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RESEARCH ARTICLE

Effect of intraperitoneal administered Deltamethrin on the fertility index of albino rats

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Abstract

Deltamethrin, a broad spectrum type II Pyrethroid is one of the most commonly and extensively used pesticides, internationally today. Its expanded use has expectedly resulted in residues in food causing toxic effects on human health. The present work was conducted to study the histomorphological changes in testis in Deltamethrin treated albino rats and its effect on fertility index. Deltamethrin in the dose of 1 mg/kg/ body weight daily for a month was administered intraperitoneally in adult wistar albino rats (150-200 g). Controls were maintained. The animals were sacrificed within 24 hour of the last injection by perfusion. Testis was dissected out, Paraffin section (8u) were cut and stained for the light microscopy. Cytoarchitecture of the seminiferous tubules were hypocellular with epithelial disorganization, sloughing with degenerating cellular debris in the lumens. The Johnsen's criteria were used for the estimation of fertility index. The mean Johnsen score were 8.58 ± 0.181 and 8.92 ± 0.099 in control group whereas 2.85 ± 0.347 and 3.14 ± 0.374 in the central and peripheral group of seminiferous tubule in the experimental group of rats respectively. Findings in the present study strongly suggested inflammatory and degenerative changes in the testis with decreased fertility index indicating hypospermatogenesis that could lead to infertility.

INTRODUCTION

Human beings are constantly exposed directly or indirectly to the most harmful chemical substances, pesticides which are added to our environment. Deltamethrin, a type II synthetic Pyrethroid is one of the most popular and widely used insecticides in the world because of its broad spectrum control (Tomlin, 2006). Deltamethrin is registered for use on various crops including cotton, corn, cereals, soybeans, and vegetables for pests such as mites, ants, weevils, and beetles (Toxicological Profile for Pyrethrins and Pyrethroid, 2004; Pesticide Products, 2007). Deltamethrin has been registered for use on areas such as golf courses, ornamental gardens, lawns, outdoor perimeter treatments, indoors as spot and crack and crevice treatments, and pet collars (Pesticide Tolerance. Fed. Regist., 2004).The common routes of Human exposure to deltamethrin are direct exposure to the vapors or consumption of polluted food and water. Deltamethrin is a suspected endocrine disruptor (Garey and Wolff, 1998). It is neurotoxic to humans and has been found in human breast milk (Bouwman et al., 2006).

Many studies have focused the possible role of deltamethrin on decreased sperm production and potentially Cryptorchidism (Gray et al., 1989; Gustafson et al., 1994; Jensenet al., 1995; Sharpe and Skakkebaek, 1993; Mc Lanchlan et al., 1998; Toppari et al., 1996). There are different reports showing trends towards decreasing sperm quality in men over the past five decades (Auger et al., 1995). Due to relatively short period of time taken for this trend to occur, it is believed that decreasing sperm quality is the result of environmental rather than genetic factors

(Giwercman et al., 1993). It has been reported that deltamethrin lowers the percentage of sperm count and sperm motility which significantly increased sperm abnormalities (Abd El- Aziz et al., 1994).
There are many gaps in the data on many aspects of the toxicity of this recent and most widely used light stable synthetic Pyrethroid, deltamethrin. The available information indicates that it may pose serious hazards to non-target organs like testis. Hence the present work was conducted to study the histomorphological changes in the testis produced by deltamethrin and its effect on fertility.

MATERIALS AND METHODS

Test and control material: The test material was Deltamethrin (type II synthetic Pyrethroid). The vehicle, physiological saline (0.9% sodium chloride) was used as the control material.

Animal and housing condition: The range of body weight at the start of dosing in inbred adult Wistar albino rats (Rattus Norvegicus) was 150-200 g. Twenty male albino rats were selected. They were divided into two groups. Therats were individually housed in compartment of wire mesh cage (W420 X L460 X H150 mm) in a barrier sustained animal room. Animals were group housed (12 h light/dark cycle) with ad libitum access to food and water. The group II rats were injected with deltamethrin without dilution (1 mg/kg body weight) intraperitoneally, for five days/week for a month. The group I were controls. The control group received equal quantity of physiological saline by the same route.

Experimental design: Animals were sacrificed within twenty four hours of the last injection by perfusion under anesthesia. Paraffin sections (8µ) were cut, and stained with haematoxylin and eosin for light microscopy.

Measurements

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 seminiferous tubules and interstitial tissue were studied with haematoxylin and eosin staining in both the groups, experimental and control.
Fertility index (Mean score): Mean score (MS) according to the Johnson (1970) was calculated by the number of tubules recorded at each score multiplied by the score and the sum of the products was divided by the total number of tubules recorded. This 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 and experimental groups.

Statistical analysis: Quantitative observations in all the rats were done in both the groups and the data was tabulated and statistically analyzed by independent sample "t" test. Statistical analysis was performed using SPSS 11.5 software. $P < 0.001$ was considered as a significant level.

RESULTS

The basement membrane of the seminiferous tubule appeared to be slightly thickened at sites. Most of the seminiferous tubules appeared to be hypo-cellular, empty look and the number of nuclei in the tubules appeared to be decreased. This cellular hypoplasia of the epithelium was more pronounced in the central tubules. The layered architecture was lost resulting in an enlarged lumen. The spermatogenic cells and supporting cells lining the tubules appeared disheveled. There was sloughing of the apical tubular epithelium into the lumen. In most tubules the seminiferous epithelium showed a loss of germ cell attachment and appearance of expanded intercellular spaces between spermatogenic cells. The spermatogenic cells had become vacuolated and a lesser compact arrangement between the epithelial cells was observed (fig. 1). The interstitial tissue appeared to be thinned out but a large empty space was visible between the seminiferous tubule's basement membrane. In the peripheral region, the interstitial tissue appeared to be more markedly increased as compared to the central region. The Leydig cells appeared to be reduced in number, with marked disruption of the intertubular stroma as compared with the control (fig. 2).
The mean score of the fertility index in group I (control) rats was 8.92 ± 0.099 and 8.58 ± 0.181 for the peripheral and the central seminiferous tubules respectively Whereas in the group II (experimental) rats was 3.14 ± 0.374 and 2.85 ± 0.347 for the peripheral and the central tubules respectively (Table). The fertility index showed marked decline in the peripheral tubule as compared to the central tubule. On statistical analysis the mean score of fertility index was significantly lower as compared to the control animals (Graph).

Graph: Comparison of mean fertility index in the central and peripheral region of the testis in control and experimental rats

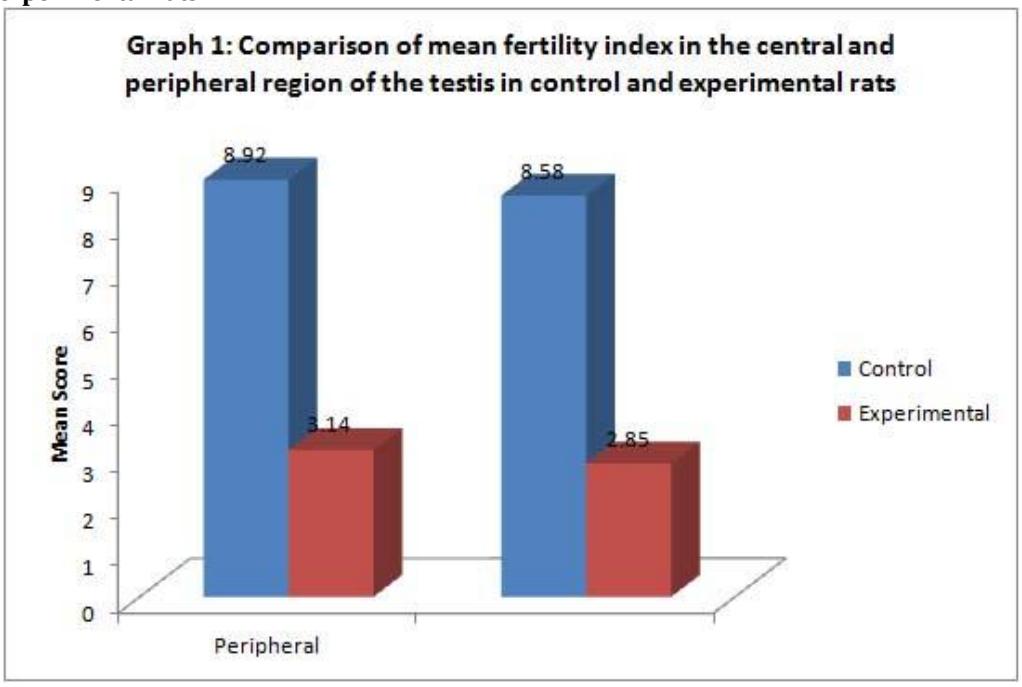

Table: Comparison of mean fertility index in the central and peripheral region of the testis in control and experimental rats.

Region	Group	Fertility index (Mean ± S.D)	p-value (one way ANOVA)	Significance (Tukey's test at 5% level)
Central	**Control**	8.58 ± 0.181	<0.001	Both groups were significantly different from each other
	Experimental	2.85 ± 0.347		
Peripheral	**Control**	8.92 ± 0.099	<0.001	Both groups were significantly different from each other
	Experimental	3.14 ± 0.374		

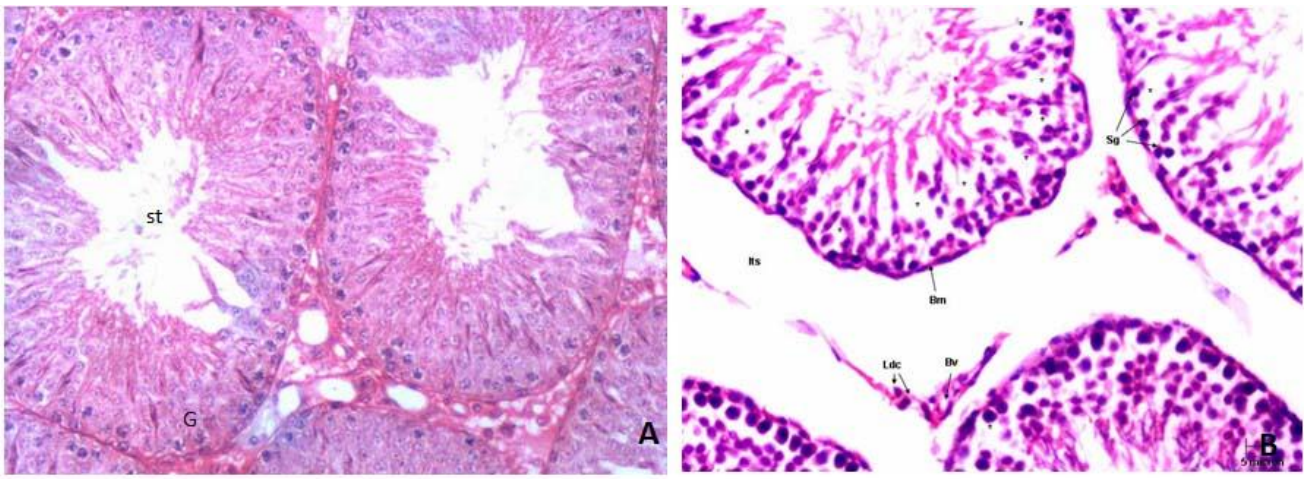

Figure 1: Comparison of the photomicrograph of the transverse section of the control (A) and Experimental (B) rat testis at same magnification: control (A) shows semineferous tubules (st.) filled with germ cells (G). While Experimental (B) shows increased vacuolization (*) ,thickening of the basement membrane (Bm) at sites in the seminiferous tubules, increased intertubular space (Its) with very little interstitial tissue containing leydig cells (Ldc) and blood vessels (Bv).

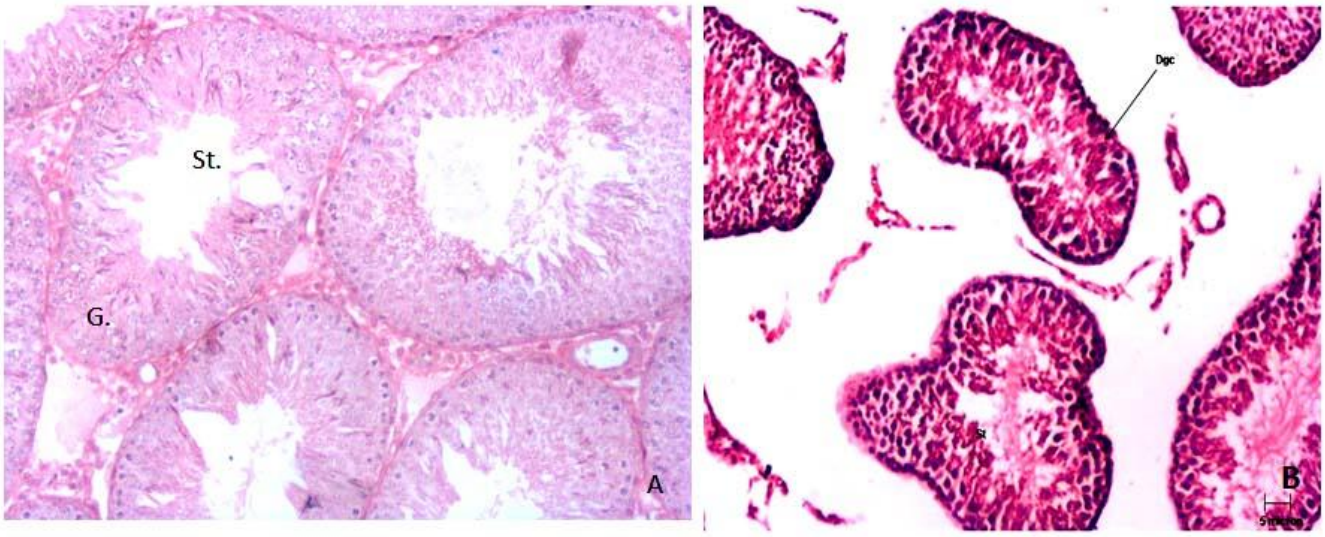

Figure 2: Comparison of the photomicrograph of the transverse section of the control (A) and Experimental (B) rat testis at same magnification: control (A) shows semineferous tubules (st.) filled with germ cells (G). While Experimental (B) shows altered and distorted seminiferous tubules (St) , intertubular edema with very little interstitial tissue. Early spermatids (Sp) seen.

DISCUSSION

The basement membrane enclosing the seminiferous tubules appeared to be slightly thickened at sites and surrounded externally by a thin layer of lamina propria containing myoepithelial cells (fig. 1). Similar observations were also reported by El-Gohary et al (1999) after administration of deltamethrin in adult male albino rats in a dose of 1mg/kg intraperitoneally for twenty-one consecutive days. The spermatogenic and supporting cells forming the tubular epithelium of these seminiferous tubules showed disorganization and appeared disheveled. The presence of a large number of debris of degenerating cells and spermatozoa in the lumen with accumulation of macrophages, and

nodules of homogeneous pink stained material is suggestive of the process of degeneration of these cells. These tubules showing sparsely spaced germ cells in the epithelium and a loss of germ cell attachment to the basal lamina with appearance of expanded intercellular spaces between spermatogenic cells and containing cellular debris in their lumen are the atrophying tubules (fig. 1). Testicular atrophy and degenerative changes of the seminiferous tubules have been reported in experimental animals with various insecticides (Chapin and Ku, 1994; Ezeasor, 1990). Our histopathological results are in agreement with the observations of Hess and Nakai (2000) who found that the administration of pyrethroids induced sloughing of germ cells and abnormal development of the head of elongating spermatids and assigned these changes to the intracellular redistribution of water and ions. The spermatogenic cells had become highly vacuolated and a less compact arrangement between the epithelial cells was observed. Many abnormal, degenerated late spermatids with random orientation were seen towards the basal and adluminal compartments of the seminiferous epithelium (fig. 1). These features are suggestive of varying degree of degeneration of spermatogenic cells in the seminiferous tubule and may affect the normal spermatogenesis causing infertility in these animals. Franca et al., (2006) also reported similar observations. Vacuolation is considered to be an early form of degeneration of a cell and might have appeared due to disturbances in cell metabolism caused by deltamethrin. The presence of multiple vacuoles displaced the germ cells towards the lumen of tubule as reported by Richburg and Boekelheide, (1996). This causes necrosis of germ cells resulting in their sloughing without an attached fragment of Sertoli cell as degenerating cellular debris into the lumen (Boekelheide 1993). Spermatozoa were absent from the lumen of these tubules. El-Gohary et al. (1999) administered deltamethrin in adult male albino rats in a dose of 1mg/kg intraperitoneally for twenty-one consecutive days and observed the Sertoli cells to be vacuolated at sites in selected tubules or even in sectors of the same tubule, They stated it is known to be an indication of suppressed spermatogenesis leading to dysfunction and infertility in future (El-Gohary et al. 1999).

Statistical analysis revealed a gross drop in the fertility index in the experimental animals (Table). No such data are available in the literature for comparison. Hassan et al. (1993) found a reduced percentage of pregnancies in untreated female rats that were mated with a Pyrethroid fenvalerate treated males. Abd El-Aziz et al. (1994), on administration of deltamethrin in oral doses as low as one milligram per kilogram per day exhibited male fertility to have reduced tremendously at the end of treatment and sixty days post-treatment. Elbetieha et al, (2001) and Andrade et al, (2002) also reported a significant decrease in fertility in a dose dependent manner after oral administration of the Deltamethrin.

There is a significant increase in the incidence of male infertility in humans in the past decade possibly due to environmental contaminants like pesticides which act as endocrine disruptors (Queiroz and Weismann, 2006). Ever since the international technological shift in industrial and agricultural development from the 20th century they are being widely used involving handling and exposure although these are harmful to human. Despite the diversity of habits and cultures around the world, various authors have reaffirmed the possible significant drop in sperm quality and consequently an increase in male infertility rates, apparently this constitutes an international phenomenon (Swan et al 1997; Golden et al 1999; Skakkebaek et al, 2001; Multigner et al, 2002 and Pasqualotto et al, 2003).

CONCLUSION

The above findings conclude that the histomorphological changes showed inflammatory and degenerative changes due to Deltamethrin toxicity. Also the mean score of fertility index in the peripheral and central region of seminiferous tubules of deltamethrin treated albino rats was statistically significantly lower indicative of Hypospermatogenesis and an infertile testis.

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