



THERAPEUTIC EFFICACY OF ALOEVERA AGAINST THE ANTIOXIDANT ENZYME EFFECT OF CYPERMETHRIN IN THE FRESH WATER FISH *CYPRINUS CARPIO*

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ABSTRACT

Current scenario 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. Phosphatase is known to be sensitive to metal exposures and can be used to predict metal toxicity. The acid phosphatase (ACP) and alkaline phosphatase (ALP), TBRAS enzyme activity, GPx, Alkalain phosphatase, AchE brought a decrease in acid and alkaline phosphatase (ACP and ALP) in Liver and Kidney when a freshwater *Cyprinus carpio* exposed to Cypermethrin concentration as compared to the control group. Alovera 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). Citrate synthase was not affected by growth rate, indicating that the level of aerobic capacity was adequate in sustaining the high energy needs associated with rapid growth early in the life of the spotted *Cyprinus carpio*. Hence, the results from present investigations may be useful in the assessment of environmental stress in the aquatic ecosystem to usefull for modern researchers, and environmentalist provide signals.

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INTRODUCTION

Aquatic ecosystems that run through agricultural areas have a high probability of being contaminated by runoff and ground water is reached by a variety of chemicals. Highly effective pesticides are used tremendously, which are entering the aquatic environment bring multiple changes in organism by altering the growth rate, nutritional value, behavioral pattern, etc (Pugazhendy *et al.*, 2008). A major part of the world food is being supplied from fish source, so it is essential to secure the health of fishes (Tripathi *et al.*, 2002). Residues of toxic chemical found in water, sediment, fish and other aquatic biota can pose a risk to organisms to predators and human being. Pesticides at high concentration are known to increase much visible pollution also recognized globally as a potential threat to both human and other animal population, which interact with the aquatic environment (Tamizhazhagan *et al.*, 2017).

Increased use of chemical pesticides results in the excess of toxic chemicals. Mainly ensuring the aquatic ecosystem. More ever pesticides exposure causes severe alterations in the tissue biochemistry of fishes (Pugazhendy *et al.*, 1999). In general the toxic effects will be more when two or more toxicants act

together in a synergistic manner (Pugazhendy *et al.*, 2007) pesticides get into water via different routes. When pesticides are applied on fields, gardens, parks and other places, a remarkable amount of the chemicals, end up as runoff. This runoff moves in streams, rivers and lakes. Similarly, when pesticides are applied on lawns in urban and suburban areas. Rain washes some of the pesticides into streets with gutters from where the pesticide contaminated water goes through drains and pipes and eventually flows into nearby creeks and rivers (Pugazhendy, 2007). Some of the pesticides also end up in groundwater systems by leaching down through the soil. Small amounts also vaporize into the atmosphere and then later fall back to the land as precipitation. As a result of all these pathways, pesticides are widely found in rivers, streams, lakes and even in drinking water (Kalavathy *et al.*, 2001),

The pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and alter the physico-chemical properties of water, (Bhalchandra *et al.*, 2001). In recent years, synthetic pyrethroids have been developed for major uses in agriculture and public health purposes. The current commercial products were evolved from the natural pyrethrins, which possess high insectical potency low mammalian toxicity and very short persistence. These are highly toxic to fish and some aquatic invertebrates (Coats *et al.*, 1989).

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Fishes are important sources of nation's diet, highly nutrients, easily digestible and its nutritional value depends on the biochemical composition (Meenambal *et al.*, 2012). Fish is extremely sensitive bioindicator of aquatic pollution and being preferred as a test species in toxicological screening of water (Venkatesan, 2012). Investigation on toxicity makes it possible to evaluate the effects of sublethal concentration on growth, behavior, physiology and biology of organisms, to determent their adaptation capabilities and to forecast possible consequences to toxic effect (Tamizhazhagan *et al.*, 2017). Short term test is useful for routine monitoring for exploratory test and for, estimating effluent discharge. These tests determine LC₅₀ which is a quick estimate of different toxicants and assessment of a toxicant to estimate toxicant concentration to be used in the intermediate and long term test. The intermediate test is conducted when a toxicity test is dealt with a long life cycle organism or longer life cycle stage which requires additional time for determination of LC₅₀ (Mohamed and Gad, 2008).

The antioxidant GSH is tripeptide- γ -glutamyl-cystenine-glycine or reduced glutathione. In living organisms GSH plays an important role in normal cell function. Extensive studies have shown that the GSH is involved in various biological reactions such as the detoxification of hydrogen peroxide, amino acid transport and scavenging of free radicals (Kumar *et al.*, 2000). Reduced glutathione (GSH) plays an important role in the regulation of blood pressure by improving the endothelial function by increasing the bioavailability of nitric oxide which acts as an antioxidant (Meenambal *et al.*, 2012). The ACP and ALP are a lysosome enzyme and the rise in its activity is probably related to the cellular damage. It is difficult, however, to relate the decrease in ACP activity with necrosis. Increase in acid phosphatase and alkaline phosphatase activities can be interpreted as a shift, which emphasis on energy break down pathway from normal ATPase system which includes phosphorylation (Kalavathy *et al.*, 2001),

The acetylcholinesterase enzyme (AChE) activity is frequently a variation after exposure to pesticides. Besides this, some authors describe AChE activation after herbicide exposure, but the activation effects are little known (Vasantharaja *et al.*, 2012; Pugazhendy *et al.*, 2008). The alteration in acetyl cholinesterase activity affects animals due to the central role of this enzyme in regulating the proper levels of the neurotransmitter acetylcholine in the central nervous system, neuromuscular junction and the sympathetic synapses. AChE inhibition could interact growth, survival, feeding and reproductive behaviors of fish exposed to different pollutants (Dutta and Arends, 2003).

MATERIALS AND METHODS

Collection and preparation of *Aloe vera*

The dried *Aloe vera* powder was collected from Thookunampakkam village near to Pondicherry. The *Aloe vera* powder was kept in carefully.

Collection and Maintenance of the experimental animal

The freshwater fish *Cyprinus carpio* were collected from the fish farm located in Pinnalur village, near Vadalur, Cuddalore district. The fishes were brought to the laboratory and transferred to the rectangular fiber glass tanks (100 X 175 cm) of 500 liters capacity containing chlorine free aerated well

water, fishes of the same size and weight were used irrespective of their sex for the experiments.

Estimation of tissues thiobarbituric acid reactive substances (TBARS)

The tissue homogenate was prepared in Tris HCl buffer (pH 7.5), 1 mL of the tissue homogenate, was treated with 2 mL of TBA-TCA_HCl reagent and mixed thoroughly. The mixture was kept in a boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant was taken for measurement. A series of standard solutions in the range 2-10 n mole concentrations was treated in a similar manner. The absorbance was at 535nm against the reagent blanks. Values were expressed as nmole/mg protein.

Assay of superoxide dismutase (SOD)

0.5 mL tissue homogenate was diluted to 1 mL with water. Then 2.5 mL of ethanol and 1.5 mL chloroform (all reagents chilled) were added. This mixture was shaken for one minute at 4⁰ C and then centrifuged. The enzyme activity in the supernatant was determined.

Assay of Catalase (CAT)

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

Estimation of glutathione peroxide (GPx)

GSH-Px activity was measured in the PMS by the method by Lawrence and Burk (1976). The reaction measured the rate of GSH oxidation by H₂O₂ 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 μ /min/ mg protein.

Estimation of acid and alkaline phosphatase

Acid and alkaline phosphatases were assayed following the procedure adopted by Tenniswood *et al.* (1976). *p*-Nitrophenyl phosphate was colorless in solution but upon hydrolysis the phosphate group liberated *p*-nitrophenyl which was highly colored in an alkaline solution. The rate of hydrolysis of *p*-nitrophenyl phosphate was proportional to the enzyme present in the tissue.

Estimation of tissue acid phosphatase

100mg of wet tissue from Gill, liver, kidney and muscle was weighted and homogenized in a glass homogenizer using 10 mL distilled water. To each test tube 0.5 mL of substrate solution (*p*-Nitrophenyl-phosphate) and 0.5 mL of 0.1 N citrate buffers was added. Test tubes with the above solution were kept in a water bath and maintained at 37⁰C for 5 min then; 1 mL O the tissue extracts was added to the test tube. The test tube with the tissue extracts was then kept in a water bath at 37⁰ C for 30 min. After completion of 30 min the reaction was arrested in the extract by adding 3.8 mL of 0.1 N sodium hydroxide. The colour formed at the end was read at 415nm grating spectrophotometer and the values expressed in μ mole/min/mg protein.

Estimation of tissue alkaline phosphatase

100mg of wet tissues from gill, liver, kidney and were weighted and homogenized using 10 mL distilled water. To each test tube, 0.5 mL of substrate solution (*p*-Nitrophenylphosphate) and 0.5 mL of glycine buffer was added. The test tube with the above solutions was then kept in a water bath and maintained at 37⁰ C for 30 min. After completion of 30 min the reaction was arrested in the extract by adding 10 mL of 0.02 N sodium hydroxide. The color formed at the end was read at 415nm in Grating Spectrophotometer (Cecil model CE-373). The values were expressed in μ mole/min/mg protein.

Estimation of acetylcholine (ACh) and acetylcholinesterase activity (AChE)

The brain tissue was isolated from the fish 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 color developed was measured at 454nm in a double beam spectrophotometer against a reagent blank. The enzyme activity was expressed in μ mole of acetylcholine hydrolyzed/mg of protein / hr.

RESULTS

The observed values of SOD activity in the fish exposed to cypermethrin (group 2) were increased when compared with control group 1. The percent increases are 28.03, 36.87, 57.45, 62.79, and 71.40, for the period of 24 to 120 hours respectively. While in fish exposed to cypermethrin along with *Aloe vera* exposed groups are decreased compared to group 2. The percent changes are 7.73, 14.54, 24.88, 24.18 and 26.88 for the period of 24 to 120 hours respectively. The *Aloe vera* 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, 1.40, 3.19, and 5.68 for a period 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.

In the present investigation *Cyprinus carpio* administered with cypermethrin (Group 2) shows an increased the activities when compared to control. The overall increased percent changes are 4.34, 9.47, 14.96, 22.94, and 26.07 for the period of 24 to 120 hours respectively. The cypermethrin along with *Aloe vera* exposure group 3, the recorded value of SOD content in the liver tissue was decreased, the percent decrease is 0.11, 4.90, 8.74, 13.17 and 14.37 for the period of 24 to 120 hours respectively. The *Aloe vera* supplemented (Group 4). SOD levels are decreased, when compared with group 2 and 3, which is near to control. Decreased percent changes are 0.27, 0.96, 0.47, 2.24 and 1.68 for the period of 24 to 120 hours respectively. The observed values SOD content in the liver tissues is statistically significant at 1% and 5% levels.

The observed Catalase activity in the gill tissue of *Cyprinus carpio* when exposed to sublethal concentration of cypermethrin 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. The cypermethrin along with *Aloe vera* exposed fish, the CAT activity was 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. The supplemented feed exposed groups 4.

CAT activity is increased when compared to control. The percent changes are 7.29, 7.25, 6.28, 8.76 and 7.91 for 24, 48, 72, 96 and 120 hours respectively. Recorded CAT activities of *Cyprinus carpio* in gill tissue for the four groups are statistically significant at 1% and 5% levels.

In the present investigation, *Cyprinus carpio* exposed to sublethal concentration of cypermethrin. Resulted increased in lipid peroxidation activity when compared with control (group 2). The increased percent changes are 8.64, 29.04, 55.80, 76.81, and 85.40 for the period of 120 hours respectively. The cypermethrin along with *Aloe vera* exposure (groups 3) increased in LPO activity when compared to group 2. The percent changes are -54.34, -20.57, -10.29, -27.73 and -36.84 up to the period 24 to 120 hours respectively. The *Aloe vera* supplemented fish shows increased when compared with group 2 and 3, near to control values. The percent changes are 7.99, 12.90, 14.06, 18.93, and 19.99 for the period of 24 to 120 hours respectively. The increased and increased levels of lipid peroxidation activity in gill tissues are statistically significant at 1% and 5% levels.

In the present investigation *Cyprinus carpio* exposed to cypermethrin (group 2), shows an increased in GSH activity when compared to control. The percent changes are -119.81, -140.27, -208.21, -229.35 and -347.59 for 24 to 120 hours respectively. The cypermethrin along with *Aloe vera* exposure (Group 3), gradually regained against cypermethrin toxicity. The recovery percent changes are -1.54, 16.57, 29.74, 22.72 and 32.05 for the period of 24 to 120 hours respectively. The percent changes of group 4 *Aloe vera* supplemented feed group shows an increased when compared with Group 1 control. The percent changes are 55.18, 61.11, 63.92, 91.29 and 103.05 for the period of 24 to 120 hours respectively. Increased and decreased levels GSH activity in gill tissue is statistically significant at 1% and 5% levels.

The administration of cypermethrin (Group 2) shows the increasing the GPx activity in gill when compared to control. The percent changes are 7.05, 30.20, 40.16, 51.65 and 58.06 for the period of 24 to 120 hours respectively. The fish exposed to cypermethrin along with *Aloe vera* (Group 3) the in response gradually recovered when compared to group 2. The recovery percent changes are -12.98, -8.77, -5.84, -8.17 and -5.10 for the period of 24, 48, 72, 96 and 120 hours respectively. In the *Aloe vera* supplemented feed exposed group 4 there are no marked changes occurring in the GPx content. The percent changes are 6.22, 5.30, 12.70, 16.94, and 7.25 for the period 24, 48, 72, 96 and 120 hours respectively. Increased and decreased values in GPx activity in gill tissue are statistically significant at 1% and 5% levels.

The observed values of ACP activity in the gill, tissue shows an increased in *Cyprinus carpio* exposed to sublethal concentration of cypermethrin (group 2). Increased percent changes are 325.6, 232.03, 163.07, 126.08, and 63.63 for 24 to 120 hours respectively. In the fish exposed to cypermethrin along with *Aloe vera* (Group 3) gradually decreased (regained), when compared with group 2. The percent changes are 41.35, 31.29, 27.48, 27.88 and 14.10 for the period of 24 to 120 hours respectively. The *Aloe vera* supplemented feed expose (group 4) are near to control. The percent changes are 12.00, 11.71, 19.23, 21.73 and 40.55 for the period of 24 to 120 hours respectively. The increased and decreased levels of ACP

activity in group 2, 3 and 4 are statistically significantly at 1% and 5% levels. (Table 1)

The level of ALP enzyme activity in the gill tissue shows a decrease in the exposed to sublethal concentration of cypermethrin (group 2). The percent changes are 28.98, 37.76, 40.14, 49.52, and 47.88 for 24 to 120 hours respectively. Group 3 ACP values are gradually increased when compared to group 2. The percent changes are -1.30, -21.77, -24.20, -67.52 and -77.99 for 24 to 120 hours respectively. The fish supplemented feed exposed group 4. Slight variation was noticed when compared to control. The percent changes are 10.03, 1.79, 2.73, 4.39, and 8.08 for 24 to 120 hours respectively. Recorded ALP activities in gill tissue for all the 4 groups of are statistically significant at 1% and 5% levels.

In the present investigation, the recorded values of ACh are increased in cypermethrin exposure (group 2) than the control. The percent changes are -44.97, -55.59, -61.67, -63.66 and -64.79 for the period of 24, 48, 72, 96 and 120 hours respectively. When the fish exposed to cypermethrin along with *Aloevera* (Group 3), the activity of ACh is increased when compared to group 2. The recoveries per cent changes are 5.52, 8.84, 11.59, 10.06 and 8.88 during the period of 24, 48, 72, 96 and 120 hours respectively. The *Aloevera* exposure group 4 the fish *Cyprinus carpio*, the ACh activities are decreased. Supplemented feed exposure (group 4) was equal to normal (near to control). The percent changes are -2.46, -2.42, -3.74, -3.62, and -3.26 for the period of 24, 48, 72, 96 and 120 hours respectively. The activities of ACh in brain tissue are statistically significant at 1% and 5% levels (Table 1).

endocrine and metabolic function as well as activity of enzymes involved in oxidative stress. Cells has a wide array of enzymatic and non enzymatic antioxidant defence system. Defence systems that tend to inhibit oxygen radicals formation include the antioxidant enzyme such as SOD, CAT, and GSH (Torres *et al.*, 1993). These enzymes play an important role in countering the oxidative stress induced by the formation of ROS. Superoxide dismutase is an enzyme that catalyzes the dismutation of superoxide to hydrogen peroxide which is detoxified to hydrogen peroxide which is detoxified CAT and GPx (Li *et al.*, 2009).

In the present investigation superoxide dismutase activity in the gill, liver and kidney tissue of *Cyprinus carpio* was increased after cypermethrin administration appears to be an adaptive response to increased generation of reactive oxygen species. It has been reported in the literature that exposure of animals to increases SOD activity in various tissues (Datta *et al.*, 1992). SOD provides the first line of defence against oxygen derived free radicals. SOD activity decreases oxidative stress by disputation of O₂. The increase in the activity of SOD in our study reflects compensatory mechanism to increased oxidative stress (Meenambal *et al.*, 2012).

SOD induction could occur owing to increased production of the O₂ radical. Therefore, an increase in SOD activity indicates an increase in O₂ production similar to other results of pollution (Wendelaar Bonga, 1997).

Table 1 Changes in the level of Super oxide dismutase (U mole / mg protein) activity in the fresh water fish *Cyprinus carpio* exposed to 120 hours sublethal concentration of cypermethrin and *Aloevera*

Organs	Groups	Hours Of Exposure				
		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	38.64 ^{NS} ± 2.31	41.39 ^{NS} ± 2.48	48.26* ± 2.41	49.36* ± 2.46	52.14 ^{NS} ± 2.60
	Cypermethrin	28.03	36.87	57.45	62.79	71.40
	III	35.65 ^{NS} ± 2.13	35.36 ^{NS} ± 1.76	36.25 ^{NS} ± 1.81	37.42 ^{NS} ± 1.87	38.12 ^{NS} ± 1.90
	CYP + ALOV	18.12	16.93	18.27	23.41	25.31
	IV	7.73	14.54	24.88	24.18	26.88
	Aloevera	30.22** ± 1.81	30.42 ^{NS} ± 1.82	31.08 ^{NS} ± 1.86	31.29 ^{NS} ± 1.56	32.15 ^{NS} ± 1.92
	I Control	0.13	0.59	1.40	3.19	5.68
	II	48.12 ± 2.40	48.65 ± 2.43	48.92 ± 2.44	49.02 ± 2.45	49.32 ± 2.46
	III	50.21 ^{NS} ± 2.51	53.26 ^{NS} ± 2.66	56.24 ^{NS} ± 3.37	60.27 ^{NS} ± 3.01	62.18 ^{NS} ± 3.73
LIVER	Cypermethrin	4.34	9.47	14.96	22.94	26.07
	III	50.15 ^{NS} ± 2.50	50.65 ^{NS} ± 2.53	51.32 ^{NS} ± 2.56	52.33 ^{NS} ± 3.13	53.24 ^{NS} ± 2.66
	CYP + ALOV	4.21	4.11	4.90	6.75	7.94
	IV	0.11	4.90	8.74	13.17	14.37
	ALOEVERA	48.25 ^{NS} ± 2.41	49.12 ^{NS} ± 2.45	49.15 ^{NS} ± 2.45	50.12 ^{NS} ± 3.00	50.15 ^{NS} ± 3.00
	I CONTROL	0.27	0.96	0.47	2.24	1.68
	II	34.12 ± 1.70	34.65 ± 2.07	34.39 ± 1.71	35.36 ± 1.76	35.49 ± 1.77
	III	37.18 ^{NS} ± 1.85	39.39 ^{NS} ± 2.36	42.65 ^{NS} ± 2.55	48.39* ± 2.41	56.32* ± 3.37
	CYPERMETHRIN	8.96	13.67	24.01	36.84	58.69
	IV	38.42 ^{NS} ± 1.92	39.65 ^{NS} ± 1.98	40.39 ^{NS} ± 2.01	41.63 ^{NS} ± 2.49	43.19 ^{NS} ± 2.15
KIDNEY	CYP + ALOV	12.60	14.43	17.44	17.73	21.69
	+	-3.33	-0.66	5.29	13.96	23.31
	ALOEVERA	35.41 ^{NS} ± 1.77	35.08 ^{NS} ± 2.10	39.34 ^{NS} ± 2.18	36.11 ^{NS} ± 1.80	36.27 ^{NS} ± 2.17
	+	3.78	1.24	5.67	2.12	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

Oxidative stress results from an increase in reactive oxygen species or an impairment of the antioxidant defence system. Antioxidant may be induced or inhibited by exposure to environmental pollutants and inhibition of antioxidants impairs the capacity to prevent ROS formation and cell damage (Vasantharaja *et al.*, 2012). Exposure to toxicants may affect

The relation between SOD activities and pollution levels, and the possible use of SOD levels as biomarkers have often been discussed. In *Cyprinus carpio*, the SOD activity is generally higher in cypermethrin and treated fish compared with control, which supports the view, those chemical toxicity results in

slightly increased SOD activity (Bhalchandra and Lomte, 2001).

The apparent increase in SOD activities in the Gill, liver and kidneys of the fish may be due to the production of superoxide anions which lead to the induction of SOD, to convert the superoxide radicals to H₂O₂. Increase in the activity of SOD is usually observed in the face of environmental pollutants against oxidative stress (Coats *et al.*, 1989). Furthermore, the increase in antioxidant enzyme in kidney demonstrates that kidney has an important role in the detoxification of cypermethrin and / or its metabolites. Induction of SOD could occur during high production of superoxide anion radical. Therefore, an increase in SOD activity indicates an increase of O₂ production.

CAT activation is the second main antioxidant enzyme, CAT, occurs due to high pollutant levels and without relation to SOD response (Vasantharaja, *et al.*, 2012 Pugazhendy *et al.*, 2008). The depletion of CAT or its tolerance along with activation of SOD may be observed (Pandy *et al.*, 2003; EIdemerdash *et al.*, 2004 and Huang *et al.*, 2007), although CAT mRNA levels were higher in the exposed fish (Sayeed *et al.*, 2003).

Lipid peroxidation is the initial step of cellular membrane damage caused by pesticides, metals and other xenobiotic (Li, *et al.*, 2009). Lipid peroxidation is considered to be a valuable indicator of oxidative damage to cellular components. Most components of cellular structure and function are likely to be potential targets of oxidative damage, and the most susceptible substrates for auto oxidation; polyunsaturated fatty acids in the cell membrane, which undergoes lipid peroxidation. Exposure of fish to cypermethrin dosages increased in (Gill, liver and kidney) tissues. These increases in MDA can most likely be ascribed to an excessive production of ROS, which could be related to antioxidant enzymes leakage. The induction of elevated ROS production can lead to oxidative injury to important cellular macromolecules such as lipid, proteins and nucleic acid (Mohamed and Gad, 2008) In the present study cypermethrin treatment induced oxidative stress, as demonstrated by compromised antioxidant defences. Our results are in agreement with the study who demonstrated a significant increase in lipid peroxidation in fish, liver kidney and gills following cypermethrin and cypermethrin along with *Aloe vera* exposure. Exposure of experimental animals to herbicide known to induce lipid peroxidation in various tissues, which is responsible for the adverse biological effects (Sharma *et al.*, 2005; EIdemerdash *et al.*, 2004, Kumboj *et al.*, 2006). Mechanism of pesticides toxicity has been usually associated with the increase in lipid peroxidation in liver (Sharma *et al.*, 2005)

A significant decrease in GSH in the liver of *Cyprinus carpio* after cypermethrin 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; Thaper *et al.*, 2002; 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 cypermethrin could be the results of either utilization of GSH for conjugation and /or participation of GSH as an antioxidant in terminating toxicity. Administrations of cypermethrin along with *Aloe vera* have

resulted in restoration to near the control value. GSH is the primary intracellular antioxidant and the conjugating agent, was shown to deplete and to have impaired function in cypermethrin toxicity. In fact, GSH serves as a primary line of cellular defence against cypermethrin compounds. (Quing, 1998).

GSH is the major thiol, which binds electrophile molecules of spices and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and detoxification of exogenous and endogenous substances. (Ketterer *et al.*, 1983). 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 in one of GSH to near to control value.

However, our results show an increase in GSH-Px level in kidney tissue, which suggests the operation of a protective response in this tissue against oxidative stress induced by cypermethrin. Glutathione reductase (GR) plays an important role in cellular antioxidant protection and adjustment processes of metabolic pathway (Worthington *et al.*, 1974). Although perhaps not involved in antioxidant defence in the 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 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 cypermethrin 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. The results of present investigation establish that cypermethrin intoxication causes dose-depend mortality. Significant decline in Alkaline phosphatase activity and significant elevated MDA level and acid phosphate activity. Alkaline phosphatase enzymes is associated with transfer of phosphates and it is linked with transportation of intermediate compounds in glycogenesis or glycogenolysis

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