



Studies on the effect of chlorpyrifos on the biochemical parameters of African catfish *Clarias garaepinus* (Burchell 1822)

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ABSTRACT

Organophosphorus pesticides are used in agricultural areas. They are one of the most commonly used pesticides having potentially harmful toxic effects, introduced into the environment and human ambient. These pesticides areas utilized widely to ensure agricultural harvests against the harm brought about by different types of agricultural pests. Biochemical factors show explicit reactions to specific types of ecological pressure and can be used as a potential biomarker to study the impact of environmental conditions on the physiological processes of the organism. Keeping in view, the present study was undertaken to investigate the effect of CPF on protein and cholesterol levels in African catfish, *Clarias gariepinus*. The results of the present study showed that there was a decrease in the level of protein and cholesterol in the fishes exposed to different sublethal concentrations of CPF for 15 days as compared to the control. Based on the results, it can be concluded that the CPF alters the metabolic activity of African catfish, *Clarias gariepinus* (Burchell, 1822) in response to stress caused by the CPF.

Keywords: Biochemical markers, *Clarias garaepinus* (Burchell 1822), chlorpyrifos, aquatic toxicity, protein, and cholesterol.

INTRODUCTION

An aquatic ecosystem is one of the most important ecosystems in our environment. A large number of pollutants are released into the aquatic ecosystem since it represents the ultimate destination for most anthropogenic contaminants. Communities of organisms that are dependent on each other and their environment live in the aquatic ecosystem. Pesticides disturb the balance between the ecosystem and the species that belong to the particular ecosystem. Fishes are an important part of the aquatic ecosystem, as they interact closely with the physical, biological, and chemical environment. Pesticides are responsible for a large number of fish

mortality in the rice field since fishes inhabiting the aquatic environment of the rice field directly come in contact with the pesticides. Pesticides not only affect the fish but also food webs related to them.

A survey was conducted to examine the influence of pesticides on the aquatic community in West Bengal (India). Many body tissues of the fish such as gills, alimentary canal, liver, and brain of carps and catfishes were found drastically damaged by pesticides. It has been reported that such a level of pesticides in fish could harm the fish consumers as well (Konar, 2011).

In humans, several acute and chronic diseases have been associated with pesticide exposure. Evaluation of acute toxicity is essential for the determination of the severity of animals to the toxicants and also to know the degree of damage to the target organs. Pesticides produce many physiological, biochemical, and behavioral changes in freshwater particularly those of fishes, by influencing the activities of several enzymes and hormones (Radhaiah *et al.*, 1987). By acting on the enzymes involved in the metabolic pathway of several biochemical reactions, these toxicants alter the biochemical and physiological processes of organisms. Thus, it is important to study the toxic effects of environmental contaminants at the biochemical level also. Chlorpyrifos is one of the most widely used organophosphorus insecticides in the world (Fig.1).

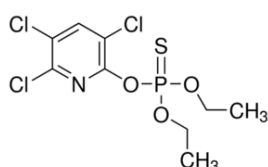


Fig. 1: Chemical structure of Chlorpyrifos.

The primary target organ for chlorpyrifos toxicity is the central and peripheral nervous system, due to the ability of the chlorpyrifos-oxon metabolite to inhibit the enzyme activity of acetylcholine-esterase, which terminates neurotransmission at cholinergic synapses. Chlorpyrifos is not considered to be teratogenic at doses that do not cause frank maternal toxicity.

Although mutagenicity and chronic animal bioassay for the carcinogenicity of chlorpyrifos were largely negative, a recent epidemiological study of pesticide applicators reported a significant exposure-response trend between chlorpyrifos use, lung and rectal cancer.

Therefore, the present study was undertaken to investigate the effect of chlorpyrifos on the protein and cholesterol concentration of freshwater African catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

For the present study, the chemical chlorpyrifos 20% EC with the commercial name Yalban was purchased from the local market.

Test animal

For the present study, the freshwater African catfishes, *Clarias gariepinus* (Burchell, 1822), were selected because of their availability in the local market and their convenient size. It could be safely transported and maintained easily under laboratory conditions because of its air-breathing habit, its hardy nature, moreover suits the experimental work. All the fishes used during the present study were brought from the local market. The bodyweight of fishes ranged between 250-350 gm and their length varied between 30-37cm. The fishes were maintained in glass aquaria containing 30L of tap water, under normal conditions of light and temperature. The fishes were fed with fish food every alternate day and water changed at an interval of one day. The fishes were acclimatized for one week by keeping 8 fishes, (4 males and 4 females) in one aquarium before their use in the experiment.

Experimental setup

The four aquaria were taken, filled with 30L tap water, and in each aquarium 4 male and 4 female fishes were kept. The fishes without exposure to any toxicant in one aquarium were treated as a control group and other aquaria exposed to toxicant were treated as experimental group and labeled accordingly. The fishes from the experimental group exposed to two sub-lethal concentrations (0.5 and 1.5 mg/L) of chlorpyrifos for 15 days.

Biochemical estimation of protein and cholesterol

For biochemical estimation of cholesterol and protein, the fishes from both the control and experimental group were anesthetized with the 2-phenoxyethanol. After anesthetization, blood was collected in a non-EDTA tube by cutting the caudal fin to collect the serum. For the estimation of serum, protein, Lowry's (1951) method and for cholesterol estimation Zake's *et al.*, (1953) method were used.

RESULTS

Effect of chlorpyrifos on cholesterol concentration

The concentration of cholesterol in serum of fish, *Clarias gariepinus* (Burchell, 1822) from the experimental group exposed to sublethal concentrations of CPF showed a decrease in the cholesterol concentration in both males and females as compared to the control group (Fig. 1 and 2). The cholesterol concentration of male fish from the control group showed 256.83 ± 20.22 mg/dl whereas the cholesterol concentration of

male fish was exposed to 0.5mg/L, and 1.5mg/L chlorpyrifos (CPF) showed 169.28 ± 20.32 mg/dl, and 122.16 ± 18.55 mg/dl respectively. (Table 1).

Similarly, the cholesterol concentration of female fish from the control group showed 302.46 ± 6.02 mg/dl whereas the cholesterol concentration of female fish exposed to 0.5mg/L, and 1.5mg/L CPF showed 250.99 ± 6.84 mg/dl and 217.5 ± 8.2 mg/dl respectively (Table 2).

Table 1: Cholesterol concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days

Group	Concentration of cholesterol (mg/dl) in Male
Control	256.83 ± 20.22
0.5mg/L CPF	169.28 ± 20.32*
1.5 mg/L CPF	122.16 ± 18.55**

(Values are expressed in Mean ± SE) N=8 for each group, p<0.05*, p<0.001**, p<0.0001***)

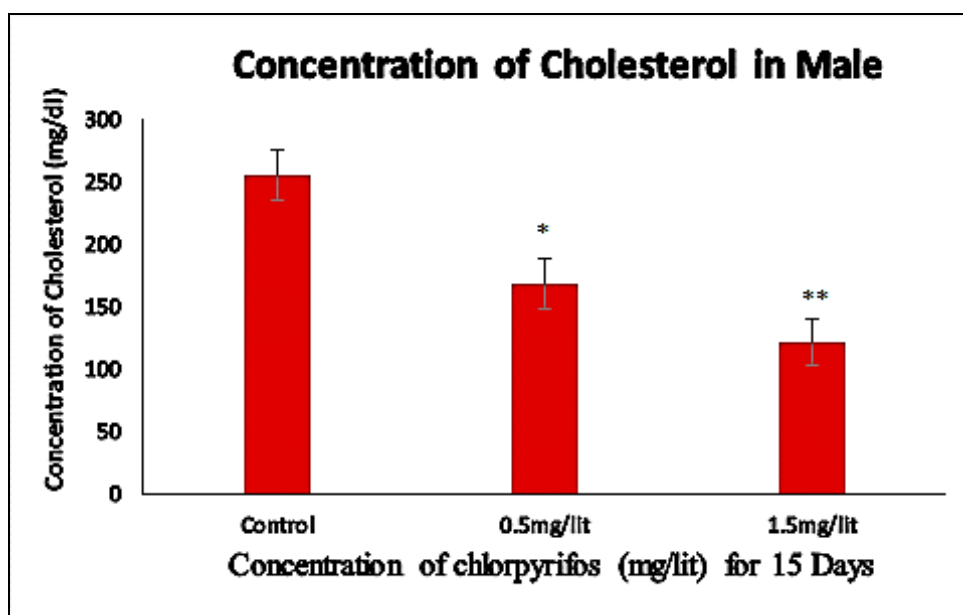


Fig. 1: Cholesterol concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days.

Table 2: Cholesterol concentration in Female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days

Group	Concentration of cholesterol (mg/dl) in Female
Control	302.46 ± 6.02
0.5mg/L CPF	250.99 ± 6.84**
1.5 mg/L CPF	217.5 ± 8.2**

(Values are expressed in Mean ± SE) N=8 for each group, p<0.05*, p<0.001**, p<0.0001***)

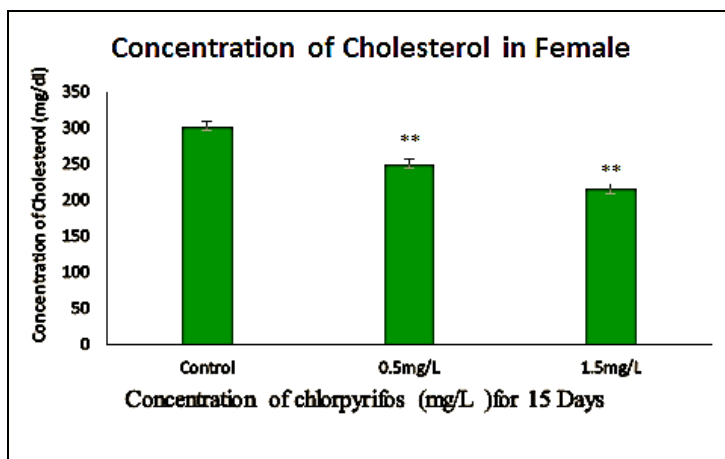


Fig. 2: Cholesterol concentration in female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days.

Effect of Chlorpyrifos on protein concentration

The concentration of protein in the serum of fish, *Clarias gariepinus* (Burchell, 1822) from the experimental group showed a decrease in the protein concentration in both males and females as compared to the control (Fig. 3 and 4). The protein concentration of male fish from control group showed 18.91 ± 2.3 mg/dl whereas the protein concentration of male fish exposed to 0.5mg/ L, and 1.5mg/ L chlorpyrifos (CPF)

showed 11.54 ± 0.88 mg/dl, and 8.01 ± 0.85 mg/dl respectively. (Table 3).

Similarly, the protein concentration of female fish from the control group showed 22.74 ± 2.53 mg/dl whereas the protein concentration of female fish exposed to 0.5mg/L, and 1.5mg/ L CPF showed 9.57 ± 0.44 mg/dl and 7.49 ± 0.78 mg/dl respectively (Table 4).

Table 3: Protein concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days

Group	Concentration of Protein (mg/dl) in Male
Control	18.91 ± 2.3
0.5mg/L CPF	$11.54 \pm 0.88^*$
1.5 mg/L CPF	$8.01 \pm 0.85^*$

(Values are expressed in Mean \pm SE) N=8 for each group, $p < 0.05^*$, $p < 0.001^{**}$, $p < 0.0001^{***}$)

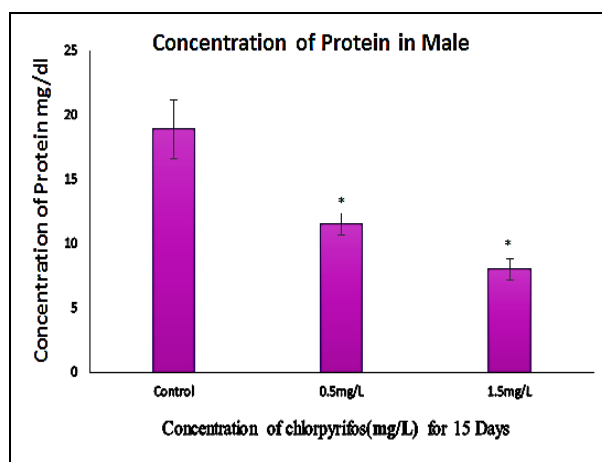


Fig. 3: Protein concentration in male fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days

Table 4: Protein concentration in female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days

Group	Concentration of protein (mg/dl) in Female
Control	22.74 ± 2.53
0.5mg/L CPF	9.57 ± 0.44**
1.5 mg/L CPF	7.49 ± 0.78**

(Values are expressed in Mean ± SE) N=8 for each group, p<0.05*, p<0.001**, p<0.0001***)

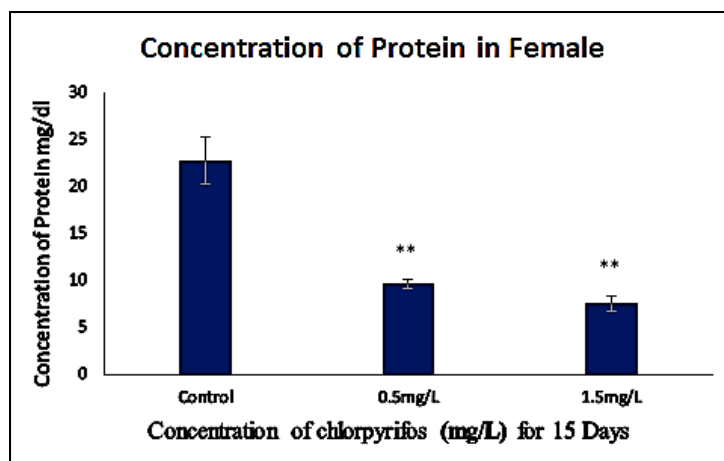


Fig.4: Protein concentration in Female fish, *Clarias gariepinus* (Burchell, 1822) exposed to different concentrations of chlorpyrifos for 15 days.

DISCUSSION

Proteins are important biomolecules in the biological system made up of a large number of amino acids. Proteins play an important role as energy precursors for fish and other organisms under stress conditions. Proteins are of prime importance to the living world not only because of their peculiarity but also since they appear to confer their biological specificity among various types of cells. Changes in each of the blood components have been employed as useful bioindicators of stress in teleosts (Das *et al.*, 2004) to investigate the susceptibility of organ systems to various contaminants including pesticides.

In the present study, it has been found that there was a decrease in the level of protein in the group of fishes exposed to different concentrations of CPF for 15 days as compared to the control. Similar results were also recorded by the Narra *et al.*, (2011), Ghayyur *et al.*, (2019), Verma and Rawat, (2017), Ramesh, and Saravanan, (2008), Revathi, *et al.*, (2020), Revathi and Krishnamurthy, (2018), Deshmukh, (2017), Agrhari *et al.*, (2006), Somaiah *et al.*, (2014), Tilak *et al.*, (2001), Tilak *et al.*, (2009), Chezhan *et al.*, (2010).

In many organisms, toxic substances bring about a kind of stress and an organism responds to that by developing necessary potential. During stress, organisms need sufficient energy which is supplied from reserve food material i.e. protein, glycogen, and lipid (Hyalij, 2013). Toxicants such as pesticides make genuine disability the physiological and wellbeing status of fish.

Carbohydrates, protein, and lipid which are the major components of the animal's body, play an important role in body construction and energy metabolism (Shoba *et al.* 2011) especially proteins. Proteins play a crucial role in virtually all biological processes. They are important biomolecules involved in a wide spectrum of cellular functions (Prasanth, 2006). These constituents are affected by many factors especially by pesticides (Jabakumar and Jayaraman 1988). Investigations on the effects of pesticides have revealed that pesticides interfere with energy reserve metabolism in different species (Babu *et al.* 1988).

Under extreme stress caused by various toxicants, glucose which is a primary source of energy, protein, and cholesterol supply energy in metabolic pathways and biochemical reactions to fulfill the demand for

energy to cope with the stress conditions (Ganeshwade, 2011). In the present study, a decrease in protein concentration might be due to the impairment of protein synthesis or an increase in the rate of its degradation to amino acid caused by the CPF (Deshmukh, 2017 and Tulasi and Rao 2013). This might be fed to TCA cycle aminotransferase to cope with high energy demands to meet the stress condition (Revathi *et al.*, 2020 and Prasad and Veeraiah, 2002). According to Saxena *et al.*, (1989), the decrease protein level after exposure to the toxicants due to the reduced protein synthesizing capacity of the liver of fish. Therefore, the CPF may alter or decrease the protein-synthesizing capacity of the liver of *C. gariepinus* which in turn limits the synthesis of protein synthesis and protein concentration in the body.

Cholesterol is an important normal body constituent used in the structure of cell membrane, synthesis of bile acid, and synthesis of steroid hormones. Measurement of the level of total cholesterol includes identifying all types or classes of cholesterol found in the system. Intestinal cholesterol absorption plays a major role in maintaining total body cholesterol homeostasis. In the present study, it has been found that there was a decrease in the level of cholesterol in the group of fishes exposed to different concentrations of CPF for 15 days as compared to the control. Similar findings were also reported by the Zade *et al.*, (2020), Logaswamy and Remia, 2009, Shakoori *et al.*, (1996), Jee *et al.*, 2005, Jipsa *et al.*, (2014), Lakshman *et al.*, (1988) and Riaz, (2018). The reduced cholesterol level due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol (Gijare and Tantarapale, 2014). It could be due to stress caused by the toxicants which suppress the lipid metabolism and leads to a decrease in the value of cholesterol (Choudhary and Gaur 2001 and Shinde *et al.*, 2002). Therefore, in the present study, the decreased level of cholesterol observed in the group of fishes exposed to different sublethal concentrations of CPF could be due to either inhibition of cholesterol biosynthesis in the liver or stress caused by the CPF.

CONCLUSION

The present study concludes that the CPF has marked effects on the biochemical parameters of the fish

which ultimately causes the disturbance in the normal physiological processes and homeostasis.

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Conflict of Interest

The author declares that there is no conflict of interest.

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