Original Article

The Effects of Trifluralin on LH, FSH and Testosterone Hormone Levels and Testis Histological Changes in Adult Rats

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Abstract

Background: Trifluralin is a herbicide and used in agriculture widely. It enters plants through developing roots and stops plant cells from division and elongation (meristemic inhibitor). Extensive application of trifluralin to control annual grasses and broadleaf weeds 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 trifluralin on reproductive parameters of the male rats including serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone levels, and changes in testicular tissue and body weight were investigated.

Materials and Methods: For this purpose male rats were randomly divided in 5 groups, to include control, sham (received normal saline as a solvent), and three experimental groups which received 500, 1000 and 2000 mg/kg oral trifluralin respectively. After 16 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 HE.

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

Conclusion: It can be concluded that oral administration of trifluralin could decrease gonadotropins and testosterone hormone levels and also this herbicide could have hazardous effects on testis tissue.

Keywords: Trifluralin, 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.

There is an acceptable description of endocrine disrupting chemicals:

"An environmental endocrine or hormone disruptor may be defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior."

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 pesticides, are released intentionally (1-3).

These days pesticide usage has become more widespread as new ways to harvest more crops and control weed are used so humans are exposed

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to a wide variety of pesticides. Therefore it is necessary to notice the possible hazardous effects of these pesticides on human health.
In this article we have investigated the effects of one of the most current pesticides, called Trifluralin, on LH, FSH and testosterone hormone levels and testis histological changes in adult rats. Trifluralin is a dinitroaniline herbicide used in agriculture widely. It enters plants through developing roots and stopes plant cells from division and elongation (meristemic inhibitor). Extensive application of trifluralin 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 Trifluralin 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 Trifluralin entering into the body via fruits and vegetable feeding by human and domestics.
3. Potential effects of Trifluralin as an endocrine disrupting chemical.

Materials and Methods

In our experimental study, the effects of Trifluralin on reproductive parameters of male Wistar rats were investigated. For this purpose, 40 mature male rats with the average age of 2.5-3 months and 250 grams weight, were randomly divided in 5 groups of 8: control, sham and three experimental groups 1, 2, 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 500, 1000 and 2000 grams Trifluralin per kilograms live weight per day (mg⁄kg/day) respectively. The control group left untreated and the sham received equivalent amount of normal saline as solvent. Everyday Trifluralin was administered orally via syringes equipped with feeder.
The rats were weighed at the end of day 16 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 RIA 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 Hematoxilin – Eosine.
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

Trifluralin 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 primary spermatocytes, spermatogonias, spermatids, sertoli cells and leydig cells in all or some of experimental groups compared to the control group (Table 3 and Fig 1-4).

Table 1: Mean body and testis weight changing in experimental group in contrast group at the end of experimental period

Group		Weight		
		Mean criteria error ± Mean body weight(gr) (X±SEM)		
		Body	Right testis weight	Left testis weight
Control		253.6±0.02	254.6±2.1	1.39±0.04
Sham		254.6±2.1	1.39±0.03	1.4±0.03
Experimental groups	500mg/kg/bw Trifluralin	230.0±3.16*	1.2±0.05*	1.19±0.04*
	1000mg/kg/bw Trifluralin	229.0±3.11*	1.15±0.08*	1.15±0.03*
	2000mg/kg/bw Trifluralin	227.38±3.67*	1.07±0.11*	1.06±0.09*

**** Indicates significant difference (p≤0.05) between control and experimental groups***

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.

Group		Hormones		
		Mean criteria error ± Mean body weight(gr) (*X*±*SEM*)		
		Testostrone	LH (Iu/lit)	FSH (Iu/lit)
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	500mg/kg/bw Trifluralin	3.41±0.29	2.63±0.14**	7.5±0.29*
	1000mg/kg/bw Trifluralin	2.67±0.49*	2.46±0.21*	7.3±0.32*
	2000mg/kg/bw Trifluralin	2.02±0.29*	1.71±0.15**	6.45±0.35*

** indicates significant difference between control and experimental groups (p≤0.05)*
*** indicates significant difference between control and experimental groups (p≤0.001)*

Table 3: Changing of mean number of testis cells in experimental groups in contrast to control group.

Group		Testis Cells				
		Mean criteria error ± Mean body weight(gr) (X±SEM)				
		Spermatogonia	Primary Spermatocyte	Spermatid	Sertoli	Leydig
Control		56.2±2.06	67.0±3.18	180.75±9.83	11.0±1.25	10.5±0.85
Sham		51.5±1.59	64.5±1.59	199.25±9.61	10.5±0.73	11.38±1.67
Experimental groups	500mg/kg/bw Trifluralin	49.0±2.24*	58.5±2.13*	157.5±8.81	11.0±1.0	9.75±0.49
	1000mg/kg/bw Trifluralin	32.25±2.92**	45.0±3.7**	141.13±9.41*	9.5±0.76	8.13±0.35
	2000mg/kg/bw Trifluralin	10.13±1.36**	22.0±2.8**	30.63±1.1**	7.38±0.5*	5.75±0.37**

** indicates significant difference between control and experimental groups (p≤0.05)*
*** indicates significant difference between control and experimental groups (p≤0.001)*

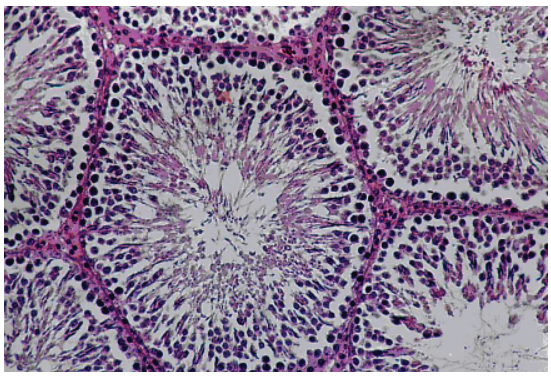

Fig 1: Testis tissue in control group (H&E staining; ×40)

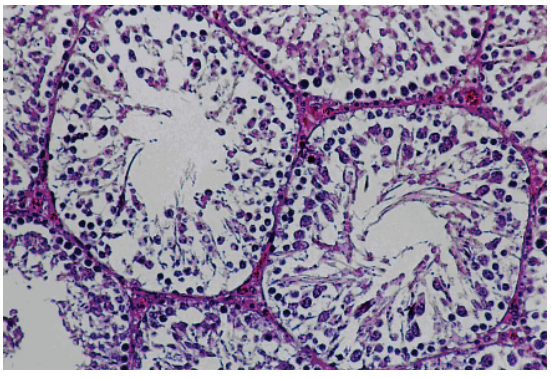

Fig 2: Testis tissue in experimental group 1 (H&E staining; ×40)

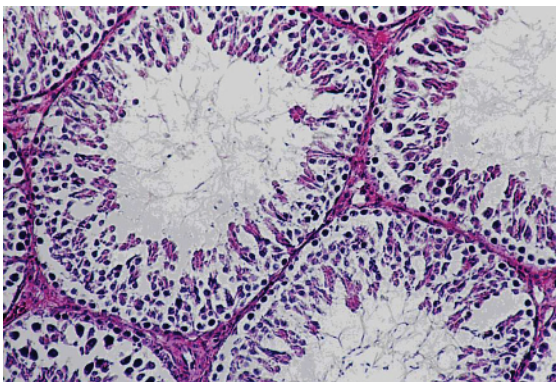

Fig 3: Testis tissue in experimental group 2 (H&E staining; ×40)

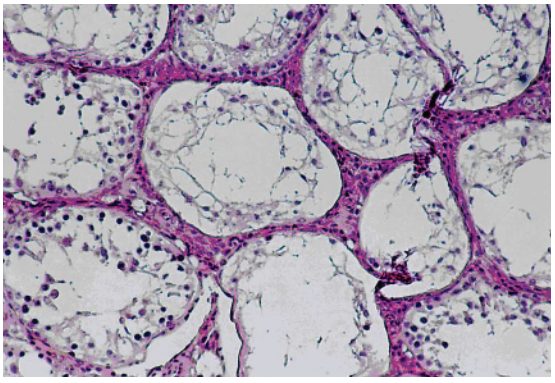

Fig 4: Testis tissue in experimental group 3 (H&E staining; ×40)

Sperm density declined significantly in all experimental groups and seminiferous tubules showed degeneration and deformation in experimental group 3 (Fig 4).

Discussion

Trifluralin effects on body weight

Our findings demonstrated that Trifluralin 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, Trifluralin 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 body mass index (BMI) (7, 8).

Some experimental studies showed that Trifluralin 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).

Trifluralin effects on testis weight

According to a previous study on rats and dogs exposed to a compound pesticide containing Trifluralin, 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 Trifluralin 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.

Trifluralin effects on LH/FSH hormones level decline

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

Trifluralin 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 Trifluralin (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).

Trifluralin effects on testosterone hormone level

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, Trifluralin can increase serum cortisol level (10). Cortisol elevation induces negative effects on testosterone synthesis, steroidogenesis, and spermatogenesis, directly (16-21).

According to recent studies, Trrifluralin leads to activation of a type of nuclear receptor in liver called PXR (Pregnane Xenobiotic Rceptor), which is a kind of steroid/xenobiotic sensor (22-24). Many hormone disrupting chemicals that activate PXR have antiandrogenic effects (23), so it could be possible that Trifluralin 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). Trifluralin 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 enzyme prevention which acts as a

cholesterol-to-pregnenolone converter (26-28).

Hazardous effects of Trifluralin on testis tissue

An experimental study demonstrated that a herbicide with 26% Trifluralin could increase chromosomal malformation in testis germinal cells (11, 29). It can be mutagenic (6). On the other hand, the published article by CDC (Cytochrome P450 type scc) 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 Trifluralin 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 Trifluralin 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, Trifluralin 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

We suggest that, applied dosage of Trifluralin herbicide during 16 days cause a decrease in serum LH, FSH and testosterone concentration and spermatogenesis.

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References

1. Brevini TA, Zanetto SB, Cillo F. Effects of endocrine disruptors on developmental and reproductive functions. Curr Drug Targets Immune Endocr Metabol Disord. 2005; 5(1): 1-10.
2. Trubo R. Endocrine-disrupting chemicals probed as potential pathways to illness. JAMA. 2005; 20; 294(3): 291-293.
3. Elobeid MA, Allison DB. Putative environmental-endocrine disruptors and obesity. Curr Opin Endocrinol Diabetes Obes. 2008; 15(5): 403-408.
4. Zitzmann M. Effects of testosterone replacement and its pharmacogenetics on physical performance and metabolism. Asian J Androl. 2008; 10(3): 364-372.
5. Wade AP, Wilkinson GS, Davis JC, Jeffcoate TN. The metabolism of testostrone, androstendione and oestrone by testes from a case of testicular feminization. J Endocrinol. 1968; 42(3): 391-403.
6. Saghir SA, Charles GD, Bartels MJ, Kan LH, Dryzga MD, Brzak KA, et al. Clark AJ. Mechanism of trifluralin-induced thyroid tumors in rats. Toxicol Lett. 2008; 30; 180(1): 38-45.
7. Iacobellis G, Ribaudo MC, Zappaterreno A, Iannucci CV, Leonetti F. Relationship of thyroid function with body mass index, leptin, insulin sensitivity and adiponectin in euthyroid obese women. Clin Endocrinol (Oxf). 2005; 62(4): 487-491.
8. Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L, et al. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab. 2005; 90(7): 4019-4024.
9. Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E, Connell JM. Cortisol effects on body mass, blood pressure, and cholesterol in the general population. Hypertension. 1999; 33(6): 1364-1368.
10. Choi SM, Lee BM. An alternative mode of action of endocrine-disrupting chemicals and chemoprevention. J Toxicol Environ Health B Crit Rev. 2004; 7(6): 451-463.
11. Rakitsky VN, Koblyakov VA, Turusov VS. Nongenotoxic (epigenetic) carcinogens: pesticides as an example. A critical review. Teratog Carcinog Mutagen. 2000; 20(4): 229-240.
12. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect. 1996; 104 Suppl 4: 741-803.
13. Debus N, Breen KM, Barrell GK, Billings HJ, Brown M, Young EA, et al. Does cortisol mediate endotoxin-induced inhibition of pulsatile luteinizing hormone and gonadotropin-releasing hormone secretion? Endocrinology. 2002; 143(10): 3748-3758.
14. Suter DE, Schwartz NB. Effects of glucocorticoids on secretion of luteinizing hormone and follicle-stimulating hormone by female rat pituitary cells in vitro. Endocrinology. 1985; 117(3): 849-854.
15. Breen KM, Billings HJ, Wagenmaker ER, Wessinger EW, Karsch FJ. Endocrine basis for disruptive effects of cortisol on preovulatory events. Endocrinology. 2005; 146(4): 2107-2115.
16. Bambino TH, Hsueh AJ. Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis in vivo and in vitro. Endocrinology. 1981; 108(6): 2142-2148.
17. Juniewicz PE, Johnson BH, Bolt DJ. Effect of adrenal steroids on testosterone and luteinizing hormone secretion in the ram. J Androl. 1987; 8(3): 190-196.
18. Norman RL. Effects of corticotropin-releasing hormone on luteinizing hormone, testosterone, and cortisol secretion in intact male rhesus macaques. Biol Reprod. 1993; 49(1): 148-153.

19. Hoogeveen AR; Zonderland ML : Relationships between testosterone, cortisol and performance in professional cyclists. Int J Sports Med. 1996; 17(6): 423-428.
20. Shankar DS, Kulkarni RS. Effect of cortisol on testis of freshwater fish Notopterus notopterus (Pallas). Indian J Exp Biol. 2000; 38(12): 1227-1230.
21. Consten D, Keuning ED, Terlou M, Lambert JGD, Goos HJT, Cortisol effects on the testicular androgen synthesizing capacity in common carp, Cyprinus carpio L. Fish Physiol Biochem. 2001; 25(2): 91-98.
22. Mikamo E, Harada S, Nishikawa J, Nishihara T. Endocrine disruptors induce cytochrome P450 by affecting transcriptional regulation via pregnane X receptor. Toxicol Appl Pharmacol. 2003; 15; 193(1): 66-72.
23. Tabb MM, Blumberg B. New modes of action for endocrine-disrupting chemicals. Mol Endocrinol. 2006; 20(3): 475-482.
24. Guillette LJ Jr. Endocrine disrupting contaminants--beyond the dogma. Environ Health Perspect. 2006; 114 Suppl 1: 9-12.
25. Toell A, Kröncke KD, Kleinert H, Carlberg C. Orphan nuclear receptor binding site in the human inducible nitric oxide synthase promoter mediates responsiveness to steroid and xenobiotic ligands. J Cell Biochem. 2002; 85(1): 72-82.
26. Del Punta K, Charreau EH, Pignataro OP. Nitric oxide inhibits Leydig cell steroidogenesis. Endocrinology. 1996; 137(12): 5337-5343.
27. Pomerantz DK, Pitelka V. Nitric oxide is a mediator of the inhibitory effect of activated macrophages on production of androgen by the Leydig cell of the mouse. Endocrinology. 1998; 139(3): 922-931
28. O'Bryan MK, Schlatt S, Gerdprasert O, Phillips DJ, de Kretser DM, Hedger MP. Inducible nitric oxide synthase in the rat testis: evidence for potential roles in both normal function and inflammation-mediated infertility. Biol Reprod. 2000; 63(5): 1285-1293
29. Pontecorvo G, Fantaccione S. Recombinogenic activity of 10 chemical compounds in male germ cells of Drosophila melanogaster. Ecotoxicol Environ Saf. 2006; 65(1): 93-101
30. Ishikawa T, Kondo Y, Goda K, Fujisawa M. Overexpression of endothelial nitric oxide synthase in transgenic mice accelerates testicular germ cell apoptosis induced by experimental cryptorchidism. J Androl. 2005; 26(2): 281-288
31. Lue Y, Sinha Hikim AP, Wang C, Leung A, Swerdloff RS. Functional role of inducible nitric oxide synthase in the induction of male germ cell apoptosis, regulation of sperm number, and determination of testes size: evidence from null mutant mice. Endocrinology. 2003; 144(7): 3092-30100
32. Garbán H, Vernet D, Freedman A, Rajfer J, González-Cadavid N. Effect of aging on nitric oxide-mediated penile erection in rats. Am J Physiol. 1995; 268(1 Pt 2): H467-475
33. Lee NP, Cheng CY. Nitric oxide/nitric oxide synthase, spermatogenesis, and tight junction dynamics. Biol Reprod. 2004; 70(2): 267-276