WITHANIA SOMNIFERA PROTECTS THE HAEMATOLOGICAL ALTERATIONS CAUSED BY SODIUM ARSENITE IN CHARLES FOSTER RATS

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ABSTRACT
This study was carried out to investigate the therapeutic role of the ethanolic extract of Withania somnifera on Sodium Arsenite induced haematological alterations in rats. Oral administration of sodium arsenite at the dose of 8 mg/kg body weight daily caused haematological alterations in rats as manifested by the significant decrease in RBC’s count, Haemoglobin percentage, Haematocrit, Mean Cell Volume of RBC’s (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and White Blood Cell Count (WBC)’s as compared with control. The Lipid peroxidation also showed elevated levels in comparison to control. But, after oral administration of ethanolic extract of Withania somnifera at the dose of 200 mg/kg body weight daily for 30 days to Sodium Arsenite pretreated rats there was an increase in the haematological parameters as well as lipid peroxidation levels decreased significantly. The study reveals that ethanolic extract of Withania somnifera possesses protective effect against haematological alterations caused by arsenic induced toxicity.

Keywords: Withania somnifera, Sodium Arsenite, Haematological parameters, Rats.

INTRODUCTION
Arsenic is one of the most dangerous occupational and environmental toxins. Both natural and anthropogenic sources are responsible for the distribution of many toxicants, mainly heavy metals throughout the environment. Arsenic is abundant in the crust of the earth and is found in all environments. It is found in soil, minerals, surface and ground water1. The arsenic contamination was also observed in three districts Ballia, Varansi and Gazipur of UP, in the upper and middle Ganga plain, India2. Approximately 20 incidents of groundwater arsenic contamination have been reported from all over the world3. Due to groundwater contamination, a large number of populations in Bangladesh, India are suffering from melanosis, leucomelanosis, keratosis, hyperkeratosis, dorsum, non petting oedema, gangrene, skin cancer and skin lesions in sole and palm4-6. Arsenite binds to cellular sulphhydryl especially the vicinal ones and interferes with energy generation7-9. In the tissue, it exerts toxic effects through several mechanisms such as reversible combination with sulphhydryl groups. It also inhibits numerous other cellular enzymes such as cellular glucose uptake, gluconeogenesis, fatty acid oxidation and production of glutathione through sulphhydryl group binding10-11. The second major toxicity caused by arsenic is termed as ‘arsenolysis’ in which oxidative phosphorylation is hampered as arsenic binds with ADP to form ADP arsenate which irreversibly results in loss of energy in cells. Thus, ROS’s are capable of damaging DNA, lipids and proteins finally, hampering the cell signaling12-14. Withania somnifera also called as Ashwagandha, winter cherry or Indian Ginseng has been an important medicinal herb in Ayurveda and indigenous medical systems for over 3000 years. Studies indicate that Ashwagandha possesses antioxidant15, antitumor16, anti-inflammatory17, immunomodulatory18, antistress19, adaptogenic20, antiulcer21 and rejuvenating properties22. However, no studies have reported the antidote effect of W. somnifera root extract against arsenic induced hematological disorders in rats. So, this novel study has been undertaken.

MATERIALS AND METHODS

Animals
Charles Foster rats (18 females), weighing 160 g to 180 g of 8 weeks old, were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA). The research work was approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. IAEC/2012/12/04. Food and water to rats were provided ad libitum (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups (2 each). The rats were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22 ± 2°C with 12 h light/dark cycle.

Chemical
Sodium Arsenite (98.5 %) manufactured by Biosol Laboratories Pvt. Limited, Kolkata, India was obtained from the Scientific store of Patna, India.

Preparation of plant ethanolic extract
In the present study, dry root of Withania somnifera were purchased from Haridwar Medicinal Store, Haridwar,
Thiobarbituric acid reactive substances (TBARS), as a marker for lipid peroxidation (LPO), were determined by the double heating method. The identity of the medicinal plant was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected roots of *Withania somnifera* were shade dried and were grinded to fine powder. The powder was then soaked in 70% ethanol for 48 h and finally extracted with 5% absolute ethanol using soxhlet apparatus for 6-8 h and the residue was concentrated and dried at 37°C. The ethanolic extract dose was calculated after LD50 estimation and finally made to 200 mg/kg body weight.

**Experimental Design**

The present study was designed as Sodium arsenite at the dose of 8 mg/Kg body weight was administered orally daily for 60 days. Upon these pre-treated groups, *Withania somnifera* ethanolic root extract was administered at the dose of 200 mg/Kg body weight orally for 30 days. No treatment was administered to control group and was designated as healthy control. At end of the treatment, blood samples were collected in small glass vials containing EDTA as an anticoagulant for haematological studies and serum for lipid peroxidation study.

**Haematological Evaluation**

The haematological parameters Red Blood Cell Count (RBC’s), Haemoglobin percentage (HGB), Haematocrit (HCT), Mean Cell Volume of RBC’s (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and White Blood Cell Count (WBC’s) were done manually.

**Lipid Peroxidation**

Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method. The principle of the method was a spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and incubated for 15 minutes at 90°C. After cooling in tap water, the mixture was centrifuged at 3000 rpm for 10 minutes and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and again incubated for 15 minutes at 90°C. The solution was then cooled in tap water and its absorbance was measured using Thermo Scientific UV-10 (UV-Vis) spectrophotometer (USA) at 532 nm.

**Statistical Analysis**

Results are presented as mean ± SD and total variation present in a set of data was analyzed through one way analysis of variance (ANOVA). Difference among mean values has been analyzed by applying Dunnett’s test. Calculations were performed with the Graph Pad Prism Program (Graph Pad software, Inc., San Diego, U.S.A.). The criterion for statistical significance was set at P < 0.05.

**RESULTS**

**Morbidity and Mortality**

The rats after arsenic exposure (8 mg/Kg b.w./day) for 60 days have shown signs of toxicity such as nausea, nose bleeding, lack of body co-ordination (11 percent of rats showed paralysis like symptoms), blackening of tongue and foot and general body weakness.

**Haematological changes**

Data of haematological parameters are shown in Table 1, the study shows significant decrease P < 0.0001 in the erythrocyte counts (RBCs), haemoglobin percentage, haematocrit percentage, MCV, MCH but significant increase in leukocyte count (WBCs) in comparison with control group after 60 days of exposure. But, after administration of *Withania somnifera* there was significant reversal in the haematological values. ANOVA showed that the sodium arsenite has more deleterious effect on time duration of exposure (P < 0.0001).

**Lipid Peroxidation**

The lipid peroxidation show significant decrease in the serum MDA levels in *Withania somnifera* treated group in comparison to arsenic treated group (Graph 1).

**DISCUSSION**

Arsenic in the present scenario in South East subcontinent region of Asia has created major health related problems through contamination in underground drinking water. Among possible target organs of heavy metals like arsenic, the kidney and central nervous system appear to be the most sensitive ones. Having been absorbed from the alimentary tract, most of the metals form durable combination with the protein thionein, forming metallothionein, which plays an important role in further metabolism of these metals.

Table 1: Changes in the haematological parameters of Charles foster rats exposed to Sodium arsenite at the dose of 8 mg/Kg body weight daily for 60 days and its amelioration by *Withania somnifera* at the dose of 200 mg/Kg body weight for 30 days

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>Control (n = 6)</th>
<th>Arsenic treated 8 mg/Kg b.w. 60 days (n = 12)</th>
<th><em>Withania somnifera</em> treated 200 mg/Kg b.w. 30 days (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10⁶/mm³)</td>
<td>7.16 ± 0.08</td>
<td>2.77 ± 0.11</td>
<td>5.08 ± 0.07</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>91.92 ± 0.50</td>
<td>53.83 ± 1.68</td>
<td>85.17 ± 1.19</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>275.8 ± 1.52</td>
<td>161.5 ± 5.04</td>
<td>255.5 ± 3.58</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>385.1 ± 2.33</td>
<td>583.4 ± 12.63</td>
<td>385.1 ± 2.33</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>128.7 ± 0.76</td>
<td>194.5 ± 4.21</td>
<td>167.6 ± 0.31</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>33.33</td>
<td>33.33</td>
<td>33.33</td>
</tr>
<tr>
<td>WBC (10⁹/mm³)</td>
<td>7714 ± 29.90</td>
<td>16333 ± 176.4</td>
<td>8667 ± 74.42</td>
</tr>
</tbody>
</table>

The data are presented as mean ± S.D, n = 6, significance at P < 0.0001.
The kidney and liver are considered to be the most susceptible organs for metals; because these organs contain most of the metallothionein binding toxic metals27-31. These toxic metals also produce free radicals such as lipid peroxides. They encounter with biomembranes and sub-cellularorganelles32-44. The study shows haematological changes after arsenic exposure as white blood cells (WBCs) level was increased after arsenic exposure denots the necrosis activities in the cells45 while decrease in the red blood cells (RBC’s) and hemoglobin levels denotes inhibition of hem-synthesis pathway. Similarly, the other haematological parameters also showed decreased levels as these are dependent on the hemoglobin and RBC levels46-47. But, in Withania somnifera treated group showed significant reversal in the haematological parameters due to its antioxidant property. Similarly, lipid peroxidation amelioration by Withania somnifera and its free radical scavenging activity have been well studied48-53. Aloe vera and Centella asiatica in the traditional medicine of Indian Ayurveda has been widely used against heavy metals / metalloid particularly in reducing alteration in haematological and biochemical parameters4. Amelioration by other molecules and plants have been well documented against arsenic induced toxicity10,34,35,54-57. As, arsenic induces cellular toxicity by damaging body’s oxidative defense mechanism, Withania somnifera has potential natural antioxidant and immunomodulatory property which eliminates the arsenic from the body by chelation therapy. It also possesses rejuvenating property by which it maintains the cellular integrity accordingly. Thus, from the entire study it can be concluded that Withania somnifera ameliorates the haematological alterations as well as lipid peroxidation in arsenic induced toxicity in rats by its various properties.

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