International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Therapeutic efficiency of *Noloxone* against atrazine toxicity on the Fresh water fish *Labeo rohita*

B. Manjunatha[1,2,*], M. Srinivasulu[2], G. Jaffer Mohiddin[2], M. Naga Raju[3], M. Selvanayagam[3], T. Selvaraj[4], M. Michael Babu[5]

[1]Department of Zoology, Sri Krishnadevaraya University, Anantapur - 515003, Andhra Pradesh, India
[2]Department of Life Sciences, Universidad de las Fuerzas Armadas-ESPE, Sangolqui- POBOX: 171-5-231B, Quito, Ecuador, South America
[3]Department of Environmental Engineering, Universidad Estatal Amazonica, Puyo - 160150, Ecuador, South America
[4]Centro de Biologia Cellular Y Molecular (CBCM), Universidad Technica Particular de Loja (UTPL), San Cayetano Alto s/n. Loja, Ecuador, C. P. 1101608, South America
[5]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam - 629 502, Kanyakumari, Tamil Nadu, India
*Corresponding author: *manjubhargav2010@gmail.com*

Abstract

The effects of atrazine, an herbicide used worldwide and considered as a potential contaminant in aquatic environments. In the present research, the effect of atrazine on antioxidant enzyme activity in the fresh water fish *Labeo rohita* is carried out.The experimental fish were treated with atrazine (0.5 mg/ L) for 120 hours. Another group (III) of fish treated with atrazine in 120 hours, and then exposed to dried *Noloxone* pellet 2 gram. The group IV fish was exposed to *Noloxone* alone for 120 hours. After the treatment fish were dissected and the following organs namely gill, liver and kidney were analyzed for antioxidant enzymes like Catalase (CAT), Superoxide dismutase (SOD), Lipid peroxidation (TBARS) level. Antioxidant enzymes are biomarkers used to indicate the atrazine toxicity. The SOD, CAT and LPO are increased during the atrazine exposure period (P>0.05). In the group III, atrazine along with *Noloxone* exposure the antioxidant enzymes was recovered (P>0.05). The present study suggests that the *Naloxone* algae might play a role in reducing the toxic effect of atrazine and Sits enzymological effects seem to mediate such a protective effect.

Keywords: Antioxidant enzymes; LPO; SOD; CAT; *Naloxone;* Atrazine*; Labeo rohita.*

Introduction

The presence of pesticides in the environment has caused significant social and scientific development anxiety worldwide, as their all over the world extensive usage can create potential risks to the environment and human health, as they can easily pollute bodies of water, resulting in extensive damage to non-target species, including fish [1]. Fish representing as bio-indicator of environmental contamination and may play an important role in the evaluation of the potential risk of pollution in aquatic environment. They may directly exposed to chemicals caused by agricultural output through runoff or indirectly by food chain of ecosystem, this may reflect the biological influences of environmental contamination in water [2].

Atrazine is one of the most currently and widely used herbicides and several studies have already detected its presence in water bodies at levels above the limits determined by local guidelines [3,4].

Despite being classified as moderately toxic, atrazine can promote toxic effects on aquatic animals [5,6]. Some studies have been conducted on fish to evaluate the toxicity of atrazine [7] and focused on several aspects such as the biochemical [8], genetic [6], histopathological [9] and physiological effects [10,11]. However, the mechanisms of toxicity of this herbicide and its metabolites are not yet fully understood [12].

Antioxidant enzymes are important in coping oxidative stress caused by the metabolism itself and environmental factors [13]. Oxidative stress results from disruption of the prooxidant and antioxidant balance by reactive oxygen species (ROS) and other radicals or oxidants [14]. While xenobiotics are able to increase ROS levels, the capacity to induce oxidative stress depends on the over whelming of antioxidant defenses. Aerobic organisms have developed antioxidant defense mechanisms that scavenge ROS or prevent ROS-mediated cellular damage [15], including enzymes sensitive to free radical proliferation such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [16]. Among the main ROS-inflicted damages is lipid membrane oxidation, known as lipid peroxidation (LPO), a process that follows exposure to a wide variety of environmental pollutants [17].

Fish are the most important aquatic organisms and are very vulnerable to such environmental stresses. Many recent laboratory and field studies have suggested that the measurement of enzymatic activities might be an effective indicator of exposure to chemical pollution; Atrazine (2-chloro-4–ethylamino–6-isopropylamino-s-triazine) is one of the most commonly used herbicides found in the rural environments. It is extensively used on corn, sorghum, sugarcane, pineapple and to some extent on landscape vegetation. Rated as moderately toxic to aquatic species, atrazine is mobile in the environment and is among the most detected pesticides in streams, rivers, ponds, reservoirs and ground waters [18]. It has a hydrolysis half-life of 30 days and relatively high water solubility (32 $mg \cdot L^{-1}$), which aids in its infiltration into ground water [19]. Atrazine concentrations of 20 $\mu g \cdot L^{-1}$ have been commonly detected in surface water runoff, while concentrations as high as 700 $\mu g \cdot L^{-1}$ have been reported [20-22]. Due to the low persistence of atrazine herbicide, repeated applications are practiced for the control of weeds in agricultural fields, and as a result, large quantities of the herbicide find their ways into water bodies. The mode of action of atrazine is blocking electron transport in photosystem II leading to chlorophyll destruction and blocking photosynthesis. When atrazine was first released for agricultural use, it was thought that since photosynthesis is limited to plants, animals would be immune to any effects of atrazine. It was soon suspected that atrazine might have non-target action in animals including genotoxic [23], clastogenic [24] and biochemical effects [25]. The indiscriminate use of this herbicide, careless handling, accidental spillage or discharge of untreated effluents into natural water ways have harmful effects on the fish populations and other aquatic organisms and may contribute to long term effects in the environment.

Noloxone is a cyanobacterium is classified as blue green algae. It has been used as a food [26] because of its quantity of proteins, vitamins, essential amino acids, minerals and essential fatty acids [27]. It has been reported that *Noloxone* have several pharmacological activities. Brown et al., [28] reported that *Noloxone* have antioxidant properties especially some phycobiliproteins such as cphycocyanin (CP) and allophycocyanin [29]. Hence, the aim of the present study is to determine the protective effect of atrazine on antioxidant enzyme activities and therapeutic efficiency of *Noloxone* in the fresh water fish *Labeo rohita*.

Materials and Methods

The fish *Labeo rohita* of 75±5g weight and 15±5cm length were obtained from the Fisheries Department, Anantapur, Andhra Pradesh, India and were transferred to large cement tanks. They were kept in the cement tank filled with dechlorinated water and supplied continuous aeration. Acclimatization to experimental condition for 15 days at room temperature, fishes were fed with artificial libitum during acclimatization and tank water was renewed every day after feeding, food was withheld from before 24 hours to the experiment.

Atrazine toxicity studies

Acute toxicity bioassays to determine the LC_{50} was calculated by the log – dose /probit regression line, sub lethal concentration 0.5 mg/ L was used for 24, 48, 72, 96 and 120 hours experimental study.

Experimental design

After acclimation the *Labeo rohita* fresh water fish were divided into four groups whereas Group I control, Group II atrazine treated, Group III Atrazine + *Noloxone*, Group IV *Noloxone* alone treated. Each group was exposed to sub-lethal concentration of atrazine for a period of 120 hours. Simultaneously, a control was maintained to compare toxic impacts. For the present study commercial formulations of atrazine (50% WP) with the trade name "Rasayanzine", manufactured by Krishi Rasayan Exports Ltd (India) was purchased from the local market.

Antioxidant enzymes assay

The concentration of TBARS in the tissues (gill, liver and kidney) was estimated by the method of Biradar and Rayburn [30]. Superoxide dismutase (SOD) activity was determined following the procedure of Pandey et al., [31]. The activity of catalase (CAT) was assayed by the method of Patlac and Chinoy [32].

Statistical analyses

The data obtained from the quantitative study were expressed as the mean±SE, percentage changes and were statistically analyzed using student 't'-test, to compare means of treated data against their control and the result were considered significant at ($P<0.05$),($P>0.05$) level.

Results

In the present study, the fish *Labeo rohita* were exposed to atrazine (group II) to study the effects of atrazine on antioxidant enzyme activity. When compared with control (group I), the antioxidant enzyme superoxide dismutase values were gradually increased ($P>0.05$). The observed group III atrazine along with *Noloxone* exposed fish showed gradually recovered. The group IV also increased ($P>0.05$) when compared with control group. The observed liver SOD (Table - 1) in fish were exposed to atrazine (group II). When compared to control groups the SOD level was gradually increased for the period of 24, 48, 72, 96 and 120 hours, respectively. The group III (atrazine along with *Noloxone*), group IV (*Noloxone* supplemented) exposed also increased ($P>0.05$), when compared with control groups. The kidney tissues show enzyme superoxide dismutase levels were increased, for the period of 24, 48, 72, 96 and 120 hours, respectively. The observed values of group III atrazine along with *Noloxone* exposed gradually recovered and the group IV (*Noloxone* supplemented) also increased when compared with control group.

The catalase activity (Table - 2) in *Labeo rohita* group II atrazine treatment increased when compared with control for the period of 24, 48, 72, 96 and 120 hours, respectively. The group III atrazine along with *Noloxone* exposed and group IV *Noloxone* supplemented also increased in the enzyme activity, when compared with control group. In the liver tissue of herbicide atrazine exposed group II, the enzyme activity was increased. The catalase activity in group III and group IV also increased when compared with control group ($P>0.05$). The catalase activity in kidney of the atrazine treatment group II were increased when compared with control group, for the period of 24, 48, 72, 96 and 120 hours, respectively. In the group III (atrazine along with *Noloxone* exposed) and group IV (*Noloxone* supplemented) also the enzyme activity was gradually increased when compared with control group ($P>0.05$).

The observed value of LPO (Table - 3) levels in *Labeo rohita* exposed to atrazine herbicide increased in lipid peroxidation activity in all tissues (gill, liver and kidney), for the period of 24, 48, 72, 96 and 120 hours, respectively. The observed group III and group II were increased in peroxidase activity, when compared with control group. The observed liver and kidney lipid peroxidase activity were increased, when compared with control groups. The group herbicide atrazine exposed group II of *Labeo rohita* induce the rapid secretion of enzyme peroxidase.

Table 1. Change in the level of Superoxide dismutase (U mole / mg protein) activity in the fresh water fish *Labeo rohita* exposed to 120 hours sublethal concentration of atrazine and therapeutic efficiency of *Noloxone.*

Organs	Exposure period					
	Groups	**24h**	**48h**	**72h**	**96h**	**120h**
	Group I	30.15±1.48	30.19±1.49	30.52±1.81	30.28±1.78	30.39±1.78
	Group II	38.46 NS 2.29	41.14 NS ±2.35	48.11 * ±2.29	49.29 * ±2.35	52.08 NS ±2.54
		28.01	36.48	57.31	62.58	71.32
Gill	Group III	35.48 NS ±2.11	35.27 NS ±1.62	36.19 NS ±1.73	37.34 NS ±1.82	38.07 NS ±1.78
		18.12	16.53	18.19	23.37	25.26
		7.59	14.39	24.74	24.58	26.82
	Group IV	30.15** ±1.65	30.35 NS ±1.68	31.03 NS ±1.75	31.23 NS ±143	32.11 NS ±1.81
		0.11	0.56	1.35	3.13	5.56
	Group I	48.15±2.36	48.69±2.41	48.95±2.43	48.98±2.44	49.26±2.45
	Group II	49.97 NS ±2.35	52.96 NS ±2.59	56.08 NS ±3.34	60.23 NS ± 2.96	62.13 NS ±3.67
		4.26	9.38	14.89	22.87	26.02
Liver	Group III	49.95 NS ±2.37	50.42 NS ±2.43	51.28 NS ±2.53	52.31 NS ± 3.09	53.21 NS ±2.57
		4.19	4.16	4.85	6.63	7.89
		0.09	4.86	8.71	13.14	14.32
	Group IV	48.13 NS ±2.34	49.11 NS ±2.39	49.17 NS ±2.41	50.09 NS ±2.97	15.11 NS ±2.92
		0.25	0.94	0.45	2.19	1.63
	Group I	34.05±1.56	34.53±1.96	34.21±1.63	35.23±1.68	35.45±171
	Group II	37.09 NS ±1.75	39.25 NS ±2.29	42.37 NS ±2.47	48.27*±2.43	56.29*±3.38
		8.87	13.53	23.74	36.79	58.62
Kidney	Group III	38.35 NS ±1.79	39.51 NS ±1.89	40.25 NS ±1.92	41.58 NS ±2.41	43.14 NS ±2.11
		12.52	14.35	17.36	17.65	21.65
		-3.31	-0.63	5.18	13.89	23.27
	Group IV	35.34 NS ±1.69	35.02 NS ±2.01	39.26 NS ±2.14	36.08 NS ±1.73	36.25 NS ±2.15
		3.65	1.19	5.56	2.08	2.15

Values are mean ± SE of six replicates percentage changes and student “t” test, Significant at * P > 0.05; ** P < 0.01 levels

Table 2. Change in the level of Catalase (µ mole / min / mg protein) activity in the fresh water fish *Labeo rohita* exposed to 120 hours sublethal concentration of atrazine and therapeutic efficiency of *Noloxone.*

Organs	Exposure period					
	Groups	**24h**	**48h**	**72h**	**96h**	**120h**
	Group I	9.82 ± 0.45	9.89 ± 0.56	9.99 ± 0.46	10.11 ± 0.57	10.21 ± 0.48
	Group II	8.97 ^{NS} ± 0.51	9.11 ^{NS} ± 0.52	8.61 * ± 0.41	7.98* * ± 0.37	7.32 * * ± 0.41
		3.67	7.62	13.65	20.74	28.09
Gill	Group III	9.32 ^{NS} ± 0.43	9.25 ^{NS} ± 0.51	9.15 ^{NS} ± 0.41	9.15 ^{NS} ± 0.51	9.11 ^{NS} ± 0.50
		5.13	0.07	8.25	9.51	10.61
		-2.85	-1.28	-6.21	-14.15	-24.29
	Group IV	9.11 ^{NS} ± 0.51	9.17 ^{NS} ± 0.43	9.35 ^{NS} ± 0.52	9.22 ^{NS} ± 0.42	9.39 ^{NS} ± 0.53
		7.25	7.21	6.25	8.72	7.87
	Group I	11.35 ± 0.75	11.79 ± 0.69	11. 87 ± 0.55	12.01 ± 0.56	12.59 ± 0.58
	Group II	11.49 ^{NS} ±0.71	10.37 ^{NS} ±0.63	9.31 ** ±0.48	8.42 ** ± 0.53	7.51**±0.46
		0.18	12.85	22.24	30.45	4.07
Liver	Group III	8.35**±0.48	8.71 ** ±0.51	9.12 ** ±0.53	9.89 * ± 0.57	10.08**±0.49
		26.28	26.13	23.19	17.68	19.91
		26.41	15.24	1.25	-18.21	-34.95
	Group IV	10.32* ±9.01	11.37 ^{NS} ±0.65	11.59 ^{NS} ±0.67	11.96 ^{NS} ±0.69	12.29 ^{NS} ±0.83
		0.49	3.51	1.98	0.32	2.27
	Group I	15.41±0.75	15.16±0.89	15.96±0.75	16.09±0.93	16.23±0.78
	Group II	15.32 ^{NS} ±0.74	14.61 ^{NS} ±0.85	13.34 * ±0.62	13.05*±0.74	12.25**±0.59
		0.56	3.51	16.43	18.81	24.42
Kidney	Group III	13.12 * ±0.62	13.65 ^{NS} ±0.79	14.42 ^{NS} ±0.69	14.97 ^{NS} ±0.71	15.37 ^{NS} ±90
		15.01	9.85	9.64	6.85	5.19
		14.51	6.53	-8.13	-14.72	25.43
	Group IV	14.39 ^{NS} ±0.83	14.85 ^{NS} ±0.71	14.71 ^{NS} ±0.84	15.35 ^{NS} ±0.88	16.29 ^{NS} ±0.95
		0.81	1.96	7.89	4.48	0.34

Values are mean ± SE of six replicates percentage changes and student "t" test, Significant at * P > 0.05; ** P < 0.01 levels, NS - Non-Significant.

Table 3. Change in the level of Lipid peroxidation (n mole / mg protein) activity in the fresh water fish *Labeo rohita* exposed to 120 hours sublethal concentration of atrazine and therapeutic efficiency of *Noloxone.*

Organs	Exposure period					
	Groups	**24h**	**48h**	**72h**	**96h**	**120h**
	Group I	18.57 ± 0.89	18.69 ± 0.89	18.80 ± 0.90	18.896 ± 0.91	18.936 ± 0.91
	Group II	20.19 ^{NS} ± 1.02	24.09 * ± 1.38	29.29 * * ± 1.41	33.37* * ± 1.88	35.06 * * ± 1.69
		8.59	28.98	55.76	76.78	85.36
Gill	Group III	31.38 * * ± 1.53	29.09 * ± 1.97	26.29 * ± 1.54	24.09 * ± 1.17	22.15 ^{NS} ± 1.08
		68.59	55.53	39.71	27.73	17.05
		-54.28	-20.52	-10.26	27.68	36.81
	Group IV	20.08 ^{NS} ± 1.17	21.09 ^{NS} ± .22	21.43 ^{NS} ± 1.46	22.45 ^{NS} ± 1.54	22.69 ^{NS} ± 1.55
		7.95	12.86	14.01	18.89	19.95
	Group I	12.08 ± 0.56	12.45 ± 0.71	12.29 ± 0.57	12.61 ± 0.72	12.93 ± 0.85
	Group II	13.31 ^{NS} ±0.75	14.38 ^{NS} ±0.81	18.07 *±1.76	16.18 ** ± 0.77	19.22 ^{NS} ±0.67
		10.11	15.49	46.94	28.11	-48.57
Liver	Group III	20.07**±1.16	18.21 ** ±0.87	17.45 ** ±1.01	22.32 ** ± 1.09	28.37**±1.38
		65.91	46.11	41.84	81.46	119.23
		-50.39	-26.45	3.44	-37.54	-47.51
	Group IV	13.03 ^{NS} ±0.75	13.44 ^{NS} ±0.91	13.72 ^{NS} ±0.79	14.05 ^{NS} ±0.81	14.08 ^{NS} ±0.96
		7.95	7.99	11.64	11.26	8.91
	Group I	9.25±0.61	9.39±0.52	9.64±0.55	9.85±0.46	9.91±0.65
	Group II	10.24 ^{NS} ±0.58	12.35 ** ±0.57	14.36**±0.82	14.21**±0.79	14.02**±0.65
		10.51	31.47	49.08	44.05	41.61
Kidney	Group III	19.08 ^{NS} ±0.81	18.41 ** ±0.89	16.07 ** ±0.93	18.21 ** ±0.87	21.35 ±1.02
		1.81	95.62	66.67	84.59	115.15
		-86.15	-48.71	-11.75	-28.11	-51.87
	Group IV	10.29 ^{NS} ±0.68	10.69 ^{NS} ±0.70	11.27 * ±0.64	11.72 * ±0.06	12.05*±0.81
		11.06	13.76	17.14	18.65	28.49

Values are mean ± SE of six replicates percentage changes and student "t" test, Significant at * P > 0.05; ** P < 0.01 levels, NS - Non-Significant.

Discussion

Fish are often used as sentinel organisms for ecotoxicological studies because they play number of roles in the trophic web, accumulate toxic substances and respond to low concentration of mutagens [33]. Therefore, the use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems [34,35]. Fishes are used as a model for the biomonitoring of the aquatic environment and as sentinel agents for pollutants [36]. The pollutants affect various organs, particularly gills, which are exposed directly to the contaminants in the environment. However, most of the pollutants are transferred to the blood and transported to the liver, which is the principal detoxifying organ for xenobiotic substances. Hepatic tissue is the place where most of the pollutants accumulate and therefore the long-term damage is most likely to occur. Most of the pollutants and their metabolites induce toxicity via oxidative stress arising from the increased production of free oxygen radicals. The antioxidant defense enzymes involved in the removal of ROS are the principal candidates for biomarkers of oxidative stress. The antioxidant defense enzymes, however, have great variability and are dependent on the organisms, organ tissues and the nature of pollutants involved [37].

The antioxidant enzymes in fish could be used as biomarkers of exposure of aquatic pollution [38]. Pesticides may significantly damage certain physiological and biochemical processes when they enter into the organ of fishes. Atrazine can cause serious impairment to physiological and healthy status [39]. Lipid peroxidation is the initial step of cellular membrane damage caused by pesticides, metals and other xenobitoics [40]. Exposure of atrazine and *Noloxone* dosage increased in gill, liver and kidney tissues. These increase described to an excessive production of ROS, which could be related to antioxidant enzyme leakage.

The present study shows atrazine treatment induced oxidative stress as demonstrated by compromised antioxidant defences. Results are in agreement with the study which demonstrated a significant increase in lipid peroxidase in fish liver, kidney and gills following atrazine administration group II and group III atrazine along with *Noloxone* exposure. Khan et al., [41]; Lu et al., [42] have registered the elevated level of LPO exposed to various toxicants. Nwani et al., [43] reported by the elevated level of lipid peroxidase in the liver of *C. punctatus* in response to the exposure to atrazine as observed that there is increased production of ROS. Increased ROS production may, thus, be associated with the metabolism of atrazine herbicide leading to the peroxidation of membrane lipids of the liver. The liver is noted as site of multiple oxidative reactions and maximal free radical generation [44-46]. Previous investigations have reported the induction of LPO by pesticides such as deltamethrin [47,48], alachlor [49], malathion [50] and butachlor [51] in fish. Lushchak et al., [52] however, did not record elevation of lipid peroxidation in the brain and liver of goldfish *Crassius auratus* exposed to sublethal concentration of Roundup®. The different responses probably are functions of species, the time of exposure, type and concentration of stressors. The observed LPO resulting from ROS generated by the atrazine may lead to cell apoptosis. ROS and oxidative stress have been demonstrated to be triggers of apoptosis [53].

In this current study atrazine caused significant increase in CAT activities in liver, kidney and gill tissues of *Labeo rohita*. CAT is an enzyme located in peroxisomes and facilitates the removal of H_2O_2 [54,55]. Herbicides induced inhibition of CAT activity has been reported in various studies in fish species. Luck [56], reported that endosulfan caused a decrease in CAT activity. In this study, CAT activity was not connecting to other markers of the oxidative stress. The low levels of CAT could be attributed to high production of O_2. This has been reported to inhibit CAT activity in decrease of excess production (Academic Press). Pesticides – induced inhibition of CAT activity has been stressed by simulation of O_2 [57]. Enhancement of CAT activity was observed in Cichlid fishes from polluted waters [58], in mullets [59], *Lepomis macrochirus* [60] and *Prochilobus lineatus* [61] exposed to herbicides. The inhibition of CAT by the superoxide radical has been reported [29,62].

The increase in superoxide dismutase activity after atrazine administration appears to be an adaptive response to increased generation of reactive oxygen species. The exposure of animals to xenobiotics increases SOD was reported and the present study reflects a compensatory mechanism to increase oxidative stress. In the present investigation,

therapeutic efficiency of *Noloxone* are important detoxifying mechanism against atrazine toxicity of *Labeo rohita*. *Noloxone* has been shown to be an excellent source of proteins, vitamins and minerals [38]. *Noloxone* is useful in human nutrition, due to the high quality and quantity of its protein. Other benefits are attributed to *Noloxone* antiarthritic effect due to the anti-inflammatory and antioxidative properties of phycocynanin [63], anti-atherogenic property [64], chemo protective and radio-protective effect [65] and antioxidant activity on lead-induced toxicity. This may cause to recover the animal from atrazine toxicity. Reported in various studies in fish species, for example, Bainy et al., [66]; Mohan Singh and Rajat Sandhir [67] reported that endosulfan caused decreases in CAT activity in liver, kidney and gill tissues of *Channa punctatus*. SOD is a group of metalloenzymes that plays a crucial antioxidant role and constitutes the primary defence against the toxic effects of superoxide radical in aerobic organism [68]. Depletion of antioxidant enzyme activity could be caused by a down regulation of transcription and translation process. However, organisms are equipped with interdependent cascades of enzymes to alleviate oxidative stress and repair damaged macromolecules, produced during normal metabolism or due to exposure to xenobiotics. In this cascade, SOD and CAT are the major enzymes in eliminating ROS formed during bioactivation of xenobiotics in the hepatic tissues [69] and the induction of SOD/CAT system provides a first line of defense against ROS. Nwani et al., [43] reported by the increase in LPO, CAT and SOD activities in the liver tissue with sublethal concentrations of atrazine induce oxidative stress in *C. punctatus* and could be an adaptive response to protect the fish from the atrazine-induced free radical toxicity.

The present study fish exposed to concentration of atrazine displayed an increase in superoxide dismutase activity. This increased observation in SOD activity could be explained by oxidative stress caused by atrazine exposure and could contribute to atrazine toxicity. As revealed in literature, this may also be the results of excessive SOD radical production or a direct action of herbicides on synthesis of the enzyme [70].

In summary, the present study clearly indicates that atrazine increased the *in vivo* SOD, CAT and LPO levels in *Labeo rohita*. Weather it is because of atrazine or its metabolite and this effect remains to be clarified. However, toxicity associated with atrazine may, in part, result from change in the antioxidant enzymes and that some protection from this toxicity could be provided by antioxidant. Furthermore, our results indicate that the *Labeo rohita* is a convenient species for monitoring pesticides in freshwater of agricultural areas.

Conclusion

We conclude that the antioxidant enzyme activity in the different organs (gill, liver and kidney) did not represent good indices of the effect of atrazine in *Labeo rohita*. However, the activity of SOD, CAT and LPO in the organs of fish potential useful bioindicators in the *Naloxone* is reducing the herbicide atrazine toxicity.

Acknowledgments

The work was supported by the University Grants Commission (UGC-RFSMS), Government of India; and in part of the National Secretariat of Higher Education, Science, Technology and Innovation (SENESCYT), Ecuador.

References

1. Moreno DH, Soler F, Míguez MP, López MP. Brain acetylcholinesterase, malondialdehyde and reduced glutathione as biomarkers of continuous exposure of tench, Tinca tinca, to carbofuran or deltamethrin. Science of the Total Environment 2010; 408: 4976–4983.
2. Ramesh M, Srinivasan R, Saravanan M. Effect of atrazine (Herbicide) on blood parameters of common carp Cyprinus carpio (Actinopterygii: Cypriniformes). African Journal of Environmental Science and Technology 2009; 3(12): 453–458.
3. Konstantinou IK, Hela DG, Albanis TA. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. Environ Pollut 2006; 41: 555–570.
4. Davis RK, Pederson DT, Blum DA, Carr JD. Atrazine in a stream-aquifer system: estimation of aquifer properties from atrazine concentration profiles. Ground Water Monit Remediat 2007; 13 (2):134–141.

5. Solomon KR, Carr JA, Du Preez LH, Giesy JP, Kendall RJ, Smith EE. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. Crit Rev Toxicol 2008; 38: 721–772.
6. Ventura BC, Angelis DF, Marin-Morales MA. Mutagenic and genotoxic effects of the atrazine herbicide in Oreochromis niloticus (Peciformes, Cichlidae) detected by micronuclei test and the comet assay. Pestic Biochem Physiol 2008; 90: 42–51.
7. Yang L, Zha J, Li W, Li Z, Wang, Z. Atrazine affects kidney and adrenal hormones (AHs) related genes expressions of rare minnow (Gobio cyprisrarus). Aquat Toxicol 2010; 97:204–211.
8. Dong X, Zhu L, Wang J, Wang J, Xie H, Hou X, Jia W. Effects of atrazine on cytochrome P450 enzymes of zebrafish (Danio rerio). Chemosphere 2009; 77:404–412.
9. Paulino MG, Sakuragui MM, Fernandes MN. Effects of atrazine on the gill cells and ionic balance in a neotropical fish, Prochilodus lineatus. Chemosphere 2012a; 86:1–7.
10. Nieves-Puigdoller N, Björnsson BT, Mccormick SD. Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. Aquat Toxicol 2007; 84: 27–37.
11. Paulino MG, Souza NES, Fernandes MN. Subchronic exposure to atrazine induces biochemical and histopathological changes in the gills of a Neotropical freshwater fish, Prochilodus lineatus. Ecotoxicol Environ Saf 2012b; 80: 6–13.
12. Graymore M, Stagnitti F, Allinson G. Impacts of atrazine in aquatic ecosystems. Environ Intern 2001; 26: 483–495.
13. Jorgensen SW. A derivative of encyclopedia of ecology. Ecotoxicology. Academic Press, London, 2010; 390.
14. Prior RL. Biochemical measures of antioxidant status. Top Clin Nutr 2004; 19: 226–238.
15. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf 2006; 64: 178–179.
16. Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82:47–5.
17. Livingstone DR. Contaminant stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar Pollut Bull 2001; 42: 656–666.
18. Battaglin WA, Rice CK, Foazio MJ, Salmons S, Barry RX. The occurrence of glyphosate, atrazine and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa and Wyoming 2005–2006. Environ Monit Assoc 2008; 155: 281–307.
19. Orme S, Kegley S. PAN Pesticide Database; Pesticide Action Network: San Francisco, CA, USA, 2004.
20. Perry C. Source, extent and degradation of herbicides in a shallow water aquifer near Hesston, Kansas. In Water-Resources Investigation Report 91–4019; US Geological Survey: Lawrence, KA, USA, 1990.
21. Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW, Williams WM. Ecotoxicological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem 1996; 15: 31–76.
22. Selim H. Retention and runoff losses of atrazine and metribuzin in soil. J Environ Qual 2003; 32:1058–1071.
23. De Ventura Campo B, de Angelis Fransceschi D, Marin-Morales MA. Mutagenic and genotoxic effects of the atrazine herbicide in Oreochromis niloticus (Perciformes, Cichlidae) detected by the micronuclei test and the comet assay. Biochem Physiol 2008; 90: 42–51.
24. Yoder J, Watson M, Benson WW. Lymphocytes chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutat Res 1973; 21: 13–17.
25. Weigand C, Krause E, Steinberg C, Pflugmacher S. Toxicokinetics of atrazine in embryos of the Zebrafish (*Danio rerio).* Ecotoxicol Environ Saf 2001; 99: 199–205.
26. Oruc EO, Uner N. Combined effects of 2,4D and azinphosmethyl antioxidant enzymes and lipid perxodation ln liver of *Oerochromis nilotes.* Comp Biochem Physicol 2002; 127: 291–296.
27. Yilmaz R, Atessahin A, Sahna E, Karahan I, Ozer S. Protective effect of lycopene on adriamycin induced cadiotoxicity and nephrotoxicity. Toxicology 2006; 218: 164–171.
28. Brown KE, Kinter MT, Oberley TD, Freeman ML, Frrierson HF, Ridu LA, Tao Y, Oberley LW, Spitz DR. Enhanced gamma glutamyl traspetion expression and selective loss of Cu/Zn superoxide

dismutase in hepatic overload. Free Radical Biol Med 1998; 24: 545–555.
29. Kono Y, Fridovich I. Superoxide radical inhibits catalase. J Biol Chem 1982; 257: 51–57.
30. Biradar DP, Rayburn AL. Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies. J Environ Qual 1995; 24: 1222–5.
31. Pandey SI, Ahmad S, Pervez B, Bin-Hafeez R, Haque S, Raisuddin. Effect of endosulfan on antioxidants of fresh water fish *Channa puntatus* Bloch. Protection against lipid peroxidation in liver by copper pre-exposure. Environ Contam Toxicol 2001; 41: 345–352.
32. Patlac D, Chinoy NJ. Synergestic action of ascorbic acid and calcium mitigation of fluoride induced toxicity in uterus of mice. Indian J Environ toxicol 1996; 7:16–9.
33. Cavas T, Ergene-Gözükara S. Micronucleus test in fish cells, a bioassay for *in situ* monitoring of genotoxic pollution in the marine environment. Environ Mol Mutagen 2005; 46: 64–70.
34. Lopez-Barea J. Biomarkers to detect environmental pollution. Toxicol Let. 1996; 88:79.
35. Van Der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharm 2003; 13: 57–149.
36. Sedeno-Diaz JE, Lopez-Lopez E. Fresh water fish as sentinel organism: from the molecular to the population level, a Review. In: TURKER, H. (Ed.). New advances and contributions to fish biology. 2013. http://dx.doi.org/10.5772/54825.
37. Sole M, Baena M, Arnaua S, Carrassonb M, Maynoua F, Cartes JE. Muscular cholinesterase activites and lipid peroxidation leves as biomarkers in several Mediterranean marine fish species and their relationship with ecological variabels. Enviromental International 2011; 36: 202–211.
38. Ahmad I, Hamid T, Fatima M, Chand HS, Jain SK, Athar M, Raisudeen S. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus*) is a biomarker of paper mill effluent exposure. Biochem Biophys Acta 2000; 15: 37–48.
39. Dillon JC, Phuc AP, Dubacq JP. Nutritional value of the algae *Spirulina*. World Rev Nutr Diet 1995; 77:32–46.
40. Mendes RL, Norbe BP, Cardoso MT, Pereria A, Palavra AF, Su Mirenda MS, Cintra RG, Barros SBM, Mancini-Filho J. Antioxidant activity of the microalgae *Spirulina maxima*. Braz J Med Res 1998; 31 (8): 1075–1079.
41. Khan M, Shoba JC, Mohan IK, Naidu MU, Sundaram C, Singh S, Kuppusamy P, Kutala BK. Effect of *Spirulina* against doxorubicinreduced cadiotoxicity. Phytother Res 2005; 19(12): 1030–1037.
42. Lu HK, Hsieh V, Hsu JJ, Yang YK, Chou HN. Preventive effects of *Spirulina platensis*on skeletal muscle damage under exercise-induced oxidative stress. Eur J Appl Physiol 2006; 98(2): 220–226.
43. Nwani CD, Wazir Singh L, Naresh Sahebrao N, Ravindra K, Basdeo K, Satish Kumar S. Toxicity of the Herbicide Atrazine: Effects on Lipid Peroxidation and Activities of Antioxidant Enzymes in the Freshwater Fish *Channa Punctatus* (Bloch). Int J Environ Res Public Health 2010; 7: 3298–3312.
44. Gül S, Belge-Kurutas E, Yildiz E, Sahan A, Doran F. Pollution correlated modifications of liver antioxidant systems and histopathology of fish. (Cyprinidae) living in Seyhan Dan Lake. Turkey Environ Int 2004; 30: 605–609.
45. Avci A, Kacmaz M, Durak I. Peroxidation in muscle and liver tissues from fish in a contaminated river due to petroleum refinery industry. Ecotoxicol Environ Saf 2005; 6: 101–105.
46. Atli G, Alptekin O, Tukel S, Canlin M. Response of catalase activity to Ag+, Cd+, Cr6+, Cu2+ and Zn2+ in five tissues of fresh water fish *Oreochromis niloticus*. Comp Biochem Physiol C 2006; 143: 218–224.
47. Atif F, Parvez S, Pandey S, Ali M, Kaur M, Rehman H, Khan HA, Raisuddin S. Modulatory effect of Cadmium exposure on Deltamethrin-induced oxidative stress in *Channa punctatus*. Arch Environ Contam Toxicol 2005; 49: 371–377.
48. Şimşek-Köprücü S, Yanar E, Seker E. Effects of Deltamethrin on antioxidant status and oxidative stress biomarkers in freshwater mussel *Unio elongatulus euchres*. Bull Environ Contam Toxicol 2008; 81: 253–257.
49. Peebua LP, Kosiyachinda P, Pokethitiyook P, Kruatrachue M. Evaluation of alachlor herbicide impacts on Nile Tilapia (*Oreochromis niloticus*) using biochemical biomarkers. Bull Environ Contam Toxicol 2007; 78: 138–141.
50. Chandra S. Toxic effect of Malathion on acetylcholinesterase activity of liver, brain and

gills of freshwater catfish *Heteropneutes fossilis*. Environ Conserv 2008; 9: 45–52.
51. Farombi EO, Ajimoko YR, Adelowo OA. Effect of butachlor on antioxidant enzyme status and lipid peroxidation in freshwater African catfish (*Clarias gariepinus*). Int J Environ Res Public Health 2008; 5: 423–427.
52. Lushchak OV, Kubrak OI, Storey MJ, Storey BK, Lushchak IV. Low toxic herbicide roundup induces mild oxidative stress in gold fish. Chemosphere 2009; 76: 932–937.
53. Shen HM, Liu ZG. JNK signaling pathway is a key modification in cell death mediated by reactive oxygen and nitrogen species. Free Radic Biol Med 2006; 40: 928–939.
54. Markulan S, Marklund G. Involvement of superoxide anion radical in autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469–474.
55. Wills ED. Mechanism of Lipid peroxide formation in animal tissue. Biochem J 1966; 99: 667– 76.
56. Luck H. Catalse. In: Bergmeyer HU, editor. Methods of enzymatic analysis. New York: Academic Press; 1971; 885–93.
57. Milton TS, Tsokos JO. Statistical methods in the biological and health science. McGraw-will Internet Book Comp 1983; 381–405.
58. Filho DW, Torresl MA, Tribessl TB, Pedresosa RC, Soares CHL. Influence of season and pollution on the antioxidant defenses on the Cichlid fish acara *Geophagus brasiliensis*. Braz J Med Biol Res 2001; 34: 719–726.
59. Karakoc FT, Hewer A, Philips DH, Gaiñes AF, Yuregir G. Biomarkers of marine pollution observed in species of mullet living in two Eastern Mediterranean harbours. Biomarkers 1997; 2: 303–309.
60. Elia AC, Waller WT, Norton SJ. Biochemical responses of Bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. Bull Environ Contam Toxicol 2002; 68: 809–816.
61. Langiano VC, Martinez CBR. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. Comp Biochem Physiol C 2008; 147: 222–231.
62. Saravanan R, Revathi K, Murthy B. Lambda cyhalothrin induced alterations in antioxidant enzyme status of various tissues in freshwater catfish *Clarias batrachus*. Asian J Microbiol Biotech Environ Sci 2008; 10: 341–349.
63. Jayachandran K, Pugazhendy K. 2010. Ph.D., thesis Chelating mechanism of atropine on certain physiological and ultrastructural changes in the fingerlings of *Labeo rohita* (Hamilton) exposed to atrazine.
64. Gamble SC, Goldfarb PS, Porte C, Livingston DR. Glutathione peroxide and other antioxidant enzyme function in marine invertebrates (Mytilus enpecten mammimus, Carcinus means and Asterias rubens). Mar Environm Res 1995; 191–195.
65. Kohen R, Nyska A. Oxidation of biological systems: oxidative phenomena, antioxidants, redox reactions, and methods for quantifications. Toxicol Pathol 2002; 30: 620–650.
66. Bainy ACD, Arisi ACM, Azzalis LA, Simizu K, Barios SBM, Jungueira VBC. Differential effects of short - term lindane administration parameters related to oxidative stress in rat liver and erythrocytes. J Biol Toxicol 1993; 8:187–194.
67. Mohan Singh, Rajat Sandhir. Effects on antioxidant status of liver following atrazine exposure and its attenuation by vitamin E. Experimental and Toxicologic Pathalogy 2011; 63: 269–276.
68. Switzer L. *Spirulina*, the whole food revolution. Proteus Corporation, USA; 1980: 1–69.
69. Sk HU, Bhattacharya S. Prevention of cadmium induced lipid peroxidation, depletion of some antioxidantive enzymes and glutathione by series of organoselenocyanates. Environ Toxicol Pharmacol 2006; 22: 298–308.
70. Ramirez D, Gonzalez R, Merino N, Rodriguez S, Ancheta O. Inhibitory effects of *Spirulina* in zymozan - induced arthritis in mice. Mediators Inflamm 2002; 11:75–79.