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Research Article

ANTIOXIDANT ENZYME ACTIVITY EFFECT OF SOLANAM VIRGINIANUM AGAINST LEAD ACETATE TOXICITY OF THE FRESH WATER FISH CYPRINUS CARPIO

Pichaimani N[1]., Pugazhendy K[2]*., Tamizhazhagan[2]., Sakthidasan V[2]., Jayanthi C[3] and Sasikala P[2]

[1]Department of Zoology, Bharathiar University, Coimbatore, Tamilnadu, India
[2]Department of Zoology, Annamalai University, Tamilnadu, India
[3]Department of Education, Annamalai University, Tamilnadu, India

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A B S T R A C T

Environmental pollution occurs when the environmental degradation crosses limit so that. It becomes lethal to living organisms. Pollution of water bodies forces them to acclimatize to various factors thus imposing a considerable amount of stress on their lives Assay of Superoxide dismutase (SOD)**,** Assay of Catalase (CAT)**,** Estimation of Reduced glutathione (GSH)**,** Estimation of Glutathione peroxidase (GPX)**,** Acetylcholine (ACh) and acetylcholinesterase activity (AChE)**,**Serum glutamate oxaloacetate transaminase (SGOT)**,** Serum glutamate pyruvate transaminase (SGPT) Liver and Kidney when a freshwater fish *Cyprinus carpio* exposed to lead acetate concentration as compared to the control group. *Solanam virginianum* act as alter the acidic and alkaline phosphatase activity in the studied organs three groups of newly hatched spotted *Cyprinus carpio* were held at three different temperatures in order to determine relationships between metabolic, digestive and growth response in rapidly developing larvae. Reduced glutathione showed a positive compensation (higher activity at a lower temperature) whereas glycolytic enzymes (pyruvate kinase and Lactate dehydrogenase) and aspartate aminotransferase (AST) showed a negative compensation (lower activity at a lower temperature).Hence, the results from present investigations may be useful in the assessment of environmental stress in the aquatic ecosystem.

INTRODUCTION

The increase in population, increased human activities, indiscriminate use of natural resources and dumping of wastes cause water pollution Vasantharaja *et al*.,2012. Pollution of aquatic ecosystem is posing a great challenge due to regular mixing of different industrial effluents agrochemicals like fertilizers, insecticides, detergents, pesticides and plant toxin in water (Pugazhendy *et al.,*2008) Water pollution is recognized globally as a potential threat to both human and other animal populations which interact with the aquatic environment (Vasantharaja *et al.,*2012) Environmental pollution is one of the most serious problem facing human beings and all other living things, today with the rapid growth in industrialization, population explosion and technological advancement, man's activities have tremendously affected the environment (Sharma *et al*., 2002). Heavy metals are extremely toxic and ubiquitous in natural environments and they occur in soil, surface water and plants, which readily mobilized by human activities such as mining and dumping of industrial waste in natural habitats such as forests, rivers, lakes and ocean (Larison *et al*., 2000). As a result, heavy metals pose a potential threat to terrestrial biota. They are known to cause profound reproductive loss in animals (Eeva and Lehikoinen, 2000).

The weathering of rocks, soil forms and increased use of metal containing fertilizers in agriculture could lead acetateto a continued rise of the concentration of metal pollutants in freshwater reservoirs as a result of water runoff, thereby representing the greatest hazard to human consumers of fish (Marr and Creaser, 1983; Gutenmann *et al*., 1988). Heavy metal constitute a serious type of pollution in fresh water and being stable compounds, they are not readily removed by oxidation, precipitation or other processes and affect the activity in recipient animal (Vasantharaja *et al*.,2012)

Lead acetate is one of the oldest known metals and also one of the most widespread toxicants, which poisoning remains a health threat (Hernberg, 2000). Lead architectonic is probably the most common form of heavy metal intoxication. It is well documented that, one of the most dangerous and insidious poisons. It continuous environmental and occupational exposure may contribute to renal, nervous, hepatic, hematological and reproductive disorders in man and animals

Corresponding author:* **Pugazhendy K
Department of Zoology, Annamalai University, Tamilnadu, India

(Flora *et al.,* 2006; El-Sayed and El-Neweshy, 2009; Ashry *et al.,* 2010). Conventional fish meal continues to be a primary protein source in formulated feeds. But due to its rising cost, uncertain availability and unreliable quality have led to the scientific search for alternative sources. The utility of Plant Protein Sources (PPS) to completely or partly replace the fish-meal is being researched meticulously (Krishnankutty and Sujatha, 2003).

Toxic effects of lead acetatealso include nephrotoxicity (Nolan and Shaikh, 1992), hepatotoxicity and cardiovascular damage (Gajawat *et al.,* 2006). The carcinogenic effect of lead acetate has been receiving increasing attention (IARC, 1993; Silbergeld *et al*., 2000). Lead acetate causes oxidative stress in the body by inducing the generation of free radicals thereby reducing the antioxidant defense system of the cells (Gurer and Ercal, 2000). The antioxidant defenses of the body come into play to nullify the generated ROS. The most important antioxidant found in cells is g*lutathione* (GSH). It is a tripeptide having sulfhydryl groups and is found in mammalian tissues in mill molar concentrations. It is an important antioxidant for quenching free radicals (Venkatesan *et al.,* 2012). Similarly, lead acetateinactivates enzymes like δ-amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase, which further depresses the glutathione levels Meenambal *et al.*, 2012.

A few other notable antioxidant enzymes that are rendered inactive by lead acetate include *superoxide dismutase* (SOD) and *catalase*(CAT). Decrease in SOD concentration reduces the disposal of superoxide radical, whereas reduction in CAT impairs scavenging of superoxide radical (O2–). Apart from targeting the sulfhydryl groups, lead acetate can also replace the zinc ion that serve as important co-factors for these antioxidant enzymes and inactivates (Flora *et al.,* 2007). Lead acetate stimulated oxidative stress is a state that involves the generation of free radicals beyond the permissible limits, deleting at the same time the antioxidant reserves and thus hampering the ability of the biological system to reverse the resulting effects. Free radicals generation starts a chain reaction that results in lipid peroxidation. It has been reported that those who take an antioxidant rich diet are at the forefront of reaping various health benefits. To boost antioxidant levels, food is always favored over supplements mainly because it contains thousands of antioxidants, in contrast to supplements, which are generally rich in a single or a few antioxidants. This review will now incorporate a detailed study of some natural antioxidants that have been investigated and put forth for the treatment of lead acetate induced oxidative stress (Pugazhendy *et al*.,2007).

MATERIALS AND METHODS

Collection and Maintenance of the Experimental Animals

Freshwater fish *Cyprinus capio* obtained of the Navarathna fish farm nearby Pinnaloor, Cuddalure district. Fishes were safely brought to the laboratory and transferred to the rectangular cement tanks (100×175cm) of 500 liters capacity containing chlorine free well water, fishes of the same size and weight were used irrespective of their sex for the experiments. The fish brought to the laboratory were acclimatized in the cement tank for a night before they were used for the experiment. The fish tanks (aquarium) were kept free from fungal infections by washing with potassium permanganate solution. The fishes were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in well aerated tap water. Prior to experimentation, they were acclimatized to experimental tanks for at least one week. Fishes measuring 15±5cm in length and 75±5g in weight were selected irrespective of their sex for the experiments. The fishes were fed daily on artificial libitum during acclimatization and tank water was renewed every day after feeding.

Selection of Plant

Whole plants of *Solanum virginianum* were cleaned and chopped into small pieces and dried under shade. The coarse powder was obtained by mechanical grinding. The powdered material (100g) was subjected to continuous hot extraction in soxhlet apparatus at a temperature of (60- 700 C) by using ethanol (95% v/v) as solvent6. After complete extraction, the extract was dried. The yield was about 5% w/w and it was stored at 4oC in desiccator.

Antioxidant enzymes

The tissues homogenate was prepared in Tris HCL buffer (pH 7.5), 1mL of the homogenate was treated with 2.0 mL of TBA-TCA-HCl reagent was added and mixed thoroughly. The mixture was kept in a boiling water bath for 15 min, after cooling, the tubes were centrifuged at 1000x g for 10 min and the supernatant was estimated.

Assay of Superoxide dismutase (SOD)

Superoxide dismutase was assayed by the method of Kakkar *et al.* (1984). The assay is based on the inhibition of the formation of NADH-phenazinemethosulphate, nitroblue tetrazolium formation. The reaction was initiated by the addition of NADH. After incubation for 90 sec, adding glacial acetic acid stops the reaction. The color developed at the end of the reaction was extracted into n-butanol layer and measured in a Spectrophotometer at 560nm.

Assay of Catalase (CAT)

Activity of Catalase was determined by the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. Chromic acetate formed was measured at 620nm. The catalase preparation was allowed to split H_2O_2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H_2O_2 as chromic acetate was determined colorimetrically. About 0.9 mL of phosphate buffer, 0.1 mL of superintendent 0.4 mL of hydrogen peroxide was added. The reaction was arrested after 60 seconds by adding 2.0 mL of dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 min, cooled and the color developed was read at 620nm. The specific activity was expressed as μmol of H2O2 consumed/ min/mg of protein.

Estimation of Reduced glutathione (GSH)

Reduced glutathione was estimated by the method of Ellman (1959). This method was based on the formation of 2-nitro-5-thiobenzoic acid (a yellow colour compound) when 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added to

compounds containing sulphydryl groups. 0.5 mL of supernatant was pipette out and precipitated with 2.0 mL of 5% TCA. 0.1 mL of supernatant was taken after centrifugation and 0.5 mL of Ellman's reagent and 3 mL of 0.3 M disodium hydrogen phosphate were added. The yellow color developed was read in a Spectrophotometer at 420nm with a blank containing 3.5 mL of buffer. The amount of glutathione was expressed as mg/g of tissues.

Estimation of Glutathione peroxidase (GPX)

The activity of GPx was measured by the method of Rotruck *et al.,* (1973). A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of GSH for a specified time period. Then the remaining GSH content was measured. 0.2 mL of tris buffer, 0.2 mL of EDTA, 0.1 mL of sodium azide, 0.5 mL of tissues of homogenate were added. the mixture, 0.2 mL of GSH followed by 0.1 mL of H2O2 was added. The contents were mixed well and incubated at 37 °C for 10 min, along with a control containing all reagents except homogenate. After 10 min, the reaction was arrested by the addition of 0.5 mL of 10% TCA. The tubes were centrifuged and the supernatant was assayed for GSH by the method of Ellman (1959). The activity was expressed as µg of GSH consumed/min/mg of protein

Acetylcholine (ACh) and acetylcholinesterase activity (AChE)

The brain tissue was isolated from the rates in the cold room and 2 per cent homogenate prepared in 0.25 m aliquot of the filtrate was taken and 0.5 mL of clear ferric chloride solution was added to each ferric chloride aliquot. The intensity of the colour developed was measured at 545mm in a double beam spectrophotometer against a reagent blank. The enzyme activity was expressed in µmole of acetylcholine hydrolysed/mg of protein/hr.

Serum glutamate oxaloacetate transaminase (SGOT)

A highly significant (104.40) elevation in serum glutamate oxaloacetate transaminase activity was observed in lead acetate group II intoxicated fish. *Solananum virginianum* alone supplementary group IV did not show any significant (4.30) alteration. However, the combined treatment of *Solananum virginianum* with lead acetateresults in gradually recovered (85.75) in SGOT activity (Table 1).

Serum glutamate pyruvate transaminase (SGPT)

A highly significant (109.31) elevation was observed in SGPT activity in lead acetate group II intoxicated fish with respect to control animals in I. Only *Solananum virginianum* supplementary group IV did not show any significant alteration (+1.04). The combined group III of lead acetate treated with *Solananum virginianum* supplementary group in significant decline

RESULTS

In the present investigation, *Cyprinus carpio* exposed to sublethal concentration of lead acetate, resulted increased in lipid peroxidation activity when compared with control (group 2). Increased percent changes are 8.64, 29.04, 55.80, 76.81, and 85.40 for the period of 120 hours respectively. Lead acetate along with *Solananum virginianum* exposure (groups 3) increased in LPO activity when compared to group 2. Lead acetate along with *Solananum virginianum* (group 3), the LPO levels are increased when compared with group 2. Increased percent changes are -50.44, -26.49, 13.06, -37.58 and -47.55 for 24 to 120 hours respectively. The supplemented feed exposed group the LPO levels are increased when compared with group 2 and 3, which is near the control values. The percent changes are 7.99, 8.01, 11.68, 11.30 and 8.95 for the period of 24 to 120 hours respectively. The present investigation LPO activity in liver tissue in group 2, 3 and 4 is statistically significant at 1% and 5% levels (Table 1).

The *Solananum virginianum* supplemented feed exposure group 4 also increased when compared with group 2 and group 1. The percent changes are 11.08, 13.80, 17.16, 18.70, and 28.52 for the period for 24, to 120 hours respectively. The increased and decreased value of LPO activity in the kidney tissues was statistically significant at 1% and 5% levels (Table 1). The *Solananum virginianum* supplemented groups are also decreased, when compared with group 2 and 3, which is near to control. The percent changes are 0.13, 0.59, 0.40, 3.19, and 5.68 for period the of 24 to 120 hours respectively. The recorded values of SOD content in the gill tissue for the four groups are statistically significant at 1% and 5% levels (Table 1).

The *Solananum virginianum* supplemented (group 4), the SOD levels are decreased, when compared with group 2 and 3, which is near to control. Decreased percent changes are 0.27, 0.96, o.47, 2.24 and 1.68 for the period of 24 to 120 hours respectively. The observed values SOD content in the liver tissues are statistically significant at 1% and 5% level (Table 1).The lead acetate along with *Solananum virginianum* exposure groups 3 the SOD activity in kidney tissue were significantly reduction when compared with group 2. The percent changes are 3.33, 0.66, 5.29, 13.96 and 23.31 during the period of 24, 48, 72, 96 and 120 hours respectively. The *Solananum virginianum* exposure (group 4) fish, the observed values of SOD activity are increased when compared with control. Decreased percent changes are 3.78, 1.24, 5.67, 2.12, and 2.19 for the period 24, 48, 72, 96 and 120 hours respectively. Recorded SOD activities in kidney tissue are statistically significant at 1% and 5% levels (Table 1).

The observed Catalase activity in the gill tissue of when exposed to sublethal concentration of lead acetate is (group 2) decreased when compared to group 1. The percent changes are 3.71, 7.66, 13.67, 20.78, and 28.12 for 24 to 120 hours respectively. Lead acetate along with *Solananum virginianum* exposed fish, the CAT activity is increased when compared to group 2. The increasing percent changes are -2.88, 1.31, -6.24, -14.17, and -24.32 for the period of 24 to 120 hours respectively. Lead acetate along with *Solananum virginianum* supplemented feed exposed (group 3) are increased when compared with group 2. Increased percent changes are 20.77, 15.28, 3.29, -18.23, and -34.97 for the period of 24 to 120 hours respectively. The supplemented feed exposed group 4 the CAT activity is near to control. The percent changes are 0.51, -3.54, 2.01, 0.33 and 2.29 for the period of 24 to 120 hours respectively. The increase and decreased CAT activity in liver tissues are statistically significant at 1% and 5% levels (Table 1).

Lead acetate along with *Solananum virginianum* exposure (group 3), gradually regained against lead acetate toxicity. The *Solananum virginianum* supplemented feed exposure fish (group 4) are increased in all other 3 groups. The percent

changes are 10.91, 11.87, 11.32, 7.90, and 10.10 for the period of 24 to 120 hours respectively. GSH activities in liver tissue for four groups are statistically significant at 1% and 5% levels (Table 1). Lead acetate along with *Solananum virginianum* exposure (group 3) is gradually regained when compared to group 2. Increased percent changes are 6.67, 6.40, 13.54, 27.59 and 31.83 for the period of 24 to 120 hours respectively. Supplemented feed exposure (group 4) the GSH activities are increased when compared with all other 3 groups. The overall percent changes are 8.21, 6.94, 4.70, 6.30 and 7.27 for the period of 24 to 120 hours respectively. The increased and decreased GSH activities in kidney are statistically significant at 1% and 5% levels. (Table 1).

Among all the tissues, liver shows a higher protein content which might be due to greater concentration of enzyme. Liver is the site of metabolism Pugazhendy *et al*., 2007. The liver plays an important role in the synthesis of proteins. Gill is the vital organs in fish which have direct contact with the medium through which pollutant enter into body (Muthulingam, 1999; Tamizhazhagan *et al*., 2017. who demonstrated a similar situation in *Clarius batrachus* exposed to decis. Pugazhendy *et al*., 2007 pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery.

Table 1 Variation of Superoxide dismutase (U min / mg protein) activity in the fresh water fish Cyprinus carpio exposed to leadacetate and Solananum virginianumfor120 hours

Organs	Hours Of Exposure					
	GROUPS	**24**	**48**	**72**	**96**	**120**
Gill	I Control	30.18 ± 1. 50	30.24 ± 1.51	30.65 ± 1.83	30.32 ± 1.81	30.42 ± 1.82
	II Lead acetate	38.64 ^{NS} ± 2.31 28.03	41.39 ^{NS} ± 2.48 36.87	48.26 * ± 2.41 57.45	49.36 * ± 2.46 62.79	52.14 ^{NS} ± 2.60 71.40
	Iii Lead acetate+ *Solananum virginianum*	35.65 ^{NS} ± 2.13 18.12 7.73	35.36 ^{NS} ± 1.76 16.93 14.54	36.25 ^{NS} ± 1.81 18.27 24.88	37.42 ^{NS} ± 1.87 23.41 24.18	38.12 ^{NS} ± 1.90 25.31 26.88
	IV *Solananum virginianum*	30.22 ** ± 1.81 0.13	30.42 ^{NS} ± 1.82 0.59	31.08 ^{NS} ± 1.86 1.40	31.29 ^{NS} ±1.56 3.19	32.15 ^{NS} ±1.92 5.68
Liver	I Control	48.12 ± 2.40	48.65 ± 2.43	48.92 ± 2.44	49.02 ± 2.45	49.32 ± 2.46
	II Lead acetate	50.21 ^{NS} ± 2.51 4.34	53.26 ^{NS} ± 2.66 9.47	56.24 ^{NS} ± 3.37 14.96	60.27 ^{NS} ± 3.01 22.94	62.18 ^{NS} ± 3.73 26.07
	III Lead acetate+ *Solananum virginianum*	50.15 ^{NS} ± 2.50 4.21 0.11	50.65 ^{NS} ± 2.53 4.11 4.90	51.32 ^{NS} ± 2.56 4.90 8.74	52.33 ^{NS} ± 3.13 6.75 13.17	53 24 ^{NS} ± 2.66 7.94 14.37
	IV *Solananum virginianum*	48.25 ^{NS} ± 2.41 0.27	49.12 ^{NS} ± 2.45 0.96	49.15 ^{NS} ± 2.45 0.47	50.12 ^{NS} ± 3.00 2.24	50.15 ^{NS} ± 3.00 1.68
Kidney	I Control	34.12 ± 1.70	34.65 ± 2.07	34.39 ± 1.71	35.36 ± 1.76	35.49 ± 177
	Ii Lead acetate	37.18 ^{NS} ± 1.85 8.96	39.39 ^{NS} ± 2.36 13.67	42.65 ^{NS} ± 2.55 24.01	48.39 * ± 2.41 36.84	56.32 *± 3.37 58.69
	III Lead acetate+ *Solananum virginianum*	38.42 ^{NS} ±1.92 12.60 -3.33	39.65 ^{NS} ± 1.98 14.43 -0.66	40.39 ^{NS} ± 2.01 17.44 5.29	41.63 ^{NS} ± 2.49 17.73 13.96	43.19 ^{NS} ± 2.15 21.69 23.31
	+ *Solananum virginianum*	35.41 ^{NS} ± 1.77 3.78	35.08 ^{NS} ± 2.10 1.24	39.34 ^{NS} ± 2.18 5.67	36.11 ^{NS} ± 1.80 2. 12	36 .27 ^{NS} ± 2.17 2.19

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

DISCUSSION

The *Solananum virginianum* also showed significant anti-inflammatory action. Compounds like bioflavinoid are reported to produce anti-inflammatory action by decreasing capillary permeability. Extracts tested might contain flavonoids and phenols. Earlier report on the anti-inflammatory activity of the constituents of *Solananum virginianum* flowers supports our results (Sethuraman, 1984). *Solananum virginianum* may have ability to scavenge free radical induced by lead and enhancing repairing mechanism. Regain trends occur in all parameters in the *Cyprinus carpio* against lead shows the presence vital phytochemical compounds in *Solananum virginianum*.

In the present investigation, the effect of sublethal concentration of lead (group 2) on the level of protein, amino acid, glucose, glycogen and enzymological parameters like SOD, CAT. $GP_{X,}$ GSH, GST, LPO, ACh and AChE level in different tissues of *Cyprinus carpio* fish has been studied. It shows a decrease in protein content and an increase in amino acid content of gill, liver and kidney from 24 to 120 hours. Protein depletion in tissues may constitute a physiological mechanism and may play a role of compensatory mechanism under lead stress, to provide intermediates to the Kreb's cycle. It has also been reported that this trend of proteins was to enhance osmolality to compensate osmoregulatory problems encountered due to the leakage of ions and other essential molecules during pyrethroid toxicity (Rafat Yasmees, 1986). Depletion of protein fraction in various tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Decreased total protein level was observed in the muscle and liver tissues of the freshwater teleost fish *C. fasciatus* exposed to sub-lethal doses of malathion and carbaryl pesticide Pugazhendy *et al*., 2009. In the present study, compared to group 2 and group 3, a significant decrease in protein content is observed in all tissues after the period of 120 hours. *Solananum virginianum* supplementary alone exposed fish (group 4) shows more towards normalcy in protein content in all tissues compared to control (group 1).

In the present observation, lead exposed (group 2) fish shows a significant increase in the free amino acid level of gill, liver and kidney tissues at 24, 48, 72, 96 and 120 hours of exposure periods. The increase in free amino acid level suggests that tissues damage probably due to the increased proteolytic activity under toxic stress. However, the elevated levels of free amino acid can be utilized for energy production by feeding them into the TCA cycle through aminotransferase reaction. The increase in the levels of free amino acid can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis. A third possibility for increased amino acid level might be their increase due to transamination and deamination of keto acid (Pugazhendy *et al*., 2007; Jayantha *et al.,* 1983).

An increase level of total free amino acids has been found in liver tissues of total free amino acids has been found in liver tissues of *Mystus vittatus* exposed to zinc sulphate (Ramesh Kumar,1989). An increase in amino acid content in liver tissue might be due to enhanced proteolysis and decreased utilization of amino acid for protein synthesis. Increased free amino acids levels have been reported in liver, muscle and brain tissue of *Cyprinus carriageway* to sublethal concentration of mercury (Sivaramakrishanan and Radhakrishniah, 1998). In the present investigation, it is suggested that the observed reduction in protein level and an increase in amino acid level may be the direct consequence of stress imposed by the insecticide, lead. Similarly, enhanced level of total free amino acid is found in the tissues of muscle and liver of fresh water teleost fish *Colisa fasciatus* exposed to lead at different exposure period (Shailendra *et al.,* 2010).

In the present study, *Cyprinus carpio* fish exposed to sublethal concentration of lead (group 2) shows a significant reduction in the glycogen level and increase glucose level of gill, liver and kidney tissues from 24 to 120 hours of exposure periods. Several investigation has been made on the effect of glycogen and glucose levels of fish (Karuppasamy, 2005; Patil and Dhande, 2000; Jayachandran, 2010). Liver and muscle are two active sites where storage and metabolism of glycogen take place. In the present study depletion of glycogen content in the tissues can be attributed to the toxic stress of lead and energy requirement due to the detoxification process. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands. In fish exposed to toxicant through glycolysis or Hexose Monophosphate pathway. It is assumed that the decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. Decrease in liver and muscle glycogen levels is in corroboration with the reports of earlier workers (Bedii and Kenan, 2005; Dubale and Punita Shah, 1981; Sastry and Subhadra, 1984)

Glucose serves as an immediate and major metabolic fuel. Increased glucose concentrations in white muscle of *Oreochromis niloticus* after lead exposure to sublethal concentration reported by Almedia *et al.* (2001). The increase in the glucose level of the tissue while decrement in tissue glycogen in exposed fish makes it clear that the glycogen reserves are being used to meet the stress caused, increase in serum glucose levels in fish under stress was reported by Bedii and Kenan (2005), Chowdhury *et al.* (2004). This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis (Almeida *et al.,* 2001). Superoxide dismutase (SOD) and catalase (CAT) activity was observed at 24, 48, 72, 96 and 120 hours. SOD activity was decreased gradually in the treated (group 2) compared to control (group 1). Recovery (group 3) SOD activity was gradually regained in liver, kidney and gill. Supplementary feed alone (group 4) observed slightly increased statistically insignificant. The enzyme SOD is known to provide cytoprotection against free radical induced damage by converting superoxide radicals (o_2^-) generated in peroxisomes and mitochondria to hydrogen peroxides. Treated group recored decrease the SOD level due to toxic effect of lead. Defense mechanism gradually failed for protection. The recovery group was increasing the SOD activity due to plant pellet supplementary feed and animal develops the defense mechanism.

The combined effect of lead and zinc caused changes in the liver SOD and CAT detoxification system of carp (Dimitrova *et al.,* 1994). In fish, modulation of antioxitant system in liver by endosulfan and modulatory effect of pre exposure to copper on the endosulfan induced oxidative stress in vivo have been reported (Pandy *et al.,* 2001; Buet *et al* (2005) have observed that heavy metals in the response of SOD and CAT have been significantly reduced by suggesting a possible deterioration of the protective defense system of fish. Activity of CAT was decreased significantly in all the organs during the lead treated period. the similar observation was made by Sayeed *et al.* (2003); Tripathi and Verma, (2004). Decrease in CAT activity is followed by an inhibition of the activity of enzyme. SOD has been reported by Fatima and Ahmad (2005). Recovery group is gradually increased in CAT activity at the end of treatment due to plant pellet feed.where as supplementary alone treated group 4 CAT increased slightly. It is equal to normal fish (group 1).

In the present study, LPO level of the fish organs increased mainly in the gill. Liver followed by kidney. Gill was directly exposed to the toxin, so that gill TBARS increased than liver and kidney. Lead induced oxidative stress in all the tissues. Lipid per oxidation has been extensively used as a marker of oxidative stress (Hugett *et.al*., 1992). Basically, the main mechanism of the toxic effect of pesticides involves the generation of a high level of free radicals, and thereby the damage of tissues and organs throughout this process (Yousef *et al.,* 2003; Manna *et al.,* 2004). These radicals attack the cell membrane and lead to destabilization and disintegration of cell membranes a result of lipid per oxidation (Stajn *et al.,* 1997). TBARS is a major oxidation product of per oxidized polyunsaturated fatty acids, and increased TBARS content is an important indicator of lipid per oxidation (Celik and Suzek, 2009).

GP_X level is gradually increased in the treated group during the exposure period. Recovery group GP_X levels are gradually decreased. Supplementary feed group shows slightly increased statistically insignificant. GP_X enzymes play a critical role in the defense against oxidative stress. The activity of GPx can be induced by xenobiotics, and detoxification of peroxides can be achieved by this induction (Hamed *et al.,* 1999). The biological function of GSH-Px is to reduce H_2O_2 and lipid hydro peroxides (Verma *et al.*, 2007). Decreased activities of antioxidant enzyme SOD, CAT and increasing of GP_X in the all tissues of lead treated fish, which indicated the failure of

antioxidant defense system to overcome the influx of ROS induced by lead.

The second line of defense includes the non-enzymatic radical scavenger GSH, which scavenges residual free radicals resulting from oxidative metabolism and escaping decomposition by the antioxidant enzymes (Leve de and Kaplowitz, 1991). During the metabolic action of GSH, its sulfhydryl group becomes oxidized resulting with the formation of the corresponding disulfide compound, GSH. The decrease in GSH levels could be due to the presence of free radicals produced by insecticides. These effects have been previously observed by other authors in vitro and in vivo (Banerjee *et al.,* 1999; Maran *et al.,* 2009; Thompson *et al.,* 2002). In addition, GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme GST Glutathione and other thiol containing proteins plays a crucial key role in cellular defense against pesticides toxicity.

In the present study of sublethal concentration of lead inhibiting acetylcholinesterase (AChE) activity in the group 2. Because lead is a neurotoxic substance, it interferes with neurotransmitter activity. Inhibition of acetylcholinesterase preventing enzyme substrate complex formation finally acetylcholine (Ach) not reduced into acetate and choline. So, nerve cell signaling process is disturbed normal function is altered. Vittozzi and Angelis (1991) have been reported as inhibition of acetylcholinesterase (AChE) that is responsible for the degradation of acetylcholine will result in the excessive stimulation of cholinergic nerves. This will result in tumors, convulsions and finally death of the aquatic organism. The inhibition of acetylcholinesterase (AChE) activity in fish can be dangerous since it will affect feeding capability, swimming activity, identification, avoidance of predators and spatial orientation of the species (Adedeji, *et al.,* 2008).

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Reference

1. Binu Kumari, S., A. Kavitha Kirubavathy and Rajammal Thirumalnesan., 2006.Suitability and water quality criteria of an open drainage municipal sewage water at Coimbatore, used for irrigation. *J. Environ. Biol.,* 27, 709-712.
2. Svensson, C.J., Jenkins, S.R., Hawkins, S.J., Myers, A.A., Range, P., Paula, J., O'Riordan, R.M. & Åberg, P.,2004.Models of open populations with density dependent recruitment in stochastic environments: the relative importance of recruitment and survival in populations of Semibalanus balanoides. *Marine Ecology Progress Series*, 275, 185-197.
3. Sharma T.R. Sharma, R.S. Chauhan, B.M. Singh, R. Paul, V. Sagar, R. Rathore.,2002. RAPD and pathotype analysis of *Magnaporthe grisea* population from North-western Himalayan region of India .*J Phytopathol,* 150 pp. 649-656
4. Larison JR Likens GE, Fitzpatrick JW, Crock JG., 2000. Cadmium toxicity among wildlife in the Colorado Rocky Mountains. *Nature* ,406:181-3
5. Eeva, T., Lehikoinen, E. & Pohjalainen, T., 1997. Pollution related variation in food supply and breeding success in two hole-nesting passerines. Ecology 78, 1120-1131
6. Marr, I.L. and M.S.Creaser.,1983. *Environmental Chemical Analysis.* Blackie and Sons Publisher Ltd, 1st Edition, pp.104.
7. Gutenmann WH, Bache CA, McCahan JB, Lisk DJ. , 1998. Heavy metals and chlorinated hydrocarbons in marine fish products. *Nutrition Reports International,* 38(6), 1157- 1161.
8. Nammaluvar, P., 1985. Heavy metal pollution in Adyar Eastraury, India Proc symp Assxes *Environ. Poll.* 235-338.
9. Chen JF, Huang Z, Ma J, Zhu J, Moratalla R, Standaert D, Moskowitz MA, Fink JS, Schwarzschild MA.,1999. A2A adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. *J Neurosci*, 19:9192-9200.
10. Cheng, Z. J ; Hardy, R. W., 2003. Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* 218 (1-4): 501-514
11. Hernberg, S., 2000. Lead poisoning in a historical perspective. *Am. J. Ind. Med*. 38, 244-254.
12. Flora SJS, Flora G, Saxena G., 2006. Environmental occurrence, health effects and management of lead poisoning. In: José S. C, José S, editors. Lead. *Amsterdam: Elsevier Science B.*V,pp. 158-228
13. El-Sayed YS, El-Neweshy MS., 2009. Impact of lead toxicity on male rat reproduction at ''hormonal and histopathological levels''. *Toxicol Lett* ,189(Suppl.1): S219-20
14. Trivedi P.R., and Gurudeepraj., 1992. Environmental management of fresh waterecology (Ist Ed n). Akashdeep Publishing house, New Delhi
15. Sheffield, J., A. D. Ziegler, E. F. Wood, and Y. Chen., 2004.Correction of the high-latitude rain day anomaly in the NCEP– NCAR reanalysis for land surface hydrological modeling. *J. Climate,* 17, 3814-3828.
16. Damek-Poprawa, M., Jang, J. Y., Volgina, A., Korostoff, J., and DiRienzo, J. M. ,2012. Localization of Aggregatibacter actinomycetemcomitans cytolethal distending toxin subunits during intoxication of live cells. Infect. *Immun.* 80, 2761-2770. doi: 10.1128/IAI.00385-12.
17. Daggett, V., Li, A., and Fersht, A.R.,1998. Combined molecular dynamics and 4-value analysis of structure-reactivity relationships in the transition state and unfolding pathway of barnase: structural basis of Hammond and anti-Hammond effects. *J. Am. Chem. Soc*. 120, 12740-12754.
18. Bonde, J.P., Joffe, M., Apostoli, P., Dale, A., Kiss, P., Spano`, M., Caruso, F., Giwercman, A., Bisanti, L., Porru, S. *et al*., 2002.Sperm count and chromatin in men exposed to inorganic lead: lowest adverse effect levels. Occup. *Environ. Med*., 59, 234-242.
19. Tamizhazhagan V. Pugazhendy K., Sakthidasan V. Jayanthi C. Barbara Sawicka. Shuuduv Gerlee. Ramarajan K., Manikandan P, 2017.The Toxicity Effect of Pesticide Monocrotophos 36% E.C on the Enzyme

Activity Changes in Liver and Muscles of *Labeo Rohita* (Hamilton, 1882) *International Journal of Pharma Sciences and Researc*h ,8 560-67.

20. Areola, O. O., Williams-Johnson M., Jadhav, A. L.1999. Relationship between lead accumulation in blood and soft tissues of rats subchronically exposed to low levels of lead. *Toxic Substance Mechanism* 18, 1-13.
21. Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO *et al*.,1999. Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. *Global Biogeochem Cycles* 13:623–645
22. Wilbur, R. B., 2009. Effects of varying rate of signing on ASL manual signs and nonmanual markers. *Lang. Speech* 52, 245-285.
23. Gajawat S, Sancheti G, Goyal PK.., 2005. Vitamin C against concomitant exposure to heavy metal and radiation: A study on variations in hepatic cellular counts. *Asian J Exp Sci* 19(2): 53-58.
24. Tamizhazhagan.V, Pugazhendy.K, Sakthidasan.V, Jayanthi.C., 2016.The Toxicity Effect of monocrotophos 36 E.c% on the histological Changes in Gill of *Labeo Rohita* (Hamilton, 1882) *International Journal for Innovative Research in Multidisciplinary Field* ,2, 11, 435-439.
25. Tamizhazhagan.V Pugazhendy .K .,2016. The Toxicity Effect of Monocrotophos 36% E.C on The Biochemical Changes *Labeo Rohita* (Hamilton, 1882) *International Journal for Scientific Research & Development* 3, 11, 802-808
26. Nolan, C.V., Shaikh, Z.A., 1992. Lead nephrotoxicity and associated disorders: biochemical mechanisms. Toxicology 73, 127-146.
27. Silbergeld EK, Sacci JB Jr, Azad AF., 2000. Mercury exposure and murine response to Plasmodium yoelii infection and immunization. *Immunopharmacol Immunotoxicol*, 22:685-695.
28. Vasantharaja, C., Pugazhendy, K., Meenambal M. Vengatesan S, and, Prabakaran S, 2012.Protective role of *Cardiiospermum halicacabum* against the cypermethrin effect on the haematological parameters of *Cirrhinus mrigala (Hamilton)*. *International Journal of Toxicology and Applied Pharmacology,* 2(2), 12-17.
29. Pugazhendy, K, Susiladevi, M., Jayanthi, C., Jayachandaran, K., and Meenkshi, V., 2008. Impact of Industrial Pollutants on the Gill of Mullet Fish, *Mugil Cephalus* in the Uppanar Estuary, (Sipcot) Cuddalore on the South East Coast of India, 27 (2):231-236.
30. Vasantharaja C, Pugazhendy K, and Meenambal M., 2012, Protective role of *Cardiospermum halicacabum* against the cypermethrin toxicity on the selected biochemical indices in serum activity in *Cirrhinus mrigala* (Hemilton). *Journal of Pharmacy Research*, 5(5), 2595-2598.
31. Vasantharaja C, Pugazhendy K., Vengatesan S, Meenambal M, Prabakaran S, and Jayachandran K., 2012. Acute Toxicity of Cypermethrin and its Impact on biochemical Alteration in the Fresh Water Fish *Cirrhinus mrigala* (Hamilton) and Protective Effect of *Cadiospermum helicacabum*.(Linn). *International Journal of Pharmaceutical &Biological Archives*, 3(1):146-152.
32. Tamizhazhagan V Pugazhendy K, Sakthidasan V, Jayanthi C, Barbara Sawicka, Agevi Humphrey, Vasanth pandiyan C, Kasinathan M, Ramarajan K, Baranitharan M.,2017. Study of Toxic Effect of Monocrotophos 36% e.c on the Biochemical Changes in Fresh Water Fish *Catla catla* (Hamilton, 1882). *International Journal of Chemical & Pharmaceutical Analysis,*4(3), 1-8.
33. 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). *International Journal of Recent Scientific Research*. 3, 3, 181-186.
34. Meenambal M, Pugazhendy K, Vasantharaja C, and Venkatesan S., 2012. Ameliorative property of *Delonix elata* supplementary feed against Cypermethrin induced serum biochemical changes in fresh water fish *Cprinus carpio* (Linn). *Journal of Pharmacy Research,* 5 (5), 2489-2492.
35. Pugazhendy K, Jayanthi C, Susiladevi M, Jayachandran K., 2007. Effect of Lead Intoxication on Acetyl Cholinesterase Activity in Fresh water Fish *Cyprinus Carpio* (Linn). Fingerlings. *Biochem Cell. Arch,* 7, 2, 309-312.
36. Pugazhendy K, Jayachandaran K, Divya Velayuthan Jayanthi C., 2007. Impact of Atrazine on Acetylcholinesterase Activity in Fresh Water Fish *Labeo rohita* (Linn). Fingerlings. *Environment& Ecology,* 25, (4):1391-1393.
37. Pugazhendy K., Meenakshi, V., Chandran, M., and Indra, N.,1999. Enzymatic changes due to chronic effect of mercuric chloride in the fresh water fish *Cyprinus carpio* (Linn) Fingerlings. *Sustainable Environment,* (ed. N. Sukumaran):209-214.
38. Pugazhendy K, Jayanthi C, and Kanaga K, 2005. Effect of Lead Intoxication Total Fresh Aminoacid and Glutamate Dehydrogenase In Liver and Muscle Tissues of a Fresh Water Fish, *Cyprinus carpio* (Linn), *J. Exp. Zool. India*,8, 2, 361-364.

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