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***Research Article** (Open Access)*

HISTOLOGICAL CHANGES BY CHROMIUM IN TESTIS OF ADULT MALE *SPHAERODEMA RUSTICUM* (HETEROPTERA: BELOSTOMATIDAE)

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ABSTRACT
Histology of the male reproductive system of Fabricius *(Sphaerodema rusticum)* consists of a pair of testis, seminal vesicle and vas deferens. The testis showed some remarkable changes in the insects treated with heavy metal chromium (14 ppm median lethal concentration). The testis of treated insects showed the presence of a ruptured testicular follicle, disintegrated Spermatocytes, pycnotic and necrotic spermatids. The toxicity impact of heavy metal chromium on *Sphaerodema rusticum* was appropriate to be comparatively higher than other insects.

Keywords: *Cytometric data, Toxicity, Sperms, Necrotic, Seminal vesicle, Pycnotic, Apical cells, Follicle lumen, Spermatocytes, Germinal epithelium, Testis*

INTRODUCTION
Histological as well as ultrastructural evaluations performed for diagnosis of environmental pathology, capable of detecting effects of pollutants even at sublethal levels (Au, 2004; Kheirallah *et al.*, 2016). Several authors reported on ultrastructural pathology of heavy metals in testis of aquatic & terrestrial insects (Kheiralla *et al.*, 2016). Insects' reproductive system is complex; spermatogenesis & testicular development examined for numerous insect species. Histological Numerous investigations in polluted aquatic environments indicated a relationship between metal concentrations in sediments as well as those in indigenous fauna (Corbi, 2010). The metals toxic effects maybe demonstrate at individual level, leading to elevated mortality in sensitive species along with altering essential processes including growth & reproduction (Chanu *et al*., 2017). Aquatic beetle faunas extensively sampled in ecological research aimed at biomonitoring & assessing species richness (Vinicius Albano Araujo *et al*., 2021).
These comparatively large insects exhibit sensitivity to environmental fluctuations, possess extensive distribution, could be readily sampled, then inhabit various guilds within aquatic & semi-aquatic ecosystems (Short, 2018). The testis are opaque white structures located at posterior median region of abdominal cavity. Each testis consists of 5testicular follicles" & encased in connective tissue that binds the follicles together (Merin Emerald & Rameshkumar, 2012). In Coleoptera, encompassing Cerambycidae, male reproductive system comprises pair of testes, accessory glands, an ejaculatory duct, pair of vasa deferentia, as well as an aedeagus (Nurcan Ozyurt Kocakoglu, 2023). The testis morphology & pigmentation, follicular count within testis, as well as morphology & number of accessory glands exhibit interspecific variation. Sperm at various developmental stages (spermatocytes, spermatids, & spermatozoa) identified in testis. In certain species, testicular development occurs from distal to proximal, whereas in others, it progresses from periphery to medial (Erbey *et al*., 2021). Histologically, each testis comprises numerous testicular follicles, where spermatozoa are generated, exhibiting varying lengths across different genera & species (Elelimy *et al*., 2017). Morphological characteristics of male tract in three species of Belostoma and their similarities with other aquatic Heteroptera are discussed (Selami Candan *et al*., 2020). Numerous authors documented structure & functions of male reproductive organs across various insect species. Belostomatidae Latreille (Igor Luiz Araujomunhoz, 2020). These considerations prompted an

investigation into impact of heavy metal chromium of adult male testis of Fabricius (*Sphaerodema rusticum*).

MATERIALS AND METHODS

Adult male insect *Sphaerodema rusticum*, along with its fat body, testis, & seminal vesicle, maintained separately after 48hrs prior to being dissected under a binocular microscope employing Ringer solution (Emphrussi & Beadle,1936). Ringer subsequently removed, as well as tissue fixed in Bouin's fluid for 24hrs. Heidenhain's iron alum haematoxylin applied to serial sections that were 6µ thick, & aqueous eosin employed as a counterstain (Gurr, 1958). Morphometric data pertaining to the nuclear diameter, nuclear volume, length of spermatids, sperms, the diameter and volume of the fat body cells, the male accessoty reproductive glands, testis and seminal vesicles and corpus allatum were obtained using an ocular micrometer and drum micrometer.

Six sections were randomly selected for measurements. From the measurements, the approximate volume of the nucleus (v) calculated by using the formula.

$V= \frac{\pi d3}{6}$ or $\frac{4}{3}\pi r^3$

RESULTS AND DISCUSSION

*Sphaerodema rusticum*s male reproductive system includes an aedeagus, two testes, seminal vesicles, vas deferentia, & two accessory glands. Testis longitudinal section in control insects reveals that follicle consists of multiple acini. The apical region comprises several primordial germ cells that are heavily stained with haematoxylin. Lumen contains numerous primary & secondary spermatocytes that are intensely stained with eosin. The secondary spermatocytes are microscopic in comparison to primary spermatocytes as a result of mitotic & meiotic divisions. The number of secondary spermatocytes appears to exceed that of primary spermatocytes (Fig. 1a-1d).

The testicles of *Sphaerodema rusticum*s are paired, however testes, vas deferens, along with seminal vesicle in a closely related species are paired. The sperm tubes in testicles are joined by connective tissue, that is milky-white in color & is generally observed close to rear of abdomen *Spilostethus pandurus* (Elelimy *et al*., 2017), *Isotomus speciosus* (Nurcan Ozyurt Kocakoglu, 2023), *Tropisternuscollaris* (Araujo, 2021). *S. rusticum's* testes have seven testicular follicles, which is more than another Hemipteran testicle counts (S. Candan *et al*., 2018). Similar to other Hemiptera, each follicle of *S. pandurus* contains germarium in apical region. Testicular follicles encased by a cellular layer (peritoneal sheath), an epithelial layer, as well as a non-cellular layer (tunica propria). (Rodrigues *et al*., 2008). Testis of *I. speciosus* comprises a pair of lobes, similar to those in *C. herbacea* along with *C. populi* (Ozyurt Kocakoglu, 2022). Each testicular lobe of *C. populi* comprises 10 testicular follicles.

Similar to the structure in pair of testes, vas deferens, seminal vesicles and testicular follicles and characteristic features are same species *C. oleosa* (Mayra Velez, 2020). Histological examinations of the reproductive system in insects exposed to heavy metals provides insights into the effects of these toxic substances on insect reproductive physiology (Merin Emerald & Rameshkumar, 2012). The male *P. ataturki* possesses internal reproductive structures comprising paired testes enveloped by a peritoneal sheath, aerated by multiple tracheoles, along with paired vas deferens & accessory glands. The testis that are positioned across body cavities are elongated ovoid in shape. Each testis is coated by an epithelium (Damla *et al*., 2021). The spermatozoa traverse to vas deferens, enter vas deferens, then subsequently conveyed to seminal vesicle. In *G. lineatum*, spermatozoa's heads situated within epithelial lining of seminal vesicles, while their tails extend posteriorly in a spiral into lumen. Histology of vas deferens & seminal vesicles is not significantly different (Nurcan Ozyurt *et al*., 2013).

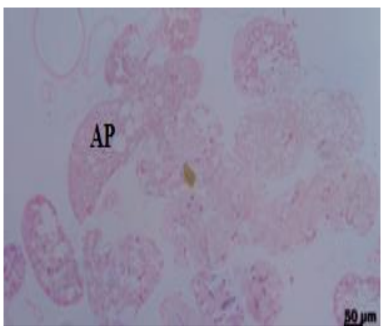
The current investigation indicates that the testicular follicles of control insects contained numerous spermatocytes, sperms, along with sperm bundles. It comprises primary & secondary spermatocytes that are intensely stained with haematoxylin. The sperm cells elongated, filamentous structures organized in bundles within follicular lumen. However, the testicular follicles of the treated insects exhibited numerous histopathological alterations, including "disintegrated apical cells, primary & secondary spermatocytes, diminished nuclear volumes of primary as well as secondary spermatocytes, lightly stained primordial germ cells, disorganized sperm with broken tails, reduced lengths of spermatids as well as sperm, necrotic spermatids as well as sperm, as well as sparsely packed spermatozoa with increased luminal space within the follicle. These alterations may be ascribed to influence of heavy metal poisoning.

Table 1: Cytometric data of control and chromium treated Testis of adult male insect *Sphaerodema rusticum*

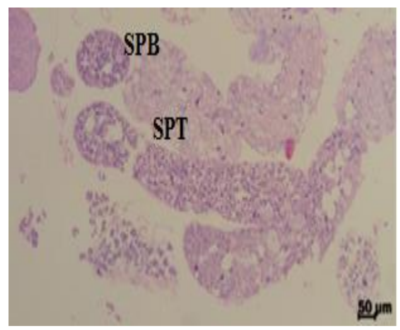
Aspects	Control	Treated	Inference
Conical cell shape	Spherical	Oval	Change of cell shape in the treated insects
Nuclear shape	Spherical	Oval	
			Change of nuclear shape due to treatment with median lethal concentration of chromium
Nuclear diameter(μ)	20.O6 ± 0.03	20.51 ± 0.01	
Nuclear volume(μ^3)	479.9 ± 0.04	509.99 ± 0.04	Change of nuclear diameter due to heavy metal intoxication
Primary spermatocyte cell shape	Spherical	Oval	Change of nuclear volume due to heavy metal intoxication
Nuclear shape	Spherical	Oval	
Nuclear diameter(μ)	22.61 ± 0.01	42.08 ± 0.02	Change in nuclear shape in the treated insects
Nuclear volume(μ^3)	510.18 ± 0.03	4080.58 ± 0.02	
			Change in nuclear shape in the treated insects
Secondary spermatocyte cell	Spherical	Oval	Change in nuclear diameter due to
Shape	20.39 ± 0.03	20.76 ± 0.01	heavy metal intoxication
Nuclear diameter(μ)			Change of nuclear volume due to heavy metal intoxication
	508.89 ± 0.02	542.47 ± 0.01	
Nuclear volume(μ^3)			
	Spherical shaped	Spindle shaped	Change in cell shape due to heavy metal treatment
Spermatid shape			
	81.47 ± 0.01	60.36 ± 0.03	Change in nuclear diameter due to heavy metal intoxication
Spermatid length(μ)			
	219.98 ± 0.25	119.99 ± 0.02	Change of nuclear volume due to heavy metal intoxication
Sperms length(μ)			
			Change in the length of spermatids due to treatment with the heavy metal, chromium
			Change the length of sperms due to treatment with the heavy metal, chromium

*Values are mean ± S.E. of different measurements; *significant at 0.01% level*

Despite insects typically possessing a high reproductive capacity, the quantity of follicles in each testicle varies among different groups (Sturm, 2018). Some species including *P. ataturki, P. parallelus* (Polat *et al*., 2019), *Poecilimoncervus*, 1950 (Orthoptera, Tettigoniidae) & *M. sanguinipes* (Jones *et al*., 2013) has a large number of follicles in their testis, while some species has much less follicle number. For example, *Orphullela punctate* (De Geer, 1773; Silva *et al*., 2018), *larval Dionejuno* (Crameri, 1779) and larval *Agraulis vanilla* (Linnaeus, 1758) male have four follicles (Mari *et al*., 2018), *Martaregabetoi* (Novais *et al*., 2017), males have two follicles (Zhang *et al*., 2016) and *M. tanacetaria* follicles (Vitale *et al*., 2011) in their testes. The reduction or absence of germ cells results in the shrinking of testicular follicles along with partial obstruction of follicle lumen, these alterations observed in our research. Similar abnormalities described by Kheirallah *et al*. (2016) in assessing adverse effect of heavy metals on the testicular structure of aquatic and terrestrial insects. The present research also examined presence of necrotic spermatids along with spermatozoa. Occurrence of necrotic cells is attributed to loss of membrane integrity, swelling of intracellular organelles, as well as depletion of ATP, resulting in an influx of calcium (Cullen, 2010). Heavy metals may be the cause of the parietal cells' enlarged cytoplasm, that is packed with necrotic sperm (Kheirallah *et al*., 2019).



(1a)



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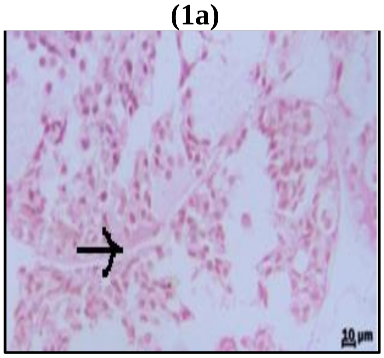

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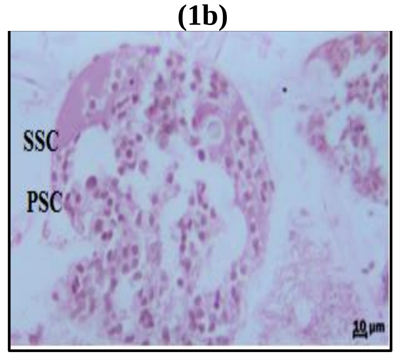


(1d)

Figure 1: Transverse section of testis follicle of control insect (a-d)

AP–Apical cells; SPB–Sperm bundle; SPT– Spermatids; → Sperms; PSC–Primary spermatocytes; SSC–Secondary spermatocytes

Histology of the testis

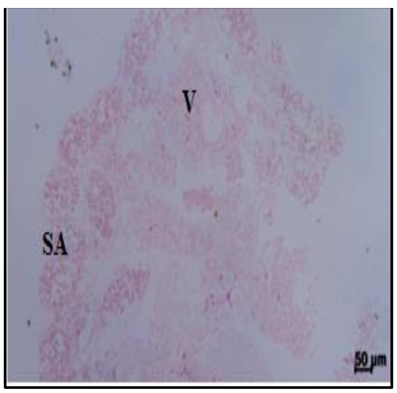
The cytometric data of volume and length of gonial cells, primary spermatocyte, secondary spermatocyte, spermatid and sperm of control and treated insects are presented in the **Table 1**.

A transverse section of the testis reveals that it consists of a large apical cell, which are mainly meant for providing nourishment for the germ cells called trophocytes (**Fig.1a**). The apical cells are found to be intensely stained. The spermatogonia are invariably found closely associated with the germinal epithelium.

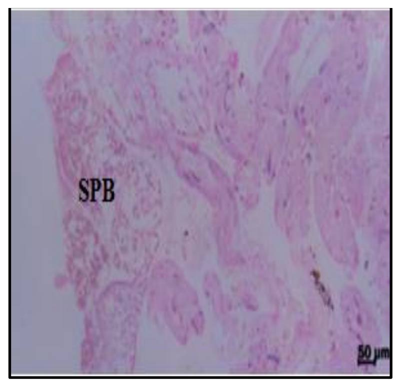
Each gonial cell is approximately 20.06 ± 0.03 µ in size and appears spherical with a large nucleus, enveloped by a thin layer of cytoplasm (**Fig.1b**). The primary spermatocytes are conspicuous with less amount of cytoplasm and centrally located nuclei, which are intensely stained with haematoxylin (**Fig.1c**). The diameter of the primary spermatocytes measures about 22.61 ± 0.01µ. The secondary spermatocytes are comparatively more in numbers than the primary spermatocytes where reduction division takes place and gives rise to spermatids and further, it develops into spermatozoa (**Fig.1d**). The secondary spermatocytes measures about 20.39 ± 0.03µ. Sperms elongated and have hair-like structure arranged in form of bundles. Head region of sperms appeared to be intensely stained with eosin, the length of the spermatid measures about 81.47 ± 0.01µ and the length of sperm measures about 219.98± 0.25µ respectively.

Histopathology of testis

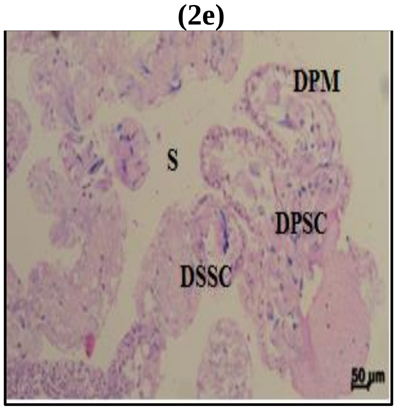
The chromium treated insect exhibit remarkable histological architecture such as the occurance of highly pycnotic and necrotic peritrophic membrane with disintegrated nutritive cells. **Fig.2e** indicate the less amount of nutrition for developing germ cells and spermatids. The gonial cells are found to be swollen with less intensely stained cytoplasm and highly pycnotic diffused nuclei (**Fig.2f**).



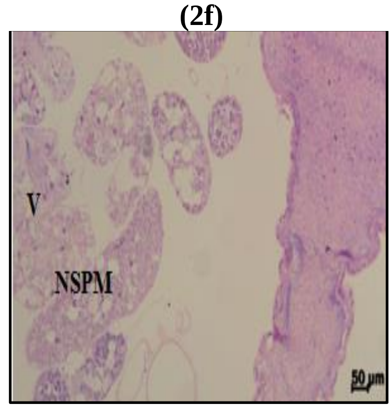
(2e)



(2f)



(2g)



(2h)

Fig.2 Transverse section of testis follicle of treated insect (e-h)

V–Vacuole; SA–Shrunken acini; SPB–Sperm bundle; DPM–Disintegrated peritrophic membrane; S–Space; DPSC–Disintegrated primary spermatocytes; DSSC– Disintegrated secondary spermatocytes; NSPM– Necrotic sperms

CONCLUSION

It has a volume of about 509.99 ± 0.04µ. The primary and secondary spermatocytes appear to be increased in their volume and measure about 4080.58 ± 0.02µ and 542.47 ± 0.01µ respectively. The spermatids seem to be less in number and clumped (**Fig.2g**). It shows an intensive reaction with eosin and appears to be clumped together, leaving more space in the follicle of the testis, which is an indication for atrophy of many primary and secondary spermatocytes (**Fig. 2h**), in the treated insects, the spermatid and sperm measured about 60.36 ± 0.03µ and 119.99 ± 0.02µ, respectively. It is concluded from above results that heavy metal chromium, analogous to several other toxicants & plant products, causes histological alteration in testis & affects reproductive potential of *Sphaerodema rusticum*s.

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