ANTIHEPATOTOXIC EFFICACY OF ACHYRANTHES ASPERA ROOTS ON SODIUM ARSENATE INDUCED HEPATIC DAMAGES IN SWISS ALBINO MICE

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ABSTRACT

Sodium arsenate (SA) is highly toxic inorganic compound. Till date no effective treatment is available to combat SA toxicity. Recognizing the toxic effect of the compound, the present study was designed to elucidate the antioxidant potential of aqueous extract of Achyranthes aspera against sodium arsenate induced hepatotoxicity in mice. Sodium arsenate ingested mice showed a significant (p<0.001/p<0.05) increase in hepatic lipid peroxidase and total cholesterol level. At the same time, decrease in hepatic antioxidant parameters like glutathione content, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and total protein content were also observed. The level of hepatic aspartate transferase, alanine transferase, acid phosphatase and alkaline phosphatase were also decreased in SA challenged mice. Pretreatment with the Achyranthes aspera showed protection in hepatic tissue to some extent. Thus, it can be inferred that Achyranthes aspera serves as a potent antioxidant agent and could be exploit to manufacture drug for the treatment of various human ailments.

KEYWORDS: Achyranthes aspera, Antioxidant, Liver, Sodium arsenate.

INTRODUCTION

Liver is the main key organ because it regulates homeostasis, and is also involved with almost all biochemical pathways, that are related to the growth, nutrient supply and energy production. Liver also fight against diseases in human body. So it is an important target for oxidative stress and other toxic effects of any compound. Various environmental factors in
the environment like heavy metals (Arsenic, Lead etc.) and other toxic compounds are responsible for hepatotoxicity.

Arsenic is a very toxic chemical and found in two forms- inorganic and organic. Sodium arsenate is a highly toxic inorganic compound. According to previous study inorganic arsenic exposure may lead to cancer of various organs (liver, kidney, lung, bladder, skin, colon, lymphoid and nasal cavity), hyper pigmentation, hyperkeratosis, maningioma and various other health problems.\[1\] Humans may be exposed with sodium arsenate via drinking water, skin absorption and inhalation. Higher concentration of arsenic in human body is responsible for increase of free radicals. These free radicals may cause oxidative stress, inhibits enzyme and mitochondrial function.\[2-3\]

Various chelating agents like *meso* 2,3-dimercaptosuccinic acid (DMSA), British Anti Lewisite (BAL; 2,3-dimercaprol) are separately or in combination with antioxidants such as ascorbic acid and some micronutrients (Zinc and Selenium) are used for the treatment of arsenic toxicity.\[4-5\] These chelating agents and synthetic antioxidants are costly and have been reported to possess various side effects.\[6\] Thus medicinal plants are better option for the treatment of arsenic hepatotoxicity.

World Health Organization (WHO) identifies all medicinal plants used globally and listed more than 20,000 species.\[7\] *Achyranthes aspera* Linn. (Hindi- Latjira, Chirchira; English- Prickly chaff flower; Sanskrit- Apamargah) belongs to Amaranthaceae family is one of the important medicinal plant. It is an erect stiff weed, available in whole India, Asia and many parts of the world such as Mexico, Central America and Africa.\[8\] It is described as bitter, pungent, purgative, heating, laxative, stomachic, carminative, digestive and is useful for the treatment of bronchitis, heart maladies, piles, itching abdominal problems, ascites, rheumatism, abdominal enlargement, rabies and enlarged cervical gland.\[9\] Whole plant possess anti-asthmatic,\[10\] anticancer,\[11\] anti-diabetic,\[12\] antioxidant,\[13\] antiulcer,\[14\] nephroprotective\[15\] and wound healing\[16\] activities.

In the light of aforementioned beneficial effects of *A. aspera*, the study was carried out to determine the protective effect of roots of *A. aspera* on sodium arsenate-induced hepatic toxicity related biochemical parameters.
MATERIALS AND METHODS

Chemicals
Sodium arsenate (Na$_2$HAsO$_4$.7H$_2$O, mol. wt. 312.02.) and all other chemicals, reagents used in current investigation were of analytical grade and were procured from reputed companies (SRL, MERCK, RANBAXY, HIMEDIA, SIGMA, S. D. Fine Chem and SUYOG).

Plant material
Achyranthes aspera roots were collected from Banasthali University campus, Tonk, Rajasthan. Plant sample was dried in shade and coarse powder was extracted by macerating 50 g in 500 ml of distilled water for 48 hours with occasional stirring. The macerated mixture was filtered and evaporated at 40ºC, using a rotary evaporator (Heidolph, Incarp Instruments Pvt. Ltd., Germany). The dried extract (1.691g / 100 g root powder) was collected and stored at 5ºC in air-tight container.

Animals
Swiss Male Albino Mice (Mus musculus) weighing 20-30 g were purchased from “Haryana Agriculture University”, Hissar (India). Ethical approval was taken from “Institutional Animal Ethical Committee” of Banasthali University to conduct experiments on animals. They were fed with standard nutritional pelleted diet (Ashirwad Ltd, India). Water was made available ad libitum.

Experimental groups
In the present study, 24 Swiss albino mice were used for hepatic biochemical and histopathological study. Mice were divided into 4 groups; each group was having 6 mice. The groups were as follows.

Group I: received drinking water; served as control (C).
Group II: received single dose of sodium arsenate (4 mg/kg body weight; once; p.o.) (SA),
Group III: received aqueous A. aspera root extract at a dose of 100 mg/kg, body weight/day for 10 days before intoxicated with sodium arsenate at a dose of 4 mg/kg body weight (AARL).
Group IV: received aqueous A. aspera root extract at a dose of 200 mg/kg, body weight/day for 10 days before intoxicated with sodium arsenate at a dose of 4 mg/kg body weight (AARH).
The dose for sodium arsenate\textsuperscript{[17]} and the plant doses\textsuperscript{[18]} were decided on the basis of previous reports. After administration of the last dose, the animals were given one day rest and then on the next day they were sacrificed. The liver tissue was removed, cleaned, rinsed in cold saline, blotted and used for various biochemical and histological assays.

**Homogenate preparation**

After removal of hepatic tissue from the animal all the adhering tissues were removed first and then washed with ice cold normal saline solution (0.9\% NaCl) until bleached of all the blood and blotted dry by filter paper sheet. Weight of liver was taken, only after drying the tissue. Tissue was mined into small pieces and homogenized (10\% w/v) in ice chilled 0.1M sodium phosphate buffer (pH 7.4), using mortal pestle and homogenate was then centrifuge (10,000 rpm) for 15 to 20 min at 4\(^{\circ}\)C. The supernatant thus obtained was used for biochemical parameters.

**Biochemical Parameters**

Hepatic lipid peroxidation (LPO) level\textsuperscript{[19]}, superoxide dismutase (SOD) activity\textsuperscript{[20]}, Catalase (CAT) activity\textsuperscript{[21]}, Reduced glutathione (GSH) content\textsuperscript{[22]}, GST\textsuperscript{[23]}, Glutathione peroxidase (GPx)\textsuperscript{[24]} activities, alkaline phosphatase (ALP) and acid phosphatase (ACP)\textsuperscript{[25]} aspartate amino transferase (AST) and alanine transferase (ALT)\textsuperscript{[26]}, Total cholesterol\textsuperscript{[27]} and Total protein\textsuperscript{[28]} content were analyzed with standard procedures.

**Histopathological Studies**

Histological evaluation of hepatic tissues was done with standard method.\textsuperscript{[29]} Collected hepatic tissues from the mice were fixed in 10\% formalin at room temperature for 4 weeks. After fixing the tissues, these were washed thoroughly under the running water and dehydrated in ascending grade of ethyl alcohol, cleared and then embedded in soft paraffin. Tissues section of about 6 \(\mu\text{m}\) were cut, stained by haematoxylin and eosin and then examined under light microscope.

**Statistical analysis**

The experimental results were expressed as mean ± standard deviation (SD). Data comparisons were carried out using one way analysis of variance (using SPSS 16.0) followed by Tukey comparison test to compare results between all groups. Results with p<0.05 and p<0.001 was regarded as statistically significant.
RESULTS
Effects of SA and A. aspera on hepatic oxidative stress and enzyme of antioxidant defense system

Effects of aqueous extract of A. aspera on lipid peroxidation (LPO) and antioxidant-enzymes (SOD, CAT, GST, GPx and GSH) in control and treated groups against sodium arsenate (SA) induced hepatotoxicity in male mice were given in table 1. Sodium arsenate exposure (group II) led to a significant increase of LPO level and significant decrease in endogenous enzymes activity like CAT, SOD, GSH, GST and GPx in comparison to the normal control group (group I). Administration of A. aspera (group III and IV) in pre-arsenic exposure was beneficial in improving the LPO level while CAT, SOD, GSH, GST and GPx level too returns to normal level in comparison to the arsenic treated group (group II).

Table 1: Effects of aqueous extract of Achyranthes aspera roots on enzymatic and non-enzymatic antioxidant profile against SA induced hepatic toxicity in mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Normal mice)</th>
<th>Group II (SA)</th>
<th>Group III (AARL + SA)</th>
<th>Group IV (AARH + SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmoles TBARS/mg tissue protein)</td>
<td>0.643±0.065</td>
<td>6.410±0.847*</td>
<td>0.803±0.006* b</td>
<td>0.833±0.014* b</td>
</tr>
<tr>
<td>SOD (min^-1 g^-1)</td>
<td>3.147±0.489</td>
<td>0.280±0.174**</td>
<td>1.468±0.153**</td>
<td>3.265±1.117 b</td>
</tr>
<tr>
<td>CAT (µmole H₂O₂/ min/mg protein)</td>
<td>5.117±0.351</td>
<td>2.240±0.030*</td>
<td>4.260±0.052** a</td>
<td>5.487±0.145 a</td>
</tr>
<tr>
<td>GSH (mg/g)</td>
<td>2.73± 0.252</td>
<td>2.27± 0.058**</td>
<td>3.1±0.10 a</td>
<td>3.37± 0.058** a</td>
</tr>
<tr>
<td>GST (nmoles of GSH- CDNB/ min/mg protein)</td>
<td>3.283± 0.042</td>
<td>2.567±0.499**</td>
<td>3.124±0.012</td>
<td>4.783±0.034** a</td>
</tr>
<tr>
<td>GPx (µmole of GSH/ min/mg protein)</td>
<td>0.214± 0.004</td>
<td>0.167± 0.005**</td>
<td>0.231±0.015 a</td>
<td>0.245± 0.013** a</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n=6). *p<0.001, **p<0.05 v/s control group (C); ap<0.001, bp<0.05 v/s compound treated group (SA). (LPO-lipid peroxidation; SOD- super oxide dismutase; CAT- catalase; GST- glutathione-s- transferase; CDNB - 1-chloro-2,4-dinitrobenzene, GPx- glutathione peroxidase; GSH- reduced glutathione; C- Control; SA- Sodium arsenate; AARL- Achyranthes aspera root low; AARH- Achyranthes aspera root high).

Effects of SA and A. aspera on hepatic enzymes

The effects of aqueous extract of A. aspera on the AST, ALT, ACP and ALP level in hepatic tissue were given in table 2. Mice exposed with sodium arsenate (group II) showed significant depletion in the level of hepatic enzymes (AST, ALT, ACP and ALP) in comparison to the control group (group I). On the other hand, administration of A. aspera
(group III and IV) produced effective recovery in the hepatic enzyme levels in comparison to the SA treated mice.

Table 2: Effects of aqueous extract of Achyranthes aspera roots on hepatic biochemical parameters against SA induced liver toxicity in mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Normal mice)</th>
<th>Group II (SA)</th>
<th>Group III (AARL + SA)</th>
<th>Group IV (AARH + SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (µmole pyruvate/min/mg)</td>
<td>0.845±0.046</td>
<td>0.532±0.022*</td>
<td>0.580±0.056*</td>
<td>0.638±0.009*b</td>
</tr>
<tr>
<td>ALT (µmole pyruvate/min/mg)</td>
<td>0.549±0.016</td>
<td>0.422±0.020*</td>
<td>0.469±0.006*b</td>
<td>0.572±0.006a</td>
</tr>
<tr>
<td>ACP (mmoles PNPP/min/mg)</td>
<td>28.733±0.208</td>
<td>18.363±0.509*</td>
<td>20.40±0.794*</td>
<td>26.70±1.825a</td>
</tr>
<tr>
<td>ALP (mmoles PNPP/min/mg)</td>
<td>5.74±0.14</td>
<td>2.143±0.357*</td>
<td>5.76±0.433 a</td>
<td>6.393±0.352 a</td>
</tr>
<tr>
<td>TP (mg/g)</td>
<td>9.247±0.015</td>
<td>5.16±0.061*</td>
<td>6.28±0.025*a</td>
<td>8.803±0.025*a</td>
</tr>
<tr>
<td>TC (µg/ml)</td>
<td>0.387±0.486</td>
<td>0.432±0.015</td>
<td>0.235±0.005</td>
<td>0.357±0.003</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n=6). *p<0.001, **p<0.05 v/s control group (C); ap<0.001, bp<0.05 v/s compound treated group (SA). (ACP- acid phosphatase; ALP- alkaline phosphatase; AST- aspartate transferase; ALT- alanine transferase; TP- total protein; TC- total cholesterol; PNPP- p-nitrophenyl phosphate C- control; SA- sodium arsenate; AARL- Achyranthes aspera root low; AARH- Achyranthes aspera root high).

Effects of SA and A. aspera on total protein and cholesterol level
Effects of SA and A. aspera on total protein and total cholesterol level of hepatic tissue were summarized in table 2. Mice treated with arsenic (group II) showed significant decrease in protein and insignificant increase in cholesterol level in comparison to the normal control group (group I). Administration of A. aspera root extracts had beneficial effect on the total protein and cholesterol level in hepatic tissue.

Histological studies
The liver of control mice showed normal hepatic lobules with central veins embedded in connective tissue. Central vein was separated by sinusoids and showed normal size. They were regular and contained a large spheroid nucleus (Figure 1). The histological observation of sodium arsenate (SA) intoxicated mice showed that the hepatic lobules were damaged and arrangement of normal liver cells was lost. The central and portal veins were overcrowded. Considerable number of hepatic cells were damaged and lost their characteristic appearance. Size of central vein was increased. Some leucocytes infiltration was also observed (Figure 2).
Mice treated with low dose of *A. aspera* aqueous extract showed that the almost all of these histopathological changes were normalized but some hepatocytes appeared with cytoplasm and the central vein appear crowded with least infiltration of leucocytes (Figure 3), while higher dose of *A. aspera* maintained normal liver architecture and was able to reduce the fibrosis, congestion, incidence of inflammatory cells infiltration (Figure 4).

Figure 1: Photomicrograph of the control normal liver section (40X). CV- Central veins and S- Sinosoids. Sinosoids lined with endothelial cells are also present.

Figure 2: Group-II treated with sodium arsenate (4mg/kg body weight) shows the dilated sinusoids (DS) dilation in central vein (DCV) and infiltration of leukocyte in liver tissue (40X).
Figure 3: Group-III treated with aqueous extract of *A. aspera* root at low dose (100mg/kg body weight) shows the normal central vein (CV), sinusoids (S) and dense cytoplasm in hepatic tissue (40X).

Figure 4: Group- IV treated with high dose (200mg/kg body weight) of aqueous extract of *A. aspera* shows the normalized central vein (CV), normal sinusoids (S) with normalized cytoplasm in hepatic tissue (40X).

DISCUSSIONS

The present study showed the hepatoprotective effect of aqueous extract of *A. aspera* roots on arsenic induced acute toxicity and oxidative stress in mice. It is evident from the above results that pre arsenic exposure treatment with roots powder of *A. aspera* significantly protected mice from the hepatotoxic effect of arsenic. To our knowledge this is the first study to demonstrate protective effect of *A. aspera* root against sodium arsenate induced intoxication. *A. aspera* has been reported to contain various antioxidants like saponin, alkaloids, triterpinoids, sapogenins, glycosides, phenols, flavonoid, flavonol,
proanthocyanidin, tannins and steroids. Antioxidant activity of A. aspera against hydrogen peroxide, superoxide radical, hydroxyl radical was also reported.\cite{13,30-32}

Reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical and superoxide anion during oxygen metabolism may be generated due to arsenic toxicity.\cite{33} Arsenic induce mutagenesis sequence (arsenic → superoxide anion → hydrogen peroxide → hydroxyl radicals → genotoxicity) was also elaborated in mammalian cells.\cite{34} The present study showed the depletion in LPO as observed by significant decrease in the thiobarbituric acid reactive substances (TBARS) level in hepatic tissues of the arsenic treated groups. Increase in lipid peroxide after SA treatment was also observed by other scientists.\cite{35} LPO is regarded as one of the basic mechanisms of cellular damage caused by free radicals. Free radicals react with lipid peroxidation, resulting in the release of products such as melondialdehyde, hydrogen peroxide and hydroxyl radicals. An increase in LPO indicate serious damage to the cell membranes, inhibition of several important enzymes, reduced cellular function and cell death.\cite{36}

Current study showed a significant decrease in SOD, CAT and GSH level in mice treated with sodium arsenate, thus rendering the hepatic tissue to the peroxidative damage. CAT and SOD are metalloprotein accomplishing their antioxidant activity by enzymetically detoxifying peroxides (‘OCH), H₂O₂ and ¹O₂. SOD, CAT and GPx are the most important enzymes against oxygen metabolism toxicity and GSH play an important role in protecting cells from free radicals.\cite{37} Superoxide radicals are converted in H₂O₂ by SOD that is known as first defense and prevents further free radicals formation. This H₂O₂ is converted in to O₂ and H₂O with the help of CAT. Lipid hydroperoxides in the presence of GSH reduced in to lipid alcohols by the help of GPx. The increase in the LPO level and decrease in the GSH level causes decrement in the GPx activity during arsenic exposure.

GSH is the major cytosolic thiol compound which plays important cellular function including destruction of hydrogen peroxide, lipid peroxides and free radicals.\cite{38} GSH also preserves the cellular level of active forms of β-carotene, ascorbic acid and α-tocopherol.\cite{39} GSH has been suggested to be cyto-protective against arsenic,\cite{40} and its decrease from the cell might be responsible for the increase on arsenic-induced stress protein by activation of stress protein gene.\cite{33} Thiol compounds contains sulfur atom so they have antioxidant capacity, sulfur can easily loss a single electron. Arsenic binds to the thiol group of GSH and interferes with the antioxidant activity of GSH, so the level of GSH was declined. Thus, the level of
GSH dependent enzymes, GST and GPx were also decreased in arsenic exposed mice. This may be due to the decrease expression of these antioxidants during cellular damage. Significant increase in LPO level and decrement in SOD, CAT, GSH, GST and GPx activity demonstrated the high toxic effect due to ROS production in the arsenic treated mice. These results are also supported by previous study.\(^{[41]}\) Arsenic induced generation of free radicals is a complex process that involves the generation of a various ROS including superoxide (\(O_2^-\)), singlet oxygen (\(^1\)O\(_2\)), peroxyl radical (ROO\(^-\)), hydrogen peroxide, nitric oxide (NO\(^-\)), dimethylarsinic peroxy radicals ([(CH\(_3\))\(_2\)AsOO\(^-\)]) and dimethylarsinic radical ([(CH\(_3\))\(_2\)As\(^-\)]).\(^{[42]}\) The reactive oxygen species formed may cause cellular and subcellular damage by peroxidation of membrane lipids, by denaturing cellular proteins and by breaking DNA strand, disrupting cellular function.\(^{[43]}\)

When \(A. \) aspera extract was given to the mice then LPO level was significantly decrease and level of other cellular antioxidant enzymes were significantly increased. \(A. \) aspera scavenges hydroxyl radicals, superoxide anion and nitrous oxide radical, also induce lipid peroxidation.\(^{[13, 30-32]}\) \(A. \) aspera contain various types of antioxidants like flavonoids, tannins, phenol etc. that are responsible for various antioxidant activities. Literature has shown medicinal plants with hepatoprotective properties mediate their protection via antioxidant activities due to their high concentration of flavonoids and alkaloids they contain.\(^{[44-45]}\) So the main reason behind restoration of cellular antioxidant enzyme level in \(A. \) aspera treated hepatic tissue is its free radical scavenging activity and high antioxidant activity.

Acute arsenic treatment decrease the level of AST, ALT, ACP, ALP and total protein content while increase total cholesterol content in the hepatic tissue, whereas \(A. \) aspera administrated restored the various values to some extent. AST, ALT, ACP and ALP are the biomarkers for the liver functioning and veracity.\(^{[46]}\) AST, ALP, ACP and ALP are vital type of enzymes; these are linked to the amino acids and carbohydrates metabolism. Arsenic administration could cause hepatic cellular damage, resulting in the release of hepatic enzymes into the blood circulation. Significant decrease in AST, ALT, ACL and ALP enzymatic level indicate arsenic-induced liver damage, increased permeability and necrosis of cell. In the present study, the changed enzymes (ACP, ALP, AST and ALT) level were restored in \(A. \) aspera pretreated mice. These results suggested that \(A. \) aspera preserve the structural integrity of the hepatic tissue and protect from arsenic-induced tissue damage.
SA toxicity caused decrease in total protein content in hepatic tissues of male mice. The level of protein content decreased in the hepatic tissue may be due to inhibition of enzymes that is involved in the DNA repairing and expression like poly ADP-ribose polymerase-I an important DNA repair enzyme and other one is production of free radicals that cause DNA damage and DNA mutation. Administration of *A. aspera* extract led to significant recovery in the DNA damage, so total protein level was normalized after the *A. aspera* treatment. These suggest the ability of *A. aspera* that it can help in regeneration of tissue.

Liver is involved in the metabolism of cholesterol and synthesis of lipoproteins.

Changes in plasma lipids level could serve as a simple marker for assessing liver disorders. The elevation of serum cholesterol in the SA administered mice may be the result of significant concentration of serum LDL–cholesterol and this is a risk factor for coronary heart disease. The reduced concentration of cholesterol in the mice pre-treated with the *A. aspera* aqueous extracts indicates that *A. aspera* prevents an increment in cholesterol, triglyceride and total lipids by inactivation of thiol group enzymes as HMG-CoA reductase the rate limiting enzymes in cholesterol biosynthesis.

Histological changes induced by SA (sodium arsenate) were prominent in hepatic tissue. The liver is the major target organ of inorganic arsenic, which is explained by the affinity of As (III) toward vicinal dithiol in hepatic cytosolic proteins. In the present investigation, SA exposure produced pronounced hepatic histopathology as evidenced by histological alternations in liver which include necrosis with hepatocyte vacuolation, swelling, leukocyte infiltration, pyknotic nuclei, dilation of central vein and sinusoids.

These finding are also supported by previous studies. In accordance with these findings in multiple doses of SA mice liver several changes were occurred like nuclear degeneration, cytoplasmic degeneration, emptied portal vein, binucleated condition and also exhibition of vacuoles in hepatocytes. *A. aspera* extract of root at both doses level reduced morphological changes produced by SA and greatly changed the microanatomy of the liver to normal which could be explained by the presence of wide range of active ingredients present in *A. aspera*.

**CONCLUSIONS**

Aforementioned results indicate the protective role of *A. aspera* extract against sodium arsenate intoxication. Changes in the all parameter level towards the normalization further support the antioxidant role of root extract of *A. aspera*. Hence, the mechanism by which *A.
*A. aspera* exerts a hepatoprotective effect against sodium arsenate intoxication could be attributed to presence of its free radical scavenging and antioxidant properties. This plant can be used safely for longer duration as a cheap source of active therapeutics for alleviation of commonly occurring ailments in the poor and under privileged people of India.

**ACKNOWLEDGMENTS**

The authors wish to acknowledge the support received from the Banasthali University to conduct the research work.

**CONFLICT(S) OF INTEREST:** There is no conflict of interest

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