

ORIGINAL RESEARCH ARTICLE

Hepatoprotective Effect of *Pisonia alba* and *Cardiospermum halicacabum* in Atrazine Toxicity on LPO and some Antioxidant Activities in the Liver Tissue of Fresh Water Fish *Labeo rohita*S. Prabakaran¹, K. Pugazhendy^{1*} A. Revathi¹ and C. Jayanthi²¹Department of Zoology, Annamalai University, Annamalai nagar- 608 002, Tamilnadu, India²Department of Education, Annamalai University, Annamalai nagar- 608 002, Tamilnadu, India

Received 05 Jan 2014; Revised 11 Apr 2014; Accepted 25 Apr 2014

ABSTRACT

The present study was undertaken to evaluate the hepatoprotective effect of *P. alba* and *C. halicacabum* against the toxicity effects of herbicide atrazine on lipid peroxidation and some antioxidant enzyme system in the freshwater fish *Labeo rohita*. In the present experimental study, *L. rohita* were exposed to sublethal concentration of atrazine (20 mg/L of atrazine) for 120 hours. The oxidative stress in the liver was evidence by increased lipid peroxidation levels. The antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) levels were decreased compared to control. During the treatment of *P. alba* and *C. halicacabum* against atrazine exposed fish were restored near normal level (Group III and IV). The observed results were discussed in detail.

Key words: Hepatoprotect, Atrazine, *Labeo rohita*, *P. alba*, *C. halicacabum*, LPO, SOD, CAT, GPx.**1. INTRODUCTION**

The liver is a vital organ and has many important functions, including metabolism and detoxification of hepatotoxicants [1]. In most cases, liver damage is a widespread pathology which involves oxidative stress and a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma [2]. Antioxidant enzymes are important in coping oxidative stress caused by the metabolism itself and environmental factors [3]. Oxidative stress results from disruption of the prooxidant and antioxidant balance by reactive oxygen species (ROS) and other radicals or oxidants [4]. While xenobiotics are able to increase ROS levels, the capacity to induce oxidative stress depends on the overwhelming of antioxidant defenses. Aerobic organisms have developed antioxidant defense mechanisms that scavenge ROS or prevent ROS-mediated cellular damage [5], including enzymes sensitive to free radical proliferation such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [6]. 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 [7].

In recent years many medicinal plants have been used for pharmacological and toxicological studies. Drugs developed from medicinal plants perform not only the role of therapeutic but as drug leads for development of new synthetic drugs based on model structures [8]. 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, pineapples 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 [9].

Pisonia grandis (Synmyn: *Pisonia alba*, *Pisonia morindifolia*) commonly known as Leechikottai kerai in Tamil. In the alternative system of medicine *Pisonia grandis* leaves are used as

analgesic, anti-inflammatory, diuretic, hypoglycemic agent and antifungal. It is also used in the treatment of ulcer, dysentery and snake bite. The leaves are edible and mostly used to treat wound healing, rheumatism and arthritis [10]. *Cardiospermum halicacabum*, commonly known as Mudakkathan in Tamil. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific; its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache and as a poultice for swellings [11]. The aim of the present study was to evaluate the toxicity of herbicide atrazine and hepatoprotective effect of *P. alba* and *C. halicacabum* on LPO and some antioxidant enzymes in liver tissue of *L. rohita*.

2. MATERIALS AND METHODS

Experimental animal collection and maintenance

The freshwater fish *Labeo rohita* were collected from the VGM fish farm located in Kurinjipadi, Cuddalore district. The fish were brought to the laboratory and transferred to the rectangular cement tanks (100 × 175) of 500 liters capacity containing chlorine free aerated well water. The fishes measuring 14-16 cm in length and 70-80 g in weight were selected irrespective of their sex for the experiments. During this time they were fed every 24 hour with a commercial diet. The physico-chemical parameters of the water were monitored throughout the acclimation period and remained constant (pH: 7.18 ± 0.5, conductivity: 118.25 ± 8.7 μS cm⁻¹, dissolved oxygen: 8.49 ± 0.9 mg O₂ L⁻¹, temperature: 21.96 ± 2.7 °C).

Experimental chemical

Experimental chemical atrazine was purchased from (TATA Atrataf 50% WP) manufacture by Rallis India Limited, Mumbai.

Supplementary feed

Healthy disease free leaves of *Cardiospermum halicacabum* and *Pisonia alba* were collected from in and around Chidambaram and Thiruvankadu, the plant was identified. The leaves were washed in running tap water for 10 minutes leaves were dried, aerial parts (1kg) of *Cardiospermum halicacabum* and *Pisonia alba* were macerated thrice at room temperature and prepared in powdered condition and equal amount

of rice brane mixed well and small amount water added and prepared small pellet as feed.

Enzymatic assay

Superoxide dismutase (SOD) activity was determined by method of [12], the in absorbance was recorded at 560 nm. The activity of catalase (CAT) was determined by the method of [13] was recorded at Spectrophotometrically read at 620 nm. Lipid peroxides in liver tissue were estimated by the method of [14] which recorded at spectrophotometrically at 540 nm. Glutathione peroxidase (GPx) activity was assayed according to the method described by [15] oxidation of NADPH was recorded spectrophotometrically read at 340 nm.

Experimental design

Group- I: Fish exposed to tap water (control)

Group- II: Fish exposed to atrazine

Group-III: Fish exposed to atrazine along with *Pisonia alba*

Group- IV: Fish exposed to atrazine along with *Cardiospermum halicacabum*

Group- V: Fish exposed to *Pisonia alba* alone

Group- VI: Fish exposed to *Cardiospermum halicacabum* alone

Statistically analyses

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student t-test [16] to compare means of treated data against their control ones and the result were considered significant at (P<0.05) and (P<0.01) level.

3. RESULTS

In the present study, observed that liver tissue antioxidant such as SOD, CAT and GPx levels are decreased significantly at 5 % level (p<0.05) in the treated group II (Table 1, 2 & 4). At the end of 120 hours SOD, CAT and GPx levels are decreased when compared to control group I. In the group III and IV SOD, CAT and GPx levels are near to normal when compared to group II. In the group V and VI SOD, CAT and GPx levels are increased significantly at 120 hours compared to group II and which was near to control group I. LPO levels were significantly increased in the group II when compared to control group I (Table 3). In the group III and IV LPO level was regained compared to group II. In the group V and VI LPO level near to normal significantly at 120 hours compared to group II and which was near to control group I.

Table 1: Changes in the level of superoxide dismutase (U/min/mg of protein) activity in the liver tissue of freshwater fish *Labeo rohita* exposed to atrazine followed by the supplementary feed of *Pisonia alba* and *Cardiospermum halicacabum* exposed to 120 hours

Groups	Hours of exposure				
	24	48	72	96	120
Group-I: Control	30.706 ± 0.780	30.644 ± 0.917	30.628 ± 0.922	30.607 ± 0.811	30.591 ± 0.762
Group-II: Atrazine % COC	29.543** ± 0.617 -3.787	28.370** ± 0.572 -7.420	27.696** ± 0.622 -9.572	26.107** ± 0.686 -14.702	24.884** ± 0.655 -18.655
Group-III: Atrazine+ <i>P. alba</i> % COC % COT	29.908* ± 0.719 -2.598 1.235	29.240** ± 0.819 -4.581 3.066	28.607** ± 0.824 -6.598 3.289	27.080** ± 0.679 -11.523 3.726	26.987** ± 0.517 -11.781 8.451
Group-IV: Atrazine+ <i>C. halicacabum</i> % COC %COT	29.955* ± 0.429 -2.445 1.394	29.447** ± 0.574 -3.612 3.796	28.809** ± 0.580 -5.452 4.018	27.377** ± 0.704 -9.834 4.864	27.006** ± 0.718 -11.202 8.527
Group-V: <i>P. alba</i> % COC	30.716 ^{NS} ± 0.720 0.032	30.725 ^{NS} ± 0.844 0.264	30.740 ^{NS} ± 0.807 0.365	30.755 ^{NS} ± 0.696 0.483	30.769 ^{NS} ± 0.980 0.581
Group-VI: <i>C. halicacabum</i> % COC	30.724 ^{NS} ± 0.924 0.058	30.740 ^{NS} ± 0.877 0.313	30.761 ^{NS} ± 0.807 0.434	30.780 ^{NS} ± 0.609 0.565	30.792 ^{NS} ± 0.655 0.657

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated), NS- Nonsignificant.

Table 2: Changes in the level of catalase (µmol of H₂O₂ consumed/ min/mg of protein) activity in the liver tissue of freshwater fish *Labeo rohita* exposed to atrazine followed by the supplementary feed of *Pisonia alba* and *Cardiospermum halicacabum* exposed to 120 hours

Groups	Hours of exposure				
	24	48	72	96	120
Group-I: Control	7.734 ± 0.074	7.711 ± 0.065	7.695 ± 0.096	7.673 ± 0.090	7.660 ± 0.064
Group-II: Atrazine % COC	7.112** ± 0.054 -8.042	6.870** ± 0.073 -10.906	6.214** ± 0.074 -19.246	5.775** ± 0.096 -24.736	5.077** ± 0.088 -33.720
Group-III: Atrazine+ <i>P. alba</i> % COC % COT	7.341** ± 0.066 -5.081 3.219	7.076** ± 0.064 -8.234 2.998	6.874** ± 0.059 -10.669 10.621	6.273** ± 0.076 -18.245 8.623	5.956** ± 0.065 -22.245 17.313
Group-IV: Atrazine+ <i>C. halicacabum</i> % COC %COT	7.503** ± 0.069 -2.986 5.497	7.344** ± 0.054 -4.759 6.899	7.078** ± 0.057 -8.018 13.904	6.844** ± 0.088 -10.804 18.510	6.347** ± 0.096 -17.140 25.014
Group-V: <i>P. alba</i> % COC	7.739 ^{NS} ± 0.090 0.064	7.742 ^{NS} ± 0.094 0.402	7.755 ^{NS} ± 0.077 0.779	7.767 ^{NS} ± 0.066 1.225	7.786 ^{NS} ± 0.069 1.644
Group-VI: <i>C. halicacabum</i> % COC	7.743 ^{NS} ± 0.086 0.116	7.259 ^{NS} ± 0.077 0.622	7.770 ^{NS} ± 0.064 0.974	7.784 ^{NS} ± 0.079 1.446	7.796 ^{NS} ± 0.058 1.775

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated), NS- Nonsignificant

Table 3: Changes in the level of lipid peroxidase (nmol/mg of protein) activity in the liver tissue of freshwater fish *Labeo rohita* exposed to atrazine followed by the supplementary feed of *Pisonia alba* and *Cardiospermum halicacabum* exposed to 120 hours

Groups	Hours of exposure				
	24	48	72	96	120
Group-I: Control	3.222 ± 0.023	3.239 ± 0.038	3.248 ± 0.054	3.254 ± 0.044	3.267 ± 0.056
Group-II: Atrazine % COC	3.528** ± 0.033 9.497	3.834** ± 0.036 18.369	3.999** ± 0.047 23.121	4.270** ± 0.049 31.223	4.544** ± 0.054 39.087
Group-III: Atrazine+ <i>P. alba</i> % COC % COT	3.473** ± 0.051 7.790 -1.558	3.596** ± 0.042 11.021 -6.207	3.716** ± 0.039 14.408 -7.076	3.910** ± 0.038 20.159 -8.430	3.995** ± 0.037 22.283 -12.081
Group-IV: Atrazine+ <i>C. halicacabum</i> % COC %COT	3.330** ± 0.029 3.351 -5.612	3.424** ± 0.033 5.711 -10.693	3.520** ± 0.039 8.374 -11.977	3.624** ± 0.054 11.370 -15.128	3.712** ± 0.041 13.621 -18.309
Group-V: <i>P. alba</i> % COC	3.227 ^{NS} ± 0.055 0.155	3.250 ^{NS} ± 0.044 0.339	3.264 ^{NS} ± 0.027 0.492	3.277 ^{NS} ± 0.034 0.706	3.285 ^{NS} ± 0.077 0.858
Group-VI: <i>C. halicacabum</i> % COC	3.233 ^{NS} ± 0.064 0.341	3.256 ^{NS} ± 0.070 0.524	3.277 ^{NS} ± 0.061 0.892	3.290 ^{NS} ± 0.075 1.106	3.318 ^{NS} ± 0.043 1.561

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P< 0.01 levels. (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated), NS- Nonsignificant

Table 4: Changes in the level of glutathione peroxidase (µg/min/mg protein) activity in the liver tissue of freshwater fish *Labeo rohita* exposed to atrazine followed by the supplementary feed of *Pisonia alba* and *Cardiospermum halicacabum* exposed to 120 hours

Groups	Hours of exposure				
	24	48	72	96	120
Group-I: Control	6.585 ± 0.064	6.571 ± 0.078	6.557 ± 0.048	6.535 ± 0.066	6.523 ± 0.055
Group-II: Atrazine % COC	6.220** ± 0.074 -5.542	6.079** ± 0.055 -7.487	5.917** ± 0.038 -9.760	5.774** ± 0.044 -12.104	5.334** ± 0.077 -18.304
Group-III: Atrazine+ <i>P. alba</i> % COC % COT	6.344** ± 0.036 -3.659 1.993	6.117** ± 0.042 -6.909 0.625	6.055** ± 0.077 -7.655 2.332	5.956** ± 0.039 -8.859 3.152	5.774** ± 0.088 -11.482 8.248
Group-IV: Atrazine+ <i>C. halicacabum</i> % COC %COT	6.447 ^{NS} ± 0.075 -2.095 3.649	6.370* ± 0.071 -3.058 4.786	6.118** ± 0.068 -6.695 3.396	6.027** ± 0.056 -7.773 4.381	5.912** ± 0.074 -9.366 10.836
Group-V: <i>P. alba</i> % COC	6.585 ^{NS} ± 0.069 0.075	6.597 ^{NS} ± 0.088 0.395	6.617 ^{NS} ± 0.080 0.915	6.622 ^{NS} ± 0.054 1.331	6.639 ^{NS} ± 0.072 1.778
Group-VI: <i>C. halicacabum</i> % COC	6.591 ^{NS} ± 0.055 0.091	6.599 ^{NS} ± 0.047 0.426	6.622 ^{NS} ± 0.046 0.991	6.635 ^{NS} ± 0.053 1.683	6.648 ^{NS} ± 0.060 1.931

Values are mean \pm S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated), NS- Nonsignificant.

DISCUSSION

SOD is a major enzyme in eliminating ROS formed during bioactivation of xenobiotics in the hepatic tissues [17] and the induction of SOD system provides a first line of defense against ROS. SOD help to dismutase superoxide radical O₂⁻ to hydrogen peroxide (H₂O₂). In the present experimental result shows that the, levels of SOD was decreased when the fish exposed to atrazine.

But the group IV (atrazine along with *Cardiospermum halicacabum*) the SOD level was gradually increases when compared to the group II. Moreover, the group III (atrazine along with *Pisonia alba*) also enhance the SOD level very slowly than the group IV. [18] pointed out that a decreased SOD activity response may accompany a first exposure to pollutants, which can be followed by an induction of antioxidant systems. [19] suggested that the superoxide radicals by themselves or after their transformation to H₂O₂ cause an oxidation of the cysteine in the enzyme and decrease SOD activity.

Catalase enzyme is located primarily in peroxisomes and together with GSH are the main cellular defences against hydrogen peroxide [20]. In the present observation, the levels of CAT was decreased when the fish exposed to atrazine. But, the group IV (atrazine along with *Cardiospermum halicacabum*) the CAT level was gradually regained when compared to the group II. Moreover, the group III (atrazine along with *Pisonia alba*) also enhance the CAT level very slowly than the group IV. Carbaryl produced a simultaneous decrease in CAT at the end of the assay period. Therefore, the GSH decrease would lead to an increase of free radicals, affecting CAT activity [21]. Inhibition as well as CAT induction has been reported in *O. mykiss*, depending on the concentration of the organochlorine pesticide endosulfan used [20]. The inhibit levels of CAT could be attributed to high production of O₂. This has been reported to inhibit CAT activity in decrease of excess production [22].

Lipid peroxidation is considered to be a valuable indicator of oxidative damage of cellular components. Lipid peroxidation is the initial step of cellular membrane damage caused by pesticides, metals and other xenobiotics. In the present observation the level of LPO was increased when the fish exposed to atrazine. But the group IV (atrazine along with *Cardiospermum*

halicacabum) the LPO level was gradually decreases when compared to the group II. Moreover, the group III (atrazine along with *Pisonia alba*) also enhance the LPO level very slowly than the group IV. [23] have registered the elevated level of LPO exposed to atrazine toxicant. [24] also point out the elevated level of lipid peroxidation in the liver of *C. mrigala* exposure to cypermethrin. The Increased LPO level may be due to the ROS production associated with the metabolism of cypermethrin leading to the peroxidation of membrane lipid of the liver [25].

GPx is considered as the key enzymes within the antioxidative defense mechanism, which directly determines the concentration of O₂ and H₂O₂ [26]. In the present experiment the level of GPx was decreased when the fish exposed to atrazine. But the group IV (atrazine along with *Cardiospermum halicacabum*) the GPx level was gradually increased when compared to the group II. Moreover, the group III (atrazine along with *Pisonia alba*) also enhance the GPx level very slowly than the group IV. [27] reported that the significant decline was noticed in the activities of GPx in the liver tissue of cyhalothrin induced fish (*Oreochromis mossambicus*). [28] also pointed out the GPx was reduced in the sublethal concentration of nickel toxicity in *Cirrhinus mrigala*. The inhibition might be due to interaction of nickel directly with metal ion, which is dependent on subcellular origin [29]. [30] also indicated the significant decreased in GPx activity in the liver tissue of fish *Onchorhynchus mykiss* after Cd and Cr exposures.

Ethanollic extract of *C. halicacabum* extract repressed the TNF- α induced DNA binding activity of NF- κ B. These indicate the anti-inflammatory activity of the plant [31]. In the anti-inflammatory test in the liver tissue, its extract amplified the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) while in liver tissue, it lessens the level of nitrite oxide (NO) and malondialdehyde (MDA). These results suggest that ethanollic extract act as a natural antioxidant and anti-inflammatory mediator [32]. Lately, the anti-inflammatory role of rutin has been recognized in this plant [33]. The gamma-glutamyl trans-peptidase and phospholipase A2 activity to

reduce the lipid peroxide content when compared to exposed group. At the same the *P. alba* having the certain important medicinal properties but it compared to the *C. halicacabum* was less. These bioactive compounds present in *C. halicacabum* and *P. alba* which may give recovery to fish in the presence of toxic stress.

5. CONCLUSION

In summary, it has been concluded from the evidence that the plants *P. alba* and *C. halicacabum* both produced significant hepatoprotection of atrazine induced toxicity on freshwater fish *L. rohita*. When compared to both plant extracts have significantly preventing the hepatic damages in fishes. At the same the *P. alba* having the certain important medicinal properties but it compare to the *C. halicacabum* was less. But the *C. halicacabum* having the more valuable therapeutic properties compared to *P. alba*.

ACKNOWLEDGMENT

The author thankful to the Professor and Head, Department of Zoology, Annamalai University for providing necessary facilities for carried out this research work.

REFERENCES

1. Altas, S., Kizil, G., Kizil, M., Ketani, A and Haris, P. I. 2011. Protective effect of Diyarbakir watermelon juice on carbon tetrachloride-induced toxicity in rats. *Food Chemical Toxicology*, 49, 2433–2438.
2. Srivastava, A., and Shivanandappa, T. 2010. Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. *Food Chemistry*, 118, 411–417.
3. Jorgensen, S.W. 2010. A derivative of encyclopedia of ecology. Ecotoxicology. Academic Press, London, 390.
4. Prior, R.L. 2004. Biochemical measures of antioxidant status. *Top Clin Nutr.* 19, 226–238.
5. Valavanidis, A., Vlahogianni, T., Dassenakis, M and Scoullou, M. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ Saf.* 64, 178–179.
6. Droge, W. 2002. Free radicals in the physiological control of cell function. *Physiol. Rev.* 82:47–95
7. Livingstone, D.R. 2001. Contaminant stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull.* 42, 656–666.
8. Balunas, M.J and Kinghorn, A. D. 2005. Drug discovery from medicinal plants. *Life Sci.* 78:431–441.
9. Battaglin, W.A., Rice, C.K., Foazio, M.J., Salmons, S and Barry, R.X. 2008. 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.* 155, 281–307.
10. Prabu, D., Nappinnai, M., Ponnudurai, K and Prabu, K. 2008. Evaluation of wound healing potential of *Pisonia grandis* R.Br: A predinical study in wister rats. *Int. J. Lower Extrem. Wounds.*, 7, 21–27.
11. Chopra, R.N and Nayar, I.C and Chopra, S.L.R. 1986. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research.
12. Kakkar. P., B. Das and P.N. Visvanathan, 1984. A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.* 21: 130–132.
13. Sinha, K.A, 1972. Colorimetric assay of catalase *Anal. Biochem.*, 47:389–394.
14. Niehaus WG and B. Samuelson, 1968. Formation of malondialdehyde from phospholipid is chidonate during microsomal lipid peroxidation, *Eur. J. Biochem.* 6:126–130.
15. Rotruck, J.T., A.L. Pope and H.E. Gantex, 1973. Biochemical role as a component of glutathione peroxidase. *Science* 179:588–590.
16. Milton, T.S and Tsokos, J.O. 1983. Statistical methods in the biological and health science. McGraw – will. Internet Book comp. 381–405.
17. Bhattacharya, S. 2006. Prevention of cadmium induced lipid peroxidation, depletion of some antioxidative enzymes and glutathione by series of organoselenocyanates. *Environ. Toxicol. Pharmacol.* 22, 298–308.
18. Doyotte, A., Cossu, C., Jacquin, M.C., Babut Vasseur, M.P. 1997. Antioxidant enzymes glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive

- gland of the freshwater bivalve *Unio tumidus*. *Aquat. Toxicol.* 39, 93–110.
19. Dimitrova, M.S.T., Tsinova, V., Velcheva, V. 1994. Combined effect of zinc and lead on the hepatic superoxide dismutase and catalase system in carp (*Cyprinus carpio*). *Comp. Biochem. Physiol. C* 108, 43–46.
 20. Dorval. J. Leblond, V.S. Hontela, A. 2003. Oxidative stress and loss of cortisol secretion in the adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed to endosulfan, an organochlorine pesticide, *Aquat. Toxicol.* 63 (2003) 229–241.
 21. Pinho, G.L.L., Rosa, C.M., Yunes, J.S., Bianchini, A. Monserrat, J.M. 2002. InXuência da butionina sulfoximina na toxicidade da microcistina no hepatopâncreas de *Chasmagnatus granulata* (Decapoda, Grapsidae), Proceedings of V Meeting of Latino-American Society of *Environ, Toxicol. Chem.*, Victoria, ES Brasil, 283.
 22. Kono, Y and Fridovich, I. 1982. Superoxide radical inhibits catalase. *J. Biol. Chem.* 257, 51-57.
 23. Venkatesan, S., Pugazhendy, K., Prabakaran, S., Sangeetha, D., Meenambal, M and Vasantharaja, C. 2012. Chelating properties of Spirulina against the atrazine toxicity on the antioxidant enzymes activities in the fresh water fish *Cyprinus carpio* (Linn). *Int. J. Rec. Sci. Res.* 3, 3, 181-186
 24. Vasantharaja, C., Pugazhendy, K., Meenambal, M., Prabakaran, S., Venkatesan, S and Jayanthi, C. 2012. Protective role of *Cardiospermum halicacabum* against the cypermethrin toxicity in the oxidative stress in the fresh water fish *Cirrhinus mrigala* (Hamilton). *Int. J. Rec. Sci. Res.* 3, 7, 601-606.
 25. Atli, G., Alptekin, O., Tukel, S and Canlin, M. 2006. Response of catalase activity to Ag⁺, Cd⁺, Cr⁶⁺, Cu²⁺ and Zn²⁺ in five tissues of fresh water fish *Oreochromis niloticus*. *Comp. Biochem. Physiol. C* 143, 218–224.
 26. Dautremepuits C., Paris-Palacios S. Betoulle S and Morales Vernet, G. 2004. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan, *Comp Biochem Physiol C Toxicol Pharmacol*, 137, 325-333.
 27. Parthasarathy, R and Joseph, J. 2011. Studies on the hepatic antioxidant defense system in cyhalothrin-induced oxidative stress in fresh water tilapia (*Oreochromis mossambicus*). *Afr. J. Envi. Scien. Tech*, 5, 530-534.
 28. Parthiban, P and Muniyan, M. 2011. Effect of heavy metal nickel on the lipid peroxidation and antioxidant parameters in the liver tissue of *Cirrhinus mrigala*. *I. J. Dev. Res.* 1, 001-004.
 29. Chandravathy, V.M and Reddy, S.L.N. 1999. Effect of lead on antioxidant enzymes activities and Lipid peroxidation in old male mice. *Mus musculus*. *J. Environ. Biol.* 20, 103-106.
 30. Orun, I., Selamoglu, Talas, Z., Ozdemir, I., Alkan, A and Erdogan, K. 2008. Antioxidative role of selenium on some tissues of (Cd²⁺, Cr³⁺)-induced rainbow trout. *Ecotoxicology and Environmental Safety*, 71, 71-75.
 31. Sheeba, M.S and Asha, V.V. 2009. *Cardiospermum halicacabum* ethanol extract inhibits LPS induced COX-2, TNF- α and iNOS expression, which is mediated by NF- κ B regulation, in RAW 264.7 cells. *J. Ethnopharmacol.* 124, 39-44.
 32. Huang, M.H., Huang, S.S., Wang, B.S., Wu CH., Sheu, M.J., Hou, W.C., Lin, S.S., Huang, G.J. 2010. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds ex vivo and in vivo. *J. Ethnopharmacol.* 133, 743–750.
 33. Babu, V and Krishnakumari, K.C. 2005. Anti-Inflammatory and Antioxidant Compound, Rutin in *Cardiospermum halicacabum* Leaves. *Ancient Sci. Life.* 25, 47-49.