RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
ISSN 2221-1896
www.roavs.com

Alterations in gonado somatic index, seminogram and testosterone profiles in rats treated with hexaconazole

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Abstract

Hexaconazole, a triazole fungicide is widely used in crop protection. The ability of the triazoles to inhibit cytochromes P450 dependent enzymes that play an important role in steroid hormone synthesis has been reported earlier. Hence, the possible effect of hexaconazole on male accessory sex organs, seminogram and serum testosterone level was studied in male rats. A total of eighty wistar rats aged 10-12 weeks, were randomly assigned to four groups, each with twenty rats. Group I served as control and received corn oil *per os* @ 1ml/kg body weight. Groups II, III and IV were given hexaconazole suspension in corn oil daily @ 22.5 55.0 and 110.0mg/kg, *per os*, orally. Ten rats in each group were sacrificed on 30th day, while the remaining ten rats were sacrificed on 60th day. Weights of testis, prostate gland and seminal vesicles were recorded in all the rats and epididymal sperm reserve was analyzed for seminogram. Testosterone levels were also studied in the serum of treated rats. Results indicated that hexaconazole significantly decreased the weights of testis, prostate and seminal glands, indicating a decrease in gonado somatic index. The seminogram was found adversely affected in terms of decreased total epididymal sperm count and motility and increased dead sperm percent and abnormal sperm percent. Serum testosterone levels were found significantly lowered by hexaconazole both after 30 days and 60 days treatments. Decreased gonado somatic index was attributed to decreased circulating testosterone levels. Adverse affects on seminogram might have occurred from lowered testosterone levels as well as alterations in epididymal milieu due to possible presence of hexaconazole and/or its metabolites.

Key words: Triazole, Hexaconazole, Fungicide, Male Reproductive System, Seminogram, Testosterone, Rats

Introduction

Hexaconazole is a triazole fungicide used in crop protection. Triazoles inhibit the biosynthesis of ergosterol, an essential component of fungal cell membrane, via inhibition of cytochrome P450 dependent enzyme lanosterol 14α-demethylase (van den Bossche et al., 1986). Depletion of ergosterol in the fungal cell membrane results in altered membrane fluidity, thereby reducing the activity of membrane associated enzymes. This leads to increased permeability and subsequent inhibition of cell growth and replication (Como and Desmukes, 1994). In addition, azoles may exhibit other direct effects on cell membrane fatty acids and can inhibit cytochrome P 450 dependent enzymes of fungal respiration chain (Uno et al., 1982). The relative binding efficiency of these azole antifungal agents to cytochrome P450 differs, resulting in variations in antifungal activity, toxicity of the agents and the relative likelihood of drug interactions with other cytochrome P450 metabolized drugs. Cytochromes P450 are found throughout the plant and animal kingdom and play a crucial role in the synthesis of steroidal hormones in mammals. Any interference in the synthesis of sex steroidal hormones will obviously affect the reproductive performance. The manifestation of reproductive toxicity may include adverse effects on sexual maturation, gamete production and transport, cycle normality, sexual behavior, fertility, gestation, parturition, lactation, pregnancy outcomes, premature reproductive senescence or modifications in other functions that are dependent on the integrity of the reproductive system (Kimmel et al., 1995) Adverse effects of hexaconazole on pregnancy and its outcome and circulating levels of estradiol and progesterone were reported by Ravi Kumar et al. (2011). The function of the male reproductive system may often be the most sensitive to the toxic effects (Meistrich, 1986). Thus, the present study is envisaged to find the effect of hexaconazole on male reproductive system in rats.

Materials and Methods

Eighty male Wistar albino rats, aged 10-12 weeks, were randomly assigned to four groups each containing twenty rats. Group I served as control and the rats were given corn oil *per os*, @ 1 ml/kg body weight. Rats in group II, III and IV were administered hexaconazole (Technical grade 92.5%, M/s Rallis India Limited, Agro Chemical Division, Mumbai-400 703) suspension in corn oil daily @ 27.5, 55.0 and 110.0mg/kg, *per os*, respectively. Ten animals in each group were sacrificed on day 30th of the treatment and the remaining ten animals were sacrificed on the 60th day of the treatment.

Blood was collected through heart puncture under light ether anesthesia and the serum was decanted off for estimation of testosterone. Serum testosterone was assayed by RIA method as described by Mukku et al. (1981) and Jagannadha Rao et al. (1984). Testes, epididymus, prostrate and seminal vesicles were collected following a vertical incision on the lower abdomen and weighed up to the nearest milligram. The organ weights were calculated per 100g body weight using the formula:

Organ weight/body weight × 100

Epididymal sperm collection was carried out as per Abd-Allah et al. (2000) and counting was done as described by Freund and Carol (1964). A drop of above collected semen was placed on a clean slide and covered with a cover slip. A total of 200 sperm per each animal were counted for motility. Live and dead sperm count was carried out by standard technique using 3% eosin and 10% nigrosin stains. Two hundred sperms for each animal were counted. A drop of diluted epididymal sperm suspension was smeared on a slide and stained with 3% rose Bengal stain and hundred sperm per animal were observed for morphological defects.

Statistical analysis

Data were analysed by analysis of variance as described by Snedecor and Cochran (1968).

Results

Relative weights of testes prostrate and seminal vesicles at the end of 30 and 60 days treatments are given in tables 1 and 2, respectively. Semen characteristics and testosterone levels in 30 and 60 days treated rats are given in tables 3 and 4 respectively.

Since, there were differences in the terminal body weights of various groups, comparison of absolute weights of the gonads and accessory reproductive organs may not give the actual effect of the drug on their development. Hence, for more realistic interpretation, the organ weights were transformed as percent of the terminal body weight.

It was evident from the results that hexaconazole caused regression of the testes and accessory reproductive organs viz. prostate and seminal vesicles by the end of 30 and 60 days at medium and high doses tested. Study of the seminogram revealed that hexaconazole at medium and high doses significantly decreased the total count and motility and significantly increased the dead and abnormal sperm proportions in the epididymal sperm reserve. These changes were dose and time dependent.

It was further observed that hexaconazole at medium and high dose levels significantly lowered serum testosterone levels both in 30 days and 60 days treated rats. However, low dose had no effect on serum testosterone levels either after 30 days or 60 days.

Discussion

In the present study triazole fungicide hexaconazole was assessed for its potential toxic effects on male reproductive system in rats. Hexaconazole was administered to the rats in three different dose levels for

Table 1: Effect of hexaconazole (30 days *per os*) on male reproductive organ weights (g; mean±SE) in rats

Dose	Terminal	Testes		Prostate		Seminal vesicle	
(mg/kg/day)	Body weight	Ab.wt.	Rel.wt	Ab.wt.	Rel.wt.	Ab.wt.	Rel.wt.
(0.0)	199.0±2.10 (100%)[a]	1.89±0.05	0.95±0.04 (100%)	0.224±0.06	0.113±0.02 (100%)	0.724±0.21	0.363±0.13 (100%)
(27.5)	205.0±1.30 (103%)	1.93±0.10	0.94±0.02 (98.9%)	0.218±0.02	0.106±0.02 (93.8%)	0.756±0.15	0.368±0.08 (101.4%)
(55.0)	179.0±1.25 (89.9%)	1.53±0.08	0.86±0.07* (90.5%)	0.183±0.08	0.102±0.03* (90.3%)	0.602±0.13	0.336±0.80* (92.6%)
(110.0)	170.0±2.03 (85.4%)	1.37±0.5	0.80±0.04** (84.2%)	0.159±0.05	0.094±0.02** (83.2%)	0.547±0.09	0.319 0.05%** (87.9%)

Ab.wt.= Absolute weight; Rel.wt. = Relative weight; a = Per cent weights calculated against the weights in control group;
*P<0.05; **P<0.01

Table 2: Effect of hexaconazole (60 days *per os*) on male reproductive organ weights (g; mean±SE) in rats

Dose	Terminal	Testes		Prostate		Seminal vesicle	
(mg/kg/day)	Body weight	Ab.wt.	Rel.wt	Ab.wt.	Rel.wt.	Ab.wt.	Rel.wt.
(0.0)	220.0±2.3 (100%)[a]	2.06±0.04	0.93±0.03 (100%)	0.254±0.88	0.115±0.30 (100%)	0.792±0.12	0.359±0.04 (100%)
(27.5)	218.0±4.50 (93%)	2.04±0.08	0.94±0.06 101.1%)	0.268±0.07	0.123±0.50 (106.9%)	0.761±0.08	0.349±0.05 (97.2%)
(55.0)	185.0±2.5 (81.2%)	1.55±0.10	0.83±0.08* (89.3%)	0.188±0.10	0.102±0.06** (88.7%)	0.607±0.05	0.328±0.04* (91.4%)
(110.0)	170.6±3.5 (77.4%)	1.23±0.12	0.72±0.07** (77.4%)	0.157±0.08	0.092±0.05** (80.2%)	0.500 ±0.06	0.2930.02%** (81.6%)

Ab.wt. = Absolute weight; Rel.wt. = Relative weight; a = Per cent weights calculated against the weights in control group;
*P<0.05; **P<0.01

Table 3: Effect of hexaconazole (30 days *per os*) on semen characteristics and serum testosterone (mean±SE) in rats

Dose (mg/kg/day)	Total epididymal Sperm count (x10^6)	Motility (%)	Dead sperm (%)	Abnormal Sperm (%)	Testosterone (ng/ml)
(0.0)	22.2±1.2	56.2±2.1	24.4±1.2	2.4±0.30	5.18±0.51
(27.5)	24.3±1.5	54.9±1.5	24.1±2.0	2.3±0.20	5.12±0.31
(55.0)	16.8±1.4*	48.3±1.8*	35.6±1.3**	12.5±0.62**	3.79±0.25*
(110.0)	14.8±1.8**	39.3±1.2**	43.5±1.7**	22.3±1.03**	2.52±0.18**

*P<0.05; **P<0.01

Table 4: Effect of hexaconazole (60 days *per os*) on semen and serum levels (mean±SE) in rats

Dose (mg/kg/day)	Total epididymal Sperm count (x10^6)	Motility (%)	Dead sperm (%)	Abnormal Sperm (%)	Testosterone (ng/ml)
(0.0)	23.3±2.1	54.3±3.1	28.3±1.8	2.20±0.51	5.37±0.42
(27.5)	23.8±1.8	52.5±2.1	27.4±2.1	2.00±0.07	5.30±0.56
(55.0)	15.5±1.7**	40.2±1.7*	39.9±2.5**	20.90±1.13**	3.34±0.36*
(110.0)	13.9±1.9**	25.8±1.4**	51.3±3.8**	32.25±1.56**	2.11±0.05**

*P<0.05; ** P<0.01

30 days and 60 days. The 60 days maximum exposure period was chosen basing on 48 days as the period of spermatogenic cycle (Clermont and Harvey, 1967) and 9-14 days for sperm passage through the epididymus (Robb et al., 1978). Christian (1997) also suggested that males be treated for 60 days, a full cycle of spermatogenesis.

The overall effects of hexaconazole on male reproductive system in the present study include decreased testicular weight, regression of accessory reproductive organs, adverse effects on sperm count, motility, viability, morphology and decreased circulating testosterone levels.

Testicular mass is a valuable index of reproductive toxicity in male animals (Amann, 1982). The reduction in testes weight can be attributed to loss of spermatogenic elements and probably reduced levels of androgen binding protein as a result of local androgen deprivation observed in the study. The synthesis and secretion of androgen binding proteins are androgen and FSH dependent (Tindall and Means, 1976). Lowered circulating testosterone might have also caused the regression of accessory reproductive organs that are essentially under the control of androgen (Liao et al., 1975). Atrophy of accessory sex glands in the hypoandrogenism was also reported by Ortiz et al. (1999) and Menjivar et al. (1997).

Lemasters and Selevan (1993) reported that alteration in epididymal sperm count and motility provides a direct measure of fertility in animals. Sperm motility is often used as a marker of chemical induced testicular toxicity (Mori et al., 1991). Depleted sperm reserves in the cauda epididymus observed in hexaconazole treated rats pointed out that the impaired spermatogenic activity in the testis. It is well known that high levels of intratesticular testosterone are necessary for the proliferation and differentiation of spermatogenic cells and spermatogenesis. Thus the reduced testosterone levels induced by hexaconazole might have contributed to this effect. Moreover, spermatogonia lie dormant until puberty and then proliferative activity resume. These rapidly dividing, developing and maturing cells are highly susceptible to chemical insult at many stages (Ecobichon, 1995).

In addition to decreased sperm number, viability, motility and the sperm morphology were also adversely

affected in the treated rats. High circulating testosterone concentration is required for androgen-dependent sperm maturation process in epididymus (Steinberger, 1971). It is further known that, the biochemical environment in the testes and epididymis are highly regulated to assure the proper development and maturation of the sperm and the acquisition of critical functional characteristics. With the chemical exposures, perturbation of this balance may occur, producing alterations in sperm properties (Zenic and Clegg, 1989). Alterations in sperm morphology and motility appear to be associated with the androgen-deprived maturational anomalies (Zenic et al., 1994; Iwasaki et al., 1995) as well as the modified epididymal milieu due to possible presence of hexaconazole and/or its metabolites.

Decreased serum testosterone levels observed in treated rats indicated the ability of hexaconazole to impair testosterone synthesis. Since, testosterone synthesis involves the active role of cytochrome P450 containing enzymes, their inhibition by hexaconazole might have resulted in altered circulating testosterone levels. Though triazoles are comparatively less potenmt than imidazole antifungal agents in inhibiting steroidal hormone synthesis, they are not devoid of this ability at higher doses (Hanger et al., 1988; Soltis and Colby, 1998). Kumar et al. (2011) also reported that hexaconazole significantly decreased the circulating levels of female sex steroidal hormones, estradiol and progesterone in female rats.

It can be concluded from the present study that hexaconazole, a triazole fungicide, when administered to male rats for either 30 or 60 days, adversely affected the gonado somatic index, seminogram and serum testosterone levels.

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