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Histopathological Changes in Malpighian Tubules of Silkworm Exposed to Selenium

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Abstract: Selenium (Se), a trace element, has evolved from its toxic properties to an essential micro nutrient. As it is known that digestion and absorption of nutrients to various tissues has acquired great significance in the animal physiology and maintenance of homeostasis, study of pathological changes and structural modifications of a cell would help in evaluating the extent of the effect of selenium on Silkworm *Bombyx mori L.* tissues. In the present investigation a progressive histopathological study of tissues such as malpighian tubules of silkworm *Bombyx mori L.* under the exposure of lethal (32.39µg/Kg. body weight) and sub lethal doses (6.47µg/Kg. body weight) of selenium was carried out. Malpighian tubules are site for storage and/or elimination of toxic substances. The results of present study on histopathology of the silkworm *Bombyx mori. L* exposed to lethal (32.39µg/Kg. body weight) and sub lethal doses (6.47µg/Kg. body weight) of selenium at different periods of exposure revealed a relationship between the histopathological changes and the progression of external symptoms. Severe destruction to the architecture of malpighian tubules of *Silkworm* exposed to lethal dose (32.39µg/Kg. body weight) of selenium was observed. Cloudy swellings, tubular cell degenerations, narrowing of the lumen of the tubules, dilated tubules, necrosis, tubular cell edema and persistence of piknosis was observed in the proximal tubules. On the other hand sub lethal dose of selenium also produced certain histopathological changes in 3, 6, 9 and 12 groups of silkworms. The physiological and biochemical alterations are very less and more or less similar to controls in the 9 and 12 groups of silkworms. Correspondingly the structural damage to malpighian tubules in these groups is very less particularly at 6 days of exposure.

Key words: *B. mori* Larvae · Histopathological Changes · Malpighian Tubules And Selenium

INTRODUCTION

Silkworm, a lepidopteron insect has a great economical interest due to its contribution in the synthesis of silk proteins. It is well known that protein synthetic activity of a cell is performed in a most efficient manner in a narrow range of conditions. The nutrients absorbed from the Mulberry leaves for protein synthesis, its digestion and absorption to various tissues acquire a great significance in the physiology of an animal. In recent findings the contribution of trace elements such as selenium, Nickel and cobalt in nutrition has been realized and the metabolic disorders due to deficiency of selenium has been recognized practically in all the livestock producing countries of the world as selenium as a micronutrient exerts their biological effects either directly or by being incorporated into enzymes and other bioactive proteins. Selenium at sub lethal doses acts as a growth stimulant [1]. Hence inadequate quantities of nutrients cause disturbances in the maintenance of homeostasis. This may lead to development of certain external symptoms due to structural modifications of a cell. In the present investigation the tissue called Malpighian tubule is selected to observe the histopathological changes, as it is an effective excretory system to eliminate all the nitrogenous and harmful undesirable waste products from the body/blood and contributes much in the reabsorption of salts and extra water for homeostasis [2]. The internal histological changes of malpighian tubules to selenium have not yet been published. This work provides a description of Malpighian tubules of silkworm larvae under the effect of selenium.

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MATERIALS AND METHODS

Silkworm *Bombyx mori. L* of V instar with parentage of PM x NB4D2 with an average weight of 1.5gm was used for the study. The silkworms were divided into groups of 50 silkworms each were reared in rearing trays, one group in each tray and were maintained at the temperature 23±1°C and relative humidity 70-75% and worms were kept at 16hrs light 8hrs dark and allowed free access to mulberry leaves. V instar silkworms were used in the present investigation. Groups 1, 2 and 3 were treated with distilled water, lethal (32.39 µ gm/Kg b.w.) and sub-lethal (6.47 µ gm/Kg b.w) doses of selenium consequently for 3 days. Groups 4, 5 and 6 were treated with distilled water, lethal (32.39 µ gm/Kg b.w.) and sub-lethal (6.47 µ gm/Kg b.w) doses of selenium consequently for 4 days. Groups 7, 8 and 9 were treated with distilled water, lethal (32.39 µ gm/Kg b.w.) and sub-lethal (6.47 µ gm/Kg b.w) doses of selenium consequently for 5 days. Groups 10, 11 and 12 were treated with distilled water, lethal (32.39 µ gm/Kg b.w.) and sub-lethal (6.47 µ gm/Kg b.w) doses of selenium consequently for 6 days. After scheduled exposures, the silkworms were sacrificed and the Malpighian tubules were dissected and fixed in aqueous Bouins fluid. The permanent preparations of the slides were made following the usual procedure of paraffin embedding and with 6µ thick sections. Delafield's haematoxylene and eosin were used as stains and counter stains respectively. All the photomicrographs were taken with Olympus microscope 450X.

RESULTS AND DISCUSSION

The malpighian tubules of silkworm exhibited maximum histological degenerations induced by selenium at its lethal dose. The external toxicity symptoms with the selenium exposure were studied in the insect. The present histological study revealed a relationship between the histopathological changes and progression of external symptoms. No unhealthy cells were observed in the different tissues of control larval sections. And their nuclei were smaller. The histological changes in the malpighian tubules are mainly expressed in the degeneration of the cells along with their nuclei. The V instar larvae of silkworm when exposed to selenium at lethal dose have shown progressive reduction in locomotors activity and onset of locomotors ataxia. This might be due to metabolic inhibition causing reduction in the nerve or muscular functions or both. The cessation of feeding activity at the onset of symptoms within half an hour to one hr exposure to lethal dose of selenium is perhaps due to nervous effect. But it can also be attributed to an indirect effect of the selenium on malpighian tubules [3]. The symptoms such as regurgitation and diarrhea are because of the expulsion of selenium from the body. When the histological degenerations are complete the nervous system also becomes badly affected and the insects suffer paralysis and ultimately die. It may further presumed that the lesion observed with the abnormality of the malpighian tubules under selenium exposure, undoubtedly affect their role in excretion and water conservation. Malfunction of the tubules might result in abnormal haemolymph pH and inorganic ion concentration which can possibly interfere with normal neuromuscular function and cause the slow onset of ataxia and prostration [4]. Hence, selenium exposure probably set forth acute physiological aspects and initiates chemical reactions which may be responsible for bringing the pathological changes in the various body tissues viz., vacuolations, contractions, exfoliations of epithelium, destruction and dislocation ofnuclei, ruptures, extrusions and many other types of degenerative changes in the tissues and ultimate death. The results are connected with the histological study of tissues of grasshopper by Misra [5]. Severe pathological changes were observed in the malpighian tubules of group 2 (Plate I b) like cloudy swellings and degenerations of tubular cells. The swollen tubular cells projected into the lumen of the tubules, which reduced the size of the lumen. Some of the tubular cells exhibited granular cytoplasm. Some of the tubules were dilated and necrosis was seen in the proximal tubules.

In group 5 (Plate II b) also malpighian tubules showed noticeable changes, the nuclei of the tubular cells were not visible and those which could be seen appear to be pyknotic (A degenerative state of the cell nucleus). The cytoplasm of many tubular cells was granular. Necrosis in proximal tubules was evident, but the extent of damage was relatively less than in group 2 (Plate I b) Silkworms. Silkworms in group 8 (Plate III b) exhibited tubular cell edema, round cell infiltration, tubular cell necrosis. But the extent of damage was more than that of group 4 (Plate II b) silkworms. Silkworms in group 11 (Plate VIII b) exhibited severe degenerative changes as seen by wide spread disintegration of malpighian tubules. Necrosis of tubular cells was most pronounced. Presence of casts in tubular lumen, aggregation of chronic inflammatory cell in the interstitium was observed. In group 3 (Plate I c) silkworms showed necrosis in the tubular cells. The nuclei of the most of the oedematus

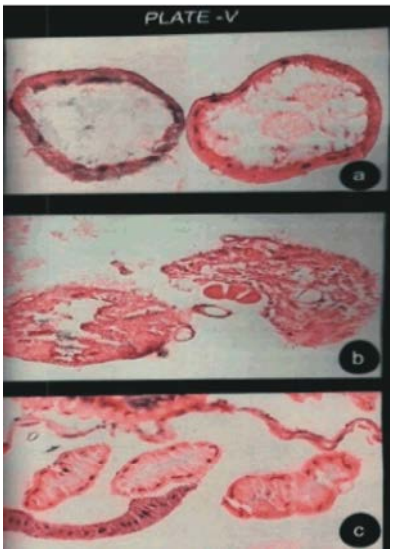

Plate I: a, b and c: Transverse sections of the malpighian tubules of V instar Silkworm *Bombyx mori L.* (PM X NB_4D_2) exposed to lethal and sub lethal doses of selenium at 3 day.(a: Group 1 (Control), b: Group 2 (Lethal) c: Group 3 (Sub lethal). (HandE). X 450

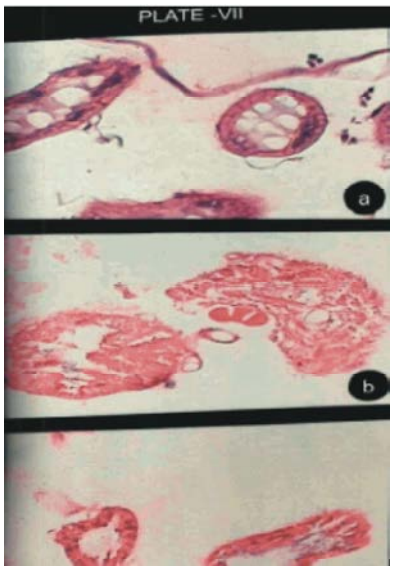

Plate III: a, b and c: Transverse sections of the malpighian tubules of V instar Silkworm *Bombyx mori L.* (PM X NB_4D_2) exposed to lethal and sub lethal doses of selenium at 5 day. a: Group 7 (Control), b: Group 8 (Lethal), c: Group 9 (Sub lethal). (HandE). X 450

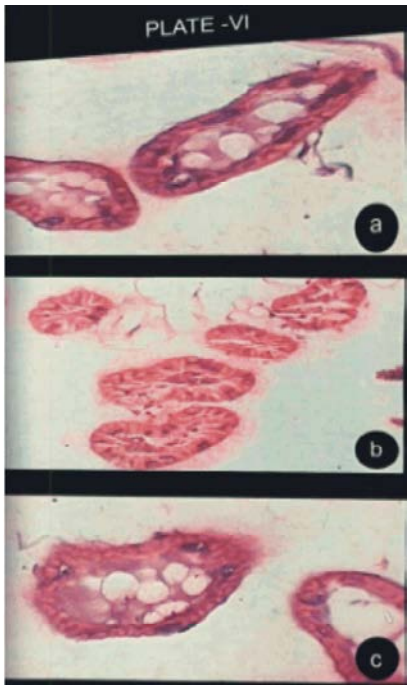

Plate II: a, b and c: Transverse sections of the malpighian tubules of V instar Silkworm *Bombyx mori L.* (PM X NB_4D_2) exposed to lethal and sub lethal doses of selenium at 4 day. a: Group 4 (Control), b: Group 5 (Lethal), c: Group 6 (Sub lethal). (HandE). X 450

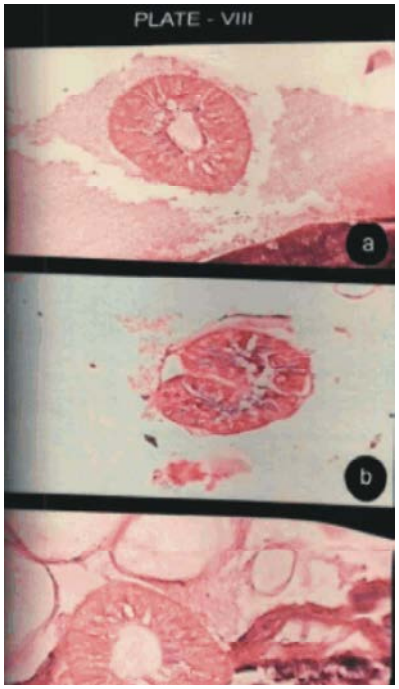

Plate IV: a, b and c: Transverse sections of the malpighian tubules of V instar Silkworm *Bombyx mori L.* (PM X NB_4D_2) exposed to lethal and sub lethal doses of selenium at 6 day. a: Group 10 (Control), b: Group 11 (Lethal), c: Group 12 (Sub lethal). (HandE). X 450

tubules were not visible and appeared pyknotic and were in the process of nuclear degeneration. In group 6 (Plate II c) silkworms, there were no appreciable changes and moderate tubular necrosis was observed. In group 9 (Plate III c) silkworms exhibited hyperplasia of tubular tufts together with necrosis in tubular epithelium and cellular infiltration in interstitial. In group 12 (Plate IV c) silkworms did not exhibit any pathological changes except limited signs of necrosis.

In a majority of insects toxic chemicals that enter the tissues are excreted often after being metabolized. The changes frequently resulted in the compound become less toxic, but the converse is sometimes true and toxicity is enhanced. Some compounds notably those that are water soluble are metabolized to components that are subsequently incorporated into the insect's primary metabolic pathways. Most of the lipophylic substances are first converted into water soluble components and are excreted. Many different enzyme systems are known to be involved in these reactions and some systems are almost certainly ubiquitous. This process may occur in a variety of tissues as there is no organ comparable with the liver i.e., the focus for comparable reactions in vertebrates. Activity of the appropriate enzymes often occurs in the midgut, fat body and malpighian tubules. Different species differ widely in their ability to metabolize toxic substances. Amongst plant feeding insects this variation contributes to host pant specificity. The caterpillar of *Manduca sexta,* for example habitually feeds on alkaloid containing plants, including tobacco and it is able to do his because it detoxifies the alkaloids [6]. Although, the end products of the metabolic process are commonly excreted, sometimes it is sequestered. Sequestration may also occur without any prior metabolism. In some species and the compound has been stored in the cuticle, perhaps minimizing the risk to the insect. But the other species store the defensive substances in glands or in the haemolymph. Similarly silkworm fat bodies accumulate most of the toxic substances for the detoxification. Greater accumulation of selenium in groups 2, 5, 8 and 11 might have resulted in extensive degeneration of structure of fat body may be due to the failure of detoxification mechanisms. The most significant changes observed are cell necrosis, fatty degeneration and hyperplasia.

Even though in the presence of sub lethal dose of selenium is decreased the selenium treated silkworm exhibited some histopathological changes were more at higher selenium dose and at 6 days of exposure. This could be attributed to sodium selenite, which is toxic due to its great solubility and stability. The lower amounts of sodium selenite resulted in very mild histopathological changes in the sub lethal treated silkworm fat body groups 3, 6, 9 and 12. Accumulation and excretion studies have also confirmed the marked decrease in the absorption of selenium in silkworm and less accumulation in the fat body, as wells as marked increase of faecal selenium excretion [7].

In insects both the malpighian tubules and hind gut function together as excretory organs. The malpighian tubules collect the filtrate from the haemolymph and pass this primary urine to the hind gut. Insect excretion has been reviewed extensively [8-10]. Since malpighian tubules are mainly concerned with the elimination of undetoxified and unwanted substances from the haemolymph of the silkworm, it is more prone to the toxicity of various toxic pollutants. Selenium treated silkworms in all the groups exhibited significant alterations in the architecture of malpighian tubules. The histopathological changes were in the direct proportion to the dosage and period of selenium administration.

Areas of cloudy swellings, degeneration and necrosis of malpighian tubules could be resulted due to increased selenium ions accumulated [7]. Similar to the histopathological changes observed in the selenium exposed silkworms in the present investigation are also reported by the earlier workers in different experimental toxicants and insecticides. Misra [5] reported that those pesticides are excreted through the malpighian tubules. He also pointed out that malpighian tubules are severely damaged in BHC, Malathion and phasmomidon experimental silkworms. He presumed that in the presence of toxic levels of pesticides in the haemolymph the tubular structure of malpighian tubules is selectively damaged by its passage. According to Misra [5] pesticide exposed malpighian tubules appear degeneration of the cells along with their nuclei, narrowing of the lumen of the tubules and hypertrophied condition. Histopathological alterations like necrosis of Microvilli, cloudy degeneration of tubular cells in malpighian tubules of poisoned insects have been noticed [3, 4, 11]. The findings of pioneer investigations showing extensive destruction to the malpighian tubules of selenium intoxicated silkworm substantiate the observations of the above studies. Our results coincides with the earlier reports that suppression of protein synthesis was observed in silkworm exposed to lethal dose indicating the breakdown of proteins in haemolymph due to active stress and sub lethal dose of selenium in trace amounts was stimulatory and did not

accept toxic effect on silkworm proteins levels [12]. Higher levels of selenium in the haemolymph and malpighian tubules also support the cytotoxicity to the tubular cells the malpighian tubules.

CONCLUSION

Silkworms on exposure to sub lethal dose of selenium did not exhibit significant changes, except mild necrotic symptoms, in conformity with recent reports reveal that low quantity of selenium is beneficial to organisms. Hence this study would be helpful in determining the safe periods/safe sub lethal doses of selenium, which directs to provide suitable quantity of selenium as a micronutrient in silkworm rearing and economic values for the maintenance of cocoon production under normal and micronutrient supplementation conditions can be expected.

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