Research Article

Testicular and Epididymal Toxicity of Carbaryl in Sprague-Dawley Strain Rats

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Abstract: In the past decade, the use of carbamate has become exceedingly popular as that of the organophosphates. There is a growing concern that the exposure to these compounds is a chronic hazard to man and animals. The present study was conducted to investigate the testicular and epididymal toxicity of carbaryl on rats. Male rats of Sprague-Dawley strain were administered orally with carbaryl dissolved in olive oil at 50 - 150mg doses/Kg body weight/day for 30 days. At the end of the treatment, rats were weighed and killed. Body weights remained unchanged at the end of the treatment. The weights of testis and epididymis decreased significantly after the treatment along with significant reductions in epididymal sperm count, viability and sperm motility. Activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase showed a significant decrease along with the reduction in the serum pituitary LH, FSH and testosterone. Carbaryl significantly decreased the activities of epididymal marker enzymes, phosphodiesterase and adenylate cyclase. These findings demonstrate the adverse effect of carbaryl on testicular steroidogenesis and epididymal functions contributing to the decline in the sperm counts of rats.

Keywords: Carbaryl; 3-beta HSD; 17-beta HSD; Testosterone; Testis; Epididymis; Rat.

1. Introduction

There is an increasing concern that environmental contaminants affect the male reproduction of humans and wildlife. Male reproductive abnormalities may be due to the increased exposure to environmental contaminants such as insecticides, pesticides, polychlorinated biphenyls, dioxins, phytoestrogens, alkylphenol ethoxylates and other xenoestrogens that enter into humans through food, drinking water, air and skin contact. These chemicals are responsible for the effects seen in humans such as concurrent increase in reproductive tract abnormalities and putative fall in sperm counts in men. 1-Naphthyl-N-methylcarbamate, also known as carbaryl or Sevin, is a contact and stomach insecticide with slight systemic properties, for use against many insect pests of cotton, fruit, vegetables and other crops. It is available for household lawn and garden pest control, and in veterinary practice, carbaryl is used on cattle, poultry and pets especially to control flies, mosquitoes, ticks and lice [1]. The general population can be exposed to carbaryl during pest control operations in both the home and recreational areas.

Studies have reported that carbaryl caused some testicular toxicity including histological changes in the seminiferous tubules and sperm shape abnormalities [2]. There is also a concern that exposure to estrogenic or anti-androgenic environmental contaminants induces major pathologic effects in the epididymis in men and experimental animals. It was also suggested that the exposure to such contaminants could affect the male hormone system [3]. The production of sexual hormones is regulated by the hypothalamus-pituitary-gonadal axis; comprising the hormone gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and inhibin B. LH and FSH are produced by the pituitary gland under the influence of pulsatile secretion of GnRH released by the hypothalamus. LH stimulates

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Leydig cells in the testes to produce testosterone, which is an important hormone for spermatogenesis through the stimulation of Sertoli cells in the seminiferous tubules. FSH controls spermatogenesis via direct stimulation of Sertoli cells and also stimulates inhibin B synthesis in the Sertoli cells. Both testosterone and inhibin B regulate GnRH and LH or FSH secretion through a negative feedback loop. For normal spermatogenesis to occur, the adequate functioning of this endocrine regulatory system and the two testicular compartments is necessary [4]. In particular, it was hypothesized that hormone disruption may block the development of Sertoli cells in the testes that "nurse" sperm cells as they develop. The number of Sertoli cells sets a cap on the number of sperm; therefore, a chemical exposure that blocked hormone involved with the Sertoli cell development would irreversibly limit sperm production [5]. Though the animal studies supported the fact that carbaryl reaches the mammalian testes, seminal vesicles and prostate the effects of carbaryl on testicular steroidogenesis and epididymal sperm dynamics, and their influence on hormone release is inconsistent. The present study was therefore conducted to correlate the effect of carbaryl on testicular steroidogenesis and the possible role of hormonal alteration in the epididymal sperm decline is discussed.

2.   Materials and Methods

2.1 Chemicals

Carbaryl (1-Naphthyl-N-methylcarbamate) 97% purity of technical grade was used. Ham's F12 medium was obtained from Himedia Laboratories, Mumbai, India. For hormone assays, ELISA kits were obtained from Diagnostic Systems Laboratories, Inc. Webster, Texas, USA. All other chemicals were of analytical grade and obtained from local commercial sources.

2.2 Animals

Male rats of Sprague-Dawley were well maintained in the Animal House. Prior to use, animals were housed in solid-bottom cages with hardwood chips and were acclimated (~1 week) in a well-regulated 12-h light/ dark cycle at 23 ± 3^0C. Standard commercial rodent feed and water were provided ad libitum.

2.3 Treatment

Animals were divided into four groups of 6 rats each and were administered orally with carbaryl dissolved in olive oil at 50-150mg/ Kg body weight for 30 days. Control group was administered orally with olive oil alone.

All the rats used in the experiments were marked by tail marking. Growth of the animals was monitored regularly and rats showing poor growth rate were discarded from the experiments. At the end of the treatment, rats were killed by using an overdose of anesthetic ether and the body weight and organ weights were recorded.

2.4 A collection of epididymal sperm and sperm function tests

Epididymal sperm were collected by the method of Gray *et al.*, [6]. Briefly, the cauda epididymides was cut into small pieces in 5ml of Ham's F-12 medium at 32^0C. The sperm obtained from left epididymis were used for the determination of sperm viability, sperm motility and sperm count. Sperm collected from the right epididymis were used for biochemical estimations. Sperm viability test was done by the method as described in the WHO Laboratory Manual [7]. The sperm viability was expressed in percentage as the number of viable sperm of total sperms counted. Epididymal sperm motility was evaluated by the method as described by Linder *et al.*, [8]. Sperm motility was expressed as a percentage of motile sperm of the total sperm counted. Epididymal sperm were counted by the method as described in the WHO Laboratory Manual [7]. The complete spermatozoa (head with tail) were counted and expressed as a percentage of the total sperm.

2.5 Preparation of tissue homogenates and biochemical assays

Testis and epididymis were dissected out and transferred to a petri dish containing cold normal saline. Epididymis was washed several times in cold normal saline in order to remove maximum number of sperm attached to the epididymal parenchyma. Only the caudal region of the epididymis was separated and homogenized in cold normal saline with the help of Glass Teflon Homogenizer. The homogenates were centrifuged at 800g for 20 min at 4^0C. The supernatants were collected and used for various biochemical assays. Protein contents were determined according to the method of Lowry *et al.*, [9]. The activities of epididymal marker enzymes, phosphodiesterase [10] and adenylate cyclase [11] were estimated. The activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase were estimated according to Bergmeyer [12] in testicular homogenate.

2.6 Collection of blood serum and hormone assays

Blood samples from control and treated groups were collected in clean glass centrifuge tubes. Blood was centrifuged at 2000g for 15 min after storing overnight at 4^0C. Serum samples were collected and stored at -20^0C in microfuge tubes until used.

2.7 Quantitative determination of serum hormone levels

Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were measured by enzyme linked immunosorbent assay (ELISA) using kits from Diagnostic System

Laboratories, Inc. Webster, Texas, USA. The assays were done strictly according to the procedure given along with the kits.

2.8 Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test. Differences were considered to be significant at $p < 0.05$ against a control group. Data are presented as mean $\pm$ SD for six animals per group. All biochemical estimations were carried out in duplicate.

3. Result and Discussion

3.1 Body weight and organ weights

Body weights of the animal remained unchanged in the carbaryl-treated rats (Table 1). The weights of testis and epididymis showed a significant ($p < 0.05$) decrease at all doses when compared with the control groups (Table 1).

3.2 Epididymal sperm function test

The spermatozoal number collected from the cauda region of the epididymis and the sperm motility decreased significantly ($p < 0.05$) at all doses of carbaryl treatment as compared with the corresponding control groups (Table 1). Epididymal sperm viability decreased significantly ($p < 0.05$) at 150mg dose of carbaryl administration (Table 1).

3.3 Enzyme activities in testis and epididymis

Administration of carbaryl significantly ($p < 0.05$) decreased the activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase at all doses of the treatment in rat testis (Fig. 1).

The activities of the epididymal marker enzymes, phosphodiesterase and adenylate cyclase decreased significantly ($p < 0.05$) at all doses in the carbaryl-treated rats as compared with the control groups (Figs. 2 and 3).

Table 1. Effect of carbaryl on the weights of testis and epididymis and epididymal sperm dynamics in rats.

Treatment	Control	Carbaryl (mg/ Kg body weight)		
		50	100	150
Body weight (g)	135.24 ± 2.07	137.31 ± 3.14	136.61 ± 2.94	134.33 ± 6.52
Testis (mg)	946.94 ± 2.66	$850.01 \pm 3.32*$	$748.75 \pm 4.68*$	$749.64 \pm 2.50*$
Epididymis (mg)	339.07 ± 4.12	$241.06 \pm 5.19*$	$238.34 \pm 1.99*$	$217.22 \pm 2.94*$
Sperm count (x10^8)	8.19 ± 0.25	$7.66 \pm 1.99*$	$6.29 \pm 0.85*$	$5.24 \pm 0.27*$
Motility (%)	98.41 ± 2.23	$80.0 \pm 1.56*$	$60.27 \pm 1.89*$	$58.64 \pm 2.57*$
Viability (%)	98.41 ± 5.37	97.32 ± 3.46	95.45 ± 2.75	$88.67 \pm 3.20*$

[(Data are expressed as mean $\pm$ SD for six-animals/ group) (Asterisks (*) denotes p values set significant at 0.05 against the control groups)]
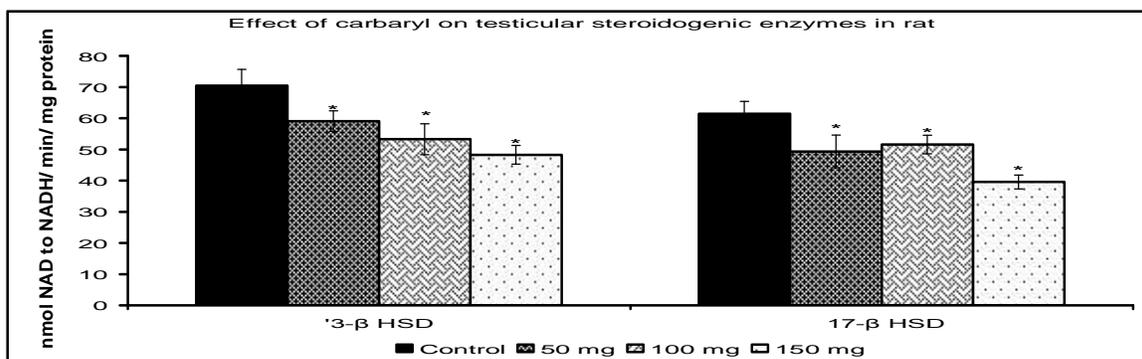

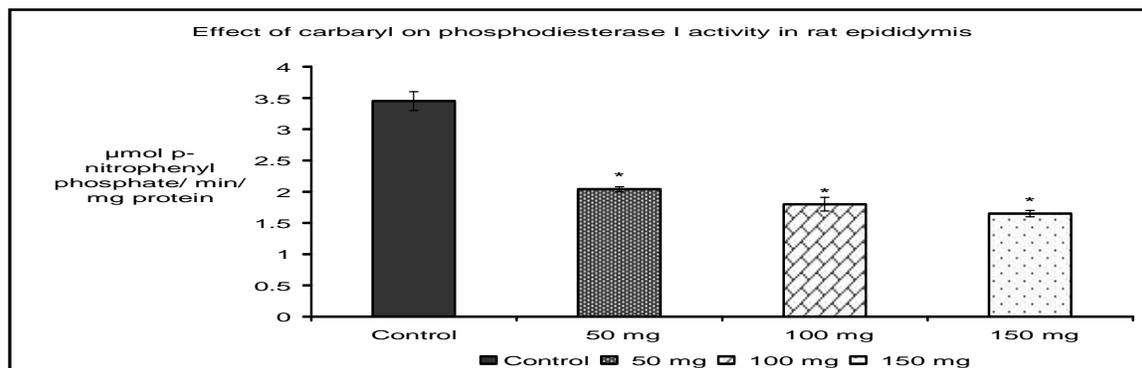**Fig. 1. Effect of carbaryl on testicular steroidogenic enzymes in rat.**

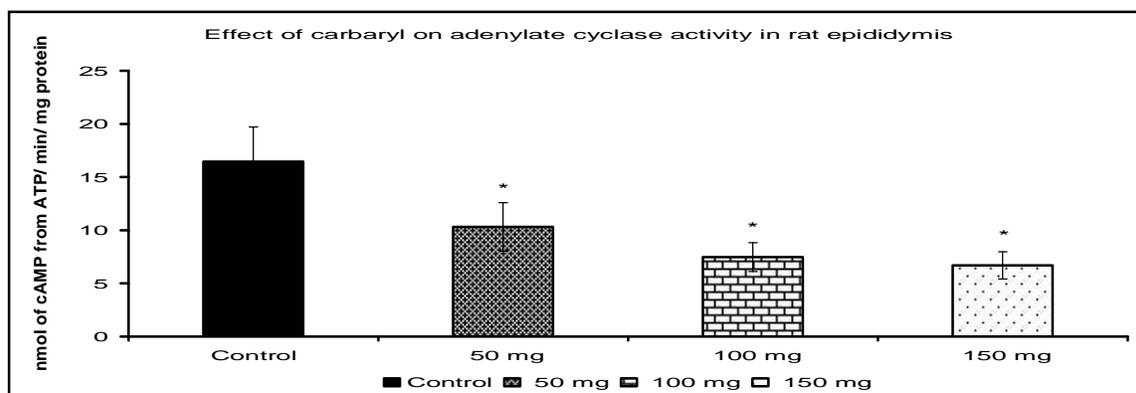**Fig. 2. Effect of carbaryl on phosphodiesterase I activity in rat epididymis.**

Fig. 3. Effect of carbaryl on adenylate cyclase activity in rat epididymis.
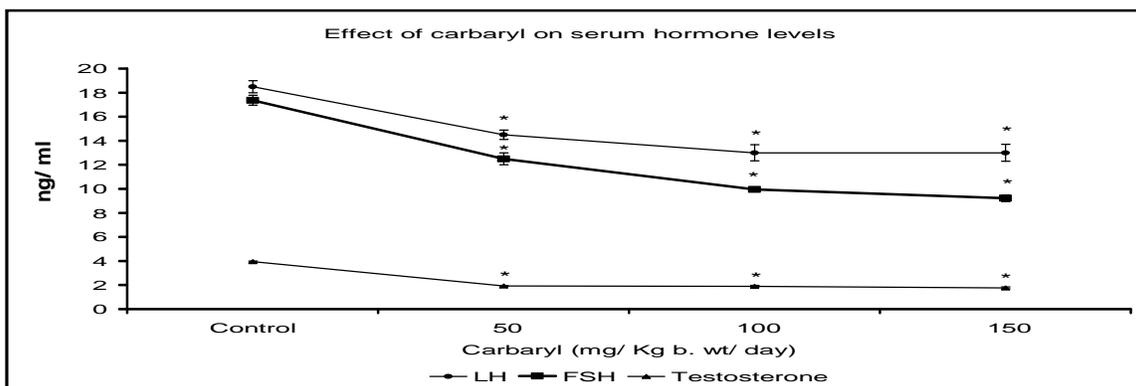

Fig. 4. Effect of carbaryl on serum hormone levels.

3.4 Serum hormone levels

A significant reduction in the levels of serum testosterone, follicle stimulating hormone and luteinizing hormone were observed after carbaryl treatment when compared with the control groups (Fig. 4).

Pesticides comprise a large number of distinct substances with dissimilar structures and diverse toxicity which act through different mechanisms. The present observations have shown that carbaryl affects the testicular steroidogenesis and caused hormonal modification and that could be the source of the decreased epididymal sperm count and infertility in rats.

In the present study, the body weight of the carbaryl-treated rats remained unchanged. However, it was reported by Pant *et al.,* [13] that a significant decrease in weight gains of rats at 100mg dose after 60 days of carbaryl exposure. Carbaryl decreased the weights of testis and epididymis at all doses. The decrease in the testicular weight of carbaryl-treated rats may indicate impairment at testicular, pituitary or at hypothalamic level. Epididymis requires a continuous androgenic stimulation for their normal growth and functions. Reduction in the weight of epididymis reflects a decreased bioavailability and production of androgen.

Carbaryl treatment showed an epididymis-specific decrease in cauda epididymal sperm counts and motility confirming a previous report [13, 14]. Exposure to carbaryl impaired sperm motility, which may result in infertility due to the failure of sperm to reach the site of fertilization as well as their inability to penetrate the zona pellucida. Cyclic nucleotides, especially cAMP have been shown to be an intrinsic regulator of sperm motility. Moreover, the acquisition of sperm motility when they traverse the epididymis is reported to be correlated with an increase in the intracellular cAMP content [15]. In the present study, carbaryl decreased the activity of adenylate cyclase enzyme in epididymis thereby it is clear that cAMP is not produced and ultimately resulted in the decreased sperm motility. The decline in phosphodiesterase activity observed in the present study may be triggered by similar factors in the epididymal fluid. Therefore, the decrease in the epididymal enzymes adenylate cyclase and phosphodiesterase is correlated to the poor motility of sperm in the rat epididymis.

Administration of carbaryl decreased the activities of steroidogenic enzymes 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase. This result was indicative of the reduced testicular steroidogenesis [16] and the exposure to carbaryl seems to impair steroid synthesis in Leydig cells which

concomitantly reduced testosterone level. It was suggested that an anti estrogenic environmental contaminant has a direct inhibitory effect on testosterone production. Thus the decrease in the testosterone production is likely to decrease the testicular steroidogenic enzymes and both are correlated. FSH and estrogen are also equally important in determining adult sperm production. Reduction in the FSH level may result in the decreased Sertoli cell and permanently decreases the sperm production. Therefore, from the present study, it is confirmed that carbaryl lowers the level of LH, FSH and testosterone and ultimately resulted in decreased sperm counts.

4. Conclusion

In conclusion, the present study indicates that carbaryl alters testicular and epididymal functions possibly by decreasing the sperm counts and thereby disrupts male reproduction in rats. The development and growth of the male reproductive system is obviously a complex process and therefore exposure to carbaryl could likely affect the male fertility in rats.

Acknowledgment

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