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Optical microscopic changes in male rat reproductive organs post-treated with Carbofuran – II

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ABSTRACT

Carbofuran (Furadan) is one of the broad-spectrum carbamate pesticide used to control insects in a wide variety of agricultural crops. This paper describes the effects of carbofuran on the male reproductive organs of the Albino rats. The male adult albino rats (n = 24) were grouped into A (control), B and C (experimental). The experimental animals (group B) were administered with 2 mg/ kg body weight/ day carbofuran in saline for 60 days and then sacrificed. The animals of group C were kept without treatment for 30 days and then sacrificed to see the delayed changes. Epididymedes were fixed and processed to see histological changes. Epithelial degeneration, reduction in spermatozoa in the tubal lumen and increased vascularity were observed in animals treated for 60 days. These structural changes persisted in the organs even after stoppage of carbofuran for 30 days.

Keywords: Carbofuran; Rats; Toxicity; Epididymis; Optical microscope.

INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), generally called as furadan, is one of the most toxic carbamate pesticides (Fig. 1) used to control insects in a wide variety of field crops. It has one of the highest acute toxicities to humans. The toxic effects of carbofuran are due to its activity as a acetyl cholinesterase inhibitor[1].

Carbofuran is also known to be highly toxic to birds, particularly in its granular form, which birds often eat, mistaking them for seeds and then die instantly. It has been documented in hundreds of avian mortality events sometimes involving large numbers of birds in each incident. US-EPA banned granular carbofuran in 1991. The liquid form of the pesticide, which is less hazardous, is still one of the few insecticides effective on soybean aphids, and is being used in most soybean-growing regions of the United States[2].

Pesticides may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation[4,5]. The widespread use of pesticides in public health

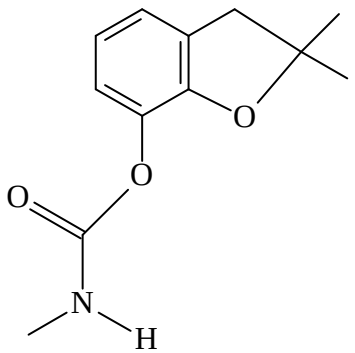

Fig. -1: Chemical structure of Carbofuran

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and agriculture has caused severe environmental pollution and health hazards including cases of severe, sub-chronic and chronic human poisoning[6-9].

This study was designed to see the toxic acute and chronic effects of carbofuran on epididymis, one of the male reproductive organs which plays an important role in male fertility.

MATERIALS AND METHODS

Materials

Pesticide (carbofuran) was supplied by FMC Corporation (Philadelphia, USA). Albeno adult male rats (n = 24), each weighing between 150 – 200 g, were taken from the Veterinary Institute, Universiti Sains Malaysia. The light microscopic observations were made with a Nikon Eclipse E-600 Image Proplus V 4.5 Dual Dimension Microscope (Japan).

Method

Albino rats (n = 24) were grouped into A, B and C, each group comprising eight animals. These groups were further subdivided into A1 and A2; B1 and B2; and C1 and C2, comprising four animals in each sub-group. Four animals of each sub-group were kept in one cage. The animals in group A (control) were given normal saline orally for a period of two months. The rats were housed under standard laboratory conditions with free access to food and water ad libitum. The group B (experimental) animals were given 2 mg/ kg body weight/ day of carbofuran powder dissolved in normal saline. Each animal was given 1 ml/ kg body weight carbofuran in saline daily for the same period as the control group. Group C was treated like group B for a period of two months and then left for another one month without treatment. The animals of group A1, B1 and B2 were sacrificed by euthanasia with sodium phenobarbitol (100 mg/ kg body weight) on day 60. Laparatomy was performed and epididymis were carefully removed and separated. The tissues were fixed in 10% formaline, embedded in paraffin and 5 µm sections were cut and stained with haematoxylin and eosin stain. The tissue sections were observed under the light microscope for qualitative changes in epididymis. The animals of group A2, C1 and C2 were sacrificed on day 90 and same tissues were removed and examined.

RESULTS

Group A (control)

Fig. 2 shows the sections of highly coiled tube with surrounding connective tissue and blood vessels. The tube is lined with pseudo-stratified columnar epithelium composed of rounded basal cells and tall columnar cells. The basal lamina is supported by smooth muscle cells and loose connective tissue rich in blood capillaries. The surface of epithelial cells is bearing numerous very long, atypical immotile and microvilli (Figs. 3, 4). These cells have absorptive or phagocytic and secretory function. The lumen of the epididymis is filled by spermatozoa (Fig. 5).

Group B (experimental)

The degeneration of epididymal epithelium both basal and columnar cells is observed in most of the sections (Fig. 6). Nuclei of the cells are pyknotic with irregular shape. The lumen is showing greater reduction in spermatozoa (Fig. 7).

Group C (experimental)

The connective tissue around the epididymis was infiltrated with inflammatory cells and dilated blood vessels (Fig. 8). The lumen was still having very few spermatozoa and cellular debris (Fig. 9). Basal cells and muscle cells were present in sufficient number. Columnar cells were occupying various positions in the epithelium. Their apical surfaces were having microvilli, while lumen was having scanty spermatozoa and desquamated cells.

DISCUSSION

Carbofuran has the potential to cause damage to the reproductive system and to health by prolonged exposure. This chemical has been shown to produce developmental toxicity in several studies at doses, which cause minimal maternal toxicity. The male reproductive organs are severely damaged at doses around 0.2 mg/ kg body weight in both rats and dog. The described effects are degeneration of seminiferous tubules, loss of spermatogenesis, degenerative changes in Sertoli cells, and depletion of a variety of cell types. The present studies also reveal similar findings.

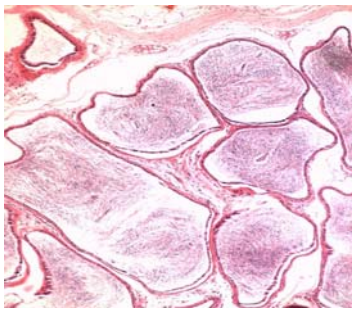

Fig. - 2: Photomicrograph of group A (control) rat epididimys showing highly coiled tube with surrounding connective tissue and blood vessels. H and E 40x

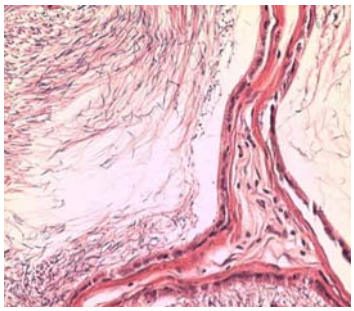

Fig. - 3: Microphotograph of sectioned epididimys of rat showing tubular epithelium resting on basement membrane with spermatozoa filling its lumen. H and E 100x

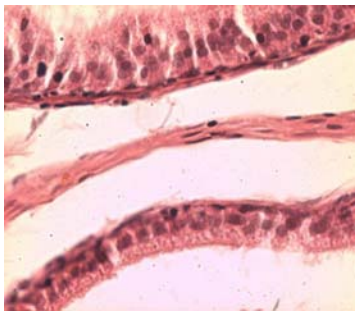

Fig. - 4: Photomicrograph of cross-section of epididimys showing basal cells and pseudostratified columnar cells with giant microvilli and circular muscle fibers H and E 40x

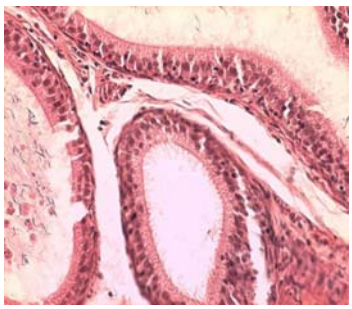

Fig. - 5: Photomicrograph of rat epidodymis showing epithelial cells and tall microvilli. H and E 400x

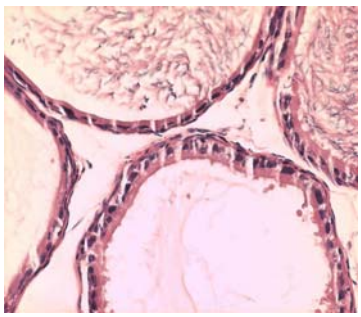

Fig. - 6: Photomicrograph of group B treated rat epididimys showing degeneration of epithelium

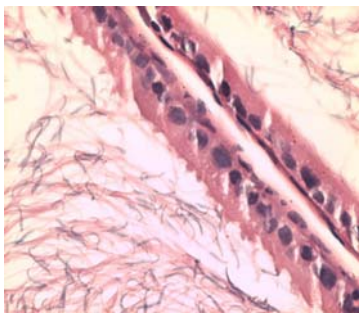

Fig. - 7: Micrograph of treated epididimys showing degenerated basal and columnar cells with short and few microvilli. H and E 400x

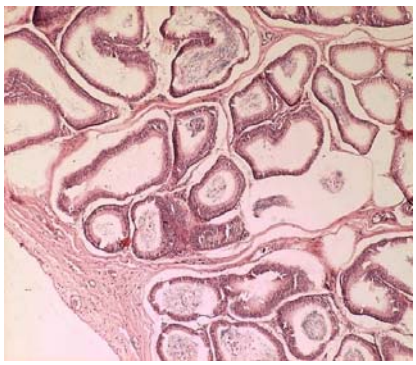

Fig. - 8: Photomicrograph of group C rat epididymis showing inflammatory cells and blood vessels in surrounding connective tissue. H and E 40x

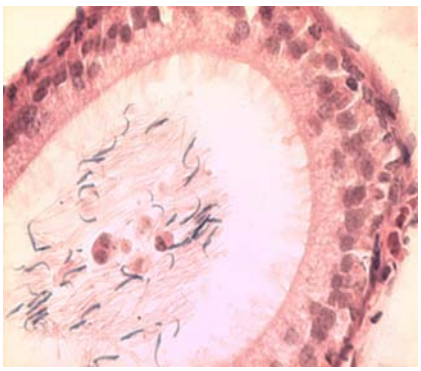

Fig. - 9: Photomicrograph of cross-sectioned epididymis showing degenerated basal and columnar cells with microvilli and few spermatozoa with desquamated cells in the lumen. H and E 400x

The importance of the androgens for normal spermatogenesis is well documented. Spermatogenesis depends on testosterone production by Leydig cells in response to stimulation by FSH and LH from pituitary gland. FSH increases Sertoli cell synthesis of an androgen binding protein needed to maintain high concentrations of testosterone. LH stimulates testosterone production by the interstitial cells of Leydig[10].

In the present study it was observed that the chronic administration of carbofuran led to significant histological changes in male reproductive organs like epididymis. Maturation of spermatozoa takes place in the epididymis, and any abnormality in the structure of epididymis may result in a defective maturation of the spermatozoa leading to male infertility. Similar changes had been reported in rat testis administered with methomyl carbamate (17 mg/ kg body weight in saline) daily for two months[11].

It has been proved by many studies that hormonal changes produced by carbofuran compounds are in favour of direct toxic effect of the insecticide or possibly through an alteration in the neuroendocrine environment resulting into acetyl cholienestrase inhibition[12].

The inhibition of plasma and erythrocyte AChE, supported by clinical signs, demonstrates a toxic effect on the peripheral nervous system. In addition, dose-related inhibition of brain AChE has been observed in many studies with rat and rabbit.

Epididymides are the site of sperm maturation and storage. It reabsorbs 90% of fluid secreted by the testes. Sperms are physiologically immature when they leave the testis but they mature as they travel through the head and body of epididymis. They are stored in its tail and remain fertile for 40 – 60 days. If not ejaculated they disintegrate and reabsorbed by epididymis.

Acid phospatase enzyme plays an important role in the process of cell metabolism, autolysis, differentiation and many related processes[12]. Dilatation of blood capillaries noted in the connective tissue outside the circular muscle layer is the result of acid phosphatase enzyme activity. The increase in acid phospahatase enzyme activity could be explained on the bases of enhancement of cell membrane permeability with disturbance in the transphosphorylation process as a result of cellular degeneration[13], whereas the dilatation and increased vasculature is most probably a compensatory mechanism to the hypoxia induced by carbofuran intoxication. Chronic insecticide intoxication may also lead into cellular disintegration. As it is clear from the results of this study that sixty days after the discontinuation of carbofuran to the group C rats, the histo-pathological changes reverted back slightly in the epididymis but persistence of these histological changes in moderate amount indicate long-lasting reproductive damage.

Present study shows that the long term use of carbofuran at the dose of 2 mg/ kg body weight orally is responsible for irreversible damage to male reproductive organs, if precautionary measures are not taken while using them. Although carbofuran has high acute oral toxicity with an oral LD-50 in rats of 8 – 14 mg/ kg body weight. These structural changes persisted in the organs even after the stoppage of carbofuran for 30 days.

Structural damage to mammalian epididimides may lead to infertility and carcinogenicity. Therefore, the utilization of pesticides must be predicted in selecting the quantities and mode of usage which will minimize the possibility of exposure of non target organisms to injurious quantities of these chemicals. This can be achieved through public health education to make people aware of the hazardous effects of these compounds. It is therefore recommended that great precautions must be taken to minimize the harmful side effects of such chemicals to the environment especially to men, animals and agriculture products aiming to avoid environmental pollution. The precautionary measures like wearing of impermeable gloves and masks to reduce the risk of inhalation of spray liquid.

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REFERENCES

1. Ecobichon D. J., Toxic effect of pesticides. In: Klaassen CD (ed), Casarett and Doull's Toxicology: The Basic Science of Poisons, 5/ed. McGraw-Hill, p. 659 (1996).
2. Hassall K. A., The Biochemistry and Uses of Pesticides, MacMillan, Toronto, p. 142 (1990).
3. Ellenhorn, M. J., Schonwald S., Ordog G. and Wasserberger J., Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning, Williams and Wilkins, Maryland, USA, 1614 (1997).
4. Banerjee B. D., Seth V., Battacharya A., Pasha S. T. and Chakrabory A. K., *Toxicol. Lett.* **107,** 33 (1999).
5. Etemadi-Aleagha A., Akhgari M. and Abdollahi M., *Mid East Pharmac.* **10,** 8 (2002).
6. Abdollahi M., Jafari A. and Jalali M., *MJIRI* **9,** 221 (1995).
7. Abdollahi M., Jafari A. and Jalali N., *Irn J Med Sci.,* **20,** 105 (1995).
8. Jalali N., Pajoumand A., Abdollahi M. and Shadnia S., *Toxicol Lett Sppl*. **1**/116, 309 (2000).
9. Pajoumand A, Jalali N, Abdollahi N, Shadnia S., *J Pharm Pract Res,* **32,** 297 (2002).
10. Kackar R., Srivastara M. K. And Raizada R. B., *Ind. Health* **35,** 104 (1997).
11. Goldman J. M., Rehnberg G. L., Cooper R. L. Grey L. E., Jr. Hein J. F. and Mc-Elroy W. K., *Toxicology* **57,** 173 (1989).
12. Sugar J., *Acta. Morpho. Acad Sci* **15,** 93 (1997).
13. Linder R. E., Rehnberg G. L., Strader L. F. and Diggs J. P., *J. Toxicol. Environ. Health,* **25,** 285 (1988).