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Original Research Article

Evaluation of the Histomorphometric Effects on Testis Following Administration of Cypermethrin in a Mammal, Albino Rat

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Abstract

Background: In India, the commonly used pesticides belong to organochlorines, organophosphates, carbamates or pyrethroids group. Pyrethroids are insecticides chemically similar to pyrethrum found in natural pyrethrum extracted from the flower of chrysanthemum. The available information indicates there is an ongoing concern that cypermethrin may be causing a variety of reproductive disorders in humans and wildlife. Despite testicular toxicity after cypermethrin administration, there is a dearth of literature on the histopathological features in the testis.

Aim: To evaluate the histomorphometric effects on testis following administration of Cypermethrin in a mammal, albino rat.

Methods and Materials: Adult male Wistar albino rats, weighing 150-200 grams were procured from animal house of UCMS and associated GTB hospital, Dilshad Garden. The animals were divided into two groups as follows: Group I: experimental: 6 evaluable rats. Group II: control: 6 evaluable rats. Animals were kept in separate cages under natural light and dark conditions. The size of the seminiferous tubules was measured in the peripheral and central regions, from four different fields of every sections of the testis. Two diameters at right angles to each other, passing through the center of the tubules were measured. One was considered the long diameter and the other called the short diameter. Hundred tubular profiles that were round or nearly round were chosen randomly and measured in each animal. There readings, obtained from experimental and control animals were tabulated and statistically analyzed by independent sample "t" test.

Results: In the control group, the mean weight of left testis was 905.83±21.77 mg and that of right testis was 902.00±22.80 mg while in the experimental animals the mean weight of testis on the left side was 989.50±111.66 mg and on the right testis was 981.00±113.07 mg. The mean vertical, anteroposterior and transverse diameters of left testis in control animals were 1.875±0.122, 0.875±0.176 and 0.845±0.016 cms respectively while the diameters of right testis were 1.875±0.029, 0.845±0.034 and 0.866±0.039 cms respectively. In experimental animals, the mean vertical, anteroposterior and transverse diameters of the left testis were 1.878±0.028, 0.858±0.031 and 0.855±0.023 cms respectively and on the other side in right testis the diameters were 1.868±0.027, 0.848±0.028 and 0.851±0.029 cms respectively.

Conclusion: Although the pyrethroid pesticides are stated to be less toxic to mammals, the present study showed distinct histomorphological changes in the testes of adult albino Wistar rats.

Keywords: Histomorphometric Effects, Testis, Cypermethrin, Mammal, Albino Rat.

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Introduction

Pesticides are defined as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any insect, rodent, fungus, nematode, weed or any form of life declared to be a pest [1]. In India, the commonly used pesticides belong to organochlorines, organophosphates, carbamates or pyrethroids group. Pyrethroids are insecticides chemically similar to pyrethrum found in natural pyrethrum extracted from the flower of chrysanthemum. In India, Cypermethrin, a synthetic pyrethroid, is being used widely for pest control at home and in agriculture. It has a higher biological activity and is more stable than its natural mode [2,3].

Due to its antiandrogenic effects, estrogenic activity of cypermethrin has been the focus of research over the past few years. Recent studies have shown that a cypermethrin, in trace amounts, may lead to serious problems in both males and females like infertility, increased mortality of offspring and behavioral changes such as aggression [4,5]. Although, testicular toxicity has been reported after the administration of cypermethrin yet its histopathological effects on the testis have not been observed. Hence the present study was conducted to evaluate the histomorphometric effects of cypermethrin on the testes of albino rats [6,7].

Oral administration of cypermethrin in male mice produced an appreciable increase in the weights of testis, seminal vesicles, prostate gland and epididymis [8] Microscopy of testicular tissue affected with cypermethrin revealed interstitial edema, vacuolation in the earlystages of germ cell layer, thickening of the basal membrane, Leydig cell atrophy and Sertoli cells with dense residual bodies [9].

The available information indicates there is an ongoing concern that cypermethrin may be causing a variety of reproductive disorders in humans and wildlife. Despite testicular toxicity after cypermethrin administration, there is a dearth of literature on the histopathological features in the testis [10] hence the present study was conducted to evaluate the histomorphometric effects on testis following administration of Cypermethrin in a mammal, albino rat

Methods and Materials

Adult male Wistar albino rats, weighing 150-200 grams were procured from animal house of UCMS and associated GTB hospital, Dilshad Garden. The animals were divided into two groups as follows:

Group I: Experimental - 6 evaluable rats

Group II: Control - 6 evaluable rats

Animals were kept in separate cages under natural light and dark conditions. The animals were fed food and water ad libitum. The body weights of the animals were recorded before the onset of the experiment and prior to the sacrifice of animals.

Group I animals were given cypermethrin dissolved in groundnut oil in a dose of 60 mg/kg/day for fifteen days by oral gavage. Group II rats were given groundnut oil in the same amount by the same route. The animals of both groups were sacrificed twenty four hours after the last dose, by perfusion with formal saline under anesthesia.

Body weight of animals

The body weight of animals was recorded before the onset of experiment and prior to the sacrifice of the animals. The data was recorded and statistically analyzed.

Size and weight of the testis

The size of the testis was measured vertically between the upper and the lower poles. The anterio-posterior and transverse diameter was also recorded in the middle of the testis with the help of vermier caliper in both the groups. Each testis was blotted and weighed in an electronic scale. The data was recorded and statistically analyzed.

Histomorphological study

Observations were done on every fifth section of the testis stained with haematoxylin and eosin on a zeiss light microscope and image Pro-Express Analysis system in both the groups. The various characteristics of the testis with regard to the state of tunica albuginea, seminiferous tubules, various cells in the seminiferous epithelium and interstitial tissue were studied with haematoxylin and eosin staining and Masson's trichrome in both experimental and control groups.

Histomorphometric Study

For all linear measurements, Abercrombie's (1946) method was used in which ocular micrometer was calibrated with stage micrometer.

Calibration of ocular micrometer for linear measurements

Calibration of ocular micrometer was done with the help of stage micrometer, separately for low power and high power objective of a light microscope, keeping the particular eye piece and particular objective, the readings were constant.

Calculations

At 20 X objective

Each small division of stage micrometer = 10 micron

4 small divisions of ocular micrometer = 1 small division of state micrometer

i.e. 4 small divisions of ocular micrometer = 10 microns

1 small divisions of ocular micrometer = 10/4 = 2.5 microns

Measurement of seminiferous tubules

The size of the seminiferous tubules was measured in the peripheral and central regions, from four different fields of every sections of the testis. Two diameters at right angles to each other, passing through the center of the tubules were measured. One was considered the long diameter and the other called the short diameter. Hundred tubular profiles that were round or nearly round were chosen randomly and measured in each animal (Franca *et al*, 2006). There readings, obtained from experimental and control animals were tabulated and statistically analyzed by independent sample "t" test.

Measurement of the epithelium thickness in the seminiferous tubules

The height of the epithelium was measured in hundred seminiferous tubules that were round or nearly round, at four sites, at right angles and opposite each other in the peripheral and central regions of the testis in each animal. The readings from all the animals in both the groups were tabulated and statistically analyzed by independent "t" test.

Tubular count

Measurement of unit area

An ocular micrometer with an engraved square grid was used to measure an area. The length of one side of the square grid was calibrated with the stage micrometer, to find out the length of one side of the grid. The area of the grid was then calculated.

Calculations

In 20 X objective

One small division of stage micrometer = 10 microns

One side of 5 small squares of ocular grid = 6 small division

i.e. one side of 5 small square = 60 microns

Therefore, one side of each small square of ocular grid = 60/5 = 12 microns

Therefore, one side of the square grid = 12 ×20 = 240 microns

20 small squares = 240 microns

Therefore, Total area of the grid = (240 μ) ² = 57600 μ²

Tubular count in a unit area

Counting of seminiferous tubular was done in sections stained with haemotoxylin and eosin by placing an ocular micrometer with an engraved square grid of an area of 57600 μ², in the peripheral & central region. Tubules falling on the right & lower border of the square were included, whereas these falling on the upper & left border were chosen randomly & measured in each animal. Quantitative observation in all the rats were done in both the groups and the data was tabulated & statistically analyzed by independent sample "t" test.

Results

In the control group, the mean weight of left testis was 905.83±21.77 mg and that of right testis was 902.00±22.80 mg (Table no: 1) while in the experimental animals the mean weight of testis on the left side was 989.50±111.66 mg and on the right testis was 981.00±113.07 mg (Table no: 2). The mean vertical, anteroposterior and transverse diameters of left testis in control animals were 1.875±0.122, 0.875±0.176 and 0.845±0.016 cms respectively while the diameters of right testis were 1.875±0.029, 0.845±0.034 and 0.866±0.039 cms respectively (table no: 3). In experimental animals, the mean vertical, anteroposterior and transverse diameters of the left testis were 1.878±0.028, 0.858±0.031 and 0.855±0.023 cms respectively and on the other side in right testis the diameters were 1.868±0.027, 0.848±0.028 and 0.851±0.029 cms respectively (Table no: 4).

In the present study, the effect of cypermethrin was observed in testis of adult albino Wistar rats. The control and experimental animals included in the study, survived well throughout the experiment. It was noticed that prior to administration of cypermethrin the animals became very aggressive and showed a lot of resistance. After each dosing the animals were hyperactive. This increase in activity was accompanied by borrowing, licking and excessive salivation which lasted for 15-20 minutes. The cypermethrin treated animals had loss of appetite and were sluggish. The experimental animals also presented with vomiting and their faeces were not formed.

The mean body weight of the animals in the control group was 175.67±6.02g before starting the experiment and 178.83±5.84g prior to sacrifice. On the other hand, mean body weight of the experimental animals was 180.00±8.36g and 162.00±26.87g before the experiment and prior to sacrifice respectively.

Histomorphological observations in control animals

Tunica albuginea was surrounding the testis which was in turn surrounded by a lot of adipose tissue. The tunica albuginea consisting of plenty of collagen fibers stained pink with haematoxylin and eosin stain, dark blue with Masson's trichrome stain. Fibroblasts were also seen embedded in collagen fiber bundles. In Masson's trichrome stained slides, the collagen fibers were stained dark blue, the muscle fibers red the nuclei were stained black. Deep to the tunica albuginea was subtunical space occupied by small to large blood vessels with some connective tissue.

The structural architecture of testis consists of convoluted seminiferous tubules supported by loose connective tissue known as interstitial tissue. The mean diameters of seminiferous tubules in control animals were 133.41±12.67μ. The mean height of germinal epithelium in control animals was 229.33±34.62μ. Thin basement membrane was enclosing each tubule and external to it was myoepithelial cells. The basement membrane stained pink with haematoxylin and eosin stain and red with Masson's trichrome stain. Each

tubule was lined by germinal epithelium which was formed of spermatogenic cells and few supporting cells. Spermatogenic cells included dark and light spermatogonia, primary and secondary spermatocytes; spermatids and mature spermatozoa. Large cells lying close to basement membrane were the spermatogonia. They had large rounded with scanty cytoplasm. Next to these cells were the primary spermatocytes.

They had large ovoid nuclei with scanty cytoplasm. As the life span of secondary spermatocytes is short as they enter into the second meiotic division to produce spermatids therefore, secondary spermatocytes were rarely seen. Small round cells with lightly stained cytoplasm and large spherical nuclei present towards the lumen of seminiferous tubule were early spermatids.

On the other side, presence of acrosomal cap with darkly stained nuclei and long tail pointing towards lumen of the tubule were older spermatids. Sertoli cells were present in between these cells, which were tall, pyramidal to columnar cells extending from the basement membrane to the lumen. These cells contained large, lightly stained basal nuclei with one or more prominent nucleoli.

The interstitial tissue consisted of connective tissue along with fibroblasts, macrophages, lymphatics, nerves, mast cells, small and large arterioles, venules and interstitial cells of Leydig. The interstitial cells of Leydig were polyhedral in shape with scanty and poorly stained cytoplasm. They were mainly found in groups close to blood vessels.

The interstitial tissue stained pink and nuclei blue with haematoxylin and eosin stain. The fibrous connective tissue stained dark blue and nuclei black with Masson's trichrome stain. The mean score of fertility in control groups was 8.672±0.279.

Histomorphological observations in experimental animals

The testes in experimental animals were smooth and encapsulated. It was observed that fatty tissue adhered on the surface of testis in patches. There was no structural deformity in the testes. The testis was covered with tunica albuginea which consisted of collagen and few muscle fibers. Many fibroblasts were also observed between the collagen fibers. Some experimental slides showed separation of bundles of collagen fibers forming tunica albuginea.

Subtunical space was present deep to tunica albuginea, which appeared to be empty and increased. It contained some loose areolar tissue and small to medium sized blood vessels. This layer showed no significant difference as compared to the control animals. Below this space, a number of irregular seminiferous tubules were noted. Basement membrane was enclosing each tubule, although it was disrupted at many places.

It stained pink with haemotoxylin and eosin stain and red with Masson's trichrome. The mean diameters of seminiferous tubules in experimental animals are 127.58± 23.43 μ ($p= 0.030$), which is statistically significant (table no 3). The mean height of germinal epithelium in experimental animals was 151.84±39.90 μ ($p=<0.001$) which is statistically significant. (Table no 4).

Many of the seminiferous tubules were hypo cellular with significant reduction of the numbers of nuclei. Some tubules were looking normal while germ cells were either completely or partially missing from other tubules. The normal architecture of germinal epithelium was lost in most of the tubules. Clumping of cells was noticed in many of the tubules. Sertoli cells were not seen in many of tubules. The sertoli cells were not touching the basement membrane and also did not maintain their normal shape. The number of spermatogonia was seen to be significantly reduced.

They were of small size and also were not touching the basement membrane. There were

only few primary spermatocytes which appeared to be smaller in size as compared to control groups. Few spermatids were noted and there was gross absence of tailed spermatids and spermatozoa in most of the tubules.The seminiferous tubules widely placed suggesting marked disruption of intertubular stroma. The Leydig cells appeared to be decreased in number. The testicular tissue was less vascular in experimental animals

Table 1: Weight (mg) of Testis in control rats

S. No.	Left testis	Right testis
1.	930	930
2.	885	880
3.	925	930
4.	920	900
5.	880	882
6.	895	890
Mean	905.83	902.00
SD	21.77	22.80

SD = Standard Deviation

Table 2: Weight (mg) of Testis in experimental rats

S. No.	Left testis	Right testis
1.	1044	1040
2.	871	873
3.	830	817
4.	1035	1010
5.	1047	1025
6.	1110	1120
Mean	989.50	981.00
SD	111.66	113.07

SD = Standard Deviation

Table 3: Mean diameter (µ) of seminiferous tubules in control and experimental rats

Group	Mean	SD	p-value	Significance
Controls	133.41	12.67	0.030	Significant
Experimental	127.58	23.43		

SD = Standard Deviation

p value <0.05 is significant

Table 4: Mean height (µ) of germinal epithelium of seminiferous tubules in control and experimental rats

Group	Mean	SD	p-value	Significance
Controls	229.33	34.62	<0.001	Significant
Experimental	151.84	39.90		

SD = Standard Deviation

p value <0.05 is significant

Discussion

The histological changes induced by a pyrethroid, cypermethrin, in the testis of adult albino Wistar rats were studied in the present study. Cypermethrin was administered by oral gavage route for fifteen days to the animals. The results were evaluated and compared with that of control animals. In the control and experimental animal's adult albino Wistar rats,

the scrotal sac was very thinand covered with white fine hair. In the sub dermal region, tunica dartos was present which formed two distinct layers, the dermal dartos and dartos proper. The superficial and deep scrotal fascia and tunica vaginalis were present underneath the dartos layer. The epididymis was present on the postero-superior aspect of testis. The testes were easily palpable in the scrotal sac. On dissection, they were pearly white in color. After administration of cypermethrin, Raj *et al* (2013) noted infrequent pawing, burrowing, chewing, licking, salivation, coarse whole body tremors, writhing, hyperactivity, abnormal gait and development of hind limb extensor tone in the experimental animals. These symptoms lasted for 6 hours.

Nagarjuna *et al* (2009) observed chewing, licking and salivation, pawing, burrowing, coarse whole body tremor, gradual development of hind limb extensor tone and choreoathetosis slow twisting or writhing movement of neck and tail in the experimental animals after administration of cypermethrin for varied durations [11,12]. The present study was consistent with the study of Raj *et al* and Nagarjuna *et al* (2009), although pawing, tremors, writhing movement, abnormal gait, choreoathetosis and development of hind limb extensor tone were not observed in the current study [13,14]. The weight of the animals in both control and experimental group was measured prior to experiment and before sacrificing. At the onset of the experiment, the mean body weight of control animals was 175.67±6.02 g and 178.83±5.84 g prior to sacrifice. This slight increase in the weight of these animals was not statistically significant. In the experimental animals the mean body weight was 180.00±8.36 g and 162.00±26.87g before the experiment and prior to sacrifice respectively. The weight of these animals had decreased after the experiment, although this decrease was not statistically significant ($p \geq 0.05$). This study is in accordance with the study of Fang *et al* (2013) who also reported an insignificant gain in the body weight of adult male Sprague-Dawley rats after the administration of cypermethrin. On the contrary, Sangha *et al* (2011) and Nair (2011) reported a significant loss of body weight in treated animals. The difference between the current study and the study of Sangha *et al* (2011), Nair (2011) may have been due to the difference in dose regimen and the durationof their study as compared to our study [15,16].

In control animals, the mean weight of left testis was 905.83±21.77 mg and that of right testis was 902.00±22.80 mg while in the experimental animals the mean weight of testis on the left side was 989.50±111.66 mg and on the right testis was 981.00±113.07 mg (Table:). The increase in the weight of testes of control and experimental animals was not statistically significant ($p \geq 0.05$). This is in contrast to the findings of Elbetieha *et al* (2001) who havereported significant increase in the weight of the testes after administration of cypermethrin. The difference between their observation and the observations in the present study may have been due to the difference in the duration of the study, their study extended to a longer duration [17-21].

Conclusion

Although the pyrethroid pesticides are stated to be less toxic to mammals, the present study showed distinct histomorphological changes in the testes of adult albino Wistar rats. Therefore, it is suggested that cypermethrin be used with caution in humans so as to avoid its toxic effects on the testis. Further studies are needed to validate this study.

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