Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

Int. J. Modn. Res. Revs.
Volume 2, Issue 11, pp 511-516, November, 2014

ISSN: 2347-8314

ORIGINAL ARTICLE

PROTECTIVE EFFICACY OF *SPIRULINA* AGAINST ATRAZINE TOXICITY ON GSH-GPx ACTIVITIES IN THE FRESH WATER FISH *Cyprinus carpio* (Linn)

***S.Venkatesan, V. Balamurugan, P. Susindren, A. Sundaresan, K. Rajkumar and K. Vasanthi.**

P.G and Research Department of Biotechnology, Sri Vinayaga College of Arts and Science, Ullundupet, Tamil Nadu, India.

Article History: *Received 2nd October,2014, Accepted 31st October, 2014, Published 1st November,2014*

ABSTRACT

The finding of this study showed that atrazine had negative effect on the antioxidant enzyme activities of *Cyprinus carpio*. The experimental fish were treated with atrazine (0.5 mg/ L) for 5 days. Another group (III) of fish treated with atrazine in 120 hours, after that fish was exposed to dried *Spirulina* pellet (2 gram). The group IV fish was exposed to *Spirulina* alone for 5 day. After the treatment fish was dissected out the organs like gill, liver and kidney were analysed enzymological parameters like GSH and GPx level. Antioxidant enzymes are biomarkers used to indicating the atrazine toxicity. The GSH and GPx are increased during the atrazine exposure period (P>0.05). In the group III atrazine along with *Spirulina* exposure the antioxidant enzymes was recovered (P>0.05). The result suggests that *Spirulina* can be effectively used to neutralize the toxic effect of atrazine on *Cyprinus carpio*.

Keywords: Atazine, GSH, GPx, *Spirulina*, *Cyprinus carpio*

1.INDRODUCTION

Herbicides are widely used for the control of water plants, which may impede the flow of water during the summer, when sudden heavy rain can cause flooding. While the direct effect of herbicides addition is the loss of macrophytes, non-target organisms such as fish may also be affected through loss of habit and food supply (Elia, 2002). Worldwide herbicide usage has increased dramatically during the past two decades, coinciding with changes in farming pesticides and increasingly intensive agriculture (Fung and Mak 2001). As a consequence, residuals amount of herbicides and their metabolites have been found in drinking water and food (Jhohnen 1999; Vander Oost *et al*., 2003).

Atrazine is pre-and post emergent broad leaf herbicide that acts an inhibiting the growth of target weeds by interfering with the normal function of photosynthesis (Chapman and Stranger; 1992). To prevent the growth functions of a wide variety of plants, including some species of algae. Atrazine is most effective in wet soils applied after significant winter rain when soils are at field's capacity. It is therefore also prone to leaching to ground water and surface runoff particularly if a storm event occurs just after the application of the herbicide. Atrazine is up to 20 times more frequently detected in groundwater of the US than any other herbicides (Belluck *et al*., 1991).

Atrazine has been suggested to induce activity of aromatase (cytochrome P_{450} (CYP) $_{19}$) (Hayes *et al*., 2002; Spano *et al*., 2004). Aromatase is the rate-limiting enzyme in the synthesis of C_{18} estrogens, such as 17 Beta-estradiol (EZ), from C_{19} androgen, such as testosterone (T). However, studies have also reported no effects of atrazine on aromatase activity (Coady *et al*., 2005; Hinfray *et al*., 2006) or even reported aromatase inhibition by atrazine (Benachour *et al*., 2007). Atrazine may also affect hepatic metabolism, phase I and phase II enzyme regulate homeostasis of sex steroids. It also remains to be established whether atrazine metabolism affects the endocrine control of maturation of fish or indeed developmental stages that involve important hormonal changes such as early development and puberty are most prone to endocrine disruption. Atrazine contamination was found in the farmer's blood and urine exposed to atrazine (Perry *et al*., 2001). Atrazine could cause damaged the gill epithelium and kidney and increase the renal excretion of sodium chloride and proteins in the rainbow trout (Fisher-Scherl *et al*., 1991) and carp (Neskovic *et al*., 1993). In addition, atrazine reduced plasma testosterone, olfactory sensitivity and salinity tolerance in mature male Atlantic salmon (Warning and Moore 2004). Thus, many European and African countries have restricted its use (Coady *et al*., 2005). The acute toxicity test indicates the relative species sensitivity and lethal concentration which can be used as a basis for

Corresponding* author: *Dr.* ***S.Venkatesan**, P.G and Research Department of Biotechnology, Sri Vinayaga College of Arts and Science, Ullundupet, Tamil Nadu, India*

long term tests to establish the requirements necessary for the well being of aquatic life. In fishes, biochemical and enzymological changes are induces by the pollutants before they acquire cellular and systemic malfunction. (Ram Narayana Ran and Sathyanesan, 1987).

Glutathione is a ubiquitous thiol-containing tripeptide that is involved in numerous processes that are essential for normal biological function, such as DNA and protein synthesis (Meister and Anderson, 1983). Among the several important functions for GSH, it contributes to the removal of reactive electrophils (such as many metabolities formed by cytochrome P-450 system) through conjugation by means of glutathione *S*-transferases. GSH also scavenging ROS directly or in a reaction catalyzed by glutathione peroxidase (GPx) through the oxidation of two molecules of GSH to a molecule of glutathione disulphide (GSSG). The relationship between the reduced and oxidized state of glutathione, the GSH/GSSG ratio or glutathione redox status, is then considered as an index of the cellular redox status and a biomarker of oxidative damage, because glutathione maintains the thiol-disulphide status of proteins, acting as a redox buffer.

2.MATERIALS AND METHODS

The fresh water fish *Cyprinus carpio* were collected from Navarathna fish farm from Pinnalur village and introduced into large cement tank (4x4) disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent the fungal infection). Fish were acclimatized for about 15 days before the commencement of the experiment. They were fed on commercial fish feed which given daily at morning hours. LC50 of atrazine was calculated by the log – dose / Profit regression line. The test fishes were classified into four groups whereas Group I control, Group II atrazine, Group III Atrazine+*Spirulina*, Group IV *Spirulina* alone. Each group are exposed to sublethal concentration of atrazine for a period of 120 hours. Simultaneously a control was maintained compare to toxic impacts. Experimental chemical atrazine was purchased from (TATA Atrataf 50% WP) mft by Rallis India Limited, Mumbai. The *Spirulina* were collected from Aurospriul commercial farm, from Aurovile village, near Pondicherry

Estimation of reduced glutathione (GSH)

Reduced glutathione was estimated by the method of Ellman (1959). A known weight of tissue was homogenized in phosphate buffer. From this 0.5 ml was pipette out and precipitated with 2 ml of 5% TCA. 1 ml of the supernatant was taken after centrifugation / 0.5 ml of plasma and added to it 0.5 ml of Ellman's reagent and 3 ml of phosphate similar manner along with a blank containing 3.5 ml of buffer. The amount of glutathione was expressed as µg/mg protein.

Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase (GSH-Px): GSH-Px activity was measured in the PMS by the method of Lawrence and Burk (1976). The reaction measures the rate of GSH oxidation by H_2O_2 catalyzed by the GSH-Px present in the PMS. The rate of GSSG formation was measured by following the decrease in absorbance at 340nm as NADPH was converted to $NADP^+$ by glutathione reductase. The results were expressed as µg/min/mg protein.

Statistical analyses

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student 't'-test Milton and Tsokos, (1983), to compare means of treated data against their control and the result were considered significant at ($P<0.05$), ($P>0.05$) level

3.RESULT

In the present investigation the fish (Table 1) *Cyprinus carpio* were exposed to atrazine (group II) when compared with control (group I). The antioxidant enzyme GSH values were gradually increased ($P>0.05$), among the all tissues Gill, Liver and Kidney. The atrazine exposed group percent increase in the gill tissue was, 4.66, 5.19, 6.75, 7.26, and 10.14 for the period of 24, 48, 72, 96 and 120 hours sublethal concentrations. The observed values of group III atrazine along with *Spirulina* exposed fish shows gradually recovered that percentages are 1.54, 16.57, 29.74, 22.72, and 32.05. The group IV values also increased ($P>0.05$) when compared with control group. The observed values of liver GSH in fish were exposed to atrazine (group II) when compared to control groups the GSH levels was gradually increased in 9.34, 12.48, 18.37, 20.35 and 23.42 for the period of 24, 48, 72, 96 and 120 hours respectively. The group III (atrazine along with *Spirulina*) gradually recovered (64.77, 7.29, 38.06, 49.23, 53.80) against atrazine toxicity. The group IV (*Spirulina* supplemented) exposed value also increased ($P>0.05$), when compared with control groups. The kidney tissues shows enzyme GSH levels was increased in atrazine treatment group the percent in the kidney increase was 16.18, 19.36, 20.15, 22.54, 23.84 for the period of 24, 48, 72, 96 and 120 hours respectively. The observed values of group III atrazine along with *Spirulina* exposed values shows gradually recovered (6.67, 6.40, 13.54, 27.59, 31.83) and the group IV (*Spirulina* supplemented) values also increased when compared with control values.

The GPx activity (Table 2) in *Cyprinus carpio* group II atrazine treatment values increased when compared with control values likewise 0.285, 0.319, 0.342, 0.367, 0.392 for the period of 24, 48, 72, 96 and 120 hours respectively. The groups III atrazine along with Spirulina exposed values are increased when compared with group II, it illustrates improving the GPx activity of the fish. The group IV *Spirulina* supplemented feed exposed values also increased, when compared with control values. Here, observed the movement of fish brisk and energetically. The observed values of GPx activity in liver tissue, herbicide atrazine exposed group II was slightly increased (0.144, 0.152, 0.153, 0.157, 0.169). The group III and group IV values also increased when compared with control group ($P>0.05$). The GPx activity in kidney were atrazine treatment group II levels were increased when compared with control values

were, 0.212, 0.226, 0.235, 0.246, 0.257 for the period of 24, 48, 72, 96 and 120 hours respectively. The group III trazine along with *Spirulina* exposed group is gradually detoxifying the atrazine toxicity in kidney. The group IV (*Spirulina* supplemented) values also gradually increased when compared with control group (P>0.05).

Finally, observed result shows the atazine seriously affected the enzyme activity in GSH and GPx in different tissues like gill, liver and kidney. The protective role of *spirulina* to improve the antioxidant defense mechanism of fish.

Table 1. Changes of GSH (µg/mg protein) activity in the fresh water fish *Cyprinus carpio* exposed to atrazine and *Spirulina* for 120 hours

		HOURS OF EXPOSURE				
ORGANS	**GROUPS**	**24**	**48**	**72**	**96**	**120**
GILL	Control	2.12 ± 0.4	2.16 ± 0.15	2.19 ± 0.15	2.18 ± 0.15	2.29 ± 0.16
	Atrazine	4.66** ± 0.27	5.19** ± 0.31	6.75** ± 0.40	7.26** ± 0.43	10.14** ± 0.61
	Atrazine + Spirulina	3.24** ± 0.19	4.33** ± 0.25	4.81** ± 0.28	5.61** ± 0.33	6.89** ± 0.41
	Spirulina	3.29 **± 0.23	3.48 **±0.24	3.59 **± 0.25	4.18** ± 0.29	4.65** ± 0.32
LIVER	Control	8.34 ± 0. 58	8.42 ± 0.58	8.44 ± 0.58	8.48 ± 0.59	8.61 ± 0.60
	Atrazine	9.34 *± 0.46	12. 48** ± 0.62	18.37** ± 0.91	20.35** ± 1.01	23.42** ± 1.17
	Atrazine + Spirulina	15.40** ± 0.92	13.39** ± 0.80	11.36 **± 0.68	10.35** ± 0.65	10.82** ± 0.54
	Spirulina	9.25** ± 0.64	9.42** ± 0.65	9.34 **± 0.65	9.15** ± 0.64	9.48** ± 0.56
KIDNEY	Control	14.25 ± 0.85	14.39 ± 0.86	14.65 ± 0.88	14.76 ± 0.88	14.85 ± 0.89
	Atrazine	16.18** ± 0.80	19.36** ± 0.96	20.15** ± 0.98	22.54** ± 1.02	23.84** ± 1.12
	Atrazine + Spirulina	15.10** ± 1.20	18.12 **± 1.08	17. 42 **±1.04	16.32** ± 0.97	16.25** ± 0.95
	Spirulina	15.42 *± 0.95	15.39** ±0.92	15.34** ± 0.76	15.69** ± 0.94	15.93** ± 0.95

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

Table 2. Changes GPx (µg/min/ mg protein) activity in the fresh water fish *Cyprinus carpio* exposed to atrazine and *Spirulina* for 120 hours

		HOURS OF EXPOSURE				
ORGANS	**GROUPS**	**24**	**48**	**72**	**96**	**120**
GILL	Control	0.241 ± 0.01	0.245 ± 0.01	0.244 ± 0.01	0.242 ± 0.01	0.248 ± 0.01
	Atrazine	0.285 **± 0.01	0.319** ± 0.01	0.342 **± 0.02	0.367** ± 0.02	0.392** ± 0.02
	Atrzine + Spirulina	0.322** ± 0.02	0.347** ± 0.01	0.362** ± 0.02	0.397** ± 0.02	0.412** ± 0.02
	Spirulina	0.256 ± 0.01	0.258 ± 0.01	0.261 ± 0.01	0.254 ± 0.01	0.266 ± 0.01
LIVER	Control	0.145 ± 0.01	0.148 ± 0.01	0.151 ± 0.01	0.149 ± 0.01	0.154 ± 0.01
	Atrazine	0.144** ± 0.01	0.152* ± 0.02	0.153 ± 0.01	0.157 ± 0.01	0.169 ± 0.01
	Atrazine + Spirulina	0.149 ± 0.02	0.154* ± 0.01	0.160 ± 0.01	0.168* ± 0.01	0.183* ± 0.01
	Spirulina	0.148** ± 0.01	0.155* ± 0.02	0.158 ± 0.02	0.156 ± 0.01	0.160 ± 0.01
KIDNEY	Control	0.172 ± 0.01	0.178 ± 0.01	0.175 ± 0.02	0.180 ± 0.01	0.182 ± 0.02
	Atrazine	0.212 ± 0.01	0.226 ± 0.01	0.235** ± 0.05	0.246 **± 0.01	0.257 **± 0.1
	Atrazine + Spirulina	0.232* ± 0.02	0.248 **± 0.01	0.254 **± 0.02	0.278** ± 0.01	0.293** ± 0.02
	Spirulina	0.175 ± 0.01	0.180 ± 0.01	0.178 ± 0.01	0.188 ± 0.01	0.192 ± 0.01

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

4.DISSCUSSION

The GSH plays an important role in the detoxifying of electrophilis and prevention of cellular oxidative stress (Benova *et a.,* 1990). The considerable decline in the GSH tissue content during exposure to atrazine may be due to an increased utilization of GSH, which can be converted into oxidized glutathione and an inefficient GSH regeneration. GSH catalyses the reduction of H_2O_2 and lipid hydroperoxides at expense of GSH. Reduced glutathione is the main nonoprotein thiol and one of the primary resultants found in cells (Moreno 2005). A significant decrease in GSH in the liver of *Cyprinus carpio* after atrazine exposure indicated pro-oxidant conditions in the liver. Decrease in GSH levels after administration of various pesticides is well documented in literature (Hazarika *et al.,* 2001; Prasanthi *et al.,* 2005). Singh *et al.* (2006) it has been reported by various researchers that GSH play an important role in protecting cells from xenobiotic-induced tissue injury (Reed and Fariss 1984; Wu *et al.,* 2004). The reduced levels of GSH in the atrazine could be the results of either utilization of GSH for conjugation and /or participation of GSH as an antioxidant in terminating toxicity. Administrations of atrazine along with *Spirulina h*ave resulted in restoration to near control value. GSH is the primary intracellular antioxidant and the conjugating agent, was shown to depleted and to have impaired function in atrazine toxicity. In fact, GSH serves as a primary line of cellular defence against atrazine compounds. (Quing, 1998).

GSH is the major thiol, which binds electrophile molecules spices and free radical intermediates. It plays a central role in the antioxidant defense system, metabolism and detoxification of exogenous and endogenous substances. (Ketterer *et al.,* 1998). GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. Because of the low activity of antioxidant enzymes in the liver and decreased content of GSH, the liver is hypothesized to be highly susceptible to oxidative stress one of GSH to near to control value. Monteria *et al.* (2006) have point out that enzyme activity can decrease by negative feedback from excess substrate or by damage induced by oxidative modification. A reduction in GSH-Px activity in a given tissue could indicate that its antioxidant capacity was exceeded by the amount of hydro peroxide products generated.

However, our results showed an increase in GSH-Px level in kidney tissue, which suggest the operation of a protective response in this tissue against the oxidative stress induced by atrazine. Glutathione reductase (GR) plays an important role in cellular antioxidant protection and adjustment processes of metabolic pathway (Wentz, 2000). Although perhaps not involved in antioxidant defence in same way the enzymes previously described. Glutathione reductase merits attention because of its importance in maintaining GSH homeostasis under oxidative stress (Winston *et al.,* 1991). Glutathione reductase is to catalyses the reduction of glutathione disulfide to reduced glutathione in an NADPH-dependent reaction (Cazenave *et al.,* 2006).

In this study, decrease in activity of GPx in the gill, liver and kidney of *Cyprinus carpio* at the highest concentration of atrazine may indicate that its antioxidant capacity was exceeded by the level of hydro peroxide products and reflects a possible failure of the antioxidant system of fish. However, Elia *et al.* (2002) have reported significant increase in the activity of hepatic GPx but no change in the activity of GPx in gills of fish following atrazine exposure. The results of the study indicate that atrazine – induced decrease in the GSH content could be due to increase in the activity of GPx. The observed increase in the activity of glutathione peroxidise upon atrazine administration might be the natural mechanism to concentrate the pro-oxidant effect of atrazine toxicity (Perottoni *et al.,* 2004).

GPx contents H_2O_2 or other lipid peroxide to water or hydroxyl lipids and in the process of GSH is converted to increase in the GPx activity following atrazine exposure. Sharma *et al.* (2005) reported an increased peroxide activity in the liver of rats after treatment with dimethote. Fatima *et al.* (2000) report a low activity of GPx in different fish tissue after exposure to paper mill effluent, indicating an in efficiency of this organ to neutralizing the peroxide impacts. A similar decrease in GPx activity in fish liver is reported after 90 days of treatment with lindane and organochlorine pesticides. GPx inhibition was reported after combined treatment with the pesticides atrazine and azinphosmethyl in the brain of carp *Cyprinus carpio* (Uner and Oruc *et al.,* 2006) and in the liver of Nile tilapia, *Oreochromis niloticus* (Oruc and Uner, 2002).

The increase in GPx activity was observed, predominantly in liver and kidney similar to the results reported by Li *et al.,* (2003) who have studied the responses of the antioxidant systems in the hepatocyte of common carp (*Cyprinus carpio* L) to microcystin. However, GPx activity decreases in gills after the longer exposure time, corroborating the LPO increase in this organ; this may be explained because gills are less efficient than kidney and liver at neutralizing the impact of peroxidative damage (Sayeed *et al.,* 2003; Ahmad *et al.,* 2004).

5.CONCLUSION

From the above observations it can be concluded that exposure of atrazine results in increased oxidative stress and altered antioxidant status of the gill, liver and kidney. Administration of spirulina along with atrazine resulted in partial normalization of the toxic effects of atrazine thus highlighting the protective effect of *Spirulina*.

6.REFERENCES

Ahmad., M. Pacheco and M.A. Santos, 2004. Enzymatic and non-enzymatic antioxidants as an adaption to phagocyte induced damage in *Anguilla Anguilla* L. following in situ harbor water exposure. *Ecotoxicol. Environ. Saf.,* **57**: 270.

Belluc, D.A., S. L. Benjamin and T. Dawson, 1991. Groundwater contamination by atrazine and its metabolities: risk assessment, policy and legal implication. In: Somasundram, L., Coats JR, editors. Pesticides transformation products: fate and significance in the environment. Washington (DC): *American Chemical Society*., *pp*.254-73.

Benachour, N., S. Moslemi., H. Sipahutar and G. E. Seralini, 2007. Cytotoxic effects and aromatase inhibition by

xenobiotic endocrine disrupters alone and in combination. Toxicol. *Appl. Pharmacol*., **222**:129-140.

Benovo, L. V., I. C. Benchev and O.H. Monovich, 1990. Thiol antidotes effect of lipid peroxidation in mercury-poisoned rats. *Chem.Biolog. Interaction*., **76:** 321.

Cazenave, J., M. A. Bistoni., S. F. Pesce and D. A. 2006. Wunderlin. Differentialdetoxification and antioxidant response in diverse organs of *Corydoras paleatus* experimentally exposed to microcystin-RR, *Aquat. Toxicol*. **76:**1-12.

Chapman, R.N and J.W. Stranger, 1992. Horticultural pesticides residues in water: a review of potential for water contamination by pesticides used in the vegetable industry in Victoria. Melbourne, Australia: *Development of Food and Agriculture, p*. 137.

Coady, K. K., M. B. Murphy., D. L. Villeneuve., M. Hecker., P. D. Jones., J. A. Carr., K. R. Solomon., E. E. Smith., G. Van Der Kraak., R. J. Kendall and J.P.Giesy, 2005. Effects of atrazine on matamorphosis, growth, laryngeal and gonadal development, aromatase activity, and sex steroid concentration in *Xenopus laevis*. *Ecotoxicol. Environ. Saf*., **62:** 160-173

Elia, A. C., W. T. Waller and S.J. Norton, 2002. Biochemical responses of Bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. *Bull. Environ. Contam. Toxicol.*, **68**: 809-816.

Ellman, G.L., 1959. Tissue sulphydryl groups. Arch. Biochem. Biophys, 82, 70-77

Fatima, M., I. Ahmad., I. Sayeed., M. Athar and S. Raisuddin, 2000. Pollutant induced over- activation of phagocytes is concomitantly associated with peroxidative damage in fish tissue. *Aquat. Toxicol*., **49**:243-250.

Fischer-Scherl, T., A. Veeser, R. W. Hoffmann., C. Kuhnhauser and R. D. Negele Ewringmann., 1991. Morphological effects of acute and chronic atrazine exposure in rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol*., **20**:454-461.

Fung, Y. S and J. L. L. Mak, 2001. Determination of pesticides in drinking water by micellar electrokinetic capillary chromatography. Electrophoresis., **22:** 2260-2269.

Hayes,T. B., A. Collins., M. Lee., M. Mendoza., N. Noriega., A. A. Stuart and A.Vonk, 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proc. Natl. Acad. Sci. USA.*, **99**:5476-5480.

Hazarika A, Sarkar s.N., M. Kateria, 2001. Subacute toxicity of anilofos, a new organophosphorus herbicide in male rats: effect on lipid peroxidation and ATPase activity. *Indian J Exp Biol*, 39, 1113-7.

Hinfray, N., J. M. Porcher and F. Brion, 2006. Inhibition of rainbow trout (*Oncorhynchus mykiss*) P450 aromatase activities in brain and ovarian microsomes by various environmental substances. *Comp. Biochem. Physiol. C: Pharmacol*. **144:**252-262.

Johnen, B. G, 1999. Herbicides and food quality a misfit? In: proceedings of the Brighton Confrerance, Weeds, vol, 3. *British Crop Protection Council., K, pp*.875-882.

Ketterer, B, 1998. Glutathione-S-transferase and prevention of cellular free radical damage. *Free Radical Research* 28:647-658.

Li, X., Y.Liu., L. Song and J.S.H.Liu, 2003. Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. *Toxicon*., **42:** 85-89.

Meister, A., Anderson, M.E., 1983. Glutathione. Annu. Rev. Biochem. 52, 711-760.

Monterio, M. C. M. Quintanerio., M.L.Pastorinho., F.Pereira., L.Morgado., A.M.Guilhermino and V.M. Soares, 2006. Acute effects of 3, 4-dichloroaniline on biomarkers and spleen histology of the common goby *Pomatoschistus microps*. *Chemosphere* **62**:1333-1339.

Moreno, S. Pichardo, L., Goomez-Amores, A. Mate, C.M. Vazquez, Camean. 2005. Antioxidant enzymes activity and lipid peroxidation in liver kidney of rats exposed to microcystin-LR administered intraoperation Toxicon, **45**, 395-402.

Nescovic, N.K., I.Elezovic., V.Karan., V.Poleksic and M. Budimir., 1993. Acute and subacute toxicity of atrazine to carp (*Cyprinus carpio* L.) *Ecotoxicol. Environ. Saf.*, **29**(2):173-182.

Oruc, E.O., N. Uner., 2000. Combined effects of 2, 4D and azinphosmethyl antioxidant enzyme and lipid peroxidation in liver *Oreochromis niloticus* Com. Biochem. Physiol, 127, 291-296

Perottoni, J., L.P. Labato., A. Silveria, J.B.T. Rocha and T. Enanuelli, 2004. Effects of mercury and selenite on alpha-aminolevulinate dehydratase activity and selected oxidative stress parameters in rats. *Environmental Research*., **95:**53-55.

Perry, M.J., D.C. Christiani, Mathew., D.Degenhardt., J.Torttorelli., J.Strauss and W.C. Sonzogni., 2001. Urinalysis of atrazine exposure in farm pesticides applications. . *Toxicol. Ind, Health*., **16** (7-8), 285-290.

Prasamthi, K., Muralidhara, R.S. Rajini., 2005. Fenvelarate induced oxidative damage in rat tissue and its attenuation by dietry sesame oil. *Food Chem Toxicol*, **43**, 299-306.

Quig, D, 1998. Cysteine metabolism and metal toxicity. A*lternative Medicine Review* **3:**262-270.

Ram, R.N and A.G. Sathyanesan, 1984. Mercuric chloride induced changes in Protein, Lipid and cholesterol levels of the liver, kidney and ovary of the fish, *Channa punctatus*. *Environ. Ecol*., **2** (2): 113-117.

Reed D.J., Fariss M.W., 1984. Glutathione depletion and susceptibility. Pharmacol Rev, 36; 25S-33S

Sayeed, I, S. Parvez, S. Pandey, B. Bin-Hafeez, R. Haque and S. Raisuddin, 2003. Oxidative stress biomakers of exposure to deltamethrin in freshwater fish. *Channa Puntatus* (Bloch). *Ecotoxicol. Environ. Saf.,* **56:**295-301.

Sharma, S and S.Sharma, 2005. Protective role of *Spirulina* feed in a freshwater fish (*Poecilia reticulata* peters) exposed to an azo dye – methyl red. *Indian J Exp Biol*, **43**:1165

Singh, M., R. Sandhir, R. Kiran, 2006. Erythrocyte antioxidant enzymes in toxicological evaluation of commonly used organophosphate pesticides. *Indian J Exp Biol*, **44**, 580-93

Spano, L, C.R.Tyler., R.Van Aerle., P.Devos, S.N.M. Mandiki., F.Silvestre., J.P.Thome and P. Kestemont., 2004. Effect of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*). *Aquat. Toxicol*. **66**:369-379

Uner, N., E.O. Oruc., Y. Sevgiler., N. Sahin., H. Durmaz and Usta, D. 2006. Effect of diazinon of acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. *Environ. Toxicol.Pharmocol*., **21**:241-245

Vander Oost,R., Beyar,J.and Vermeulen,N.P.2003.Fish bioaccumulation and biomarker and environmental risk assessment:*A review*, *Environ.Toxicol. Pharmacol*.,57-149.

Waring, C.P and A. Moore, 2004. The effect of atrazine on Atlantic salmon (Salmo salar) smolts in fresh water and after sea water transfer. *Aquat. Toxicol*., **66:**93-104.

Wentz, P.W, 2000. (LabCorp, Burlington, NC). Chelation therapy conventional treatments. Advanced Magazines/Administration of the Loboratory, May, Merion, King of Prussia, PA.

Winston, G.W and R.T. Giulio, 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquat. Toxicol*., **19**: 116-3

Wu G, Fang, Y.Z., Yang, S., Lupton, J.R., N.D. Turner, 2004. Glutathione metabolism and its implications for health J.Nutr: 134; 289-292.

*****