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ORIGINAL ARTICLE

Assessment of Lipoproteins in *Channa punctatus* under Toxic Stress of Mancozeb and Malathion in Combination

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ABSTRACT

Pesticides are related chemicals; destroy the fragile balance between species that characterizes a functioning ecosystem. Pesticides are economical way of controlling pests. Pesticides are often wont to stop the spread of pests in imports and exports, preventing weeds in gardens and protecting house and furniture from destruction. Pesticides include a good sort of chemicals with great difference in their mode of action, uptake by the body, metabolism and elimination from the body and toxicity to focus on and non-target organisms. Poisoning risks depend upon dose, toxicity, duration of exposure and sensitivity.

Key words: *Mancozeb, Malathion, Channa punctatus, Lipoproteins*

INTRODUCTION

Insecticidal use in agriculture gained momentum round the mid twentieth century. Fungicides also are utilized in agriculture for the prevention of mycosis in seed corn. Later these compounds discharge in nearby water bodies and consumed by fishes and other aquatic life. These fat soluble contaminants concentrate within the fat of fishes by bioaccumulation and bio-magnification. The fishes, best indicator of water body pollution, are the foremost sensitive of all the aquatic animals towards the pollutant. The buildup of effluents becomes hazardous to the aquatic organism because they're the foremost important factors of organic phenomenon. The desperate and uncared use of fungicides in agriculture practices has further enhanced the matter to the worldwide importance. As fish are the simplest source of protein and mineral salts but they're facing the environmental contamination. The injurious effect of certain fungicide on various vitals and their accumulation within the white muscles of the inhabitants has attracted the eye of variety of workers. The fish selected is usually utilized in laboratory because it's hardy and simply available throughout year. This is often valued as a fish due to its ability to survive extended period out of water. Walking catfish are often sold and treated accept ease, ensuring fairly fresh foods product.

MATERIALS AND METHODS

SELECTION OF ANIMAL: *Channa punctatus* (Bloch.) was selected as a test animal. It has an elongate body that is broader at the head, tapering towards the tail. It is commonly available in fresh water. It is hard fish and stand in the aquarium condition very well.

COLLECTION OF FISH: The fishes were collected during September to October, when the room temperature from 25^{0}C to 30^{0}C. Adult live specimen of fish *Channa punctatus* (Bloch.) with the size ranges 16-18 cm and weight 40 to 70 g were collected from the local market.

MANAGEMENT AND FEEDING OF FISH: They were carefully examine for any injury and then kept in 0.2% $KMnO_4$ solution for few minutes to get rid of any dermal infection. Finally they were stored in large glass aquarium in laboratory condition for 15 days. The dechlorinated water used and changed every alternate day. Various physiochemical characteristics of test water such as temperature, pH, and hardness were regularly reported.

EXPERIMENT COMPOUNDS: Mancozeb and Malathion
Mancozeb is a dithiocarbamate non-systemic agricultural fungicide with multi-site, protective action on contact. It is a combination of two other dithiocarbamates: maneb and zineb. The mixture controls many fungal diseases in a wide range of field crops, fruits, nuts, vegetables, and ornamentals.

EXPERIMENTATION: The experiment was conducted in five aquariums one was used for control and rests are used for pollution study. Each aquarium contains 10 fishes, which were exposed to sub lethal concentration of mancozeb and malathion in combination at different time interval (24, 48, 72 and 96 hour). The sub lethal concentration was selected on the basis of LC_{50} value.

COLLECTION OF BLOOD: Five fishes from each set control and treated were sacrificed for the studies after 24, 48, 72 and 96 hours of exposure to combination of pesticides. The blood was collected after the severing the caudal peduncle of the living fish by scissor.

SEPARATION OF SERUM
The centrifuge tubes containing blood samples were allowed to stand in slanting position for about one hour at room temperature and were centrifuged at 2500rpm for 30minutes.

ESTIMATION OF HIGH DENSITY LIPOPROTEIN (HDL): High density lipoprotein was estimated by the Wybenga and Pileggi method (1970).

PRINCIPLE: Chylomicrons, LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatent and is estimated using ERBA cholesterol reagent.

$$\text{Serum/plasma} \xrightarrow[Mg^{2+}]{\text{Phosphotungstate}} \underset{\text{(supernatent)}}{\text{HDL}} + \underset{\text{(Precipitate)}}{\text{VLDL + Chylomicrons}}$$

REAGENTS
- Cholesterol reagent
- Standard solution
- Precipitating reagent

PROCEDURE: HDL Cholesterol Separation- 0.25ml of serum sample and 0.5ml of precipitating reagent were taken into centrifuge tubes. Mixed well and allowed the reaction mixture to stand for 10minutes at room temperature. The contents were centrifuged at 4000rpm for 10minutes to obtain a clear supernatent. Use the supernatent to determine the concentration of HDL cholesterol in the sample.
Three test tubes were marked as 'Test', 'Standard' and 'Blank'.

Test: 1ml of cholesterol reagent and 0.05ml of supernatent (*vide supra*) were taken in a test tube marked as 'Test'.

Standard: 1ml of cholesterol reagent and 0.05ml of standard solution were taken in a test tube marked as 'Standard'.

Blank: 1ml of cholesterol reagent and 0.05ml of distilled water were taken in a test tube marked as 'Blank'.
Mixed well and incubated for 10minutes at 37^0C. Optical density of 'Test' and 'Standard' was measured by photoelectric colorimeter at 505nm after setting the zero with 'Blank'.

CALCULATION

$$\text{Serum HDL (mg/dl)} = \frac{\text{O.D. of 'Test'}}{\text{O.D. of 'Standard'}} \times 75$$

ESTIMATION OF LOW DENSITY LIPOPROTEIN (LDL): Low density lipoprotein (LDL) was calculated from the values of serum cholesterol, very low density lipoprotein (VLDL) and high density lipoprotein (HDL) by using following formula given by Friedwald *et al*. (1972).

$$LDL = CHOLESTEROL - (VLDL + HDL)$$

ESTIMATION OF VERY LOW DENSITY LIPOPROTEIN (VLDL): Very low density lipoprotein (VLDL) was calculated by the following formula given by Friedwald *et al*. (1972).

$$VLDL = \frac{\text{Triglyceride (TG)}}{5}$$

RESULTS AND DISCUSSION

The low density lipoprotein, very low density lipoprotein have been observed to be increased, while a decrease in high density lipoprotein has been observed after 24hrs, 48hrs, 72hrs and 96hrs exposure to mancozeb+malathion in experimental fish *Channa punctatus* (Table-1-3, Fig. 1-3).

Table 1: High density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb + malathion intoxication

HDL	Control	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	55.67	52.50	46.67	42.30	38.50
±S.Em.	±0.45	±0.37	±0.33	±0.38	±0.28
Significance level	-	P> 0.05	p< 0.05	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Fig. 1: High density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

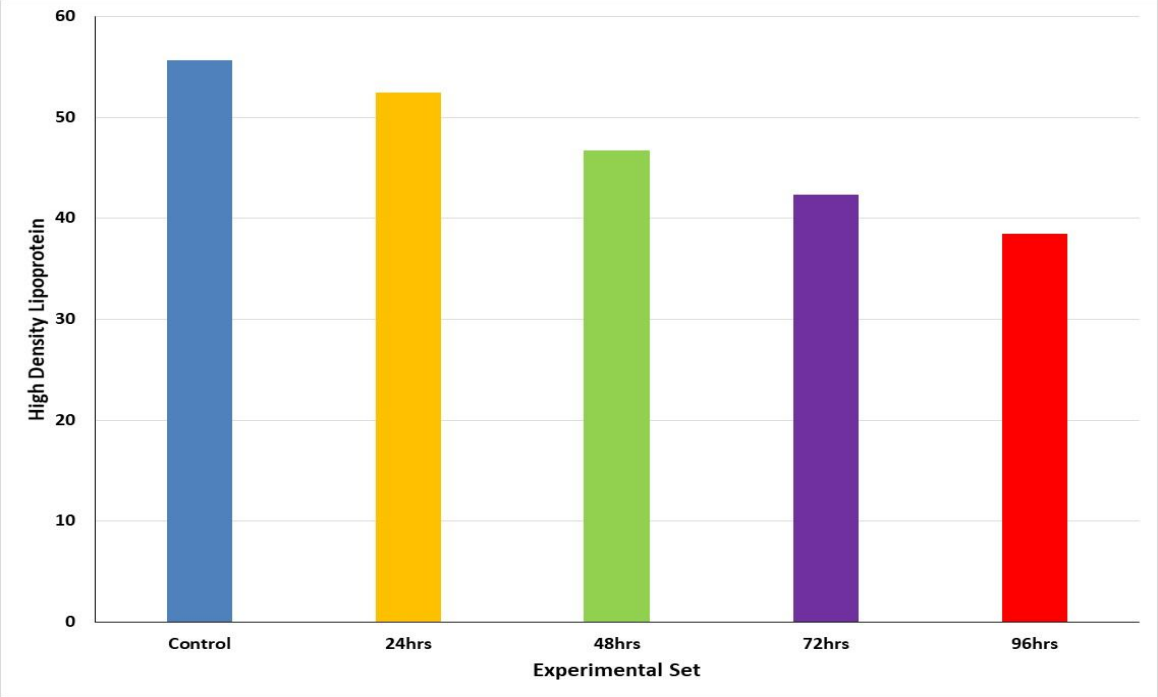


Table 2: Low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb + malathion intoxication

LDL	Conrol	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	72.40	75.37	81.50	86.70	91.57
±S.Em.	±0.50	±0.45	±0.33	±0.37	±0.62
Significance level	-	P> 0.05	p< 0.05	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Fig. 2: Low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

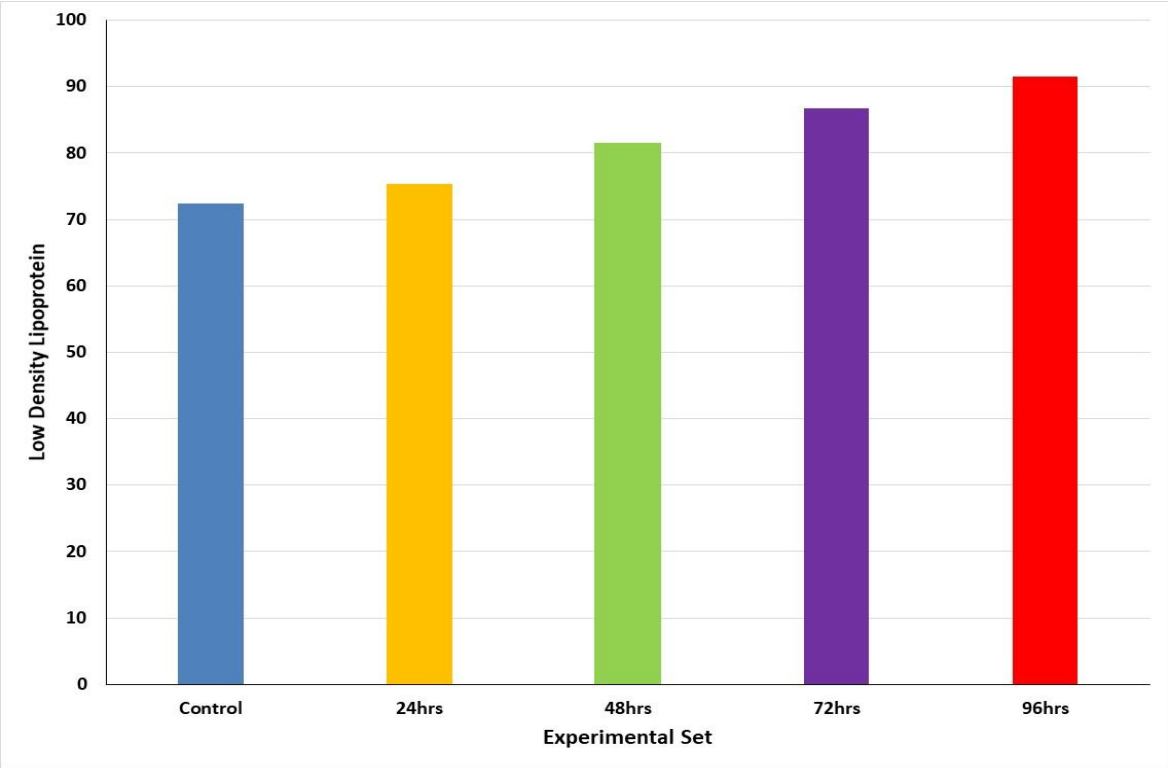
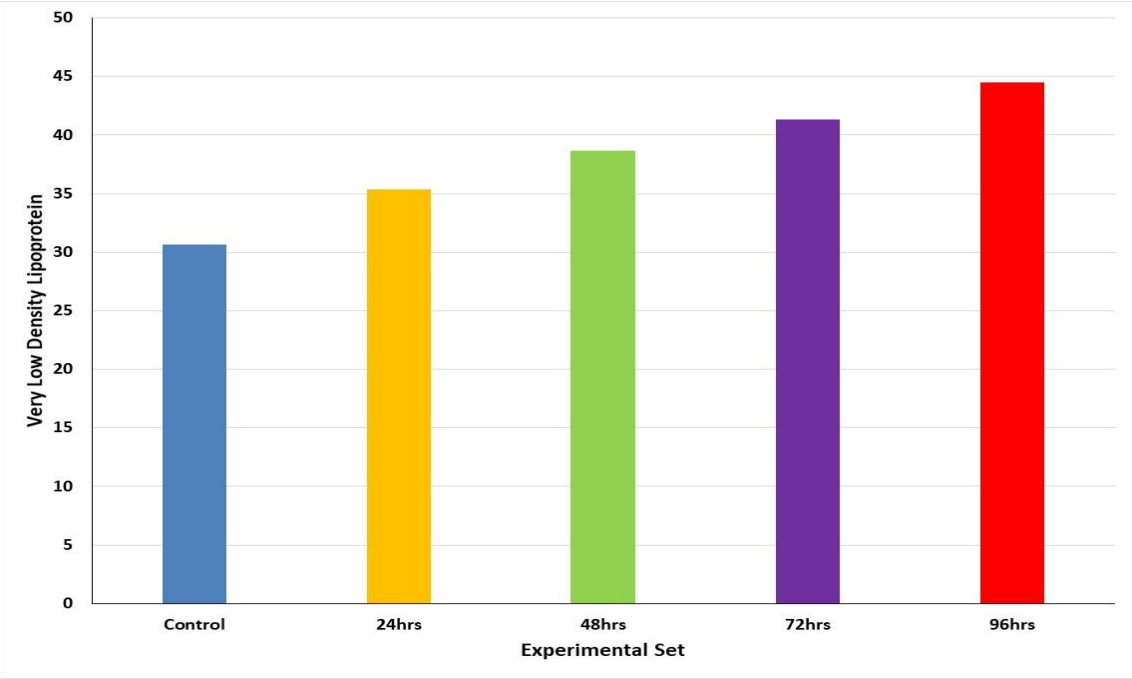


Table 3: Very low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb + malathion intoxication

VLDL	Control	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	30.66	35.33	38.65	41.30	44.50
±S.Em.	±0.18	±0.19	±0.25	±0.33	±0.35
Significance level	-	P> 0.05	p< 0.05	p< 0.05	p< 0.01

S.Em. = Standard error of mean

Fig. 3: Very low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb + malathion intoxication



Aquatic pollution has become a global problem and is posing a serious threat to the survival of aquatic organisms. This pollution is because of many reasons of which anthropogenic, agricultural are main causes. Pesticide use if extensive now days in agriculture to enhance production and protect crops from pests various categories of pesticides are used in crops. The toxic studies are mainly done on single pesticide while farmers use more than one pesticide in one crop which results in harmful combinations.

The alterations are significant after treatment. It may be due to utilization of cholesterol and other lipid fractions in treated fish to counteract toxic stress and stabilize the molecules of toxicants and their secretion in blood increases the serum levels. Further, this may also be due to hindrance in lipid metabolism which results in accumulation of lipid content in blood. In accordance to the present findings, similar increased lipid profile has been reported by Ghosh (1988) who observed the alterations of cholesterol in blood of *Channa punctatus* over the influence of Chromium, Sivaramakrishna and Radhakrishna (1998) in *Cyprinus carpio*, Rani *et al*. (2001) in *Tilapia mossambuca*, Radha *et al*. (2005) in *Cyprinus carpio,* Shankar and Kulkarni (2007) in *Notopterus notopterus* and Karthikeyan *et al*. (2007) in *Cirrhinus mrigala*. These findings are in favour of the explanation of the present work. The changes are due to alteration in enzymes governing lipid, lipoprotein and triglyceride metabolism.

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