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Antioxidant Potential of *Ocimum sanctum* in Arsenic Induced Nervous Tissue Damage

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Abstract

The present study aimed to assess the antioxidant potential of hydro-alcoholic extract of Ocimum sanctum leaves (HAEO) in Wistar rats intoxicated with arsenic. The experimental animals (n=75) were divided randomly into five groups of 15 rats each. Rats in group I received distilled water, whereas rats in groups II, III, IV and V received arsenic as sodium arsenate salt @ 25 ppm in drinking water daily for 45 days. In addition, rats from groups III, IV and V received HAEO @ 50, 100 and 200 mg/ kg body weight, respectively, once daily for a period of 45 days. Rats of group I and II acted as negative and positive controls, respectively. At the end of the experiment, all rats were sacrificed; blood and tissues were assayed for various biochemical indicators of oxidative stress. Cytopathological changes in brain and spinal cord tissues were studied in arsenic intoxicated animals. The free radical scavenging and intracellular antioxidant activities of HAEO were determined from its radical scavenging ability and antioxidant power assay. HAEO was found to inhibit lipid peroxidation in brain tissue in a dose dependent manner. Administration of HAEO @ 100 mg/kg body weight was found to be more effective in restoring action on discrete regions of the brain than dose rate of 50 and 200 mg. Non-significant effect of HAEO on blood and tissue arsenic concentrations was observed except some moderate depletion of blood arsenic concentrations. Mild to severe necrosis and degenerative changes were observed in brain tissues of arsenic intoxicated animals. It is concluded that administration of HAEO could provide specific protection from oxidative injury in arsenic intoxicated rats by accelerating the recovery of antioxidant enzymes to normal levels but it did not have protective effect on brain cellular structures. The proposed mechanism for this protection is the free radical scavenging ability of Ocimum sanctum plant leaves.

Key Words: *Ocimum sanctum*, sodium arsenite, oxidative stress, antioxidant, brain.

Introduction

Arsenic is a naturally occurring element that has been recognized as a human poison since ancient times. Higher level of arsenic in ground water is associated with sedimentary deposits derived from volcanic rocks, hence mineral leaching appears to be the source of arsenic concentration (21). Arsenic is ubiquitously distributed in the environment in a number of organic and inorganic forms and thus exposure to this metalloid has become inevitable for both man and animals (2). Because of the abundance of arsenic, humans experience daily exposures via ingestion as major route through drinking water, and inhalation and skin absorption as a minor route (34). Episodes of arsenic poisoning, caused by arsenic contaminated water, have already been reported in many countries of the world including Bangladesh, India, Nepal, Cambodia, Myanmar, Taiwan, Mongolia, Vietnam, China, Afghanistan, Pakistan, Argentina, Mexico, Chile and the United States (5). Besides the natural sources, arsenic contaminating herbicides, insecticides and rodenticides are also potential vehicles of arsenic toxicity (8). Chronic exposure to arsenic due to consumption of contaminated water gives rise to several ill health effects in man and animals raising public health concern globally (10). Epidemiological studies have demonstrated that arsenic causes neurotoxicity including impairment of learning and

concentration (27). Several studies have reported arsenic-induced brain damage (33) as arsenic crosses the blood–brain barrier and has a wide range of effects on brain white matter (29). Epidemiological and experimental studies indicate that cerebral and cerebellar cortexes may be affected with arsenic (22). Brain damage is closely associated with oxidative stress induced by arsenic (3). Because of its high metabolic rate and relatively reduced capacity for cellular regeneration compared with other organs, the brain is believed to be particularly susceptible to the damaging effects of ROS (Reactive Oxygen Species). The brain has a limited capacity to detoxify ROS owing to the lack of glutathione-producing capacity in neurons, (35). Therefore, neurons are the first cells to be affected by the increase in ROS and shortage of antioxidants and, as a result, are most susceptible to oxidative stress.

Diet rich in B-vitamins, antioxidants or micronutrients was found to provide adequate protection from arsenic toxicity in human beings (20). Several chelators alone or with antioxidants were tried successfully in treatment of arsenic toxicity. Ascorbic acid, thiamine and methionine greatly enhance the prophylactic potential of chelators and help in the removal of heavy metals like arsenic, lead etc (31). In recent years, the clinical importance of herbal drugs has received considerable attention. Many synthetic drugs or antioxidants have been shown to have one or more side effects; there has been increasing interest in the therapeutic potential of plants having antioxidant properties in reducing free radical induced tissue injury leading to better clinical recovery. The leaf extract of Oscimum sanctum has potent free radical scavenging activity in vitro and lipid peroxidation activity in vivo (38). The aim of the present study was to establish the chronic effects of arsenic on central nervous system, possible target for arsenic toxicity and to evaluate the effect of feeding hydro alcoholic extract of Oscimum sanctum leaves (HAEO) on oxidative changes induced by sodium arsenate in the brain tissues of Wistar rats.

Materials and methods

Chemicals

Bovine serum albumin (BSA), Ammonium sulfate, 2,4-dinitrophenylhydrazine (DNPH), 5,5- dithiobis (2-nitrobenzoic acid) (DTNB), ethylene diamine tetra-acetic acid (EDTA), nitro blue tetrazolium (NBT), potassium dihydrogen phosphate, reduced glutathione (GSH), sodium arsenite ($NaAsO_2$), sodium azide, sodium pyrophosphate, trichloro acetic acid (TCA), thiobarbituric acid (TBA), were procured from Sisco Research Laboratory, India. All chemicals and reagents used in present experiment were of analytical grade.

Preparation of Extract

Mature leaves of Ocimum sanctum plant were collected from nearby areas of Durg district, India. The leaves were washed thoroughly, shade dried, blended to form a fine powder. Hydro-alcoholic extract of Ocimum sanctum plant leaves (HAEO) was prepared by dissolving 250 g leaf powder 187.5 ml of methanol and 62.5 ml of distilled water. The mixture was filtered through Whatman No.1 filter paper in a Buchner funnel under vacuum. The filtrate was concentrated by evaporation with a vacuum rotary evaporator at 45°C to yield 17.92 g of solid mass. The dried extract was stored at 4°C for further use. The desired doses of HAEO were prepared by dissolving the required quantity of extract in triple distilled water.

Experimental Animals

Seventy five healthy adult Wistar rats, weighing between 200 and 250 gm were acclimatized under laboratory conditions for 2 weeks before the start of the experiment. They were provided with standard diet and water ad libitum and maintained under standard conditions of temperature (30–370C) and humidity (50%) with alternating 12 h light / dark cycles. The protocol of the study was in conformity with the guidelines of the Institutional Ethical Committee.

Pilot Study

A pilot study was conducted on rats to select the dose of sodium arsenate which could produce the minimum toxic effect. The estimated dose was used in experimental rats, which could induce arsenic toxicity but did not produce mortality.

Approximate LD50 of HAEO was found to be 4505 ± 80 mg/ kg body weight (bw) on administration by oral route and mortality was observed for a period of 72 hours. The administration of HAEO did not produce any acute toxic symptoms (100% survival) at dose rate up to 4.5 gm/ kg bw. Median effective dose of HAEO was the criteria for selection of graded doses of HAEO for the experiment.

Experiment Schedule

The experimental animals were divided randomly into five groups of 15 rats each to study oxidative stress indices, histopathological changes, and arsenic burden in tissues and ameliorative potential of HAEO during oral exposure of arsenic in rats through drinking water. Rats of group I were kept as healthy controls and they received distilled water ad libitum for a period of 45 days, whereas rats of groups II, III, IV and V received arsenic as sodium arsenate salt @ 25 ppm in drinking water daily for 45 days. The animals of group II acted as positive control, while animals of groups III, IV and V received HAEO @ 50, 100 and 200 mg/ kg bw, respectively, once daily in drinking water for a period of 45 days. Groups III, IV and V constituted the treatment groups and were designated as $HAEO_{50}$, $HAEO_{100}$ and $HAEO_{200}$.

Collection of Blood and Brain Tissue Samples

On day 46, blood samples (3.0 ml) were collected from all experimental rats under anesthesia from the retro-orbital plexus through a fine capillary tube to estimate arsenic concentration and oxidative stress markers in blood. RBC hemolysate of each blood sample was prepared at 10% concentration.

After sacrificing all experimental rats by cervical dislocation, different parts of brain and spinal cord tissues were excised: some were sliced for histopathological studies, others were washed and homogenized in a mixture of 0.1M Tris HCl and 0.001M EDTA buffer (pH 7.4) and centrifuged at 12,000 g for 30 min at 4^0C. The supernatant was collected and used for the analysis as tissue homogenate.

Biochemical Assay

In all assays of present experiment equal volume of distilled water was used as blank to that of RBC hemolysate or brain tissue homogenate.

Arsenic Concentration in Blood and Brain

Blood and tissue samples were digested immediately after collection by adding concentrated Nitric acid and 30% Hydrogen peroxide (3:1). Digestion was carried out in bomb at 100^0C for 6 h in hot air oven. Dilution of known concentration was finally made with triple distilled water. Samples were finally stored at -20^0C up to analysis. Arsenic was estimated using a Hydride Vapour Generation System (Perkin Elmer model MHS-10) fitted with an atomic absorption spectrophotometer.

Lipid Peroxidation in Blood

Lipid peroxide level in 10% RBC hemolysate was determined using the method of Placer et al. (23). Briefly, the reaction mixture consisted of 0.2 ml of RBC hemolysate, 1.3 ml of 0.2 M Tris-0.16M KCl buffer (pH-7.4) and 1.5 ml of TBA reagent. The mixture was heated in boiling water bath for 10 min using glass beads as condenser. After cooling, 3 ml of pyridine/ n-butanol (3:1 v/v) and 1 ml of 1N NaOH were added to it and mixed by shaking vigorously. The absorbance was read at 548 nm. The nmol of malonaldehyde (MDA) per ml of RBC hemolysate was calculated using 1.56×10^5 as the extinction coefficient (39). Lipid peroxide level in the RBC hemolysate was expressed as nmol of MDA/mg of haemoglobin.

Lipid Peroxidation in Brain Tissue Homogenate

Lipid peroxide level in brain tissue homogenate was estimated by adding 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of thiobarbituric acid (TBA) and 0.6 ml of distilled water to 0.2 ml of homogenate. The mixture was heated at 90^0C for 1 hr in a water bath and then cooled under tap water. One ml of distilled water and 5 ml mixture of n-butanol and pyridine (15:1, v/v) was added and shaken vigorously. The absorbance of organic layer was measured at 532 nm after centrifugation at 4000 g for 10 min and level of endogenous lipid peroxide was expressed in nmol of MDA/ mg of protein using 1.56×10^5 as the extinction coefficient (39).

Non-Enzyme Antioxidant: Glutathione

Reduced glutathione (GSH) was estimated in hemolysate and tissue homogenate by dithio-bis-2-nitro benzoic acid (DTNB) method as per the procedure outlined by Prins and Loos (24). To 200 µl of hemolysate, 4000 µl of 0.08N H_2SO_4 was added and kept at room temperature. Sodium tungustate solution (500 µl) was added to it and shaken vigorously for 5 min. The mixture was centrifuged at 2000 g for 20 min; 2000 µl of supernatant was taken in a separate test tube and Tris buffer and DTNB reagent were added and mixed well. The absorbance was recorded spectrophotometrically at 412 nm against blank. Similar procedure was adopted for brain tissue homogenate to estimate GSH.

Catalase Activity

Catalase activity was measured spectrophotometrically in hemolysate and tissue homogenate after appropriate dilution following the method of Cohen et al. (6). The reaction commenced with the addition of 50 µl of diluted sample to 3 ml of phosphate buffer-H2O2 solution. Initial absorbance was read after 20 seconds. Time required for initial absorbance to decrease by 0.05 unit was recorded between 0.6 to 0.7 unit at 240 nm. Catalase present in assay mixture was expressed in unit/ mg of haemoglobin and protein for blood and brain tissue, respectively.

Superoxide Dismutase

Superoxide dismutase (SOD) activity was measured in hemolysate and tissue homogenate using nitroblue tertazolium as substrate as per the method of Marklund and Marklund (17) with certain modifications suggested by Minami and Yoshikawa (18). Briefly, the assay mixture consisted of 50 mM of tris cacodylic acid buffer (pH - 8.2), and 0.2 nM of pyragallol in a total volume of 3.0 ml. The increase in absorbance due to auto-oxidation of pyragallol was recorded at 30 sec and 90 sec using 420 nm wavelength. One unit of SOD activity was defined as the amount of enzyme, which inhibited the auto-oxidation of pyragallol by 50% under the given laboratory condition and the value was expressed in unit/ mg of haemoglobin and protein for blood and brain tissue respectively.

Protein Determination

Protein concentration in erythrocyte hemolysate and tissue homogenates were determined photometrically using Folin-Phenol reagent (14) and bovine serum albumin (BSA) at 660 nm.

Hemoglobin Determination

Hemoglobin in erythrocyte hemolysate was determined by cyanomethamoglobin method and values were expressed in mg/ml of hemolysate (4).

Histological Studies

The brain and spinal cord tissues of all experimental rats were fixed in 10% buffered formalin and were processed for paraffin embedding. Sections of about 5 µm thickness were stained with hematoxylin and eosin to study the histology of brain of all experimental rats.

Statistical Analysis

Data were subjected to one-way ANOVA for analysis of variance and significant differences were analyzed by Duncan's multiple range test using a SPSS computer programme version 10.0. (36).

Results

Arsenic Concentration in Tissues

Arsenic tissue concentration in hemolysate and homogenate of brain and spinal cord are presented in table 1. The concentration of arsenic in the blood, cerebral cortex, and cerebellum increased significantly in rats of group II whereas non-significant increase in arsenic concentration was observed in medulla oblongata and spinal cord. Administration of HAEO (50, 100 and 200) reduced the arsenic accumulation in cerebral tissues and maximum restoration was observed with HAEO100.

Blood Oxidative Stress

Table 2 depicts the level of oxidative stress markers in arsenic exposed animals. Administration of sodium arsenite caused significant (P< 0.05) increased levels of LPO in blood and decreased levels of GSH, CAT and SOD values were observed in animals of group II (positive control) as compared to that of group I(negative control). Treatment with HAEO resulted in significantly (P< 0.05) reduced LPO and significant increase in GSH, CAT and SOD concentrations in blood. Maximum restoration was observed with HAEO100**.**

Effect on Lipid Peroxidation in Brain Tissue

In brain and other nervous tissues except spinal cord, arsenic exposure resulted in significantly (P<0.05) increased LPO levels as compared to negative control group. HAEO treatment resulted in significant (P< 0.05) reduction in LPO levels and maximum reduction was recorded with HAEO100 group (Table 3).

Table1. Effect of arsenic and HAEO on the levels of arsenic concentration (ppm) of the control and experimental animals.

Groups	Blood	Cerebral cortex	Cerebellum	Medulla Oblongata	Spinal cord
Negative control	0.17±0.04	0.14±0.05	0.27±0.25	0.17±0.13	0.07±0.03
Positive control	4.08±0.04*	3.84±0.06*	2.62±1.30*	0.28±0.19	0.14±0.03
$NaAsO_2$+HAEO(50mg/kg)	3.77±0.04	2.81±0.04	1.96±1.22	0.11±0.11	0.12±0.04
$NaAsO_2$+HAEO(100mg/kg)	3.52±0.04	2.34±0.03	1.73±1.16	0.10±0.14	0.13±0.05
$NaAsO_2$+HAEO(200mg/kg)	3.36±0.04	3.17±0.04	1.30±1.32	0.18±0.16	0.12±0.06
$NaAsO_2$+HAEO(200mg/kg)	3.36±0.04	3.17±0.04	1.30±1.32	0.18±0.16	0.12±0.06

Negative control=Healthy animals, Positive control= Arsenic exposed ($NaAsO_2$) @25ppm, Values are Mean±SE; * p < 0.05 compared to Negative control

Table2. Levels of the oxidative markers in the blood of the control and experimental rats.

Groups	LPO **(nmol MDA/mg of protein)**	GSH **(nmol/mg of protein)**	CAT **(units/mg of protein)**	SOD **(units/mg of protein)**
Negative control	2.77±0.41	10.49±1.56	6.13±2.16	2.32±0.46
Positive control	6.32±0.36*	7.47±2.30*	2.18±2.39*	0.52±0.31*
$NaAsO_2$+HAEO(50mg/kg)	5.21±0.47	8.13±2.38	4.69±1.63	1.60±0.27
$NaAsO_2$+HAEO(100mg/kg)	3.72±0.40#	9.84±1.08#	5.90±2.30#	2.29±0.30#
$NaAsO_2$+HAEO(200mg/kg)	4.16±0.28	8.95±1.06	5.86±1.80#	2.46±0.36#
$NaAsO_2$+HAEO(200mg/kg)	3.36±0.04	3.17±0.04	1.30±1.32	0.18±0.16

Negative control=Healthy animals, Positive control= Arsenic exposed ($NaAsO_2$) @25ppm, Values are Mean±SE; n = 15; * P < 0.05 compared to Negative control and # compared to positive control animals.

Table3. Effect of arsenic and HAEO on the levels of LPO (nmol /mg of protein) of the control and experimental animals.

Groups	Cerebral Cortex	Cerebellum	Medulla Oblongata	Spinal Cord
Negative control	3.66±0.31	2.58±0.30	3.67±0.25	2.75±0.40
Positive control	8.43±0.37*	5.37±0.22*	6.62±0.33*	3.36±0.44
$NaAsO_2$+HAEO(50mg/kg)	7.54±0.22	4.87±0.49	4.13±0.53	3.42±0.49
$NaAsO_2$+HAEO(100mg/kg)	4.12±0.21#	3.76±0.47#	2.44±0.50#	2.39±0.31
$NaAsO_2$+HAEO(200mg/kg)	4.73±0.42#	4.22±0.42	3.87±0.36#	3.55±0.25

Negative control=Healthy animals, Positive control= Arsenic exposed ($NaAsO_2$) @25ppm, Values are Mean±SE; n = 15; * P < 0.05 compared to Negative control and # compared to positive control animals.

Effect on GSH

Arsenic administration significantly (P<0.05) decreased GSH level in cerebral cortex, cerebellum, medulla oblongata and spinal cord. HAEO treatment was found effective in significant (P<0.05) elevation of GSH level with HAEO100 and HAEO200 in brain tissue (Table 4)

Effect on Antioxidant Enzymes

The changes in CAT and SOD activity, which is indicative of oxidative stress following exposure to arsenic either alone or in combination with different doses of Ocimum sanctum are presented in Tables 5 and 6, respectively. Activity of CAT follow declined trend in cerebral cortex, medulla oblongata and cerebellum however in spinal cord non-significant decrease in CAT activity was observed. Animals of III, IV & V groups

responded to HAEO treatment but HAEO100 was found most effective and CAT activity increased significantly as compared to positive control group (group II) animals in affected tissues. In Group II animals significantly ($P<0.05$) decreased SOD activities in cerebral cortex and cerebellum and non-significant decreased activity was observed in medulla oblongata and spinal cord. HAEO administration increased SOD activities in all treatment groups compared to positive control group. However maximum increase in SOD activity was observed in animals treated with HAEO100.

Table4. Effect of arsenic and HAEO on the levels of GSH (nmol/mg of protein) of the control and experimental animals

Groups	Cerebral Cortex	Cerebellum	Medulla Oblongata	Spinal Cord
Negative control	12.76±0.31	14.68±0.30	13.67±0.25	12.75±0.40
Positive control	8.53±0.37*	8.27±0.22*	7.62±0.33*	8.06±0.44*
$NaAsO_2$+HAEO(50mg/kg)	10.54±0.22	8.46±0.49	8.13±0.53	9.42±0.49
$NaAsO_2$+HAEO(100mg/kg)	11.62±0.21$^{\#}$	11.96±0.47$^{\#}$	12.34±0.50$^{\#}$	10.79±0.31$^{\#}$
$NaAsO_2$+HAEO(200mg/kg)	11.87±0.42$^{\#}$	10.22±0.42$^{\#}$	11.67±0.36$^{\#}$	9.85±0.25

Negative control=Healthy animals, Positive control= Arsenic exposed ($NaAsO_2$) @25ppm, Values are Mean±SE; n = 15; * $P < 0.05$ compared to Negative control and $^{\#}$ compared to positive control animals

Table5. Effect of arsenic and HAEO on the levels of CAT (Units /mg of protein) of the control and experimental animals

Groups	Cerebral Cortex	Cerebellum	Medulla Oblongata	Spinal Cord
Negative control	40.22±1.50	28.72±0.51	42.39±0.30	40.26±0.45
Positive control	23.41±1.29*	15.63±0.42*	27.33±0.42*	38.44±0.32
$NaAsO_2$+HAEO(50mg/kg)	30.29±1.38	17.72±0.53	30.49±0.38	37.32±0.59
$NaAsO_2$+HAEO(100mg/kg)	36.44±1.62$^{\#}$	22.62±0.59$^{\#}$	35.65±0.40$^{\#}$	37.69±0.40
$NaAsO_2$+HAEO(200mg/kg)	32.72±1.46$^{\#}$	18.31±0.47$^{\#}$	33.15±0.26$^{\#}$	36.25±0.33

Negative control=Healthy animals, Positive control= Arsenic exposed ($NaAsO_2$) @25ppm, Values are Mean±SE; n = 15; * $P < 0.05$ compared to Negative control and # compared to positive control animals.

Table6. Effect of arsenic and HAEO on the levels of SOD (units/mg of protein) of the control and experimental animals

Groups	Cerebral cortex	cerebellum	Medulla Oblongata	Spinal cord
Negative control	6.22±2.11	8.27±0.35	7.22±0.52	5.66±0.43
Positive control	2.69±1.59*	3.65±0.28*	6.60±0.31	4.22±0.34
$NaAsO_2$+HAEO(50mg/kg)	3.53±1.22	6.57±0.36	6.38±0.44	4.76±0.57
$NaAsO_2$+HAEO(100mg/kg)	5.86±1.85$^{\#}$	7.59±0.50$^{\#}$	7.83±0.26	5.02±0.36
$NaAsO_2$+HAEO(200mg/kg)	3.20±1.33	4.12±0.41	6.76±0.33	5.23±0.25

Negative control=Healthy animals, Positive control= Arsenic exposed ($NaAsO_2$) @25ppm, Values are Mean±SE; n = 15; * $P < 0.05$ compared to Negative control and # compared to positive control animals.

Histopathological Findings

Histopathological changes in different nervous system areas were examined by haemaoxylin and eosine staining. Accumulation of serous fluid and neuronal degeneration and necrosis were observed in cerebrum (Figure 1) and spinal cord (Figure 2). No pathological alteration was reported in medulla oblongata in arsenic exposed rats. Administration of either dose of HAEO did not provide any protective effect on cellular architecture of nervous tissues including brain and spinal cord.

Discussion

The present study evaluated the effects of hydro-alcoholic extract of Ocimum sanctum on arsenic-induced oxidative stress in blood and brain tissues of Wistar rats. Samson et al. (32) reported abundance of phytochemicals such as phenolics and flavonoids in Ocimum sanctum plant leaves. Arsenic concentrations significantly increased in blood and different parts of brain tissues upon arsenic exposure for a period of 45 days. Arsenic intoxication was further witnessed by histopathological changes observed in different parts of brain and spinal cord. Arsenic can cross the blood-brain barrier and accumulate in nervous tissues (28); similarly increased arsenic concentration has been reported in blood upon arsenic exposure in rats (12).

Arsenic exposure produces free radicals, which cause damage to lipid, protein and DNA (16). It is evident from the present study that arsenic exposure at the dose rate of 25 ppm resulted in alteration of oxidative stress markers in blood and brain tissues. Arsenic intoxication induced a high degree of lipid peroxidation in brain tissues of the experimental rats due to the susceptibility of brain tissues toward oxidative damage (40). Increased lipid peroxidation is thought to be the consequences of oxidative stress, which occurs when the dynamic balance between peroxidant and antioxidant mechanism is impaired. Administration of HAEO resulted in significant reduction in LPO level in brain tissues probably by

suppressing lipid peroxidation through different chemical mechanisms, including free radical quenching, electron transfer, radical addition, or radical recombination. Arsenic exposure led to severe depletion of GSH levels in blood and nervous tissues. Arsenic decreases the available pool of reduced glutathione through direct union to its sulfhydryl groups, but more importantly through its metabolism, since glutathione is required for several steps of arsenic methylation and excretion (11, 37). An increase in brain arsenic concentration resulted in marginal decrease in GSH level and an increase in lipid peroxidation in RBC hemolysate as well as in brain homogenate. Present observation approximates with earlier report of reduction in glutathione level in arsenic exposed rats (19). Treatment with HAEO could prevent the arsenic induced alteration in concentrations of non-enzymatic antioxidant markers probably due to its free radical scavenging activity.

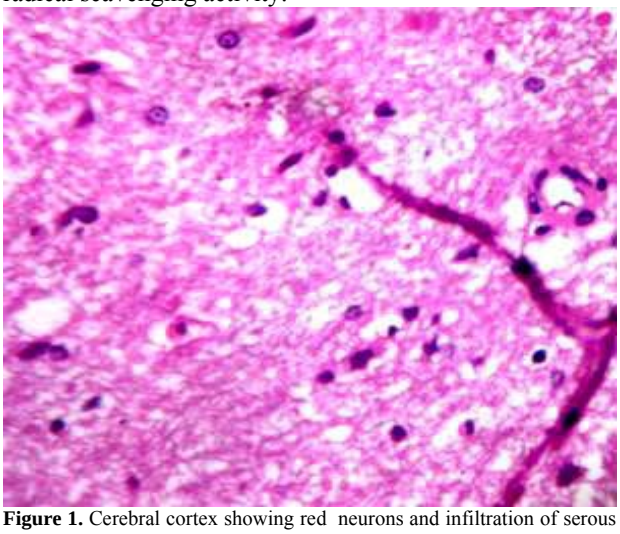

Figure 1. Cerebral cortex showing red neurons and infiltration of serous exudate.(HE, 200X).

Antioxidant enzymes are considered to be the first line of cellular defense that prevents cellular components from oxidative damage. Among them SOD and CAT mutually function as important enzymes in the elimination of ROS. Reduction in SOD activity in arsenic-exposed rats may be due to the over production of super oxide radical anions (41). Flora and Tandon (7) and Ramanathan et al. (25) also found pronounced depression of antioxidant enzymes associated with increased lipid peroxidation in arsenic intoxicated rats.

Arsenic intoxication significantly reduced the CAT activity in the brain tissues of experimental rats. The paucity of NADH accumulation during arsenic metabolism might decrease the catalase activity, since NADH is required for the activation of CAT (13). CAT is a hemoprotein, which reduces hydrogen peroxide to molecular oxygen and water (9). Dimethylated metabolites of arsenic react with molecular oxygen, which can induce generation of free radicals (1). Reduced activity of antioxidant enzymes such as SOD and CAT upon arsenic exposure in nervous tissues was recorded in the present study. In the present experiment HAEO100 extract treatment for 45 days in arsenic intoxicated rats improved the activity of antioxidant enzymes, SOD and CAT. HAEO50 in the pilot study proved to be ineffective to ameliorate the oxidative stress in arsenic exposed rats whereas under HAEO200 exposure two rats died.

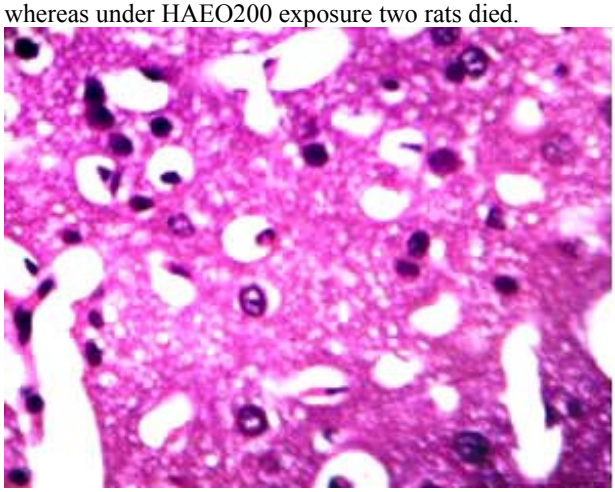

Figure 1. Spinal cord with infiltration of serous fluid and red neurons.(HE, 400X).

Nervous tissues, particularly neurons, are severely affected with arsenic induced toxicity as described in this paper, which induces pathological changes through oxidative damage. Roy et al. (30) reported similar observation in brain tissues of arsenic intoxicated goats. Recent studies have suggested that low levels of glutathione and increased oxidative stress may play a significant role in the brain pathology due to arsenic poisoining.

From the present study, it may be concluded that arsenic intoxication resulted in severe oxidative impairment in the nervous tissues of the experimental animals and that could be minimized by oral administration of HAEO. Treatment with HAEO resulted in reduction of lipid peroxidation and acceleration in recovery of antioxidant enzymes to normal levels in rats intoxicated with arsenic. HAEO100 showed most effective antioxidant potential among three doses used in present study. However, HAEO administration did not act in restoring cellular architecture of nervous tissues, which were damaged due to arsenic intoxication.

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