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Research Article

# *In vivo* inhibitory effect of Diclofenac on Succinate dehydrogenase and Acetylcholinesterase enzymes in the tissues of *Channa punctatus*

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## ABSTRACT

Pharmaceuticals have emerged as priority pollutants of the environment all over the world in recent times. Diclofenac is a non-steroidal anti-inflammatory drug that has been usually detected in surface waters in the range of ng/l to µg/l. There are many experimental evidences on its toxicity in the aquatic flora and fauna. The objective of the present study is to investigate the acute toxic effect of the drug, Diclofenac in the fresh water fish, *Channa punctatus*. The fish were exposed to ten different concentrations of Diclofenac for 96 hours. The median lethal concentration value was evaluated by Probit analysis. Later the fish were exposed to sub lethal and lethal concentrations of diclofenac for a period of 96 hours. The effect of Diclofenac was observed in five different tissues like gill, kidney, brain, liver and muscle. Succinate dehydrogenase enzyme was estimated by Nachlas *et. al.*, method (1960). Acetylcholinesterase enzyme activity was estimated by Ellman method (1961). There was remarkable inhibition in the activity of the two enzymes studied. The inhibition was found to be significant and dose dependent. This study signifies that enzymes are the effective biomarkers to find out the biochemical alterations in toxic studies. It also assumes that pharmaceutical residues in the water deteriorate the health of the fish.
**Key words**: Diclofenac, acute toxicity, enzymes, *Channa punctatus*

## 1. Introduction

Pharmaceuticals are essential elements for the betterment of human and animal health. The extensive usage of these pharmaceuticals for various purposes has made their entry into nearby fresh water systems. The pharmaceutical residues have emerged as priority emerging pollutants of the aquatic environment in recent times. Their residues have been detected in various aquatic systems in the range of ng/L to µg/L all over the world. They are continuously released into water through various pathways. The main route is through excretion of the unabsorbed drugs into the drains. The improper disposal of the unused drugs and the effluents from the manufacturing industries are the other routes of pollution. There are various reports on the aquatic toxicity of pharmaceuticals on non-target organisms like algae, crustaceans, molluscans and fish.

Diclofenac is a commonly prescribed non-steroidal anti-inflammatory drug (Skoutakis *et. al.*, 1988). The annual consumption of the drug is in millions of tons all over the world. It is used to reduce pain and inflammation in several disorders like rheumatoid arthritis, osteoarthritis, spondylarthritis, and dysmenorrhea (Brogen *et. al.*, 1980). The drug is available in the form of tablets, capsules, gels, creams and injections.

Diclofenac has been frequently detected in surface waters of rivers, lakes, seas, ground water, drinking water, effluents of waste water treatment plants in the range of ng/L to µg/L (Buser*et al.*, 1998). The chronic toxicity studies have revealed that diclofenac is harmful to fish at the environmental relevant concentrations of 1µg/L (Cuckle*et al.*, 2011). The acute toxicity data in fish is very sparse.

Fish play a significant role in assessing the contamination in aquatic environment. They are directly exposed to chemicals from surface runoff and indirectly through food chain. *Channapunctatus* is the best species for evaluation of toxicity due to its wide distribution in fresh water, availability throughout the year, high commercial importance and easy acclimatisation to laboratory conditions.

Enzymes are the macromolecules that catalyse the metabolic processes in the cell. They are sensitive molecules and are easily affected by even small changes in external and internal media. Succinate dehydrogenase is the only enzyme that participates both in tricarboxylic acid cycle and electron transport system in cellular respiration and helps in energy generation. It also plays a role in oxygen level sensing and tumor suppression. Acetylcholinesterase occurs throughout the central and peripheral nervous system of vertebrates. Acetylcholinesterase is the most vital enzyme for the normal function and coordination of the body. The inhibition of AChE results in build-up of acetylcholine within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission.

The present investigation has been undertaken considering two vital enzymes SDH and AChE as biomarkers to evaluate biochemical alterations induced by Diclofenac in the different tissues of *Channa punctatus.*

## 2. Experimental section

The experimental fish, *Channa punctatus* were purchased from the local market of the village Hasanparthy of Warangal district, Telangana, India. The healthy fish with an average weight of 110-120g and 25 cm length were collected and transported to laboratory kept in a large plastic water tank. The fish were acclimatized for a week in dechlorinated tap water. They were fed *ad libitum* with groundnut oil cake and standard commercial feed. The water was renewed for every 12 hours and proper aeration was supplied. The dead fish were removed immediately to avoid depletion of dissolved oxygen level which adversely affects other fish (Nagaraju and Venkatarathnamma*et al.,* 2013). The fish were starved one day before the experiment in order to lessen the metabolic differences due to differential feeding (Yadav Rao and Wani 2014).

Diclofenac (2- [(2-6 Dichlorphenyl ) amino] benzene acetic acid sodium salt, 99% pure (CAS 15307- 86-5) of analytical grade quality was obtained from Sara Exports Limited, Ghaziabad, Uttar Pradesh, India. Diclofenac stock solution was prepared in acetone.

### *2.1 Acute Toxicity*

The fish were exposed to ten different concentrations of Diclofenac ranging from 5ppm, 10ppm, 15ppm, 20ppm, 25ppm, 30ppm, 35ppm, 40ppm, 45ppm and 50ppm for 96 hours. A control group was maintained and the mortality was recorded in all concentrations at specific time interval. The median lethal concentration value ($LC_{50}$) was calculated by probit analysis using microsoft excel.

One third of the lethal concentration was taken as sub lethal concentration and the fish were exposed to both concentrations of Diclofenac and evaluated against control. All the experiments were carried out in triplicate. The live fish were sacrificed and the vital tissues like brain, liver, muscle, kidney and gill were isolated from all groups. The tissues were studied for Succinate dehydrogenase and Acetylcholinesterase enzyme activity with respect to control.

### *2.2 Succinate dehydrogenase enzyme activity*

SDH activity in the organs of fishwas estimated using Nachalas *et. al.*, method. A 5% homogenate (w/v) of the tissue was prepared in 0.25M ice cold sucrose solution, centrifuged at 3000 rpm for 10 minutes and the supernatant was taken as the source of the enzyme. The incubation mixture consisted of 0.2 ml of 0.4M phosphate buffer (pH 7.7), 0.2 ml of 0.2M sodium succinate, 1.0 ml of 0.004M 2-(p-indophenol)-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT), 0.1 ml of 0.005M phenazinemethosulphate and 0.5 ml of enzyme preparation. The mixture was incubated at 37$^{o}$ C for 30 minutes and the reaction was stopped by adding 6.0 ml of glacial acetic acid. The formazone formed was extracted into 6.0 ml of toluene by keeping overnight at 0$^{o}$C and the optical density of the colour developed was read at 495 nm in a spectrophotometer. A blank taking 0.5 ml of distilled water and control by taking 0.5 ml of boiled enzyme were also run simultaneously. INT standards were prepared alongside for comparison. The enzyme activity was expressed as µM of formazone formed /mg protein/hr.

### *2.3 Acetylcholinesterase Activity*

AChE activity in the tissues of the fish was estimated by the method of Ellman*et al.,* method (1961). The live fish were sacrificed and the tissues were isolated. The homogenates were prepared in 0.1 M phosphate buffer at 8 pH. The homogenates were centrifuged for 20 min at 8000 rpm at 4$^{o}$C. The supernatants were collected and stored in strong vials at –80$^{o}$C. The homogenate was added to sodium phosphate buffer and incubated at room temperature for 5 minutes. Then 10 ml of DTNB was added followed by acetylthiocholine iodide. The increase in absorbance was recorded for 10 min at 412 nm in colorimeter. The enzyme activity was expressed as µ moles of Ach hydrolysed /mg protein/hr.

## 3. Results and Discussion

### *3.1 Measurement of Acute Toxicity*

Diclofenac has caused 100% mortality of fish at 50ppm concentration. There was no mortality at 5ppm of Diclofenac and 50% mortality was found at 30ppm. The $LC_{50}$ value of Diclofenac for 96 hours was found to be 25.28ppm. One third of this value 8.42 ppm was taken as sublethal concentration.

### *3.2 Succinate Dehydrogenase Enzyme*

Succinate dehydrogenase enzyme is found in bacterial cells and inner mitochondrial membrane of eukaryotes. It catalyses the oxidation of succinate to fumarate in Kreb's cycle and provides electrons to the respiratory chain ubiquinone pool.

The present study has shown inhibition in the Succinate dehydrogenase enzyme activity in different tissues as the concentration of diclofenac has increased. The results are summarized in Table 2. The order of inhibition in the enzyme activity is liver followed by gill, muscle, kidney and brain. Several studies have reported similar decreasing trend in Succinate dehydrogenase enzyme activity on exposure to various toxicants in different fish species.

**Table 1: Fish exposed to different concentrations for calculation of $LC_{50}$ value**

| S.No. | Concentration of Diclofenac | Log Concentration | No. of fishes exposed | No. of fishes died at 96 hr | Probit Kill | Percent Kill |
|---|---|---|---|---|---|---|
| 1 | 5ppm | 0.698 | 10 | 0 | 0 | 0 |
| 2 | 10ppm | 1. | 10 | 1 | 3.72 | 10 |
| 3 | 15ppm | 1.176 | 10 | 1 | 3.72 | 10 |
| 4 | 20ppm | 1.301 | 10 | 2 | 4.16 | 20 |
| 5 | 25ppm | 1.397 | 10 | 4 | 4.75 | 40 |
| 6 | 30ppm | 1.477 | 10 | 5 | 5.00 | 50 |
| 7 | 35ppm | 1.544 | 10 | 6 | 5.25 | 60 |
| 8 | 40ppm | 1.602 | 10 | 8 | 5.84 | 80 |
| 9 | 45ppm | 1.653 | 10 | 9 | 6.28 | 90 |
| 10 | 50ppm | 1.698 | 10 | 10 | 7.33 | 100 |

Sastry and Siddiqui (1982) have reported inhibition of SDH activity in liver and brain of *Channa punctatus* exposed to sub lethal concentrations of seiven. There was a significant decrease in the succinate dehydrogenase activity in muscle, liver and brain tissue of fish *Cyprinus carpio* exposed to different sub lethal concentrations of distillery effluent (Ramakritina *et. al.,* 2005). Singh *et. al*., (2010) have reported decline in the SDH activity exposed to cypermethrin in the fish, *Colisa fasciatus*. Suneetha (2012) has observed decreased SDH activity in the gill, liver and muscle of the fresh water fish, *Labeo rohita* after exposing to sublethal and lethal concentrations of two pesticides endosulfan and fenvalarate for 24hr and 15 days.

The fish exposed to nickel chloride have shown decrease in the succinate dehydrogenase activity in gill, liver, kidney, brain and muscle of *Labeo rohita* (Moorthikumar and Muthulingam, 2011). The exposure of Chloropyrifos has caused inhibition of Succinate dehydrogenase enzyme in kidney, gill, muscle and liver of *Clarius batrachus*(Reddy *et. al*., 2011). There was decrease in SDH activity exposed to lead nitrate in the liver extracts of *Cirrhinus mrigala* (Fernandes *et. al*., 2016). Lakshmaiah (2016) has noticed decrease in the succinate dehydrogenase enzyme in gill, liver, muscle, kidney and brain of *Cyprinus carpio* on exposure to phorate.

**Table 2: Inhibitory activity of SDH eznyme expressed in µmoles of formazon formed /mg of protein /hour.**

| Tissues | Control | 8.42ppm | 25.28ppm |
|---|---|---|---|
| Liver | 0.312±0.03 | 0.228±0.05<br>26.92% | 0.164±0.05<br>47.43% |
| Muscle | 0.264±0.03 | 0.236±0.04<br>10.60% | 0.158±0.08<br>40.15% |
| Gill | 0.228±0.08 | 0.176±0.07<br>22.80% | 0.132±0.03<br>42.10% |
| Brain | 0.180±0.02 | 0.160±0.04<br>11.11% | 0.142±0.02<br>21.11% |
| Kidney | 0.216±0.03 | 0.168±0.01<br>22.22% | 0.132±0.06<br>38.88% |

The inhibition of succinate dehydrogenase (SDH) activity in different tissues has indicated depression of cellular metabolism resulting in a shift to anaerobic metabolic pathway to meet the increased energy demand under stress (Tripati and Verma 2004, Lakshmaiah 2016). More *et. al*., (2005) have suggested that anaerobic activity of the cells due to pollution stress has reversed the physiological and biochemical adaptation. This decrease in succinate dehydrogenase activity might be suggestive of the weakening of biochemical differences which in turn could be the results of tissue damage. The decreased SDH activity has suggested the inhibition of mitochondrial oxidation of succinate which results in decreased energy production (Fernandes *et. al*., 2016).

The decrease in SDH activity has also indicated the impairment of oxidative metabolism in the mitochondria as a consequence of hypoxic conditions under pesticide exposure, most probably by disrupting the oxygen binding capacity of the respiratory pigment. The decrease in this enzyme activity may be probably due to mitochondrial damage affecting enzymes of Kreb's cycle and diminished state of respiration (Bag *et. al*., 1999). The drug might have caused disintegration of the mitochondrial respiratory mechanism which has led to prevention of transfer of electrons to molecular oxygen. This ultimately results in inhibition of SDH activity and shifts the aerobic metabolism to anaerobic metabolism. As SDH is the oxidative enzyme involved in Kreb's cycle any disruption in this enzyme activity will affect Kreb's cycle. Since Kreb's cycle represents a central oxidative pathway for carbohydrates, fats and amino acids, any disturbance in this cycle may likely affect the whole metabolism.

### *3.3 Acetylcholinesterase Enzyme*

Acetylcholinesterase is an essential enzyme for the normal functioning of nervous system. It is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction and the target tissues of the parasympathetic system. The inhibition of acetylcholinesterase enzyme in these tissues causes structural disintegration and malfunctioning of the organs. AChE catalyses the breakdown of ACh into choline and acetic acid and is responsible for the removal of the neurotransmitter ACh from the synaptic cleft through hydrolysis and terminates synaptic transmission (Kopecka *et. al*., 2004).

Several investigations have reported inhibition in the acetylcholinesterse enzyme activity in fish on exposure to various toxicants. The present study has shown inhibition in the enzyme activity in both the concentrations studied. There

was greater inhibition in the brain tissue followed by muscle, gill, liver and kidney. The results of the study are given in the Table 3.

Coppage *et al*., (1975) have observed similar inhibition of acetylcholinesterase activity in the brain tissue of fish exposed to malathion. Singh and Kumar (2000) have reported decrease in acetylcholinesterase activity in *Catla catla* exposed to malathion. Glusczak *et. al*., (2007) have observed reduction in acetylcholinesterase activity in brain and no change was seen in muscle tissue of *Rhamdia quelen* exposed to glyphosate. There was significant reduction in the AchE activity in brain of fish, *Leporinus obtusidens* exposed to 5ppm of Roundup, but there was no change in muscle (Glusczak *et .al*., 2006).

**Table 3: AChE activity levels (µm acetylcholine hydrolyzed / mg / protein /h) in the tissues of *Channa punctatus* on exposure to sublethal and lethal concentrations of Diclofenac**

| Tissues | Control | Sublethal | Lethal |
|---|---|---|---|
| Brain | 6.55 ± 0.019 | 5.12 ± 0.022<br>21.83% | 3.81 ± 0.034<br>41.83% |
| Muscle | 5.67 ± 0.036 | 5.24± 0.029<br>7.58% | 4.42 ± 0.031<br>22.04% |
| Gill | 4.83 ± 0.021 | 4.61 ± 0.030<br>4.55% | 3.98 ± 0.052<br>17.59% |
| Liver | 4.76 ± 0.024 | 4.57 ± 0.033<br>3.99% | 3.94 ± 0.017<br>17.22% |
| Kidney | 4.25± 0.045 | 4.13 ± 0.037<br>2.82% | 3.86 ± 0.032<br>9.17% |

Diafuran has inhibited the acetylcholinesterase activity in the brain and muscle of three carps *Cyprinus carpio*, *Ctenopharyngodon idella*, *Aristichthys nobilis* at all the set concentrations (Golombieski *et. al*., 2008). There was reduction in the acetylcholinesterase activity in brain, muscle, gill and liver on day 14 in both sublethal concentrations of Chloropyriphos in *Cyprinus carpio* (Halappa and David, 2009). Thirumalavan and Sankar (2010) have noticed decrease in the acetylcholinesterase activity in the brain tissue of the arsenic treated *Catla catla*. Palaniswamy *et. al*., (2011) have noticed decline in the activity of acetylcholinesterase enzyme in muscle, liver and gill of *Channa striata* after exposure to plant extract of *Cleistanthus collinus*. There was significant deacrease in the activity of AChE exposed to Chloropyriphos and Carbendazim (Palaniswamy*et. al.,* 2014). Neelima *et. al*., (2015) have noticed maximum inhibition of AChE in brain and minimum in kidney on exposure to cypermethrin in tissues of *Cyprinus carpio.*

The inhibition of this enzyme in the liver may be due to the synergistic action of the parent compound and its metabolites (Casida *et. al.,* 1983). The inhibition of the AChE activity results in buildup of acetylcholine causing prolonged excitatory postsynaptic potential. The effect of toxicant on the neurotransmitter may result from their action or sub cellular process such as interference with mechanism regulating calcium distribution in nerve terminals and anabolic effect that may occur as a result of impairment of energy production or inhibition of enzymes involved in the synthesis and storage of transmitters. This might be due to alterations in cholinergic system in the tissues exposed to toxicant (Sarkar *et al*., 1998).

The inhibition of AchE was accompanied by an increase in acetylcholine levels. This condition could lead to the increase of catecholamines which would affect the activity of the enzymes involved in carbohydrate metabolism. The stress caused due to this may affect the synthesis site of AchE or decrease the levels of excess AChE. The inhibition of AChE results in build-up of acetylcholine within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission (Meilson *et. al.,* 1998).

The results in the present study suggests that drug might have blocked the active site of the enzyme and inhibited its synthesis. The decline in the AChE activity may be due to the alterations in the cholinergic system induced by drug toxicity. The inhibition in acetylcholinesterase activity may be due to decrease in cholinergic receptor binding due to the effect of the toxicant.

## 4. Conclusion:

Diclofenac enters into body of fish and causes changes in the metabolic activities of the enzymes. The study has revealed that there was inhibition in the activities of both the succinate dehydrogenase and acetylcholinesterase enzymes. The pharmaceutical residues in the aquatic environment alter the biochemical composition of the non-target organisms like fish.

## Competing Interests

The authors have declared that no competing interests exist.

## References


[1]. Skoutakis V.A, Carter C.A, Mickle T.R, Smith V.H. and Arkin C.R, Alissandratos J, Petty D.E., 1988; Review of diclofenac and evaluation of its place in therapy as a nonsteroidal antiinflammatory agent..Drug Intell&Clin Pharm, 22 (11): 850-859.

[2]. Brogen R.N, Pakes G.E, Speight T.M, Avery G.S. 1980; Diclofenac sodium: a review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin. Drugs, 20: 24-48.

[3]. Buser H.R, PoigerT, and Müller M.D. 1998; Occurrence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake. Environ. Sci. Technol, 32 (22): pp 3449- 3456.

[4]. Cuklev F, Kristiansson E, Fick J, Asker N,Förlin L, Larsson D.G.J. 2011; Diclofenac in fish: Blood plasma levels similar to human therapeutic levels affect global hepatic gene expression. Environ toxicology and chemistry, 30 (9): pp 2126-2134.

[5]. Nagaraju B. and Venkatarathnamma V. 2013; Effect of Profenofos an organophosphate on protein levels in some tissues of fresh water fish, *Labeorohita* (Hamilton). International Journal of Pharmacy and Pharmaceutical Sciences. 5(1), pp 276-279.

[6]. SavithaYadav Rao W and Wani G.P. 2014; Effect of polo (an insecticide) on protein contents of liver and gonad of fresh water fish, *Labeorohita*. International research Journal of Pharmacy. 5(10) pp 792-794.

[7]. Nachlas MM, Margulius SD and Seligman AM. A colorimetric method for estimation of succinic dehydrogenase activity. Journal of Biological Chemistry, 1960; 235: 499-504.

[8]. Ellman GL, Courtney KD, Andres VJ, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. BiochemPharmacol 1961; 7:88-95.

[9]. Sastry KV, Siddiqui AA (1982) Chronic toxic effects of the carbamate pesticide Sevin on carbohydrate metabolism in a freshwater snakehead fish,*Channa punctatus*. ToxicolLett 14:123–130.

[10]. Ramakritinan CM, Kumaraguru AK, Balasubramanian MP. Impact of Distillery Effluent on Carbohydrate Metabolism of Freshwater Fish, *Cyprinus carpio* Ecotoxicology, 2005, 14 (7), 693–707.

[11]. Singh KS, Singh SKS, Yadav RP. Toxicological and biochemical alterations of cypermethrin (synthetic pyrethroid) against freshwater teleost *Colisafasciatus*at different seasons. World J Zool 2010; 5(7):25-32.

[12]. Suneetha K. Effects of endosulfan and fenvalerate on carbohydrate metabolism of the fresh water fish, *Labeorohita* (Hamilton). Int J Pharm PharmSci, 2012; 4 (1): 262-268.K. Moorthikumar and M. Muthulingam. Impact of heavy metal nickel chloride on enzyme succinate dehydrogenase of freshwater fish *Labeorohita*(Hamilton). International Journal of Current Research 3( 7), .115-119, 2011.

[13]. Madhusudan Reddy N , Ghousia Begum, Rajender K and Venkateshwara Rao J. 2012. Toxic impact of two organophosphate insecticides on biochemical parameters of a food fish and assessment of recovery response. 28 ( 4) : 343-352.

[14]. Fernandes S, Fernandes S, Seema Das, Gupta RP. Toxicity study of lead nitrate on freshwater fish *Cirrhina mrigala.* International Journal of Advanced Research (2016), 4, (6) 1601-1608.

[15]. Lakshmaiah G. Concentration related differences in the inhibition of acetylcholinesterase activity in the common carp *Cyprinuscarpio*by Phorate, an organophosphorous insecticide. *International Journal of Advances in Scientific Research* 2016; 2(04): 083-088.

[16]. Tripathi G and Priyanka V . Fenvalerate induced changes in a catfish, *Clarias batrachus*: metabolic enzymes, RNA and protein. Comparative Biochemistry and Physiology part C., 2004; 138(1): 75-79.

[17]. More, T.G., Rajput, R.A. and Bandela, N.N. 2005. Effect of heavy metal on enzyme succinic dehydrogenase of freshwater bivalve, *Lamellidenus marginalis. Poll. Res*.,24: 675-679.

[18]. Bag S, Vora T, Ghatak R, Nilufer I, D'mello D, Pereira L, Pereira J, Cutinho C, Rao V. A study of toxic effects of heavy metal contaminants from sludge-supplemented diets on male Wistar rats. Ecotoxicol Environ Saf. 1999 ;42(2):163-70.

[19]. Kopecka J, Rybakowas A, Barsiene J, Pempkowiak J. AChE levels in mussels and fish collected off Lithuania and Poland (Southern Baltic). Oceanologia 2004; 46:405-418.

[20]. Coppage DL, Mathew G, Cook GH, Knight J. Brain acetylcholinesterase inhibition in fish as a diagnosis of environmental poisoning by malathion, o, o–dimethyl s-(1.2 dicarbetaoxyethyl) Phosphoro–dithoate. *Pesticide Biochemistry and Physiology* 1975; 5 (6): 536-542.

[21]. Manju, S and Santosh, K (2000). Effects of sublethal concentrations of dimethoate and malathion on AChE activity and enzyme kinetics of liver of *Catlacatla* (ham.). Uttar Pradesh J. Zool., 20: 131-135

[22]. Glusczak L, Miron Ddos S, Moraes BS, Simões RR, Schetinger , Morsch VM, Loro VL. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*) Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 146 (4) 2007, 519-524.

[23]. Glusczak L, dos Santos Miron D, Crestani M, Braga da Fonseca M, de Araújo Pedron F, Duarte MF, Vieira VL. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinusobtusidens*). Ecotoxicol Environ Saf. 2006 ;65(2):237-41. 2005.

[24]. Golombieski JI; Marchesan E; Camargo ER ; Salbego J; Baumart JS; Loro VL; de Oliveira Machado SL; Zanella R; Baldisserotto B. Acetylcholinesterase enzyme activity in carp brain and muscle after acute exposure to diafuran Sci. Agric. (Piracicaba, Braz.), v.65, n.4, p.340-345, 2008.

[25]. Halappa R, David M. In vivo inhibition of acetylcholinesterase activity in functionally different tissues of the freshwater fish, *Cyprinuscarpio*, under chlorpyrifos exposure. Drug Metabol Drug Interact. 2009; 24(2-4):123-36.

[26]. Thirumavalavan, R and Sankar Samipillai, S. Arsenic induced biochemical changes in the brain tissue of fresh water fish, *Catlacatla.* International Journal of Current Research Vol. 1, pp. 030-034, January, 2010.

[27]. Palanisamy P, Mallikaraj D, Sasikala G, and Natarajan GM. Acetylcholine esterase, acetylcholine, succinic dehydrogenase and lactic dehydrogenase changes in the fresh water food fish *Channa striata* after exposure to *Cleistanthus collinus* suicidal plant extract. 9, (1) 2011; 017.

[28]. Palanikumar ,L, . Kumaraguru AK, . Ramakritinan CM, Anand M. Toxicity, biochemical and clastogenic response of chlorpyrifos and carbendazim in milkfish, *Chanos chanos*. Int. J. Environ. Sci. Technol. (2014) 11:765–774.

[29]. Neelima P, Govinda Rao K, Gopala Rao N, Chandra Sekhara Rao J. Enzymatic alterations as biomarkers of Cypermethrin (25%EC) toxicity in a freshwater fish, *Cyprinus carpio*(Linn.) International Journal of Fisheries and Aquatic Studies 2015; 3(1): 149-158.

[30]. Casida JE, Gammon DW, Glickman AH, Lawrence LJ. Mechanisms of selective action of pyrethroid insecticides. Annual Review Pharmacology and Toxicology 1983; 23:413-438.

[31]. Sarkar, S., Yadav, P. and Bhatnagar, D. 1998. Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: a study with relation to time. Biometals, 11: 153- 157

[32]. Mileson BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT. Common mechanism of toxicity: A case study of organophosphorus pesticides. Toxicol Sci. 1998; 41:8-20.