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Histological alteration in ovary of the freshwater prawn, *Macrobrachium kistensis* exposed to cuprous oxide

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ABSTRACT

Among the various biomarkers, the histological analysis of different organs may indicate the biological responses to an unfavorable situation, because often the prolongs exposure of the organism to toxic agents does not provoke death directly, but it affects the structure and function of organs of freshwater prawn, *Macrobrachium kistensis*. The experimental prawn treated with lethal concentration (LC_{50}value) 24, 48, 72 and 96 hours and sublethal concentration ($1/10^{th}$ of LC_{50} of 48 hr) for 7, 14 and 21 days of cuprous oxide shows many histological changes in ovary. Histological observations of ovary following exposure cuprous oxide showed destruction of epithelial layer, degeration of oocytes, disorganization of nucleus, vacuolization at periphery, rupturing of follicular epithelium, shrinkage in ooplasmic material and damage of ovarian structure was observed after exposed to lethal and sublethal concentration.

Introduction

Heavy metal causes severe adverse effects on the health of organisms. They act at cellular and molecular level which ultimately lead into physiological and biochemical disorders leading to death.

Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues of animals exposed to toxic stress of heavy metals [Sprague, 1971; Andhale, *et al.*, 2011].

Reproduction is a physiological process and is an essential biological need of animals for the continuity of the generation which is known to dominate all other physiological processes. This main function of reproduction is to replace population losses due to death and migration (warren, 1971).

Sarojini *et al.*, (1988) observed the effects of pesticide on different stages in the oogenesis in the freshwater prawn, *Macrobrachium kistensis*, when exposed to pesticide. Yadav and Sarojini (1989) studied the lethal and sublethal effect of endosulfan on the ovary of the freshwater prawn, *Caridina weberi*.

A histological change in the ovary of the freshwater crab, Barytelphusa guerini exposed to cuprous oxide was carried out by Machale *et al.*, 1990. Chourpagar and Kulkarni (2011) observed histological changes in the tissues of freshwater female crab, *Barytelphusa cunicularis* exposed to heavy metal pesticides.

The available literature reveals that practically less work has been carried out on the effects of copper compound on histological changes, so present work is undertaken to study the lethal and sublethal effects of cuprous oxide on ovary of the prawn, *Macrobrachium kistensis.*

Material and Methods

The prawn, *Macrobrachium kistensis* were collected from Kham River near Aurangabad and kept in aerated plastic troughs under laboratory conditions for 3-4 days. During experiment other conditions were also maintained.

Adults and intermoult prawns were selected for histopathological studies exposed to LC_{50} values of cuprous oxide for 24, 48, 72 and 96 hours and sublethal concentration ($1/10^{th}$ of LC_{50} of 48 hr) for 7, 14 and 21 days. Simultaneously control group of prawns were also maintained. The test media was changed daily to maintain cuprous oxide concentration. The animals were fed during chronic exposure with pellets of wheat bran twice a week.

At the end of exposure period, the ovary was dissected from control and experimental prawns, and fixed in bouin's fluid, dehydrated, cleared in xylol and embedded in paraffin wax (M.P. 58-60˚C) serial sections were cut at 7 to 8 μ and stained in haematoxilin- Eosin.

Results

Histological structure of control ovary:-

The ovary of M. kistnensis (Fig 1) is covered with an outer epithelial membrane followed by connective tissue and inner germinative epithelium. In the early stage of development the germinative epithelium. In the early stage of development the germinative zone or zon of proliferation is distinguished by the presence of compact mass of oogonial cells which undergo meiotic division and give rise to primary oocytes (previtellogenic oocytes). Each vitellogenic oocytes is covered with a thin layer of follicle cells. The mature oocytes or vitellogenic oocytes are completely filled with yolk globules and granules. The nutritive cells are present in close vicinity of the oocytes and supply the nutritive material to the developing oocytes. The degenerating ova are surrounded by nutritive phagocytes which increase in their size with the increase in vacuolization. In fully matured ovary all the above described stages of oocytes as well as follicular cells and phagocytes are observed.

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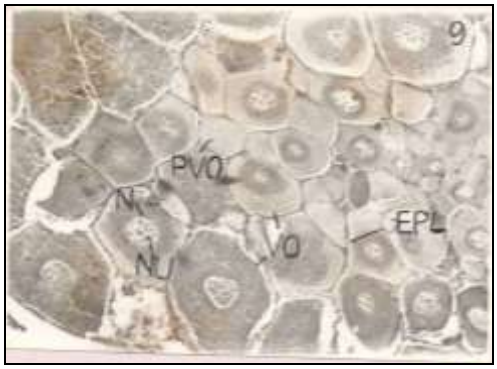


Fig 1 [Control] H & E X 100

Fig 1 .T.S. of control ovary of freshwater prawn, *Macrobrachium kistensis* showing normal structure of vitellogenic oocytes [VO], Nucleus [N], Nucleolus [NU], epithelial layer [EPL], Previtellogenic oocytes [POV].

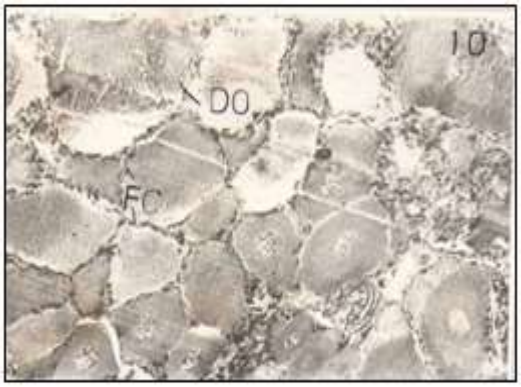


Fig 2 A: [24 h] X 100.

Fig 2 B: [48 h] X 100.

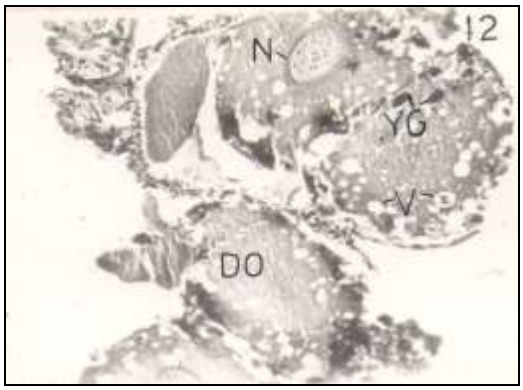


Fig 2 C: [72 h] X 100.

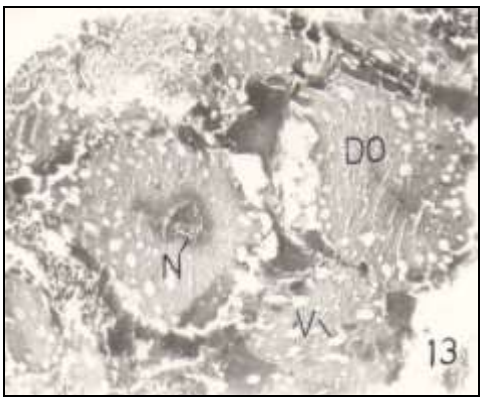


Fig 2 D: [96 h] X 100.

Fig 2 [A-D] T.S. of experimental ovary of freshwater prawn, *Macrobrachium kistensis* exposed to lethal concentration of cuprous oxide showing degeration of oocytes [DO], Damage of nuclei [N], damage in yolk granules [YG] and vacuolization [V] after 24, 48, 72 and 96 h exposure. H& E X 100

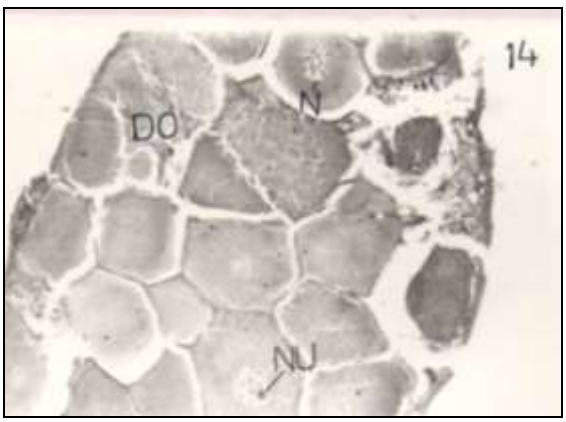
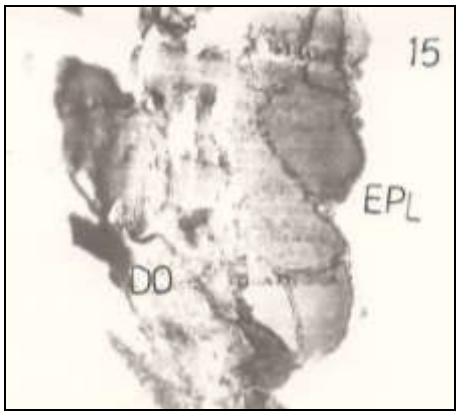


Fig 3 A: [7 Days] X 100



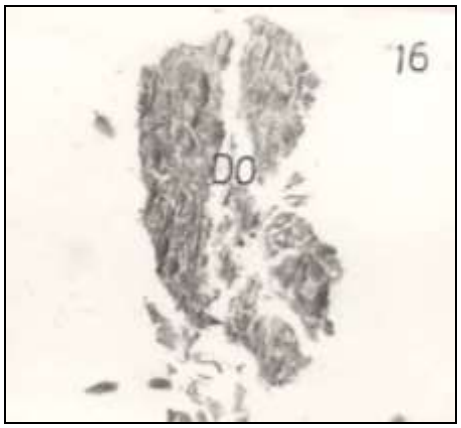


Fig 3 B: [14 Days] X 100. **Fig 3 C: [21 Days] X 100.**

Fig 3 [A-C] T.S. of experimental ovary of freshwater prawn, *Macrobrachium kistensis* exposed to sublethal concentration of [1/10th of 48 h]cuprous oxide showing shrinkage of ooplasmic material, disappearance of nuclei, damage structure was observed after 7, 14 and 21 days of exposure. H & E X 100

Histological changes in the ovary of experimental prawn exposed to lethal concentration of cuprous oxide

Histological observations of ovary following exposure cuprous oxide after 24 h showed destruction of epithelial layer and evidence of degeration of oocytes (Fig 2 A). After 48 h of exposure oocytes degeration and disorganization of nucleus was observed (Fig 2 B). After 72 h exposure to cuprous oxide, vacuolization at periphery, rupturing of follicular epithelium was seen (Fig 2 C). At the end of 96 h maximum number of degerating oocytes with disintegrated nucleoli and vacuolization was observed (fig 2 D).

Histological changes in the ovary of experimental prawn exposed to sublethal concentration of cuprous oxide

After 7 days exposure complete shrinkage in ooplasmic material was observed (Fig 3 A) after 14 days shrinkage in ooplasmic material and vacuolization in nucleus was noticed (fig 3 B). After 21 days of exposure increased number of degerating oocytes and disintegration of nucleus and damage of ovarian structure was observed (Fig 3 C).

Discussion

The pathological changes induced due to cuprous oxide caused specific changes or abnormality at tissue level. In the present study, cuprous oxide treatment caused distinct changes in Gonadal histology.

Cuprous oxide exposure induced significant alteration in the ovary of M. kistnensis, such as damage of germinal zone, changes in cell shape, rupture of oocytes membrane, vacuolization, degeneration of tissue, disappearance of nucleus and nuclei.

After acute and chronic treatment increased in exposure period led to increase in damage to the ovarian tissue. The observed cellular deformities following the cuprous oxide exposure may be due to the direct effects on the developing oocytes through general metabolism and growth or through hormones controlling ovarian growth. The disappearance of nucleus and nucleolus result in the decline of the reproductive activity.

The ooplasmic vacuolization may be due to the sudden alteration in membrane permeability leading to the active transport of cuprous oxide molecules. Such structural damage is caused by the alteration of intercellular ionic composition. Sastry and Miller (1981) reported destabilization of intercellular lysosomes which result in the release of hydrolyses into cytoplasm in response to the toxic stress thus resulting in autolytic cellular damage. A similar mechanism might be working in the present study on *M. kistnensis.*

The observed alteration in the ovarian structure of *M. kistnensis* after exposure to cuprous oxide collaborates the earlier reports of several investigators. Gyananath (1982) reported changes in cell shape, degeneration of oocytes in the ovary of *M. lamerrii* after exposure to dimecron. Deshpande (1985) observed shrinkage in ooplasm, changes in cell shape, vacuolization, and degeneration of tissue necrosis of nuclei and increase in number of phagocytes in the freshwater prawn, *M. kistnensis* after exposure to pesticide. Gangshettiwar (1986) observed the effects of phenol on *M, lamerrii* and noted rupture of oocytes membrane, changes in cell shape, vacuolization and degeneration of nucleoli and nucleus in oocytes.

Rao *et al.,* (1987) reported that the inhibition of germinative zone, lack of distinct nucleolus, in oocytes of freshwater prawn, *M. lamerrii* after exposure to mercuric chloride. Kharat, *et.al,* (2011) observed the histological changes in ovary of freshwater prawn, *Macrobrachium kistensis* exposed to TBTCL. Rajkumar (2012) reported effect of heavy metals in growth of tiger prawn, *Penaeus monodon* (Far. 1978).

Martin *et al.,* (1989) observed destruction of proliferating zone and changes in structure of ovary of the freshwater prawn, *C. weberi* after exposure to methyl parathion. Yadav and Sarojini (1989) observed that disintegration of follicle epithelium, vacuolization dearrangement of oocytes, pycnosis of nutritive cells of nucleus of oocytes in the ovary of freshwater prawn, *C. weberi* after exposure to endosulfan.

Machale *et al.,* (1990) studied the effect of cuprous oxide on the ovary of *B. guerini* and noticed shrinkage in ooplasm, vacuolization on peripheral side, rupture of oocytes, structural damage, necrosis of nuclei and nucleoli and disintegration of oocytes. Sarojini *et al.,* (1990) observed the degeneration of oocytes, vacuolization and replacement of oogonia with fibrous tissue in the ovary of freshwater crab, *B. guerini* after exposure to zinc sulphate. Rao *et al.,* (1990) reported the shrinkage of ooplasm.

The present Histopathological study on ovary showed progressive damage and degeneration and it is clearly evident with the progress of exposure period i.e. extent of tissue damage increases with the increase of cuprous oxide exposure of *M. kistnensis.* Damage to ovary after cuprous oxide exposure lead to decline in reproductive activity.

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