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Spermatotoxic Effect of Hexaconazole in Adult Rats

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ABSTRACT
Hexaconazole is a systemic triazole fungicide used in agricultural practices. The aim of the present investigation is to study the toxic effect of Hexaconazole on the epididymal spermatozoa.
The adult male rats were exposed to Hexaconazole at the dose of 25 mg/kg body weight, orally daily for 60 days. The control group received corn oil alone as vehicle. Third group of rats were administered with Hexaconazole for 60 days and left untreated for further period of 60 days. Hexaconazole treatment resulted in a significant decrease in the epididymal sperm count, motility and viability and increased incidence of sperm abnormalities. However, the toxic effect of Hexaconazole was reversible in the withdrawal group. Thus, the present investigation suggests that the chronic low dose treatment of Hexaconazole is capable of inducing epididymal sperm toxicity.
***Keywords**: Hexaconazole; Fungicide; Toxicity; Spermatozoa*

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INTRODUCTION

Hexaconazole(α-butyl-α-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole-1-ethanol) (HC) is a systemic Triazole fungicide used in crop protection. Triazole fungicides are used agriculturally to control rust and mildew of fruit, vegetables, cereals and seeds, residential and commercial turf, and in pharmaceutical applications for the treatment of local and systemic fungal infections.

Triazoles inhibit the biosynthesis of ergosterol, an essential component of fungal cell membranes, via. inhibition of cytochrome P450 dependent enzyme lanosterol 14α-demethylase [1]. Cyp51 is evolutionarily conserved between plants, fungi and animals and in animals is critical for cholesterol synthesis and therefore steroid biosynthesis [2].Besides Cyp51, triazoles also modulate the gene expression and enzyme activity of multiple cytochrome P450 (CYP) and other metabolic enzymes in mammalian liver and other tissues [3-7]. CYPs are phase I metabolizing enzymes that increase hydrophilicity and facilitates subsequent elimination of xenobiotics. CYP enzymes also necessary for biosynthesis and catabolism of sterols, vitamin D and other endogenous biochemistry [2,8].

Numerous reports have focused on various aspects of adverse trends in reproductive health, such as low and probably declining semen quality, sexual behavior, fertility, gestation, parturition,lactation, premature reproductive senescence or modification in other functions that are dependent on the integrity of the reproductive system [9,10].To the best of our knowledge, little is known about the effects of Hexaconazole on the epididymal spermatozoa. Therefore, the present investigation was undertaken to determine the spermatotoxic effect of Hexaconazole in the adult rats.

MATERIALS AND METHODS

Animals

Male albino rats (*Rattus norvegices*) weighing 200-210 gms were used in the present study. They were obtained from Ragavendra Enterprises, Bangalore. The animals were housed in a clean polypropylene cages under controlled conditions of temperature 28 ± 2 °C and 50 ± 5 % humidity with constant 12h/12h dark and light cycle. They were fed with standard rat pellet diet and water *ad libitum*.The animals were maintained and handled as per the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (845/ac/04/2004) and Institutional Animal Ethic Committee (IAEC).

Hexaconazole (Technical grade - 93.39 %) was obtained as a gift from Tagros Chemicals India Ltd., Cuddalore, Tamil Nadu.

Experimental Design

Rats were divided into three groups and each group consists of six animals.
Group I -Control : Rats were given corn oil as vehicle orally, daily for 60 days.
Group II -Hexaconazole treatment (HC): Rats were treated with Hexaconazole dissolved in Corn oil at a dose of 25 mg/kg body weight orally, for 60 days.
Group III- Hexaconazole Withdrawal treatment (HC-WD): Rats were treated with Hexaconazole in corn oil at a dose of 25 mg/kg body weight, orally, for 60 days and left untreated for another 60 days to observe the withdrawal effect.

Spermatological studies

The sperm collected from epdidiymis were used for the determination of sperm viability, sperm motility and sperm counts. **Sperm viability** test was done by the method as described in the WHO Laboratory Manual [11]. An aliquot of 100µl of epididymal sperm was mixed with 100µl of 0.5% eosin solution. A drop of the above mixture was put on a micro-slide covered with a cover slip and examined after 30 sec at 200 x with a help of light microscope. Randomly two hundred spermatozoa were counted. The live spermatozoa were unstained and dead sperm were stained. The sperm viability was expressed in percentage as the number of viable sperms of the total sperms counted.

Sperm motility was evaluated by the method of Joshi and Sharma [12]. The known weight of cauda epididymis was gently teased in a specific volume of physiological saline (0.9 % Nacl) to release the spermatozoa from the tubules. The sperm suspension was examined within five minutes after their isolation form epididymis. The results were determined by counting both motile and immotile sperms in at least ten separate and randomly selected fields. The results were finally expressed as percent motility [13].

Epididymal sperms were counted by the method as described in the WHO Laboratory manual [11]. An aliquot of 5 µl of epididymal sperm was diluted with 95µl of dilutes (Addition of 50 g sodium bicarbonate, 10 ml 35% formalin and 0.25 g trypan blue in a volumetric flask and made up to a final volume of 1 L with distilled water). A cover slip was placed on the counting chambers of the improved Neubauer type Haemocytometer. Approximately 10 µl of thoroughly mixed diluted specimen transferred to each of the counting chambers of the Haemocytometer and allowed to stand for 5 min in a humid chamber to prevent drying out. Sperm cells sedimented during this time and were then counted with the help of light microscope at 200x. Complete spermatozoa, head with tail, were counted.

Statistical Analysis

Single way Analysis of Variance (ANOVA) was followed to analyse the data according to Zar[14]. If the 'F' ratio was significant, Student-Neuman-Keul's (SNK) test was followed.

RESULTS AND DISCUSSION

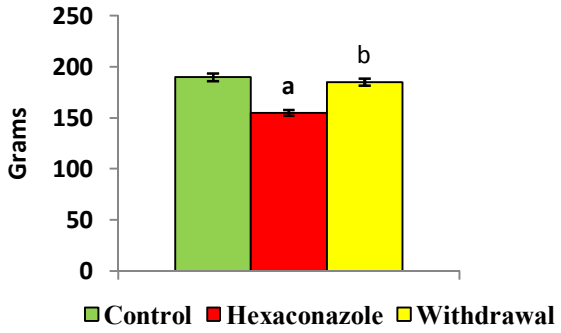
Impaired spermatogenesis is a common feature under the most pesticides and fungicides toxicities. Hexaconazole, a triazole fungicide widely used in crop protection. The variations in the body weight, reproductive organs weights of animals subjected to Hexaconazole treatment (25 mg/kg body weight, orally for 60 days) are shown in Figs. (1-3). Based on 48 days as the period of spermatogenic cycle and 9-14 days for sperm passage through epididymis the maximum exposure period of 60 days was chosen in the present investigation. There was a significant decrease ($P < 0.05$) in the body weight and weights of testis, epididymis, seminal vesicle and ventral prostate as compared to control group. However, withdrawal of Hexaconazole restored the normal weight in the body and reproductive organs. The reduction in the body weight may be due to high rate of protein breakdown which might be needed to fulfill energy requirement during detoxification. Testicular mass is a valuable index of reproductive toxicity in male animals [15]. The reduction in testicular weight in Hexaconazole treated rats may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis [16].

The structural and functional integrity of male accessory sex organs (epididymis, seminal vesicle and prostate) are androgen dependent [17]. Atrophy of accessory sex glands in the hypo androgenism was also reported by Ortiz et. al., [18] and Menjivar et.al., [19]. In the present study, the weight of male accessory sex organs were significantly reduced, which might be due to lowered bioavailability of androgens [20]. Similar results were obtained by Ravikumar et al [10], who reported that Hexaconazole at the dose level of 55 and 110 mg/kg body weight for 30 days and 60 days decreased significantly the weight of testis and accessory sex organs.

The effect of Hexaconazole on the sperm count, motility, viability and abnormalities in adult rats have been presented in Figs (4-6). The present study reveals that the Hexaconazole (a triazole fungicide) significantly ($P < 0.05$) decreased the sperm count, motility and viability. Further, the dead and abnormal sperm proportions in the epididymal sperm reserves were found to be significantly increased by

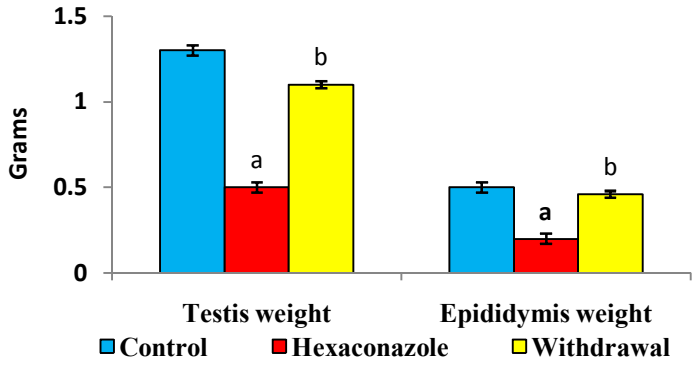
Hexaconazole.The sperms acquire motility and fertilizing ability only during their epididymal transit; Testosterone (T) and Dihydrotestosterone (DHT) are involved in the maturation of sperm within the epididymis as well as the transit of sperm through the duct [21,22]. Any alteration in epididymal sperm count and motility provides a direct measure of fertility [23]. Decreased sperm count is an indicator of reduced spermatogenesis as a result of the toxicity of any agent [24,25].

Fig: 1 Effect of Hexaconazole treatment on the body weight in adult male rats.



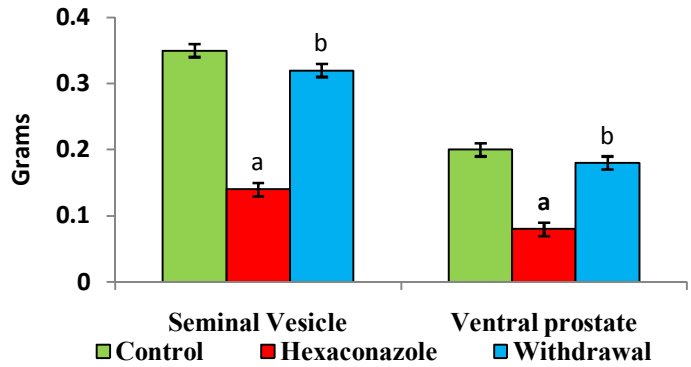
Each value is Mean ± SEM of 6 animals
a and b represent statistically significant at P< 0.05 level - Control Vs. Hexaconazole; Hexaconazole Vs.Withdrawal respectively.

Fig: 2 Effect of Hexaconazole treatment on the testicular weight and epididymal weight in adult male rats.



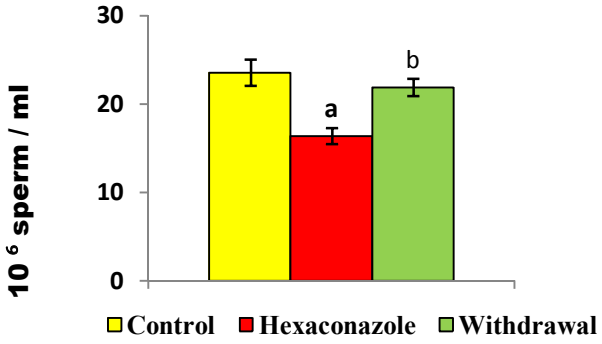
Each value is Mean ± SEM of 6 animals
a and b represent statistically significant at P< 0.05 level - Control Vs. Hexaconazole; Hexaconazole Vs.Withdrawal respectively.

Fig: 3 Effect of Hexaconazole treatment on the seminal vesicular and prostatic weight in adult male rats.



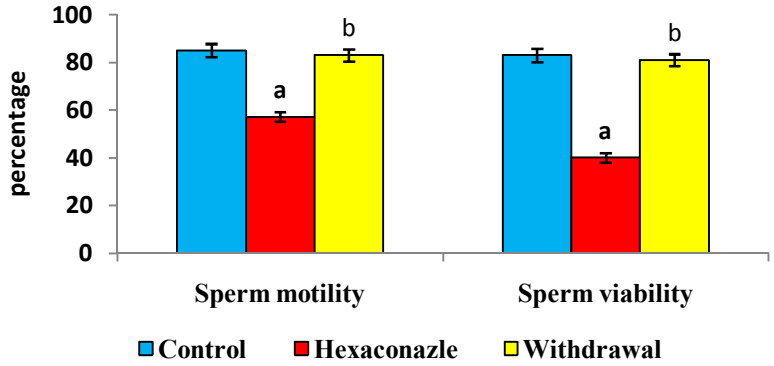
Each value is Mean ± SEM of 6 animals
a and b represent statistically significant at P < 0.05 level - Control Vs. Hexaconazole; Hexaconazole Vs.Withdrawal respectively.

Fig: 4 Effect of Hexaconazole on epididymal sperm count in adult rats.



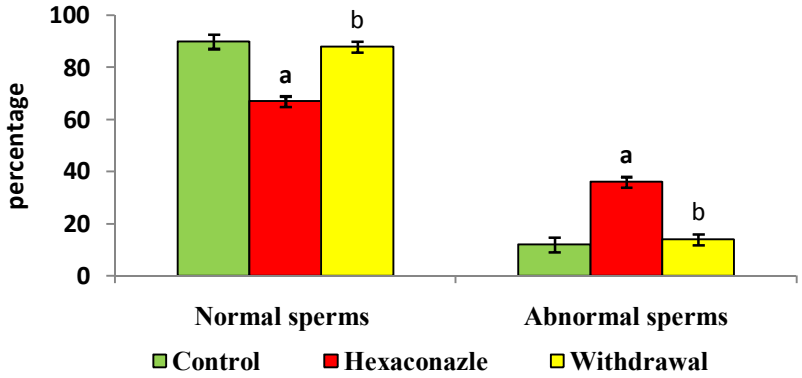
Each value is Mean ± SEM of 6 animals
a and b represent statistically significant at P< 0.05 level - Control Vs. Hexaconazole; Hexaconazole Vs.Withdrawal respectively.

Fig: 5 Effect of Hexaconazole on epididymal sperm motility and sperm viability in adult rats



Each value is Mean ± SEM of 6 animals
a and b represent statistically significant at P< 0.05 level - Control Vs. Hexaconazole; Hexaconazole Vs.Withdrawal respectively.

Fig: 6 Effect of Hexaconazole on epididymal normal and abnormal sperms in adult rats



Each value is Mean ± SEM of 6 animals
a and b represent statistically significant at P< 0.05 level - Control Vs. Hexaconazole; Hexaconazole Vs.Withdrawal respectively.

In the present study, the reduction in the sperm count, motility and viability observed in the Hexaconazole treated rats indicates the impaired spermatogenic activity in the testis and maturation process of the sperms in the epididymis. Our findings are in agreement with the earlier report of Ravikumar et. at., [10]; Their studies on adults rats (aged 10-12 weeks) treated with Hexaconazole at 55

and 110 mg/kg body weight for 30 and 60 days revealed that this fungicides significantly decreased the sperm count and motility with an increase in the dead and abnormal sperm proportions in the epididymal sperm reserves. They suggested that the high levels of intra testicular testosterone are necessary for the process of spermatogenesis and the reduced testosterone levels induced by Hexaconazole might have contributed to these adverse effects; High circulating testosterone concentration is required for androgen dependent sperm maturation process in epididymis [26]; Alterations in sperm morphology and motility appear to be associated with the androgen deprived maturational anomalies as well as the modified epididymal milieu due to possible presence of Hexaconazole and or its metabolites. It has also been reported that the decreased serum testosterone levels in the Hexaconazole treated rats indicated the ability of Hexaconazole 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 [10]. In the present study also the reduction in sperm count, motility and viability in the Hexaconazole treated rats may be due to the direct action of Hexaconazole or due to an indirect, manifestation through altered epididymal function.

The present study also reveals that there was an appreciable increase in the epididymal sperm count, motility and viability observed in the withdrawal group showing that the rats had recovered from the deleterious effect of Hexaconazole. Thus the present investigation suggests that the treatment of Hexaconazole is capable of inducing adverse effects on the epididymal spermatozoa in adult rats. However, all the parameters that were affected by Hexaconazole were restored to normalcy upon withdrawal of Hexaconazole treatment indicating that these effects are transient and reversible.

REFERENCES

1. Ghannoum,MA., Rice, LB. (1999). Antifungal agents: mode of action, mechanism of resistance, and correlation of these mechanisms with bacterial resistance. Clin.Microbiol.Rev, 12:501-517.
2. Zarn, J.A., Bruschweiler, B.J., and Schlatter,J.R. (2003). Azole fungicides affect mammalian steroidgenesis by inhibiting sterol 14 alpha-demethylase and aromatase. Environ. Health perspect, 111,255-261.
3. Barton, H.A., Tang, J., Sey, Y.M., Stanko, J.P., Murrell, R.N., Rockett, J.C. and Dix, D.J. (2006). Metabolism of myclobutanil and triadimefon by human and rat cytochrome P450 enzymes and liver microsomes. Xenobiotica 36, 793-906.
4. Goetz, A.K., Bao, W., Ren. H., Schmid, J.E., Tully, D.B., Wood, C.R., Rocjertt, J.C, Narotsky, M.G., Sun,G., Lambert, G.R.,et al. (2006). Gene expression profiling in the liver of CD-1 mice to characterize the heaptotoxicity of triazole fungicides. Toxicol. Appl. Pharmacol. 215, 274-284.
5. Ronis, M.J., Ingelman-Sundberg, M., and Badger, T.M., (1994). Induction suppression and inhibition of multiple hepatic cytochrome P450 isozymes in the male rats and bobwhite quail (Colinusvirginianus) by ergosterol biosynthesis inhibiting fungicides (EBIFs). Biochem. Pharmacol. 48, 1953-1965.
6. Sun, G., Thai, S., Tully, D.B., Lambert, G.R., Goetz, A.K., Wolf, D.C., Dix, D., and Nesnow, S.(2005).Propiconazole-induced cytochrome P450 gene expression and enzymatic activities in rat and mouse liver. Toxicol. Lett. 155, 277-287.
7. Tully,D.B., Bao, W., Goetz, A.K., Ren, H., Schmid, J.E., Strader, L.F., Wood, C.R., Best., D.S., Narotsky, M.G., Wolf, D.C., et al. (2006). Gene expression profiling in liver and testis of rats to characterize the toxicity of triazoles fungicides. Toxiciol. Appl. Pharmacol. 215, 260-273.
8. Nelson, D.R., Zeldin, D.c., Hoffman, S.M, Maltais, L.J., Wain, H.M., and Nebort, D.W. (2004). Comparison of cytochrome P450 genes from the mouse and human genomes, including nomenclature recommendation for genes, pseudgenes and alternative-splice variants. Pharmacogenetics 14, 1-18.
9. Kimmel, G.L., Clegg, E.D. and Crisp, T.M. (1995). Reproductive toxicity testing: A risk assessment perspective. In: Witorsch, R.J. (editor). Reproductive Toxicology. 2nd edition. Raven press Ltd., New York.: 75-98.
10. Ravikumar, P., Kanniappan, M., Mathuram, L.N., Selvasubramanian, S., and Sriram, P.(2011). Alterations in gonad somatic index, seminogram and testosterone profiles in rats treated with hexaconaozle. Research opinions in Animal and Veterinary sciences, 1(2),78-82.
11. WHO Laboratory Manual (1999). The examination of human semen and sperm-cervical mucus interaction. Cambridge university press.
12. Joshi, S.C and Sharma, P. (2012). Effect of acephate on sex hormones, sperm dynamics and fertility in male albino rats. International Journal of Research in Pharma. and Bio medical science, 3(1), 286-292.
13. Prasad, M.R.N., Chinoy N.J and Kadam K.M. (1972). Changes in succinate dehydrogenase levels in the rat epididymis under normal and altered physiological conditions. Fertil. Steril, 23: 186-190.
14. Zar, J.H. (1974). Biostatical Analysis prentice-Hall, New York.
15. Amann , R.P. (1982). A critical review of methods for evaluation of spermatogenesis from seminal characteristic. Journal of Andrology. 2: 37-38.
16. Kaur, C. and Mangat, H.K. (1980). Effects of estradiol dipropionate on the biochemical composition of testis and accessory sex organs of adult rats. *Andrologia*, 12(4): 373-378.

17. Klinefelter, G.R. and Hess, R.A. (1988). Toxicology of the male excurrent ducts and accessory sex glands. In: Reprod.and Dev. Toxicology. 553-591.
18. Ortiz, G., Vilchis, F., Cardenas, M.,Cruz, C., Pedraza-Chaverri, J., and Menjivar, M. (1999).Reproductive function in male rats with chronic nephrosis. *Journal of Reproduction and Feritlity*, 117: 223-228.
19. Menjivar et al.,Vilchis, F., Cardenas, M., Cruz, C., Pedraza-Chaverri, J., and Perz-palaios. (1997). Transient alteration of the reproductive function in nephritic rats.
20. Sujatha,, R.,Chitra, K.C., Latchoumycandane, c and Mathur, (2001). Effect of lindane on testicular antioxidant system and steroidogenic enzymes in adult rats. *Asian J. Androl.,* 3:135-138.
21. Bedford, J.M. (1979). Evolution of the sperm maturation and sperm storage functions of the epididymis in the spermatozoan (D.W. Fawcert and J.M. Bedford. Eds.). 7-21. Urban &Schwarzenberg, Baltimore.
22. Brooke, D.E. (1979). Biochemical environment of maturing spermatozoa. In the spermatozoa (D.W. Fawcert and J.M. Bedford. Eds.), 23-24. Urban &Schwarzenberg, Baltimore.
23. Lemasters, G.K and Selevan, S.G.(1993). Toxic exposures and reproduction: a view of epidemiology and surveillance. In: Scialli, A.R and Zinaman, M.J. (editors). Reproductive Toxicology and Infertility. McGraw Hill. 307-321.
24. Sobarzo, C. and Bustoos-orregn, E. (2000). Sperm quality in mice acutely treated with parathion. *Asian Journal of Andrology*. 2:147-150.
25. Poon, R., Rigden, R., Chu, I. and Valli, V.E. (2004). Short-term oral toxicity of pentyl ether, 1,4-diethoxybutane, and 1,6-dimethoxyhexane in male rats*. Toxicol. Sci.* 77: 142-150.
26. Steinberger, E. (1971). Hormonal control of mammalian spermatogenesis. *Physiological Reviews*, 51: 1-22.

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