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RESEARCH ARTICLE

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SPERMATOTOXICITY EVALUATION OF DELTAMETHRIN 1% + CHLORPYRIPHOS 35% EC BY ORAL GAVAGE IN WISTAR RATS

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ABSTRACT

Effect of a combination of pesticides, viz. Delatmethrin 1% + Chlorpyriphos 35% EC, has been studied on the reproductive toxicology of wistar rats. Male rats were exposed to test substance at the dose level of 15, 25, and 35-mg/kg body weight for a consecutive period of 20 weeks. At the end of the experimental period, all the animals were subjected for necropsy, sperm parameters such as motility, testicular sperm-head count, epididymal count and morphological evaluations were carried out. The treatment is reported to induce significant alteration in sperm motility, testicular sperm-head count and epididymal count at 35 mg/kg body weight dose group. Results suggested that the pesticide combination, viz (Delatmethrin 1% + Chlorpyriphos 35% EC) used in the present investigation inhibit the sperm production and motility either directly or indirectly.

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KEY WORDS

Deltamethrin, Chlorpyriphos, Sperm motility, Testicular Sperm-head count, Epididymal count, Wistar rats, Spermatotoxicity

INTRODUCTION

In the present scenario, all over the globe, use of pesticides for agriculture, industrial and domestic purposes has become a common and integral need of human life style. Result of which, the pesticides through different ways getting accumulated in the human body causing different types effect on the physiology of the humans.

Reproductive systems of majority of the living beings of the earth (both terrestrial and aquatic organisms) are most sensitive system to almost all types of chemical interactions in addition to the climatic variations. Hence the frequently affected organs are being gonads.

Several industrial and environmental chemicals including pesticides are known to cause ologospermia, necrazospermia and many times azospermia in man and numerous other chemicals are known to have affected the spermatogenesis in experimental animals.

Pesticides like organophosphate (Chlorpyriphos is a synthetic organophosphate, non-systemic, broad-spectrum insecticide and acaricide, acting as a cholinesterase inhibitor) through skin contact, GI route and through respiration, affect the male and female reproductive systems in various aspects. Commercial manufacture of chlorpyrifos started in 1969, since then it has been used for agriculture and industrial purposes. The major use of chlorpyrifos in farming is to protect corn, cotton and fruit trees against insects) and synthetic Pyrethroids have been individually reported to be spermatotoxic, affecting the spermatogenesis process (IARC Working Group[8], 1991; Akbarsha *et al*., 2000[1]; Okamura *et al*., 2004[14]) and Epididymal sperm counts.

The most sensitive method of assessing the toxic effect on male reproductive system is to assess the effect at epididymal and/or testicular site.

After careful dissection of the tissues of the testicle and further processing, the sperm suspensions are counted using a hemacytometer and analyzed for the effect. But no studies have been reported on their effect in combination. Therefore, the present investigation was designed to evaluate the spermatotoxic potential of the pesticide combination, Deltamethrin1% + Chlorpyriphos 35% EC, in Wistar rats which gives a fair idea of the cumulative effect of the pesticides widely used in agriculture and industries passed through the food chain reaching the human and causing the deleterious effect on the human reproductive system.

MATERIALS AND METHODS

The pesticide combination, Deltamethrin 1% + Chlorpyriphos 35% EC: (emulsifiable concentrate) was procured from the local market of Dharwad, Karnataka, India, under the tread name Hyban, supplied by Hyderabad Chemical Supplies Limited, Hyderabad, India. The Batch number, manufactured date and expiry date of the test substance was checked prior to initiation of the treatment was found suitable for the exposure hence stored as per the manufacturer's instructions till use and while using.

Male Wistar rats (weight range 230-250 g) were received from the Animal Breeding

Facility, Karnataka University's Research Laboratory, EMTL (Environmental and Molecular Toxicology Laboratory), Department of Zoology, Karnatak Science College, Dharwad – 580 001, Karnataka, India.

SAFETY PRECAUTIONS

Gloves, cap, face mask and goggles were used in addition to protective body garments and rubber slipper to ensure adequate personnel health and safety and to avoid inhalation and skin contact with the test item.

ACCLIMATIZATION

The animals were acclimatized to the laboratory conditions for a minimum period of five days before starting of the experiment. At the end of acclimatization period, the animals were weighed, randomized and allotted to four groups of 5 animals each as follows. The animals were identified by picric acid body marking and were maintained at 22 ± 3 ºC with the relative humidity at 30-70% throughout the experimental period. The animals were provided with the standard laboratory rodent diet (rat/mice feed) procured from (Sai Durga Feeds & Foods) Bangalore, Karnataka, India.

DOSE SELECTION

The dose levels of 15, 25 and 35 mg/kg were selected for the present study based on the results of the acute oral toxicity study.

TREATMENT

Total of 20 rats were divided into four groups as control, low, middle and high dose groups. Control group animals were administered aqua-guard water (lead and pesticide free water)-G1, and served as control. Other three groups were treated with Deltamethrin 1% + Chlorpyriphos 35% EC - G2 (low dose), G3 (mid dose), and G4(high dose) at the dose levels indicated above.

At the termination of study, different sperm parameters such as motility, epididymal count[18], testicular sperm-head count[3] and morphological parameters evaluations were evaluated [17]

RESULTS AND DISCUSSION

In the present study a dose-dependent reduction in the cauda epididymal sperm count was observed in rats exposed to Deltamethrin 1% + Chlorpyriphos 35% EC which is attributed to decreased spermatogenesis.

It is well known that, cytotoxic drugs depress spermatogenesis in mammals[19] by causing death of developing germ cells in the seminiferous tubules. This results in the elimination of active cells of spermatogenesis and thereby results in reduction in daily sperm production[13].

In the present investigation definite alterations were observed in sperm motility, epididymal counts, testicular sperm-head count and sperm morphology of rats subjected to Deltamethrin 1% + Chlorpyriphos 35% EC. Results showed a concomitant inhibition of sperm motility with increase in the dose levels. The percentage of motile sperm inhibited at high dose group was significantly ($p \leq 0.01$) lower when compared to concurrent control group. A decrease in the number of sperms counted in cauda epididymis in Deltamethrin 1% + Chlorpyriphos 35% EC treated groups was found to be statistically significant at 25-mg/kg body weight ($p \leq 0.05$) and 35-mg/kg body weight dose levels ($p \leq 0.01$) when compared with control group. A treatment related decrease in the number of testicular sperm-head count was observed in the all the Deltamethrin 1% + Chlorpyriphos 35% EC treated groups when compared to the control group. The observed decrease in testicular sperm-head count was significant at all the dose levels. Morphological examination of sperms revealed various abnormalities in them related to head and tail such as no head, no hook, blunt hook, broken tail, no tail, etc.

The percentage of abnormal sperms observed in various treated groups (between groups), however, did not show any statistical significance including dose dependency (Table 1; Fig. 1 to 4). The functions of male reproductive system include a cascade of events like Spermatogenesis, sperm maturation, storage and ejaculation. Where in the product of such event yields spermatozoa has to travel along female genitalia and reach the ova and result in fertilization.

Table 1

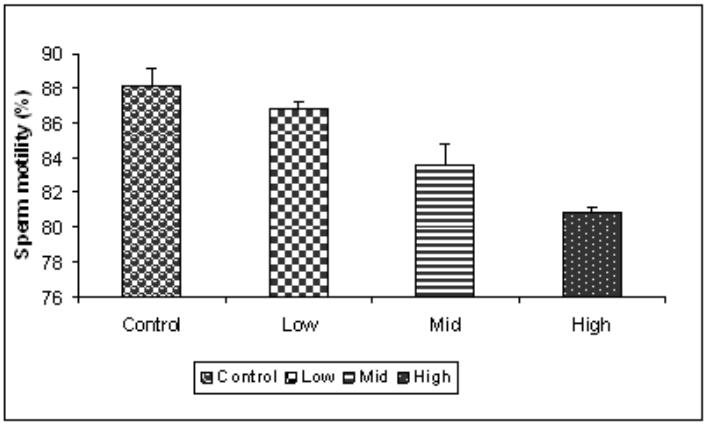
Sperm evaluation in male (wistar rats) at the termination of study

Parameters	Group number and dose level (mg/kg body weight)			
	G1 (0)	G2 (15)	G3 (25)	G4 (35)
Sperm motility (%)	88.17 ± 0.98	86.78 ± 0.49	83.67 ± 1.11	80.83 ± 0.32**
Epididymal count (10^6/g)	1568.9 ± 9.17	1493.6 ± 11.75	1465.08±29.80*	876.83±58.83*
Sperm-head count (10^6/g)	146.54±2.62	134.07±3.45*	125.36±1.27**	99.67±4.54**
Sperm abnormality (%)	6.4±1.73	7.29±1.41	7.38±1.9	8.07±1.69

Mean + SE, * P ≤ 0.05, ** P ≤ 0.01

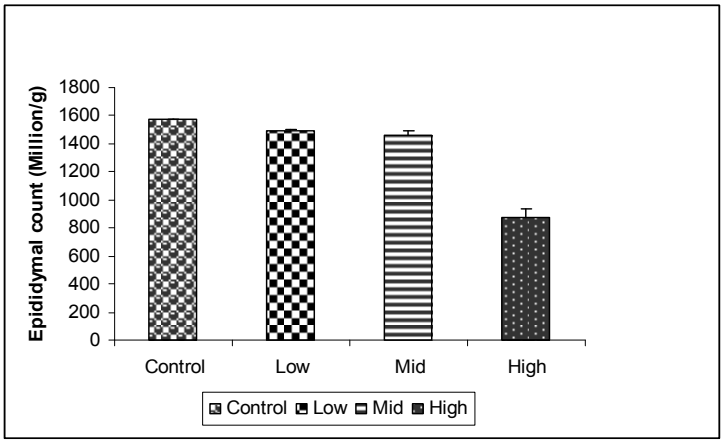
Graph 1

Sperm motility (%)



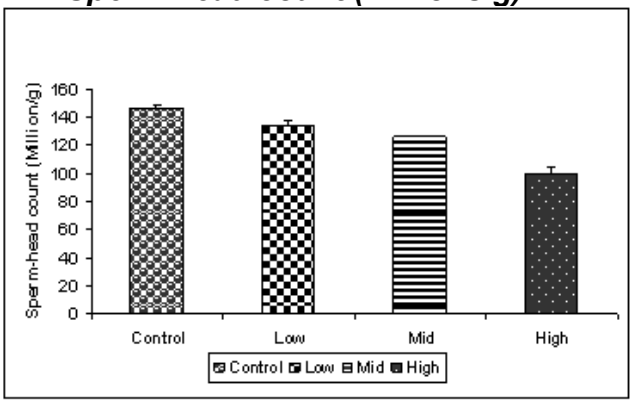
Graph 2

Epididymal count(Millions/g)



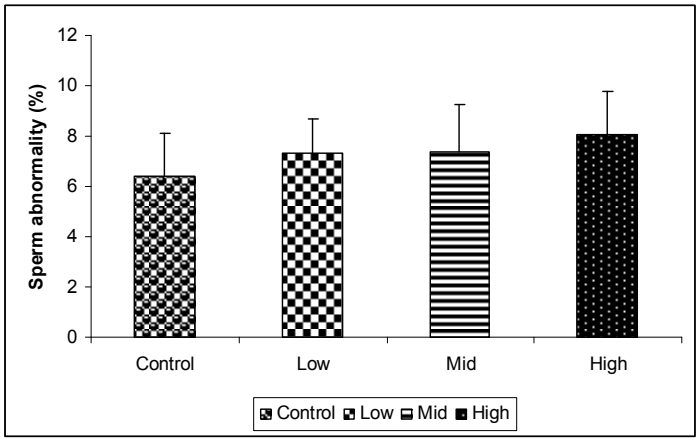
Graph 3

Sperm head count (Millions/g)



Graph - 4

Sperm abnormality (%)



As a supporting role, it also involved in the synthesis male sex hormone – testosterone that influences the development of secondary sex characters in males. There are two critical points of concern in the male reproductive toxicity. The first is production of sufficient spermatozoa, which are capable of fertilizing an ovum and the second is production of sperm with normal chromosome number, structure and genetic material. A toxic agent may produce alteration in spermatogenesis via an effect on the hypothalamus, pituitary, gonadal axis or effect on accessory sex gland function or sexual function (libido, potency, ejaculation), which may result in failure of fertilization. However, a toxicant may cause genetic or chromosomal abrasion in germ cells and the sperm carrying abraded genetic material fertilizes an ovum may result in embryonic resorptions, fetal structural/functional abnormality/s and fetal death[15].

Multiple end points of male reproductive toxicities were investigated in a number of chemicals that are reported to produce mild to severe reproductive effect in Sprague Dawley rats. Most toxicologically sensitive end points used for the assessment of a potential male reproductive toxicant are testicular histology testicular sperm-head counts, cauda epididymal sperm counts, sperm morphology and sperm velocity [12].

In the present experiment, Wistar rats were treated with Deltamethrin 1% + Chlorpyriphos 35% EC revealed significant inhibition of motile sperm in the high dose group. This finding is in agreement with the observations of [14], who have reported significant decrease in sperm motility in Wistar rats on treatment with organophosphorus insecticide (dichlorvos) at 2-mg/kg subcutaneously for a period of 9 weeks[4] had observed a decrease in motility in rats treated with alpha-chlorohydrins (ACH) and had suggested that this could most likely due to its direct toxicity on developing spermatocytes / spermatids and matured spermatozoa at epididymis or both. One of the obvious effects of epididymal maturation of spermatozoa is that spermatozoa display changes in the pattern and effectiveness of their flagellar motion. The alteration in the sperm motility during epididymal maturation might be due to changes in ATP metabolism, ion concentration and enzymatic activity in the spermatozoon[5 10] had also suggested the decline in sperm motility due to altered epididymal physiology since epididymis was responsible for sperm maturation, which was controlled by circulating androgen.

It has been clearly established that mammalian spermatozoa leaving the testis contain a Cytoplasmic droplet. Generally, a greater proportion of spermatozoa lose its Cytoplasmic droplet during its transit from corpus to cauda epididymis, hence, the loss of Cytoplasmic droplets is considered as an index of spermatozoa maturation in mammals[2].

On leaving the testis, the Cytoplasmic droplet is located in the region near to the head of spermatozoon. Subsequently it moves posterior along the midpiece until it reaches the point of annulus and then is shed when spermatozoon leaves the corpus epididymis[7]. The loss of the Cytoplasmic droplet from the spermatozoon brings an additional reduction in cell volume occurring extra-testicular and also after a considerable amount of the time once the spermatozoa are released from the sertoli cells [6]. The Cytoplasmic droplet plays a role in estrogen biosynthesis in the lumen of male reproductive tract. Janulis et al. in 1998 have demonstrated the activity of P-450 aromatase, the enzyme that converts androgen to estrogen in the Cytoplasmic droplet. Akbarsha *et al*. in 2000 have found that 60 – 95% of spermatozoa residing in the lumen of cauda epididymis of Wistar rats treated with organophosphate insecticide for 48 days, retained Cytoplasmic droplet and hence the motility of spermatozoa released from cauda epididymis was inhibited. Therefore, it is logistic to conclude that one of the mechanisms of action of this toxicant on male

reproductive function may be the retention of the Cytoplasmic droplet and the resultant impairment of sperm motility.

Testicular homogenization-resistant spermatids data are particularly useful for confirming reduction in sperm production. Deltamethrin 1% + Chlorpyriphos 35% EC administered rats exhibited significantly reduced testicular homogenization-resistant spermatids and epididymal sperm count.

A decrease in testicular homogenization-resistant spermatids indicates reduction in the number of elongated spermatids. This could be due to either direct effect of test article on these cells or maturation depletion following effect on an earlier cell type. Exposure of Deltamethrin 1% + Chlorpyriphos 35% EC also exhibited significant reduction in epididymal sperm count in this experiment which may be due to antispermatogenic nature of this combination. Lanning *et al*. in 2000 have suggested that a reduction in testicular homogenization-resistant spermatids coincides with a decrease in epididymal sperm count.

CONCLUSION

The current study evidenced that the pesticide combination of Deltamethrin 1% + Chlorpyriphos 35% EC resulted in reduction of sperm production and increased motility either directly or indirectly. However, the exact mechanism through which this is achieved needs to be studied further.

REFERENCES

1. Akbarsha, M. A., Lakha, P. N. L.and Murugain, P. (2000). Retention of Cytoplasmic droplet by rat cauda epididymal spermatozoa after treatment with cytotoxic and xenobiotic agents. *J. Reprod. Fertility*, 120: 385-390.
2. Bedford, J. M. (1975). Maturation transport and fate of spermatozoa. In: *Handbook of Physiology*, (Eds) Greep, R. O. and Hamilton, D. W., American Physiology Society, Washington, *Endocrinol*, 5: 303.
3. Blazak, F. W., Treinen, A. K. and Juniewicz, E. P. (1993). Application of sperm-head counts in the assessment of male reproductive toxicity. In: *Methods in Toxicology.* Volume 3, Part A, Male Reproductive Toxicology. (Eds) Chapin E.R. and Heindel J.J., Academic Press, Inc, San Diego, California, USA, pp. 86-94.
4. Buttar,H. S., Moffatt, J. H., McMahon, A. and Foster, W. G. (1997).Sperm motility analysis, sperm chromatin structure assay and serum hormone assessment in alpha-chlorohydrin treated rats. *Teratology,* 55(1): 68.
5. Eddyy, E. M. and O'Brienda, D. A. (1989). Biology of the gamete maturation, transport and fertilization. In: *Toxicology of the Male and Female Reproductive System.* (Eds) Peter K. Working, Hemisphere Publishing Corporation, USA, pp. 31-100.
6. Gist, H. D., Hess, R. A. and Thurston, R. J. (1992). Cytoplasmic droplet of painted turtle spermatozoa. *J. Morphol.,* 214: 153-158.
7. Hermo, L., Dwerkin, J. and Richard, O. (1988). Role of epithelial clear cell of the rat epididymis in the disposal of the content of the Cytoplasmic droplet detached from spermatozoa, *Am. J. Anat.,* 183: 107-124.
8. IARC Working Group (1991). Deltamethrin. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans,* pp. 251-266.
9. Janulis, L., Hess, R.A., Bunick, D., Nitta Janssen, S., Asawa, Y. and Bahr, J.M. (1998). Mouse epididymal sperm counting

active P 450 aromatase which decreases as sperm traverse the epididymis. *J. Androl.,* 1965: 71.

10. Knobil, E. Neil, J., Greenwald, G., Markert, C. and Pfaff, D. (1994). *The Physiology of Reproduction,* 2nd edition, Raven Press, New York.
11. Lanning, L.L., Creasy, D.M., Chapin, R.E., Mann, P.C., Barlow, N.J., Regan, K.S. and Goodman, D.G. (2002). Recommended approaches for the evaluation of testicular and epididymal toxicity. *Toxicol. Pathol.,* 30(4): 507-508.
12. Linder, R. E., Strader, L. F., Slott, V. L., and Suarez, J. D. (1992). Endpoints of spermatotoxicity in the rats after short duration exposures to fourteen reproductive toxicants. *Repord. Toxicol.,* 6: 491-505.
13. Lu C. C, Meistrich M. L. (1979). Cytotoxic effect of chemotherapeutic drugs on mouse testis cells.Cancer Res; 39: 3575- 3582.
14. Okamura, A., Kamijima, M., Ohtani, K., Watanabe, Y., Takagi, K., Shibata, E., Wang, H., Ichihara, G. and Nakajima, T. (2004). Male reproductive toxicity assessment of dichlorvos. *Toxicol. Appl. Pharmacol.,* 197(3): 353-354.
15. Raticliff, J. M., McElhatton, P. R. and Sullivan, F. M. (1995). Reproductive toxicology. In: *General and Applied Toxicology.* Eds.: Ballantyne B. Marrs, T. and Turner P., Macmillan Press Limited, New York, pp. 925-956.
16. Rattner, B. A., Sileo, L. and Scaners C. G. (1982). Hormonal responses and tolerance to cold of female quail *Colinus-virginianus* following parathion ingestion. Pestic. Biochem. Physiol., 18: 132-135.
17. Ron, Filler (1993). Methods for evaluation of rat epididymal sperm morphology. In : *Methods in Toxicology,* Vol. 3, Part A, Male Reproductive Toxicology, Eds: Chapin, E.R. and Heindel, J.J., Academic Press, Inc. San Diego, California, USA.
18. Williams, J. (1993). Semen analysis and fertility assessment in rabbits. In: *Methods in Toxicology.* Vol. 3, Part A, Male Reproductive Toxicology, Eds: Chapin, E.R.. and Heindel, J.J., Academic Press, Inc. San Diego, California, U.S.A, pp. 344-361.
19. Wyrobek A, Gordon L, Bukhart J G, Francis M W, Kapp R W, Letz G., Malling H V, Topham J. C, Whorton M. D (1983). On evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. Mut. Res; 115: 1-72.