Protective Effect of Vitamin C against Carbofuran-Induced Testicular Toxicity in Albino Mice

Wael M. Al-Amoudi

Department of Biology, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, Saudi Arabia

Abstract: The effect of the carbamate insecticide "carbofuran" on testes of male albino mice and the possible protective role of vitamin C against the insecticide toxicity was assessed. Treating mice with carbofuran for 8 weeks induced a significant decrease in the diameters and germinal epithelial heights of the seminiferous tubules. The histological evidence showed inhibition of spermatogenesis. There was also a decline in sperm count. Histochemical results revealed that animals given carbofuran had decreased contents of carbohydrates and total proteins in the testicular tissue. Treating mice with carbofuran and vitamin C showed an improvement in the testicular damage. The seminiferous tubules appeared normal and the different stages of spermatogenic cells showed an advanced degree of activation.

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1. Introduction

The increased agricultural uses of pesticides have introduced serious, novel hazards to the human and their livestock. Studies have shown that prolonged exposure to some of these contaminants causes chronic or persistent neurologic syndrome, immunosuppressive effects, malignant tumors, teratogenic action, abortion and reproductive failure (Clary and Ritz,2003). Carbofuran (2,3-dehydro-2,-2-dimethyl-7 benzofuranylmethylcarbamate), is a systemic N-methyl carbamate pesticide used as a soil applied chemical to control soil dwelling and foliar feeding insects and nematodes on a variety of agricultural crops, including maize, corn, rice, potatoes, alfalfa and grapes (Gupta, 1994). Carbofuran is a potent cholinesterase inhibitor and is highly toxic to humans and wildlife through the oral and inhalation routes of exposure (Baron,1991). Yousef et al, (1995) have reported that the carbofuran decreased libido and sperm number in rabbits. This pesticide also disrupts testicular morphology and alters activities of enzymes associated with specific cell types of testes (Pant et al, 1995). In utero or lactational exposure of carbofuran to male rats caused testicular and spermatotoxicity (Pant et al, 1997).

Vitamin C is a major circulating water-soluble antioxidant. It is well absorbed by the gastrointestinal tract and required for multiple biological functions and biochemical reactions in humans and animals and it is an important element for the body (Li and Schellhorn, 2007). In addition, it is an essential nutrient for the biosynthesis of collagen, L-Carnitine and norepinephrine. In the male reproductive system vitamin C is known to protect spermatogenesis and it plays a major role in semen integrity and fertility both in men (Agarwal et al. 2005; Eskenazi et al. 2005) and animals, increases testosterone levels (Sönmez et al. 2005) and prevents sperm agglutination. It is an important chain-breaking antioxidant, contributing up to 65 percent of the total antioxidant capacity of seminal plasma found intracellulary and extracellulary (Makker et al. 2009). Shrilatha and Muralidhara (2007) reported the protective effect of vitamin C on testicular oxidative stress, sperm oxidative stress and genotoxic effects using a diabetic mice model. Similarly, Naziroğlu (2003) concluded that vitamin C acted as antioxidant in reproductive milieu. The effect of vitamin C against reproductive toxicity of pesticides were investigated (Allhaza and Bashandy, 1998; Uzunhisarcikli et al. 2007). The present work studied the effect of vitamin C on testicular toxicity of carbofuran.

2. Materials and Methods

Animals

Sexually mature male albino mice (*Mus musculus*) weighing 20±5 g were used in the present study. The animals were kept in the laboratory under constant temperature (22±1^{o}C) for at least one week before and along the period of the experimental work. They were maintained on a standard rodent diet composed of 55% corn starch, 20% casein, 15% corn oil, 5% salt mixture and 5% vitaminized starch Water available *ad libitum*.

Experimental Design

All the experiments were done in compliance with the guide for the care and use of laboratory animals. Animals were divided into 4 groups.

Group **1**: Animals of this group (20 mice) were orally given carbofuran by gastric intubation at a dose level of 1/10 LD_{50} (25mg/kg body weight) three times per week for continuous 8 week.

Group **2**: Animals in this group (20 mice) were given

the same dose of carbofuran given to animals of group 1 followed by vitamin C (16 mg/kg body weight) three times weekly for 8 weeks.

Group **3**: Animals of this group (20 mice) were orally given vitamin C.

Group **4**: This group is a control, in which animals (20 mice) were orally given water.

Histological and Morphometrical Examination

Ten rats were sacrificed from treated and control groups after 8 weeks. Their testes were excised. For histological study testes were fixed in Bouin's fluid, dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of five micrometers thickness were cut and stained with haematoxylin and eosin for histological examination. The diameter and germinal epithelial height of seminiferous tubules were measured from the spermatogenic cells on the inner surface of the basement membrane through the most advanced cell types lining the lumen of the tubules. All data were obtained from 10 random microscopic fields per animal at X 100 objective.

Histochemical Studies

For histochemical study testes of the different animal groups were fixed in Carnoy's fluid. Periodic acid Schiff's reaction (Kiernan, 1981) was used for demonstration of polysaccharides. Total proteins were detected using the mercury bromophenol blue method (Pearse, 1972).

Testicular Sperm Count

One testis of each mouse was placed in 1 ml of phosphate buffer saline immediately after dissection. The tunica albuginea was cut by surgical blades and removed, and the remaining seminiferous tubules were mechanically minced by using surgical blades in 1 ml of phosphate buffer saline. The testicular cell suspension was pipetted several times to form a homogenous cell suspension. One drop of the suspension was placed on a ''Makler Counting Chamber" and the testicular sperm concentration was determined under a phase contrast microscope at 200X magnification and expressed as million sperm cells per ml of suspension.

Statistical Analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, PA). Values of $P < 0.05$ were considered statistically significant

3. Results

Morphometrical Results

Data in table (1) revealed that the diameter of the seminiferous tubules was significantly decreased in mice treated with carbofuran. There was likewise a significant decrease in the height of the tubule epithelium. Animals treated with carbofuran and vitamin C showed a significant increase (in comparison with those treated with carbofuran) in both the diameter of the seminiferous tubules and the height of the epithelium.

Table (1): Effect of carbofuran and/or vitamin C on the diameter and the height of the epithelium of seminiferous tubules of mice testes.

Animal group	Diameter in μm (mean ± SD)	Epithelial height in μm (mean ± SD)
Control	184 ± 12.3	67 ± 4.3
Vitamin C	189 ± 11.6	66 ± 3.3
Carbofuran	130 ± 5.6*	28 ± 4.6*
Carbofuran + Vitamin C	167.5 ± 6.6	51 ± 6.1

(*) Significant at $P < 0.05$ in comparison with control.

Histological Observations

Histological examination of testis of control mouse showed normal testicular architecture with well-organized seminiferous tubules and Sertoli cells. All stages of transformation of the seminiferous epithelium from spermatogonia to mature spermatozoa could be seen in the tubules (Fig.1A). The histopathological examination of mice treated with vitamin C showed normal testicular figure. Treating animals with carbofuran showed various histopathological changes. The seminiferous tubules were widely separated and their boundaries were irregular. The interstitial tissue was degenerated (Fig. 1B). The spermatogonia appeared with cytoplasmic vacuolation with pyknotic nuclei (Fig.1C). There was reduction in the spermatogenic cells and when present it was degenerated (Fig.2A). Treating animals with carbofuran and vitamin C led to increase in the cellularity of germ cells (Fig.2B).

Histochemical Results

Carbohydrates

PAS-positive materials appeared in tunica albuginea and in the intertubular connective tissue of testes of control mice. The spermatogenic cells exhibited weak reaction while the sperms showed strong reaction (Fig.3 A). Testes of the carbofuran-treated animals revealed a decrease of PAS-positive materials. In these specimens, tunica albuginea, the boundaries of the seminiferous tubules as well as the intertubular connective tissue had weak PAS-positive materials (Fig. 3B). Also more or less normal carbohydrate contents were observed after treatment with carbofuran and vitamin C (Fig.3C).

Total Proteins

The total proteins appeared in the testicular tissues of control mice as deeply stained granules inside the nuclei and cytoplasm of all spermatogenic cells. The tunica albuginea, intertubular connective tissue as well as the boundaries of seminiferous tubules showed strong reaction (Fig.4A). Animals treated with carbofuran showed a noticeable decrease in the proteinic content of the spermatogenic cells in both the nucleus and the cytoplasm (Fig.4B). The treatment with carbofuran and vitamin C showed increase in total proteins in the spermatogenic cells (Fig.4C).

Sperm count

Data in table (2) showed that sperm count was significantly decreased in mice given carbofuran. On the other hand, animals treated with carbofuran and vitamin C showed an increase in sperm count in comparison with those given carbofuran.

Table (1): Effect of carbofuran and/or vitamin C on sperm count

Animal group	Sperm count ($x10^6$) (mean ± SD)
Control	1.2 ± 0.01
Vitamin C	1.1 ± 0.3
Carbofuran	1.02± 0.01*
Carbofuran + Vitamin C	1.06± 0.02

(*) Significant at $P < 0.05$ in comparison with control

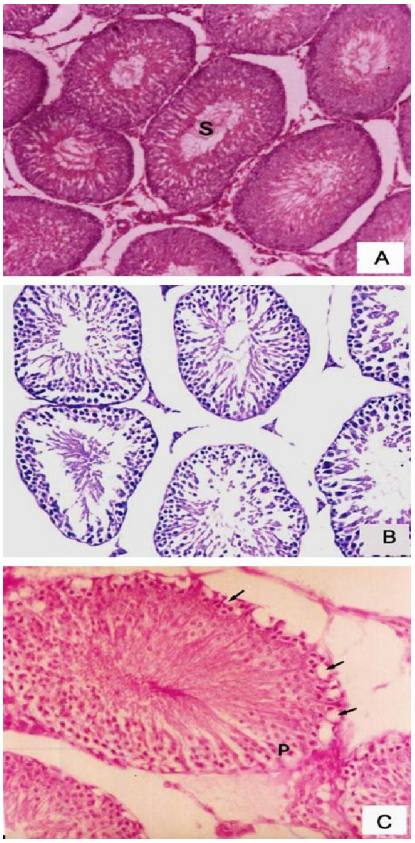

Fig.1. (A). Section in the testis of a control mice showing seminiferous tubules with lumen filled with sperms (S), X12, **(B).** Testis of carbofuran-treated mice showing irregular seminiferous tubules widely separated from each other, X120, **(C).** Seminiferous tubules with cytoplasmic vacuolation (arrows) and pyknotic nuclei (P) of spermatogonia, X300.

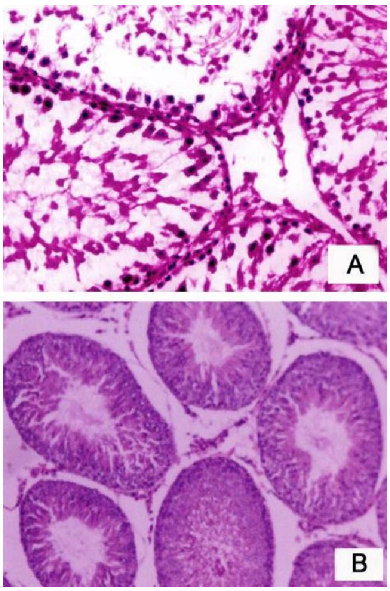

Fig.2. (A). Seminiferous tubules of a treated mouse showing degenerated spermatogenic cells, X300, **(B).** Testis of a mouse treated with carbofuran and vitamin C showing increase of cellularity, X120.

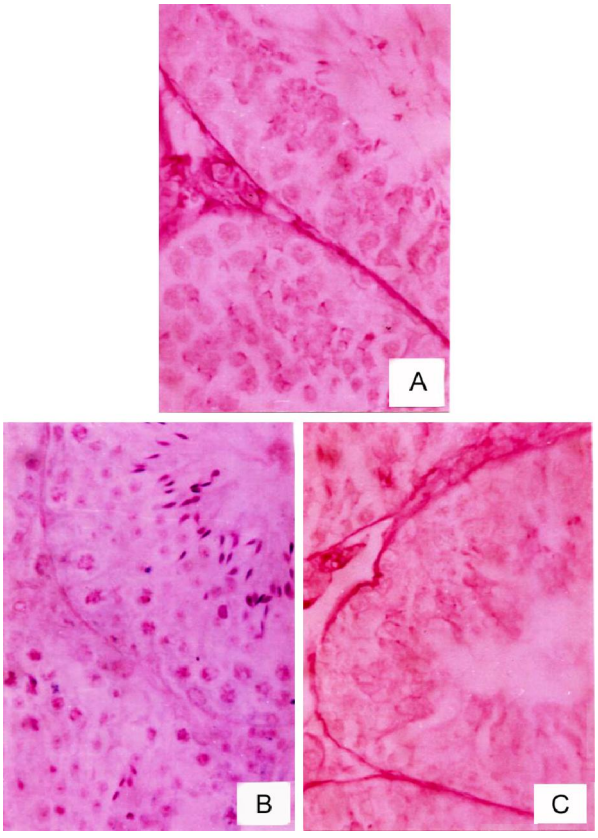

Fig.3. (A). Seminiferous tubule of a control mouse showing normal distribution of PAS-positive materials, X300, **(B).** Seminiferous tubules obtained from a mouse treated with carbofuran showing decrease of the PAS-positive materials, X300, **(C).** Seminiferous tubules of a mouse treated with carbofuran and vitamin C showing a somewhat normal appearance of PAS-positive materials, X300.

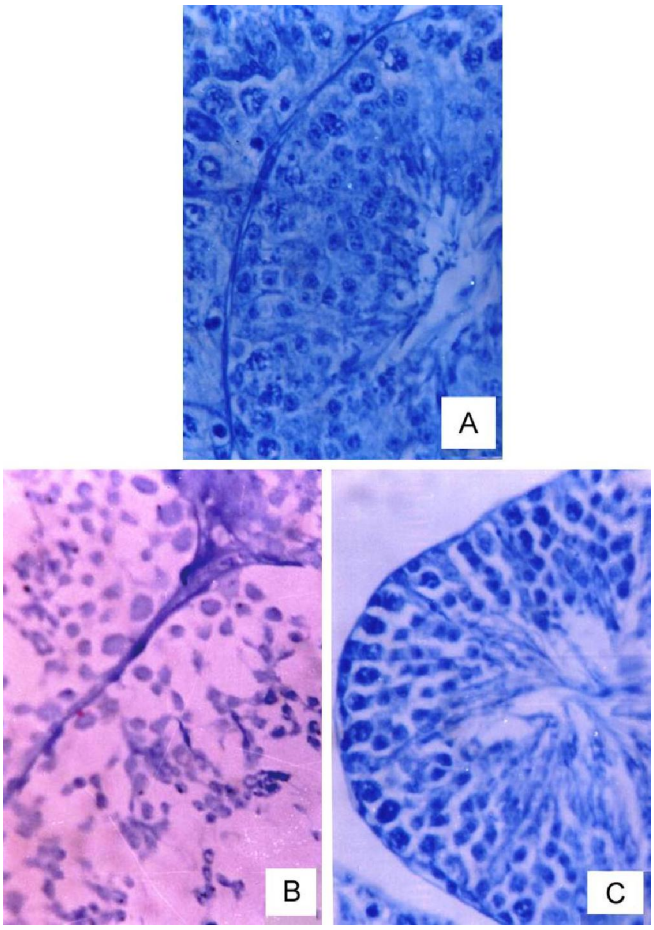

Fig.4. (A). Seminiferous tubules of control mouse showing normal content of proteins, X300, **(B).** Seminiferous tubules of carbofuran–treated mouse showing reduction of proteins, X300, **(C).** An increase of proteins in seminiferous tubules of a mouse treated with carbofuran and vitamin C, X300.

4. Discussion

The wide use of pesticides in recent years has contaminated the environment. Alarming reports on the influence of insecticides on animal reproduction give reasons to believe that such chemicals have an unfavourable influence on the male reproductive system. Results observed in the present work indicated that carbofuran caused harmful effects in testes of albino mice. Histological as well as morphometric results revealed arrest of spermatogenesis. These results are in agreement of Tejada et al, (1988) who reported that carbofuran disrupts testicular morphology and alters activities of enzymes associated with specific cell types of testes. In utero or lactational exposure of carbofuran to male rats caused testicular and spermatotoxicity (Pant et al, 1998). It has been reported that pesticides can cause various histopathological changes in the reproduction system of male mammals (Mahgoub and El-Medany, 2001; Uzunhisarcikli et al, 2007). These changes include decreased spermatogenesis and sperm counts. Farag et al, (2000) observed that acephate, an organophosphate insecticide, decreases the number of spermatogenic cells in the testes and Khan et al, (2001) reported that phosphorothionate, inhibits spermatogenesis. Xu et al, (2004) have found that male rats exposed to phoxim, along with fenvalerate, show decreased daily sperm production. In addition, insecticides not only decrease sperm counts, but also reduce sperm motility (Uzunhisarcikli et al, 2007). Long term exposure of male animals with carbendazim revealed the decreased testicular, epididymal weights, altered sperm morphology, testicular atrophy and thus infertility (Gawande et al, 2009).

Histochemical results showed that carbofuran led to reduction of total carbohydrates as well as total proteins in testicular tissue of mice. Similarly, Sakr and Okdah (2004) reported decrease of carbohydrates and proteins in testes of mice intoxicated with benomyl. Ivanova and Izmirova (1977) reported that

oral administration of the fungicide, cause an inhabitation in protein synthesis in testes and liver of rats. The fungicide, mancozeb was found to decrease protein content in testis, thyroid and adrenal of rat (Nicolau, 1982). Ksheerasagar et al, (2010) has reported that treatment with mancozeb caused significant decrease in the levels of proteins and glycogen in testes of mice.

It was reported that most of the pesticides exert its toxic actions through formation of free radicals. Free radicals resulted in structural abnormalities, some degenerative changes in reproductive system and cross linkage of the nucleic acids (Tuzmen et al, 2008). The testicular alterations recorded in the present study might be due to free radical reactions.

Treating animals with carbofuran and Vitamin C improved the testicular histological and histochemical alterations induced by carbofuran. The protective effect of vitamin C against insecticide toxicity was studied by several investigators. Allhaza and Bashandy (1998) reported that vitamin C alleviates "Pifpaf"" which contain permethrin induced testicular toxicity and decrease of sperm count in rats. Uzunhisarcikli et al, (2007) have also reported that administration of vitamins C and E improves sperm counts, motility, and morphology. Treatment of intoxicated rats with antioxidant vitamins (A,C and E) mixtures reduced the harmful influences of tefluthrin. The frequency of chromosome aberrations in bone marrow cells of intoxicated rats treated with vitamins as well as the frequency of sperm abnormalities were decreased and readjusted near to that of the healthy control animals, also MI and sperm counts were increased significantly near to the control group after vitamins ingestion as treatments (Salah et al, 2009).

Vitamin C can prevent the uncontrolled formation of free radicals and activated oxygen species or inhibit their reaction with biological structures, which affected the immuno-system. It can prevent genetic changes by inhibiting DNA damage induced by ROS (Verma et al, 2007). The observed protection by vitamin C supplementation against carbofuran toxicity might be due to its antioxidant activity.

Corresponding author
Wael M. Al-Amoudi
Department of Biology, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, Saudi Arabia.
Email: wmamoudi@uqu.edu.sa

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