ABSTRACT

The modulatory potentials of aqueous extracts of garlic and Viscum album leaves on sodium-arsenite induced clastogenicity were examined in male wistar rats. Animals were randomly divided into 8 groups. Group 1 (Distilled water), Group 2 (Sodium arsenite), Group 3 (Garlic alone), Group 4 (Sodium arsenite and Garlic), Group 5 (Viscum album only), Group 6 (Viscum album and Garlic), Group 7 (Viscum album and Sodium arsenite) and Group 8 (Viscum album, Garlic and Sodium arsenite). Aqueous extracts of garlic at 100mg/kg body weight (bwt) and Viscum album leaves (25mg/kg bwt) were fed to the rats for 30 consecutive days. Sodium arsenite (2.5mg/kg bwt) was given once weekly. Aqueous extracts was administered through oral gavage for 28 days. Clastogenicity activity was evaluated by studying micronuclei formation in polychromatic erythrocyte cells in bone marrow. Plasma levels of gamma-glutamyl transferase (γ-GT), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alanine aminotransferase(ALP) compared to the negative control. The results suggest that pretreatment of rats with either garlic or Viscum album extracts reduced the elevated plasma levels of liver enzymes and clastogenicity induced by sodium arsenite in rats.

Keywords: Clastogenicity, toxicity, micronuclei, Viscum album, polychromatic erythrocytes

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. A number of evidence has been accumulated to demonstrate promising potentials of medicinal plants. Medicinal plants are considered to be important source of antioxidant compounds and have therapeutic benefits. Garlic (Allium Sativum) a small bulbous perennial herb is a recognized and valuable spice for foods and is a popular remedy for various ailments and physiological disorders [3]. It has antioxidant, antibiotic, antihelminthic, fungicidal, antiarteriosclerotic and anti thrombic properties. In-vivo studies show that garlic and its associated sulfur components suppress the incidence of tumors in rodents models [15]. Viscum album (mistletoe) is a member of the plant family Loranthaceae. [11] It is a semi parasitic woody perennial plant commonly found growing on oaks and other deciduous trees like cocoa, apple and kola trees. Mistletoe extracts are often applied to tumour patients because of their cytotatic or apoptotic and immunomodulatory effects[6].They have been shown to enhance body immunity modulated cardiovascular dysfunctions, and also possesses immunostimulatory[8], cytotoxic activity on tumour cells with possible adjuvant role in cancer therapy[4]. Arsenic, an extremely toxic heavy metal is a common environmental pollutant. with sodium arsenite (NaSO2) showed in several studies to be the most toxic of all arsenic compounds. [5]. For most people, food is the largest source of arsenic exposure (about 25 to 50 micrograms per day [µg/d]), with lower
amounts coming from drinking water and air. Among foods, some of the highest levels are found in fish and shellfish; however, this arsenic exists primarily as organic compounds, which are essentially nontoxic.[1] Arsenic with genotoxic, hepatic tumorigenic, and carcinogenic potentials have been severally implicated in the onset of chromosomal aberration, micronucleus and sister chromatid exchange in mammalian cells[10]. Arsenics is frequently used in the production of herbicides, insecticides, rodenticides, and food preservatives. Yamanaka (2001)[18] showed that the biotransformation end products of arsenic such as dimethyl arsenic radicals play important roles in initiating carcinogenesis in organs such as liver, lungs and urinary bladder. Recently arsenic intoxication in experimental animals has been associated with hepatic tumors[17]. In view of above background information, this study aims to investigate the hepatoprotective and immunomodulatory effects of aqueous extracts of Viscum album and garlic on Sodium arsenite-induced toxicities in albino Wistar rats.

EXPERIMENTAL SECTION

Chemicals
Sodium arsenite purchased from Sigma Chemical Co. USA. (γ-GT), Alkaline Phosphatase (ALP), Alanine amino transferase (ALT) and Aspartate amino transferase (AST) kits were obtained from Randox Laboratories Ltd United Kingdom. All other reagents and chemicals are of analytical grade and were obtained from Sigma Chemical Co. St. Louis, MO, USA.

Plant materials
Viscum album leaves were collected from Cocoa trees in the Cocoa Research Institute of Nigeria, (CRIN) Idi-Ayunre, Ibadan Oyo-State, Nigeria. Cold extraction of the 20g of fresh leaves of mistletoe was achieved by steeping in 500ml of distilled water overnight. A dose of 25mg/kg body weight was administered orally to the experimental animal. Freshly sliced cloves of garlic was grinded into paste and then dissolved in double distilled water and filtered. A dose of 100mg/kg body weight was fed to the experimental animals.

Experimental animals
Male adult albino rats (average weight about 150g) were purchased from Physiology Department, University of Ibadan, Ibadan. The animals were acclimatized for one week in the Animal House of the Department of Biochemistry, University of Ibadan. They were fed with pellets from Ladokun feeds and water adlibitum and maintained under 12hour light and 12hour dark cycle.

Experimental protocol
The experimental rats were randomly divided into eight treatment groups of five animals each. Group I rats served as negative control and were treated with distilled water only for thirty consecutive days. Those rats given sodium arsenite were given normal diet for thirty consecutive days with 2.5mg/kg body weight of Sodium Asernite on the 7th, 14th, 21st and 30th day respectively.

Liver function test:
γ – GT activity was determined following the method of Szasz. ALT and AST activities were determined using the ALT and AST following the method of Reitman and Frankel, 1957.

Micronuclei test.
Genotoxic effects were evaluated in the rat bone marrow using micronuclei test described by Schmid. Bone marrow cells from both femurs were flushed with 0.5ml fetal bovine serum( FBS).This was centrifuged at 2000 rpm for 5 minutes to obtain bone marrow cell pellet ,which was suspended again in 0.5ml FBS for another round of 5 minute centrifugation at 2000pm. The pellet containing the cells were smeared onto a glass slide, and the air dried, fixed in methanol and stained in May-Gruenwald and Giemsa stains.

STATISTICAL ANALYSIS
Results were express as mean + standard error of the mean. One- way analysis of variance (ANOVA) was used for data analysis followed by Duncan Multiple Range test. P values less than 0.05 were considered statistically significant.
RESULTS AND DISCUSSION

The frequency of micronucleated polychromatic erythrocytes (mPCEs) induced in rat bone marrow cells after administration of sodium arsenite is shown in Table 1.

Table 1: The frequency of micronucleated polychromatic erythrocytes (mPCEs) per 1000 polychromatic erythrocytes in test and control animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>MPCES/1000PCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water</td>
<td>3.33±5.77</td>
</tr>
<tr>
<td>2</td>
<td>Sodium arsenite</td>
<td>26.67±5.77</td>
</tr>
<tr>
<td>3</td>
<td>Garlic alone</td>
<td>13.33±5.77</td>
</tr>
<tr>
<td>4</td>
<td>Sodium arsenite + garlic</td>
<td>16.67±11.55</td>
</tr>
<tr>
<td>5</td>
<td>Viscum Album only</td>
<td>13.33±5.77</td>
</tr>
<tr>
<td>6</td>
<td>VA + Garlic</td>
<td>20±10</td>
</tr>
<tr>
<td>7</td>
<td>VA + SA</td>
<td>33.33±11.55</td>
</tr>
<tr>
<td>8</td>
<td>VA + G + SA</td>
<td>56.67±11.55</td>
</tr>
</tbody>
</table>

Values are Mean ± S.EM

Administration of sodium arsenite, significantly induced mPCEs formation in the rat bone marrow cells when compared to the control. Pretreatment of rats with extracts of Garlic or Viscum album only decreased the induction of mPCEs by sodium arsenite.

Enzyme assay analysis:

Results of the liver enzymes activities is shown in Table 2. Treatment with sodium arsenite alone significantly increased the \( \gamma \)-GT activity in the plasma (P < 0.05) as compared with the negative control.

Table 2: Activities of liver enzymes

<table>
<thead>
<tr>
<th>Groups</th>
<th>( \gamma )-GT</th>
<th>ALP</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.88±2.66</td>
<td>14.12±0.71</td>
<td>32.11±2.35</td>
<td>40.81±1.88</td>
</tr>
<tr>
<td>3</td>
<td>19.39±2.32</td>
<td>21.17±0.54</td>
<td>40.44±2.47</td>
<td>48.35±3.82</td>
</tr>
<tr>
<td>4</td>
<td>22.00±2.21</td>
<td>14.80±0.35</td>
<td>48.00±1.60</td>
<td>51.18±2.78</td>
</tr>
<tr>
<td>5</td>
<td>17.08±2.20</td>
<td>49.32±0.43</td>
<td>33.35±1.67</td>
<td>46.30±3.56</td>
</tr>
<tr>
<td>6</td>
<td>19.40±2.56</td>
<td>37.17±0.43</td>
<td>55.85±3.54</td>
<td>47.30±2.88</td>
</tr>
<tr>
<td>7</td>
<td>18.23±3.83</td>
<td>23.19±0.40</td>
<td>30.89±3.14</td>
<td>40.19±3.49</td>
</tr>
<tr>
<td>8</td>
<td>21.71±2.89</td>
<td>30.89±3.14</td>
<td>35.28±2.14</td>
<td>49.19±2.28</td>
</tr>
</tbody>
</table>

DISCUSSION

Arsenic, an extremely toxic heavy metal is a common environmental pollutant. With sodium arsenite (sNaSO2) showed in several studies to be the most toxic of all arsenic compounds. Arsenic and many of its compounds are especially potent poisons. Arsenic disrupts ATP production through several mechanisms. At the level of the citric acid cycle, arsenic inhibits pyruvate dehydrogenase and by competing with phosphate uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production is also increased, which might form reactive oxygen species and oxidative stress. These metabolic interferences lead to death from multi-system organ failure probably from necrotic cell death, not apoptosis. Inorganic arsenic in humans has been linked to a form of skin cancer and also to bladder, liver, and lung cancer. Inorganic exposure to arsenic in humans, by the inhalation route, has been shown to be strongly associated with lung cancer while ingestion of inorganic arsenic in humans has been linked to a form of skin cancer and also to bladder, liver, and lung cancer.

The activities of \( \gamma \)-GT for the animals administered sodium arsenite significantly increased when compared to the negative control. Increased activity is indicative of oxidative stress and cyogenetic damage in animal exposed to sodium arsenite. Table 2 shows that pretreatment of rats with either aqueous extract of Garlic or Viscum album before sodium arsenite administration, reduced \( \gamma \)-GT. The ALP, AST and ALT activities were similar to the pattern observed with \( \gamma \)-GT activity. Elevation of these enzymes in the plasma might be due to the increased permeability of plasma membrane, increased synthesis of enzyme by the liver inflammation, cellular necrosis and cholestatis in the liver.
Table 1 shows that Sodium arsenite significantly increased (P<0.05) the frequency of micronucleated Polychromatic Erythrocytes (mPCEs) when compared to negative control which is an indicative that sodium arsenite is clastogenic. There was a significant increase in the frequency of mPCEs in rats bone marrow when sodium arsenite, Garlic and Viscum album extracts were administered. The frequencies of aberrations and damaged cells were significantly reduced in the rats administered sodium arsenide and either Garlic extract or Viscum album extract only. Mistletoe extracts are often applied to tumour patients because of their cytostatic or apoptotic and immunomodulatory effects.[6] The mild clastogenic activity observed when these extracts were administered suggested that both extract can ameliorate the effect of sodium arsenite in the rats bone marrow. Also it has been shown that sodium arsenite intoxication can compromise the integrity of the liver in mouse, rat, fish and goat[13,14]. Increased activity of gamma glutamyl has been associated with hepatotoxic, oxidative stress and chromosomal aberration in cells.

CONCLUSION

The results suggest that pretreatment of rats with either garlic or Viscum album extracts is effective in reducing sodium arsenite induced hepatic damage and clastogenic effects. This observation may be due to several interaction between the different constituents of the extracts though both plants have therapeutic potentials.

REFERENCES