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IMPACT OF HEPTACHLOR ON ANTIOXIDANT ENZYME MARKERS OF FISH *CATLA CATLA*

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ABSTRACT

Heptachlor is an Organochlorine insecticide used world wide in Agriculture and Aquaculture due to its activity against a broad spectrum of Insect pests. Acute toxicity of an organochlorine insecticide is characterized by their persistence and ability to accumulate in aquatic organisms. Our present study is aimed to elucidate the effect of heptachlor and antioxidant system of carp fish *Catla catla*. $1/5^{th}$ sub lethal concentration of heptachlor is 1.46mg/L (LC_{50} 7.86 mg/L for 96hrs) was used for the toxicity experiment. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. The shift in the balance between oxidants and antioxidants in favour of oxidants is termed "oxidative stress". Heptachlor is also induced a gradual and significant ($P < 0.05$) increase in Lipid Peroxidation and antioxidant enzymes like SOD, GST and CAT activities is gradually increased throughout the study period. The modified activities of antioxidant enzymes imply the activation of physiological mechanism to scavenge the reactive oxygen species produced by the toxicant exposure. The antioxidant enzymes like Superoxide dismutase, Glutathione-S-transferase and Catalase can be used as biomarkers for non specific immune responses caused by acute exposure of pesticides or other similar aquatic pollutants. The present study explained that even at sub lethal concentration level of heptachlor is harmful to *Catla catla* like aquatic culture organisms and applications of heptachlor close to bodies of water lead to havoc to aquatic life, then to the humans.

KEYWORDS: *Catla catla*, Heptachlor, Antioxidant Enzymes, Superoxide dismutase (SOD), Catalase (CAT), Glutathione -S - Transferase (GST) and Lipid Peroxidation (LPO).

INTRODUCTION

India is the world's third largest producer of fish and next only to China in the area under fish production, but it is facing serious challenges that are hampering realization of full potential of the sector. Besides being a major source of revenue, fisheries sector is increasingly contributing to nutritional security of the country.[1,8]

During the last six decades, Indian fisheries has grown more than thirteen folds with an increase in fish production from 0.752 million tonnes in 1950-51 to 10.07 million tonnes in 2014-15 (DAHDF, Govt. of India 2014), of which the share of the aquaculture sector alone is around 4.15 million tons.[8] Aquaculture was about 36% (of total fish production) in the 1980s and now it has increased up to 65% in 2014-15 contributing more than half of the total fish production.

The fisheries sector is an important player in all over socio-economic development of India. The sectors contribution to employment generation, food and nutritional security and foreign exchange earnings is now well recognized.[5] The fisheries sector has also been one of the major contributors of foreign exchange earnings. During 2014-15, export value of marine products reached Rs. 30, 213 crores.[8]

Fisheries sector has been identified as a Growth Engine for social economic development of the new State of Andhra Pradesh. AP stands first in total fish and prawn/shrimp production in India since 2013-14 both in terms of production and value. The contribution of fisheries sector is 6.01% in Andhra presh GSDP, whereas the fisheries contribution is about 0.83% of GDP of the nation. The overall fish production has more than doubled in the past one decade from 8.14 lakh tonnes in 2005-06 to 19.64 lakh tonnes in 2014-15.[18] The share of Andhra Pradesh in India's sea food exports has increased from about 20% in 2009-10 to about 40% in 2013-14. During 2009-10 the exports from Andhra Pradesh was Rs. 2,100 crores but by 2013-14 exports have increased to Rs. 12,100 crores. During 2014-15, the marine exports have been increased to an estimated value of Rs. 16,000 crores.[6]

Fish are used extensively for the environmental monitoring, because they uptake contaminants directly from water and diet.[28] Generally the ability of fish to metabolize organochlorines is moderate. Organochlorine (OC) Pesticides are hydrophobic molecule which disrupts ionic channels like Na^+ - K^+ pumps in nervous cell membrane leading to automatic stimulation of neurons and involuntary contraction of muscles. The fish is a good

indicator and highly sensitive in such ecosystem where the water gets contaminated with toxic chemicals and pollutants from the chemical industries.

Heptachlor is an organochlorine (cyclodiene) insecticide which was first isolated from technical chlordane in 1946. Heptachlor epoxide is a man-made compound that looks like a white powder. Heptachlor epoxide is created when a substance called heptachlor is released to the environment and mixes with oxygen. The organochlorine insecticide 3-Chlorochlordene, commercially available as Heptachlor (OC), is used as a treatment against ectoparasite and as an insecticide for crops.

Pesticides liberated into the aquatic environment causes deleterious effect on fish and subsequently to man. Heptachlor is poorly hydrolyze and as such, it biodegrades slowly in the environment. So, this compound persists for longer time in the food chain and cause severe effects at different levels of food chains. Heptachlor entered in to the aquatic environment cause serious threatening to various aquatic organisms and also cause severe metabolic abnormalities in non target species like fish and freshwater mussels.[7, 24]

Heptachlor is a stimulant to central nervous system of all vertebrates. The liver is the other organ significantly affected by heptachlor. Heptachlor is converted to heptachlor epoxide and other degradation products in the environment. Heptachlor epoxide degrades more slowly and, as a result, is more persistent than heptachlor.[14] Heptachlor epoxide has been found in food crops grown in soils treated with heptachlor many years before. Heptachlor adsorb strongly to sediments, and both are bioconcentrated in aquatic and terrestrial organisms. Biomagnification of heptachlor in aquatic food chains is significant. Because heptachlor is readily metabolized to heptachlor epoxide by higher trophic level organisms, biomagnification of heptachlor itself is not significant. Because of the more persistent nature of heptachlor and its lipophilicity, biomagnification of heptachlor in terrestrial food chains is significant.[4]

Heptachlor is a manufactured chemical that was used in the past for killing insects in homes, in buildings, and on food crops. There are no natural sources of heptachlor or heptachlor epoxide. Trade names for heptachlor include Heptagran®, Heptamul®, Heptagranox®, Heptamak®, Basaklor®, Drinox®, Soleptax®, Gold Crest H-60®, Termide®, and Velsicol 104®. Heptachlor is both a breakdown product and a component of the pesticide chlordane (approximately 10% by weight). Pure heptachlor is a white powder.

Synthesis of Heptachlor

Analogous to the synthesis of other cyclodienes, heptachlor is produced via the Diels-Alder reaction of hexachlorocyclopentadiene and cyclopentadiene. The resulting adduct is brominated followed by treatment with hydrogen chloride in nitromethane in the presence of aluminum trichloride or with iodine monochloride.[17] Compared to chlordane, it is about 3-5 times more active as an insecticide, but more inert chemically, being resistant to water and caustic alkalies.[17]

Metabolism of Heptachlor

Soil microorganisms transform heptachlor by epoxidation, hydrolysis, and reduction. When the compound was incubated with a mixed culture of organisms, chlordene (hexachlorocyclopentadine, its precursor) formed, which was further metabolized to chlordene epoxide. Other metabolites include 1-hydroxychlordene, 1-hydroxy-2, 3-epoxychlordene, and heptachlor epoxide. Soil microorganisms hydrolyze heptachlor to give ketochlordene (Fig-1).

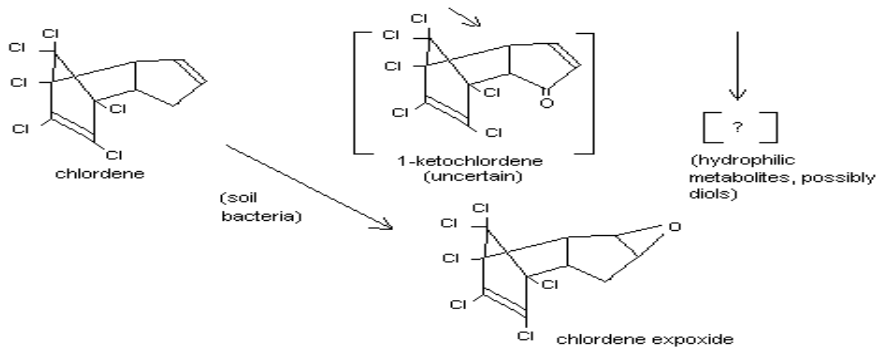

Figure 1
Metabolism of heptachlor

Technical-grade heptachlor is a tan powder and has a lower level of purity than pure heptachlor. Technical-grade heptachlor was the form of heptachlor used most often as a pesticide.

The present study is aimed to evaluate the effect of sub lethal concentration of heptachlor on Antioxidant enzymes in various tissues of carp fish, *Catla catla* over an exposure period of 45 days. These parameters have been used to assess the health of fish, monitoring stress responses and forebode systematic relationship and physiological adaptations of animals. They quickly reflect the weak condition of fish. The cells have a complex defense system to protect themselves from ROS, including main antioxidant enzymes such as Lipid Peroxidation, Catalase (CAT), Superoxide dismutase (SOD), Glutathione -S- transferase

(GST) and enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS.[23]

The primary defense is offered by enzymatic antioxidants, which have been shown to neutralize ROS. Fish tissues, specifically the liver and kidney are endowed with antioxidant defense system consisting of catalase (CAT), superoxide dismutase (SOD), and Glutathione – S-transferase to protect them from oxidative stress. Antioxidants contributes to the maintenance of relatively low level of the reactive and harmful hydroxide radical, the superoxide radical and hydrogen peroxide in the presence of Cu^{2+} and/or Fe^{3+}. Such markers measured at the molecular or cellular level in fish have been identified as sensitive "early warning" tool in environmental quality assessment tests.[27]

MATERIALS AND METHODS

Experimental Animal

Live specimens of *Catla catla* of (30.0 ± 1.6g) were collected from AP Govt. Fish Breeding and Hatchery Centre, Kalyani dam, near Tirupati, Chittoor district and immediately transferred to transparent polypropylene tank of 500L capacity filled with filtered, well aerated and dechlorinated bore well water. The fish were fed with a commercial pelletized formulated fish feed twice a day. The water quality is maintained constantly throughout the experimental period in control medium and also in pesticide treated aquatic medium.

Experimental Chemical

Heptachlor an organochlorine pesticide obtained from Kisan Agro Chemicals, Anantapur district, Andhra Pradesh. Heptachlor smells somewhat like camphor. Heptachlor does not burn easily and does not explode. It does not dissolve easily in water but are attracted to fats. Heptachlor is very stable in both fresh and salt-water environments. LC_{50} of heptachlor for 96h is calculated by the static bioassay method.[9] Fish were starved 24h prior to the experimental period to nullify the metabolic changes. Five replicates of each containing twenty fish were subjected to heptachlor at various concentrations for 96 hours. The lethal concentration of heptachlor (LC_{50}/96hrs) and identified as 7.86 mg/L and 1.46 mg/L was selected as $1/5^{th}$ sub lethal concentration for the further analysis.

Collection of tissues

After the experimental period the fish were killed by pithing (by damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle) and the

tissues viz gill, liver and muscle were removed from its body. They were washed in ice-cold 0.33M sucrose and blotted dry and the desired amounts of tissue were weighed and used. The tissues are homogenized in 6 volumes of homogenizing buffer (50mM Tris-HCl mixed with 1.15% KCl and pH adjusted to 7.4) using Teflon homogenizer. The resulting homogenate was centrifuged at 16,000 g for 15min in a cold centrifuger at -4 °C. The supernatant was decanted and stored at –20 °C in a deep freezer for until enzymatic analysis.

CAT activity is determined by the method of Xu et al.[31]which is based on the first-order reaction of CAT with H_2O_2. The CAT activity was dictated by spectrophotometry at 240 nm. SOD activity is determined by the method of Zou et al.[33] based on inhibition of SOD by auto-oxidation of 1, 2, 3-benzenetriol. The SOD activity was dictated by spectrophotometry at 325 nm. Glutathione -S-transferase is determined by the method of Habig et al. (1974a). Lipid Peroxidation (LPO) is determined by the Thiobarbituric Acid Reactive Substances (TBARS) assay used to deliberate lipid peroxidation by measuring the malondialdehyde (MDA) concentration in each tissue lysate.[25]

STATISTICAL ANALYSIS

The Probit mortality was found out using SPSS software 16.0 and biochemical data processed using SPSS 13 statistical program. All data were expressed as arithmetic mean ± SD, for the analysis of the experimental parameters Student's -t test was used. Mean values are significant at $P < 0.05$.

RESULTS AND DISCUSSION

In the present study the Probit analysis confirms the LC_{50} value of heptachlor for 96hrs was estimated experimentally as 1.46mg/L and the upper and lower confidence limits were calculated as 1.35mg/L and 1.74mg/L respectively, indicating that heptachlor is toxic to fish at a low concentration. Vittozzi and Angelis[30] reported 0.78 mg/l and 0.79 mg/l as 96 h LC_{50} values of Azodrin for bluegill and trouts respectively. Attempts were also made in the present study to observe carefully the demeanour conditions of the fish during the 96 h exposure of heptachlor. Behavioural changes such as curling of spine gradual increase in colour fading, a thick mucous film was formed on whole body and gills, of all experimental fish and Vertical movement of the fish was also observed during the experimental period. This may be due to loss of labyrinthine sense at high inebriation which makes the fish to turn upside down and finally died.[21]

In the present study, observed modulations in antioxidant enzymes such as Superoxide dismutase, Catalase, Glutathione-S-transferase and Lipid Peroxidation activities in different tissues of fish after treated with heptachlor and there was an increased Lipid Peroxidation, Superoxide dismutase, Glutathione-S-transferase and Catalase activity on heptachlor 45 days exposure period.

Glutathione - S - transferase (GST) is a group of multifunctional enzyme involved in biotransformation and detoxification of xenobiotics.[16, 26] Highly reactive electrophilic components can be removed before they covalently bind to tissue nucelophilic compounds which would lead to toxic effects. It plays an important role in protecting tissue from oxidative stress.[2,10] The increased GST activity in liver observed in the present study after exposure to Heptachlor suggests the increase in detoxification process in *Catla catla*. GST has been reported as a biomarker for assessing the environmental impact of organic pesticides generating oxidative stress.[22] The GST was more active in hepatic tissue than in white muscle and gill, which indicates the effective role of liver in detoxification of organochlorine pesticides.[3]

The enzyme glutathione - S - transferase was found to be increased in the liver during the heptachlor exposure periods. The kidney enzyme activity slowly increased on the successive exposure days and later showed decreased activity on the 45th day like other antioxidants (Table-1). The higher glutathione - S- transferase activity observed in the liver of the carp after pesticide toxicity indicates an augmented detoxification activity in the liver tissue. The kidney also shows prominent response in glutathione - S- transferase activity, but less when compared to the liver. The glutathione-S-transferase detoxifies a number of environmental carcinogens, reactive nucleophile, and epoxides intermediates. The increased glutathione-S-transferase assay was suggested as a useful tool for bio monitoring oxidative stress.

The percent changes of catalase activity in various tissues are represented in Table - 2. The increased catalase activity indicates a reduced activity to protect the cells against H_2O_2. It was reported that the enhanced SOD and catalase activities in the hepatocytes of the carp fish, could be induced by microcystine.[20,29] The induction of Catalase in the liver and other tissues was an adaptive response of these cells to mitigate to toxicity for the prolonged exposure of 45 days.

Increased levels of ammonia under stress conditions may be due to augmented oxidative dismutation process, which may lead the formation of either ammonia or peroxides. It is well known that when peroxide production is more, the elevation in catalase activity is also high. This may be another plausible reason for increased catalase activity in the present study. Increased catalase activity was in response to a gradual increase of SOD activity at all days during heptachlor toxicity stress, observed in the present study. This is inconsonance to the reports of Yarsan et al[32] and Janardana Reddy and Reddy.[32]

SOD activity was found that a gradual and significantly increased in all tissues of heptachlor treated fish at all exposure periods. The present increase was also increased with increase of exposure periods and the highest percent increase was recorded in liver of fish on day 45 (Table - 3) over to the control. The increase activity of SOD and Peroxidase under toxic condition may be for counteracting Lipid peroxidation and removing toxic H_2O_2 or the organic hydroperoxide formed during heptachlor exposure. The results obtained in the study demonstrate that the sub lethal concentration of heptachlor can cause changes in biochemical responses and antioxidant activity in fish, this alteration may be potentially disruptive on the survivability and non-functioning of *Catla catla*. The stress created by the sub lethal concentration of heptachlor leads to increased activity of antioxidant enzymes such as peroxidase and SOD.[12,13]

In the present study increase of Lipid peroxidation levels of different tissues of *Catla catla* treated with sub lethal concentration of heptachlor reflects the extent of oxidative damage in the respective tissues of the fish (Table- 4). A significant increase in Lipid oxidation marker may specify the susceptibility of lipid molecules to reactive oxygen species and the elongation of oxidative damage imposed on this molecules. Lipid peroxidation levels significant increase in different tissues *Catla catla* may be upon oxidative stress in different tissues of fish, in levels in fish such as *Geophagus brasillensis* are also reported in response to oxidative stress were made earlier.[19] Maintenance of high constitutive levels of antioxidant enzymes like Superoxide dismutase and Catalase is crucial to prevent oxyradical mediated lipid peroxidation.

Table 1: Variations in Catalase (CAT) Activity in various tissues of fish treated with sublethal concentration of heptachlor over an exposure period of 45 days.

Parameter Tissue	Control	Days of exposure with heptachlor				
		3d	7d	15d	30d	45d
Gill	165.16± 1.79	169.32± 1.79	188.18± 1.80	215.16± 1.82	245.16± 1.85	299.93± 1.87
% Change	--------	(2.51)	(13.93)	(30.27)	(48.43)	(81.59)
Liver	231.08± 2.26	268.13± 2.27	342.10± 2.28	388.01± 2.30	473.21± 2.34	517.16± 2.39
% Change	--------	(16.0)	(48.04)	(67.91)	(104.78)	(123.8)
Kidney	160.05± 2.93	174.03± 2.93	221.32± 2.95	254.13± 2.97	315..22± 2.30	339.21± 2.32
% Change	-------	(8.734)	(38.28)	(58.78)	(96.95)	(111.94)
Muscle	171.42± 2.15	182.31± 2.16	208.21± 2.18	259.68± 2.20	299.31± 2.22	322.67± 2.25
% Change	--------	(6.352)	(21.46)	(51.48)	(74.60)	(88.23)

Mean values are significant at $P < 0.05$.

Values are mean ± SD of 6 individual observations.

Table 2: Variations in Superoxide dismutase (SOD) Activity in various tissues of fish treated with sublethal concentration of heptachlor over an exposure period of 45 days.

Parameter Tissue	Control	Days of exposure with heptachlor				
		3d	7d	15d	30d	45d
Gill	0.534± 0.04	0.569± 0.03	0.598± 0.04	0.687± 0.05	0.884± 0.06	0.954± 0.09
% Change	--------	(6.55)	(11.98)	(28.65)	(65.54)	(78.65)
Liver	0.764± 0.03	0.799± 0.04	0.851± 0.05	0.932± 0.04	1.219± 0.06	1.432± 0.07
% Change	--------	(4.58)	(11.38)	(21.98)	(59.55)	(87.43)
Kidney	0.621± 0.02	0.654± 0.02	0.729± 0.03	0.845± 0.04	0.969± 0.05	1.148± 0.06
% Change	--------	(5.314)	(17.39)	(36.07)	(56.03)	(84.86)
Muscle	0.421± 0.03	0.447± 0.03	0.489± 0.05	0.593± 0.06	0.689± 0.06	0.768± 0.07
% Change	--------	(6.17)	(16.15)	(40.85)	(63.65)	(82.42)

Mean values are significant at $P < 0.05$.

Values are mean ± SD of 6 individual observations.

Table 3: Variations in Glutathione-S-transferse (GST) Activity in various tissues of fish treated with sublethal concentration of heptachlor over an exposure period of 45 days.

Parameter / Tissue	Control	Days of exposure with heptachlor				
		3d	7d	15d	30d	45d
Gill	0.214± 0.03	0.236± 0.02	0.289± 0.03	0.310± 0.04	0.343± 0.05	0.384± 0.05
% Change	--------	(10.2)	(35.04)	(44.85)	(60.28)	(79.4)
Liver	0.461± 0.01	0.532± 0.02	0.667± 0.02	0.785± 0.03	0.853± 0.03	0.932± 0.04
% Change	--------	(15.4)	(44.68)	(70.28)	(85.03)	(102.1)
Kidney	0.340± 0.03	0.375± 0.04	0.435± 0.04	0.547± 0.05	0.589± 0.05	0.654± 0.04
% Change	--------	(10.29)	(27.94)	(60.88)	(73.23)	(92.3)
Muscle	0.284± 0.02	0.313± 0.02	0.346± 0.04	0.425± 0.05	0.501± 0.05	0.523± 0.06
% Change	--------	(10.2)	(21.83)	(49.64)	(76.40)	(84.15)

Mean values are significant at $P < 0.05$.

Values are mean ± SD of 6 individual observations.

Table 4: Variations in Lipid Peroxidation (LPO) Activity in various tissues of fish treated with sublethal concentration of heptachlor over an exposure period of 45 days.

Parameter / Tissue	Control	Days of exposure with heptachlor				
		3d	7d	15d	30d	45d
Gill	29.21± 0.02	25.14± 0.04	35.18± 0.05	42.38± 0.06	49.71± 0.06	53.23± 0.09
% Change	--------	(13.93)	(20.43)	(45.08)	(70.18)	(82.23)
Liver	82.15± 0.08	96.19± 0.08	124.32± 0.09	138.37± 0.09	141.32± 0.10	165.13± 0.11
% Change	--------	(17.0)	(51.33)	(68.43)	(72.02)	(101.01)
Kidney	59.16± 0.04	62.14± 0.05	78.39± 0.06	85.31± 0.06	98.71± 0.07	111.32± 0.09
% Change	--------	(5.03)	(32.50)	(44.20)	(66.85)	(88.16)
Muscle	35.19± 0.05	39.32± 0.06	42.15± 0.07	52.13± 0.07	58.83± 0.08	65.03± 0.11
% Change	--------	(11.73)	(19.77)	(48.123)	(67.17)	(84.79)

Mean values are significant at $P < 0.05$.

Values are mean ± SD of 6 individual observations.

CONCLUSION

In the present study we conclude that the sublethal concentration of heptachlor caused to induce significant modifications in antioxidant enzymes of *Catla catla*. The findings of the present study also provide a better understanding of the toxicological endpoint of aquatic

pollutants and to ascertain a safer level of these chemicals in the aquatic environment and protection of aquatic habitats.

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