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Effect of *Aloe vera* juice on toxicity induced by arsenic in *Labeo rohita* (Hamilton)

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Abstract: An attempt has been made to study the effect of *Aloe vera* juice on the toxicity induced by arsenic on *Labeo rohita* fingerlings, exposed to sub–lethal concentration of a combination of arsenic and *Aloe vera* juice for 21 days. The study of bioaccumulation pattern of arsenic supplimented with Aloe vera juice was carried out on selected parts like liver and muscle tissues. In both the liver and muscle tissues a pronounced effect of arsenic and *Aloe vera* juice was noted on the activities of Glutamate - oxaloacetate transaminase (GOT), Glutamate - pyruvate transaminase (GPT), acid and alkaline phosphatase (ACP and ALP) enzymes. Also the levels of total proteins, total lipids, protease, free amino acids and glycogen were assessed. A significant decrease in enzymatic activity of GPT, GOT, ACP and ALP was noted in liver and muscle tissues. The level of protein, lipids and glycogen also decreased, whereas the amount of protease and free amino acids profoundly increased. The results of present study suggest that *Aloe vera* juice has an effective hepatoprotective and tissue protective property against arsenic toxicity. The results further suggest that the *L.rohita* fingerlings could be suitable for monitoring the bioavailability of water bound metals in fresh water habitats.

Keywords: Fresh water, Heavy metal, Bioaccumulation, Pollution

INTRODUCTION

Increased industrialization, urbanization, population growth and overall man's greed to overexploit Mother Nature has created a serious threat to all kind of life in the form of pollution which has now become a global problem. One of the major environmental problem is the heavy metal pollution of water. With the advent of agricultural and industrial revolution, most of the water resources are getting contaminated (Khare and Singh; 2002). Industrial discharges containing toxic and hazardous substances, including heavy metals (Gbem *et al.*, 2001; Woodling *et al.*, 2001) contribute tremendously to the pollution of aquatic ecosystem causing cytotoxic, mutagenic and carcinogenic effects in animals (More *et al.*, 2003). Among the various toxic pollutants, heavy metals are particularly serving their action due to tendency of bioaccumulation in the food chain (Khare and Singh, 2002).

Fish are often at the top of aquatic food chain and may concentrate large amounts of metals from the water (Mansour and Sidky, 2002). Metal bioaccumulation is largely attributed to differences in uptake and depuration period for various metals in different fish species (Tiwari-Fufeyn and Ekaye, 2007). Multiple factors including season, physical and chemical properties of water (Kargin, 1996) can play a significant role in metal accumulation in different tissues of a fish.

The arsenic compounds are used in pigments and dyes, as a preservative of animal hides, in glass manufacture, agricultural pesticides and various pharmaceutical substances (ATSDR, 2006). Arsenic exerts its toxic effects through an impairment of cellular respiration by inhibiting various mitochondrial enzymes and uncoupling oxidative phosphorylation. Arsenic interacts with sulfahydryl group of proteins and enzymes and substitutes phosphorus in a variety of biochemical reactions resulting in toxicity (Pattlolla *et al.*, 2005).

Aloe vera has been used by many countries for its curative and therapeutic properties. Although over 75 active ingredients from the inner gel have been identified, many of the medicinal effects of aloe leaf extracts have been attributed to the polysaccharides found in the parenchymatous tissue of inner leaf (Ni *et al.*, 2004 a; b), but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained there in rather than a single chemical substance (Dagne *et al.*, 2004).

Important pharmaceutical properties have recently been discovered for the *Aloe vera* gel and its whole leaf extracts (Vinson *et al.*, 2005).These include promotion of wound healing and antifungal activity, hypoglycemic or antidiabetic effects, antiinflammatory and anticancer effects, immunomodulatory and gastroprotective properties. *Aloe vera* extracts have been reported to cause an increased in bile flow and bile solids because of

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stimulated secretory activity of the liver cells. The hepatoprotective action is also attributed to preserving the metabolizing enzymes of the liver through an antioxidant activity (Chandan *et al.*, 2007).

Information on the sub- lethal toxic effects of arsenic on survival and physiology of fishes are limited in Indian context (Ambrose *et al.*, 1994; Vutukuru *et al.*, 2000; Sornaraj *et al.*, 1995; Abbasi *et al.*, 1995; Sastry *et al.*, 1984; Mahipal Singh,1995). Effects of arsenic with *Aloe vera* juice supplementation on the widely consumed Indian major carp, *Labeo rohita* which forms an important link in aquatic food chain have not been studied so far. In view of this, short - term sub - lethal toxicity tests were performed on *Labeo rohita* over a period of 21 days to elucidate the sub - lethal toxicity of arsenic with *Aloe vera* juice supplementation on the biochemical parameters of fish. The corresponding results are discussed in this paper and compared with those in other fishes exposed to arsenic and various other metallic and environmental stressors.

MATERIALS AND METHODS

The laboratory experiments were conducted at the Patkar -Varde College, Mumbai, India. Fingerlings of major carp *Labeo rohita* were obtained from Aarey fish farm Goregaon (East), Mumbai. The specimens (7-8 ± 2 cm long and 8-9 ± 0.6 g. wt.) were transported to the laboratory in appropriately aerated plastic bags. The plastic bags were placed in maintenance plastic pools for about 30-35 minutes for acclimatization, and then were transferred to a big plastic pool of 100 liters capacity containing well-aerated de-chlorinated aged tap water (water stored before 24 hours). The fishes were maintained at an oxygen level of 6.2 to 6.14 mg / L, 7.2 – 7.5 pH and 24- 26°C temperature. These conditions were sustained during the entire length of the experiments and the fishes were fed with commercial fish pellets. After the acclimatization, the healthy fingerlings of *Labeo rohita* were divided in four groups, each group containing 10 fishes and transferred to an aquarium measuring 18 X 12 X14 cm. and containing 10 liter aged water.

Group A : Control group, not exposed to any chemicals. Group B : exposed to 0.5 ml/L *Aloe vera* juice.Group C : exposed to 0.5 ml/L *Aloe vera* Juice and 100 µg/ L arsenic. Group D : exposed to 100 µg/ L arsenic. The sub-lethal dose of arsenic was prepared by dissolving one gm. of arsenic oxide in 100 mL of distilled water. Dilutions were made to get the required concentration of arsenic. The test media was changed daily with fresh addition of the toxicant. After 21 days, all the fishes were sacrificed and liver and muscle tissues were excised. The tissues were blotted, weighed and then homogenized in chilled distilled water, 95% ethanol and 5% and 10% trichloric acid using a glass homogenizer. Thereafter, they were centrifuged at 10000 rpm for 20 minutes.

Biochemical analysis: Biochemical investigations were carried out to assess the sub-lethal toxicity to liver and muscle tissue on exposure to different combinations of *Aloe vera* juice and arsenic. The enzymes, glutamate - oxaloacetate transaminase (GOT) and glutamate - pyruvate transaminase (GPT) were quantified using colorimetric determination of supernatants with 2,4-dinitrophenylhydrazine (Bergmeyer, 1956). The amounts of acid and alkaline phosphatase (ACP, ALP respectively) were determined by using p-nitrophenyl phosphate (Bergmeyer, 1956). Protein levels were estimated following the method of Lowry *et al.* (1951). Total lipids were determined by the methodology described by Schmit (Schmit, 1964.) The protease activity was estimated using the method of Moore and Stein (Moore and Stein, 1954). The total free amino acids were estimated based on the method of Spices (Spices, 1957). Glycogen activity was estimated following the Anthrome method of Van der Vies (Van der Vies, 1954) as modified by Mahendru and Agarwal (Mahendru and Agarwal, 1982) for snail. A total of three replicates were performed to assess the biochemical activities. All the chemicals and reagents were used of AR grade.

RESULTS AND DISCUSSION

The metal accumulation in fish depends on both the structure of tissues and organs, and the interaction of heavy metals in the environment on tissues and organs. The bioaccumulation of arsenic in the selected tissues (liver and muscle tissues) of *L. rohita* fingerlings with *Aloe vera* juice supplementation has been presented in Table 1.

In both the liver and muscle tissues effect of arsenic and *Aloe vera* juice was noted on the activities of enzymes such as, Glutamate - oxaloacetate transaminase (GOT), Glutamate - pyruvate transaminase (GPT), acid and alkaline phosphatase (ACP and ALP), and on total proteins, total lipids, protease activity, and free amino acids and glycogen activity.

Increasing trends in the activities of enzymes such as GPT, GOT, ACP and ALP were observed in liver and muscle tissues of the fish exposed to control and with *Aloe vera* juice, whereas the enzymes activities was found to decrease in the liver and muscle tissues when the fishes were exposed to arsenic only. A little elevation in GPT, GOT, ACP and ALP was observed in the liver and muscle tissues when the fishes were treated with a combination of *Aloe vera* juice and arsenic as compared to the fishes treated with arsenic only. An increase in the level of total protein content was observed in liver and muscle tissues when the fishes were treated with *Aloe vera* juice. The level of total protein content was found to decrease in the liver and muscle tissues when the fishes were exposed

Table 1. Effect of sub lethal concentration of *Aloe vera* juice on toxicity induced by arsenic on GPT, GOT, ACP, ALP, total proteins, total lipids, protease activity, free amino acids and glycogen on liver and tissue of *Labeo rohita.*

S. No:	Group A	Group B	Group C	Group D
GPT (IU/ L)	L=67.10±2.03	L= 66.91±1.22	L= 56.11±0.92	L= 41.13±0. 62
	T= 65.32±1.08	T= 66.22±1.67	T= 54.21±1.67	T=42.34±0.81
GOT (IU/L)	L=41.50±2.08	L= 41.25± 2.40	L=35.20±0.48	L=19. 70±1.20
	T=42.10±1.42	T=41.23±2.33	T= 34.24±1.22	T= 19.56±0.86
ACP (IU/L)	L=11.384±0.531	L=10.768±0.262	L=8.348±0.243	L=5.891±0.683
	T=10.538±0.580	T=10.835±1.343	T=7.826±1.421	T=6.652±0.321
ALP (IU/L)	L=98.88±2.46	L=98.34±1.89	L=78.69±1.34	L=48.91±0.38
	T=97.68±2.81	T=96.64±2.38	T=62.83±2.92	T=42.57±0.42
Total Proteins	L=7.362±0.581	L=7.162±1.247	L=5.684±0.361	L=4.052± 0.219
µg / g dry wt.	T=6.823±1.702	T=6.342±1.465	T=4.891 ±0.140	T=4.921 ±1.021
Total Lipids	L=98.08±1.22	L=98.77±1.54	L= 55.71±2.41	L=51.87±2.81
µg /g dry wt.	T=101.61±2.83	T= 100.62±2.73	T= 51.24±2.21	T=48. 56±1.28
Protease Activity	. L=0.764±0.031	L=0.761±0.061	L=0.818±0.033	L=0.982±0.071
µg /g dry wt	T=0.759±0.041	T=0.756±0.018	T=0.921±0.042	T=0.939±0.022
Free Amino acids	L=6.132±0.087	L=6.821 ±0.091	L=7.721±0.062	L=7.474±0.051
µg /g dry wt.	T=6.601±0.067	T=6.712±0.071	T=7.016±0.029	T=8.512±0.110
Glycogen Activity	L=12.86±1.12	L=12.68±1.94	L= 9.22±0.98	L= 6.27±1.12
µg /g dry wt.	T=12.61±1.53	T= 11.81±0.34	T= 7.94±0.48	T=6.87±1.08

SD = ± Average readings = 03; L = Liver; T = Muscle tissue

to arsenic. A little elevation of total proteins was observed in the liver and muscle tissues when the fishes were treated with a combination of *Aloe vera* juice and arsenic as compared to the fishes treated with arsenic only. Increasing trends in the level of total lipids content were observed in liver and muscle tissues of the fish exposed to control and with *Aloe vera* juice, whereas the level of total lipid content was found to decrease in the liver and muscle tissues when the fishes were exposed to arsenic only. A little elevation of total lipids was observed in the liver and muscle tissues when the fishes were treated with a combination of Aloe *vera* juice and arsenic than the fishes were treated with arsenic only. The level of total free amino acid and protease activity decreased to a greater extent when the fishes were treated with *Aloe vera* juice as compared to the once treated with arsenic. Depletion of glycogen activity was observed in liver and muscle tissues when the fishes were exposed to arsenic whereas the level of glycogen was found to increase in liver and muscle tissues when the fishes were exposed to *Aloe vera* juice. A little elevation in the glycogen activity was observed in the liver and muscle tissues when the fishes were treated with a combination of *Aloe vera* juice and arsenic when compared to the fished treated with arsenic only.

It has been shown that the liver is the prime organ for removing xenobiotics and biocides in fishes (Roy, 2002).However, various pollunts, including organic and inorganic chemicals may alter the cellular enzymatic activities in the liver and other organs affecting the metabolic pathways responsible for removal of toxicants. In present studies, the decreased activities of GOT, GPT, ACP and ALP may indicate disturbance in the cell organelles. Such damage to cell organelles has been reported in various studies (Karata and Kalay, 2002 and Roy, 2002). Furthermore, accumulation of arsenic in liver and muscle tissues could be a possible reason for varying enzyme activities. It has been further reported that arsenic, in the form of arsenate can displace phosphate in enzymes or signaling proteins thus blocking the energy production and normal cell signaling (Dartmouth toxic metal research, 2005). Decrease in phosphatase activity (ACP and ALP) levels shown in the study might be due to the increased arsenic level in the water and its accumulation in the liver and muscle tissues of fish.

As metal toxicity induced oxidative stress, the antioxidant enzymes (especially the glutathione-dependent enzyme), react to defend against arsenic toxicity. Allen and Rana (2004) showed that activities of glutathione-s-transferase, glutathione peroxidase; glutathione reductase and catalase in the liver and kidney decreased, as a result of arsenic toxicity in the liver and kidney. This can be correlated with the decrease in GOT and GPT activities in the fishes exposed to arsenic.

In our studies decrease in tissues lipid and proteins was also observed in *L.rohita* exposed to arsenic. Earlier studies have also shown that the lipid and protein concentration of vital organa like gills, liver, muscles and kidney depleted when exposed to chromium (Arillo *et al.,* 1982; Shastry and Sunita, 1984; and Ambrose *et al.*, 1994). This might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins which are important cellular constituents of cell membranes and cell organelles present in cytoplasm

(Htarpear, 1963). The decrease in the total protein level and increase in the total free amino acid level observed in both tissues suggest the high protein hydrolysis activity due to elevation of protease activity.

It has been reported that the increase in free amino acid levels results from the breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.,* 1996). It is also attributed to lesser use of amino acids (Rao *et al.*, 1987) and their involvement in the maintenance of an acid base balance (Moorthy *et al.,* 1984). Natarajan (1985) suggested that stress conditions induce elevation in the transamination pathway.

Depletion of glycogen observed in our investigations may be due to direct utilization for energy generation, a demand caused by active compound induced hypoxia. Several reports suggest liver glycogen energy reserves in the fish get depleted due to muscular exercise (Black *et al.,* 1962; Nath and Kumar, 1987) during acute hypoxia or physical disturbances in fish (Health and Fritechard, 1965). Finally stress causes increased secretion catecholamine resulting in glycogenolysis leading to decrease in glycogen content (Radhakrishaiah *et al.,* 1992)

Conclusion

Aloe vera has been reported to have a hepatoprotective effect in animals. In the current study, we found that *Aloe vera* at the higher dose levels prevented a decrease in enzymatic activity as GPT, GOT, ACP and ALP in liver and muscle tissues. The levels of protein, lipids and glycogen were also found decreased, whereas protease and free amino acids were found increased. The hepatoprotective effect of *Aloe vera* juice supplementation with arsenic shall increase the secretion of bile and detoxifies the toxins by maintaining the liver functions. The results of present study suggest that *Aloe vera* juice has an effective hepatoprotective and tissue protective property against arsenic toxicity. This was confirmed by biochemical investigations.

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