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SUSTAINED REDUCTION OF SERUM TESTOSTERONE BY D-ALLETHRIN ADMINISTRATION IN ALBINO RATS

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ABSTRACT

This study was carried out to examine the effect of prolonged oral administration of D-allethrin on the testes and serum testosterone levels in male albino rats. A total of 60 healthy adult male albino rats (Rattus norvegicus) were used. They were separated into four groups of 15 rats each. D-allethrin was orally administered to all rats in group II, group III and group IV at doses of 25, 100 and 175 mg/kg/day for four weeks respectively. Rats in group I served as control and were given the vehicle olive oil only. The rats in each of the groups were further separated into sub-groups of A and B of 9 and 6 rats respectively. Sub-group B (6 rats) were punctured at the tail region and blood samples taken once every week (7 days) and analyzed to determine the serum testosterone levels. Each time blood samples were collected from sub-group B rats, three rats from sub-group A were euthanized and testes excised, measured and fixed in 10% formalin for subsequent processing and histological studies. At the end of the fourth week, all the rats in sub-group B were also euthanized and their testes excised, measured and fixed in 10% formalin for subsequent processing and histological studies. Hormone profile analysis showed that serum testosterone levels decreased significantly ($p<0.05$) in rats treated with D-allethrin which indicates that the germ cell population of their seminiferous tubules were also affected since testosterone is required for the synthesis of germ cells. However, the testicular weights and testicular circumferences of the rats were not significantly affected. These findings suggest that chronic exposure to pesticides containing D-allethrin may disrupt gonadal steroidogenesis in male rats. Thus, investigations to further study the mechanism of D-allethrin toxicity and the reversibility/irreversibility of its pathological effects is highly recommended.

INTRODUCTION

D-allethrin, a mixture of the cis and trans isomers of Allethrin, and an active component of mosquito repellent coils in many countries (Meister, 1992) was used in this study. Studies (Melissa *et al*, 2007) have indicated that pyrethroids can interact competitively with human androgen receptors and androgen binding proteins, suggesting that chronic exposure to pyrethroids may disrupt endocrine functions. Eil and Nisula (1990) reported that pyrethrin and Bioallethrin but not D-allethrin were tested in human genital skin fibroblast.

Studies (Golla and Suresh, 2012) have also indicated that prolonged exposure to allethrin-based mosquito coil emissions resulted in testicular interstitial edema, structural damage of the seminiferous tubules and epithelial cells and evidence of dead spermatozoa in the tubular lumen in rats. Allethrin-based mosquito coils also

have been associated with sperm abnormality (Idowu *et al* 2015).

The present study was to examine the effects of prolonged oral administration of D-Allethrin on the testes morphology and serum testosterone in albino rats.

MATERIALS AND METHODS

Sixty male albino rats (*Rattus norvegicus*) weighing an average 160+6 gm were used for these experiments. Following acclimation for two weeks at 25 $\pm$ 3^{o}C animals were placed in 4 groups of 15 rats.
Research-grade allethrin (1000 mg, 93% purity) was suspended in 100ml olive oil (Goya En Espana, SAU, Sevilla, Spain). Thereafter, the rats were separated into 4 groups of 15 rats each. From the 10% stock solution, 25, 100, and 175 mg/kg of allethrin was prepared and orally administered to groups II, III, and IV respectively while Group I received olive oil only. Six rats from each group were separated bled through the tail vein once every week for serum testosterone assay while three were killed each week and their testes removed and fixed in 10% formalin for subsequent histological studies.
At the end of four weeks (28 days) of treatment, all the bled rats were also sacrificed, their testes removed and measured and fixed in 10% formalin for subsequent processing and histological studies.

Testosterone assay

The blood samples from the six rats were centrifuged at 2000g for 5 minutes and the serum separated for hormonal analysis as described by Tietz (1995). Using testosterone Enzyme Immunoassay kit from (Biocheck Inc., Foster City, CA).

Testicular Weight and Circumference

Following sacrifice, testes were removed, cleaned with normal saline, blotted on a filter paper and weighed on an electronic balance (Metlar, MT-501). The testicular circumference (mm) of each testis was determined using a thread and a meter rule.

Assessment of the Germ Cell Population of the Seminiferous Tubules

The testes were removed from formalin, washed and dehydrated in ascending grades of alcohol and prepared for histological sections as described by Singh (2010). Slides were examined using Olympus microscope (Olympus Corporation, CX31RTSF, Japan).

Statistical Analysis

All data were expressed as means $\pm$ SEM and analyzed using one-way and three-way ANOVA for both treatment and time effects and their interactions. Where a significant F was obtained, means were compared using Tukey's Multiple Range test ($p < 0.05$).

RESULTS

Fig.1. Shows that the serum testosterone levels of group I (control) rats orally administered Olive oil, increased significantly ($p<0.05$) with increase in days of the experiment.

Whereas, the serum testosterone levels of group II, group III and group IV rats orally administered D-allethrin + Olive oil at doses of 25, 100 and 175mg/kg/day respectively, decreased significantly ($p<0.05$) with increase in the duration of administration.

Fig.2. Shows the cumulative effect of treatment (dose) and time (week) on the serum testosterone levels of rats in group II-IV over a period of four weeks. The chart indicates that serum testosterone levels decreased significantly ($p<0.05$) with

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increase in dose and duration (time) of administration when compared with the control.

Fig.3. Shows that there was no significant difference in mean testicular weight of group I (control) rats administered Olive oil at week one, week two, week three and week four.

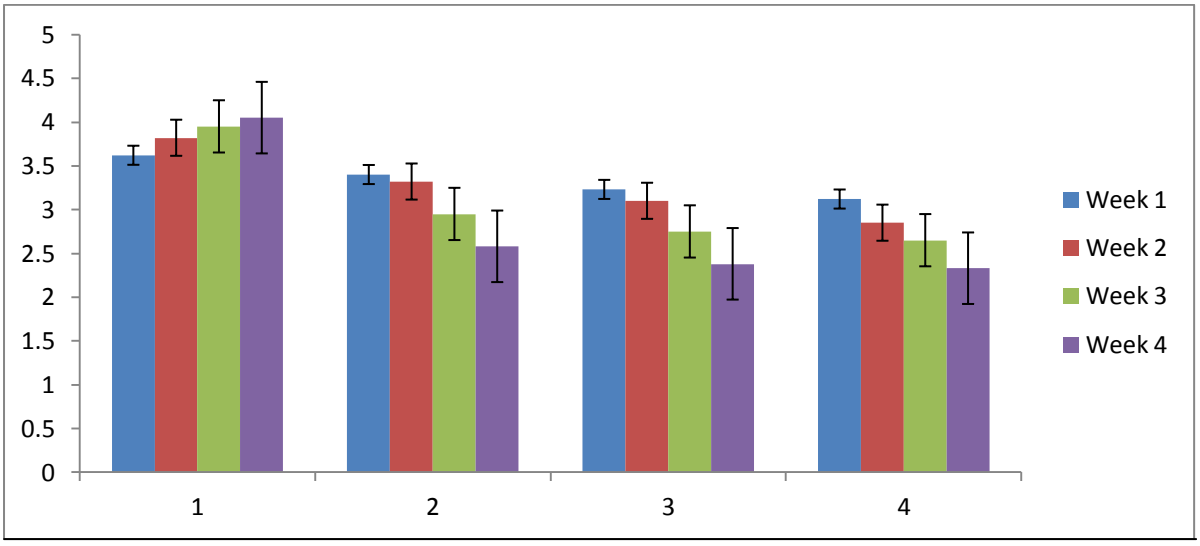
Also, there were no significant differences in mean testicular weight per week of group II, group III, and group IV rats orally administered D-allethrin + Olive oil at doses of 25, 100 and 175mg/kg/day respectively.

Fig.4. indicates that there were no significant effects of treatment (dose) and time (week) on the testicular weights of rats in all the groups.

Fig.5 and fig.6. Show that the mean testicular circumference of rats in all the groups, increased significantly ($p<0.05$) at week three and week four. But, no significant differences in mean testicular circumference were observed at week one and week two.

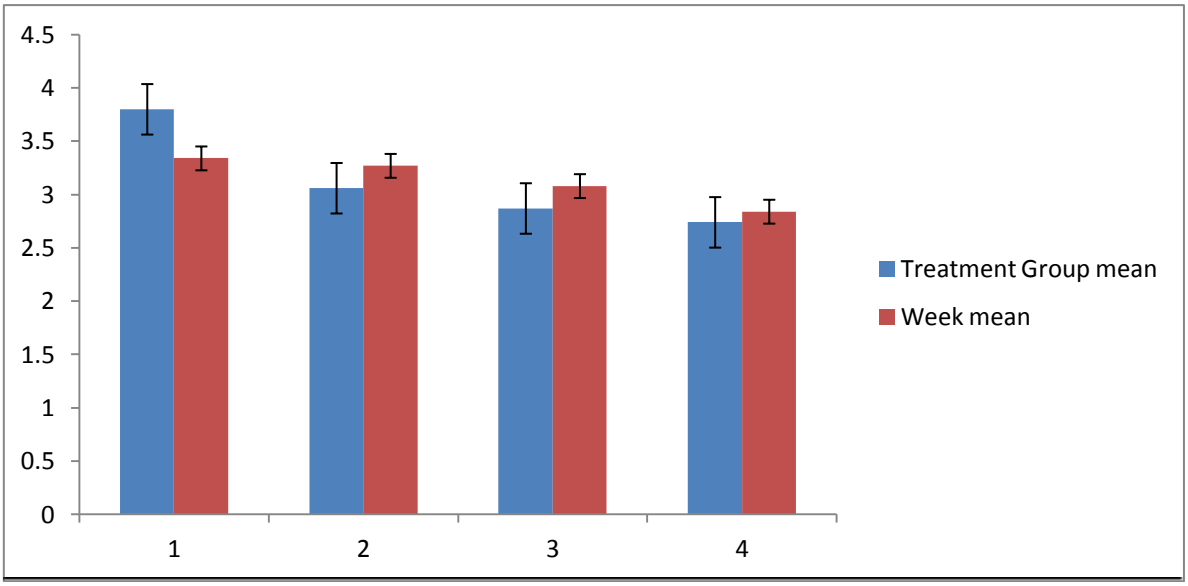
Results from the histological examination of the testes of rats in group I (control) showed apparently normal seminiferous tubules with spermatogenic cells at different stages of development, and the cumulative effect of treatment (dose) and time (week) on the rats in group II-IV over a period of four weeks (Fig. 7 and Fig. 8).

The Photomicrographs revealed that the germ cell population of the seminiferous tubules of animals treated with D-allethrin for one week reduced with increase in dose of administration when compared with the control. Also, many spermatocytes were exfoliated in the lumen of some tubules, and the connective tissue stroma appeared to be loosely packed around the seminiferous tubules (Fig. 9). These pathological changes were exaggerated in animals treated with D-allethrin for four weeks (Fig. 14).

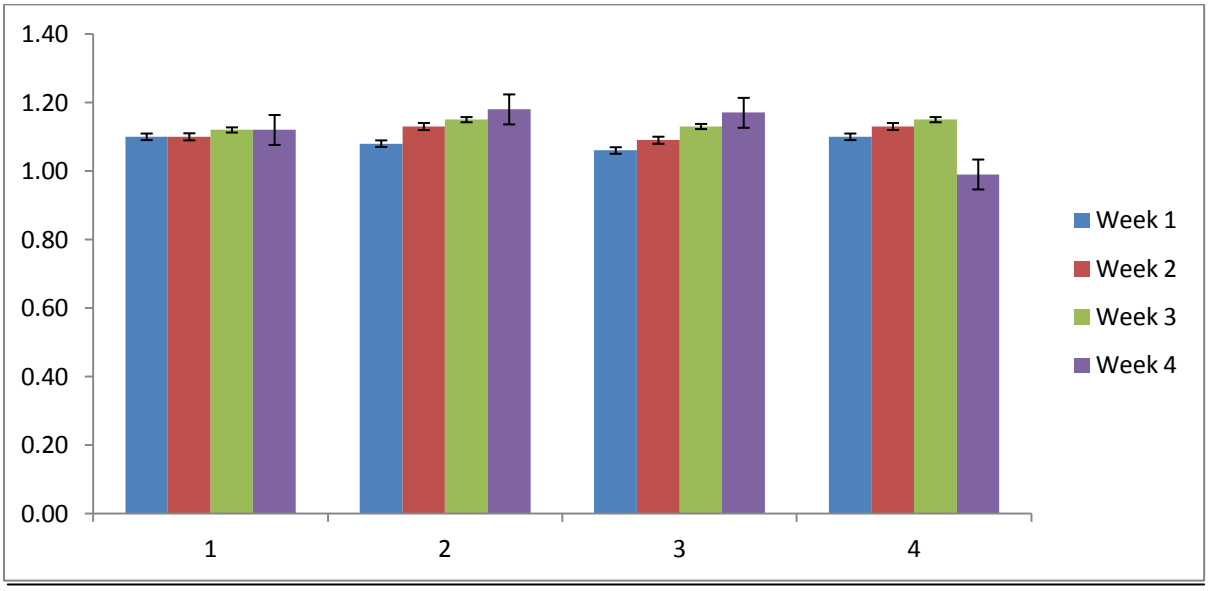


1 = Group I, 2 = Group II, 3 = Group III, 4 = Group IV

Figure 1. Serum testosterone levels (mean + standard error of mean (SEM)) of rats in group I-IV. Blood samples were collected once every week for four weeks (n=6).



Fiure 2. Cumulative effects of treatment and time (week) on the serum testosterone levels (mean + standard error of mean (SEM)) of rats in group I-IV over a period of four weeks.



1 = Group I, 2 = Group II, 3 = Group III, 4 = Group IV

Figure 3. Testicular weights (mean + standard error of mean (SEM)) of rats in group I-IV. Samples were collected once every week for four weeks (n=3 for sub-group A rats and n=6 for sub-group B rats).

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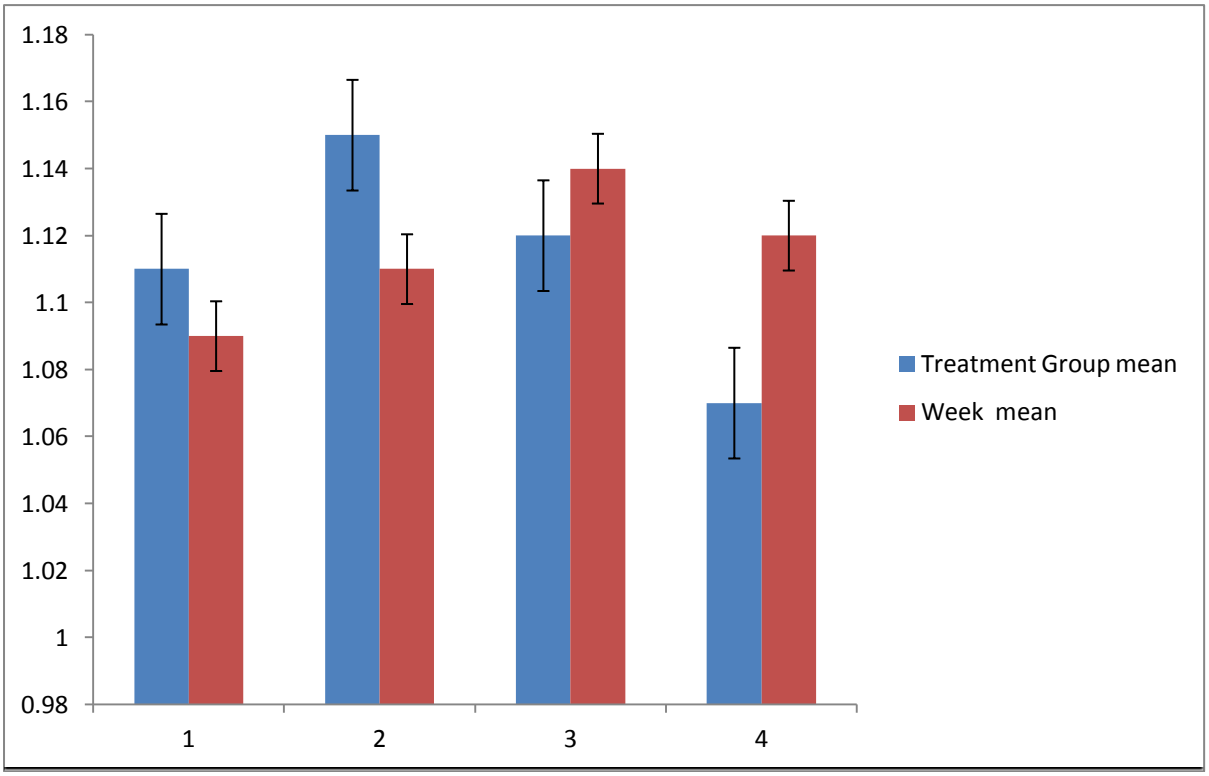
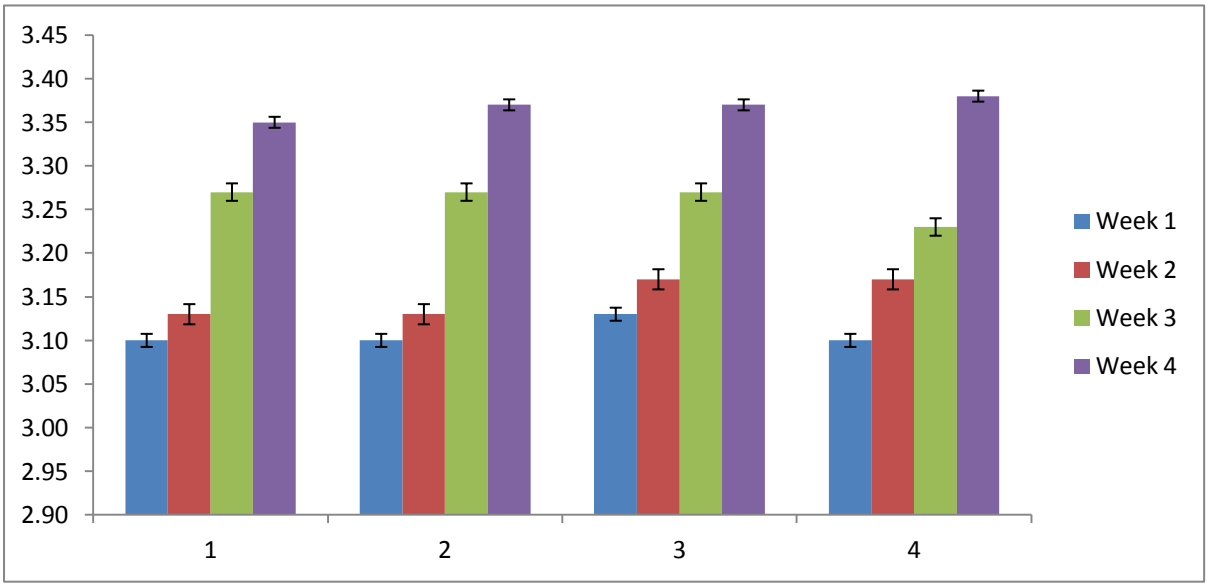


Figure 4. Cumulative effect of treatment and time (week) on the testicular weights (mean + standard error of mean (SEM)) of rats in group I-IV over a period of four weeks.



1 = Group I, 2 = Group II, 3 = Group III, 4 = Group IV

Figure 5. Testicular circumferences (mean + standard error of mean (SEM)) of rats in group I-IV. Samples were collected once every week for four weeks (n=3 for sub-group A rats and n=6 for sub-group B rats).

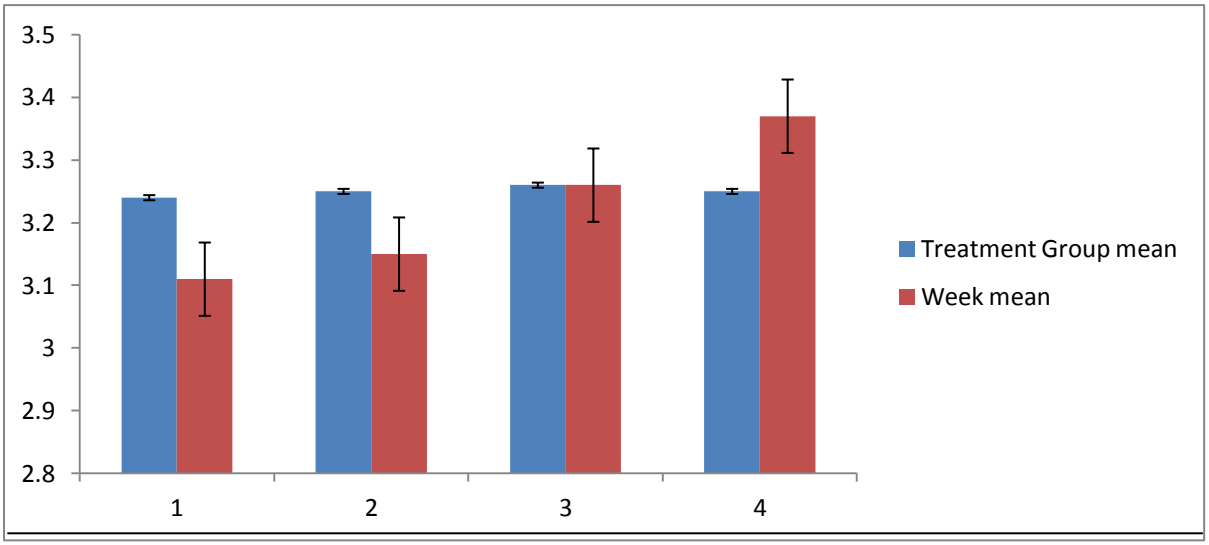


Figure 6. Cumulative effect of treatment and time (week) on the testicular circumferences (mean + standard error of mean (SEM)) of rats in group I-IV over a period of four weeks

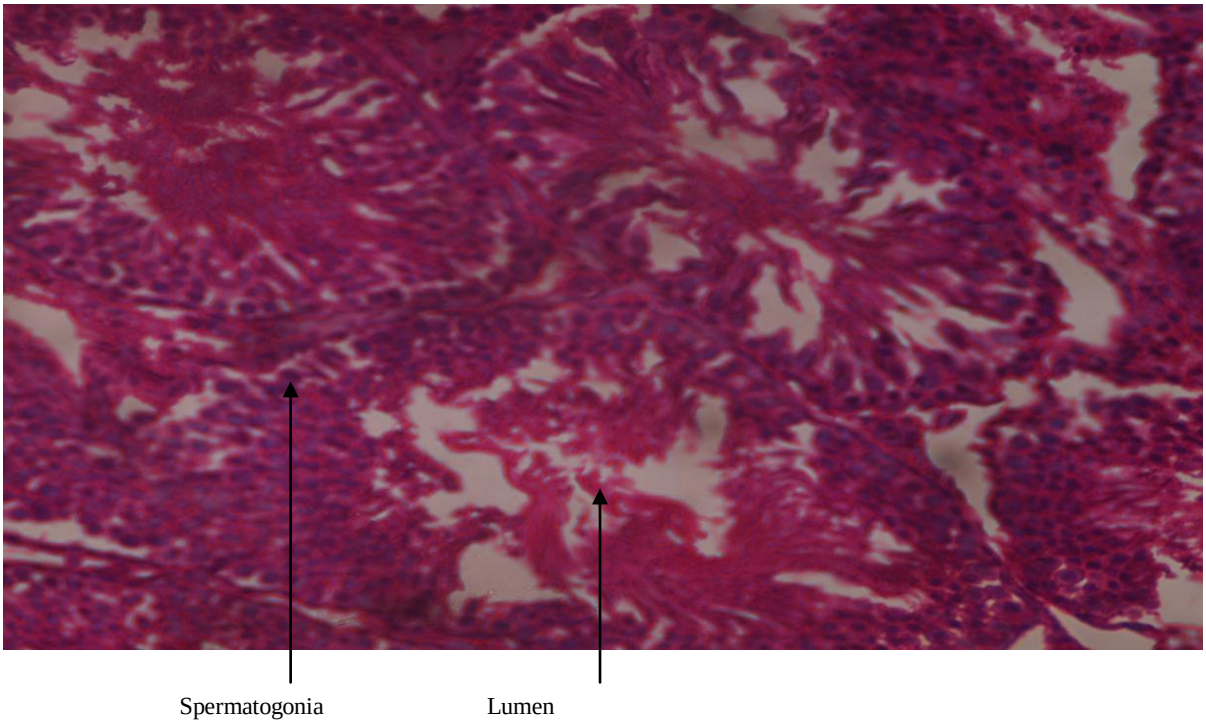


Figure 7: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules of a group I (control) rat administered Olive oil for one week showing apparently normal seminiferous tubules with spermatogenic cells at different stages of development. H and E, x 40

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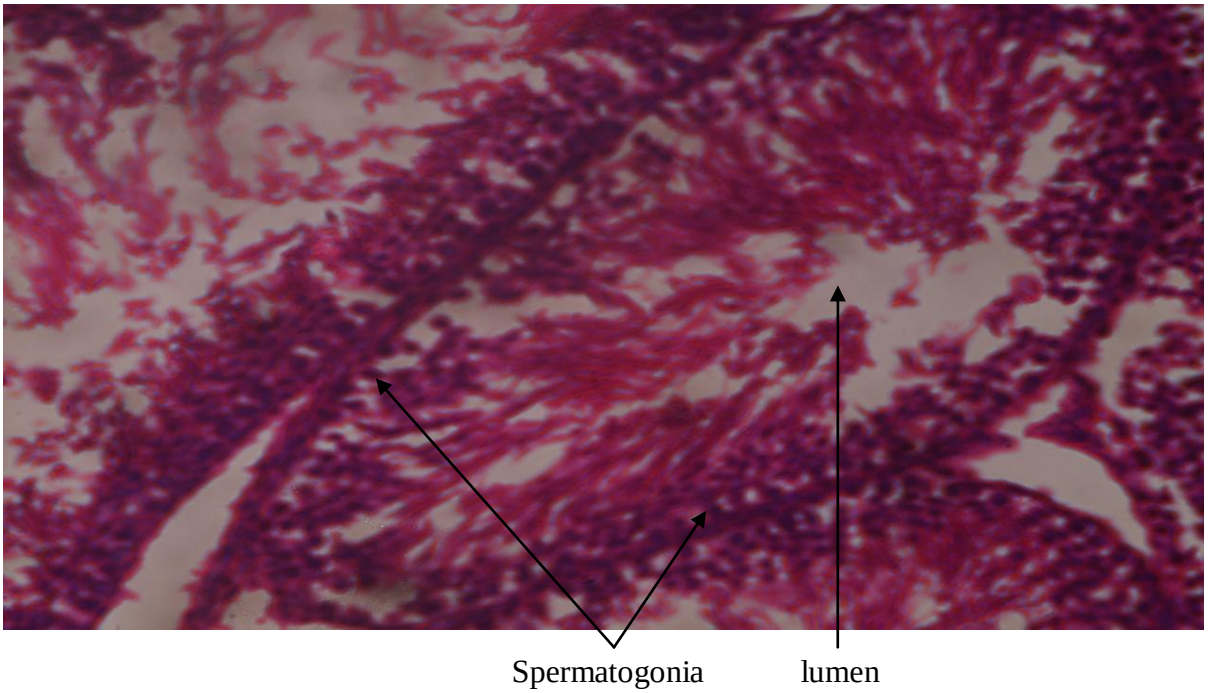

Figure 8: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules of a group I (control) rat administered Olive oil for four weeks showing apparently normal seminiferous tubules with spermatogenic cells at different stages of development. H and E, x 40

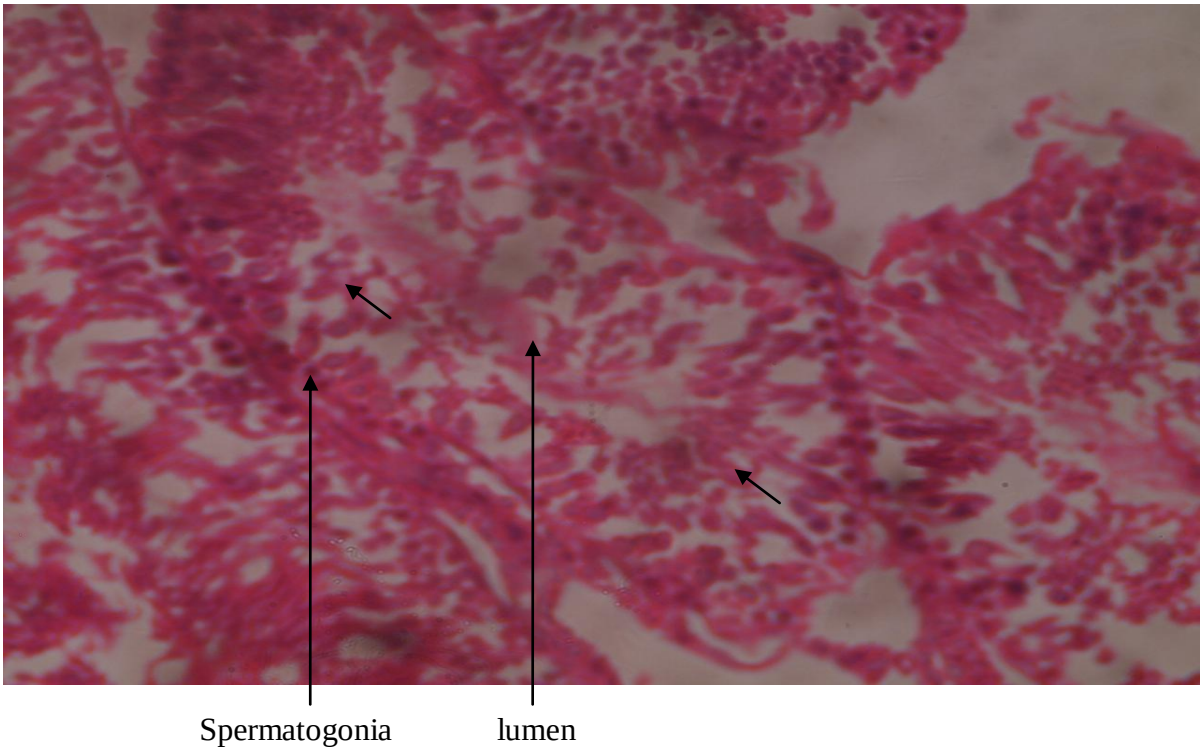

Figure 9: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules of a group II rat administered D-allethrin + Olive oil at the dose of 25mg/kg/day for one week showing exfoliated spermatocytes (arrows) in the lumen of the tubules. H and E, x 40

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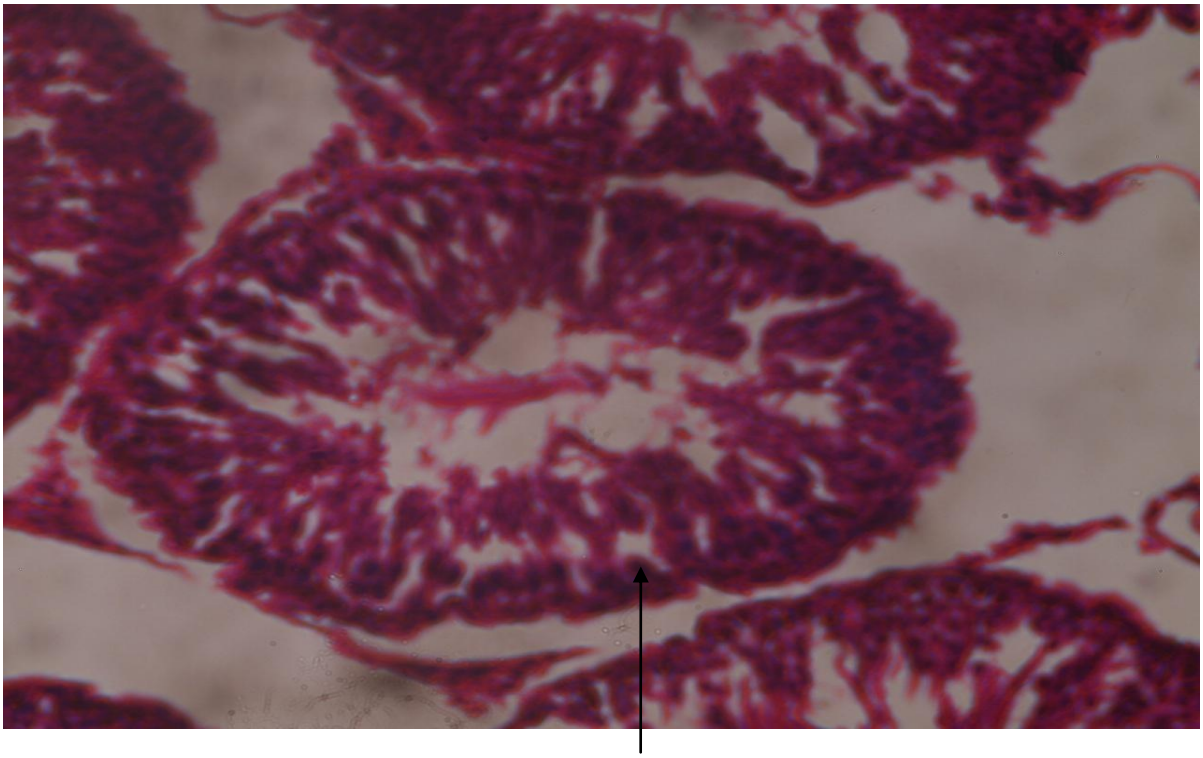

Figure 10: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules of a group II rat administered D-allethrin + Olive oil at the dose of 25mg/kg/day for four weeks. H and E, x 40

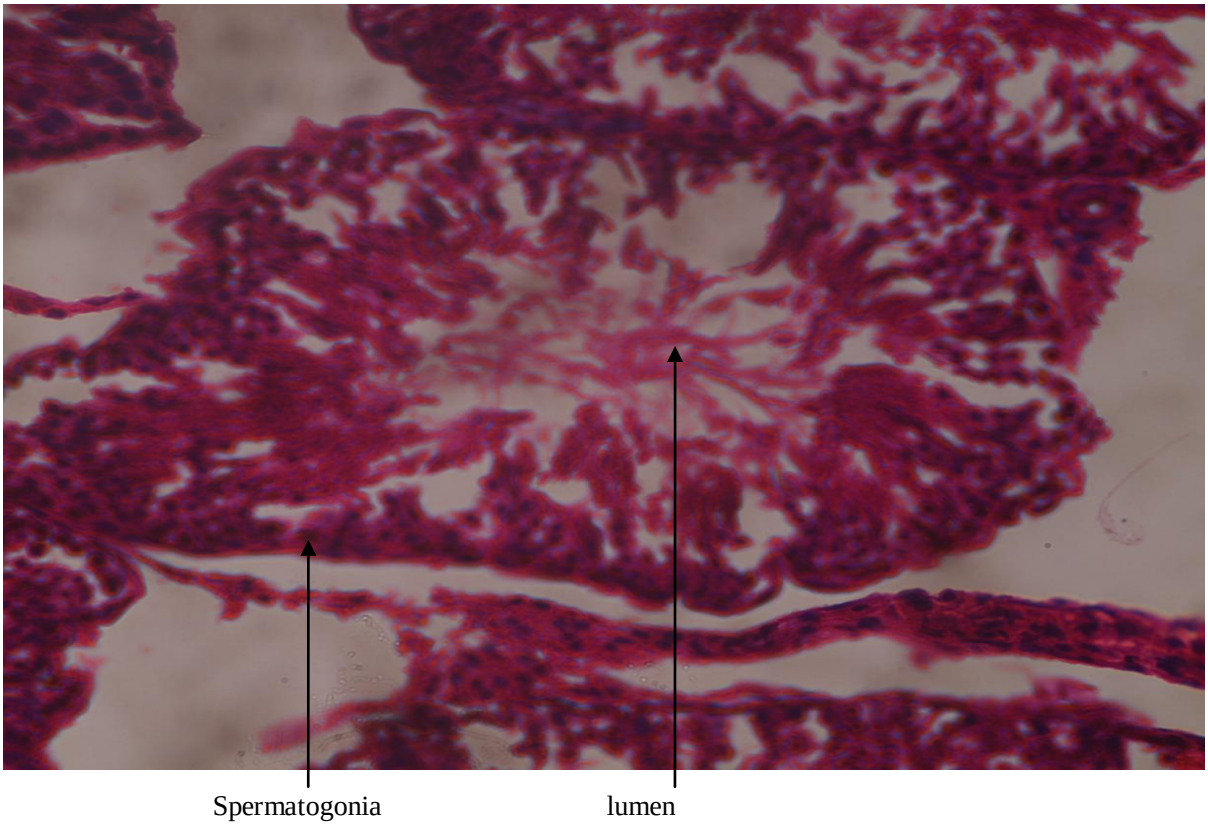

Figure 11: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules of a group III rat administered D-allethrin + Olive oil at the dose of 100mg/kg/day for one week. H and E, x 40

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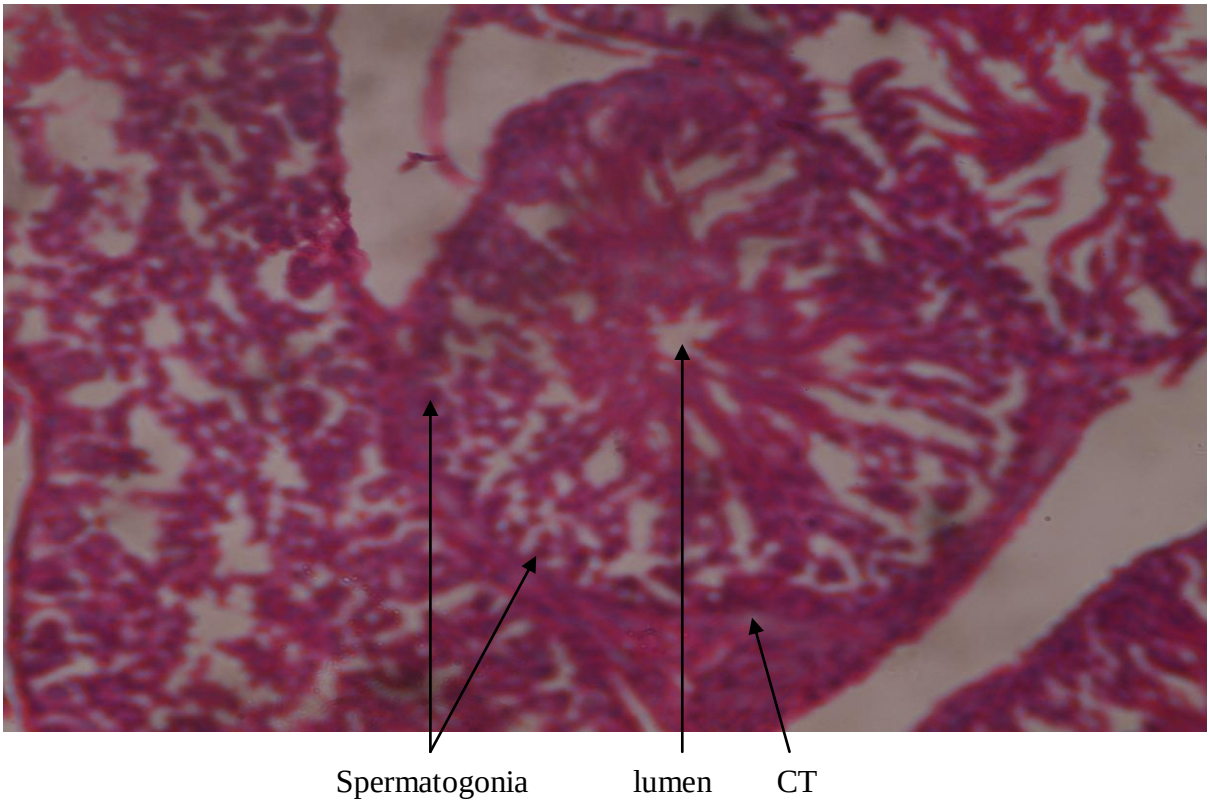

Figure 12: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules and the loosely packed connective tissue (CT) stroma around the seminiferous tubules of a group III rat administered D-allethrin + Olive oil at the dose of 100mg/kg/day for four weeks. H and E, x 40

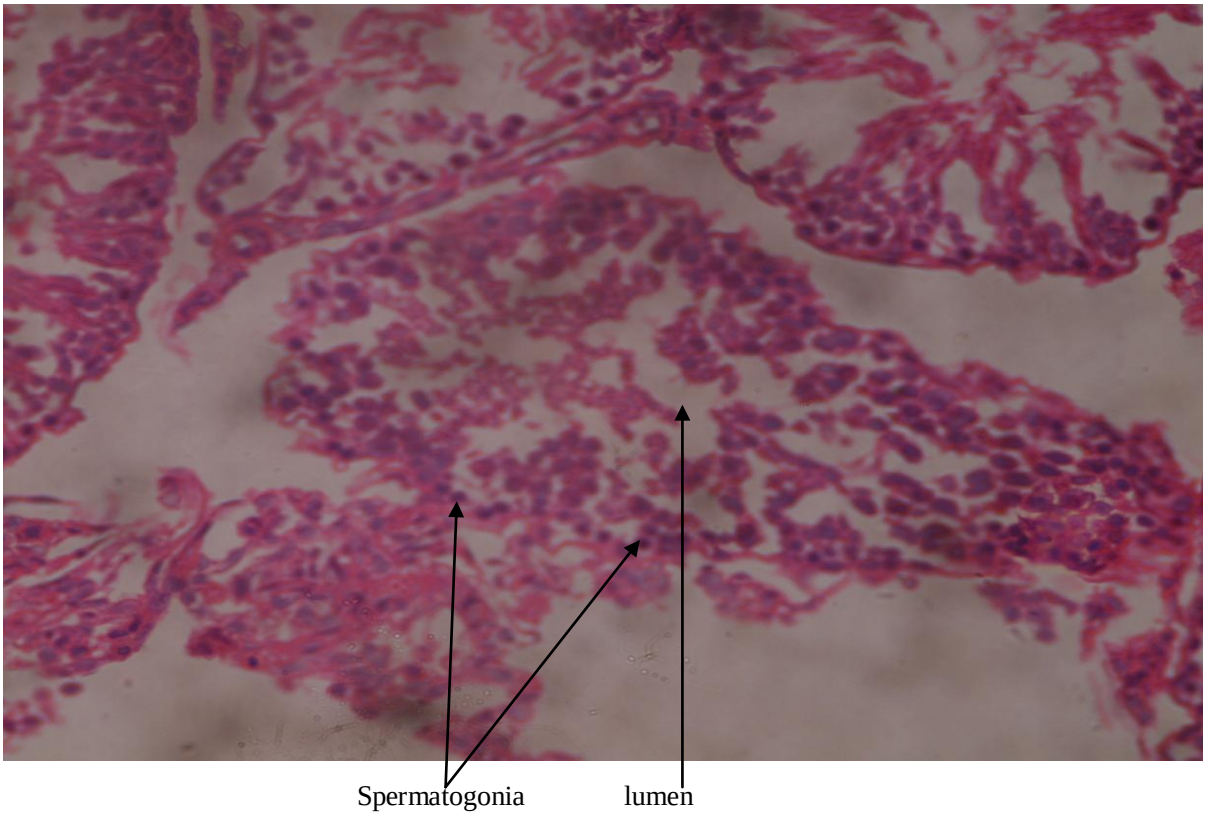

Figure 13: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules of a group IV rat administered D-allethrin + Olive oil at the dose of 175mg/kg/day for one week. H and E, x 40

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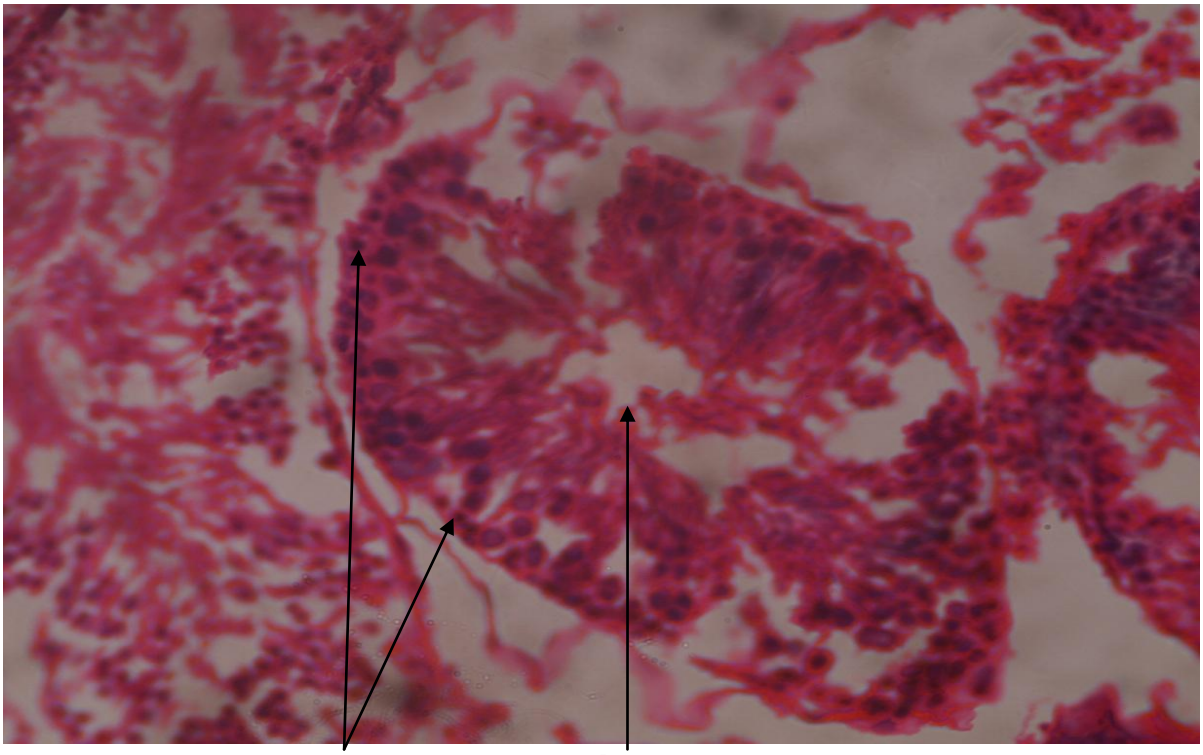

Figure 14: A Photomicrograph of a section in the testis of a rat showing spermatogonia, the germ cells of the seminiferous tubules of a group IV rat administered D-allethrin + Olive oil at the dose of 175mg/kg/day for four weeks. H and E, x 40

DISCUSSION

The present study demonstrated that oral administration of D-allethrin at doses of 25, 100 and 175 mg/kg/day decreased serum testosterone levels in male albino rats in a dose-dependent manner, beginning with the first week of administration of the pesticide. This agrees with the results of Sahar, *et al*. (2011) who reported significantly lower level of testosterone in pyrethroid-exposed workers. Zhang *et al*. (2007) also reported that permethrin caused reproductive damage in mice by interrupting the early stages of testosterone synthesis.

Treatment with permethrin did not significantly alter testicular weight and circumference in these rats. Therefore, the observed increase in testicular circumference was probably due to general growth of the animal. This observation does not agree with the report of Golla and Suresh (2014) that oral administration of allethrin for 60 days was associated with a decrease in testicular size in rats. Our results also differ from the report of Sakr and Azab (2001) that inhalation of tetramethrin for six weeks decreased testicular weight and tubular diameter. Anil and Mahindra (2014) also reported significant reduction in testicular size when rats were treated with deltamethrin, a type II synthetic pyrethroid.

Morphologically, the seminiferous tubules of the rats in this study appeared normal, with spermatogenesis at different stages of development. The basement membrane also showed a layer of healthy population of germ cells and microscopic examination of

cells showed no defects of the germ cell population of animals treated with D-allethrin.

The reports of Sakr and Azab (2001) that treatment of rats with tetramethrin significantly decreased the germ cell population, and the report of Golla and Suresh (2014) on the degeneration of the tubular lining of rats that received allethrin for 60 days, support the present report. We therefore, conclude that oral administration of D-allethrin despite its reported half-life of 6-12 hours in mammals (Bradbury and Coasts, 1989), significantly decreased serum testosterone in the albino rats. The target of action by D-Allethrin may be the cellular lining of the seminiferous tubules, the source of testosterone production.

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