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THE HEAVY METAL MERCURY IS A THREAT TO ANIMAL LIFE: A STUDY ON MALE GAMETOGENESIS IN *GESONULA PUNCTIFRONS*

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ABSTRACT

The effect of mercury was investigated on the animal model, *Gesonula punctifrons*, concerning their gamete producing activity. Mature male grasshoppers treated with 1 mg of mercuric chloride per kg of fodder, exhibited a drastic impact on male gametogenesis in this insect species. It was found that due to intake of mercury, a damaging effect on the chromatin material of the cell could be noticed. A devastating effect on the process of spermiogenesis could be noticed on the treated animals. Compared to the normal spermiogenesis, the treated organisms showed notable abnormal features in spermatid maturation. Spermatids showed less condensation of the chromatin along the phases of maturation of the spermatids and accumulation of vacuolar zones within the nuclear chromatin. Eventually this abnormality often led to production of less number of mature sperms with normal morphology. Thus, the toxic effect of mercury may have some damaging effect on the chromatin materials.
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Key words : *Gesonula punctifrons*, spermatogenesis, spermiogenesis, chromatin, meiosis.

INTRODUCTION

Mercury as a polluting agent is known to common man from the remote past. For this reason, most of the people are conscious about exposure to mercury. But how mercury affects the physiology and the genetic system of the living organism, is a subject to be explored in details. The toxic mercury is a heavy metal, which binds to the thiol groups of polypeptide chain and causes oxidation. It acts as a spindle inhibitor and generates reactive oxygen species leading to glutathione depletion in cultured mammalian cells (De Flora et al., 1994). Mercury can be obtained from laboratory compounds as well as fungicides, dyes, disinfectants, drugs etc. Toxic effect of mercury includes damage to the brain, kidney and lungs. Mercury poisoning causes several diseases including Pink disease, Hunter-Russell syndrome and Minimata disease. Symptoms include sensory impairment (vision, hearing and speech) causing disturbed sensation and a lack of co-ordination.

Recent reports proclaim that methyl mercury induces free radical stress and results in early aging (Zahir et al., 2006). The present study deals with the changes in the testicular activity of a model organism, grasshopper belonging to species *Gesonula punctifrons*.

MATERIALS AND METHODS

Twenty matured male grasshoppers (Gesonula punctifrons) were collected from nature and they were kept in a glass cage with fresh grass as fodder. They were allowed to live in the laboratory condition for about 15 days for acclimatization. In another cage one kg of fresh grass was taken and the grass was mixed with 1mg of mercuric chloride in 100ml aqueous solution. In this cage 10 matured male grasshoppers out of the twenty already reared in the laboratory were set free and they were sacrificed after 4 days of treatment with mercury. The grasshoppers were supplied with water every day and the survivors were collected on the 4^{th} day. The testicular tissue were taken out from both the groups and testis- lobes were isolated from both treated and non-treated grasshoppers. The collected tissues were fixed with aceto-methanol and then stained with 2% aceto-orcein for 15 minutes. The stained tissue materials were washed properly with 45% acetic acid and then they were squashed over clean glass slides with clean cover glass. The stained tissue materials were then observed under microscope to study the cellular features in the testis.

RESULTS

The testicular tissue stained with aceto-orcein showed details of the process of spermatogenesis including cellular association. A comparison of the tissues from the untreated grasshoppers and that of the treated ones indicated some landmark observations. The testicular lobules were found to be well differentiated with large number of cell population with the display of sequential events of spermatogenesis. The post meiotic spermatid differentiation exhibited distinct metamorphosis of the spermatids to form matured spermatozoa. Altogether 11 different stages of spermatid differentiation could be noted (Fig. 1) in the untreated animals. A spermatid at the initial stage was found to contain very little amount of cytoplasm and the cytoplasm portion was found to move towards the posterior part of the cell during spermatid differentiation. Along with this nuclear elongation appeared to be prominent as spermatid progressed more and more towards the maturing spermatid stage. From the mid spermatid stage the cytoplasmic part was found to be almost disappeared (Fig.1, stage 7-9). The 11^{th} stage marked the maturity of the spermatozoon when it appeared like a long very slender needle like head (Fig.1, stage 11) with a long extremely slender tail.. The elongated compact chromatin head was found to highly flexible.

The treated animals in their testicular tissues exhibited landmark feature as chromatin swelling of the nuclear mass of the spermatids during their differentiation. All the stages of spermatid differentiation were marked with nuclear swelling with reduced stain ability of a spermatid. The mature spermatozoa that were developed following spermateleosis appeared thicker in comparison to those formed during normal gametogenesis in the testis of the grasshopper (Fig. 2, stage 12). The swelling of the spermatids were also accompanied by accumulation of vacuolated structures (Fig. 2, stage 5–10). Some developing spermatids were found to be quite stubby with wavy outline and; less condensed chromatin. The bodies appeared to be the aberrant forms of spermatids those might not be transformed into functional spermatozoa. Further, number of spermatozoa in the lobules of the treated animals was found to be extremely less compared to that in the normal animals.

DISCUSSION

Mercury being a highly toxic pollutant attracted many investigators to study its effects on cellular and physiological functions of the living organisms. Japanese medaka (*Oryzias latipes)* after the exposure to the methyl mercuric chloride showed high concentration of methyl mercury accumulation in the liver and in the brain, while low accumulation in the muscle. Histopathological changes such as edema, vacuolization, pychnotic nucleus in the cell, degenerative sperms were observed in the slices from liver, gill and male gonad respectively. Inhibition of cholinesterase activity was also another common feature (Liao et al., 2006). When catfish (*Clarias batrachus*) was treated with mercury, histological study of testicular tissue showed that the seminiferous tubules were smaller in size and contained mostly non-dividing cells in comparison to the control fish in which they were greatly distended and full of

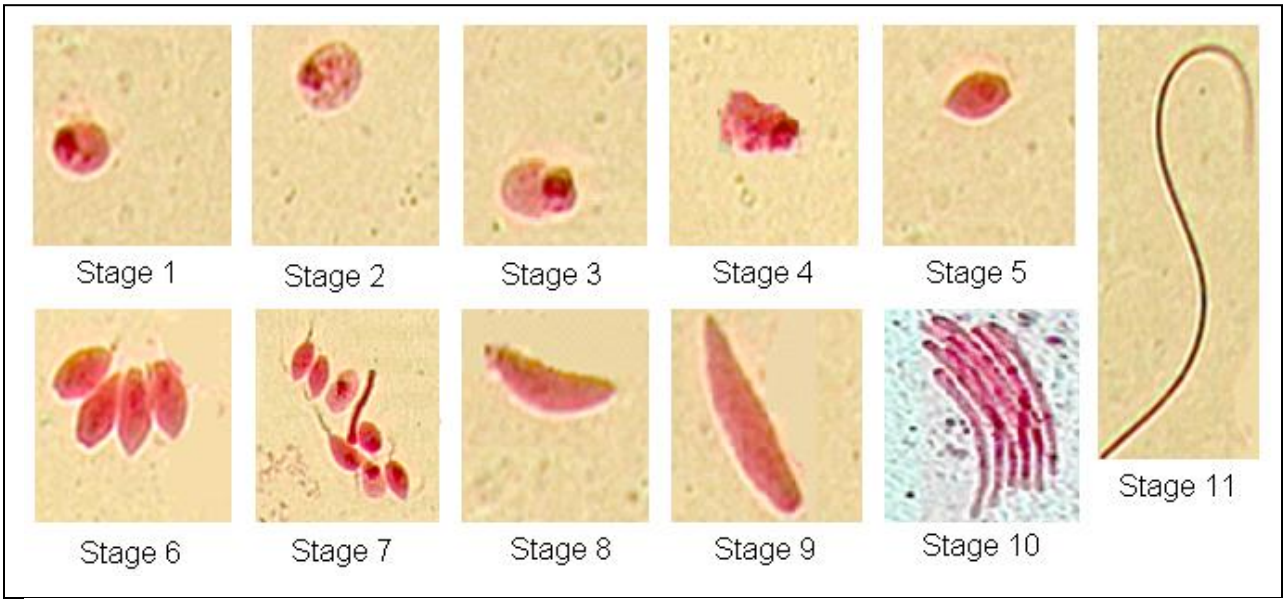

Figure-1. Spermiogenesis in Gesonula punctifrons
Stage 1-10 represents different stages of metamorphosis of the spermatids and stage 11 appears as the mature spermatozoon with very compact slender and long head

spermatozoa. Leydig cells showed pychnotic changes in mercury treated fish. Levels of phospholipids and free cholesterol registered significant reduction. The observation suggested that impairment of testicular lipid metabolism by mercury is one of the possible factors leading to inhibition of steroidogenesis and spermatogenesis (Kirubagaran and Joy, 2005). Mercuric chloride if administered into male mouse produced a reduction in epididymal sperm account, sperm motility and sperm viability. Co-administration of vitamin E with mercuric chloride prevented the changes in sperm, proving that vitamin E has some protective effect against mercuric chloride, causing retention of the reproductive activity (Rao and Sharma, 2001).

In the present study, tissue configuration of the treated grasshopper showed some detectable changes which could not be found in control animals (Meistrich, et al., 1976). .Due to the intake of the toxic material, cellular and nuclear integrity was not properly maintained. The spermatogenic cells underwent a few uncomfortable situations to cope up with the presence of the toxic metal and thus they might have accumulated more water from outside to be swelled in order to reduce the damaging effect of mercury. Some investigators (Schmidt et al., 1992, and Smidt & Ibrahim, 1994) observed accumulation of mercury in the gut and testes of a species of grasshopper when they were fed with food treated with mercuric chloride and they also observed chromatin fragmentation in the treated animals. Mercuric chloride exposed monkey *Macaca fascicularis* showed changes in spermatogenesis (Mohamed et al, 1986). Effects of exposure to sublethal mercuric chloride on the testis and the fat body of the frog *Rana cyanophlyctis* was also reported (Kanamadi and Saidapur, 1992). Methyl mercury hydroxide showed some impact on meiotic chromosomes of the grasshoppers, *Stethophyma grossum* (Klasterska and Ramel, 1978). Fragmentation of chromatin material during meiotic divisions as observed in the meiotic plates appeared probably due to the toxic effect of the chemical. The appearance of the vacuoles in the nucleus of the spermatids appeared due to the accumulation of water within the nuclear mass. Reduction in the number of mature sperms as observed may be due to impaired spermiogenesis which might have a negative impact on fertility of the grasshoppers.

The experimental result appeared to be constintent with the results of some other

Figure 2 : Stages of spermiogenesis as observed in Grasshopper treated with $HgCl_2$.
Distinct cytoplasmic movement during differentiation of spermatids could not be detected in the treated cases. Further a distinct swelling of the nuclear mass is prominent in all the developing spermatids. Some spermatids showed accumulation of vacuolated zones within the nucleus (Stage 5, 6 & 7). Spermatids even at their advance stage of appeared to be aberrant being shorter and thicker compared to normal spermatids

previous work (Kirubagaran.and Joy, 2005, Klasterska and Ramel, 1978, Liao. et al., 2006, Meistrich et al., 1976, Mohamed et al., 1986, Wester & Canton, 1992).

CONCLUSION

The present finding indicates that mercury as a heavy metal is very seriously toxic in context of human life in which human may face a sub-fertility state on being exposed to pollution with mercury from various sources. An indication to such effects is reflected through the condition of the treated grasshopper testis.

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