GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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CARBARYL INDUCED ALTERATIONS IN HISTOLOGY AND CERTAIN BIOCHEMICAL PARAMETERS IN LIVER OF *CLARIAS BATRACHUS*

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ABSTRACT
Despite proven toxicity to non target organisms, farmers still find the pesticide as the most effective means to protect the crop. Carbaryl (1-naphthyl methylcarbamate) is among the most widely used carbamate pesticide and appears frequently in surveys of aquatic systems. Hence effects on the histological and some biochemical parameters of liver in fishes were investigated. Healthy *Clarias batrachus* was collected from nearby wetland and acclimatized in laboratory conditions. LC_{50} of carbaryl was determined through probit analysis. Fishes were exposed to two sub lethal doses (2mg/l & 4mg/l) of the pesticide. Usual procedure for histological examination was followed. Biochemical estimation was done with autoanalyzer. After exposure the histological examination revealed necrosis, hepatic cord distortion, cellular damage etc. Consistent with these changes were the alterations in serum levels of enzymes found in the liver. SGOT, SGPT and alkaline phosphatase showed significant elevation in their levels in the serum. Corresponding to the increase in the activities of these enzymes there occurred reduction in protein content including albumin and globulin. A small change in the cholesterol level was also noticed indicating hepatic insufficiency.

KEY WORDS: *Carbamate, Genetic base, Xenobiotics, Hepatocytes, Necrosis.*

INTRODUCTION

One of the several contradictions prevailing in modern society is the application of pesticides to ward off the pests from plants. Pesticides are a group of heterogeneous compounds with proven toxicity and serious implications for man, animals, and the environment, still they are used regularly world over in agriculture and health programmes. Of course its contribution in increasing the yield has been great. It is also true that more than 2 lakh people die of pesticide poisoning every year. Furthermore despite caution from scientific world, it is used indiscriminately by farmers under the notion that "if little is good, a lot more will be better". The greed to obtain the optimum yield induced rampant use of pesticides and this played havoc with environment as well as with human and other forms of life. These have penetrated into each and every part of the environment in one or other form and have led to the degradation of environment including the aquatic system. If the credits of pesticides include enhanced economic potential in terms of increased production of food and fibre and amelioration of vector borne diseases then their debits have resulted in serious health implications for man, animals, and the environment. The aquatic ecosystem as greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman *et al*., 2002).It is clear from the kinetics of pesticides that a large part of it applied in field reaches the water bodies by surface run off, sedimentary transport and inflicts injuries to its biodiversity. Fishes are among the most vulnerable fauna in the water bodies that are affected by chemical pollutants. They are particularly sensitive to the influence of pesticides and other toxic pollutants because they are able to uptake and retain the dissolved xenobiotic in water and thus a good indicator of the health status of aquatic bodies. However now it has been possible to assess the relative well being of the fishes and use them as indicator of the relative well being of the aquatic system.Assessment can be performed at different levels of organisation, from whole fish communities (*e.g.* fish assemblages) down to the molecular level (*e.g.* gene expression).However, many of the tests conducted are subjective and do not prove sufficient in environmental hazard evaluation. In terms of present utility biochemical tests are ranked higher and crucial in determining changes that may occur in fishes. The changes may be of some value in assessing the impact of exposure under natural conditions and may also serve as tools for biological monitoring.

MATERIALS & METHODS

The experimental fish *Clarias batrachus* were collected from a nearby wetland in Samastipur (Bihar). The fishes were sorted out by visual examination for their maturity. They were acclimatized in the laboratory conditions for 15 days in the glass aquarium (120 cm×45 cm×45 cm) filled with unchlorinated, tap water (pH 7.0 ± 0.13, free carbon dioxide 10.57 ± 2.1 mg/1, dissolved oxygen 5.85 ± 0.92 mg/l, total alkalinity 150 ± 5.07 mg/l as CaCO3 and hardness 116 ± 6.70 mg/l as CaCO3) & having aeration facilities. The fishes were fed with a mixture of trash fish and rice bran at 9:1 proportion at about 10% of the body weight of the stocked fish daily. Technical grade samples of Carbaryl (99% W/W) was used. Since it is not completely soluble in water little of acetone was used as solvent. The dose mortality studies were conducted for 96

hrs and LC_{50} value was calculated using Probit method. The LC_{50} values of Carbaryl to fish *Clarias batrachus* through other methods are almost similar. Hence value obtained through computer was employed for all further derivations. Two sub-lethal concentrations were selected for experiment. For conducting biochemical studies fishes were exposed to sub-lethal concentrations of the test chemical. Two aquaria were set up for each concentration and each aquarium contained 10 fish in 60 liter dechlorinated tap water. Fishes were left (Monteiro *et al*., 2006) to discard the metabolic wastes (Roy and Bhattacharya, 2006). Similar number of fishes was maintained as control. After exposure for 96 hrs and before sacrifice signs of toxicity like inactive movement, increase in ventilation rate and decrease in sensibility to external stimulus were recorded. Blood samples were collected from fish by cardiac puncture with 1ml non-heparinized injection needle. Blood was collected in appendorf tubes and was allowed to coagulate. Samples were centrifuged at 3000 rpm for 15 minutes and the supernatant serum was used for biochemical analysis. All biochemical tests were determined using the automatic analyzer. 10 ml of serum sample was loaded for each test. Quantification of protein was done through Folin-Lowry's method and determined spectrophotometrically at 750nm. Liver was excised, cut into pieces fixed and subjected to usual procedure for histological examinations

RESULTS

Carbaryl, a carbamate pesticide is most frequently used by farmers for control of varied pests. The LC_{50} value obtained for the experimental fish was 15.08 mg/l for 96 hr .The exposure to sub-lethal concentrations (2 and 4 mg/l) of the carbaryl for 96 hr induced perturbations in the levels of certain biochemical components including the activities of some enzymes in the blood and liver of the catfish used. The liver of the control showed normal histology whereas in *Clarias* exposed to a safe dose of the carbamate pesticide it exhibited varying degrees of histopathological changes including cytoplasmolysis, nuclear pyknosis, and necrosis. In some regions of liver, there occurred extensive degeneration of proliferated hepatocytes, in close proximity to blood sinuses. Apart from this, the rupturing of blood sinus causing invasive infiltration of leukocytes was also observed. Corresponding to cellular damage, a significant decrease in hepatosomatic index was also recorded (fig.1)

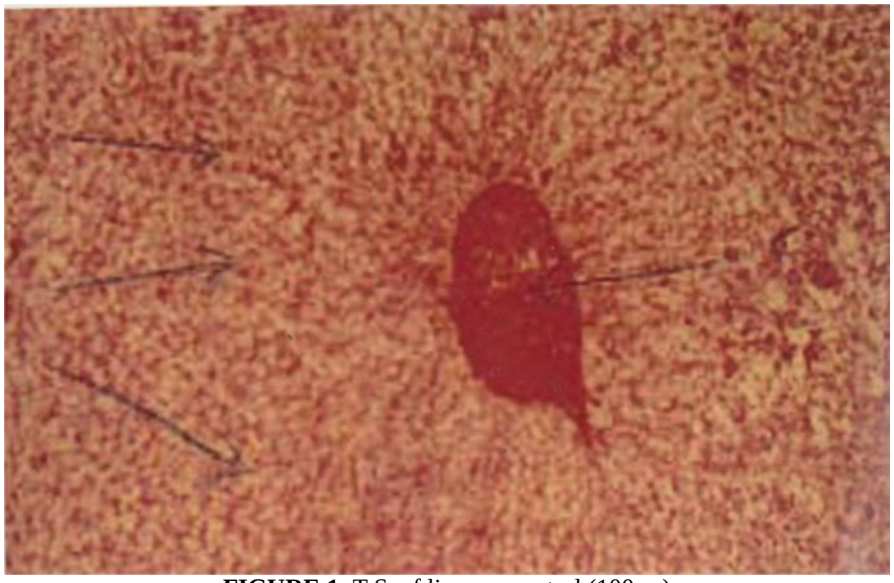

FIGURE 1: T.S. of liver on control (100 xs)

Most remarkable of alterations in biochemical profile has been the changes in the levels of some crucial enzymes and fall in the protein and cholesterol levels. The SGOT in control fish was 150.33 ±7.50 IU/l but it increased to a level of 159.52 ± 8.01 IU/l after with 2mg/l and 162/l with 4mg treatment. The increase in SGPT value is even more pronounced. The values obtained with 2mg/l and 4mg/l treatments were 47.30 ± 4.83IU/l and48±7.22IU/l respectively while in control forms it was recorded to be 27.45IU/l. (Table 1& 2). On the other hand the rise in transaminases has been reciprocated with decrease in the protein content throughout the exposure period. In comparison to 6.2 ± 0.49 g/dl recorded in control fishes the protein content receded down to 4.00 ± 0.59 & 3.12 ± 0.49 after 2mg/l & 4mg/l carbaryl treatment in experimental groups respectively. In line with protein the albumin level also decreased from 2.10 ±0.28 in control group to 1.10±0.25 in fishes treated with 2mg/l carbaryl and to 0.78 ± 0.55 after exposure to 4mg/l carbaryl. Similarly the amount of globulin recorded in control group was 5.12 ± 0.39 but after exposure to two sub lethal concentrations (2 & 4mg/l) of the pesticide it receded to 3.0 ± 0.59 and 1.76 ± 0.68 respectively. However, very little change was recorded in the serum cholesterol level during exposure period. The cholesterol level in fishes of control group was 218.35 ± 5.08 whereas after exposure to 2 mg/l carbaryl the value receded to 206.45 ± 7.04 and fishes treated with 4mg/l carbaryl showed a value of 195 ± 6.45

TABLE 1. Biochemical parameters of *Clarias batrachus* on exposure to Carbaryl at 2mgl/1

Indices	Unit	Groups	N	Mean ± SD
SGOT	IU/l	Control	10	150.33 ±7.50
		Experimental	10	159.52 ±8.01
SGPT	IU/l	Control	10	27.45 ± 5.12
		Experimental	10	47.30 ± 4.83
Serum alkaline phosphatase	IU/l	Control	10	42.97 ±3.88
		Experimental	10	135.38 ±5.67
Serum Protein	g/dl	Control	10	6.2±0.49
		Experimental	10	4.00 ±0.59
Serum albumin	g/dl	Control	10	2.10 ±0.28
		Experimental	10	1.10 ±0.25
Serum globulin	g/dl	Control	10	5.0 ±0.69
		Experimental-	10	3.1 ±0.59
Serum cholesterol	mg/dl	Control	10	218.35 ±5.08
		Experimental	10	206.45 ± 7.04

TABLE 2. Biochemical parameters of *Clarias batrachus* on exposure to Carbaryl at 4 mgl/1

Indices	Unit	Groups	N	Mean ± SD
SGOT	IU/l	Control	10	150.33 ±7.50
		Experimental	10	154 ±9.39
SGPT	IU/l	Control	10	27.45 ± 5.12
		Experimental	10	48 ± 7.22
Serum alkaline phosphatase	IU/l	Control	10	42.97 ±3.88
		Experimental	10	138 ± 4.34
Serum Protein	g/dl	Control	10	6.2± 0.49
		Experimental	10	3.12 ± 0.49
Serum albumin	g/dl	Control	10	2.10 ±0.28
		Experimental	10	0.78 ±0.55
Serum Globulin	g/dl	Control	10	5.0 ±0.69
		Experimental-	10	1.76 ±0.68
Serum cholesterol	mg/dl	Control	10	218.35 ±5.08
		Experimental	10	195 ± 6.54

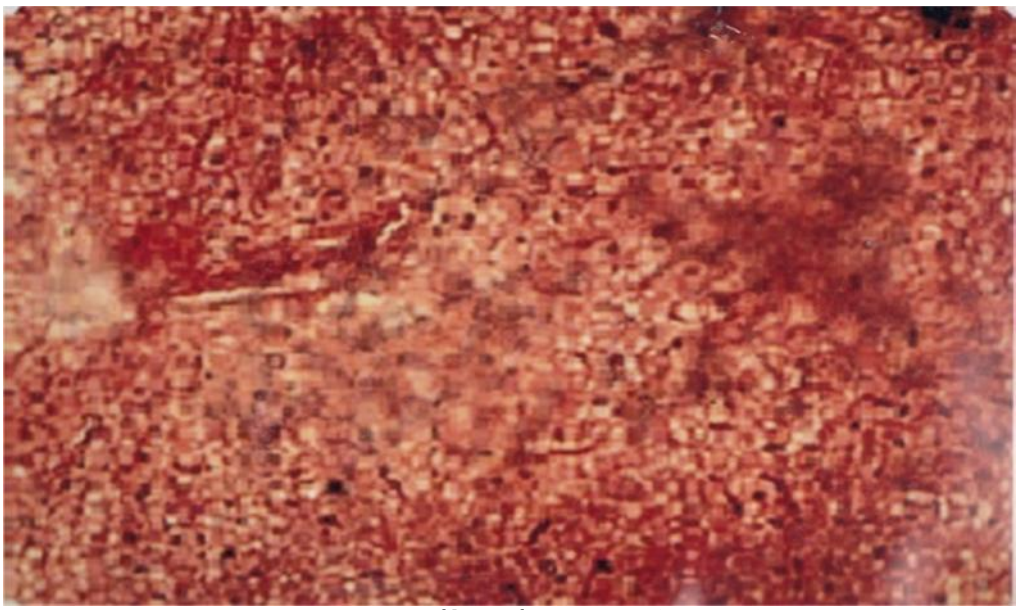

FIGURE 2: T.S. of liver after exposure (HE 400x)

DISCUSSION

Carbaryl (1-naphthyl methylcarbamate) is among the most widely used carbamate pesticides and appear in surveys of aquatic system and atmospheric transport (Munn *et al*., 2006, Daly et al 2007) . The pesticide has been reported to hurt the aquatic fauna in varied ways and at varied liver. Such is the case with fishes too. The liver plays a vital role in the metabolic processes of the body. These processes can broadly be classified as synthesis, storage and

excretion. It is clear from the present investigation that the pesticide could cause extensive damage to liver and put the fish under acute stress. From the present investigation it is clear that the changes in the biochemical parameters are consistent with damages at histological levels. The treatment of the fish with carbaryl led to a marked increase in the activities of transaminases (SGOT and SGPT), phosphatases (acid and alkaline) in the fish serum, the magnitude of the effect being dependent on the pesticide concentration and duration of exposure. These enzymes are found in liver and are clear indication of liver dysfunction. Serum Glutamate Oxaloacetate Transaminase (SGOT) is a mitochondrial enzyme whereas SGPT is a cytosolic enzyme. Their rise in the serum level is the testimony of the hepatic insufficiency consequent upon the cellular damage observed in course of histological examination. This is in agreement with the findings of Patil and Radhakrishnamurty (1979) who observed that these enzymes are enhanced when there is inflamation, degeneration and neoplastic lesions in liver. However, contrary to observation of Lusokova *et al*. (2002) on *Lepidocephalichthys thermalis* with diazinone and Sastry *et al*. (1988) in Channa punctatus, there has been a significant increase in alkaline phosphatase in the present investigation with carbaryl on *Clarias batrachus* and this trend continued till 96h hour of exposure.

The two sub-lethal exposure results also revealed that the sliding down of the protein content is related to the concentration of the insecticide. There occurred progressive decrease in the protein content with increase in concentration. Similar observation has been made by Singh and Bhati (1994). Decrease in protein may be due to the impairment of protein synthesis or increase in the rate of its degradation to amino acids. The insufficiency of liver is further corroborated by decrease of Albumin and globulin in experimental group (Table 1 and 2). The reduction in protein content observed in the experiment is in agreement with the reports of Singh (1988), Borah and Yadav (1995) and Muley *et al.*(2007).The extent of decrease is dependent upon the concentration of the pesticide and duration of treatment. In the present investigation also it is seen that the protein content changed with the changes in the levels of two transaminases. The activities of these enzymes are often related to the direction of protein metabolism, and would have enhanced protein catabolism. Similar are the inferences of Ghousia Begum and Vijaya Raghawan (1995), Khare and Singh (2002), Muley *et al*. (2007) regarding reduction in protein content. The change in cholesterol was probably an outcome of histological damages observed in liver which subdued its capacity to store cholesterol. Khan *el al.* (1992) observed significant decrease of cholesterol in liver of Cd treated fish *Garra mullya* and stated that it may be due to general damage in liver. Shakoori *et al.* (1996) studied effect of sublethal dose of fenvalevate on the liver of fish *Ctenopharyngodon idella* and observed decreased level of cholesterol. Virk and Sharma (1999) studied biochemical changes induced by nickel and chromium in the liver of *Cyprinus carpio* and observed significant decline in the cholesterol level of liver and stated this may be due to toxicity stress which suppresses the activity of a number of enzymes responsible for lipid transformation ultimately causing disturbance in lipid metabolism and leads to decrease in values of cholesterol. These results thereby suggest that carbaryl is capable of inducing histopathological and biochemical alterations in liver of fishes that may be translated into physiometabolic dysfunction in this species..

ACKNOWLEDGEMENT

We are thankful to Fisheries department , Government of Bihar who allowed us the collect the fishes from restricted areas and also to Dr.B.S.Jha, Professor, for his valuable suggestions and all those whose papers helped us in investigations.

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