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## EFFECT OF PYRIPROXYFEN ON FUNCTION AND TISSUE OF TESTIS IN ADULT RAT

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### ABSTRACT

**Objective:** Pyriproxyfen is a pyridine insecticide and used in agriculture widely. Extensive application of pyriproxyfen to control annual insect pest in agriculture, horticulture and horn garden, leads to environmental pollution and its entrance into the food chain could have determined effects on human and other species. In this research the effects of pyriproxyfen on reproductive parameters of the male rats including serum LH, FSH, testosterone levels, and changes in testicular tissue and body weight were investigated.

**Methods:** For this purpose 250 ± 5g male Wistar strain rats (n = 50) were randomly collected and divided into 5 group of 10, including control, sham (received distilled water 70%+ethanol 20%+aceton 10%as a solvent), and three experimental groups which received 1000, 2000 and 4000 mg/kg/bw pyriproxyfen orally. After 28 days, body and testis weight were measured and blood samples were taken from heart and used for measurement of LH, FSH and testosterone levels. To evaluate histological changes, testes were removed and weighed and, after obtaining tissue section, stained by H and E method.

**Results:** Serum testosterone, FSH, and LH levels showed significant decreasing in experimental groups ( $p \leq 0.05$ ). There was significant decreasing in the number of germinal and somatic cells in testis in experimental groups. There was also a significant decreasing in body and testis weight in experimental groups, as compared to control group.

**Conclusion:** It can be concluded that oral administration of pyriproxyfen could reduction gonadotropins and testosterone hormone levels and also this insecticide might have hazardous effects on testis tissue.

**Keywords:** Pyriproxyfen, Testosterone, Gonadotropins, Testis, Rat

### INTRODUCTION

One of the most disquieting discoveries in recent years concerns the possible roles of environmental chemicals on endocrine systems (1-3). Endocrine systems coordinate and regulate many important body functions such as growth and maturation, behavior, reproduction and embryo development. They do this by making and releasing hormones which act as "chemical messengers"(1-3) Endocrine systems can be affected by certain substances outside of the body, both naturally-

occurring and artificial. By interfering with the normal communication between the messenger and the cell receptors, the chemical message is misinterpreted, generating abnormal response(s) in the body.

The number of substances believed to act as endocrine disruptors is wide and varied, including both natural and synthetic materials. Concern arises because potential endocrine disruptors may be present in the environment, unrecognized but

possibly able to cause effects at low concentrations.

Synthetic chemicals suspected as endocrine disruptors may reach humans and animals in a variety of ways. Some, such as insecticides, are released intentionally (1-3).

These days insecticide usage has become more widespread as new ways to harvest more crops and control weed are used so humans are exposed to a wide variety of insecticides. Therefore it is necessary to notice the possible hazardous effects of these insecticides on human health.

In this article we have investigated the effects of one of the most current insecticides, called pyriproxyfen, on LH, FSH and testosterone hormone levels and testis histological changes in adult rats. Pyriproxyfen is a pyridine insecticide used in agriculture widely. It enters insects and inhibits insect maturation process from cell division to elongation (a juvenile hormone mimic). Extensive application of pyriproxyfen to control annual grasses and broadleaf weeds in agriculture, horticulture and home gardens leads to environmental pollution and its entrance into the food chain could have detrimental effects on human and other species.

We selected pyriproxyfen because of the following important points:

- 1- Excessive usage of this pesticide in recent years.
- 2- Low solubility in water and high stability in the environment may lead pyriproxyfen entering into the body via fruits and vegetable feeding by human and domestics.
- 3- Potential effects of as pyriproxyfen an endocrine disrupting chemical.

## MATERIALS AND METHODS

In our experimental study, the effects of pyriproxyfen on reproductive parameters of male Wistar strain rats were investigated. For this purpose, 40 adult male rats with the average age of 2.5-3 months and 250 grams weight were

randomly divided in 5 groups of 10: control, sham and three experimental groups 1, 2, and 3.

The 5 groups of rats were kept in special polycarbonate cages under the same conditions of unlimited water and special squeezed food. The temperature range and light and dark cycle were between 20-25°C and 12 hours respectively.

The rats in the experimental groups 1, 2, and 3 received 1000, 2000 and 4000 grams pyriproxyfen per kilograms live weight per day (mg/kg/day) respectively. The control group left untreated and the sham received solvent (distilled water 70%+ethanol 20%+acetone 10%). Everyday pyriproxyfen was administered orally via syringes equipped with feeder.

The rats were weighed at the end of day 28 and then under anesthesia by ether, blood was taken from the heart. Each group's blood samples were centrifuged at the rate of 5000 per min. in order to separate blood serum.

Serum concentrations of LH, FSH and testosterone were measured by special rat kits using the ELISA method. For histological studies of testis tissue, the testes were removed, weighed and kept in formalin for 17-18 hours and then microscopic slides were prepared and stained by Hematoxylin – Eosin method.

Finally, the data were statistically analyzed using the SPSS software. The means of the sham and experimental groups were compared with the control group using the ANOVA and Duncan's multiple range tests.  $P < 0.05$  was considered as a significant difference.

## RESULTS

Pyriproxyfen treatment caused a significant decrease in body and testis weights in the experimental groups (Table 1). It also decreased serum LH and FSH levels in all experimental groups. There was also a significant decrease in experimental group 3 compared to the control group (Table 2). Testosterone decreased significantly in experimental groups 2 and 3 compared to the control group (Table 2).

Histological studies of testis tissue demonstrated significant decrease in the number of spermatogonia, primary spermatocytes, spermatids, sertoli cells and leydig cells in all or some of experimental groups compared to the control group (Table 3 and Figures 1,2,3,4). Sperm density declined significantly in all experimental groups and seminiferous tubules showed degeneration and deformation in experimental group 3 (Figure 4).

## DISCUSSION

Our findings demonstrated that pyriproxyfen could decrease serum testosterone hormone concentration. Many studies have proven that testosterone has a direct effect on protein synthesis in all tissues and organs of the body and can increase bone and muscle mass in males (4, 5); so in experimental groups decreased testosterone could lead to body weight loss via decreasing protein synthesis and bone/muscle mass.

According to previous studies, pyriproxyfen is a thyroid hormone disruptor and can induce tumor formation or hypertrophy of thyroid follicular cells (6), so probable significant increase in serum T3 and T4 levels lead to proteolysis and decreasing protein mass, followed by severe muscle catabolism (7). Recent data demonstrated that increasing serum T4 and decreasing TSH decrease only BMI (7,8).

Some experimental studies showed that pyriproxyfen increases cortisol significantly. Cortisol increases under following physiologic and pathologic situations:

In physiologic situations (stress, fight and flight,) increasing cortisol leads to increased gluconeogenesis and protein catabolism and decreased glucose oxidation to supply the required energy. Following alleviation of this condition, appetite increases leading to increased weight (9). This probably happened in the sham group under daily stress (see table 1). But pathologic elevation of cortisol leads to decreased protein synthesis,

increased protein catabolism, decreased bone mass and finally decreased body weight (9).

According to a previous study on rats and dogs exposed to a compound pesticide containing pyriproxyfen, prostate, seminal vesicle and androgen sensitive organ weights decreased (10).

Researches have demonstrated that physiologic concentrations of testosterone, LH and FSH play an important role in spermatogenesis (4), so a significant decrease of these hormones in our study could decrease the number and function of somatic and germinal cells of testis followed by testis weight reduction.

Some experimental studies have demonstrated that pyriproxyfen can generate free radicals (10) and chromosomal malformations in cells (11), so this could occur in sensitive cells in the testis and result in cell death and testis weight reduction.

Experimental studies on rats demonstrated that pyriproxyfen can decrease pituitary gland weight and size (12), which could be the result of decreasing pituitary secreted hormones like LH and FSH.

Pyriproxyfen causes disorder in adrenal and significant increase in serum cortisol level (5). Findings demonstrated cortisol elevation reduces pulsatile secretion of GnRH and gonadotropins specially (13-17), so it can potentially reduce testis endocrine function (16,17).

Tumor and hypertrophy in follicular cells of thyroid by pyriproxyfen (6) can increase serum T4 and then TRH through negative feedback and therefore causes a long-term increase in prolactin level. Prolactin elevation decreases GnRH and FSH/LH secretion (9)

Significant decrease in interstitial cells number and LH hormone concentration in experimental groups could lead to decreased testosterone secretion from interstitial cells (4,5). As previously states, pyriproxyfen can increase serum cortisol level (10). Cortisol elevation induces negative effects on testosterone synthesis, steroidogenesis, and spermatogenesis, directly (16-21).

According to recent studies, pyriproxyfen leads to activation of a type of nuclear receptor in liver called PXR<sup>1</sup>, which is a kind of steroid / xenobiotic sensor (22-24). Many hormone disrupting chemicals that activate PXR have anti androgenic effects (23), so it could be possible that pyriproxyfen has the same effect too. Moreover, PXR induces CYP3A enzyme. This enzyme metabolizes a great number of steroids like testosterone and increases these steroids' clearance from plasma. Also, CYP3A induction would produce some metabolites which have endocrine disrupting function (24). Recent hypotheses express that PXR activation via xenobiotics leads to iNOS (human gene promoter inducing nitric oxide synthesis) upregulation and then increase nitric oxide synthesis (24-25). Pyriproxyfen elevates blood platelets level (25) and causes release of serotonin and histamine inflammatory factors. These factors can start inflammatory reactions and increase nitric oxide synthesis (9). Nitric oxide prevents steroidogenesis in interstitial cells by the mechanism of CP450scc<sup>2</sup> enzyme prevention which acts as a cholesterol-to-pregnenolone converter (26-28).

An experimental study demonstrated that a herbicide with 26% pyriproxyfen could increase chromosomal malformation in testis germinal cells (11,29). It can be mutagenic (6). On the other hand, the published article by CDC<sup>3</sup> has demonstrated that this herbicide can pass through cytoplasmic membrane and produce free radicals, oxidative stress and damage to biomolecules like DNA and protein (10), so these properties of pyriproxyfen can cause severe damage to or kill sensitive testis cells, especially spermatogonia, primary spermatocytes and spermatids.

Decreasing LH, FSH, and testosterone hormones concentration could be effective in decreasing

spermatogenesis and the number of testis germinal cells (4, 5).

Significant increase in serum cortisol and nitric oxide (e.g. by pyriproxyfen in our study) decreases steroidogenesis and spermatogenesis (16,20,21); also nitric oxide is able to decrease spermatocytes and spermatids number and sperm content, due to induction of apoptosis (18,30-32) and can damage seminiferous tubules epithelium(33) too. Moreover, according to some studies, cortisol elevation decreases LH pulsatile secretion and causes oligospermia (13-16).

According to experimental studies on rats, pyriproxyfen has hazardous effects on prostate and seminal vesicle glands (10,17), which have a crucial role in generating most of semen contents and supplying suitable milieu for sperm life (4,5), so this could be an important result of severe oligospermia in the experimental groups in our study.

## CONCLUSION

The results obtained from the present study indicated that different doses of pyriproxyfen showed significant reduction in levels of FSH, LH and testosterone. In addition, this compound eliminated spermatogenesis process in seminiferous tubules. Hence, based on foregoing evidence, the present study suggest that usage of pyriproxyfen must be done with consider to different levels of hazard analysis. However, to achieve to this information more investigations are needed.

<sup>1</sup>- Pregnane Xenobiotic Rceptor

Cytochrome P450 type scc -<sup>2</sup>

<sup>3</sup> - Control Diseases Center

**Table 1- Mean body and testis weights changing in experimental groups in contrast to control group at the end of experimental period**

Weights		Mean criteria error±		
		Mean body weight(g) $(\bar{X} \pm SEM)$		
Groups		Body weight	Right testis weight	Left testis weight
Control		253.6 ±0.02	1.36±0.02	1.39±0.04
Sham		254.6±2.1	1.39±0.03	1.4±0.03
Experimental groups	Pyriproxyfen $\frac{mg}{kg/bw}$ 1000	230.0±3.16*	1.2±0.05*	1.19±0.04*
	Pyriproxyfen $\frac{mg}{kg/bw}$ 2000	229.0±3.11*	1.15±0.08*	1.15±0.03*
	pyriproxyfen $\frac{mg}{kg/bw}$ 4000	227.38±3.67*	1.07±0.11*	1.06±0.09*

Between control and experimental groups. )0.05  $P \leq$  (\* indicates significant difference

**Table 2- Changing of Mean serum levels of testosterone, LH and FSH in experimental groups in contrast to control group at the end of experimental period**

Hormones		Mean criteria error±		
		Mean serum level of hormones $(\bar{X} \pm SEM)$		
Groups		Testosterone (ng/ml)	LH (mIu/ml)	FSH (mIu/ml)
Control		4.14±0.24	4.01±0.26	7.58±0.24
Sham		4.3±0.43	3.89±0.2	7.3±0.29
Experimental groups	Pyriproxyfen $\frac{mg}{kg/bw}$ 1000	3.41±0.29	2.63±0.14**	7.5±0.29
	Pyriproxyfen $\frac{mg}{kg/bw}$ 2000	2.67±0.49*	2.46±0.21**	7.3±0.32
	Pyriproxyfen $\frac{mg}{kg/bw}$ 4000	2.02±0.29**	1.71±0.15**	6.45±0.35*

\* indicates significant difference between control and experimental groups ( $P \leq 0/05$ ).

\*\* indicates significant difference between control and experimental groups ( $P \leq 0/001$ ).

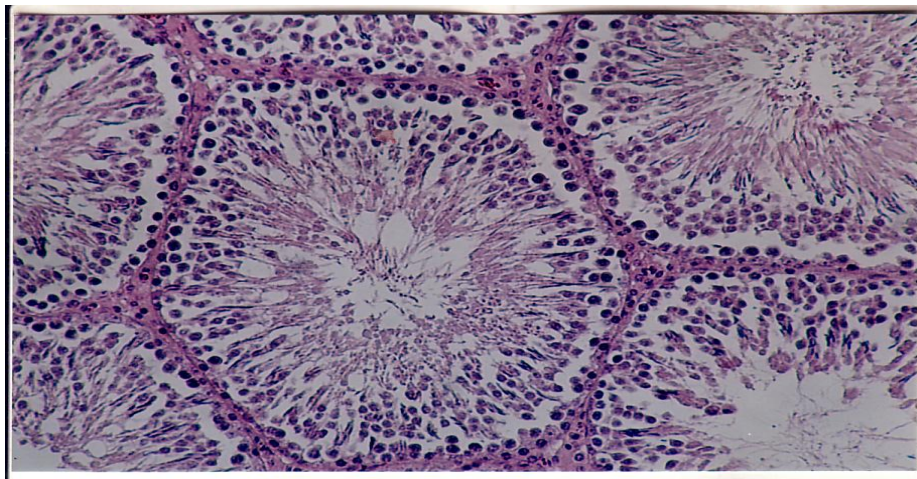


**Table 3- Changing of mean number of testis cells in experimental groups in contrast to control group**

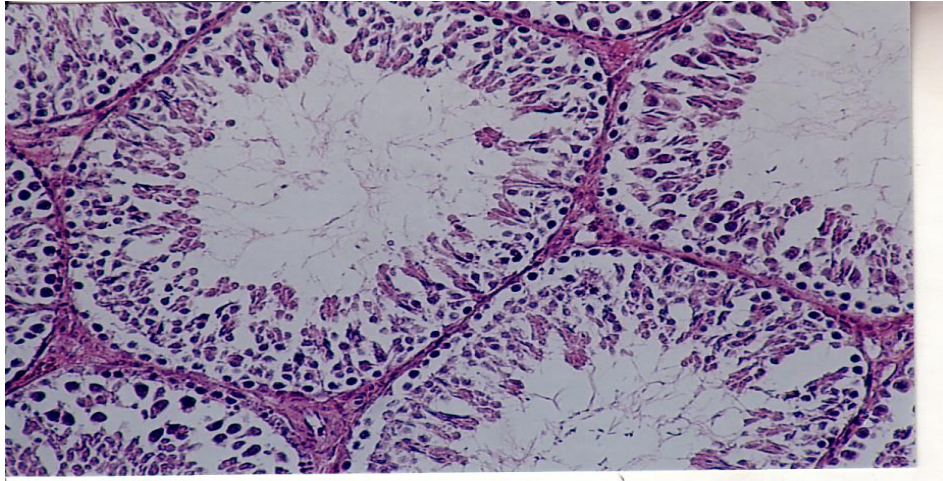
Testis cells		Mean criteria error ± Mean number of testis cells ( $\bar{X} \pm SEM$ )				
		Spermatogonia	Primary spermatocyte	Spermatid	Sertoli	Leydig
<b>Groups</b>						
<b>Control</b>		56.5±2.06	67.0±3.18	180.75±9.83	11.0±1.25	10.5±0.85
<b>Sham</b>		51.5±1.59	64.5±1.59	199.25±9.61	10.5±0.73	11.38±1.67
<b>Experimental groups</b>	Pyriproxyfen $\frac{mg}{kg/bw}$ 1000	49.0±2.24*	58.5±2.13*	157.5±8.81	11.0±1.0	9.75±0.49
	Pyriproxyfen $\frac{mg}{kg/bw}$ 2000	32.25±2.92**	45.0±3.7**	141.13±9.41*	9.5±0.76	8.13±0.35
	Pyriproxyfen $\frac{mg}{kg/bw}$ 4000	10.13±1.36**	22.0±0.8**	30.63±1.1**	7.38±0.5*	5.75±0.37**

\* indicates significant difference between control and experimental groups ( $P \leq 0/05$ ).

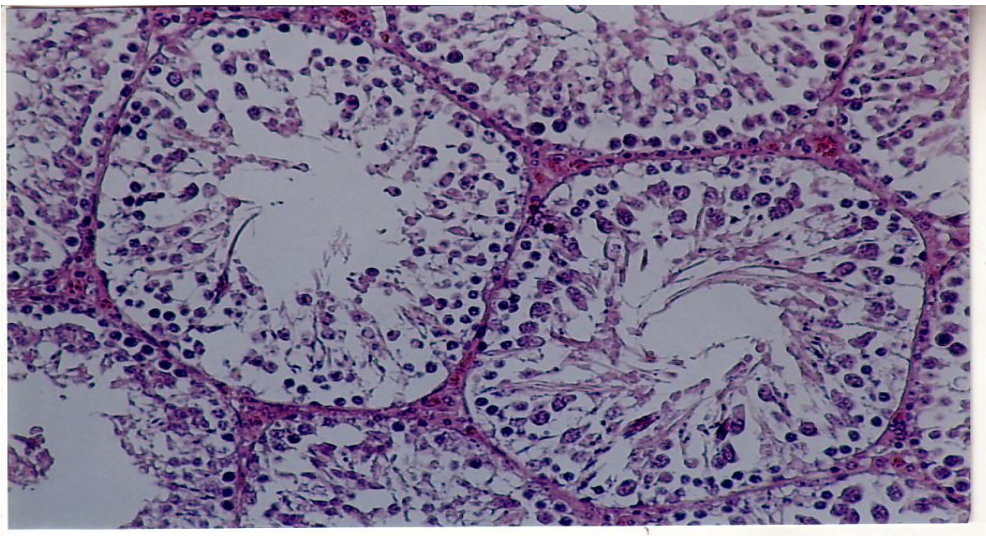
\*\* indicates significant difference between control and experimental groups ( $P \leq 0/001$ ).



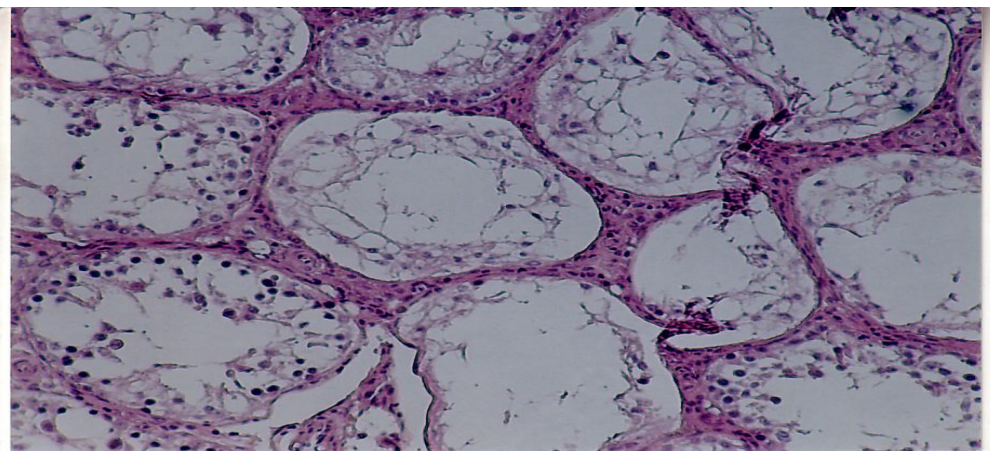
**Figure 1- Testis tissue in control group (H&E staining; 40X)**



**Figure 2- Testis tissue in experimental group 1(H&E staining; 40X)**



**Figure 3- Testis tissue in experimental group 2(H&E staining; 40X)**



**Figure 4- Testis tissue in experimental group 3(H&E staining; 40X)**



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