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Effect of hexaconazole on reproductive performance of female rats

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Abstract

Triazole fungicides inhibit the biosynthesis of fungal ergosterol via inhibition of CYP450 dependent enzyme. The CYP450 enzymes are also found throughout the plant and animal kingdom and any interference in their synthesis will adversely affect important physiological functions in mammals. Hence, hexaconazole, a triazole fungicide, was studied for its effect on repductive performance in female rats. Hexaconazole was administered (either for 30days or ≥ 73days) *per os* @ 0.0, 27.5, 55.0 and 110mg/kg/day for four groups, each with 20 rats. In ten rats, estrous cycles were monitored for 15 days, beginning from 15th day after commencement of treatment. The rats that were used for estrus cycle monitoring were sacrificed after the study. The remaining ten rats continued to receive the treatment and on 30th day, they were allowed to mate with untreated male rats. Treatment was continued through out mating period, gestation period and postnatally until the end of lactation and weaning on day 21. Apart from estrus cyclicity, average litter size, average pup weight on postnatal day 21 and reproductive indices were studied. All rats were sacrificed at the end of treatment and serum estradiol and progesterone levels were assayed. It was observed that medium and high doses significantly reduced the estrus cyclicity, and serum estradiol and progesterone levels. A significant reduction in average litter size and weight was also observed. These doses also significantly affected various reproductive indices *viz*, fertility, parturition, gestation and viability indices. The study indicated the ability of hexaconazole to impair the mating and ovulatory processes along with fetotoxic potential in female rats.

Key words: Triazole, Hexaconazole, Fungicide, Female Reproductive System, Rats

Introduction

Triazoles are one of the widely used fungicides in agricultural and horticultural practices. Triazoles inhibit the biosynthesis of ergosterol, an essential component of fungal cell membrane, via inhibition of cytochrome P-450 dependent enzyme lanosterol 14α–demethylase. Lanosterol 14α-demethylase is necessary for the conversion of lanosterol to ergosterol. At the molecular level, N-14 nitrogen atom of the triazoles binds to the heme iron of cytochrome P-450, there by inhibiting cytochrome activation and enzyme function (van den Bosshe et al., 1986). Cytochrome P-450, the ubiquitous heme containing proteins are found through out 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. Milne et al. (1987) observed that ovulation in the rat was delayed by a single administration of the substituted triazole R151885 (1,1-di (4-fluorophenyl)-2- (1,2,4-triazole-l-yl)-ethanol). Plasma levels of estradiol were also found markedly reduced 6-12 hrs after administration of R151885. It was further observed that granulosa cells isolated from rat ovaries that produce estradiol and progesterone *in vitro* in the presence of follicle stimulating hormone and testosterone, failed to produce estradiol at normal level, when the triazole R151885 was added to the cultures. Since, hexaconazole, a triazole fungicide, being extensively used in crop protection, was studied for its effect on female reproductive system in rats.

Materials and Methods

Adult Wister strain rats aged 90-100 days were used in the study. All the female rats were previously screened for normal estrus cyclicity and were randomly divided into four groups, each with 20 rats. One group served as control and received the vehicle corn oil, *per os*, @ 1ml/kg body weight and the remaining three groups received hexaconazole (Technical grade 92.5%, M/s Rallis India Limited, Agro Chemical Division, Mumbai-400 703) suspension in corn oil @ 27.5, 55.0

and 110.0mg/kg *per os*, once daily until the sacrifice. Of the twenty rats in each group, ten rats were subjected to daily estrus cycle monitoring from day 16 to 30. Following this, these ten rats were sacrificed on the mid day of proestrus. The remaining ten rats in all the groups were used for further studies. Thirty days after commencement of treatment, the rats were allowed to mate with untreated male rats in the ratio of 1:2. Every morning vaginal smears were examined for the presence of sperm. Those found negative were returned to the male. A female was considered mated if sperm were found in the vaginal smear and that day was designated '0' day of pregnancy (Manson and Kang, 1989). Irrespective of the outcome of the mating, the mating was discontinued after two cycles. Treatment was continued through the mating, gestation and postnatally, until the end of lactation and weaning on day 21. The total duration of treatment for the rats that were used for estrus cycle monitoring was 30 days and for the rats that were used for mating and reproductive indices study the duration was ≥ 73 days (i.e., 30 days before mating, ≤1 day mating period, 21 days of gestation period and 21 days of lactation period).

Sterile cotton swabs, soaked in sterile normal saline, were introduced into the vagina, rotated gently along the vaginal walls and the swabs were then squeezed on clean glass slide for microscopic examination of cytology as per Manson and Kang (1989).

All the dams were allowed to deliver naturally. The pups were examined for litter size, viability and weight on postnatal day 21. Reproductive indices were calculated as per Lu (1996), as shown below:

Fertility index= No. of pregnant animals/No. of females mated × 100

Parturition index= No. of females delivered/No. of pregnant animals × 100

Gestation index= No. of pups born alive/No. of total pups born × 100

Viability index= No. of pups alive at day 4/No. of pups born alive × 100

Lactation index= No. of pups alive at day 21/No. of pups alive at day 4 × 100

Levels of estradiol and progesterone were estimated in the serum collected from rats sacrificed on the mid day of proestrus by RIA method as described by Mukku et al. (1981) and Jagannadha Rao et al. (1984) respectively.

Statistical analysis

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

Results

In control and low dose groups, three cycles were observed with an average of 5 days per cycle, while in medium and high dose groups only 2.1 and 1.6 cycles were observed respectively (Table 1). The decrease in both these groups was significant. Serum estradiol and progesterone levels in rats exposed to hexaconazole for 30 days and in rats that were exposed to hexaconazole for ≥73 days are shown in table1. It was evident that hexaconazole significantly reduced both these hormones in medium and high dose group rats while low dose of the drug had no effect even at the end of the study.

The number of animals found pregnant, number of animals delivered, number of total pups born, average litter size and average pup body eight on postnatal day 21 are shown in table 2. The average litter size in all the treated groups was found decreased significantly, while the average pup body weight on postnatal day 21 was significantly decreased in medium and high dose groups. Among the reproductive indices (Table 3), fertility, parturition, gestation and viability indices were found decreased at medium and high dose levels of hexaconazole. Gestation index was lowered even at low dose of hexaconazole. However, low dose did not affect the fertility, parturition and viability indices. All the three tested does of hexaconazole had no significant adverse effect on lactation index.

Discussion

Triazole fungicides inhibit the fungal growth through inhibition of ergosterol biosynthesis in fungal cell membrane via cytochrome P450 containing enzyme inactivation. Several cytochrome P450 containing enzymes take part in mammalian steroid hormone synthesis. Hence, hexaconazole, a triazole fungicide was studied for its possible effect on female reproductive function.

Table 1: Effect of hexaconazole on estrus cycle and serum estradiol (mean±SE) and progesterone (mean±SE) levels

Dose (mg/kg/day)	No. of estrus cycles in 15 days	Serum estradiol (pg/ml)		Serum progesterone(ng/ml)	
		30th day	≥73 days	30th day	≥73 days
(0.0)	3.3±0.21	45.28±4.51	42.56±2.18	52.46±3.57	55.12±4.50
(27.5)	3.2±0.25	41.32±3.67	44.35±3.46	49.36±2.19	47.35±2.63
(55.0)	2.1±0.34*	23.52±4.15**	20.28±3.52**	24.72±2.23**	23.78±3.21**
(110.0)	1.6±0.42**	20.18±2.78**	18.47±2.16**	21.64±2.1**	22.35±3.48**

*P<0.05 **P<0.01

Table 2: Effect of hexaconazole on pregnancy and its outcome in rats

Dose (mg/kg/day)	No. of females Mated	No. of females pregnant	No. of females delivered	No. of total pups born	Average litter size	Average pup body wt. on day 21
(0.0)	10	10	10	92	9.20	31.4±2.1
(27.5)	10	10	10	70	7.00*	28.5±2.3
(55.0)	10	8	7	41	5.86**	22.2±1.9**
(110.0)	10	7	5	23	4.60**	20.2±1.6**

*P<0.05; **P<0.01

Table 3: Effect of hexaconazole on reproductive indices in rats

Dose	Index				
(mg/kg/day)	Fertility	Parturition	Gestation	Viability	Lactation
(0.0)	100.00	100.00	98.91	92.31	85.71
(27.5)	100.00	100.00	92.86*	90.77	88.14
(55.0)	80.00**	87.50*	90.24*	83.78*	80.65
(110.0)	70.00**	71.43**	86.96**	80.00**	81.25

*P<0.05; **P<0.01

Out of twenty rats in each group, ten rats in each group were used for estrus cycle monitoring and these were avoided for further reproductive studies because, continuous manipulation of vagina for such a prolonged period could possibly result in pseudopregnancy and give false interpretation of results (Manson and Kang, 1989). In this study, hexaconazole in medium and high doses significantly impaired the estrus cyclicity in rats. The earliest biomarker that reflects ovotoxicity identified in laboratory animals is disruption in estrus cyclicity (Hoyer and Sipes, 1996). Since estrus cycle is a reflection of circulating sex steroids, it appears that hexaconazole caused disruption of normal estrus cycle in rats through impaired steroidogenesis. Estimation of serum etradiol and progesterone levels showed that these hormones were significantly lowered in medium and high dose groups.

Hexaconazole significantly decreased the average litter size in all the three treated groups indicating its fetotoxic effect. Reproductive indices revealed that, hexaconazole affected the fertility, parturition, gestation and viability indices at medium and high dose levels, while gestation index was lowered even at low dose level also. These effects indicate the potential of hexaconazole to impair the mating and ovulatory process along with fetotoxicity. Impairment of ovulation and fetotoxic potentials were previously recorded with other triazole members (Vergieva, 1990; Machera et al., 1995)

Decrease in circulating estradiol and progesterone levels by the administration of substituted triazoles was reported by Middleton et al. (1986). Hanger et al. (1988) noted that, at high doses, triazoles can inhibit the mammalian steroidal hormone synthesis. One important enzyme in steroid synthesis is CYP19 aromatase, which catalyses the conversion of androgens to estrogens. *In vitro* inhibition of this aromatase by triazole fungicides, propiconazole, triadimefon and triadimenol was reported by Vinggaard et al. (2000).

In conclusion, the study revealed that, hexaconazole, a triazole fungicide has the potential to induce changes in estrus cyclicity and reduce levels of circulating serum estradiol and progesterone levels in female rats. It was also evident that hexaconazole can adversely affect the pregnancy and its outcome including various reproductive indices in female rats.

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