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PROTECTIVE EFFECT OF *EICHHORNIA CRASSIPES* AFTER ARSENIC INTOXICATION IN THE LIVER IN ALBINO RAT

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ABSTRACT

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. Chronic exposure to arsenic due to consumption of contaminated water gives rise to several ill health concerns globally. Plants are conceived as source of antioxidants due to presence of phenol, which posses wide biological properties. Recent studies showed that phenolic compounds help in ganging the antioxidant potential of tuberous plants. Carotenoids and phenols were reported to be non enzymatic antioxidants which take part in defence mechanism by scavenging reactive oxygen species.

INTRODUCTION

Poisoning from arsenic in drinking water is a very serious problem in many regions of the world. Upto 50 million people world wide have been severely affected. The group of diseases caused by drinking water with high levels of arsenic daily is called arsenicosis. People with arsenicosis initially develop sores on the palms of their hands and on the soles of their feet, known as keratosis, which make daily chores both challenging and painful. Although the disease is not contagious the physical manifestations are such that people with arsenicosis are socially excluded.Eventually, arsenicosis leads to death, usually caused by internal cancer. Suffering of large human population from arsenicosis, particularly arsenic related skin lesions due to consumption of contaminated drinking water . For a prolonged period has been reported from many countries of the world, including Argentina, Bangladesh, India, Mexico, Thailand and Taiwan. The generation of reactive oxygen species and development of oxidative stress in the target organs in one of the several mechanism through which arsenic exerts its toxicity. Oxidative stress due to acute arsenic toxicity in rats has been ameliorated by therapeutic supplementation of nutritional antioxidants like ascorbic acid, alpha tocopherol or cystine. Deficiency of methionine, a precursor amino acid needed for glutathione synthesis, in normal diets of rabbits makes them more susceptible to arsenic toxicity.

Many synthetic antioxidant compounds have shifted the attention on the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging activity and thus maintaining the health. There is also extensive evidence implicating that the free radical damage to cell leads to pathological changes associated with aging. Free radicals may be a contributory factor in a progressive decline in function of immune system. Water hyacinths (*Eichhornia crassipes*) are free

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floating aqueous weeds that multiply very quickly. With pinkish-purple flowers, they grow in dense mats in tropical and subtropical freshwater rivers, lakes and reservoirs. The plant originated in the Amazon Basin and was introduced into many parts of the world as an ornamental garden pond plant due to its beauty. The plant *Eichhornia crassipes* belongs to the family Pontedericeae, closely related to the Liliaceae. The mature plant consists of long, pendant roots, rhizomes, stolons, leaves, inflorescences and fruit clusters. The roots are very fibrous and provide the plant with all of their nutrients from the water. The plants are up to 1 metre high, although 40cm is the more usual height. The inflorescence bears 6 - 10 lily-like flowers, each 4 - 7cm in diameter. The stems and leaves contain air-filled tissue which give the plant its considerable buoyancy. The plant itself, although more than 95% water, has a fibrous tissue and a high energy and protein content. It has been used for the removal or reduction of nutrients, heavy metals, organic compounds and pathogens from water. Since the plant has abundant nitrogen content, it can be used as a substrate for biogas production and the sludge obtained from the biogas can be used as compost. However, due to easy accumulation of toxins, the plant is prone to get contaminated when used as feed. Parts of the plant are also used in the production of traditional handicrafts in Southeast Asia. In Bangladesh, farmers have started producing fertiliser using Water hyacinth.

Antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) involves the rapid conversion of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) in order prevent the former from participating in the formation of highly pernicious hydroxyl radicals. The H_2O_2 generated in this manner is a powerful membrane permeant oxidant in its own right that has to be rapidly eliminated from the cell in order to prevent the induction of oxidative damage to lipids, proteins and DNA. The GSTs are ubiquitous multifunctional enzymes, which play a key role in cellular detoxification. The enzymes protect cells against toxicants by conjugating them to glutathione, there by neutralizing their electophiles sites, and rendering the products more water soluble. This activity is critical in the detoxification of peroxidised lipids as well as the metabolism of xenobiotic. The phospholipid hydroperiode GPx (PHGPx) is one of the most important GPx isoforms in a testicular context and is highly expressed in both spermatogenic and leydig cells. The mammalian liver is the largest gland of the body. Liver is responsible for performing variety of function independently

MATERIALS AND METHODS

The present investigations have been made on acclimatized specimens of albino rat, *Rattus norvegicus* (Berkenhout), under the good laboratory practices. The colony of albino rats was bred in the animal house of zoology Department, School of Life Sciences, Khandari Campus, Agra. Thirty five male albino rats of almost equal size and weight 120 ± 25 gm and eight weeks aged were selected for the present investigations. The albino rats were housed in polypropylene cages measuring 45 x 25 x 15 cm and maintained in controlled temperature (25 ± 2ºC), humidity (65 ± 10%) and proper circadian rhythm. The cages were regularly cleaned to avoid abnoxious odours and infections. They were fed with Goldmohar brand feed (manufactured by Lipton India Ltd., New Delhi) and tap water *ad libitum*. The albino rats were maintained under good laboratory practices (GLP) and guidelines of committee for the purpose of control and supervision on experiments on animals (CPCSEA) were followed. The arsenic water was collected from Sikandra area of Agra. The water samples were collected directly from

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different as usual water sources like hand pumps in polypropylene bottles and were carried for estimations.

Selection of Doses: We selected the arsenic water sample of Sikandra area of Agra. The maximum limit of arsenic concentration was found 0.102mg/l among the tested samples. This arsenic water sample of above area was given to rats as drinking water daily for 7, 14, 21 days respectively.

Dose of *Eichhornia crassipes:* Because of wide variations in preparation techniques, the optimum dose of *Eichhornia crassipes* has not been determined. In some previous studies *Eichhornia crassipes* was administered orally to dogs and human (Khandare, *et al*., 2000) and 2002) at 10 gm/kg body weight in juice formulation. In these studies having observed their effective outcome, we selected the dose of *Eichhornia crassipes* at 10 gm/Kg body weight for the present study.

Experimental Protocol: The selected twenty albino rats of almost equal weight and size were divided into four groups of five rats each. The one group of albino rats were treated as control group, while rest three groups were treated with arsenic water for 7, 14 and 21 days respectively.

Biochemical estimations: lipid perioxidation, superoxide dismutase, chloramphenicol acetyltransferase, glutathione s-transferases, glutathione reductase were estimated by standard kit methods.

Statistical Calculations: Standard protocols with the help of statistical software were used after Fischer and Yates (1950).

RESULTS AND DISCUSSION

Superoxide dismutase (SOD), catalase (CAT and glutathione reductase (GR) shows decreasing trend, while lipid peroxidation (LPO) and glutathione-s-transferase (GST) shows increasing trend after administration of arsenic which comes to normalized values after *E. crassipes* treatment after 3 days, 7 days and 14 days arsenic treatments (Table-1, Fig. 1-5)

Table – 1

Beneficial effects of *E. crassipes* on liver biochemical parameters of albino rat after Arsenic water intoxication

Experimental Period	Lipid peroxidation (nmol/ml)	Superoxide dismutase (nmol/ml)	Chloramphenicol acetyltransferase (nmol/ml)	Glutathione-s-transferase (nmol/min/ml)	Glutathione reductase (nmol/min/ml)
Control	3.302±0.018	3.032±0.037	18.68±0.08	0.21±0.01	22.70±0.25
7 days As	3.866±0.038***	2.62±0.061***	16.28±0.33***	0.296±0.15^{NS}	18.04±0.13***
3 days *E. crassipes* treatment after 7 days As	3.092±0.021***	2.988±0.039^{NS}	18.83±0.12***	0.262±0.01^{NS}	20.87±0.06***
15 days As	4.54±0.067***	2.118±0.0729***	15.29±0.03***	0.08±0.07***	16.44±0.05***
7 days *E. crassipes* treatment after 15 days As	3.242±0.024***	3.02±0.052***	19.30±0.09***	0.314±0.04***	21.64±0.09***

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21 days As	6.678±0.061***	0.908±0.0672***	12.82±0.15***	0.048±0.01***	11.70±0.21***
14 days *E. crassipes* treatment after 21 days As	3.37±0.054***	4.982±0.168^{NS}	21.70±0.04***	0.454±0.04***	22.83±0.10***

Number of rats in each set= 5; As = Arsenic water ingestion; ± S.Em. = Standard Error of mean.
NS = non significant; * = significant; ** = highly significant; *** = very highly significant

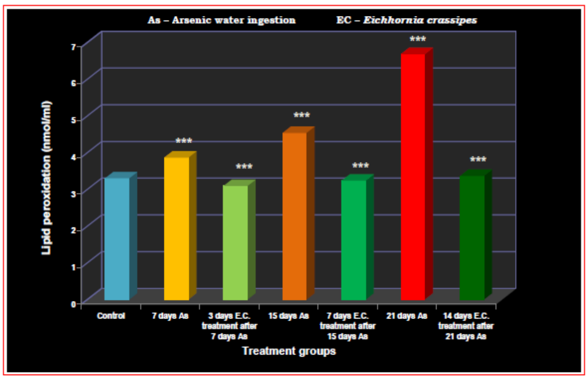

Fig. 1

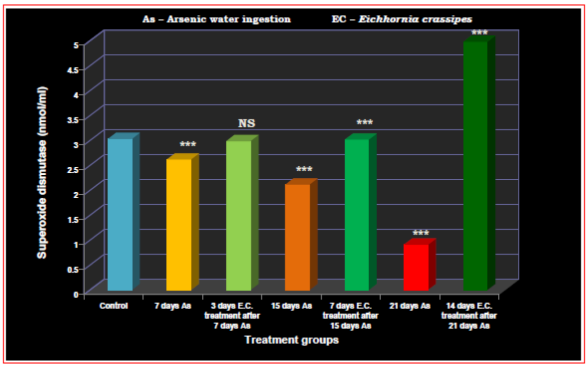

Fig. 2

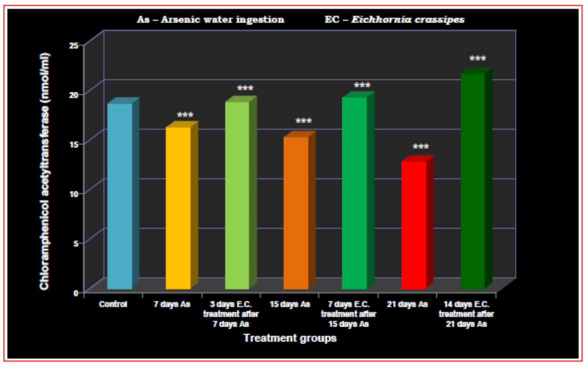

Fig. 3

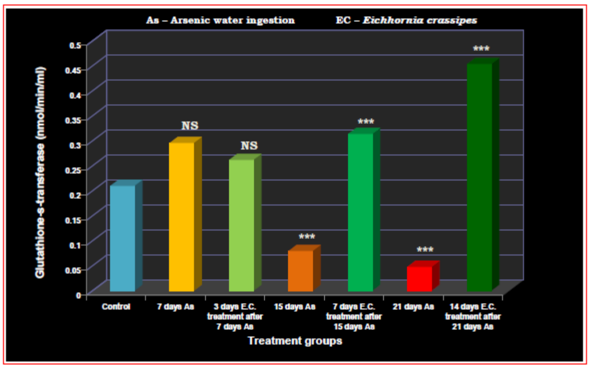

Fig. 4

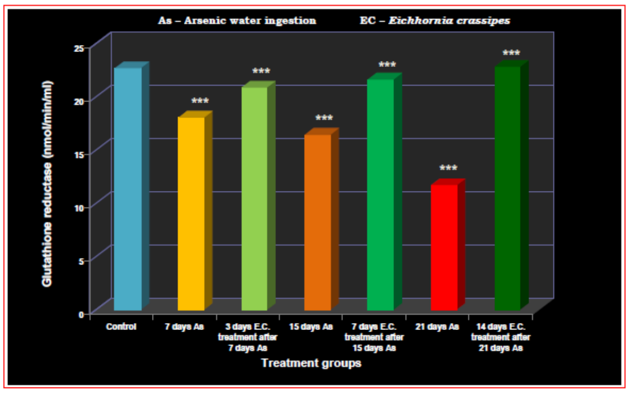

Fig. 5

Arsenic, being an environmental toxicant, leads to development of serious health hazards affecting human being. People are exposed to arsenic mainly via drinking water, food, industrial processes and other sources. Various types of health hazards like acute gastrointestinal symptoms,

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subacute sequela resulting in polyneuropathy (Zhen *et al*., 2005) and chronic symptoms like degenerative, inflammatory and neoplastic changes of skin, and cardiovascular, nervous and reproductive systems are observed in arsenic toxicity (Wang *et al*., 2006). Adverse effects of arsenic on tissue metabolic activities through generation of reactive oxygen species (ROS) were reported earlier (Susheela and Bhatnagar, 2002). Reactive oxygen species causes oxidative damage to cellular components including DNA, tissue proteins, fatty acids etc. (Sarkar and Banerjee, 2004). Arsenic-induced lipid peroxidation through generation of ROS may lead to production of various lipid peroxides like conjugated dienes, alkenes and aldehyde products (Raghu *et al*., 2009). This abnormal lipid metabolism may be associated with cellular dysfunctions leading to carcinogenesis and cardiovascular diseases (Manna *et al*., 2006). Arsenic causes overt endothelial cell injury, cell proliferation and changes in monolayer binding of labeled low-density lipoprotein and permeability of albumin. Arsenic is considered as one of the risk factors, associated with cardiovascular diseases. It has been established that chronic ingestion of arsenic can lead to cardiovascular disorder with a resulting peripheral vascular disease, atherosclerosis, coronary artery disease, myocardial infarction, ischemic rat disease, and diabetes. Further studies by Raghu *et al*. (2009) demonstrate abnormal electrical potential after arsenic trioxide exposure.

Glutathione exhibits significant role in the detoxification of arsenic because GR is required as a co-factor for the optimal activity of methionine adenosyltransferase which facilitates methylation of arsenic (Liu *et al*., 1999). Depletion of tissue glutathione level has been found to be a causative factor in arsenic-induced oxidative damage (Jeji *et al*., 1985). Perturbation of glutathione content in tissue was reported earlier (Manna *et al*., 2008; Das *et al*., 2010). They also reported that short-term arsenic toxicity in rats produces a significant decrease in GR concentration associated with increased lipid peroxidation level. An inverse correlation between arsenic-induced changes in lipid peroxidation and GR content in rat was observed in earlier studies. These findings on rat are also supported by our present study, where GR depletion was associated with increased lipid peroxidation in that tissue. Cytotoxic effects of lipid peroxidation have been established. Overproduction of lipid peroxide causes destabilization in cellular lipid substances, especially membrane structures. The decreased glutathione reductase activity in the rat after arsenic exposure may be a contributory factor for diminished GR concentration in tissue in addition to increased utilization of GR for neutralizing free radicals generated by arsenic treatment.

The present study also demonstrates that exposure to arsenic significantly decreased the SOD activity in tissue. This finding suggests that there was an excess accumulation of superoxide anion in this tissue after arsenic exposure, which may be responsible for increased free radical formation in tissue. The production of free radicals due to arsenic exposure depends on the dose and duration of exposure as well as the detoxifying capacity of cells against arsenic-induced oxidative stress. Arsenic treatment in our present experiment decreased the catalase activity in rat. Earlier few reports have been suggested that arsenic-induced decreased catalase activity may be due to its adverse effects on cellular antioxidant defense system. Consequently, over accumulation of H_2O_2 in tissue of arsenic-exposed animals may enhance the free radical production and promotes arsenic-induced oxidative damage.

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Eichhornia crassipes, commonly known as Common Water Hyacinth, is an aquatic plant native to the Amazon basin, and is often considered a highly problematic invasive species outside its native range. Its habitat ranges from tropical desert to subtropical or warm temperate desert to rainforest zones. It tolerates annual precipitations of 8.2 dm to 27.0 dm (mean of 8 cases = 15.8 dm), annual temperatures from 21.1°C to 27.2°C (mean of 5 cases = 24.9°C), and its pH tolerance is estimated at 5.0 to 7.5. It does not tolerate water temperatures >34°C. Leaves are killed by frost and salt water, the latter trait being used to kill some of it by floating rafts of the cut weed to the sea. Water hyacinths do not grow when the average salinity is greater than 15% that of sea water. In brackish water, its leaves show epinasty and chlorosis, and eventually die. The roots of *Eichhornia crassipes* naturally absorb pollutants, including lead, mercury, and strontium-90, as well as some organic compounds believed to be carcinogenic, in concentrations 10,000 times that in the surrounding water. Water hyacinths can be cultivated for waste water treatment.

Water hyacinth, *Eichhornia crassipes* (Mart) Solms, originated in the state of Amazon, Brazil, spread to other regions of South America, and was carried by humans throughout the tropics and subtropics. It is now widespread and recognized as one of the top 10 weeds in the world. Water hyacinth has invaded Africa, Asia, North America and occurs in at least 62 countries by 2010. It causes extremely serious ecological, economical and social problems in regions between 40° North and 45° South (Inkielewiez *et al*., 2006). *E. crassipes* forms dense monocultures that can threaten local native species diversity and change the physical and chemical aquatic environment, thus altering ecosystem structure and function by disrupting food chains and nutrient cycling. The large, dense monoculture formed by this species covers lakes and rivers, blocking waterways and interfering with the water transport of agriculture products, tourism activities, water power, and irrigation of agricultural fields. Dense mats of water hyacinth can lower dissolved oxygen levels in water bodies leading to reduction of aquatic fish production. Water hyacinth is very efficient in taking up calcium, magnesium, sulfur, ferric, manganese, aluminum, boron, copper, molybdenum, zinc, nitrogen, phosphorus, and potassium favoring its growth over other aquatic species (Halliwell and Gutteridge, 1985). When this macrophyte (water hyacinth) dies, sinks and decomposes, the water becomes more eutrophic due to the large release of nutrients (Gupta *et al*., 2007). Water quality deteriorated, clean drinking water can be threatened and human health impacted. Aggressive growth of *E. crassipes* was correlated with increased temperature, high solar radiation and sunshine duration which may result in an intensive plant growth during summer that may be increased by global warming. High biomass production of water hyacinth corresponded with large amounts of phenolic allelochemicals in the water, which may also help in the process of invasion. High air temperatures in summer (35°C) caused an increase in the rate of evapo-transpiration leading to decrease in water level and consequently a possible increase in allelochemical concentration in the aquatic habitats (Bharti *et al*., 2012).

The obtained results could be concluding that, water hyacinth (*Eichhornia crassipes*) was shown to be an abundant source of useful active components against free radicals. The active compounds were complex in structure and so would be difficult and expensive to synthesize chemically. Controlling the wide spread of the water hyacinth in the our country may be achieved by harvesting it for pharmaceutical uses. Extracts and fractions used pharmaceutically could require the harvest of millions of tones/year.

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REFERENCES

- Bharti VK, Srivastava RS, Sharma B, Malik JK. 2012. Buffalo (Bubalus bubalis) epiphyseal proteins counteract arsenic-induced oxidative stress in brain, heart, and liver of female rats. Biol Trace Elem Res.;146(2):224–229.
- Das A.K., Sahu R., Dua T.K. 2010. Arsenic-induced myocardial injury: protective role of Corchorus olitorius leaves. Food Chem. Toxicol.; 48:1210-217.
- Guo, XY., G. F. Sun, and Y. C. Sun, 2003. Oxidative stress from fluoride–induced hepatotoxicity in rats. Fluoride, 36: 25-29.
- Gupta R, Dubey DK, Kannan GM, Flora SJS. 2007. Concomitant administration of Moringa oleifera seed powder in the remediation of arsenic induced oxidative stress in mouse. Cell Biol Int.;31(1):44–56.
- Halliwell, B., and JMC Gutteridge, 1985. Oxygen radicals and the nervous system. Trends Neurosci; 8: 22-6.
- Inkielewiez, I., M. Rogowska, and J. Krechmiak, 2006. Lipid peroxidation and antioxidant enzyme activity in rats exposed to fluoride and ethanol. Fluoride, 39: 53-9.
- Jeji, J., R. Sharma, S. S. Jolly, and S. Pamnani, 1985. Implication of glutathione in endemic fluorosis. Fluoride, 18: 37-8.
- Liu K. T., G. Q. Wang, L. Y. Ma, P. Jang, B. Y. Xiao, and C. Zhang, 1999. Adverse effects of combined arsenic and fluoride on liver and kidney in rats. Fluoride, 32 (4): 243-247.
- Manna P., Sinha M., Sil, P.C. 2008. Arsenic-induced oxidative myocardial injury: protective role of arjunolic acid. Arch. Toxicol.; 82:137-49.
- Manna, P., M. Sinha and P.C. Sil, 2006. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. BMC Compl. Albern. Med., 6: 33.
- Raghu K.G., Yadav G.K., Singh R. 2009. Evaluation of cardiac effects induced by arsenic trioxide, a potent anti-APL drug. J. Environ. Pathol. Toxicol. Oncol.; 28:241-52.
- Sarkar, M., and A. Banerjee, 2004. Cause, Effect and Remedial options for fluoride in Drinking water. Middle Pomeranian Sci. Soc. Environ. Prot., 123-129.
- Susheela, A. K. and M. Bhatnagar, 2002. Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. Mol. Cell. Biochem., 234-235: 335-340.
- Wang L., Xu Z. R., Jia X. Y., Jiang J. F., Han X. Y. 2006. Effects of arsenic (AsIII) on lipid peroxidation, glutathione content and antioxidant enzymes in growing pigs. Asian-Australasian Journal of Animal Sciences. 19(5): 727-733.
- Zhen, X., Z. Xu, J. Li, and M. Wang, 2005. Effects of fluorosis on lipid peroxidation and antioxidant system in young pigs. Fluoride, 38: 157-161.