clinically relevant parameters. This study examined the effects of Maalox plus® antacid and PureCal® calcium which are rich in magnesium and calcium, respectively, on the haematological and biochemical profiles of rats exposed to acute alcohol intoxication. Alcohol was administered orally at a dosage 5 g/kg body weight for five days and supplements for two days of the week in duration of 28 days. Haematological and biochemical profiles were determined using automated analyzer. Statistical comparison was done using one way ANOVA followed by Tukey’s test. Alcohol caused neutrophilia, eosinophilia and clinically relevant parameters. This study examined the effects of Maalox plus® and PureCal® were ineffective against neutrophilia but effective in normalizing eosinophilia. Basopenia was normalized by 4.25 mg/kg magnesium and calcium at 4.25 mg/kg and 8.5 mg/kg. Macrocytic anaemia was another alcohol disorder that was reversed by Maalox plus® and PureCal® except in animals given 8.5 mg/kg Mg and those co-treated with Ca and Mg. The alcohol induced thrombocytopenia was unaffected by the drugs. Alcohol did not significantly affect liver enzymes, lipids, creatinine and BUN levels. However, the drugs lowered BUN levels in animals given 8.5 mg/kg Mg, 4.25 mg/kg calcium and those co-treated with calcium and magnesium. Alcohol did not influence serum levels of Mg$^{2+}$ but it significantly increased Ca$^{2+}$ and lowered K$. Maalox plus® and PureCal® normalized the hypercalcemia in a dose-dependent manner. Alcohol induced hypokalemia was normalized by 4.25 mg/kg Mg and 17 mg mg/kg Ca. Maalox plus® and PureCal® had alleviated the alcohol induced adverse effects. These findings suggest that the drugs are useful agents that could have applications in the management of adverse effects associated with acute alcohol intoxication.

Introduction

Alcohol is an addictive drug whose dangers were recognized early and surviving literature of the Greeks shows that around 1700 BC there was warning against excessive consumption of alcohol [1]. Consumption of alcohol starts early during adolescence and this has been associated with poor outcomes in adulthood [2]. Health strategies to reduce or delay early substance exposure thus focus on adolescents [3]. Alcohol consumption in moderation has been reported to lower the risk of contracting coronary heart disease which is a major contributor of mortality in developed countries [1]. Beneficial effects of alcohol on the cardiovascular system can be attributed to alcohol elevating high density lipoprotein, fibrinolysis and endothelial function while on the other hand reducing plasma viscosity, fibrinogen concentration, platelet aggregation and coagulation [4].

On the contrary excessive alcohol consumption could lead to serious health consequences. It is the third largest causative agent for disease contributing 4% to the global disease burden [5]. An estimated 2.5 million deaths are attributed directly to alcohol yearly [6]. Harmful effects of alcohol include damage to the liver [7], brain [8] and the gastrointestinal tract [9]. Abuse of alcohol is also known to cause fetal abnormalities, cardiovascular disease and inhibition of CNS activity [10]. Excessive consumption of this xenobiotic has also been implicated in increase of susceptibility to lung and respiratory infections [11]. Malnutrition is also a common feature of excessive indulges and includes deficiencies of proteins, vitamins and minerals [12].

Many toxic effects of alcohol appear to be mediated through electrolyte imbalances especially hypomagnesaemia and hypocalemia. Magnesium deficiency is a common episode among alcoholics and this is because the body excretes 260% more magnesium within minutes of drinking alcohol [13]. Other factors contributing to hypomagnesemia include diarrhea, vomiting, ketosis and starvation [14]. Magnesium deficiency can cause a wide variety of features including hypocalcemia, hypokalemia, cardiac and neurological manifestations [15]. Animal studies have shown that magnesium deficiency aggravates the hepatic damage that is caused by alcohol [16].

Consequences of alcohol consumption include a direct toxic effect on the bone marrow, blood cell precursors and mature white blood cells thus compromising the immune system [17]. Alcohol intoxication has detrimental effect on many organs in the body with promotion of organ damage being attributed to aldehyde forming protein and DNA adducts [18].

The resultant alteration of electrolyte balance together with acid-base disorders as a consequence of alcohol intoxication have a hand in morbidity and mortality observed in alcoholics [19]. Oral magnesium supplementation is necessary for restoration of magnesium balance.
and concomitant correction of hypocalcemia [20]. Studies suggest that magnesium treatment in alcoholics may help normalize elevated enzyme activities such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma Glutamyltransferase (GGT) and some clinically relevant parameters [21].

Disturbances in serum concentration of calcium and magnesium are associated with disturbances of physiologic functions manifested by numerous clinical symptoms and signs. It has been suggested that balancing calcium and magnesium intake ratios has an alleviatory effect on coronary vascular disease. The current study uses magnesium and calcium singly and in combination to come up with a prevention strategy towards management of acute alcohol toxicity.

Materials and Methods

Materials

Alcohol (Smirnoff Vodka 37.5%, UDV Kenya Ltd., Nairobi, Kenya) was purchased from the Kenya Breweries staff shop. Maalox plus® antacid (Winthrop Pharmaceuticals Ltd, Guilford, UK) suspension containing 200 mg magnesium hydroxide per 5 ml was used as the source of magnesium. Calcium supplementation was achieved through administration of PureCal® calcium (Fourtill Life Sciences Ltd., Mumbai, India) that contained 160 mg calcium per 5 ml. Both the antacid and calcium supplement were procured from local chemists.

Experimental animals

A total of 50 Wistar rats aged between 7-8 weeks were used in this study. The animals were bred and housed at the Department of Biochemistry and Biotechnology animal house. They were housed in cages at conventional conditions at a temperature of 22-25°C and a 12 h light and dark cycle. The animals were fed with commercially available standard chow diet and water ad libitum. Animal studies were done following the ethical guidelines of the Guide for the Care and Use of Laboratory Animals (Institute of laboratory Animal Resources, 1996).

Experimental design

The animals were allocated randomly to ten groups of five animals each. The negative controls received water only while the positive control group was treated with alcohol. The other groups were first treated with alcohol followed by either Maalox plus® antacid, or PureCal® calcium or a combination of both. Details of the treatment regimens are as presented in Table 1. Alcohol was administered at a dose 5 g/kg body weight for five days in a week. The drugs were administered for the subsequent two days after alcohol ingestion [22]. Maalox plus was diluted 1:40, 1:20 and 1:10 to achieve dosages of 4.25 mg/kg, 8.5 mg/kg and 17 mg/kg magnesium, respectively. On the other hand PureCal® was diluted 1:32, 1:16 and 1:8 to come up with dosages of 4.25 mg/kg, 8.5 mg/kg and 17 mg/kg calcium, respectively. All the solutions were administered through the oral gavage [23] using a cannula and treatment was continued for 28 consecutive days.

Sampling

48 h after the final day of alcohol administration venous blood was collected from the tail of the rats and used for hematological analysis. The animals were euthanized using diethyl ether and blood drawn through cardiac puncture. Serum was processed and used for biochemical analysis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Water (negative control)</td>
</tr>
<tr>
<td>B</td>
<td>5 g/kg alcohol (positive control)</td>
</tr>
<tr>
<td>C</td>
<td>5 g/kg alcohol+4.25 mg/kg magnesium</td>
</tr>
<tr>
<td>D</td>
<td>5 g/kg alcohol+6.5 mg/kg magnesium</td>
</tr>
<tr>
<td>E</td>
<td>5 g/kg alcohol+17 mg/kg magnesium</td>
</tr>
<tr>
<td>F</td>
<td>5 g/kg alcohol+4.25 mg/kg calcium</td>
</tr>
<tr>
<td>G</td>
<td>5 g/kg alcohol+8.5 mg/kg calcium</td>
</tr>
<tr>
<td>H</td>
<td>5 g/kg alcohol+17 mg/kg calcium</td>
</tr>
<tr>
<td>I</td>
<td>5 g/kg alcohol+4.25 mg/kg magnesium+4.25 mg/kg calcium</td>
</tr>
<tr>
<td>J</td>
<td>5 g/kg alcohol+4.25 mg/kg magnesium+8.5 mg/kg calcium</td>
</tr>
</tbody>
</table>

Table 1: Details of the treatment regimen.

Hematological analysis

The first drop of venous blood was wiped off and the following five drops collected in EDTA vials. A full haemogram was conducted using a haematological analyzer (Mindray Beckman Coulter 6800, Shenzhen Mindray Bio-Medical Electronics Co Ltd. Shenzhen, and China) [24]. The parameters determined were total White Blood Cells counts (WBC), Neutrophils (NEUT), Eosinophils (EOSI), Basophils (BASO), Lymphocytes (LYMP), Red Blood Cells (RBC), Platelets (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Biochemical analysis

Blood was obtained with the aid of a syringe through cardiac puncture and transferred to a plain tube. The blood was allowed to clot at room temperature and then centrifuged at 1500 g for 10 min. The supernatant was then transferred into a polypropylene tube. Biochemical parameters were analyzed using a chemical analyzer (Selectra ProS, ELITechGroup, Milsebeek Netherlands) as per the manufacturer's recommendation. The parameters analyzed were Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Gamma Glutamyl Transferase (γ-GT), Creatinine (CREAT), Total Cholesterol (T-CHOL), High Density Lipoproteins (HDL), Blood Urea Nitrogen (BUN), Magnesium (Mg²⁺), Calcium (Ca²⁺) and Potassium (K⁺).

The reagents ALT/GPT 4+1 SL, AST/GOT 4+1 SL, GAMMA GT SL were used to assay ALT, AST and γ-GT respectively. Total cholesterol, HDL, BUN and CREAT were determined using CHOLESTEROL PAP SL, CHOLESTEROL HDL SL 2G, UREA UV SL and CREATININE PAP SL stable liquid reagents, respectively. Magnesium, calcium and potassium were determined by the use of ion selective electrodes. All reagents and the standards were supplied by ELITech Clinical Systems, Netherlands.
Data management and statistical analysis

Experimental data on the haematological and biochemical parameters was obtained and results were presented as means ± standard error of mean. One-way Analysis of Variance (ANOVA) was performed to determine whether there were statistically significant differences among the ten experimental groups. This was followed by Tukey’s post hoc test for multiple comparisons to compare individual groups and to determine which means differed significantly (p<0.05). All statistical analysis was performed using WINKS Statistical Data Analysis (SDA) and graphs software. Data was presented as text, tables and figures.

Results

Effects of alcohol, Maalox plus® and PureCal® on hematological parameters

Alcohol at a dose of 5 g/kg body weight resulted in the increased proliferation (p<0.05) of neutrophils and eosinophils and the suppression (p<0.05) of basophils (Table 2). Treatment with Maalox Plus® antacid and PureCal® calcium was ineffective in reversing the alcohol induced neutrophilia. On the contrary Maalox Plus® and PureCal®, either singly or in combination, were effective in normalizing the alcohol induced eosinophilia. Magnesium at a dose of 4.25 mg/kg and calcium given at 4.25 mg/kg and 8.5 mg/kg were also able to normalize the basophil levels. All other treatments had no influence on the alcohol induced basopenia.

On the other hand alcohol had no significant effects on the lymphocyte and monocytes levels (p>0.05). Surprisingly, administration of Maalox Plus® and PureCal®, either individually or in combination, was found to cause lymphopenia (p<0.05). For Maalox Plus® this effect was dose dependent but that for PureCal® it was dose independent.

Alcohol was responsible for causing macrocytosis and anaemia (Table 2). Maalox Plus® and PureCal® were able to prevent anaemia from developing except for the treatments where magnesium was given at a dose of 8.5 mg/kg and one where a combined dose of magnesium and calcium was administered in a ratio of 1:1.

Table 2: Comparison of hematological parameters of Wistar rats subjected to treatment regimens of alcohol, magnesium and calcium.

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (10⁵/L)</th>
<th>NEUT (%)</th>
<th>LYMP (%)</th>
<th>MONO (%)</th>
<th>EOSI (%)</th>
<th>BASO (%)</th>
<th>RBC (10⁶/L)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>PLT (10³/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>11.26 ± 4.07</td>
<td>13.28±1.82</td>
<td>66.76±2.98</td>
<td>5.46±1.61</td>
<td>1.66±0.68</td>
<td>12.84±2.63</td>
<td>7.00±1.43</td>
<td>68.62±4.90</td>
<td>20.38±1.54</td>
<td>29.72±0.7</td>
<td>951.80± 91.97</td>
</tr>
<tr>
<td>Positive control</td>
<td>6.78 ± 1.56</td>
<td>37.14±14.48</td>
<td>49.72±6.45</td>
<td>3.94±4.26</td>
<td>3.80±0.99</td>
<td>5.40±5.04</td>
<td>3.87±1.32</td>
<td>78.86±9.06</td>
<td>20.58±0.93</td>
<td>26.36±3.63</td>
<td>610.40±93.91</td>
</tr>
<tr>
<td>Mg 4.25 mg/kg</td>
<td>7.04 ± 0.57</td>
<td>44.78±1.60</td>
<td>10.42±2.40</td>
<td>5.06±2.06</td>
<td>2.32±1.07</td>
<td>7.72±1.22</td>
<td>5.87±1.03</td>
<td>60.68±3.58</td>
<td>18.14±0.80</td>
<td>29.78±0.79</td>
<td>721.40±26.46</td>
</tr>
<tr>
<td>Mg 8.5 mg/kg</td>
<td>12.88 ± 4.18</td>
<td>53.12±15.91</td>
<td>37.14±13.40</td>
<td>1.26±0.86</td>
<td>2.40±1.26</td>
<td>6.08±4.81</td>
<td>4.20±1.11</td>
<td>66.78±2.81</td>
<td>17.50±1.47</td>
<td>26.16±0.13</td>
<td>481.40±94.37</td>
</tr>
<tr>
<td>Mg 17 mg/kg</td>
<td>12.26 ± 2.84</td>
<td>68.18±9.03</td>
<td>23.18±8.30</td>
<td>1.50±0.96</td>
<td>2.34±1.23</td>
<td>4.80±2.91</td>
<td>4.95±0.78</td>
<td>65.12±1.67</td>
<td>18.52±0.45</td>
<td>28.42±1.14</td>
<td>535.40±48.90</td>
</tr>
<tr>
<td>Ca 4.25 mg/kg</td>
<td>8.46 ± 1.53</td>
<td>47.40±11.61</td>
<td>35.76±7.57</td>
<td>5.92±3.28</td>
<td>3.18±0.62</td>
<td>7.74±2.52</td>
<td>5.15±1.50</td>
<td>63.28±4.94</td>
<td>18.56±0.44</td>
<td>29.24±2.38</td>
<td>719.00±70.66</td>
</tr>
<tr>
<td>Ca 8.5 mg/kg</td>
<td>8.74 ± 1.30</td>
<td>36.84±4.63</td>
<td>43.76±7.36</td>
<td>6.72±3.93</td>
<td>3.18±0.88</td>
<td>9.50±2.03</td>
<td>6.20±1.10</td>
<td>62.70±3.60</td>
<td>18.70±1.04</td>
<td>29.66±1.07</td>
<td>762.20±67.09</td>
</tr>
<tr>
<td>Ca 17 mg/kg</td>
<td>8.46 ± 0.83</td>
<td>51.26±9.21</td>
<td>35.72±10.66</td>
<td>4.36±3.21</td>
<td>2.80±0.25</td>
<td>5.86±1.71</td>
<td>4.79±0.48</td>
<td>63.66±4.20</td>
<td>18.60±1.63</td>
<td>28.94±0.93</td>
<td>658.80±52.97</td>
</tr>
<tr>
<td>Mg+Ca (4.25)</td>
<td>12.64 ± 4.57</td>
<td>53.82±9.60</td>
<td>38.94±7.48</td>
<td>1.56±1.15</td>
<td>1.88±0.39</td>
<td>3.80±1.28</td>
<td>4.49±1.14</td>
<td>67.32±2.96</td>
<td>18.28±1.60</td>
<td>27.08±1.98</td>
<td>467.60±67.08</td>
</tr>
<tr>
<td>Mg 4.25+Ca 8.5</td>
<td>11.20 ± 2.84</td>
<td>56.02±5.99</td>
<td>34.08±4.12</td>
<td>1.14±0.64</td>
<td>3.46±1.07</td>
<td>5.30±3.34</td>
<td>5.95±1.11</td>
<td>61.28±1.03</td>
<td>17.30±0.52</td>
<td>28.44±1.11</td>
<td>501.00±83.11</td>
</tr>
</tbody>
</table>

The values are expressed as mean±SD for five animals per group. The statistical comparison was between the negative control and the other groups; “p<0.05. WBC: White Blood Cells; NEUT: Neutrophils; LYMP: Lymphocytes; MONO: Monocytes; EOSI: Eosinophils; BASO: Basophils; RBC: Red Blood Cells; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelets; NC: Negative Control; PC: Positive Control; Mg: Magnesium; Ca: Calcium

Maalox Plus® and PureCal® drugs, either individually or in combination, were effective (p<0.05) in reversing macrocytosis (Table 2). Alcohol did not have significant effect on the mean corpuscular hemoglobin and the mean corpuscular hemoglobin concentration (p>0.05). However, treatment with 8.5 mg/kg Mg and a combined dose of Mg and Ca in a ratio of 1:2 did significantly (p<0.05) lower the mean corpuscular hemoglobin values. Alcohol was also found to suppress the platelet levels (p<0.001) and treatments with Maalox plus® and PureCal® did not influence the alcohol induced thrombocytopenia (Table 3).
Alcohol did not influence serum levels of Mg\(^{2+}\) (p>0.05) but it significantly increased Ca\(^{2+}\) and lowered K\(^+\) levels (p<0.05). Treatment with Maalox plus’ and pureCal’, either singly or in combination, was able to normalize the alcohol induced hypercalcemia in a dose-dependent manner. On the other hand Magnesium at 4.25 mg/kg and calcium at 17 mg/kg were able to normalize the alcohol induced hypokalemia but all other doses were ineffective.

### Table 3: Comparison of biochemical parameters of Wistar rats subjected to treatment regimens of alcohol, magnesium and calcium.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
<th>T (mmol/L)</th>
<th>CHOL (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>BUN (mmol/L)</th>
<th>CREAT (µmol/L)</th>
<th>Mg(^{2+}) (mmol/L)</th>
<th>Ca(^{2+}) (mmol/L)</th>
<th>K(^+) (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>115.00 ± 16.08</td>
<td>170.20 ± 52.02</td>
<td>4.49 ± 1.32</td>
<td>1.60 ± 0.30</td>
<td>1.02 ± 0.15</td>
<td>4.93 ± 0.69</td>
<td>55.28 ± 10.30</td>
<td>0.19 ± 0.06</td>
<td>0.26 ± 0.08</td>
<td>9.34 ± 2.04</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>126.60 ± 22.74</td>
<td>200.40 ± 49.34</td>
<td>4.80 ± 0.65</td>
<td>1.92 ± 0.05</td>
<td>1.77 ± 0.02</td>
<td>4.63 ± 0.19</td>
<td>59.34 ± 5.69</td>
<td>0.13 ± 0.01</td>
<td>0.62 ± 0.19</td>
<td>6.70 ± 1.77</td>
<td></td>
</tr>
<tr>
<td>4.25 mg/kg Mg</td>
<td>117.40 ± 15.32</td>
<td>139.20 ± 7.33</td>
<td>2.54 ± 0.71</td>
<td>1.11 ± 0.21</td>
<td>0.54 ± 0.36</td>
<td>4.76 ± 0.66</td>
<td>56.15 ± 9.56</td>
<td>0.41 ± 0.58</td>
<td>0.24 ± 0.05</td>
<td>7.02 ± 0.43</td>
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</tr>
<tr>
<td>8.5 mg/kg Mg</td>
<td>129.40 ± 20.53</td>
<td>166.00 ± 13.08</td>
<td>5.00 ± 1.04</td>
<td>1.08 ± 0.33</td>
<td>0.57 ± 0.27</td>
<td>3.63 ± 0.36</td>
<td>47.10 ± 4.89</td>
<td>0.28 ± 0.28</td>
<td>0.29 ± 0.05</td>
<td>6.20 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>17 mg/kg Mg</td>
<td>115.80 ± 19.32</td>
<td>164.60 ± 22.10</td>
<td>3.58 ± 1.22</td>
<td>1.27 ± 0.22</td>
<td>0.84 ± 0.46</td>
<td>3.42 ± 0.27</td>
<td>54.88 ± 4.73</td>
<td>0.14 ± 0.04</td>
<td>0.34 ± 0.09</td>
<td>6.72 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>4.25 mg/kg Ca</td>
<td>137.40 ± 15.96</td>
<td>174.60 ± 20.57</td>
<td>6.63 ± 1.47</td>
<td>1.19 ± 0.34</td>
<td>0.66 ± 0.47</td>
<td>3.02 ± 0.39</td>
<td>52.34 ± 5.13</td>
<td>0.16 ± 0.04</td>
<td>0.029 ± 0.02</td>
<td>6.82 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>8.5 mg/kg Ca</td>
<td>146.80 ± 5.31</td>
<td>162.20 ± 23.60</td>
<td>5.52 ± 1.43</td>
<td>1.13 ± 0.33</td>
<td>0.53 ± 0.52</td>
<td>427 ± 0.27</td>
<td>52.84 ± 8.17</td>
<td>0.16 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>6.40 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>17 mg/kg Ca</td>
<td>136.00 ± 16.63</td>
<td>167.00 ± 23.92</td>
<td>6.08 ± 4.06</td>
<td>1.17 ± 0.13</td>
<td>0.29 ± 0.25</td>
<td>5.25 ± 0.79</td>
<td>47.91 ± 10.31</td>
<td>0.20 ± 0.03</td>
<td>0.28 ± 0.06</td>
<td>7.78 ± 0.98</td>
<td></td>
</tr>
<tr>
<td>4.25 mg/kg Mg+4.25 Ca</td>
<td>112.40 ± 19.39</td>
<td>149.40 ± 14.54</td>
<td>3.52 ± 1.03</td>
<td>1.14 ± 0.22</td>
<td>0.86 ± 0.41</td>
<td>3.12 ± 0.44</td>
<td>54.28 ± 3.08</td>
<td>0.27 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>5.76 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>4.25 mg/kg Mg+8.5 mg/kg Ca</td>
<td>107.60 ± 11.19</td>
<td>138.60 ± 5.68</td>
<td>3.58 ± 1.24</td>
<td>1.49 ± 0.29</td>
<td>1.18 ± 0.42</td>
<td>3.51 ± 0.27</td>
<td>56.70 ± 4.68</td>
<td>0.17 ± 0.02</td>
<td>0.42 ± 0.14</td>
<td>5.88 ± 0.88</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Aspartate amino transferase to alanine amino transferase ratio.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST: ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>1.47 ± 0.35</td>
</tr>
<tr>
<td>Positive control</td>
<td>1.60 ± 0.39</td>
</tr>
<tr>
<td>4.25 mg/kg Mg</td>
<td>1.20 ± 0.18</td>
</tr>
<tr>
<td>8.5 mg/kg Mg</td>
<td>1.30 ± 0.15</td>
</tr>
<tr>
<td>17 mg/kg Mg</td>
<td>1.44 ± 0.27</td>
</tr>
<tr>
<td>4.25 mg/kg Ca</td>
<td>1.30 ± 0.30</td>
</tr>
<tr>
<td>8.5 mg/kg Ca</td>
<td>1.11 ± 0.07</td>
</tr>
<tr>
<td>17 mg/kg Ca</td>
<td>1.23 ± 0.16</td>
</tr>
<tr>
<td>4.25 mg/kg Mg+4.25 mg/kg Ca</td>
<td>1.34 ± 0.13</td>
</tr>
<tr>
<td>4.25 mg/kg Mg+8.5 mg/kg Ca</td>
<td>1.30 ± 0.10</td>
</tr>
</tbody>
</table>

**Discussion**

Alcohol treatment resulted in increased proliferation of neutrophils and eosinophils and suppression of basophils. Neutrophilia is

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associated with alcoholic hepatitis and it occurs as a result of inappropriate activation and homing of neutrophils to the microvasculature [25]. In this study the biomarkers of liver function were normal so liver disease is ruled out as the cause of neutrophilia. It is however known that low magnesium levels have been associated with inflammatory response that leads to leukocytosis especially eosinophilia and neutrophilia [26].

Maalox Plus’ antacid and PureCal® had no effect on neutrophilia but were able to normalize eosinophilia while Maalox Plus’ normalized basopenia. Eosinophilia plays an important role in destroying invading parasitic pathogens. However, a marked increase is undesirable as it is implicated with various disorders such as allergies and asthma [27]. This data suggests that the two drugs could be used to treat alcohol induced eosinophilia. In addition they could be used for managing idiopathic eosinophilia that is currently treated using corticosteroids which are known to cause multiple side effects including habituation. On the other hand Maalox Plus’ could have applications in managing basopenia especially one that is associated with acute hypersensitivity reactions.

Alcohol was found to significantly increase the mean corpuscular volume and decrease the total number of red blood cells. This observation is in agreement with several studies that have linked common alcohol use with macrocytosis which may or may not be associated with anaemia [28]. Macrocytosis and anaemia occurs as a result of the direct damage of alcohol to erythroid precursors in the bone marrow [29]. The present findings show that MCV is a sensitive marker for detecting excessive alcohol consumption thus supporting its use as part of the screening procedure for detecting alcohol abuse [30].

Maalox Plus’ antacid and PureCal® were generally effective in reversing the alcohol induced macrocytic anaemia. A review of the literature does not offer direct evidence on the role of calcium and magnesium in alleviation of the condition. However, both micronutrients are known to play a key regulatory role in various cellular processes. One of the biochemical functions of calcium in human and animals is its requirement for membrane permeability [31]. Low intracellular magnesium in the regulation of potassium chloride co-transport has also been implicated [32]. It is thus evident that both magnesium and calcium play a role in the integrity of the RBC membrane and this could explain their therapeutic effect towards the macrocytic anaemia encountered in this study.

Treatment with Maalox Plus’ and PureCal®, either individually or in combination, was found to cause lymphopenia in rats and this was considered a side effect. Supplementation with magnesium has also been reported to alleviate lymphocytosis in rats [33], where importance of distinguishing the proportion or percentage of the varied subsets of lymphocytes which include T and B cells was stressed. It should be noted that magnesium has the potential of suppressing B cell proliferation and consequently a reduction in antibody production [34]. The lymphopenia observed in this study suggests that the synthesis of a particular subset might have been decreased or catabolism decreased. It is impossible to discern which among the subsets was affected since the level of lymphocytes is reported as a whole.

Alcohol given at 5 g/kg for 5 days a week for 4 weeks did not significantly affect the levels of ALT, AST, GGT, T-CHOL, HDL, BUN creatinine and magnesium. There are many reports showing that alcohol has significant toxic effects on blood biochemistry and liver morphology. Equally there are numerous studies that have reported contrary views. There are various factors that can contribute to these contrasting differences including duration of alcohol consumption and dosage [35-42].

The adverse effects of short term (binge and sporadic drinking, also termed as acute intoxication) and long-term (chronic abuse) are well documented [43]. Both acute and chronic excessive alcohol consumption deliver unique pathological consequences with biochemical markers being more pronounced following chronic abuse. This is in agreement with the current study where biochemical markers were not elevated in this acute model that involved giving 5 g/kg of alcohol for 5 days followed by 2 days of abstinence over a four week period. Although there is no universal definition as to what constitutes acute or chronic treatment in laboratory models the findings of the present study seem to support that acute treatment would constitute feeding of alcohol for a period of four weeks or less [43].

Ethanol is known to increase the activity of acetaldehyde dehydrogenase and increases concentration of nicotinamide adenine dinucleotide thus affecting fatty acid oxidation and the tricarboxylic acid cycle consequently elevating synthesis of triacylglycerol and ultimately liver steatosis [44]. Consumption of 10 g/day alcohol has been proved to lead to high triglyceride levels with a dosage greater than 50 g/day minimizing development of low level of HDL while maximizing attainment of high levels of cholesterol [45]. Just like in our findings significant change in serum cholesterol in concomitance with significant depreciation in high density lipoprotein levels in ethanol fed groups have been achieved in prior studies [37]. The findings of the present study in conjunction with other observations [37,45] suggest that alcohol doses above 5 g/kg are required to observe an effect on triglyceride levels.

On the other hand alcohol caused significant (p<0.05) increase in serum calcium and a decrease in potassium levels. Published reports show diverse findings on the effect of alcohol on serum calcium and potassium levels with results showing increase, decrease and no effect [46-51]. The fate of potassium and calcium in the body is controlled by magnesium [52]. In the present study alcohol treated rats had lower magnesium than the negative control. Magnesium deficiency has been cited as a causative factor of increased serum calcium levels due to increased intestinal absorption [53]. On the other hand the hypokalemia seen in alcoholics has been attributed to the increase in excretion of non reabsorbable bicarbonates that induces high potassium excretion through urine [19].

Maalox Plus’ and PureCal® were effective in normalizing calcium levels but were ineffective in correcting the alcohol induced hypokalemia. Oral supplementation with magnesium for a period of at least 6 months is required for complete normalization of potassium levels [54]. This length of time could explain the reason why our oral magnesium supplementation did not yield a correction of the potassium levels.

Blood urea nitrogen is a kidney degeneration biomarker that is indicative of oxidative stress leading to kidney injury [55]. Maalox Plus’ and PureCal® were found to significantly reduce the BUN levels and similar findings have been reported [56]. It was hypothesized that micronutrients are able to counteract the effect of the free radicals in a dose dependent manner by significantly elevating the superoxide dismutase levels [57]. Magnesium and calcium therefore have the capacity to alleviate oxidative stress hence the observed reduction of BUN upon supplementation.
Conclusion

Alcohol caused neutrophilia, eosinophilia, macrocytosis, anaemia, basopenia and thrombocytopenia. Maalox plus® and PureCal® were able to normalize the eosinophilia and macrocytic anaemia that was experienced by the alcoholic subjects.

Alcohol was found not to affect most of the biochemical parameters but it disrupted the electrolyte balance by significantly increasing serum calcium levels and reducing the serum potassium. Oral supplementation with both Maalox plus® and PureCal® normalized the serum calcium with the former drug at a dose of 4.25 mg/kg being able to normalize the serum potassium levels.

References