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RESEARCH ARTICLE

IMPACT HEAVY METAL MERCURY ON OVARIAN TISSUES OF LACCOTREPHES RUBER (LINN) (HELEROPTERA: NEPIDAE)

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ABSTRACT

An attempt was made to find out the effect of sub lethal concentration of heavy metal mercury (25 ppm for 48h) on the ovarian tissues of *Loccotrophes ruber* the insects the ovaries are composed of several ovarian tubes termed ovarioles . The classification of the ovary is essentially based on the general architecture of the ovariole. The female reproductive systems of the *Loccotrophes ruber* is composed of two ovaries, each consists of five telotophic type of ovarioles. The ovary during the fully mature turn into yellow in colour due to the heavy accumulation of yellow spheres in the terminal oocyte .Telotrophic ovarioles contain a single huge cluster of germ cells. The cluster consists of several oocyte that are linked in a common ovarioles, The ovary showed some remarkable changes in the insects treated with sub lethal concentration of mercury (25ppm for 48h). In treated ovaries the tunicapropri and other epithelial sheath of the terminal filament exhibited disintegration of oogonial cells, nutritive cord, trophocytes and cytoplasmic vacuolization.

INTRODUCTION

Pollution is an unfavorable alteration of our surroundings, largely as a result of our own actions, through direct or indirect effects of changes in energy pattern, radiation levels, chemical and physical constitution and abundance of organisms. Today, environmental pollution has become an international problem. Harmful chemicals are dumped into the natural environment by the activities of human beings, either directly or indirectly through the rapid development of industrialization and urbanization (Sharma and Devis 1980). Among the various kinds of pollution, heavy metal pollution possibly the most persistent one, pose a threat to the aquatic organisms and they reach high tropic levels through food chain (Harvey *et al.*, 1974). Mercury is known to be the most toxic of all heavy metals (Taylor, 1979). Toxic effects of mercury were reported initially by Gold water (1936) and it is still viewed as one of the most hazardous pollutants. The heavy metal polluted water may lead to the destruction of the beneficial species either directly by affecting the aquatic forms of life, or indirectly through breaking the biological food chain. Mercuric chloride is an inorganic compound that has been used in agriculture as fungicides in medicine as tropical antiseptic and disinfectants, and in chemistry as an intermediate in the production of other mercury compounds. However, the toxicity of mercury was known as early as 16th century. Toxic metals released into the environment affect the reproductive system directly or indirectly. Primary targets of metal toxicity include the neuroendocrine system and sexual functions (Schrader 1997). Mercury is a systematic poison affecting the reproductive system directly. It is evident from several studies that mercury passes through blood-testis barrier to affect steriodogenesis as well as blood-epididymal barrier to affect spermatogenesis (Rao 1989). Mercury a divalent group II B metal, is the potential biocide to aquatic fauna even at fairly low concentrations. The concentration of it in freshwaters world-wide is increasing rapidly, almost doubling every decade, with the discharge of effluents from the industries. Mercury can readily accumulate in the soft tissues of aquatic fauna and has a high affinity for thiol groups, resulting in increased bio-transport, distribution and toxicity (Patel *et al.*, 1990). It is also able to bind to high and low affinity sites of the DNA molecule and produce conformational changes (Lavie and Eviater, 1988). Mercury is widely used in industries, which produce electrical equipments, paints, pesticides, pulp and paper, domestic, thermometers, medicines, batteries, dental amalgams and mercury vapour lamps. Emission of mercury from these products into the environment has been estimated to be about 25 to 50 per cent of the world's production of the metal (NRCC 1979). Mercury is taken up by most of the

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aquatic organisms either directly from the water column or indirectly through the food chain. It has been suggested that insects accumulate mercury from phytoplankton as well as directly from the water column (Jernelor 1972). The effects of mercury and its compounds have become the subject of a large number of experimental investigations in animals an mean after the out-break of minamata disease (Takeuchi *et al.,* 1962). In India, the high rate of increase in human population and rapid pace of industrialization have caused a great disposal problem of waste products. As such, the domestic waste and the industrial effluents, by and large, are being indiscriminately discharged into rivers, reservoirs, lakes, tanks, seas including the adjoining garbage field without any pre-treatment, which have led to more pollution hazards to the aquatic and human life. In female insects, the reproductive organs are visibly affected either as a direct or as indirect effect of chemicals. Induced sterility in treated females may be due to complete cessation of ovarian development, resulting in the loss of fecundity and non-hatchability due to the presence of dominant lethal (Whitten and Foster, 1975 and Labrecque and Fye, 1978).

Insect ovaries are composed of several ovarian tubes termed ovarioles. The classification of the ovary type is essentially based on the general architecture of the ovariole but most of all on the analysis of the ultimate fate of the developing germ cells (for reviews see: King and Buning, 1985; Buning, 1994, 1998). In panoistic ovarioles all germ cells can become oocytes and consequently egg cells. In meroistic ovaries divisions of gonial cells are followed by incomplete ctokineses so that clusters (clones) of sibling cells (cystocyte) are formed. the cystocytes within the cluster remain connected by intercellular bridges forming a specialized syncytium. Nevertheless their forming germ cells do not share a common fate but become diversified into oocytes and nurse cells (trophocytes). The trophocytes are usually polyploidy and synthetically active. Their primary function is to supply the growing oocytes(s) with various macromolecules (mainly rnps). within meroistic ovaries two basic categories have been distinguished: polytrophic and telotrophic. The major difference between these two types is in the way the clusters are formed and spatially organized. in polytrophicovaries each ovariole houses several distinct clusters that together with their somatic follicular coverings form separate functional subunits, termed egg chambers. Within each egg chamber only one oocytes develops, being connected with a group of its own nurse cells. telotrophic ovarioles contain a single, usually huge, cluster of germ cells. The cluster consist of several oocytes that are linked to a common, anteriorly located, trophic compartment (tropharium), usually including numerous trophocytes.

A great deal of work has been undertaken to understand the structural organization of insect ovaries. This includes the investigation of Rajendran (1981), Samal (1982), Dhanam (1984), Kalavathy (1988), Nagappan (1989) and Rajasekarapandian (1994) on Panoistic ovaries, King and Devine (1958), Ramalingam (1971) and Matsuzaki (1978) on polytrophic ovaries and teresa Szklarzewioz *et al.* (1992) on telotrophic ovaries of insects. These studies have elucidated the histoarchitecture of ovarian tissues, the role of follicle cells, nurse cells and oocytes in vitellogenesis and the neuroendocrine regulation of ovarian development. Several studies had been undertaken to understand the histopathological changes in different tissues of insects due to treatment with toxicant such as endosulfan (Sabesan and Ramalinga, 1979), malathion (Kabeer *et al.*,1980), Dimercon (Ilavazhagan,1984), monocrotophos and bendicarb (Rajender,1984, 1986a,b), sevie (Khillare and Wagh,1989), and endosulfan (Sumathi *et al.,* 2001).

Nature of mercuric chloride

Chemical name	: Mercuric chloride II
Chemical formula	: $HgCl_2$
Molecular weight	: 271.50
Colour	: White Powder
Specific gravity	: 9.44
Melting point	: 277°C
Boiling point	: 302°C
Solubility in cold water	: Sparse
Solubility in warm water	: Freely soluble
Nature	: poison

MATERIAL AND METHODS

The adult female insect ovary was exposed by vivisecting under the binocular using the insect Ringer solution (Ephrussi and Beadle 1936). The tissues were immediately fixed in Bouin's fluid. After 24 hours of fixation, the tissues were dehydrated in graded series of alcohol, cleared in xylol and embedded in paraffin wax. Serial sections of 6 thickness were stained in Heidenhain's iron alum haematoxylin and counter stained with aqueous eosin (Gurr 1958).

RESULTS AND DISCUSSION

The female reproductive system of the adult *Laccotrephes ruber* is composed of two ovaries, each consist of five telotrophic type of ovarioles. The two lateral oviducts leading from each ovary unite to form the medium common oviduct. The common oviduct runs backwards and opens to the exterior behind the 8th sternum.

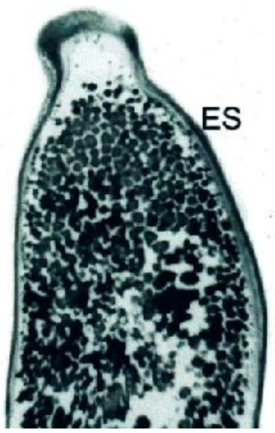

Fig .1. Photomicrograph of the longitudinal section of the anterior region of germarium of control insect loccotrephes ruber during fully mature stage of ovarian development showing the smaller sized cells. ES-Epithelial sheath

Fig 2. Longitudinal section of the anterior region of the germarium of control insect. (NC- nutritive cord)

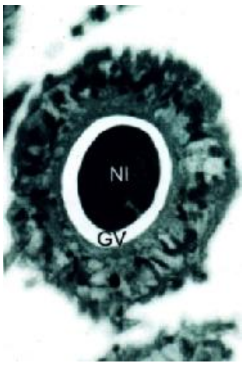

Fig 3. Photomicrograph of the cross section of the terminal oocytes of control insect loccotrephes ruber

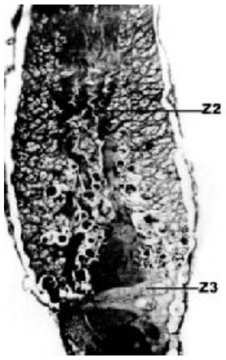

Fig 4. Longitudinal section of the posterior region of the germarium of control insect. (Z2- zone 2

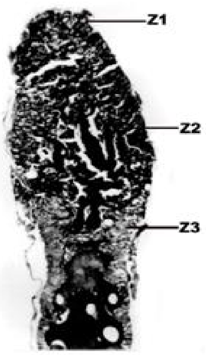

Fig 5. Longitudinal section of the germarium of neem gold treated insect. (Z1- zone 1; Z2- zone 2; Z3- zone 3)

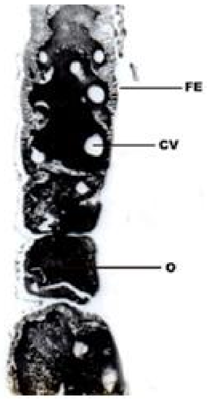

Fig 6. Longitudinal section of the vitellarium of control insect. (CV- cytoplasmic vacuolization; FE- follicular epithelium; O- oocyte)

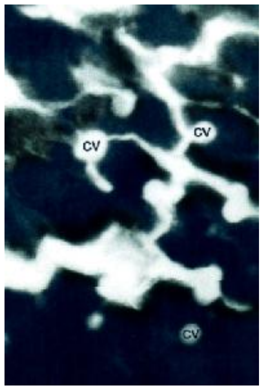

Fig. 7. photomicrograph of the section of the oocytes from heavy metal mercury treated insect, laccotrephes ruber, showing cytoplasmic vacuoles after 48 hours of exposure to sub lethal concentration. CV-Cytoplasmic vacuoles

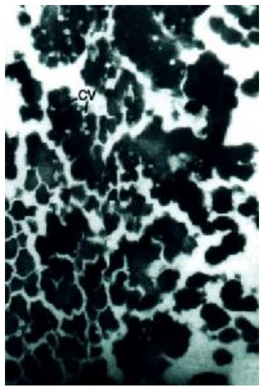

Fig.8. Photomicrograph of the longitudinal section of ovary from heavy metal mercury treated insect, laccotrephes ruber showing cytoplasmic vacuoles pycnosis of nuclei and disintegration of cells 48 hours of exposure to sub lethal concentration. CV-Cytoplasmic vacuoles; N –Nucleus

There is a spermatheca attached at the posterior part of the common oviduct. The ovarioles of the ovary are connected together anteriorly by their terminal filaments and posteriorly by their pedicles. The terminal filaments of the ovarioles join to form suspensory ligments which extend forward and end in tissues beneath the prothoraric terga. The ovary during the fully mature turn into yellow in colour due to heavy accumulation of yolk spheres in the terminal oocyte Ovary of *Laccotrephes ruber* have shown that it belongs to telotrophic type and is similar to that of *Odontopus varicornis* (Saradha1985), *Chrysocoris purpureus* (Madhavan1964) and *Belostoma* *indicum* (Kausthik 1970). The ovarian tissues of *Laccotrephes ruber* show the remarkable histopathological changes such as disintegration of oogonial cells, degeneration of nutritive cord, trophocytes and cytoplasmic vacuolization. The heavy metal mercury has a significant degenerative effect on the oogonial cells, oocytes, follicle cells and trophocytes when exposed to 48 hours of sub lethal concentration. The nutritive cords also exhibit signs of degeneration. The degenerative effect has also been noticed in the interfollicular plugs (Fig. 5). In addition to these changes, oocytes are seems to have fused with each other to form a mass of cells when treated with sublethal concentration for 48 hours of duration (Fig. 5 and 6). Saradha (1985) has reported the trophic core and nutritive cords have been damaged due to treatment with the pesticide dimethoate in *Odontopus varicornis*. This degenerative change of the follicular epithelium due to treatment with the chemosterilant tepa and the chemical substance calcium chloride has been reported for *Locusta migratoria* (Nath and Sharma, 1977). Several other studies have revealed the occurrence of this histopathological change in different insects such as *Cadea cautell a*exposed to 2 per cent apholate (Gangrade and Pant, 1970), *Poekilocerus pictus* exposed to aldrin for 6 days (Janaki Ahi 1988a), *Periplaneta americana* exposed to DDT (Mangla Bhide 1986), *Odontopus varicornis* exposed to dimethoate (Saradha1985) and *Gryllotalpa africana* exposed to monocrotophos (Radhika1992).

Further, it was shown in *Laccotrephes ruber* that the cytoplasmic organizations of oocytes have been affected by the formation of vacuoles of different sizes. Insects treated with sub lethal concentration of 48 hours of duration also exhibit signs of cytoplasmic vacuoles in oogonial cells and follicle cells. Disorganization of cytoplasm and the formation of cytoplasmic vacuoles in the cells of ovarian tissues due to treatment with heavy metal and other chemicals have been reported for few species of insects.The formation of cytoplasmic vacuoles has been reported for the ovary of *Gryllotalpa africana* exposed to monocrotophos (Radhika, 1992), *Odontopus varicornis* exposed to monocrotophos and *Cadea cautella* exposed 2 per cent apholate for 48 hours (Gangrade and Pant, 1970) In the present investigation, insects treated with sub lethal concentration for 48 hours of exposure have shown the existence of chromatin clumping and nuclear pynosis in oogonial cells, oocytes, trophocytes and follicle cells. Thus, similar histopatholgoical changes have been reported to occur in the ovary of *Musca domestica* exposed to sodium oxide solution (Thakur and Mann, 1982) and *Odontopus varicornis* exposed to dimethoate (Saradha, 1985). Other toxicant such as SAN 322 and DDVP have also produced similar changes in the germarium of *Mylabris pustulata* (Sanjeewani *et al.,* 1988). Thus, the heavy metal mercury seems to exert its action directly on ovarian tissues reducing drastically the reproductive potentials of *Laccotrephes ruber*.

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