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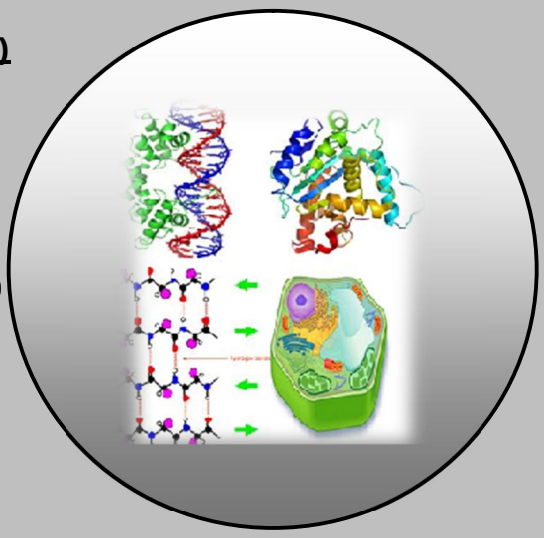

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jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

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Effect of Carbaryl on Sperm Count and Sperm Motility of Albino Rats

Archana Rani, A. Sahai, A.K. Srivastava, Anita Rani, Jyoti Chopra, A.K. Pankaj, R.K. Verma and R.K. Diwan

Department of Anatomy, King George's Medical University, Lucknow, India

ABSTRACT

Concern about the susceptibility of the male reproductive system to drugs or environmental agents has assumed an increasing extent. The outcome of such exposures have included not only reduced fertility but also embryo/fetal loss, birth defects, childhood cancer and other postnatal or functional deficits. Carbaryl, one of the most widely used broad-spectrum insecticides, was chosen to see its effect on the sperm count and sperm motility of albino rats. Three groups of animals were taken for the present study i.e. control, carbaryl low dose (100mg/kg /day) and carbaryl high dose (200 mg/kg /day). After 60 days of experimental period, rats were sacrificed. Testis along with epididymis was taken out. Sperms were obtained by mincing the epididymis in normal saline and filtering through nylon mesh. The sperm motility was assayed microscopically while sperm counting was done using Neubauer chamber.

Sperm count and sperm motility was found to be decreased in both carbaryl treated groups as compared to control. The total sperm count (per ml) in control group was $100x10^6$± 0.39 while $50x10^6$± 0.52 in low dose and $30x10^6$±0.11 in high dose. The sperm motility in control group was 81.0± 4.8%, in low dose group 38.1±6.09% and in high dose group 11.3±1.27%.

Therefore, it seems that carbaryl adversely affects the fertility by producing oligospermia and decreasing the sperm motility.

Key words: Carbaryl, Testis, Sperm Count and Sperm Motility.

INTRODUCTION

Pesticides, though present in the environment in small quantities as compared to other contaminants such as industrial wastes and fertilizers, account for public and scientific concern due to their high biological activity. Carbaryl is 1-Naphthyl methyl carbamate, also known as Sevin is a popular broad spectrum insecticide used since 1956. Pesticides have a tendency to persist and have potential to bio accumulate in the body (Kamrin 1997).

Of the potential health risks associated with exposure to chemical or physical agents, a prominent concern is that these agents may interfere with the ability of individuals to produce normal, healthy children. A large number of chemicals that have been released into the environment are known to interfere with the endocrine system.

Pesticides have the potential to cause reproductive toxicity in animals and several compounds are known to affect human reproduction too (Mattison et al. 1990, Hileman 1994). Epidemiological studies postulated that in the past 50 years the sperm number and sperm quality in human had been decreased (Bendvold et al. 1991, Carlsen et al. 1992). Pathological effects of pesticides on the reproductive system of experimental animals were recorded by many authors (Afifi et al. 1991, Abou Salem et al. 1997, Okamura et al. 2005, Presibella et al. 2005). Keeping in mind the pivotal role of testis in reproduction this experimental work was done.

MATERIAL AND METHODS

24 male albino rats weighing 50-80 g were used for the present experiment. The rats were maintained under standard laboratory conditions in an air conditioned room and housed in stainless steel cages one per cage at temperature 22±3ºc and relative humidity 30-70%. They were fed on the standard pellet diet and tap water ad libitum. Animal care was as per Indian National Science Academy (INSA) guidelines for Care and Use of Animals in Scientific Research. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC). After acclimatisation for two weeks in laboratory conditions, rats were divided into 3 groups of 8 rats each.Group-1 served as control. Group 2 & 3 received 100 mg carbaryl/ Kg body weight (low dose group) and 200 mg carbaryl/ Kg body weight (high dose group) respectively in 0.2 ml of groundnut oil orally, 6 days/ week for 60 days. After 60 days of experimental period all treated rats along with their controls were anaesthetized by intraperitoneal administration of Nembutol (30 mg/Kg body weight). Rats were sacrificed, the scrotum was skinned and an incision was given to take out the testis along with epididymis for sperm count and sperm motility. Sperms were obtained by mincing the epididymis in normal saline and filtering through nylon mesh. The sperm motility was assayed microscopically while sperm counting was done using Neubauer chamber.

For sperm counting, the specimen was mixed gently after liquefaction and the semen was drawn to the 0.5 mark of a WBC pipette.

The semen diluting fluid (consisted of sodium bicarbonate 5.0 gm, formaline 1.0 ml and distilled water 99.0 ml) was drawn to the 11 mark and mixed well. Neubauer chamber was loaded and the sperms were allowed to settle for about 5 min. Sperms were counted in the four corner squares.

Sperm motility was assayed microscopically within 5 min. following their isolation from epididymis at 37°C. A small drop of liquified semen was placed on a glass slide and covered with a cover slip. Slide was examined under the high power with reduced illumination. The number of active motile sperms (%) was counted out of the total count of 200.

RESULTS

The mean sperm count (per ml) of control rats was found to be $100x10^6 \pm 0.39$ while $50x10^6 \pm 0.52$ in low dose and $30x10^6 \pm 0.11$ in high dose group. The values were decreased as compared to control and were highly significant (Table 1, Fig.1). The percent sperm motility was also found to be decreased in carbaryl treated groups as compared to control. The sperm motility in control group was 81.0± 4.8%, in low dose group 38.1±6.09% and in high dose group 11.3±1.27%. Statistically these values were highly significant (Table 2, Fig. 2).

Table 1. Effect of carbaryl treatment on sperm count after 60 days.

Treatment	Dose (mg/kg/day)	Sperm count (mean±SD)	p-value
Control	-	$100x10^6 \pm 0.39$	-
Carbaryl (low dose)	100	$50x10^6 \pm 0.52$	<.001
Carbaryl (high dose)	200	$30x10^6 \pm 0.11$	<.001

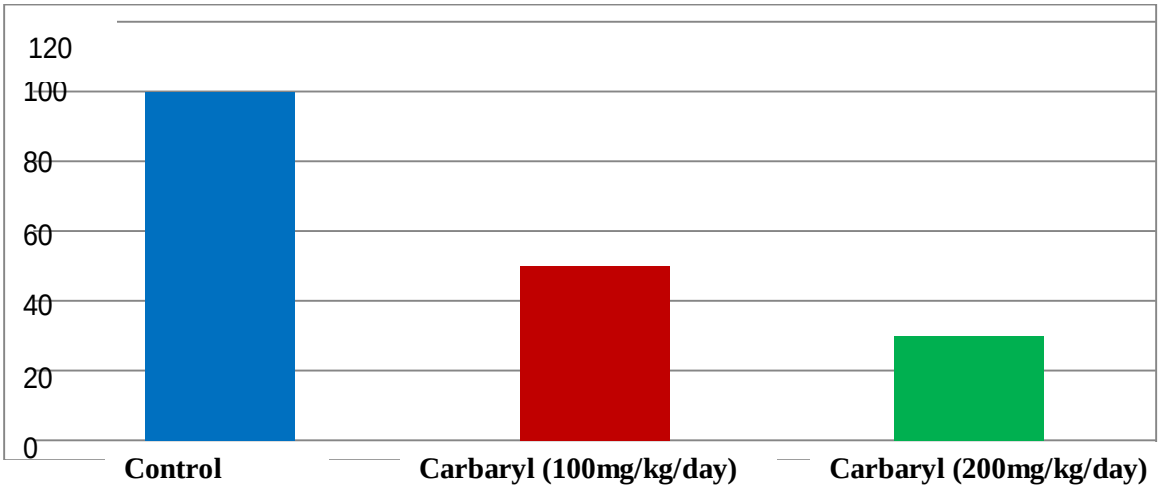

Figure 1. Bar diagram showing sperm count in control and carbaryl treated rats.

Table 2. Effect of carbaryl treatment on sperm motility after 60 days.

Treatment	Dose (mg/kg/day)	Sperm motility (mean±SD)	p-value
Control	-	81.0± 4.8%	-
Carbaryl (low dose)	100	38.1±6.09%	<.001
Carbaryl (high dose)	200	11.3±1.27%	<.001

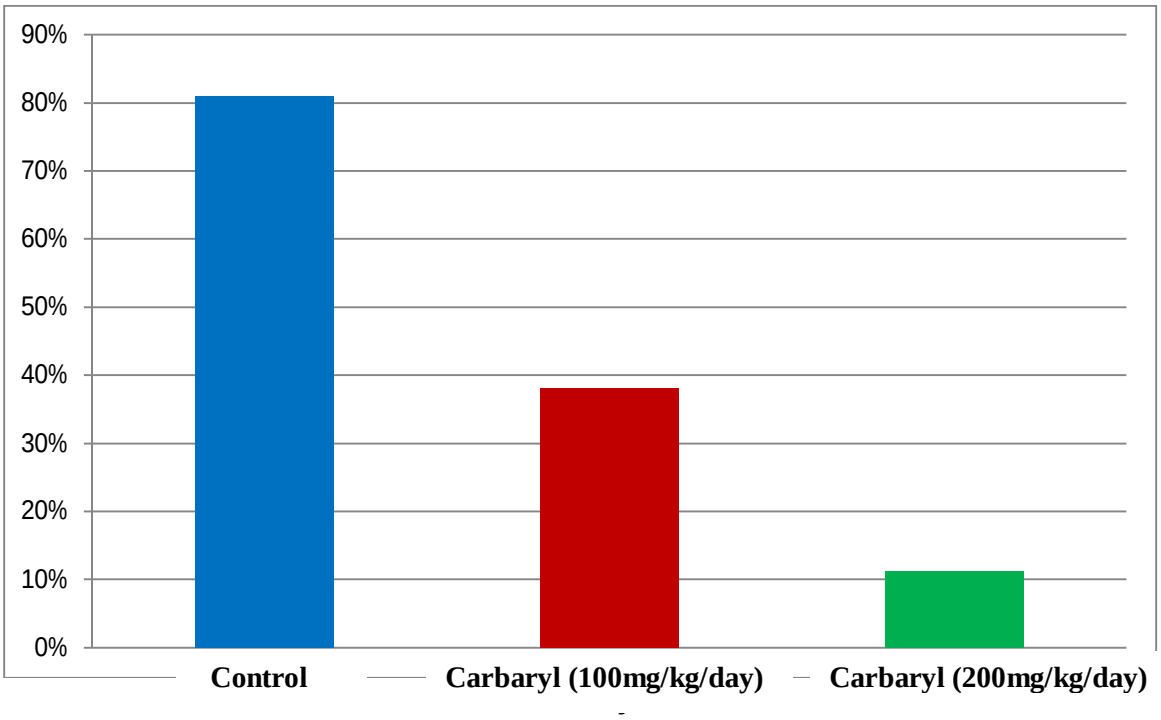

Figure 2. Bar diagram showing sperm motility in control and carbaryl treated rats.

DISCUSSION

Though animal studies support the fact that carbaryl reaches the mammalian testes, seminal vesicles and prostate (Thomas 1974), the reported effects of carbaryl on spermatogenesis are inconsistent. Several studies have shown no testicular effect attributable to carbaryl (Lillie 1973, Weil et al. 1973, Jordan et al. 1975, Dikshith et al. 1976). However, these studies generally focused on fertility and did not quantitate the effects on germ cells. Chronic oral administration of 3 mg of carbaryl/kg of body weight in rats reduced numbers of spermatogonia and testicular spermatozoa (Kitagawa et al. 1977).

An extremely complex mechanism underlies the effects of various substances on reproductive components and functions. Various chemicals may interfere in different ways with components of reproductive system. They may affect directly by interference of the substance with reproductive components or indirectly by altering hormonal regulations.

The carbamate insecticides, one of which is carbaryl, exert their insecticidal action by inhibiting cholinesterase enzymes. This inhibition is the primary mechanism by which these insecticides cause toxicity in mammals. The cholinesterase enzymes hydrolyze acetylcholine and other choline esters; consequently, their inhibition leads to the accumulation of endogenous acetylcholine and other choline esters. Probably most of the biologic effects of anticholinesterase agents, including carbaryl, are due to the inhibition of acetylcholinesterase which leads to the accumulation of endogenous acetylcholine, the principal choline ester that has demonstrated physiologic significance in humans.

Sperm count is one of the important parameter for testing testicular function. It is a direct method for assessing reproductive capability for an individual. Any factor which is involved in altering the homeostasis of reproductive biology will lead to alteration in final output of number of sperms. Sperm count is liable to decrease either due to decreased sperm production in seminiferous tubules or decreased sperm maturation in epididymis under effects of some chemicals (Sever & Hessol 1985). Dalsenter et al (1996), was in opinion that decreased sperm count may be due to damage to Sertoli cells. Other authors also reported same findings with carbaryl exposure to rats (Kitagawa et al. 1977, Rybakova 1966, Vashakidze 1975, Pant et al. 1995 & 1996). Several studies have shown no testicular effect attributable to carbaryl (Martin 1982, Osterloh et al. 1983). Reduction in sperm count, observed in the present study clearly suggests a detrimental effect of carbaryl on germinal epithelium, that too in a dose dependent manner suggesting damage.

Sometimes sperm count may be adequate but still the individual is infertile. The reason is number of active or motile sperms which decide fertility. Sperm motility was found to be significantly decreased with both low and high dose of carbaryl exposure. This finding runs parallel with that reported by Rybakova (1966), Vashakidze (1975) and Pant et al (1995 &1996). These findings do not correlate with the findings seen by Orlova and Zhalbe (1968), Benson and Dorough (1984), Weil et al. (1972), Dougherty et al. (1971) who did not observe significant effects of carbaryl on fertility, gestation and viability of rats. Decreased sperm motility may be due to androgen deprived maturational anomalies of sperms at epididymal level (Zenick et al. 1994, Iwasaki et al. 1995). According to our opinion, degeneration of Leydig cells leading to reduction in circulating as well as intratesticular testosterone level may be the probable cause for this finding.

CONCLUSION

Considering the effects of carbaryl on the testis in the present study and based on the findings of earlier studies, this compound may be designated as moderately toxic. This may affect spermatogenesis resulting in the production of decrease number of sperms.

REFERENCES

Abou Salem, M.E., El-Mashad AI and Moustafa, S.A. 1997. Pathological male reproductivity and residues of dimethoate toxicity in albino rats. *Alex J. Vet. Sci.* 13: 119-140.

Afifi N.A., Ramadan, A., El-Aziz, M.I., Saki, E.E. 1991. Influence of dimethoate on testicular and epididymal organs, testosterone plasma level and their tissue residues in rats. *Dtsch TierarztI Wochenschr* 98: 419-423.

Bendvold, E., Gottlieb, C., Bygdeman, M. and Eneroth, P. 1991. Depressed semen quality in Swedish men from barren couples: a study over three decades. *Arch Androl* 26: 189-194.

Benson, W.H. and Dorough, W. 1984. Comparative ester hydrolysis of carbaryl and ethiofencarb in fourth mammalian species. *Pestic Biochem Physiol* 21: 199-206.

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N.E. 1992. Evidence for decreasing quality of semen during past 50 years. *BMJ* 305: 609-613.

Dalsenter, P.R., Faqi, A.S., Webb, J., Merker, H.J. and Chahoud, I. 1996. Reproductive toxicity and tissue concentrations of lindane in adult male rats. *Hum Exp Toxicol* 15: 406-410.

Dikshith, T.S.S., Gupta, P.K., Gaur, J.S., Datta, K.K. and Mathur, A.K. 1976. Ninety-day toxicity of carbaryl in male rats. *Environ. Res*. 12: 161.

Dougherty, W.J., Golberg, L. and Coulston, F. 1971. The effect of carbaryl on reproduction in the monkey (Macacca mulatta). *Toxicol Appl Pharmacol* 19: 365.

Hileman, B. 1994. Environmental estrogens linked to reproductive abnormalities and cancer. *C&EN* 31: 19-23.

Iwasaki, H., Fuse, H. and Katayama, T. 1995. Histological and endocrinological investigations of cyclosporine effects on the rat testis. *Andrologia*. 27(3): 185-189.

Jordan, M., Srebro, Z., Pierscinska, E., and Wozny, L. 1975. Preliminary observations on the effects on the organs of mice of administering some carbamate pesticides. *Bull. Environ. Contam. Toxicol*. 14: 205.

Kamrin, M.A. 1997. Pesticides Profiles: Toxicity, Environmental Impact and Fate. Chemical Rubber Company (CRC) Press, USA.

Kitagawa, K., Wakakura, M. and Ishikawa, S. 1977. Light microscopic study of endocrine organs of rats treated by carbamate insecticide. *J. Toxicol. Sci*. 2: 53.

Lillie, R.J. 1973. Studies on the reproductive performance and progeny performance of caged white leghorns fed malathion and carbaryl. *Poultry Sci*. 52: 266.

Martin, A.R. 1982. Evaluation of pesticide effects on parameters of male reproduction in the mouse. *Diss. Abstr. Int.* B 42: 4279.

Mattison, D.R., Bogemil, R.J., Chapin, R., Hatch, M. and Hendricks, A. 1990. Reproductive effects of pesticides. In "The Effects of Pesticides on Human Health" Published by Princeton Scientific, New Jersey, USA.

Okamura, A., Kamijima, M., Shibata, E., Ohtani, K. and Takagi, K. 2005. A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. *Toxicology*, 213:129-137.

Orlova, N.V. and Zhalbé, E.P. 1968. Experimental material contributive to the problem of maximal permissible amounts of sevin in food products. *Vopr Pitan* 27: 49-55.

Osterloh, J., Letz, G., Pond, S. and Becker, C. 1983. An assessment of the potential testicular toxicity of 10 pesticides using the mouse-sperm morphology assay. *Mutat Res* 116: 407-415.

Pant, N., Srivastava, S.C., Prasad, A.K., Shankar, R and Srivastava, S.P. 1995. Effects of carbaryl on the rat's male reproductive system. *Vet Hum Toxicol* 37: 421-425.

Pant, N., Shankar, R. and Srivastava, S.P. 1996. Spermatotoxic effects of carbaryl in rats. *Hum Exp Toxicol* 15: 736-738.

Presibella, K.M., Kita, D.H., Carneiro, C.B., Andrade, A.J. and Dalsenter, P.R. 2005. Reproductive evaluation of two pesticides combined (deltamethrin and endosulfan) in female rats. *Reprod Toxicol* 20: 95-101.

Rybakova, M.N. 1966. Toxic effects of Sevin on animals. *Gig. Sanit*. 31: 402-407.

Sever, L.W. and Hessol, N.A. 1985. Toxic effects of occupational and environmental chemicals on the testis. In "Endocrine Toxicology" Target Organ Toxicology Series. New York: Raven Press, USA.

Thomas, J.A. 1974. Actions of pesticides and other drugs on the male reproductive system. USNTIS PB Rept. No. 237381/9GA.

Vashakidze, V.I. 1975. Effects of small doses of sevin (NMC) on gonad function following its repeated effect on white rats. Sb–Tr NII Gisieny Truda Profzabolevanii Gruz SSR 14: 253-266.

Weil, C.S., Woodside, M.D., Carpenter, C.P. and Smyth, H.F. Jr. 1972. Current status of tests of carbaryl for reproductive and teratogenic effect. *Toxicol Appl Pharmacol* 21: 390-404.

Weil, C.S., Woodside, M.D., Bernard, J.B., Condra, N.I., King, J.M. and Carpenter, C.P. 1973. Comparative effect of carbaryl on rat reproduction and guinea pig teratology when fed either in the diet or by stomach intubation. *Toxicol. Appl. Pharmacol*. 26: 621.

Zenick, H., Clegg, E.D., Perreault, S.D., Klinefelter, G.R. and Gary, L.E. 1994. Principles and methods of toxicology, edited by A. Wallace Hayes, 3rd ed. Raven Oress, New York. 937.

Corresponding author: Dr. **Archana Rani**, Department of Anatomy, King George's Medical University, Uttar Pradesh, Lucknow, India**. Email id:** archana71gupta@yahoo.co.in