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Impact of Lead Nitrate on Serum Cholesterol of Cat-fish – Heteropneustes fossilis

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Abstract: **Heteropneustes fossilis was exposed to sub-lethal concentration of Lead nitrate (131.23 mg/L) for 15 days and 30 days in order to find out its toxic effect on serum cholesterol. A significant (P<0.001) loss equivalent to 11.6 % and 13.26 was recorded on 15 days and 30 days treatment period.**

Keywords: **Heteropneustes fossilis, sub-lethal concentration, cholesterol, lead nitrate.**

1. INTRODUCTION

In recent years, heavy metal pollution has become a subject of considerable interest in the field of aquatic toxicology. The toxic metals are dispersed in the biosphere. They can be carried to places far away from source by winds depending on whether they are in gaseous form or as particulates. These are ultimately washed out by rain on the land or the surface of water ways. Their discharge into aquatic ecosystem (both fresh water and marine) leads to the accumulation of those toxic elements within the food chain thereby seriously affecting the life of birds and mammals which consume them as food. There are numerous reports explaining different effects of various heavy metals and these have been reviewed by several authors (Forstner and Wittmann, 1979; Nisha et al; 1984). Fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants. They can sensitive to contamination and the pollutants may damage some physiological and biochemical processes when they enter the organs of fishes (Tulasi et. al; 1992)

2. MATERIALS AND METHODS

Eatimation of total serum cholesterol:-

Total serum cholesterol was estimated by sackett's method (varley,1976)**.**

Reagents;-

Following rteagents were used:

(1) Alcohol –Ether mixture:

3 volumes of alcohol and 1 volume of ether were mixed. (9 Ml abs. alcohol & 3 ml of ether)

(2) Acetic anhydride – Sulphuric acid mixture:

20 ml of acetic anhydride mixed with 1 ml of conc. H_2So_4 .It was prepared freshly just before use.

(3) Chloroform

(4) Stock standard solution of cholesterol:

200 ml of pure cholesterol was dissolved in chloroform and was made upto 100 ml with chloroform. It was kept well stoppered and was used as a standard solution.

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1 ml of stock standard solution was diluted to 25 ml with Chloroform. 5 ml of that solution contained 0.4 mg of cholesterol.

Procedure:-

The sample blood was taken in a centrifugal - tube and centrifuged for 10 minutes at 7000 R.P.M. 0.2 ml of serum from the centrifugted blood was added to 10 ml of absolute alcohol – Ether mixture taken in another centrifuge- tube. The tube was then corked tightly and shaken thoroughly for a minute. There after, the tube was laid horizontally for half an hour for even distribution of the precipitate. The mixture in the tube was then centrifuged for 10 minutes and the supernatant fluid was decanted in a hard-glass test-tube marked 'T' (Test). That test-tube was placed in a boiling water- bath and the supernatant fluid was then allowed to evaporate completely upto dryness. Then 5 ml of chloroform was added to dry substance i.e. cholesterol residue. Meanwhile, working standard solution was prepared by mixing 1 ml stock standard solution of cholesterol with 25 ml chloroform. Of the prepared working solution, 5 ml was taken in another hard glass test- tube marked's' (standard). Then 2 ml of a mixture of acetic anhydride and conc. H_2So_4 (ratio 2:1) was added in both the test-tube marked 'T' & 'S' The solution in the test-tube was mixed and kept in the dark for 15 minutes for the complete development of colour.

The photoelectric colorimeter was switched on and left for five minutes and chloroform as 'Blank' solution was used to set for 100 per cent transmission, i.e. '0' optical density using the red filter (680 nm). Then the readings of the optical density of the standard and unknown solution were taken and the result was finally calculated by the following formula:

Mg of Cholesterol per 100 ml of blood

$$\frac{Reading\ of\ unknown}{Reading\ of\ standard} \times 200$$

TABLE I: Profiles of Serum cholesterol in normal and lead nitrate treated fish, Heteropneustes fossilis (Values are ± SE of Five fish in each group).

Parameter	15 Days		Student 't' Test P Value	% Increase(-) Or Decrease(-)	30 Days		Student 't' Test P value	% Increase(+) Or Decrease(-)
	Control	Treated			Control	Treated		
Serum Cholesterol (mg/100ml)	162.5 ±0.90	144.36 ±1.57	<0.001	-11.16%	160.5 ±0.65	139.2 ±2.09	<0.001	-13.26%

3. RESULT

15 days treatment period:

The results are shown in (Table I) The serum cholesterol in control fish has been estimated to be 162.5 ±0.90 mg/100 ml of blood as against. This value a significant (P<0.001) loss equivalent to 11.16 % has been recorded in exposed fish (Value being 144.36 ± 1.57 mg/100 ml of blood.

30 days treatment period:

Similar to 15 days observation the fish of group also become hypo cholesterolemic. The serum cholesterol level lowers down from 160.5 ± 0.65 in control to 139.21 ± 2.09 mg/100 in treated group. The depletion in serum cholesterol level has been statistically calculated to be significant at P<0.001 and is equivalent to a loss of 13.26 % (Table I)

4. DISCUSSION

The present study exhibited a reduction in serum cholesterol under the toxic influence of lead nitrate. The decrease in serum cholesterol level after 15 and 30 days of exposure was: 11.16 and 13.26%; It is in conformity with the findings of Dutta and Haghighi (1986) and Tewari et al; (1987).

Metals have been reported to induce both dsecrease and increase in the amount of cholesterol in blood of fishes. Sastry and Sharma (1980) found elevated cholesterol level in Channa punctatus exposed to $Hgcl_2$**.** contrary to these methylmercury has been reported to decrease the serum cholesterol level in blue gill fish, *Lepomis* macrochirus (Dutta and Haghighi 1986). Tiwari et al; (1987) recorded depleted cholesterol level in lead exposed barbas conchonius and Ruparelia et al; (1989) in Oreochromis mossambicus.

Since cholesterol is an important constituents of cell membrane and precursor for steroid hormone. The decrease as observed during present study may be related either to disruption of plasma membrane or to the altered steroidogenesis. The depressed cholesterol level may also be related to its enhanced utilization in corticosteroidogenesis or a decreased de novo synthesis suggested by Tewari et al; (1987). Wassermann et al; (1970) have suggested that thyroid hormones are also involed in cholesterol metabolism and enhanced break down in hyperthyroidism in hypocholeterolemia.

Dutta and haghighi (1986) with mercury have opined that this metal stimulate enhance protein synthesis in liver with resultant increased level of serum heavy density lipo protein and this is the major cause of hypocholesterolemia.

5. CONCLUSION

Heteropneustes fossilis was exposed to sub-lethal concentration of Lead nitrate (131.23 mg/L) for 15 days and 30 days in order to find out its toxic effect on serum cholesterol. A significant ($P<0.001$) loss equivalent to 11.6 % and 13.26 was recorded on 15 days and 30 days treatment period. The results are shown in (Table I) The serum cholesterol in control fish has been estimated to be 162.5 $\pm$0.90 mg/100 ml of blood as against. The present study exhibited a reduction in serum cholesterol under the toxic influence of lead nitrate.

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