**ACHYRANTHES ASPERA HAVE THE POTENTIAL ANTIOXIDANT PROPERTY IN SCAVENGING FREE RADICALS PRODUCED AS A RESULT OF OXIDATIVE STRESS INDUCED BY ARSENIC.**

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**ABSTRACT**

The current investigation was undertaken to evaluate antioxidant potential role of *Achyranthus aspera* in arsenic induced oxidative stress in swiss albino mice. In this study, impact of two different plant part (root and leaves) of *Achyranthes aspera* on adult female mice which were treated with various plant extracts following exposure to arsenic was investigated. 48 Swiss albino mice weighing 20-30g were obtained from Haryana Agricultural University, Hissar, India and were divided into 6 groups each containing 8 mice and received various doses of leaf and root extracts following sodium arsenate treatment. Liver was obtained from the mice and tissue homogenates were used for the estimation of SOD, CAT, GSH, GPx, GST and LPO. Arsenic exposure led to significant rise TBARS (0.313± .002 nmols TBARS/h/g), fall in SOD (0.190± .003 U/mg protein/h), decrease in CAT activity (0.329 ± .001 nmol H₂O₂ consumed/min/mg protein), rise in GSH (0.072± .005 nmol GSH / g tissue) and significant decrease in GST activity (2.81 ± .14 nmols CDNB conjugates formed/min/mg protein) as compared to control group. From the above findings, it can be concluded that, *Achyranthes aspera* have the anti-oxidant potential in scavenging free radical produced as a result of oxidative stress induced by arsenic.

**KEYWORDS:** *Achyranthes aspera*, anti-oxidant potential.

**INTRODUCTION**

Arsenic (As) is a ubiquitous element in the environment. Weathering of rocks converts arsenic sulfides to arsenic trioxide, which enters the arsenic cycle as dust or by dissolution in rain, rivers, or groundwater.[1] It is also introduced into the environment through mine...
tailings, industrial wastes discharge, fertilizers, agricultural employments of pesticides, smelting of metals, and burnings of fossil fuels. Also, arsenic can enter food chain causing wide spread distribution throughout the plant and animal kingdoms. Arsenic occurs in both organic and inorganic forms in nature but inorganic species of arsenic [As (III) and As (V)] represent a potential threat to the environment, human health, and animal health due to their carcinogenic and other effects. Arsenic can result in acute and chronic toxicity. The characteristics of severe acute arsenic toxicity include gastrointestinal discomfort, vomiting, diarrhea, bloody urine, anuria, shock, convulsions, coma and death. Chronic effects are degenerative inflammatory and neoplastic changes of the skin and respiratory, haematopoietic, cardiovascular, nervous, hepatic, endocrine and renal systems. Arsenic is a carcinogen to both humans and animals. Inorganic arsenic is classified by the International Agency for Research on Cancer and the US Environmental Protection Agency as a known human carcinogen. This classification is based on epidemiological studies which show an association of exposure to arsenic and the development of cancer. The evidence for arsenic carcinogenicity in animals, however, was very limited until Ng et al established a two year mouse model successfully to demonstrate arsenic carcinogenicity and mutagenicity.

The mechanism(s) by which arsenic induces cancer now remains poorly understood. Many different mechanisms of action have been proposed and some potential mechanisms include genotoxicity, cell proliferation, altered DNA repair and DNA methylated oxidative stress, cocarcinogenesis, and tumor promotion. Among them, the oxidative damage is considered to play an important role in arsenic carcinogenesis. Arsenic initiates cytotoxicity by introducing oxidative damage. Oxidative stress arises when reactive oxygen species such as free radicals, lipid hydroperoxides, aldehydes, hydrogen peroxides are generated, which can react with cellular constituents such as thiols and lipids and alter the antioxidant defense systems.

*Achyranthes aspera* (Amaranthaceae) has long been used in different systems of medicine in the treatment of cancer, leprosy, hepatitis, renal disorders, dermatological disorders, diabetes, toothache etc. The plant has been used as antimicrobial, hypoglycemic, antioxidant, analgesic. It is distributed as weed throughout India, tropical Asia and other parts of the world. Ayurvedic, yunani practitioners use different parts of the plant to treat leprosy, asthma, fistula, piles, arthritis, wound and insect bite and snake bite, renal and cardiac dropsy, kidney stone, diabetes, dermatological disorders, gynecological disorders, malaria. The plant is a popular folk remedy in traditional system of medicine throughout the tropical Asian and
African countries. The plant is reported to be used as antimicrobial, antifertility, Hypoglycemic, immunostimulant, anti-inflammatory, antioxidant, diuretic, Hypersensitive and analgesic. Phytochemical investigations revealed the presence of sterols, alkaloids, saponins, etc. from different parts of the plant. Keeping the above beneficial role of Achyranthus aspera in mind, the current investigation was undertaken to evaluate antioxidant potential role of Achyranthus aspera in arsenic induced oxidative stress in swiss albino mice.

MATERIALS AND METHOD

Maintenance and Procurement of Animals
Female Swiss albino mice, each weighing 20-30 g were obtained from Haryana Agricultural University, Hissar, India. The animal ethics committee of Banasthali University, Banasthali, India approved the study. All experiments were conducted on female albino mice, when they weighed 22-30g. They were housed in polypropylene cages in an air conditioned room at 25 C ± 3ºC, relative humidity of 50% ± 5% and 12 h alternating light and dark cycles. The cages were washed with standard detergent regularly. The mice were provided with chow diet (Hindustan lever limited, India) and drinking water ad libitum.

Collection of Plant Material
Achyranthes aspera was selected for study. Roots and leaves of Achyranthes aspera were collected seasonally (December and January, 2011-2012) from Banasthali University Campus, Tonk Rajasthan, India. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra, Banasthali University.

Preparation of Aqueous Plant Extract (Non-Sequential)
The parts of plant were washed, shade dried. For preparation of different type extracts, shade dried plant parts (50g) were subjected to size reduction to a coarse powder using electrical grinder. The powder so obtained was dipped in water for two days, to prepare non-sequential extracts (root and leaf respectively).

Experimental Group Formation
In the experiment, 48 adult Swiss albino female mice (mus musculus) weighing 25-30 g (3-4 months old) were used for oxidative stress parameters. One group contains 8 mice that served as control (n=8) and another contained 40 mice that received sodium arsenate 0.1mg/kg bodyweight for alternate 7 days.
The groups were as follows

**Group 1**: Received 1ml distilled water, served as control

**Group 2**: Received sodium arsenate 0.1 mg/ kg bodyweight, dissolve in distilled water, for alternate 7 days.

Then the second group was further divided into another 4 sub- groups. The subgroups were as follows

**Group 2a**: Low dose root extract of *Achyranthes aspera* (100mg/kg b wt.) for 15 days regularly after sodium arsenate exposure.

**Group 2b**: High dose root extract of *Achyranthes aspera* (200 mg/kg b wt) for 15 days regularly after sodium arsenate exposure.

**Group 2c**: Low dose leaf extract of *Achyranthes aspera* (100 mg/kg b wt.) for 15 days regularly after sodium arsenate exposure.

**Group 2d**: High dose leaf extract of *Achyranthes aspera* (200 mg/kg b wt) for 15 days regularly after sodium arsenate exposure.

The dose of sodium arsenate was decided on the basis of experiments conducted in the laboratory and the concentration of sodium arsenate used in the experiment was .1mg/kg body weight.

The plant doses were decided on the basis of experiment conducted in the laboratory and on the basis of published report (Hughes and Thompson, 1996). Total duration of treatment for each group was of 22 days. After 22 days of duration the mice were fasted overnight and then on next day they were sacrificed under light chloroform anesthesia. Liver tissues were dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The homogenate that was centrifuged at 1,000 g for 20 min at 4°C was used for the estimation of malondialdehyde (MDA) and reduced glutathione (GSH). The supernatant obtained was further centrifuged at 12,000 g for 20 min at 4°C to obtain the postmitochondrial supernatant, which was used for the assays of superoxide dismutase (SOD), catalase activity (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST).

The lipid peroxidation was determined by measuring the amounts of MDA via the thiobarbituric acid color reaction.\textsuperscript{[11]} The results were expressed as nmol MDA formed per milligram of protein (nmol/mgprot). The GSH level was determined as described by Ellman\textsuperscript{[12]} and expressed as mg per gram of protein (mg/gprot). The SOD activity was
determined according to the method of Asada et al.\cite{13} by monitoring the rate of inhibition of reduction of nitroblue tetrazolium (NBT) by the enzyme. The activity of SOD was expressed as unit per milligram of protein (U/mgprot). One unit represents the amount of enzyme required to produce 50% inhibition of NBT reduction per minute. The CAT activity was measured spectrophotometrically by calculating the rate of degradation of H$_2$O$_2$\cite{14} and expressed as unit per milligram of protein (U/mgprot). One unit represents 1 μmol H$_2$O$_2$ degraded per min. The GPx activity was measured spectrophotometrically and expressed as unit per milligram of protein (U/mgprot). One unit of GPx represents 1 μmol oxidized NADPH per min.\cite{15} The GST was measured by using 1-Chloro-2-4- dinitrobenzene (CDNB)\cite{16} and expressed as unit per milligram of protein (U/mgprot). One unit was defined as 1 μmol CDNB-GSH conjugate formed per minute.

Statistical Analysis
The data was analyzed using the Statistical Package for Social Science Program (S.P.S.S. 16). For comparison between different experimental groups, one way analysis of variance (ANOVA) was used followed by post hoc Tukey’s test. The results were expressed as Mean ± S.D (Standard deviation). Level of significance between group was set at P<0.05. / P<0.001.

RESULTS
The present study was planned to investigate the ability of the Achyranthes aspera to scavenge free radicals generated during arsenic toxicity. Arsenic exposure led to significant rise in Thiobarbituric acid reactive substances (TBARS) and fall in SOD, CAT, and GST due to damage of liver tissue. Achyranthes aspera was found to show protective effect by lowering down the content of TBARS and enhancing the activities of CAT, GST, and SOD.

Estimation of Lipid Per oxidation (LPO), Enzymatic (CAT, SOD, Gpx, GST) and Non-Enzymatic Antioxidant parameters
Reduced activities of enzymatic (CAT, SOD, GST) and rise in the level of lipid per oxidation in liver homogenate are summarized in (Table 1).

The level of CAT, SOD, and GST is increased and LPO decreased towards normal value in plant extract treated, arsenic intoxicated mice. The antioxidant activity or the inhibition of the generation of free radical is important in the protection against Arsenic induced liver lesion. The level of lipid peroxide is a measure of membrane damage alterations in its structure and
function. The level of MDA, which is one of the end products of lipid per oxidation in liver tissue was found to be high in As-treated mice (Shown in Table1) implying enhanced lipid per oxidation leading to tissue damage and failure of antioxidant defense mechanisms against free radicals.

Treatment with extracts of *Achyranthes aspera* significantly reversed these changes. Our body has an effective defense system against free radical induced damage. It consists of a set of endogenous antioxidant enzymes, two of the key components of which is catalase (CAT) and Superoxide dismutase (SOD).

Regarding non-enzymatic antioxidants, reduced glutathione (GSH) is a critical determinant of tissue susceptibility to oxidative damage. It is an intracellular reductant which is extensively found in cells. It protects cells against electrophilic attacks by xenobiotics such as free radical peroxide.

**Liver Oxidative Stress and Antioxidant Defense Related Parameters**

1. **Effect of Extracts of *Achyranthes aspera* on the Level of TBARS in the Liver of Mice Treated with Arsenic**

Arsenic exposure led to significant rise in thiobarbituric acid reactive substances (0.313±0.002 nmols TBARS/h/g) as compared to control group (0.195±0.004 nmols TBARS/h/g). When all the doses of *A.aspera* such as root low (0.192±0.004 nmols TBARS/h/g), root high (0.192±0.002 nmols TBARS/h/g), leaf low (0.185±0.004 nmolsTBARS/h/g), leaf high (0.299±0.002 nmols TBARS/h/g) were given, significant decrease in LPO level was observed as compared to arsenic treated groups.

2. **Effect of Extracts of *Achyranthes aspera* on SOD Activity in the Liver of Mice Treated with Arsenic**

Arsenic exposure led to the fall in SOD (0.190±0.003 U/mg protein/h) as compared to control group (0.230±0.001 U/mg protein/h). When all the doses of *A.aspera* extracts were administered followed by sodium arsenate, it was found that root high (0.231±0.021 U/mg protein/h) showed significant result as compared to arsenic treated group. However, administration of leaf high (0.201±0.001 U/mg protein/h) showed moderate results.
3. Effect of Various Extracts of Plant on CAT Activity in the Liver of Mice Treated With Arsenic

In the current study, following arsenic exposure led to the decrease in CAT activity (0.329±0.001 μmol H₂O₂ consumed /min/mg protein) as compared to control group (0.407±0.003 μmol H₂O₂ consumed/min/mg protein) P<0.05. Administration of the doses to some extent played a role in normalization of CAT activity. Administration of root low (0.446±0.005), root high (0.428±0.009), leaf low (0.405±0.002) showed significant results as compared to arsenic treated group (P<0.05).

4. Effect of Various Extracts of Plant on GSH Level in the Liver of Mice Treated with Arsenic

In the current study, arsenic exposure led to rise in GSH (0.072±0.005 nmol GSH /g tissue) as compared to control groups (0.024±0.000 nmols GSH /g tissue). Administration of the plant doses did not showed significant results in normalization of GSH level as compared to arsenic treated group.

5. Effect of Various Extracts of Plant on GST Activity in the Liver of Mice Treated with Arsenic

In the current study, arsenic exposure led to significant decrease in GST activity (2.81±0.014 nmols CDNB conjugates formed/min/mg protein) as compared to control group (2.92±0.63 nmols CDNB conjugates formed/min/mg protein). Administration of leaf low dose (3.86±0.028 nmols CDNB conjugates formed/min/mg protein) show significant results as compared to control group in normalization of GST level (P< 0.05). Other doses did not showed significant results in normalizing GST level.

6. Effect of Various Extracts of Plant on GPx Activity in the Liver of Mice Treated with Arsenic

In the current study, arsenic exposure led to the significant fall in GPx activity (0.770±0.016 μg of glutathione utilized/min/mg protein) as compared to control group (0.80±0.007). Administration of root low dose (0.744±0.020 μg of glutathione utilized/min/mg protein), root high dose (0.705±0.034) showed significant results as compared to normal group.

DISCUSSION

Arsenic is a widespread constituent of earth’s crust, which has been disposed by man into the environment. Although the exact mechanism of arsenic induced toxicity is not completely
understood but data showed that oxidative stress plays an important role in its toxicity. Arsenic administration induces overproduction of ROS and lowers the cellular antioxidant capacity. The roles played by natural compounds in the modulation of toxic effects of arsenic are meager.

In the present study, *A. aspera* is found to have an inhibitory role over arsenic induced toxicity.

**Effect on Oxidative Stress Related Parameters**

Results indicate a significant alteration in the peroxidative process following arsenic exposure. The increase in lipid peroxidation level and decrease in the endogenous antioxidant enzymes e.g. SOD, CAT were observed in the present study after arsenic administration. The interesting finding is that the *A. aspera* was able to scavenge the oxidative damage produced due to arsenic toxicity as evidenced by decreased lipid peroxidation process and increased antioxidant status of the body (Table 1).

Cellular system is well protected from ROS-induced cell injuries by an array of defense systems, which are composed of various antioxidants with different functions. Whenever the ROS present in the cellular system overpower the defense system, they cause oxidative stress or cell injury, leading to the development of diseases. Proposed mechanisms for arsenic induced oxidative stress are reviewed by addressing their role in the generation of ROS plus their effect on the antioxidant defense system. Arsenic binds to thiol group of enzymes and damages the antioxidant defense system leading to the generation of free radicals which in turn causes the lipid peroxidation, protein oxidation and oxidized nucleic acids, ultimately leading to membrane damage, protein dysfunction and impaired DNA repair.¹⁷

Arsenic is known to produce oxidative damage by enhancing peroxidation of membrane lipids and lipid peroxidation is a deleterious process solely carried out by free radicals. In fact, lipid peroxidation is an outcome of the chain of events involving initiation, propagation and termination reaction.¹⁸ Unchecked peroxidative decomposition of membrane lipids is catastrophic for living system.

The lipid peroxides, produced are degraded to a variety of products, including alkanals, hydroxyl alkanals, and ketones alkenes.¹⁹ All these products inactivate cell constituents by oxidation or cause oxidative stress by undergoing radical chain reactions, ultimately leading
to loss of membrane integrity. Lipid peroxidation can also adversely affect the function of membrane bound proteins such as enzyme and receptor.

Several studies have focused on the possible toxic effects of arsenic on membrane components and identified a correlation between these effects and arsenic induced oxidative damage.\cite{17} Usually the deleterious effects of oxidative stress are counteracted by endogenous antioxidant enzymes, mainly Superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH).\cite{18} The present study is in accordance to previous reports as discussed above, the activities of SOD, CAT and antioxidants were reduced by arsenic, thus rendering the tissues to the peroxidative damage. CAT and SOD are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying the peroxides, hydrogen peroxide and superoxide anions. These antioxidant enzymes depend on various essential trace elements and prosthetic groups for proper molecular structure and enzymatic activity. The pathogenesis of arsenic is multifactorial, as arsenic directly interrupts enzyme activation, competitively inhibits trace mineral absorption and binds to SH proteins.

Furthermore, studies reported that Sodium arsenate treatment increased GSH levels (as our present findings) and SOD activity, slightly decreased GPx activity and significantly decreased CAT activity.\cite{6} Maithi and Chatterjee, 2000 reported that the liver and kidneys have different adaptive cellular protective mechanisms against arsenic exposure. The kidneys, in general, were observed to be more vulnerable to arsenic-treatment in male rats that had been exposed to sodium-arsenate (3.33 mg/kg b.wts) for 14 days. Significantly increased lipid peroxidation and decreased SOD and CAT activities were identified in the mice kidneys. On the other hand, lipid peroxidation and SOD activity in their liver remained unchanged following arsenate treatment. The liver showed significantly increased GSH levels and GST were reported to be protected from arsenite-induced oxidative damage by some antioxidant components.\cite{20}

Interestingly, \textit{A. aspera} application in the present study lead to decrease in lipid peroxidation and increase in the cellular antioxidant enzyme (Table 1).

**Implication**

The present study shows that high arsenic can markedly alter MDA content and some enzymes activities associated with free radical metabolism in liver of swiss albino mice. The findings indicate that high arsenic can induce oxidant stress in swiss albino mice. The
Mechanism of arsenic on antioxidant defense system in the body seems to involve lipid peroxidation, depletion of glutathione and decreased activities of some enzymes, such as SOD, CAT, GPx, and GST, which associate with free radical metabolism. Achyranthes aspera to some extent played the role in repairing damage caused as a result of oxidative stress induced by arsenic.

Table 1- Effect of various extracts of A. aspera on enzymatic, non-enzymatic parameters in the liver of mice treated with Sodium arsenate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 Control, distilled water</th>
<th>Group 2a Root low dose extract of A. aspera(100mg/kg b.wt)</th>
<th>Group 2b Root high dose extract of A. aspera(200mg/kg b.wt)</th>
<th>Group 2c Leaf low dose extract of A. aspera(100mg/kg b.wt)</th>
<th>Group 2d Leaf high dose extract of A. aspera(200mg/kg b.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO</td>
<td>0.195±0.004</td>
<td>0.313±0.002</td>
<td>0.192±0.004</td>
<td>0.185±0.004</td>
<td>0.299±0.002</td>
</tr>
<tr>
<td>SOD</td>
<td>0.230±0.001</td>
<td>0.190±0.003</td>
<td>0.198±0.004</td>
<td>0.210±0.003</td>
<td>0.201±0.001</td>
</tr>
<tr>
<td>CAT</td>
<td>0.407±0.003</td>
<td>0.329±0.001</td>
<td>0.446±0.005</td>
<td>0.428±0.009</td>
<td>0.405±0.002</td>
</tr>
<tr>
<td>GSH</td>
<td>0.0245±0.000</td>
<td>0.072±0.005</td>
<td>0.068±0.005</td>
<td>0.054±0.002</td>
<td>0.057±0.009</td>
</tr>
<tr>
<td>GST</td>
<td>2.92±0.063</td>
<td>2.81±0.063</td>
<td>2.98±0.014</td>
<td>3.38±0.678</td>
<td>3.86±0.028</td>
</tr>
<tr>
<td>GPx</td>
<td>0.80±0.007</td>
<td>.770±0.016</td>
<td>0.744±0.020</td>
<td>0.705±0.034</td>
<td>0.778±0.005</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.D

LPO: Lipid peroxidation (nmol of MDA formed/ g tissue); SOD: Superoxide dismutase (U/ml of tissue homogenate); CAT: Catalase (Micromoles of hydrogen peroxide degraded/min/mg protein); GSH: Reduced glutathione (mg/g of tissue); GST: nmols CDNB conjugates formed/min/mg protein; GPx: (microgram of glutathione utilized/min/mg protein)

Data are means±S.D (n=6).

aP < 0.05, as compared to the control( group 1); bP < 0.05, as compared to arsenic treated (group 2).

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