The aim of the present study is to elucidate the toxicity induced by sodium arsenite on differential leucocyte count and serum transaminases and therapeutic role of tomato and spinach extract. Arsenic is a toxic element present worldwide and gaining attention due to its health hazards. Arsenic enters in living beings via ground water, anthropological activities, industrial products and use of herbicides and pesticides. From last few decades fruits and vegetables are being used to cure and prevent various body ailments. Antioxidants, vitamins and minerals present in them help to combat and reduce the oxidative stress generated by the free radical species produced as a result of various metabolic activities occurring in the body. In the present study rats were divided into 8 different groups with 5 rats in each. The following dose pattern was used in different groups of rats. Group I was kept as a control. Group II received 10mg/kg b. wt. of As, Group III were administered 50mg/kg b. wt. of TE, Group IV had 50mg/kg b. wt. of SE, Group V was given 10mg/kg b. wt. of As + 50mg/kg b. wt. of TE, Group VI received 10mg/kg b. wt. of As + 50mg/kg b. wt. of SE, Group VII had 10mg/kg b. wt. of As + 50mg/kg b. wt. of TE + 50mg/kg b. wt. of SE and Group VIII received 50mg/kg b. wt. of TE + 50mg/kg b. wt. of SE. A marked significant (p<0.001, p<0.05) increase in the activities of serum ALT and AST were observed in sodium arsenite treated rats showing hepatic dysfunction. However, supplementation of tomato extract and spinach extract showed significant reduction in their activities. Upon arsenic administration WBC count, eosinophil count and neutrophil count were elevated significantly (p<0.001, <0.05). Antioxidant activity which maintained antioxidant/proxidant balance disturbed by arsenic toxicity. No alterations were observed in monocytes and basophils count.

Keywords: Sodium arsenite, Tomato extract, Spinach extract, WBC and Serum transaminases.

INTRODUCTION

Increasing pollution is one of the major concerns of climatic issue which is enhancing day by day because of excessive use of hazardous chemicals, xenobiotics and heavy metals containing synthetic products (Jagadeesan et al1). Heavy metals enter the environment via industrial effluents, agricultural products such as pesticides and herbicides. Their presence in environment can cause harm to all living creatures on earth i.e. birds and mammals including human beings and aquatic organisms alike (Akinyeye & Okorie 2, Miquei3, Picot & Proust4). Out of numerous substances present in the environment, arsenic is found naturally and ubiquitously and belongs to group V of the periodic table (Sackett5). Arsenic is found profusely in earth’s crust as well as in minerals, soil and water (Antman6). Arsenic enters in human body through dermal contact, contaminated water and food or by inhaling polluted air with arsenic dust particles (Jomova et al7, Qiu et al8). Chronic exposure to inorganic arsenic can cause major health issues such as skin lesions on feet and palm, skin blackening, enlargement of spleen, neuro and cardiological disorders, hypertension, cancers of various organs and skin (Centeno et al9, Saha et al10, Sharma et al11). In the last two decades people have shown keen interest in having fruits and vegetables loaded with antioxidants which are essential to prevent and manage arsenic toxicity (Wang et al12, Flora et al13). Antioxidants help to mitigate and cure oxidative stress caused by reactive oxygen species generated by arsenic and have potential to damage biomolecules like proteins, nucleic acids and lipids (Shi et al14). Antioxidants are necessary to maintain the redox potential or neutralize the oxidised conditions which could occur in the body due to various metabolic activities (Velioglu et al15). These play important role to protect the body from mutagenesis, carcinogenesis and other harmful effects arising due to oxidative stress (Cook & Samman16, Huang et al17). Spinach is a vegetable consumed widely either raw or boiled. Fresh leaves of spinach contain approximately 1000mg/kg of flavonoids (Weatherby & Cheng18). Spinach leaves also contain many active phytochemicals with several properties such as antiproliferative, antioxidative, anticancerous, anti-inflammatory and antiaging (Lomnitski et al19). The most important flavonoids the spinach leaves exhibit are patuletin and spinacetin (Zane & Wender 20). Flavonoids...
possess anti-allergic, anti-oxidative and anti-viral properties (Verma et al21, Deschner et al22, Middleton et al23, Ishisaka et al24). Further flavonoids are used in the chelation therapy due to their structural confirmation which has potential to trap and nullify reactive oxygen species (Bors et al25). Tomatoes contain various natural antioxidants in the form of flavonoids, carotenoids, phenolic compounds, vitamins and glutathione which have the potential to scavenge the free oxygen radicals (Maisuthisakul et al26, Sarada et al27).

**METHODOLOGY**

Female wistar albino rats weighing 135±5g were brought from animal house of LUVAS Hisar. Rats were subjected to acclimatization for 2 weeks before the commencement of experiment. Rats were segregated into 8 groups on the basis of their body weight. Each group consisted of 5 rats. Rats had free access to standard rat feed and water ad libitum. Their body weight was monitored regularly. Rats were kept in polypropylene cages bedded with dried rice husk throughout the entire experimental period. Each cage was tagged using tag card. The cages were washed and cleaned frequently and bedding was replaced often. Water bottles were cleaned daily.

**Experimental chemicals, spinach and tomato:** Sodium arsenite was obtained from Himedia Pvt. Ltd. Spinach (Spinacea oleracea) and tomatoes (Solanum lycopersicum) were obtained from the organic agriculturist and brought to laboratory for the preparation of extract.

**Extract preparation of spinach and tomato:** Tomato extract was prepared by using the method of salawu28. Tomato puree prepared was incubated for 45 min at 80°C. Solution was filtered and stored as a stock solution.

Spinach extract was prepared according to the method of Islam et al29. Leaves were washed properly to remove soil particles under the tap water, dried in shade and a fine powder was made using a blender. Stock solution was prepared by soaking the powder in the distilled water overnight.

**Ethical approval:** Experiment was conducted after getting the permission from ethical committee with an approval number 107/GO/ReBi/S/99/CPCSEA/2017-23. Animals were maintained and handled according to the guidelines provided by the ethical committee.

**Collection and analysis of haematological parameters:** Blood was collected in anticoagulant coated tubes from retro-orbital plexus of anesthetized rats. Differential leucocyte count was analysed using automated haematological analyzer. Serum transaminases SGPT and SGOT were estimated using the commercially available kits.

**Experimental design and treatment:**

<table>
<thead>
<tr>
<th>Interval Days</th>
<th>Group I (control)</th>
<th>Group II (As)</th>
<th>Group III (TE)</th>
<th>Group IV (SE)</th>
<th>Group V (As+TE)</th>
<th>Group VI (As+SE)</th>
<th>Group VII (As+TE+SE)</th>
<th>Group VIII (TE+SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>133.3</td>
<td>133</td>
<td>132.8</td>
<td>135</td>
<td>137.7</td>
<td>139.0</td>
<td>134.5</td>
<td>132.1</td>
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<tr>
<td>5</td>
<td>142.2</td>
<td>136.5</td>
<td>141.6</td>
<td>144.1</td>
<td>142.6</td>
<td>147.5</td>
<td>143.2</td>
<td>147.6</td>
</tr>
<tr>
<td>10</td>
<td>148.3</td>
<td>144.4</td>
<td>150.1</td>
<td>150.9</td>
<td>148.1</td>
<td>153.1</td>
<td>150.8</td>
<td>155.8</td>
</tr>
<tr>
<td>15</td>
<td>151.8</td>
<td>148.2</td>
<td>158.3</td>
<td>157.3</td>
<td>153</td>
<td>158.9</td>
<td>159.1</td>
<td>164</td>
</tr>
<tr>
<td>20</td>
<td>164.7</td>
<td>155.1</td>
<td>165.2</td>
<td>162.9</td>
<td>159.9</td>
<td>163.4</td>
<td>166.8</td>
<td>170.0</td>
</tr>
<tr>
<td>25</td>
<td>170.5</td>
<td>162.8</td>
<td>173</td>
<td>174.2</td>
<td>166.8</td>
<td>169</td>
<td>172.5</td>
<td>178.1</td>
</tr>
<tr>
<td>30</td>
<td>182.2</td>
<td>169.3</td>
<td>180.9</td>
<td>185.1</td>
<td>173.4</td>
<td>177.4</td>
<td>179.1</td>
<td>182</td>
</tr>
</tbody>
</table>

**Table 1:** Body weight of rats.
Figure 1: Total WBC count of rats.

Figure 2: Lymphocyte count of rats.

Figure 3: Monocyte count of rats.

Figure 4: Eosinophil count of rats.

Figure 5: Neutrophil count of rats.

Figure 6: Basophil count of rats.

Figure 7: Serum ALT of rats.

Figure 8: Serum AST of rats.
The present study was conducted to evaluate the ameliorative potential of tomato and spinach on blood parameters as well as transaminases in sodium arsenite exposed rats. Due to paucity of literature on spinach and tomato extract we tried to explore the ameliorative potential of tomato and spinach on blood parameters as well as transaminases in sodium arsenite exposed rats. Upon administration of tomato extract along with spinach extract alone or in combination with sodium arsenite reduced the leucocyte count towards normal range. Group III (TE), V (As+TE), VI (As+SE), VII (As+TE+SE), VIII (TE+SE) (P<0.05) and Group IV (p<0.01) showed significant decrease in TLC in comparison to only sodium arsenite treated group. In the present work (Table 2) TLC count was positively correlated with monocytes (0.143), eosinophil (0.228) and neutrophil (0.122).

RESULTS AND DISCUSSION

The present study was conducted to evaluate the antioxidant efficacy of phytochemicals present in extract of tomato and spinach on differential leucocyte count and serum transaminases in sodium arsenite exposed rats. Due to paucity of literature on spinach and tomato extract we tried to explore the ameliorative potential of tomato and spinach on blood parameters as well as transaminases.

Exploding industrialization is a vital cause to create and spread pollutants which have become a threat to survival of animals and human beings (Akinbore et al30). Humans are exposed to the arsenic through well water, industrial effluents, different mining projects and agrochemicals (Klibet et al31). All rats seemed to be healthy with good morphology. The rats did not show any symptoms e.g. secretion from different parts such as nose, eyes, ears, genitalia and anal opening. The rats had normal diet and were not lethargic. Table 1 showed the body weight of rats. All rats in each group showed normal growth and weight gain but the gain was less pronounced in only sodium arsenite treated group as compared to control group.

Table 2: Represents the correlation coefficient among differential WBC count of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WBC (10⁹ x L)</th>
<th>Lymphocytes (%)</th>
<th>Monocytes (%)</th>
<th>Eosinophils (%)</th>
<th>Neutrophils (%)</th>
<th>Basophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10⁹ x L)</td>
<td>1</td>
<td>-0.111</td>
<td>0.044</td>
<td>0.228</td>
<td>0.122</td>
<td>-0.150</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>0.01</td>
<td>1</td>
<td>0.005</td>
<td>0.110</td>
<td>0.460</td>
<td>1</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>0.000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Differential leucocyte count and serum transaminases (ALT and AST) of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (CONT.) (n = 5)</th>
<th>Group 2 (As) (n = 5)</th>
<th>Group 3 (TE) (n = 5)</th>
<th>Group 4 (SE) (n = 5)</th>
<th>Group 5 (As+TE) (n = 5)</th>
<th>Group 6 (As+SE) (n = 5)</th>
<th>Group 7 (As+TE+SE) (n = 5)</th>
<th>Group 8 (TE+SE) (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC (10⁹ x L)</td>
<td>8.180±1.443</td>
<td>12.10±0.374</td>
<td>9.640±0.719</td>
<td>9.88±0.409</td>
<td>9.60±0.860</td>
<td>9.54±0.880</td>
<td>9.58±0.670</td>
<td>9.04±1.050</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>62.09±2.10</td>
<td>36.50±2.79</td>
<td>59.14±4.03</td>
<td>62.55±1.87</td>
<td>43.77±2.73</td>
<td>47.03±4.88</td>
<td>61.83±2.66</td>
<td>63.93±2.65</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.06±0.15</td>
<td>2.36±0.30</td>
<td>2.42±0.27</td>
<td>2.46±0.28</td>
<td>2.30±0.23</td>
<td>2.12±0.30</td>
<td>2.28±0.42</td>
<td>2.16±0.29</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.89±0.06</td>
<td>1.73±0.27</td>
<td>0.92±0.06</td>
<td>0.95±0.08</td>
<td>0.91±0.09</td>
<td>0.90±0.06</td>
<td>1.00±0.25</td>
<td>0.88±0.12</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>34.55±1.97</td>
<td>59.00±2.71</td>
<td>34.90±2.48</td>
<td>33.73±1.95</td>
<td>52.60±3.01</td>
<td>49.50±4.75</td>
<td>34.51±2.35</td>
<td>32.59±2.77</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.40±0.13</td>
<td>0.40±0.09</td>
<td>0.46±0.12</td>
<td>0.30±0.08</td>
<td>0.42±0.12</td>
<td>0.44±0.12</td>
<td>0.36±0.12</td>
<td>0.42±0.07</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>60.49±2.15</td>
<td>86.67±3.86</td>
<td>57.78±4.73</td>
<td>59.90±3.49</td>
<td>75.13±1.93</td>
<td>75.33±2.28</td>
<td>68.23±2.83</td>
<td>57.48±2.44</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>151.10±10.11</td>
<td>324.78±14.57</td>
<td>146.95±11.23</td>
<td>147.50±14.57</td>
<td>236.24±32.69</td>
<td>238.14±32.76</td>
<td>202.14±17.01</td>
<td>155.45±15.32</td>
</tr>
</tbody>
</table>

Data is presented as Mean±SEM. As = Sodium arsenite, TE = Tomato extract, SE = Spinach extract, WBC = White blood cell, ALT = Alanine aminotransaminase, AST = Aspartate aminotransaminase.

a<p<0.05, 0.01, 0.001 significant changes in comparison to control group.
b<p<0.05, 0.01, 0.001 significant changes among sodium arsenite treated group.
increase in total leucocyte count (TLC) upon sodium arsenite intoxication and the results are similar to the findings of Arhul et al\textsuperscript{32}, Amer et al\textsuperscript{33}, Witeska\textsuperscript{34}. Leucocytes strengthen the immunological function of organisms and increase in WBC specified the immune responses to combat stress (Kotsanis et al\textsuperscript{35}). A study conducted by suradkar et al. demonstrated that rats administered with lead acetate orally at different concentrations showed leucopenia as well as lymphopenia which might be due to the degradation of cells by toxic material or decreased production of these cells by lymphoid organs (Kumar et al\textsuperscript{36}). Similarly rats treated with polluted water showed an increase in WBC count and neutrophils which have major phagocytic and biochemical function (Al-Terehi et al\textsuperscript{37}). Opium treated rats also showed rise in the total leucocyte count. Various conditions such as alteration in the differentiation of WBC in bone marrow, infectious and inflammatory responses and changes in the endothelial cell’s adhesion molecules lead to fluctuation in WBC count in blood (Asadikaram et al\textsuperscript{38}). Administration of sodium arsenite generated significant increase in WBCs while pretreatment with A. Conyzaoides restored the number of WBCs in animals. This increase was because of the action against the entry of foreign particles (Adebayo et al\textsuperscript{39}). The increase in the WBC may be due to the release of the leucocytes from the immune organs into the blood upon triggering of the immune system by any foreign substances (Ola-Davies & Akinrinde \textsuperscript{40}).

Table 2 fig 2 revealed the lymphocyte count in various groups of rats in the present study. Moreover, significant (p<0.001) decrease in lymphocyte count of (Group II and V) and Group VI (p<0.05) was noticed in comparison to control group. Whereas, a non significant change was observed in all other treated groups. After administration of only tomato extract or spinach extract and co-administration of both alleviated lymphocyte count significantly (p<0.001) in Group IV, VII and Group VIII, (p<0.01) and group III but non significant results were observed in groups V and VI (p<0.05). Lymphocyte count showed (Table 2) negative correlation with eosinophil (-0.486) and neutrophil (-0.988). A significant decline in lymphocyte count indicated the adverse effect on lymphopoiesis by arsenic administration.

Table 2 fig 3 represents the monocyte count. No alteration was observed in monocytes of control and all the treated groups. In this study monocyte count (Table 2) was positively correlated with eosinophils (0.110) and basophils (0.077) whereas, negatively correlated with neutrophils (-0.070).

Table 2 fig 4 show eosinophil count of sodium arsenite intoxicated rats increased significantly (p<0.05) in reference to control group. But non-significant changes were observed in all treated groups with respect to control group. A significant (p<0.05) reduction in eosinophil count of rats of groups III, IV, V, VI and group VIII was observed after supplementation of tomato extract and spinach extract. In Group VII eosinophil count dropped non-significantly (p>0.05) as compared to group II. Eosinophils showed (Table 2) positive correlation with neutrophils (0.460) and were negatively correlated with basophils (-0.143).

Table 2 fig 5 depict neutrophil count of control and other groups of rats. Neutrophil count of Group II (p<0.001) and Group VI (p<0.05) increased significantly as compared to control group. On the contrary, other treated groups showed non-significant (p>0.05) elevation. Rats administered the tomato extract and spinach extract showed significant (p<0.001) decrease in neutrophil count and almost matched the control values as compared to arsenic group. However, Group VI and Group VII (p>0.05) remained non significant. Neutrophils (Table 2) were positively correlated with basophils (0.078). In the present work, arsenic treatment increased the neutrophil count of rats. These results are supported by work of various workers (Fiati et al\textsuperscript{41}, Jalaludeen et al\textsuperscript{42}).

Table 2 fig 6 show the basophil count in control and other experimental groups of rats. A non-significant (p>0.05) dissimilarities were witnessed in all treated group in comparison to Group I and Group II.

Evaluating serum and tissue enzymes helps in assessing hepatic health by pointing hepatocellular toxicity as well as related diseases (Awe et al\textsuperscript{43}). AST and ALT are well known hepatic marker enzymes and leak into circulation during cellular destruction. Liver biotransforms arsenic to reduce its toxic properties (Lin et al\textsuperscript{44}). It binds with the thiol group of enzymes and proteins present in the hepatic cells, thereby, disfunctioning the hepatocytic plasma membrane leading to increased AST and ALT activity (Goyer & Clarkson\textsuperscript{45}).

Table 2 fig 7 depict significant (p<0.001, p<0.01) increase in ALT activity as compared to control group. When rats received antioxidant rich extracts activity of ALT was found almost near to control values showing statistically significant changes.

Table 2 fig 8 depict AST activity in serum of rats. A significant (p<0.001) elevation in activity of serum AST was observed in group II and (p<0.05) in groups V, VI and VII in comparison to group I. whereas, other groups showed non-significant (p>0.05) differences in AST activity. Supplementation of tomato and spinach extracts restore the AST activity significantly (p<0.001) in groups III, IV, VII and VIII but (p<0.05) in groups V and VI when compared to sodium arsenite exposed group. Thus, based on the results obtained from our study and considering the modulatory properties of spinach and tomato extracts it is logical to think that phytoconstituents present in spinach and tomato executed antioxidant activity which maintained antioxidant/proxidant balance disturbed by arsenic toxicity. The results of our study are consistent with the previous findings (Yasmin et al\textsuperscript{46}, Mehta & Hundal\textsuperscript{47}, Goudarzi et al\textsuperscript{48}).
CONCLUSION

It can be concluded from this study that acute arsenic exposure can cause alterations in various parameters like differential leucocyte count and serum transaminases (ALT and AST). Biomethylation of the arsenicals is carried out in liver which in turn causes damage to hepatocytes leading to leakage of ALT and AST into the circulation. Arsenic exposure activates the immune responses which may elevate leucocyte count in blood. However, active phytochemicals present in extracts of tomato and spinach have antioxidant potential to prevent the arsenic induced toxicity.

Conflict of Interest: Authors declared no conflict of interest.

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