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Original Research Article

The Effect of Cypermethrin on the Fertility Index of Adult Albino Rats

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Abstract

Background: When given orally to male mice, cypermethrin significantly increased the weights of the testicles, seminal vesicles, prostate gland, and epididymis. Interstitial edoema, vacuolation in the early stages of the germ cell layer, thickening of the basal membrane, Leydig cell atrophy and Sertoli cells with dense residual bodies were all visible under the microscope in the testicular tissue that had been exposed to cypermethrin.

Aim: This study was conducted to analyze the effect of cypermethrin on thé fertility index of adult albino rats.

Methods and Materials: In this study 12 adult male Wistar albino rats, weighing 150-200 grams were procured from animal house of UCMS and associated GTB hospital, Dilshad Garden. Fertility index: (Johnson; 1970) was used to measure fertility. The seminiferous tubules in every tenth section of the testis were recorded. Means score (MS) was calculated by the number of tubular recorded at each score multiplied by the score and the sum of the products were divided by the total number of tubules recorded. The mean score was called fertility index which was taken as a parameter for the spermatogenic activity in the testis.

Results: The mean score of fertility in control groups was 8.672±0.279. Marked decrease in fertility index was observed in experimental animals. The fertility index was 2.944±12.67 ($p = <0.001$), which is statistically highly significant. In experimental animals, 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.

Conclusion: The study revealed arrest of physiological process of spermatogenesis and tubular atrophy resulting in gross decrease in healthy sperm formation which in turn leads to infertility in adult albino Wistar rats.

Keywords: Cypermethrin, Fertility Index, Adult Albino Rats.

Introduction

In today‘s world the use of pesticides has substantially improved the economic and social wellbeing of the inhabitants of developing countries by increasing the food production and by effectively controlling the vector borne diseases. At the same time, the

adverse effects caused by pesticides on human and animal health have increased. Pyrethroids are insecticides chemically similar to pyrethrum found in natural pyrethrum extracted from the flower of chrysanthemum. They can be divided into two groups: Type 1 and Type 2. Type 1 do not contain cyano group in their molecules which includes allethrin, tetramethrin, permethrin and phenothrin. Type 2 contains cyano group at the- carbon position and it includes newer compounds like deltamethrin, cypermethrin and fenvalerate [1,2].

These two types cause different set of symptoms. While Type 1 causes hyper excitation, ataxia, convulsions and eventually paralysis, type 2 causes hypersensitivity, choreoathetoses, tremors and paralysis. Despite having differences in the symptoms, both the types of pyrethroids target the same site i.e. sodium channel of nerve membrane which is the channel directly responsible for action potential. Nowadays, synthetic versions of the naturally occurring pyrethroids have been developed [3,4].

They have been modified to increase theirpesticidal activity and stability in the environment. According to the WHO task group, pesticide poisoning accounts for a significant morbidity and mortality all over the World especially in developing countries, like India. They state that every year, in the Asia Pacific region alone, one million serious unintentional poisoning cases occur due to pyrethroid. Out of this, three hundred thousand people die each year [5,6].

Liver and kidney weights were found to be increased in test animals which were fed with cypermethrin for a long term. In test animals the adverse changes in liver tissues noted were - moderately enlarged sinusoids, congested central veins and mild hemorrhage in the hepatic tissues, degranulation of hepatocytes. Rabbits that were fed repeatedly with high doses of cypermethrin showed pathological changes in the cortex of the thymus, liver, adrenal glands, lungs and skin [7]. when given orally to male mice, cypermethrin significantly increased the weights of the testicles, seminal vesicles, prostate gland, and epididymis. Interstitial edoema, vacuolation in the early stages of the germ cell layer, thickening of the basal membrane, Leydig cell atrophy, and Sertoli cells with dense residual bodies were all visible under the microscope in the testicular tissue that had been exposed to cypermethrin [8,9].

The estrogenic action of cypermethrin has drawn attention in recent years due to its antiandrogenic properties. Cypermethrin has been linked in recent research to major issues in both males and females, including infertility, increased child mortality, and behavioural changes including aggression. This study was conducted to analyse the effect of cypermethrin on thé fertility index of adult albino rats.

Methods and Materials

In this study 12 adult male Wistar albino rats, weighing 150-200 grams were procured from animal house of UCMS and associated GTB hospital, Dilshad Garden. The animals belong to:

Class: Mammalian
Order: Rodentia
Suborder: Myomorphia
Family: Muridal.

The procured animals were divided into two categories. Category 1: It included 6 albino rats taken as control. Category 2: It consisted of 6 albino rats taken as experimental animals.

Cypermethrin was administered orally to Category 1 animals in a dose of 60 mg/kg/day for fifteen days while it was dissolved in groundnut oil. Groundnut oil was administered to Category 2 rats in the same quantity and manner. Twenty-four hours

following the final treatment, the animals in both groups were sacrificed by formal saline perfusion while under anaesthesia.

Animals were anaesthetized by keeping them in an inverted glass jar containing large piece of cotton soaked in ether. Animals were anaesthetized in 5-10 minutes. Animals were pinned up on a dissection board and a midline incision was made on the skin extending from xiphoid process to jugular notch. Sternum was lifted after cutting the ribs along the sides of sternum. The heart and the ascending aorta were then exposed by removing the overlying fat and thymus. A ligature was passed under the ascending aorta and a small nick was made in the left ventricle through which a cannula was inserted into the aorta and tied with the help of a ligature. A small nick was also made in the right auricle through which the blood was allowed to clear out of the system when normal saline was injected through the cannula. Initially blood stained fluid came out of the nick in the right auricle when the normal saline was pushed into the cannula. After that, when clear fluid started to come out of the auricle, then about 200 ml of 10% formal saline was injected under low pressure with the help of a syringe till the animals became pale and stiff. The perfused rats were kept in formalin solution for 7 days.

Preparation of tissue for microscopy

The testis were dissected, weighed and observed for gross changes. Eight microns thick sections were cut. It was then kept in tissue capsule and was washed in running tap water to remove surplus fixation. The tissue was dehydrated by changing in ascending grades of ethyl alcohol. Clearing of tissue was done in cedar wood oil followed by xylene (15 minutes) and then in a mixture of xylene and paraffin wax in the ratio of 1:1 for half an hour. Tissue was further given three changes in paraffin wax (melting point 60˚C) for one and half each and then embedded in wax. Blocks were prepared with the help of Leukarts "L" shaped bars. The bars were trimmed, labeled and mounted on a block holder. Eight micron thick sections were cut using a rotator microtome. Sections were picked up by the floatation method on a glass slide smeared with egg albumin, glycerin and thymol mixture. The slides were allowed to stand upright to drain and allow the sections to dry completely. They were then kept in the incubator at 37˚C over night to prevent displacement of sections during the staining process. The sections were later stained with following stains:

1. Haematoxylin and eosin (H & E)
2. Masson's trichrome

Fertility index: (johnson; 1970)

The seminiferous tubules in every tenth section of the testis were recorded as follows:

1. Complete spermatogenesis with many spermatozoa & leaving an open lumen – 10
2. Many spermatozoa but germinal epithelium disorganized with marked sloughing & obliterated lumen -9
3. Only few spermatozoa but many spermatocyte present – 8
4. No spermatozoa but few spermatids present – 7
5. No spermatozoa, no spermatids but many spermatocyte – 5
6. Only few spermatocyte (less than five) with no spermatids or spermatozoa – 4
7. Spermatogonia the only germ cell seen – 3
8. No germ cells but many Sertoli cells present – 2
9. No cells seen in the tubular cross section – 1

Means score (MS) was calculated by the number of tubular recorded at each score multiplied by the score and the sum of the products were divided by the total number of tubules recorded. The mean score was called fertility index which was taken as a parameter

for the spermatogenic activity in the testis. The fertility index was calculated in control & experimental groups. The data was tabulated & statistically analyzed by independent sample "t" test.

Procedure

1. The sections were deparaffinized with two changes of xylene for 15 minutes each.
2. Hydration was done by passing through descending grades of alcohol.
3. Sections were then thoroughly washed under running tap water for five minutes.
4. Sections were kept in haematoxylin for fifteen minutes at room temperature.
5. Washed under running water for five minutes each. Each section was examined microscope to confirm sufficient degree of staining.
6. Differentiation was done by dipping in acid alcohol solution (1% hydrochloric acid in 70% alcohol).
7. Sections were washed in running water for five minutes.
8. Sections were kept in working eosin solution for few seconds.
9. Differentiated in 95% alcohol.
10. Cleared with two changes of xylene for fifteen minutes each.
11. Sections were mounted in DPX mountant (a mixture of Distyrene, a plasticizer and xylene

Results

Table 1: Mean fertility index in control and experimental rats

Group	Mean	SD	p-value	Significance
Controls	8.672	0.279	<0.001	Significant
Experimental	2.944	0.547		

SD = Standard Deviation
p value <0.05 is significant

The mean score of fertility in control groups was 8.672±0.279. Marked decrease in fertility index was observed in experimental animals. The fertility index was 2.944±12.67 (p = <0.001), which is statistically highly significant. (Table 1).

In experimental animals, 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. 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.

In control group, 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.

Discussion

In our study, the fertility index of the control group animals was 8.672±0.279 and 2.944±12.67 in the experimental animals. The decrease in fertility index was statistically significant ($p<0.001$). No data was available for comparison of this finding. Elbetieha *et al* (2001) [7] studied the toxic potential of cypermethrin on reproductive and fertility parameters in Sprague-Dawley male rats. They administered tap water orally containing 0; 8,571; 17,143 or 34,286 ppm of cypermethrin for twelve weeks to rats. Based on water consumption per animal per day the rats received 13.15, 18.93, and 39.66 mg cypermethrin [10,11]. Fertility was reduced in male rats ingesting cypermethrin at a concentration of 13.15 and 18.93 mg in that the number of females impregnated by them was significantly reduced. Females mated with males that had ingested cypermethrin at a concentration of 39.66 mg showed decreased number of implantation sites. Reduced number of viable fetuses was noted in females impregnated by the exposed male rats at all three doses of cypermethrin [12,13].

The body weight gain was decreased in the cypermethrin treated male rats. Ingestion of cypermethrin at a concentration of 18.93 or 39.66 mg per day resulted in a significant increase in the weights of testes and seminal vesicles. Preputial gland weights were increased at all three concentrations of cypermethrin [14,15]. The testes of treated rats showed congestion with marked hemorrhage and accumulation of connective tissue surrounding the seminiferous tubules, which had large number of immature spermatids. Daily sperm production was decreased in exposed male rats. The serum levels of testosterone, follicle-stimulating hormone and luteinizing hormone were decreased in males exposed to 39.66 mg per day. They were of the opinion that cypermethrin clearly had adverse effects on the fertility and reproduction in male rats [16-18].

There has been significant increase in infertility in humans in the past decade probably due to environmental contamination with pyrethroids like cypermethrin which act as endocrine disruptors. The actual cause of increasing infertility remains controversial but researchers suggest that in a developing country like India males are constantly exposed to pyrethroids which may be a leading cause of infertility in them [19].

Although, testicular toxicity after exposure to cypermethrin is a known fact, yet there is a dearth of availability of literature on the histopathological features of the testis after exposure to cypermethrin. The present study is one such attempt with cypermethrin in a mammal, albino Wistar rat.

Some of the tubules appeared normal in architecture but in most of the tubules spermatogenic and supporting cells forming the germinal epithelium showed disorganization and appeared disrupted. The spermatogonia appeared to be smaller in size and at many places were detached from the basement membrane and probably were in the process of moving towards the lumen of the tubules. The mean height of germinal epithelium in experimental animals was 151.84±39.90 µ and in control animals, it was 229.33±34.62 µ ($p=<0.001$).

The germinal epithelium height in experimental animals was significantly reduced statistically as compared to control animals. Some seminiferous tubules appeared empty as the cells were sparsely

placed in them. 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. Intertubular stroma was markedly disrupted making the seminiferous tubules widely placed. The number of Leydig cells had decreased in these animals as compared to control rats in the present study. Kumar (2014) suggested that detachment of spermatogenic cells from the basement membrane resulted from decreased vascularity causing sloughing of these cells in to the lumen of seminiferous tubules leading to decrease in epithelial height in cypermethrin treated animals. Ahmad *et al* (2008) and Nagarjun and Doss (2009) made a similar observation in their study [20,21].

Ahmad *et al* (2008), Nagarjun and Doss (2009) and Elbetieha *et al* (2001) observed increased amount of intertubular connective tissue. On the contrary, loss of interstitial tissue was observed in the present study. The difference between their observations and the observations of the current study may be due to different duration of study and the dosage used by them.

Maqbool Ahmad *et al* (2008) studied the deleterious effects of cypermethrin on semen characteristics and testes of dwarf goats (Capra hircus). They conducted a study on 30 male dwarf goats. The animals were divided randomly into five groups. Group I was control and was dipped in 0%, Group II was dipped in 0.1%, Group III was dipped in 0.4%, Group IV was dipped in 0.8%, and Group V was dipped in 1.6% of cypermethrin on days 0 and 15. The semen of each animal was collected at day 0 and then fortnightly till 75 days. It was evaluated for physical characteristics, ejaculatory volume and concentration of the sperms, their morphology, motility percentage and massactivity. They noted no changes in samples collected on days 0 and 15. From day 30, effect of cypermethrin on semen characteristics started appearing which were more prominent from days 45 to 75. They found that the pH of semen became more alkaline with cypermethrin treatment. Semen color changed from creamy in control group to milky white or straw colored in treated animals. Decreased weight of testis and cyanotic epididymis were observed only in bucks which were treated with 1.6% cypermethrin [19-22].

Dead spermatozoa rose significantly in the experimental groups from days 30 to 75 as compared to the control group. All these changes were dose dependent. Histopathologically, degenerative changes and loss of spermatogonia, spermatocyte, Sertoli cells, spermatids and spermatozoa in seminiferous tubules were seen. Tail less, bent tailed or coiled tailed spermatozoa were seen in experimental groups. They concluded that cypermethrin caused dose-dependent effects on all the parameters of semen seen above and these effects are high with cypermethrin solution of 0.8% and 1.6% concentration, though this effect seemed to be temporary as these parameters improved at day 75. They suggested that cypermethrin in goats should be used with great care.

Conclusion

The study revealed arrest of physiological process of spermatogenesis and tubular atrophy resulting in gross decrease in healthy sperm formation which in turn leads to infertility in adult albino Wistar rats. This study may be extrapolated to human beings. Therefore, it is suggested that cypermethrin be used with caution in humans so as to avoid its toxic effects on the testis and increasing the chances of infertility. Further studies are needed to validate this study.

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