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*Full Length Research Paper*

# The effects of lihocin toxicity on protein metabolism of the fresh water edible fish, *Channa punctatus* (Bloch)

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*In vivo* evaluations were made to assess the pesticide activity of lihocin against fresh water fish *Channa punctatus* and its ultimate mode of action on fish protein metabolism. Biochemical studies show that after exposing the fish to sub lethal dose of lihocin, total protein levels significantly decreased while FAA, glutamine, alkaline phosphatases, acid phosphatases, AlAT, AAT, GDH, AMP deaminase and adenosine deaminase were significantly enhanced in the liver, brain and kidney tissues of *C. punctatus*. The alterations in all the aforementioned biochemical parameters were significantly ($p < 0.05$) time and dose dependent. As such, the negative impact of lihocin was shown on the respiratory, as well as, energy production of the fish.

**Keywords:** *Channa punctatus,* enzyme activity, $Lc_{50}$, lihocin (OC) pesticidal impact, protein metabolism.

## INTRODUCTION

*Channa punctatus* is a common predatory fish which have low food value and due to its predatory nature, it engulfs the fingerlings of cultured carps at several stages of their rearing (Jhingran, 1975). Thus, it adversely affects the cultured carp production and set a great loss to the fish farmer. The indiscriminate and extensive use of insecticides to protect crops poses a serious threat to humans and the surrounding environment. The pesticides which are liberated into aquatic environment have a deleterious effect on fish and subsequently on man (Metelev et al., 1983).

The organochlorine insecticide 2-Chloro N,N,N, trimethyl ethano ammonium, commercially available as lihocin (OC), is used as a treatment against ectoparasite and as an insecticide for crops. Lihocin 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. The toxicity of the aqueous latex extract of *N. Inidcum* on fresh water snails (*Lymaaea acuminate* and *Indoplanoribs excustus)* and fish (*Channa punctatus* and *Colisa fasciatus)* has been established by Singh et al. (1993) and Singh and Singh (2000). A review of the toxicological literature reveals that the exposure to toxic chemicals can produce unexpected effects in non target animals (Veronica and Collins, 2003; Gonzalez et al., 2004; Lehtonen and Leimio, 2003; Salah, 1983). Most of the OC compounds and their derivatives adversely affect the nervous system.

The present study was undertaken to identify the effects of lihocin on liver, brain and gill tissues of the insecticide exposed fish *C. Punctatus.*

## MATERIALS AND METHODS

Healthy fresh water fish, *Channa punctatus* (15 to 25 cm in length and 80 to 120 gr in body weight), were collected locally from the Nirmal of Adilabad district and used as the test animal. These fish were kept in cement tanks (6 x 3 x 3 feet), at least 3 weeks, for acclimatization under continuous water flow. The average temperature of water was 22 ± 1.0C. The tank water was aerated continuously and food was provided in the form of dried groundnut cake, powdered small prawn, goat liver, etc., and water was changed every 24 h. The dead animals, if any, were removed as soon as possible from the test container to prevent water fouling.

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The physico-chemical parameters of water were as follows: atmospheric temperature (29 to 35°C, pH 7.2), electrical conductivity (0.052 mnhos), calcium (5 mg/L), sodium (2.1 mg/L), bicarbonates (142 mg/L), total alkalinity (69 mg/L), sulphates (7.1 mg/L), nitrates (3.4 mg/L), iodine (0.01 ppm), chlorides (37 mg/L), dissolved oxygen (4.2 mg/L), BOD (1.6 mg/L), COD (0.008 mg/L) and fluoride (0.03 ppm).

Toxicity experiment was performed according to Bayne et al. (1977). The fishes were exposed for 24, 48, 72 and 96 h at four different concentrations of tap water. Six tubs were set up for each concentration and each tub contains six fishes in 6L- de-chlorinated tap water. Control animals were kept in similar condition without any treatment. Mortality was recorded at every 24 h up to 96 h exposure period. Fishes were considered dead if any failed to respond to stimulus provided with glass rod.

The $Lc_{50}$ of values were evaluated according to Finney (1971). The formula was used to assess the mortality of the fish and this was recommended by the WHO and FAO.

*Channa punctatus* were kept in a plastic containing 6 L of tap water. Each tub contains six experimental animals. Fishes were exposed for 24 h or 96 h exposure period to sub-lethal concentration of $LC_{50}$ doses of lihocin (6.61 ppm). Control animals were kept in a similar condition without any treatment. After completion of treatment, fishes were removed from tubs, washed with water and killed by severe blow on the head. The liver, brain and gill tissues were quickly dissected out in ice tray and used for biochemical analysis. However, each experiment was repeated at least six times.

Protein levels were estimated according to Lowry et al. (1951) using bovine serum albumin as a standard. Homogenates (2 ml w/v) cold distilled water was prepared in 30% TCA. As such, values have been expressed as mg/100 mg wet.wt of tissue.

Free amino acids were estimated using the method of Moor and Stein (1954). Homogenates (5% w/v) were prepared in 10% (w/v) TCA and centrifuged at 300 rpm, whereas supernatant was used for amino acid estimation. FAA has been expressed as mg/100 mg wet.wt of the tissue.

Glutamine was estimated using the method of acid hydrolysis described by Colowick and Kaplan (1967). Homogenates (10% w/v) cold distilled water were prepared in 10% $H_2SO_4$, and so, their values were expressed as moles of glutamine/g wet.wt of the tissue.

AAT and AlAT were estimated using the method of Reitman and Frankel (1957). Homogenates (10% w/v) tissue were prepared in cold 0.25 M sucrose solution and centrifuged at 3000 rpm for 15 min to obtain a clear supernatant which was used as enzyme source. Values were expressed in micro moles of pyruvate formed/mg protein /h.

Glutamate dehydrogenase (GDH) activity levels were estimated following the method of Lee and Lardy (1965). Homogenates (10% w/v) were prepared in 0.25 M sucrose solution and centrifuged at 3000 rpm, while supernatant was used for the enzyme source. As such, values were expressed as micro moles of formazan formed / mg protein / hr of tissue.

AMP deaminase was estimated using the method of Weil-Malherbe and Green (1955) modified by Wagelin et al. (1978). Homogenates (10% w/v) were prepared in cold distilled water and centrifuged at 3000 rpm, while supernatant was used for enzyme source. AMP deaminase was expressed as micro moles of ammonia formed / mg protein / h.

For assaying adenosine deaminase activity, the method of Agarwal and Parks (1978) was used. Homogenates tissue (10% w/v) were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 min to obtain a clear supernatant which was used as enzyme source.

Alkaline and acid phosphatases were estimated using the method developed by Kind and King (1954). The enzyme assays were made after preliminary standardization regarding linearity with respect to time of incubation of enzyme concentration.

## RESULTS AND DISCUSSION

Exposure of lihocin caused significant behavioral changes in the fish *C. punctatus*. On the introduction of lihocin, all the fish immediately settled down at the bottom of the tub. Within 10 to 15 min, the fish felt suffocation and they came to the water surface to gasp for air. As exposure period increased, the surfacing phenomenon of fish also increased. Also, the rates of operculum movement, mucous secretion from skin and respiration through gill also increased. After some time, the opercula movement of fish slowed down, although they tried to stay at the upper water surface, but the loss of body equilibrium was pronounced. Finally, all the body activity decreased and they settled down at the base of the aquaria and died. Moreover, control animals were free from such behavioral changes.

The protein content decreased in the liver, brain and kidney tissues during lihocin treatment (Tables 1, 2 and 3). According to Nelson and Cox (2005) and Sathyanarayana (2005), the physiological status of animal is usually indicated by the metabolic status of proteins. Jrueger et al. (1968) reported that the fish can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction in liver, brain and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh et al., 1996). The toxicants may have effect on hormonal balance, which could directly or indirectly affect the tissue protein levels (Murthy and Priyamvada, 1982; Khilare and Wagh, 1988).

The free amino acid (FAA) pool was increased in the tissues of the fish during exposure to lihocin, while the elevated FAA levels were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh et al., 1996). It is also attributed to lesser use of amino acids (Seshagiri et al., 1987) and their involvement in the maintenance of an acid-base balance (Moorthy et al., 1984). Natarajan (1985) suggested that stress conditions induce elevation in the transanimation pathway. The increase in FAA levels of tissues indicates stepped up proteases activities and fixation of ammonia into keto acids (Srinivasa et al., 1986). Tripathi and Singh (2003) reported that the enhanced FAA may be due to depletion of reserved glycogen, so the fish can try to yield metabolic

**Table 1.** Effect of lihocin on the protein metabolism in the liver tissue of *Channa punctatus* (Bloch).

| Parameter | Control | Lihocin | | | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h |
| Total proteins | 163.6 ± 2.11 | 156.16 ± 1.34*<br>PC = -4.29 | 149.83 ± 2.79*<br>PC = -8.16 | 132.81 ± 3.61<br>PC = -18.82 | 124.84 ± 2.63<br>PC = -23.69 |
| Free amino acids | 719 ± 8.96 | 742 ± 42.14<br>PC = 3.19 | 796 ± 30.29<br>PC = 10.70 | 891 ± 81.62<br>PC = 23.92 | 978 ± 94.36<br>PC = 36.02 |
| Glutamine | 84.60 ± 6.42 | 87.29 ± 5.22*<br>PC = 3.17 | 93 ± 6.45*<br>PC = 9.92 | 98.30 ± 4.85<br>PC = 16.19 | 117.38 ± 9.85<br>PC = 38.74 |
| Alkaline phosphatases | 9.33 ± 0.99 | 10.46 ± 0.76*<br>PC = 12.11 | 12.63 ± 0.54<br>PC = 35.36 | 20.64 ± 0.94<br>PC = 121.22 | 21.55 ± 2.61<br>PC = 130.97 |
| Acid phosphatases | 8.36 ± 0.36 | 9.24 ± 0.11*<br>PC = 10.52 | 13.08 ± 0.85<br>PC = 56.45 | 15.08 ± 0.28<br>PC = 80.38 | 17.75 ± 0.85<br>PC = 112.32 |
| AlAT | 23.5 ± 1.60 | 28.43 ± 1.62<br>PC = 20.97 | 32.33 ± 2.13<br>PC = 37.57 | 33.20 ± 1.85<br>PC = 41.27 | 40.16 ± 1.46<br>PC = 170.89 |
| AAT | 19.75 ± 0.75 | 22.78 ± 1.18<br>PC = 15.34 | 28.36 ± 1.36<br>PC = 43.59 | 31.46 ± 4.65<br>PC = 59.29 | 33.84 ± 1.71<br>PC = 71.34 |
| GDH | 0.71 ± 0.02 | 0.83 ± 0.01<br>PC = 16.90 | 1.06 ± 0.02<br>PC = 49.29 | 1.12 ± 0.12<br>PC = 57.74 | 1.28 ± 0.23<br>PC = 80.28 |
| AMP deaminase | 0.39 ± 0.02 | 0.42 ± 0.01*<br>PC = 7.69 | 0.62 ± 0.01<br>PC = 58.97 | 0.68 ± 0.03<br>PC = 74.35 | 0.74 ± 0.042<br>PC = 89.74 |
| Adenosine deaminase | 0.132 ± 0.04 | 0.145 ± 0.016*<br>PC = 9.84 | 0.196 ± 0.023<br>PC = 48.48 | 0.203 ± 0.001<br>PC = 53.78 | 0.283 ± 0.002<br>PC = 114.39 |

Each value is mean SD of 6 observations. All values are statistically significant from control at 5% level ($p < 0.05$). PC denotes percent change over control. * Not significant.

**Table 2.** Effect of lihocin on the protein metabolism in the brain tissue of *Channa punctatus* (Bloch).

| Parameter | Control | Lihocin | | | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h |
| Total proteins | 103.83 ± 2.26 | 96.16 ± 1.34*<br>PC = -7.39 | 92.33 ± 1.88*<br>PC = -11.07 | 86.32 ± 2.80<br>PC = -16.86 | 69.83 ± 3.49<br>PC = -32.74 |
| Free amino acids | 562 ± 20.46 | 571 ± 13.22<br>PC = 1.60 | 598 ± 14.65<br>PC = 6.40 | 617 ± 8.44<br>PC = 9.78 | 645 ± 19.04<br>PC = 14.76 |
| Glutamine | 58.34 ± 5.99 | 62.80 ± 6.43*<br>PC = 7.64 | 69.85 ± 5.65<br>PC = 19.72 | 71.823 ± 6.42<br>PC = 23.10 | 79.88 ± 8.09<br>PC = 36.92 |
| Alkaline phosphatases | 7.03 ± 0.93 | 8.20 ± 1.09<br>PC = 16.64 | 10.93 ± 0.72<br>PC = 55.47 | 12.11 ± 0.32<br>PC = 72.26 | 12.59 ± 0.86<br>PC = 79.08 |
| Acid phosphatases | 6.25 ± 0.73 | 7.24 ± 0.07<br>PC = 15.84 | 9.40 ± 1.20<br>PC = 50.40 | 12.80 ± 0.15<br>PC = 104.8 | 14.66 ± 1.11<br>PC = 134.56 |

**Table 2.** Contd.

| | | | | | |
|---|---|---|---|---|---|
| AlAT | 12.16 ± 1.21 | 14.35 ± 0.73<br>PC = 18.00 | 19.16 ± 1.34<br>PC = 57.56 | 22.80 ± 1.10<br>PC = 87.50 | 27.00 ± 1.64<br>PC = 122.03 |
| AAT | 8.48 ± 0.95 | 9.48 ± 0.99<br>PC = 11.79 | 12.55 ± 1.35<br>PC = 47.99 | 16.45 ± 1.89<br>PC = 93.98 | 19.53 ± 1.24<br>PC = 130.30 |
| GDH | 0.43 ± 0.02 | 0.62 ± 0.01<br>PC = 44.18 | 0.81 ± 0.01<br>PC = 88.37 | 0.921 ± 0.01<br>PC = 114.18 | 1.03 ± 0.02<br>PC = 139.53 |
| AMP deaminase | 0.29 ± 0.01 | 0.36 ± 0.02<br>PC = 24.14 | 0.37 ± 0.021<br>PC = 27.58 | 0.44 ± 0.03<br>PC = 51.72 | 0.64 ± 0.001<br>PC = 120.68 |
| Adenosine deaminase | 0.098 ± 0.001 | 0.108 ± 0.011*<br>PC = 10.20 | 0.123 ± 0.023<br>PC = 25.51 | 0.143 ± 0.012<br>PC = 45.91 | 0.193 ± 0.001<br>PC = 96.93 |

Each value is mean SD of 6 observations. All values are statistically significant from control at 5% level ($p < 0.05$). PC denotes percent change over control. * Not significant.

**Table 3.** Effect of lihocin on the protein metabolism in the kidney tissue of *Channa punctatus* (Bloch).

| Parameter | Control | Lihocin | | | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h |
| Total proteins<br>Micro grams/100 mg wet.wt | 213.83 ± 2.60 | 204.5 ± 1.25*<br>PC = -4.36 | 195.83 ± 1.77*<br>PC = -8.41 | 182.24 ± 0.73<br>PC = -28.80 | 164.23 ± 0.65<br>3.191.29 |
| Free amino acids<br>Micro grams/100mg wet.wt | 342 ± 12.59 | 362 ± 14.09<br>PC = 5.84 | 379 ± 12.49<br>PC = 10.81 | 399 ± 12.49<br>PC = 16.66 | 399 ± 15.09<br>PC = 21.92 |
| Glutamine<br>Micro grams/100mg wet.wt | 36.85 ± 6.21 | 39.11 ± 3.84*<br>PC = 16.13 | 43.29 ± 5.11<br>PC = 17.47 | 47.82 ± 44.10<br>PC = 29.76 | 53.86 ± 3.17<br>PC = 46.16 |
| Alkaline phosphatases<br>ip formed/mg protein/hr | 9.64 ± 1.08 | 10.42 ± 0.14*<br>PC = 8.09 | 11.36 ± 1.87<br>PC = 17.84 | 13.80 ± 0.16<br>PC = 43.15 | 15.03 ± 0.98<br>PC = 55.91 |
| Acid phosphatases<br>ip formed/mg protein/hr | 5.50 ± 0.53 | 6.62 ± 0.12<br>PC = 20.36 | 8.07 ± 0.15<br>PC = 46.72 | 9.42 ± 0.28<br>PC = 71.27 | 10.24 ± 0.87<br>PC = 86.18 |
| AlAT<br>Micro grams/100mg wet.wt | 17.33 ± 1.37 | 18.29 ± 0.24*<br>PC = 5.53 | 20.83 ± 1.34<br>PC = 20.19 | 24.22 ± 0.18<br>PC = 39.75 | 26.5 ± 2.14<br>PC = 52.91 |
| AAT<br>Micromoles of pyruvate formed/mg protein/hr | 14.29 ± 1.99 | 15.42 ± 0.86<br>PC = 7.90 | 18.07 ± 0.53<br>PC = 26.45 | 19.34 ± 0.46<br>PC = 35.33 | 21.91 ± 1.53<br>PC = 53.32 |
| GDH<br>Micromoles of formazon formed/mg protein/hr | 0.09 ± 0.01 | 0.13 ± 0.01<br>PC = 44.44 | 0.22 ± 0.01<br>PC = 144.44 | 0.263 ± 0.02<br>PC = 192.22 | 0.312 ± 0.03<br>PC = 246.66 |
| AMP deaminase<br>Micromoles of ammonia formed/mg protein/hr | 0.24 ± 0.001 | 0.29 ± 0.03<br>PC = 20.83 | 0.35 ± 0.017<br>PC = 45.83 | 0.38 ± 0.06<br>PC = 58.33 | 0.41 ± 0.025<br>PC = 70.83 |
| Adenosine deaminase<br>Micromoles of ammonia formed/mg protein/hr | 0.045 ± 0.002 | 0.072 ± 0.012<br>PC = 60.00 | 0.096 ± 0.002<br>PC = 113.33 | 0.120 ± 0.01<br>PC = 166.66 | 0.136 ± 0.001<br>PC = 202.22 |

Each value is mean SD of 6 observations. All values are statistically significant from the control at 5% level ($p < 0.05$). PC denotes percent change over control. * Not significant.

energy by gluconeogenesis process. Similar findings were observed by Vijuen and Steyn (2003) in various animals during different toxic conditions.

The elevated levels of glutamine in liver, brain and kidney tissues reveal an enhancement of the biosynthesis of glutamine (Tables 1, 2 and 3). The elevated glutamine may be utilized in the formation of amino acids in protein synthesis. According to Narsaimha and Raman (1985), the organochlorine pesticides may initiate the synthesis of glutamine during toxic conditions. The present finding suggests that the fish has inherent tissue specific resistance on the potentiality to withstand ambient pesticide toxicity by suitably modulating its metabolic profiles.

The increase in AMP deaminsae and adenosine deaminase activities could contribute ammonia to the tissue through the purine nucleotide metabolism. According to Nelson and Cox (2005), these deaminases contribute little amount of nucleotides in the different tissues through purine nucleotide metabolism. Enhanced activity of deaminases may be due to tissue damage under xenobiotic action.

The lihocin pesticide caused increase in GDH activity in the tissues during initial periods of exposure. The important function of GDH is that the amino group of most amino acids is transferred to α-ketoglutarate to produce glutamate. According to Nelson and Cox (2005) and Sathyanarayana (2005), increased GDH activity may indicate an increased rapid utilization of amino acids. The oxidation of glutamate in Kreb's cycle leads to increased energy, though it is small (Narasimha and Rama, 1985).

The activities of AAT and AlAT during the toxic exposure of lihocin pesticide were enhanced (Tables 1, 2 and 3). The elevated activities of AAT and AlAT were observed by Narasimha et al. (1986) in *Anabas testudineus* during treatment of organophosphates pesticides in *T. mossambica* under linden toxicity and by Sajal et al. (1988) in gastropoda, *Thiara lineata*, during methyl parathion toxicity.

The alkaline and acid phosphatases were enhanced during the toxic exposure period and under stress conditions. The elevated levels of phosphatases may indicate an increase in the rate of phosphorylation and transport of molecules across the cell membrane. As such, the enhanced phosphatases activity revealed an increase in the transportation of metabolites through the cellular cellular membrane (Venkateshwarlu et al., 1990) and it was reported that the pesticides cause significant increase in the cellular damage which enhanced the activity of phosphatases activity. Carla et al. (2005) reported that phosphatases activity was altered in kupffer-melanomacro phagic cells of *Rana esculenta* during environmental pollution. As such, the fish can utilize stored proteins to overcome toxic stress. During toxic stress, the levels of key enzymes involved in protein metabolism are changed.

The present study concludes that lihocin, in sub lethal concentration, affects tissue protein metabolism in *Channa panctatus*. As a consequence of lihocin toxicity, the fish shifts to alternate methods of metabolism to overcome the toxic stress and maintain its survival in the polluted environment.

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