

IMPACT OF ASCORBIC ACID SUPPLEMENTATION TO COUNTERACT EFFECT OF MERCURY POLLUTION.

Dr. Manju Sharma

Principal, Lord Buddha Institute of Technology & Science, Kota.

Abstract:Mercury cycling in the environment is increasing due to its increased uses in industrial activities. Mercury chloride in water is absorbed by fishes. By eating infected fishes mercury is absorbed by human body which may result in death. The present investigation aimed to determine the protective role of ascorbic acid in diet to counteract the effects of mercury chloride. The experiment was set in 3 groups. *Gambusia affinis* were exposed to mercury chloride and 0.5% of vitamin C supplemented in diet. Experiment was carried for 90 days and then fishes were restored to control water conditions for 30 days. The general feeding behaviour, morphological survival and growth were studied. The histopathological changes in ovary showed necrotic, degeneration in oocytes.

The result shows that the negative effects of mercury was neutralized by vitamin C diet.

Index Terms- Immature ovaries (im), necrosis, ascorbic acid (vitamin C), Ovigerous lamella (ol), vacuolated (v), atretic follicle (at)

I. INTRODUCTION

There are two ways of counteracting pollution viz; either to prevent pollution from its very source or to find methods of neutralizing pollutant effect when pollution has already taken place. As some reports suggest that intake of ascorbic acid with diet in animal species results in significant beneficial effects over biochemical stress including those due to the toxicity of heavy metals and pesticides (Agarwal et.al;1978, Hadson et.al;1980). Vitamin C counteract mercury when induced in Rats (Hug et.al;2008, Somaya et.al;2014). Effect of copper also neutralized by vitamin C in fish *Tilapia zilli* (Ghazaly K,1994). Histopathology of ovary in *Heteropneustis fossilis* was effected by paper mill effluent (Baruah et.al;2002). Protective role of Vitamin C reported in *Channa orientalis* (Ramdas et.al;2013). Thus in view of above, it was thought desirable to investigate the effect of supplementation of ascorbic acid diet on *Gambusia affinis* exposed to mercury pollution.

II. MATERIAL AND METHOD

Gambusia affinis (av. weight 25.5 ± 0.5 mg; av. length 32.0 ± 0.3 mm) were selected. They were conditioned in glass aquaria for a period of 15 days before starting the experiment and fed with pelleted diet @ 3% body weight. After acclimatization the fishes were weighed and divided into three groups of 70 each.

Experiments were designed at sub lethal concentration at (60% of the 48 hrs LC₅₀ value i.e. 0.34 mg/l).

Group 1- Control (fed normal pelleted diet and water free of mercuric chloride).

Group 2- Fed on normal diet and exposed to 0.02 mg/l of mercury chloride.

Group 3- Fed diet supplemented with 0.5% ascorbic acid and exposed to 0.02 mg/l of mercuric chloride.

This experiment was continued for 90 days. After 90 days G2 fishes were subdivided into two subgroups as follow for testing possible recovery.

G2a: Fed normal diet and exposed to water free from mercury pollution.

G2b: Fed diet supplemented 5.0% ascorbic acid and exposed to water free from mercury pollution.

Possibilities of recovery were also tested in G3 by restoring the fishes to mercury free water.

Day to day record of change in general behaviour, feeding pattern, morphological changes, and mortality were maintained.

Histopathological changes in ovary were observed on 7, 15, 30, 45, 60, 90 and 120 days.

III. RESULTS

(i) **General and feeding behaviour-**When fishes were transferred to G2 and G3, they showed avoidance, disturbed swimming. Increased opercular movement which continued for few hours but after 48 hrs the situation was normalised. In G2

after few days fishes were seen lethargic lying on the bottom of the aquarium. After 90 days fishes showed varying degree of recovery. In G2 and G3 feeding was avoided for 48 hrs, subsequently they normalised and started normal feeding.

(ii) **Morphological Changes-** Copious mucous secretion was observed in G2 and G3 after 48 hours. In G2 dermatitis evidenced by loosing and derooting of scales on the caudal peduncle, by 45th day body colour change was observed. When fishes were restored to normal water and normal diet after 90 days signs of recovery could be observed.

Survival and Growth- In G2 retarded growth as compared to G1 and G3 and similarly mortality was higher in G2. The supplementation of vitamin in diet decreased mortality rate and enhanced growth.

Histopathological changes-

The effect of mercury pollution on ovary will be described in the following order of pathological changes-

Stage I: Initial effects, showing hypertrophy of cell.

Stage II: Progressive changes of pathological nature.

Stage III: Advanced pathological symptoms.

Stage IV: Highly necrotic and wide spread degeneration, if any.

A maturing healthy ovary (Fig 1.1a) consists of three layers (i) an outermost thin peritoneum.

(ii) A thicker tunica albugenia made up of connective tissues and blood capillaries.

(iii) Germinal epithelium which projects into the ovocoel in the form of lamellae. These ovigerous lamellae are the seat for the development of oocytes, which can be seen in various stages of development. The Oogonia are found in clusters Oogonium has large nucleus and thus become ripe ovum.

Stage I (Fig 1.1b) – The cells constituting the ovigerous lamellae were seen to hypertrophied and less translucent. Immature (im) Oocytes also showed similar changes.

Stage II (Fig 1.1c, 1.2c) – The tunica albugenia (ta) becomes thickened and in germinal epithelium degenerative changes at various foci (indicated by arrow) could be seen. Immature oocytes (im) degenerate whereas maturing oocytes were seen becoming more opaque rather dark. In later part of Stage II the germinal epithelium was hypertrophied and vacuolated (V). In ovigerous lamellae (Ol) wall lysis could be observed. In matured oocytes the follicular wall was thickened and coalescence of lipid yolk globules was observed (indicated by arrows).

Stage III (Fig 1.1d, e, f) . The ovigerous lamellae O1 showed degeneration and lysis and increased intermolar space (ii). Immature oocytes showed marked degeneration. Maturing oocytes were few and unhealthy. Number of matured oocytes were reduced with thick follicular wall (fw) and yolk globules clumped (indicated by arrow).

Stage IV (Fig 1.2a) - The Ovigerous lamellae showed advanced necroses (n). The follicular wall of maturing oocytes was broken and number of atretic follicles (af) increased interfollicular spaces were further seen to widen.

It is obvious that when fishes were restored to normal conditions in G2 after 90 days, recovery signs appeared. Fishes transferred to G2b normal water with 5.0% vitamin C supplementation in diet (fig 1.2b). More recovery was seen in G2a fishes exposed to normal water and fed normal diet (Fig 1.2c). The protective role was obvious in G3 which had received vitamin C supplementation along with mercury pollution all through 90 days experimental period. When mercury free water was restored for a month (90-120 days) the ovarian follicles appeared more or less normalised except that interlamellar spaces were still conspicuous (Fig 1.2d).

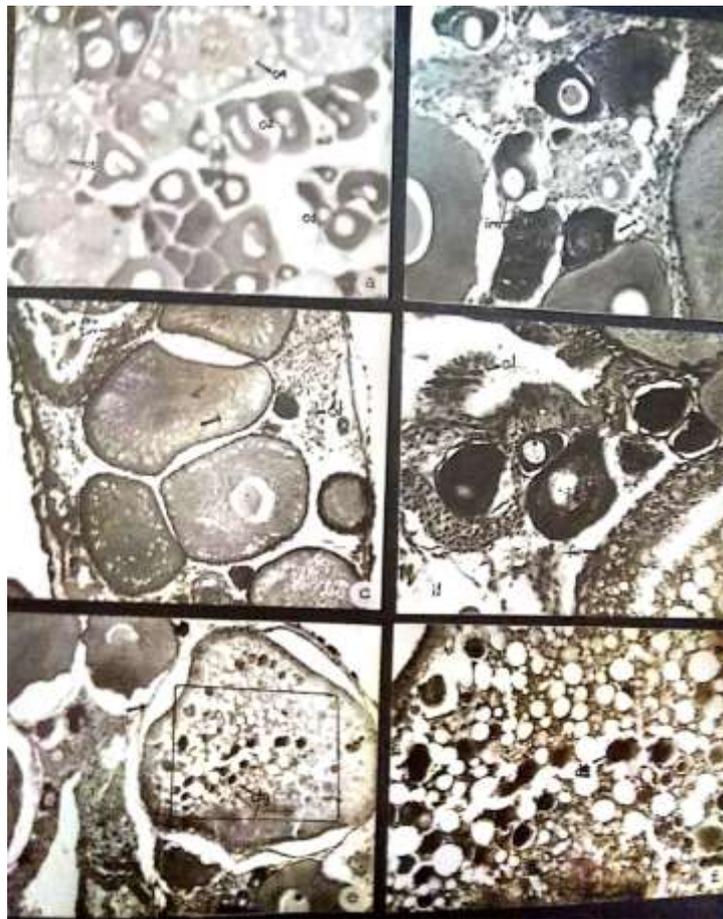


Fig1.1: Cross sections of the ovary of *Gambusiaaffinis* (a) Healthy control (b) 7 days (c) 15 days (d) 30 days (e) 60 days (f) enlargement of rectangle.

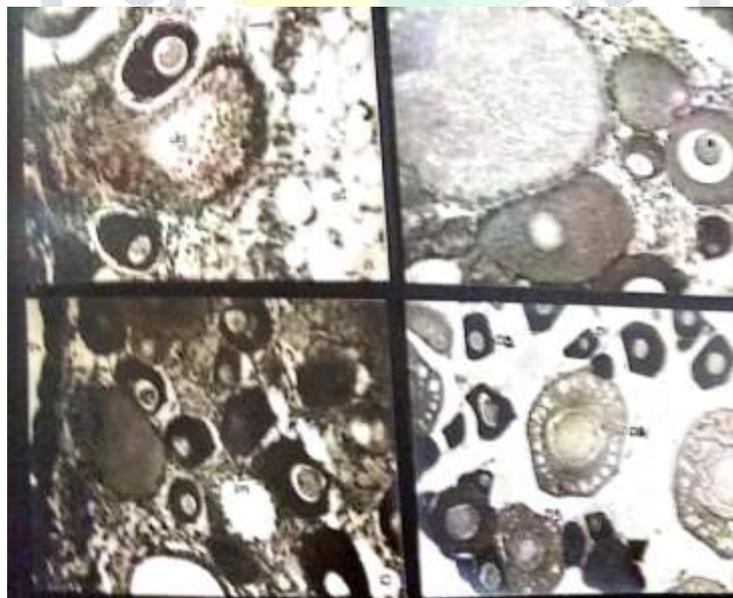


Fig1.2: Cross sections of the ovary of *Gambusiaaffinis* of 90 days (a)Control diet (b)Fed 5.0% Vitamin C diet (c) Control diet (d) 0.5% Vitamin C diet

IV. DISCUSSION

The results showed that the response of *Gambusiaaffinis* to mercury pollution with regard to biological parameters showed retarded growth ,with increased exposure time mortality increased and recovery was observed when restored to normal water

conditions of the control. Vitamin C supplementation in diet has considerable detoxifying effect resulting in 4.12% of increased growth and 19.5% reduction in mortality at the end of 90 days.

Histopathological changes in ovary induced due to mercury pollution shows significant decrease in oocytes. The number also reduced as the time of exposure increased (Day et.al;1989). Necrosis, degenerative change results in damage of tissue, vacuolated oocytes increased with the advanced experimental period (Kirubagaram et.al;1988)..

Mercury effect the reproductive capacity by reducing number and size of oocytes. Atretic oocytes were observed and effect the production of fish.(Kling et.al;1981,Sharma2017). Similar effects were observed in ovary on different fishes by different pollution (Mishra et.al;2008,Pawar et.al;1983, Sioson et.al;1996).

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