The Journal of Animal & Plant Sciences, 24(1): 2014, Page: 167-171
ISSN: 1018-7081

EFFECTS OF PROFENOFOS AN ENDOCRINE DISRUPTING CHEMICAL ON LEYDIG'S CELLS IN RABBITS

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ABSTRACT

This study was conducted to know the toxic effects of organophosphate (Profenofos) pesticide an endocrine disruptor on leydig's cells in rabbits. In this context, a total of 40 male rabbits, *Oryctolagus cuniculus*, were used as two test groups and one group labeled as control group. One tenth of LD_{50} Profenofos 500 EC, was orally administrated in animals for 11 and 15 days. Decline in the body weight was highly significant ($P < 0.001$) in treated animals. At the termination of experimental studies animals dissected and testes were removed in order to evaluate the testicular weight and histological parameters. Weight of the testes in the experimental animals exposed to profenofos for eleven and fifteen days was decreased highly significant (3.05 ± 0.12 and 2.93 ± 0.12 gms ($P < 0.001$) respectively as compared to 4.10 ± 0.12 gms. in the control group. Histopathological observations revealed that the leydig's cells of the rabbits exposed to profenofos were totally vanished or regressed. Even the interstitial space became compressed and completely hypertrophied and tumor like mass was also seen in few seminiferous tubules. This indicates the intensity of the toxic effect of Profenofos. It is concluded that exposure of profenofos cause endocrine disruption and shown drastic effects on the testicular tissues in rabbits.

Key words: Profenofos, Rabbits, Leydig's cell, Endocrine disruptor

INTRODUCTION

This is considered that various harmful pests exerting serious problems in agriculture fields and variety of agro pesticides is used to kill such harmful pests to improve the yield/ hectare. Controlled and proper use of these pesticides is not harmful but inappropriate and indiscriminately use is causing severe health problems to non targeted organisms. These agrochemicals penetrated in the environment and exert drastic toxic effects on all kinds of animals including domestic animals and bio agents (insects and birds). It was reported that "Pesticides used against agricultural pests and ecto- parasite infestation in animals may also induce injurious effects in pets and farm animals (Maffini *et al*., 2006). The indiscriminate and over recommended dose of insecticides like profenofos is increasing to kill the harmful pests like mealy bug (*Phenococcus Gossypiphilous*), army worm (*Spodoptera Litura*) to protect the cotton crops in Sindh and Punjab, Pakistan. While the over use of hazardous insecticides are exerting highly toxic effects in our ecological system and day by day these harms are increasing (Tarique *et al*., 2007).

The exposure of pesticides exerts toxic effects on reproductive system and disrupts the hormonal pathway in animals (Abro *et al*., 2005). The organophosphate (Profenofos) is a broad spectrum insecticide and frequently is used in agriculture throughout the world. The toxic effects of profenofos disrupt the endocrine system so that it is called Endocrine Disrupting Chemical (EDC). Oral and dermal exposure of profenofos exerts disruption in male reproductive organs of animals (Moustafa *et al.*, 2007).

Earlier studies on the endocrine disrupting chemicals with anti androgenic activity suggested that exposure to EDC's during sexual differentiation may induce atrophy of the male reproductive organs which causing hyperplasia of the leydig's cells due to disruption of the pituitary testicular feedback mechanisms (Kelce *et al.*, 1995; Maness *et al*., 1998; Ostby *et al*., 1999).

Definite effects of these chemicals on reproduction and sex organs have been reported in wild life and different in *vivo* studies (Ahmed, 2000). "Various substances including pesticides and different chemicals damages the health and exerted disruption or alters the functions of endocrine system of animals" (EEC, 1999).

Organophosphate (profenofos) is commonly used insecticide in agriculture for crop protection especially in cotton growing areas of Pakistan. This study was aimed to evaluate the testicular toxicity because lack of toxicological information with particular reference to endocrine disrupting effects of profenofos on male reproductive organs is causing infertility problems. Therefore mature male rabbits were exposed to this compound to assess the potential effects on testes with a view of a laboratory model for humans.

MATERIALS AND METHODS

Experimental Design and Animals: For evaluation of the effects on leydig's cells, a total of 40 male Rabbits *Oryctolagus Cuniculus*, were used in this study. These rabbits were purchased from different villages and were brought to the laboratory at Department of Zoology, University of Sindh, Jamshoro, Pakistan and housed in wooden cages with metal nets (12´ x 16´) sq. ft. These animals were kept under biological observation and acclimatized for 15 days before starting of the experimental studies. Animals were maintained three times a day on grass Alfalfa (*Medicago sativa*) which is commonly known as Lucerne and water.

After acclimation the rabbits were housed to the experimental cages for the application of Profenofos. Before start of the experiment body weight of rabbits were noted. Eight animals were assigned as control group whereas two groups each of fourteen animals were assigned as test groups by labeling procedure.

Test Chemical Preparation and Application: The solution of profenofos was applied one tenth of the $\mathbf{LD_{50}}$ concentration of technical products and this was prepared by adding in 10ml distilled water. The once daily dose 70 mg/kg body weight was orally administrated by disposable syringe to each test group animal for consecutive 11 and 15 days. Commercially available profenofos 500 EC was purchased from authorized dealers of agrochemical companies. Animals were feeding on for 11 and 15 day by giving same quantity (Lucerne) and water. After administration of dose observations were made on daily basis for 3 to 4 hours. During observations the parameters of adverse effects, diet intake, behavioral changes and mortality were assessed. At the termination of the experiment on day 11 and 15 body weight of all treated and control rabbits were recorded then killed through anesthetized procedure by using chloroform. All the male Rabbits (control and treated) were dissected and testes of each rabbit were exposed. Thereafter testicular weight was also noted for the treated and control animals. Removed testes from control and treated rabbits were processed for histopathological studies.

Histological Procedure: Pieces of removed organs (testes) were fixed in Bouin's solution for 24 hours. After that pieces of testes were passed through alcohol series for dehydration procedure. Tissues were infiltrated in Benzene for 10-15 minutes to remove alcohol until these became transparent. Tissues were embedded in paraffin wax blocks. Rotary microtome machine (RIECHERD JUNG LEICA 820 HISTOCUT ROTARY MICROTOME) was used for section cutting at a thickness of 6µm. in shape of ribbon. Harris's hematoxylin (Gurr, 1956) & Eosin (Putt, 1948) staining procedure was used. After selection of studied slides micro photography were made on Leica DM 2500 (Transmitted light microscope with digital camera 4LAS software).

Statistical analysis: Statistical analysis for the experimental data was performed by one way ANOVA on (IBM SPSS, 19) program followed by post hoc Least Significant Difference (LSD) test for the comparisons between groups. Values were described as mean ± SD. P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

Effects on food intake, health and mortality: test group animals were observed for 3-4 hours after the administration of profenofos during the experimental studies. It was observed that animals temporarily stopped eating food provided to them after the administration of insecticide. Few clinical symptoms like fatigue, silent, Tremor, convulsion, dizziness, occasionally diarrheas, dragging their hind limbs, nasal dripping and trembling were individually noted in rabbits after oral doses of test chemical. And same findings like trembling, decreased movement, diarrheas were observed in the animals exposed to pesticides (Najafi *et al*., 2010). Mortality was occurred in four test group rabbits during the experimental period.

Effects on body weight and reproductive organs: In present study ANOVA results indicated that when animals exposed to Profenofos for 11 and 15 days their body weight was decreased significantly ($p < 0.001$) as compared to control group animals as shown in (Table.1) with F- value 135.618. Exposure to organophosphate insecticide may cause decrease in body weight in the treated animals in comparison to control animals (Takizawa and Horrii, 2002; Joshi, *et al.,* 2007). Exposure to pesticides revealed reduction in body weight, induce many other health problems in animals and these might be reproductive disorders (Aly *et al*., 2009). In present work all animals in the treated groups for 11 and 15 days were observed highly significant ($p < 0.001$) decline in their testes weight as compared to control group (Table.1) and their F- value was 551.028, whereas (LSD) test showing comparison of mean values between control and each of the treated group. Values not sharing same letters are significantly different with each other. Mean ± SD body and testes weight in both treated groups was highly significant different than controls and result was same between mean values in treated groups. Profenofos exerted toxic effects on testicular tissues and disrupting the testicular function in treated animals and this associated with significant reduction in testes weight (El-Kashoury, 2009).

Table 1: Showing Parameters Examined in the Control and Treated Rabbits

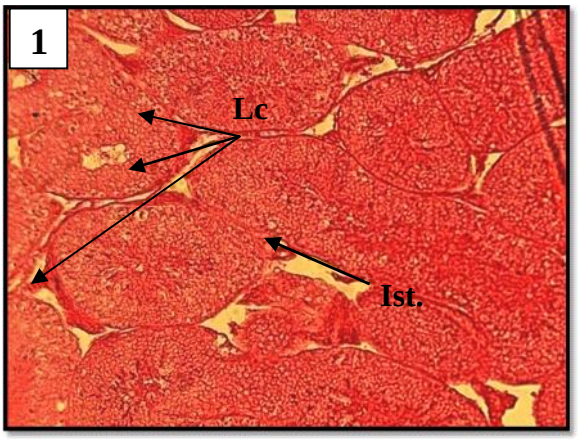
Animals (Rabbits)	Body weight (Mean±S.D) grams	Testes weight (Mean±S.D) grams
Controls	1475±25[a]	4.10±0.12[a]
Test Group (11 Days)	1400±32[b***]	3.05±0.12[b***]
Test Group (15 Days)	1310±14.43[c***]	2.93±0.12[c***]

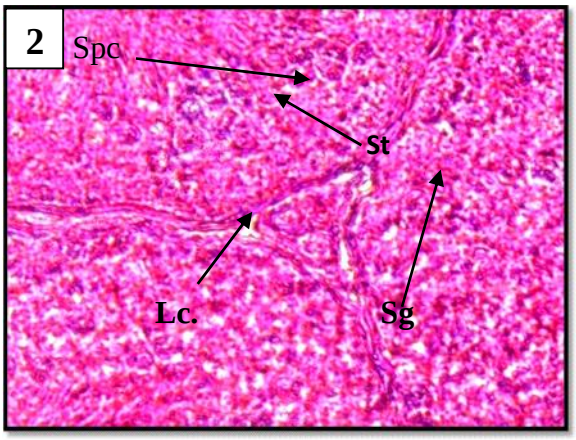
Table indicates values with (Mean ± SD) body & testes weight in treated rabbits with highly significant decrease ($P < 0.001$) *** as compared to control group. Values not sharing same letters are showing significance.

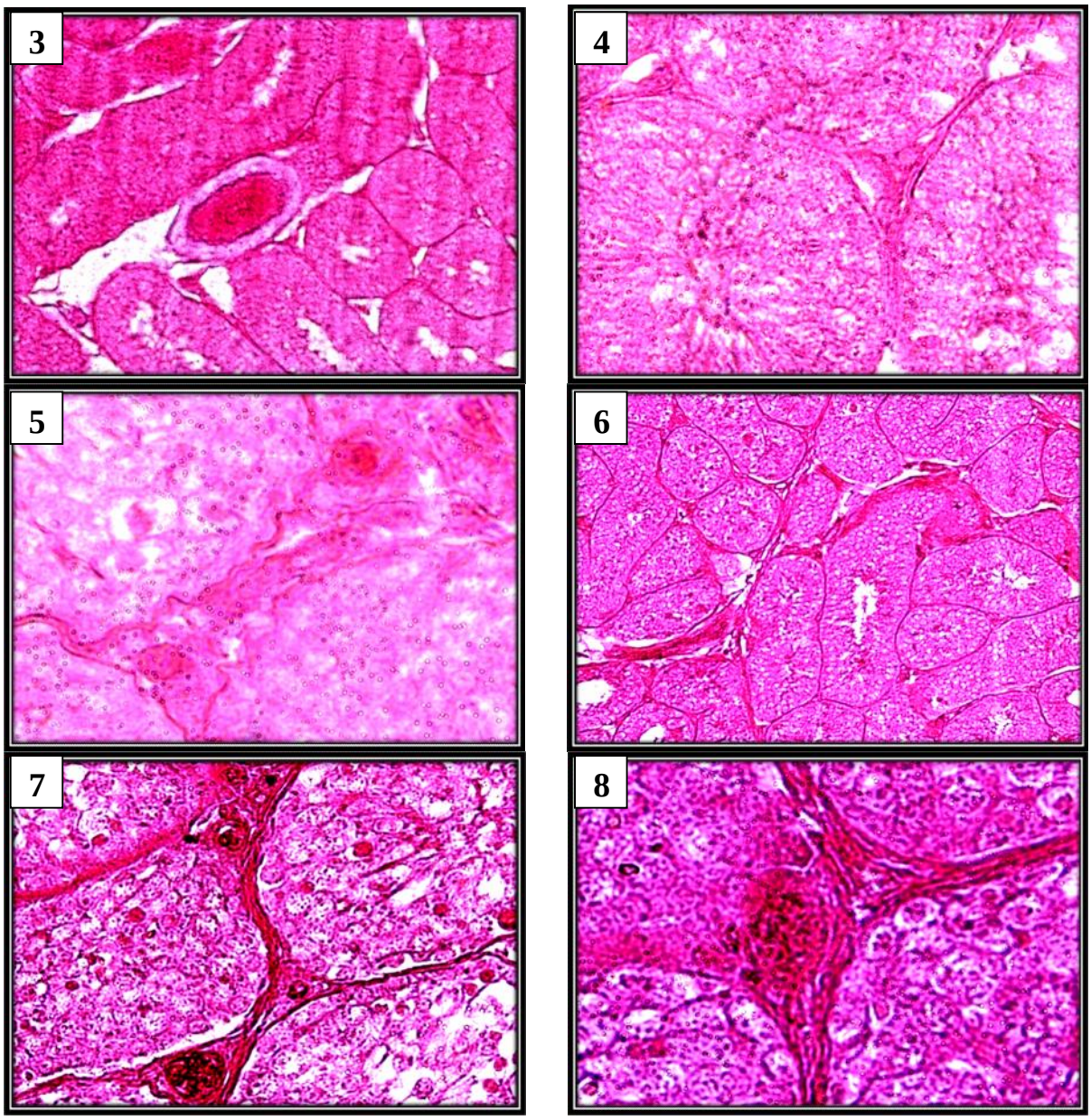
Histological Parameters: The histological structure of testes in control animals consist of seminiferous tubules with rounded /oval shaped and spermatocytes were also noted in scattered position throughout the tubules. Sertoli cells were also present inside the seminiferous tubules along with leydig's cells within interstitial space (Zidan, 2009). In the present study histological structure of testis in the control group animals showed proper sized seminiferous tubules containing different types of spermatogenic cells in stages of spermatogonia and spermatocytes with appearance of proper and narrow interstitial space and leydig's cells of normal size were also present in the interstitial space (Figs 1-2). However histopathological changes were observed in the treated animals exposed to profenofos for 11 days. Tumor like mass was present in few tubules along with few other changes like vacuolation in seminiferous tubules and reduced/suppressed number of leydig's cells were also noted. The seminiferous tubules were found with abnormal size and shape indicated in (Fig. 3). Moreover our investigations revealed that, the sizes of seminiferous tubules were further reduced along with condensed interstitial space. The number of spermatogenic cells was regressed and leydig's cells have been either hypertrophied or eliminated (Fig. 4). Destruction in leydig,s cells results, disrupt the functioning of the testes to release testosterone hormone for the development of spermatogenic cells stages (Saunders, 2003) and any change in the serum testosterone creates fertility problems (Thomas *et al*., 2008). Therefore in the present study hypertrophy was clearly seen in interstitial space and no spermatocytes were found in the seminiferous tubules. The leydig's cells were totally regressed or vanished, that shows drastic endocrine disrupting effects of profenofos on leydig's cells and testes (Fig. 5). Earlier this has been reported that animals are vulnerable to the endocrine disrupting effects of organophosphate (OP) pesticides (Lyons, 1999 and Sinha *et al*., 1997).

Exposure of pesticides exhibited pathological changes in testicular tissues and this alteration occurred as antiandrogenic effects (Garry, *et al* 2001; Lu *et al*., 2004 and Dallegrave *at al*., 2007). In accordance to previous findings, histopathological observations revealed that 15 days profenofos treated animals showed empty lumen and vacuolation with highly developed hypertrophy/tumor formation. Interstitial space totally tumorized that clearly showing effect on leydig's cells elimination or hypertrophy (Fig. 6 & 7).

It was observed that seminiferous tubules were ruptured and leydig's cells hypertrophied followed by disintegration and vacuolation occurred in tubules (Okamura, 2005 and Farag *et al*., 2007) and same histological changes were observed in the 15 days profenofos treated animals (Fig. 8). The applications of these agro chemicals besides controlling the growth of pests, damages other fauna which being confronted to these insecticides like profenofos. Looking at the significant changes and drastic effects on testicular tissues (Leydig's cells) in the rabbits and treatment related mortality, profenofos is considered highly toxic insecticide.







Figs.1&2. Histological section of Testes from control male rabbit showing nomal structure of seminiferous tubules (St) interstitial space (Ist), leydig's cells (lc), spermatogenic cells (Ssg) and spermatocytes (Sc), (H & E, x10 and x63).

Figs. 3-5. Histological section of Testes from 11 days Profenofos treated male rabbit showing vacuolation and reduced size of seminiferous tubules (St), tumor like mass present in and out side tubules, eliminated or hypertrophied leydig's cells (lc), hrpertrophied or reduced number of spermatogenic cells (Ssg) and elimination of spermatocytes (Sc), (H & E x10,40 and 63).

Figs. 6-8. Histological section of Testes from 15 days Profenofos treated male rabbit showing congestion in seminiferous tubules (St) with reduced size and area, tumor/hypertrophied itnterstitial space (Ist), spermatogenic cells (Ssg) & leydig's cells (lc), multinucleated spermatogenic cell with intra tubular vacuolation and ruptured seminiferous tubules sheath along with tumor/hypertrophy (H & E x 10, 40 and 63).

Conclusion: This is concluded that profenofos induced disruption of testicular tissues in the exposed rabbits. The severity of toxic effects was significantly higher on leydig's cells together with reduction in body and testicular weight. Thus present study provides the understanding about hazardous endocrine disrupting effects of profenofos on male reproductive organ (testes) and this can cause infertility problems.

Acknowledgments: We are thankful to Prof. Dr. Shafi Muhammad Nizamani, National Centre of Excellence in Analytical Chemistry (*NCEAC*) University of Sindh, Jamshoro, Pakistan for his valuable suggestions.

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