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HEPATOPROTECTIVE EFFECT OF *CHLOROPHYTUM BORIVILIANUM* ROOT EXTRACT AGAINST ARSENIC INTOXICATION

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Summary

The present study was conducted to find out the hepatoprotective effect of *Chlorophytum borivilianum* root extract against arsenic induced toxicity. Three groups were made: Control (DDW), Arsenic intoxicated group (4 mg/kg b.w) and Combination group (NaAsO₂ (4 mg/kg b.w) + cb root extract (800 mg/kg b.w)). Animals received their respective doses daily for 30 days orally. Body weight, liver weight, hepato pathological changes and level of ATPase were observed. Result showed significant decrease in body and liver weight along with disturbed hepatoarchitecture and decline in ATPase activity in arsenic intoxicated group as compared to control. In combination group, increased body and liver weight along with increased ATPase levels and almost normal hepatoarchitecture were observed as compared to arsenic treated group. Thus it can be concluded that *Chlorophytum borivilianum* root extract has potential to decrease the toxic effects of arsenic.

Keywords: Cb; sodium arsenite; body weight; liver weight; ATPase.

Introduction

Arsenic, a ubiquitous metalloid which occurs naturally is a toxic pollutant. It is ranked first in a list of 20 hazardous substances¹. Its exposure occurs from inhalation, absorption through skin, by ingestion of contaminated drinking water and by food². Exposure to arsenic leads its accumulation in tissues such as skin, hair and nails, resulting in various clinical symptoms such as hyperpigmentation and keratosis³. It affects nearly all organs. Inorganic arsenic exposure may lead to cancer of liver, kidney, bladder, prostate and skin as well as to Black foot disease, heart disease, maningioma and other adverse health effects⁴,⁵,⁶.
Arsenic causes a significant increase in the rate of formation of reactive oxygen species (ROS) such as $O_2^-\cdot OH$ and $H_2O_2$. These are generated during redox cycling and metabolic activation process. It also causes lipid peroxidation, oxidation of proteins and enzymes as well as DNA and DNA adducts. ATPase forms a large family of membrane proteins which couple ATP hydrolysis to the active transport of cations or other compounds such as phospholipids across cell membranes. and is also considered as a master enzyme that controls many important functions at cellular and organ level including active transport and electric potential across plasma membrane, intracellular pH regulation, cell division and cell elongation.

Chlorophytum borivilianum (Safed musli) is a traditional rare Indian medicinal herb. Its roots are widely used for various therapeutic applications in the Ayurvedic and Unani medicinal systems. Major phytochemical compounds reported from the roots of Chlorophytum borivilianum are saponins, fructans, galactans, phenolic compounds and fructo-oligosaccharides (FOS). Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals.

In the present study, the objective is to elucidate hepatoprotective role Cb root extract on arsenic intoxicated liver damage in mice.

Materials and methods

Animals: Random-bred, male Swiss albino mice, (7-8 weeks) were used for experiments. These animals were maintained in the animal house at temperatures of 24 ± 3°C and a light of 12:12 hours of light and dark. These animals were housed in polypropylene cages and fed standard mice feed from Hindustan Lever Ltd. India. Tap water was provided to the animals ad libitum and tetracycline was given to the animals against any infections. The ethical committee of Department of Zoology, University of Rajasthan, Jaipur (India) has approved to carry out the experiments.

Chemical:- Heavy metal Arsenic in the form of Sodium arsenite (NaAsO$_2$, trivalent) CAS No. 7784-465 used in the present study and was obtained from Himedia, Mumbai, India (Batch No. 3-1621 RM-1847. The salts of Arsenic was dissolved in double distilled water (DDW) and was administered orally. Different dose of arsenic were administered and dose was determined on basis of $LD_{50/30}$ (Fig.1)
Preparation of *Chlorophytum borivilianum* root extract (drug): The roots were collected locally, air dried in shade and powdered. Powder was distilled in Soxhlet apparatus (for 36 hours using DDW) at 40⁰ C. The remaining material was dried in oven at 36⁰ C and was used as drug.


Figure 1. Different dose of arsenic administration and the dose selection determination on basis of LD50/30.

Figure 2. The reducing power assay of *Chlorophytum borivilianum* and its comparison with ascorbic acid. Ascorbic acid served as positive control.
Drug Tolerance study and Selection of Dose: Mice were divided into various groups to receive 100, 200, 400, 800 mg/kg body weight of root extract orally for seven consecutive days. The animals were observed for 30 days. After 30 days, lipid per oxidation (LPO) and GSH content were measured in the liver in all groups and according to the highest GSH and minimum LPO level, the dose was decided.

Treatment of animals

The following experiment was designed to examine the effects of Chlorophytum borivilianum on arsenic induced toxicity.

**Group I (Control Group):** Animals in this group were not given plant extract or heavy metal. Only vehicle (DDW) was given orally.

**Group II (Arsenic treated group):** Arsenic 4mg/kg bodyweight was given orally up to 30 days in the form of sodium arsenite (III).

**Group III (Combination Group):** Sodium arsenite and Chlorophytum borivilianum root extract both were given up to 30 days orally.

**Autopsy Intervals:** The Animals from the above groups were autopsied at various intervals i.e. 1, 3, 7, 15 and 30 days.

**Parameters to studied:**

- **Body weight and Liver weight changes:** The animal from each group were weighed and killed by cervical dislocation on days 1, 3, 7, 15 and 30 days and liver was carefully excised, trimmed free of extraneous tissue, blotted dry and weighed quickly and used for histopathological and biochemical studies (for ATPase).

- **Histopathological preparations** – Excised liver was fixed in Bouin's fixative for 24 hrs. The fixed tissue was further processed by standard method and sections were cut at 5 µ and stained with Haematoxyline and Eosine.

- **Total ATPase assay:** Liver homogenate was made. The total ATPase was determined according to the method of Akagawa and Tsukada (1979).21

**Statistical analysis**

The statistical significance in the different parameters between control and experimental were assessed by one way ANOVA.

**Results**

**Body weight and Liver weight Changes:** The body weight and liver weight in NaASO₂ treated group were found to be significantly lower with respect to DDW (control) from day 1 to 30 whereas both were recovered in combination group with respect to arsenic intoxicated group (Fig. 3, 4).
Figure 3. Variation in body weight changes in different experimental groups. Each value represents as mean ± SD and n = 5. Significance level was set up p < 0.05 (Almost Significant), p < 0.01 (Significant) and p < 0.001 (highly significant). \[a=0.05, b = p < 0.01, c = p < 0.001\]. Statistical comparison were done as control Vs arsenic and arsenic Vs combination group.

Figure 4. Variation in liver weight changes in different experimental groups. Each value represents as mean ± SD and n = 5. Significance level was set up p < 0.05 (Almost Significant), p < 0.01 (Significant) and p < 0.001 (highly significant). \[a=0.05, b = p < 0.01, c = p < 0.001\]. Statistical comparison were done as control Vs arsenic and arsenic Vs combination group.

**Histopathological alteration:** Control group showed normal cellular architecture with distinct hepatocytes (with prominent nucleus), sinusoidal spaces and central vein. In group 2 during 30 days exposure, arsenic caused various pathological alterations such as expanded sinusoidal spaces, karyolysis, karyorrhysis in hepatocytes, cytoplasmic vacuolization lymphocyte
infiltration and enucleation (Fig 5, 6 & 7) in hepatocytes as compared to control (DDW) group. In combination group, it showed recovery in the form of maintained hepatic histoarchitecture.

Figure 5. Photomicrograph of arsenic treated group at 400X showing Cytoplasmic Vacuolization, Expanded sinusoidal space.

Figure 6. Photomicrograph of control treated group at 400X showing central vein, portal vein, binucleated cells and normal sinusoidal space.
Figure 7. Photomicrograph of combination treated group at 400X showing hepatocytes and sinusoidal space.

Total ATPase Assay: The total ATPase activity in NaASO$_2$ treated group was found to be significantly lower with respect to DDW (control), whereas combination group showed significant elevation in total ATPase with respect to their control (arsenic treated group) during 30 days experimental period (Fig.8).

Figure 8. Variation in ATPase activity in different experimental groups. Each value represents as mean ± SD and n = 5. Significance level was set up p< 0.05 (Almost Significant) , p< 0.01 (Significant) and p< 0.001 (highly significant). [a=0.05, b = p< 0.01 c = p< 0.001]. Statistical comparison were done as control Vs arsenic and arsenic Vs combination group.
Discussion

Reduction in body weight is used as an indicator for the deterioration of general health status. It has been reported that arsenic could induce toxicological effects and biochemical dysfunctions representing serious health hazards. The findings from the present study indicate that arsenic exposure caused decrease in the body and liver weight which are in support of the findings by Yousef et al. (2008) and El-Demerdash et al. (2009) who reported that high arsenic exposure have significantly induced disturbances the total body weight and liver weight. Oral administration of arsenic is associated with severe gastrointestinal and liver side effects. Arsenic increases permeability of intestinal lining and causes the gut leaky. This increased permeability of intestinal lining may be responsible for improper absorption of nutrients and loss of appetite and weakness, and reduction in body weight. Reduction in liver weight is due to damaged hepatic histoarchitecture. Arteel et al. suggested that arsenic exposure caused a change in the balance between cell death and proliferation coupled with loss of body weight. Arsenic produces ROS during its cycles between different oxidation states, which appears to be involved in the mechanism of various types of cell injury. Liver cells have particularly high probability of being subjected to ROS induced toxicity because hepatocytes produces large amount of ROS during the deoxygenation of xenobiotics and toxic substances. Arsenic induced reactive oxygen species and subsequent depletion of antioxidant cell defenses can result in disruption of the pro-oxidant/antioxidant balance in mammalian tissues. Consequently, ROS directly react with cell biomolecules, causing damages to lipids, proteins and DNA, and hence leading to cell death.

Present study revealed decreased ATPases activity after arsenic intoxication at all autopsy intervals. Arsenic induced reactive oxygen species impairs cell membrane stability and damages mitochondrial membrane severely. It is well established that mitochondria are the major site of utilization of oxygen and many of the mitochondrial enzymes contain essential sulfhydryl groups. In addition, since the inner and outer mitochondrial membranes contain unsaturated lipids, mitochondria are more susceptible to arsenic attack as well as by the free radicals produced by it than other organelles. The damaged membrane cannot develop a proton motive force that is preliminary requirement of cellular energy production. Arsenic disrupts mitochondrial membrane potential and increases ROS generation that causes depletion of ATP. It uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. Thus ATPase activity is significantly reduced after arsenic intoxication.

In combination group modulation by Cb root extract was observed in terms of increased body and liver weight, less damage in hepatocytes and increased ATPase level. Cb root extract contains Saponins, Gallotannins and Fructans. Saponins, Gallotannins and Fructans have antioxidant activity. Saponins
inhibit intracellular ROS formation, reduces the level of the lipid peroxidation (MDA) and maintains cellular antioxidant enzymes activities\(^\text{42}\). Saponins have hypocholesteric properties\(^\text{19}\). Cholesterol enrichment was shown to have an inhibitory effect on many membrane ATPases, as it may directly interact with the boundary lipids of ATPase and alter the intermolecular hydrogen bonds of the protein\(^\text{43,44}\). Saponin (Ginsenosides) interacts with membrane cholesterol and displace it from surrounding lipids environment of ATPases. Removal of cholesterol will lead to an increase in membrane fluidity which facilitates conformational changes of ATPases during their transport cycle that controls the enzyme activity of biological membrane and has important role in ion transport.\(^\text{45}\) Saponins maintains Na\(^+\)-K\(^+\)-ATPase activity against ROS induced reaction.\(^\text{46}\) Saponins reduces ROS formation due to their antioxidant property. Since cell membrane is protected, GIT is not leaky, so body weight is increased. Hepatohistoarchitecture injuries induced by ROS, are reduced due to antioxidant property of saponins. The body and liver weight are also recovered. Thus it can be concluded that cb root extract can ameliorates arsenic induced toxicity in terms of increased body and liver weight, lessens the hepatotoxicity and maintains ATPase level.

References


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Conferences Attended

1. Sunil Kumar Sharma, Garima Sharma, Ambika Sharma and Madhu Kumar "Modulatory effect of Chlorophytum borivilianum against arsenic induced toxicity" (Oral Presentation No. 28) presented in International Conference on Free Radical and Natural Products. 10th Annual Meeting of Society of Free Radical Research (SFRR) India held at Hyderabad from 11-13th Jan, 2010.

2. Garima Sharma, Sunil Kumar Sharma and Madhu Kumar "Protective effect of Chlorophytum borivilianum against arsenic induced reproductive toxicity in male Swiss albino mice" poster presented in International Conference on Reproductive Health & 20th Annual Meeting of the Indian Society for the study of Reproduction & Fertility February 8-10, 2010 held at Jaipur, Rajasthan.
PROTECTIVE EFFECT OF CHLOROPHYTUM BORIVILIANUM AGAINST ARSENIC INDUCED REPRODUCTIVE TOXICITY IN MALE SWISS ALBINO MICE

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Arsenic, a metalloid is a recognized reproductive toxicant, found in drinking ground water and soil. An impaired antioxidant defence mechanism followed by oxidative stress is the major cause of arsenic induced toxicity, which can lead to reproductive failure. Herb Chlorophytum borivilianum root extract contains saponins, sapogenins, gallotannins and fructans which exhibited good free radical scavenging activity as evidenced in the present study by DPPH assay. To see its modulatory role against the arsenic toxicity Swiss albino mice were divided into three groups. Group-I served as control and received DDW. Group-II received sodium arsenite (4 mg/kg body weight) up to 7 days orally. Group-III received combination of sodium arsenite and Chlorophytum borivilianum root extract (800 mg/kg body weight) for 7 days orally. The biochemical estimation of LPO, GSH, acid phosphatase, alkaline phosphatase were done in testis. The present results showed significant increase in the activity of GSH and decrease in LPO, acid phosphatase and alkaline phosphatase level in combination group as compared to arsenic treated group. The results indicate that the root extract have the potential in reducing the toxic effect of arsenic.