CHAPTER – IV

INDUCTION OF GONADAL, ACCESSORY REPRODUCTIVE ORGANS AND BIOCHEMICAL CONSTITUENTS OF TESTIS, LIVER AND KIDNEY TOXICITY TO MICE AFTER CHRONIC EXPOSURE TO CARBOFURAN
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INTRODUCTION

A review of vast array of therapeutic agents, environmental and industrial chemicals is reported to affect the male reproduction through various mechanisms (Dixon, 1984; Newman, 1984). Impaired spermatogenesis can occur by indirect or direct mechanisms. Indirect impairment involves interference with hormones that promote spermatogenesis, namely testosterone and the gonadotropins (Sundram and Witorsch, 1995). Sperm counts in healthy men around the world have fallen about 50 percent in the last 50 years. Detailed studies of how sperm counts have changed over time in a particular area show the same pattern, with a few exceptions. Researchers hypothesize that exposure to toxic chemicals may be an important cause for the decline (Cox, 1996).

Organophosphorus pesticides such as phosphomidon, methyl parathion, edifenphos and dimethoate affects the testis leading to male reproductive toxicities like degeneration of spermatogenic cells, cause sperm shape abnormalities and decrease in the sperm count (Bhatnagar et al., 1990; Mathew et al., 1992; Jayashree et al., 1994; Mahadevaswami, 2002).

It has been reported that chlorinated pesticide endosulfan and dicofol adversely affects the male reproductive system in mice and rats (Hiremath, 2000; Jadaramkunti and Kaliwal, 2001). The oral administration of carbamate fungicide benomyl in the pubertal and past pubertal rats resulted in decreased testicular weight and decreased sperm count in the epididymis and vas deference (Carter et al., 1984). In rats carbendazim has rapid and direct effects on meiotic spermatocytes and latent effects on spermatids, leading to morphological abnormalities and failure of
spermatogenesis and induces chromosome aberrations in spermatids (Nakai et al., 1997; Matsuo et al., 1999). Recently, it has been reported that a carbamate fungicide mancozeb, affects reproduction by causing gonadal toxicity to male and female rats (Kackar et al., 1997; Mahadevaswami et al., 2000; Baligar and Kaliwal, 2001; Bindali and Kaliwal, 2002; Ksheerasagar, 2001).

Carbofuran has been listed as a potential endocrine disrupter by the German Federal Environment Agency (ENDS, 1999). Yousef et al., (1995) have reported that the exposure of carbofuran caused decline in the sperm counts released in the rabbits. It has been reported that administration of carbofuran causes a dose-dependent decrease in the weight of the body and organs, including epididymidis, seminal vesicles, ventral prostate, coagulation glands and decrease in sperm count and increase in morphological abnormalities of sperm in adult male rats (Pant et al., 1995; 1997). The effect on biochemical constituents on exposure to carbofuran on experimental animals is limited.

In the present study, chapter I has revealed that carbofuran affects the estrous cycle, decreases the number of healthy follicles and increases the number of atretic follicles and also changes the biochemical content of protein, glycogen and total lipids in the ovary, uterus, liver and kidney. Chapter II of the present study revealed that carbofuran affects the estrous cycle, inhibits the compensatory ovarian hypertrophy, decreases the number of healthy follicles with increased number of atretic follicles in hemicastrated mice. Chapter III of the present study has shown the inhibition of implantation while it has not affected the later part of the pregnancy.
Therefore, in this chapter, the investigation has been undertaken to know the effect of carbofuran on histologic and histometric analysis of the testis, epididymis and biochemical constituents such as protein, glycogen and lipids of the testis, liver and kidney in the albino mice.
MATERIALS AND METHODS

Male, Swiss albino mice aged 80-90 days, weighing 25-30 g were used for the experiment. The maintenance of mice is explained in Chapter I. Daily body weight was recorded in the morning before the treatment of carbofuran. The doses were given below the acute LD$_{50}$ level of oral intoxication, according to their body weight. The mice were divided into five groups, each group consisting of ten animals in each experiment.

Experiment I

Carbofuran in doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d was administered orally for 30 consecutive days. The experiment was designed to determine the effective dose of carbofuran on testes, epididymidis and other accessory reproductive organs. Olive oil treated mice served as controls.

Experiment II

To find out the temporal effect of carbofuran on spermatogenesis and accessory reproductive organs, an effective dose of 1.3 mg/kg body weight/d carbofuran was administered orally for 5, 10, 20 and 30 days. Olive oil treated mice served as controls.

All mice were autopsied on 31st day. The testes, epididymidis, vasa deferentia, seminal vesicles, prostate glands, Cowper’s glands and Coagulatory glands were dissected out. The adherent tissues and blood vessels were removed blotted free of blood and mucous and weighed to the nearest milligram in single pan electrical balance. Kidney, adrenal, liver, spleen, thymus and thyroid were also dissected out and weighed.
Histology and Histometry

The testes and epididymis were fixed in aqueous Bouin’s fluid for 24 hours and dehydrated by placing them in 30%, 50%, 70%, 90% and absolute alcohol, cleared in benzene and embedded in paraffin wax. Sections of 5 μm thickness were obtained and stained in Harris haematoxylin eosin (Humoson, 1979). The detailed procedure of histological process is described in chapter I. Histologic measurements were made using ocular and stage micrometer from the sections of each group.

Counting of spermatogenic elements

Randomly chosen 10 good sections of testis from each group were observed under the microscope. The spermatogenic elements like spermatogonia, spermatocytes and spermatids were counted from different round seminiferous tubules of each sections of each group and then the average of each spermatogenic element was calculated.

Biochemical estimations

Protein, glycogen and total lipids were estimated after autopsy on 31st day of the organs like testes, kidney and liver as per the methods mentioned in Chapter I.
OBSERVATIONS

Experiment I

Effect of carbofuran on the testes, accessory reproductive organs, body weight, weight of the organs and biochemical constituents of the testis, liver and kidney in albino mice

Testis (Table 4.1; Graph 4.1)

The mean weight of the testes is 738.96 mg in control mice. The mean weight of the testes with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 739.99, 736.99, 711.02 and 704.44 mg respectively. There is no significant change in the weight of the testes with 0.4 and 0.7 mg carbofuran treatment. However, there is a significant decrease in the weight of the testes with 1 and 1.3 mg carbofuran treatment when compared to that of the control mice.

Number of spermatogenic cells (Table 4.1; Graph 4.2)

The mean number of spermatogonia is 78.80 in control mice. The mean number of spermatogonia with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 77.95, 69.70, 61.95 and 54.55 respectively. There is no significant change in the number of spermatogonia with 0.4 mg carbofuran treatment. However, there is a significant decrease in the number of spermatogonia with 0.7, 1 and 1.3 mg carbofuran treatment when compared to that of the control mice.

The mean number of spermatocytes is 83.65 in control mice. The mean number of spermatocytes with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 81.00, 75.05, 69.85 and 64.45 respectively. There is no significant change in the number of spermatocytes with 0.4 mg carbofuran treatment. However, there is a significant
decrease in the number of spermatocytes with 0.7, 1 and 1.3 mg carbofuran treatment when compared to that of the control mice.

The mean number of spermatid is 114.45 in control mice. The mean number of spermatids with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 110.30, 86.25, 65.20 and 46.20 respectively. There is no significant change in the number of spermatids with 0.4 mg carbofuran treatment. However, there is a significant decrease in the number of spermatids with 0.7, 1 and 1.3 mg carbofuran treatment when compared to that of the control mice.

Diameter of the testes, seminiferous tubules and spermatogenic cells (Table 4.2; Graphs 4.1, 4.3)

The mean diameter of the testes is 3.45 mm in control mice. The mean diameter of the testes with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 3.42, 3.46, 3.40 and 3.35 mm respectively. There is no significant change in the diameter of the testes of all the carbofuran treated mice when compared to that of control mice.

The mean diameter of the seminiferous tubule is 221.48 μm in control mice. The mean diameter of the seminiferous tubule with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 218.56, 221.12, 217.99 and 216.99 μm respectively. There is no significant change in the diameter of the seminiferous tubule of all the carbofuran treatment when compared to that of the control mice.

The mean diameter of spermatogonia is 7.88 μm in control mice. The mean diameter of the spermatogonia with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 7.92, 7.52, 7.42 and 6.99 μm respectively. There is no significant change in the diameter of the spermatogonia with 0.4, 0.7 and 1 mg carbofuran treatment.
However, there is a significant decrease in the diameter of spermatogonia with 1.3 mg carbofuran treatment when compared to that of the control mice.

The mean diameter of spermatocytes is 9.85 μm in control mice. The mean diameter of the spermatocytes with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 9.91, 9.88, 9.55 and 8.89 μm respectively. There is no significant change in the diameter of the spermatocytes with 0.4, 0.7 and 1 mg carbofuran treatment. However, there is a significant decrease in the diameter of spermatocytes with 1.3 mg carbofuran treatment when compared to that of the control mice.

The mean diameter of spermatids is 6.46 μm in control mice. The mean diameter of the spermatids with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 6.34, 6.32, 6.29 and 5.78 μm respectively. There is no significant change in the diameter of the spermatids with 0.4, 0.7 and 1 mg carbofuran treatment. However, there is a significant decrease in the diameter of spermatids with 1.3 mg carbofuran treatment when compared to that of the control mice.

The results in the present study indicate that there is a significant decrease in the weight of the testes with 1 and 1.3 mg carbofuran treatment. However, there is no significant change in the weight of the testes with 0.4 and 0.7 mg carbofuran treatment. Treatment with 0.7, 1 and 1.3 mg carbofuran shows a significant decrease in the number of spermatogonia, spermatocytes and spermatids. However, there is no significant change in the number of the spermatogonia, spermatocytes and spermatids with 0.4 mg carbofuran treatment. Treatment with 1.3 mg carbofuran shows a significant decrease in the diameter of the spermatogonia, spermatocytes and spermatids. However, there is no significant change in the diameter of the
spermatogonia, spermatocytes and spermatids with 0.4, 0.7 and 1 mg carbofuran treatment. There is no significant change in the diameter of the testes and seminiferous tubules of all the carbofuran treated mice. It is dose dependent (Tables 4.1, 4.2; Graphs 4.1, 4.2, 4.3).

**Histologic observations**

Histologic observations of the testes of the control mice consist of seminiferous tubules and inter-tubular elements. The seminiferous tubules show normal spermatogenesis with all cell types and well developed interstitial cells. Each seminiferous tubule shows the tubular wall with the outermost basement membrane. Resting on the basement membrane are the spermatogonia and the Sertoli cells. Towards the lumen, the primary spermatocytes, secondary spermatocytes and spermatids adhere to the Sertoli cells. Sperms are seen with heads embedded in the Sertoli cells and the tails lying in the lumen (Fig.1).

T.S. of the testis of the mouse treated with 0.4 mg carbofuran shows normal spermatogenesis. The seminiferous tubules are closely packed. The inter-tubular spaces are packed with interstitial tissue, containing clusters of Leydig cells (Fig.2).

T.S. of the testis of the mouse treated with 0.7 mg carbofuran shows symptoms of the arrest of spermatogenesis. The seminiferous tubules are closely packed. The spermatogonia and spermatocytes are affected (Fig.3). T.S. of the testis of the mouse treated with 1 mg carbofuran show symptoms of arrest of spermatogenesis. The seminiferous tubules are closely packed. The epithelium, spermatogonia, spermatocytes and spermatids are affected. Leydig cells are highly compact (Fig.4).

T.S. of the testis of mouse treated with 1.3 mg carbofuran shows complete arrest of
spermatogenesis. The seminiferous tubules are disorganized. The epithelium, spermatogonia, spermatocytes and spermatids are severely damaged and degenerated. Leydig cells are highly compact on one side leading to the disruption on the other side. The degenerative changes are observed, wherein the tubules show necropsied spermatogenic cells and the lumen was empty of active sperms. The degenerative tubule and spermatogenic arrest is seen (Fig.5).

**Accessory reproductive organs (Table 4.3; Graph 4.4)**

**Epididymidis**

The mean weight of the epididymidis is 312.63 mg in control mice. The mean weight of the epididymids with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 316.33, 313.30, 307.37 and 298.99 mg respectively. There is no significant change in the weight of the epididymidis with 0.4, 0.7 and 1 mg carbofuran treatment. However, there is a significant decrease in the weight of the epididymidis with 1.3 mg carbofuran treatment when compared to that of the control mice.

The histologic structure of the caput epididymis of the control mice consists of highly contorted tubules with almost uniform diameter. The epithelium consists of typical pseudostratified tall columnar cells. Prominent spherical nuclei are found close to the basement membrane. Beyond the level of the nuclei, towards the lumen, the cells are vacuolated. The lumen is packed with a compact mass of sperms with a clear zone between the sperms and the epithelium (Fig.6). Treatment with 0.4 mg carbofuran shows the normal sperm concentration in the lumen and the inter-tubular spaces with compact tubules as that of controls (Fig.7). Treatment with 0.7 and 1 mg carbofuran shows a slight decrease in the sperm concentration in the lumen of
the tubules (Fig. 8 and 9). Treatment with 1.3 mg carbofuran shows significant decrease in the sperm concentration in the lumen of the tubules (Fig. 10).

**Vasa deferentia**

The mean weight of the vasa deferentia is 100.66 mg in control mice. The mean weight of the vasa deferentia with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 98.33, 97.62, 97.88 and 96.64 mg respectively. There is no significant change in the weight of the vasa deferentia of all the carbofuran treated mice when compared to that of the control mice.

**Seminal vesicles**

The mean weight of the seminal vesicles is 623.17 mg in control mice. The mean weight of the seminal vesicles with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 616.06, 617.02, 614.42 and 610.54 mg respectively. There is no significant change in the weight of seminal vesicles with 0.4, 0.7 and 1 mg carbofuran treatment. However, there is a significant decrease in the weight of the seminal vesicles with 1.3 mg carbofuran treatment when compared to that of the control mice.

**Prostate glands**

The mean weight of the prostate glands is 83.66 mg in control mice. The mean weight of the prostate glands with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 84.38, 82.33, 78.66 and 73.99 mg respectively. There is no significant change in the weight of the prostate gland with 0.4 and 0.7 mg carbofuran treatment. However, there is a significant decrease in the weights of the prostate glands with 1 and 1.3 mg carbofuran treatment when compared to that of the control mice.
Coagulatory glands

The mean weight of the coagulatory glands is 25.19 mg in control mice. The mean weight of the coagulatory glands with 0.4, 0.7, 1 and 1.3 mg carbofuran is 25.59, 24.58, 23.78 and 19.04 mg respectively. There is no significant change in the weight of the coagulatory glands with 0.4, 0.7, and 1 mg carbofuran treatment. However, there is a significant decrease in the coagulatory glands with 1.3 mg carbofuran treatment when compared to that of the control mice.

Cowper’s glands

The mean weight of the Cowper’s glands is 26.66 mg in control mice. The mean weight of the Cowper’s glands with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 25.99, 2307, 24.94 and 19.58 mg respectively. There is no significant change in the weight of the Cowper’s glands with 0.4, 0.7 and 1 mg carbofuran treatment. However, there is a significant decrease in the weight of the Cowper’s glands with 1.3 mg carbofuran treatment when compared to that of the control mice.

The results of the present study indicate that there is a significant decrease in the weight of the epididymidis, seminal vesicles, prostate glands, coagulatory glands and Cowper’s glands with 1.3 mg carbofuran treatment. Treatment with 1 mg carbofuran reveals a significant decrease in the weight of the prostate gland. However, there is no significant change in the weight of all the accessory reproductive organs with 0.4 and 0.7 mg carbofuran treatment (Table 4.3; Graph 4.4).
Body weight (Table 4.4; Graph 4.5)

Change in the mean body weight is 3.40 g in control mice when compared to that of the initial body weight. Change in the mean body weight with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 3.20, 3.10, 2.20 and 1.50 g respectively. There is no significant change in the body weight with 0.4 and 0.7 mg carbofuran treatment. However, there is a significant decrease in the gain of the body weight with 1 and 1.3 mg carbofuran treatment when compared to that of the control mice.

Organs weight (Table 4.4; Graphs 4.5, 4.6)

Kidneys

The mean weight of the kidney is 1.64 g in control mice. The mean weight of the kidneys with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 1.55, 1.51, 1.47 and 1.38 g respectively. There is no significant change in the weight of the kidneys of all the carbofuran treated mice when compared to that of the control mice.

Adrenals

The mean weight of the adrenals is 42.33 mg in control mice. The mean weight of the adrenals with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 41.73, 41.93, 40.31 and 39.31 mg respectively. There is no significant change in the weight of the adrenals of all the carbofuran treated mice when compared to that of the control mice.

Spleen

The mean weight of the spleen is 438.66 mg in control mice. The mean weight of the spleen with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 446.66, 443.33, 439.99 and 434.99 mg respectively. There is no significant change in the
weight of the spleen of all the carbofuran treated mice when compared to that of the control mice.

Liver

The mean weight of the liver is 5.18 g in control mice. The mean weight of the liver with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 4.99, 5.38, 5.12 and 4.66 g respectively. There is no significant change in the weight of the liver of all the carbofuran treated mice when compared to that of the control mice.

Thymus

The mean weight of the thymus is 83.69 mg in control mice. The mean weight of the thymus with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 85.00, 81.33, 80.33 and 79.99 g respectively. There is no significant change in the weight of the thymus of all the carbofuran treated mice when compared to that of the control mice.

Thyroid

The mean weight of the thyroid is 13.86 mg in control mice. The mean weight of thyroid with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 13.07, 14.07, 14.09 and 15.00 g respectively. There is no significant change in the weight of the thyroid of all the carbofuran treated mice when compared to that of the control mice.

The results of the present study indicate that the treatment with 1 and 1.3 mg carbofuran causes a significant decrease in the body weight. However, there is no significant change in the weights of the kidney, adrenals, spleen, liver, thymus and thyroid in all the carbofuran treated mice (Table 4.4; Graphs 4.5, 4.6).
Biochemical constituents

Testes (Table 4.5; Graph 4.7)

The levels of the testes protein, glycogen and total lipids are 89.64, 7.02 and 37.84 µg respectively in the control mice. The levels of testes protein, glycogen and total lipids are 87.92, 6.62 and 36.4 µg with 0.4 mg carbofuran treatment. There is no significant change in the levels of the protein, glycogen and total lipids of all the carbofuran treated mice when compared to those of the corresponding parameters of the control mice.

The levels of the testes protein, glycogen and total lipids are 85.66, 6.50 and 40.92 µg respectively with 0.7 mg carbofuran treatment. There is no significant change in the levels of protein, glycogen and total lipids of all the carbofuran treated mice when compared to those of the corresponding parameters of the control mice.

The levels of the testes protein, glycogen and total lipids are 82.57, 5.60 and 44.28 µg respectively with 1 mg carbofuran treatment. There is a significant decrease in the levels of protein and glycogen, but there is a significant increase in the levels of the total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the testes protein, glycogen and total lipids are 80.14, 4.26, 46.68 µg respectively with 1.3 mg carbofuran treatment. There is a significant decrease in the levels of protein and glycogen, but there is a significant increase in the levels of total lipids when compared to those of the corresponding parameters of the control mice.
The results indicate that the treatment with 1 and 1.3 mg carbofuran causes a significant decrease in the levels of the testes protein and glycogen with a significant increase in the levels of the total lipids. There is no significant change in the levels of testes protein, glycogen and total lipids with 1 and 1.3 mg carbofuran treatment. It is dose dependent (Table 4.5; Graph 4.7).

Liver (Table 4.6; Graph 4.8)

The levels of the liver protein, glycogen and total lipids are 180.40, 4.48 and 40.76 μg respectively in the control mice. The levels of the liver protein, glycogen and total lipids are 182.20, 4.74 and 40.10 μg respectively with 0.4 mg carbofuran treatment. There is no significant change in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the liver protein, glycogen and total lipids are 178.80, 4.32 and 37.94 μg respectively with 0.7 mg carbofuran treatment. There is no significant change in the levels of the protein and glycogen. However, there is a significant decrease in the levels of the total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the liver protein, glycogen and total lipids are 175.50, 3.20 and 35.46 μg respectively with 1 mg carbofuran treatment. There is no significant change in the levels of the protein. However, there is a significant decrease in the levels of the glycogen and total lipids when compared to those of the corresponding parameters of the control mice.
The levels of the liver protein, glycogen and total lipids are 171.20, 2.96 and 32.90 µg respectively with 1.3 mg carbofuran treatment. There is a significant decrease in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The results indicate that the treatment with 1.3 mg carbofuran causes a significant decrease in the levels of the liver protein, glycogen and total lipids. Treatment with 1 mg carbofuran causes a significant decrease in the levels of the liver glycogen and total lipids, but there is no significant change in the level of the liver protein. Treatment with 0.7 mg carbofuran causes a significant decrease in the levels of the total lipids of the liver. However, there is no significant change in the levels of the liver protein, glycogen and total lipids with 0.4 mg carbofuran treatment. It is dose dependent (Table 4.6; Graph 4.8).

Kidney (Table 4.7; Graph 4.9)

The levels of the kidney protein, glycogen and total lipids are 92.36, 4.56 and 33.86 µg respectively in control mice. The levels of the kidney protein, glycogen and total lipids are 92.54, 4.28 and 33.56 µg respectively with 0.4 mg carbofuran treatment. There is no significant change in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the kidney protein, glycogen and total lipids are 89.50, 3.78 and 31.90 µg respectively with 0.7 mg carbofuran treatment. There is no significant change in the levels of the protein and total lipids. However, there is a significant decrease in the level of the glycogen when compared to that of control mice.
The levels of the kidney protein, glycogen and total lipids are 87.72, 3.40 and 29.08 μg respectively with 1 mg carbofuran treatment. There is a significant decrease in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the kidney protein, glycogen and total lipids are 84.86, 2.42 and 26.80 μg respectively with 1.3 mg carbofuran treatment. There is a significant decrease in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The results of the present study indicate that the treatment with 1 and 1.3 mg carbofuran causes a significant decrease in the levels of the kidney protein, glycogen and total lipids. Treatment with 0.7 mg carbofuran causes a significant decrease in the kidney glycogen, but protein and total lipids are not changed significantly. However, there is no significant change in the levels of the protein, glycogen and total lipids with 0.4 mg carbofuran treatment. It is dose dependent (Table 4.7; Graph 4.9).

Experiment II

Temporal effect of carbofuran on the testis, accessory reproductive organs, body weight, weight of the organs and biochemical constituents of the testis, liver and kidney in albino mice

Testis (Table 4.8; Graph 4.10)

The mean weight of the testis is 738.96 mg in control mice. The mean weight of the testis with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 735.66, 733.99, 730.33 and 704.45 mg respectively. There is no significant change in the
weight of the testis with 1.3 mg carbofuran treatment for 5 and 10 days. However, there is a significant decrease in the weight of the testis with 1.3 mg carbofuran treatment for 20 and 30 days when compared to that of the control mice.

Number of spermatogenic cells (Table 4.8; Graph 4.11)

The mean number of the spermatogonia is 78.80 in control mice. The mean number of spermatogonia with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 75.25, 70.25, 63.57 and 54.55 respectively. There is no significant change in the number of spermatogonia with 1.3 mg carbofuran treatment for 5 days. However, there is a significant decrease in the number of the spermatogonia with 1.3 mg carbofuran treatment for 10, 20 and 30 days when compared to that of the control mice.

The mean number of the spermatocytes is 83.65 in control mice. The mean number of spermatocytes with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 80.00, 78.35, 76.60 and 64.45 respectively. There is no significant change in the number of the spermatocytes with 1.3 mg carbofuran treatment for 5 and 10 days. However, there is a significant decrease in the number of spermatocytes with 1.3 mg carbofuran treatment for 20 and 30 days when compared to that of the control mice.

The mean number of spermatids is 114.45 in control mice. The mean number of spermatids with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 113.75, 107.50, 74.60 and 46.20 respectively. There is no significant change in the number of spermatids with 1.3 mg carbofuran treatment for 5 and 10 days. However, there is
a significant decrease in the number of spermatids with 1.3 mg carbofuran treatment for 20 and 30 days when compared to that of the control mice.

**Diameter of the testis, seminiferous tubules and spermatogenic cells (Table 4.9; Graphs 4.10, 4.12)**

The mean diameter of the testis is 3.45 mm in control mice. The mean diameter of the testis with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 3.43, 3.38, 3.38 and 3.35 mm respectively. There is no significant change in the diameter of the testis when compared to that of the control mice.

The mean diameter of the seminiferous tubules is 221.48 µm in control mice. The mean diameter of the seminiferous tubules with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 220.98, 221.81, 218.46 and 216.99 µm respectively. There is no significant change in the diameter of the seminiferous tubules when compared to that of the control mice.

The mean diameter of the spermatogonia is 7.88 µm in control mice. The mean diameter of the spermatogonia with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 7.68, 7.43, 7.12 and 6.99 µm respectively. There is no significant change in the diameter of the spermatogonia with 1.3 mg carbofuran treatment for 5 and 10 days. However, there is a significant decrease in the diameter of the spermatogonia with 1.3 mg carbofuran treatment for 20 and 30 days when compared to that of the control mice.

The mean diameter of the spermatocytes is 9.85 µm in control mice. The mean diameter of the spermatocytes with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 9.71, 9.55, 9.24 and 8.89 µm respectively. There is no significant
change in the diameter of the spermatocytes with 1.3 mg carbofuran treatment for 5, 10 and 20 days. However, there is significant decrease in the diameter of the spermatocytes with 1.3 mg carbofuran treatment for 30 days when compared to that of control mice.

The mean diameter of the spermatids is 6.46 μm in control mice. The mean diameter of spermatid with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 6.51, 6.22, 6.03 and 5.78 μm respectively. There is no significant change in the diameter of the spermatids with 1.3 mg carbofuran treatment for 5, 10 and 20 days. However, there is a significant decrease in the diameter of the spermatids with 1.3 mg carbofuran treatment for 30 days when compared to that of the control mice.

The results of the present study indicate that there is a significant decrease in the weight of the testis with 1.3 carbofuran treatment for 20 and 30 days. However, there is no significant change in the weight of the testis with 1.3 mg carbofuran treatment for 5 and 10 days. Treatment with 1.3 mg carbofuran for 10, 20 and 30 days causes a significant decrease in the number of spermatogonia, but there is no significant change with 1.3 mg carbofuran treatment for 5 days. Treatment with 1.3 mg carbofuran for 20 and 30 days causes a significant decrease in the number of spermatocytes and spermatids, but there is no significant change with 1.3 mg carbofuran treatment for 5 and 10 days. Treatment with 1.3 mg carbofuran for 20 and 30 days causes a significant decrease in the diameter of the spermatogonia, but there is no significant change in the diameter of the spermatogonia with 1.3 mg carbofuran treatment for 5 and 10 days. Treatment with 1.3 mg carbofuran for 30 days caused a significant decrease in the diameter of the spermatocytes and
spermatids but, there is no significant decrease in the diameter of the spermatocytes and spermatids with 1.3 mg carbofuran treatment for 5, 10 and 20 days. Treatment with 1.3 mg carbofuran for 5, 10, 20 and 30 days show that there is no significant change in the diameter of the testis and seminiferous tubules (Tables 4.8, 4.9; Graphs 4.10, 4.11, 4.12).

**Histologic observations**

Histologic observations of the testis of the control mice consist of seminiferous tubules and inter-tubular elements. The seminiferous tubules display spermatogenesis with all cell types and well-developed interstitial cells. Each seminiferous tubule shows the tubular wall with the outermost basement membrane. Resting on the basement membrane are the spermatogonia and Sertoli cells. Towards the lumen, the primary spermatocytes, secondary spermatocytes and spermatids are connected to the Sertoli cells. Sperms are seen with heads embedded in the Sertoli cells and the tails lying in the lumen (Fig.11). T.S. of the testis of the mouse treated with 1.3 mg carbofuran for 5 days shows normal spermatogenesis. The seminiferous tubules are seen closely packed. The inter-tubular spaces are packed with interstitial tissue, containing the clusters of the Leydig cells (Fig.12). T.S. of the mouse treated with 1.3 mg carbofuran for 10 days shows normal spermatogenesis. The seminiferous tubules are seen closely packed. However, the inter-tubular spaces are highly increased (Fig.13). T.S. of the testis of the mouse treated with 1.4 mg carbofuran for 20 days shows symptoms of arrest of spermatogenesis. The seminiferous tubules are closely packed. The epithelium, spermatogonia and spermatocytes are affected (Fig.14). T.S. of the testis of the
mouse treated with 1.3 mg carbofuran for 30 days shows symptoms of arrest of spermatogenesis. The seminiferous tubules are closely packed. The epithelium, spermatogonia, spermatocytes are severely affected and degenerated. Leydig cells are highly compact (Fig. 15).

**Accessory reproductive organs (Table 4.10; Graph 4.13)**

**Epididymis**

The mean weight of the epididymis is 312.63 mg in control mice. The mean weight of the epididymis with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 311.70, 306.66, 301.66 and 298.99 mg respectively. There is no significant change in the weight of the epididymis with all the 1.3 mg carbofuran treatment for 5 and 10 days. However, there is a significant decrease in the weight of the epididymis with 1.3 mg carbofuran treatment for 20 and 30 days.

The cauput epididymis of the control mice consists of highly contorted tubules with almost uniform diameter. The epithelium consists of typical pseudostratified tall columnar cells. Prominent spherical nuclei were found close to the basement membrane. Beyond the level of the nuclei, towards the lumen, the cells were vacuolated. The lumen was packed with a compact mass of sperms with a clear zone between the sperms and the epithelium (Fig. 16).

Treatment with 1.3 mg carbofuran for 5 and 10 days shows the normal sperm concentration in the lumen, and inter-tubular spaces with compact tubules as that of the controls (Fig. 17 and 18). Treatment with 1.3 mg carbofuran for 20 days shows decrease in the sperm concentration in the lumen of the tubules (Fig.19).
with 1.3 mg carbofuran for 30 days shows significant decrease in the sperm concentration in the lumen of the tubules (Fig.20).

**Vasa deferentia**

The mean weight of the vasa deferentia is 100.66 mg in the control mice. The mean weight of the vasa deferentia with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 102.66, 100.99, 97.97 and 96.64 mg respectively. There is no significant change in the weight of the vasa deferentia of all the carbofuran treated mice when compared to that of the control mice.

**Seminal vesicles**

The mean weight of the seminal vesicles is 623.17 mg in control mice. The mean weight of the seminal vesicles with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 621.20, 616.99, 613.44 and 610.54 mg respectively. There is no significant change in the weight of seminal vesicles of all the carbofuran treated mice when compared to that of the control mice.

**Prostate glands**

The mean weight of the prostate glands is 83.66 mg in control mice. The mean weight of the prostate glands with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 88.33, 82.66, 78.33 and 73.99 mg respectively. There is no significant change in the weight of the prostate glands with 1.3 mg carbofuran treatment for 5, 10 and 20 days. However, there is a significant decrease in the weight of the prostate glands with 1.3 mg carbofuran treatment for 30 days when compared to that of the control mice.
Coagulatory glands

The mean weight of the coagulatory glands is 25.19 mg in control mice. The mean weight of the coagulatory glands with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 24.46, 21.66, 20.92 and 19.04 mg respectively. There is no significant change in the weight of the coagulatory gland with 1.3 mg carbofuran treatment for 5, 10 and 20 days. However, there is a significant decrease in the weight of the prostate glands with 1.3 mg carbofuran treatment for 30 days when compared to that of the control mice.

Cowper’s glands

The mean weight of the Cowper’s glands is 26.66 mg in control mice. The mean weight of the Cowper’s glands with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 23.66, 24.33, 22.96 and 19.58 mg respectively. There is no significant change in the weight of the Cowper’s glands with 1.3 mg carbofuran treatment for 5, 10 and 20 days. However, there is a significant decrease in the weight of the Cowper’s glands with 1.3 mg carbofuran treatment for 30 days when compared to that of the control mice.

The results of the present study indicate that the mean weight of the epididymidis, seminal vesicle, prostate glands, coagulatory glands, Cowper’s glands are significantly decreased with 1.3 mg carbofuran treatment for 30 days. There is also a significant decrease in the weight of the epididymidis with 1.3 mg carbofuran treatment for 20 days. However, there is no significant change in other accessory reproductive organs with 1.3 mg carbofuran treatment for 5, 10 and 20 days. (Table 4.10; Graph 4.13).
Body weight (Table 4.11; Graph 4.14)

Change in the mean body weight is 3.40 g in control mice when compared with that of their initial body weight. The change in mean body weight with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 3.20, 2.90, 2.80 and 1.50 respectively. There is no significant change in the weight of the body with 1.3 mg carbofuran treatment for 5, 10 and 20 days. However, there is a significant decrease in the weight of the body with 1.3 mg carbofuran treatment for 30 days when compared to that of control mice.

Organs weight (Table 4.11; Graphs 4.14, 4.15)

Kidneys

The mean weight of the kidneys is 1.64 g in control mice. The mean weight of the kidneys with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 1.62, 1.61, 1.55 and 1.47 g respectively. There is no significant change in the weight of the kidney of all the carbofuran treated mice when compared to that of the control mice.

Adrenals

The mean weight of the adrenals is 42.33 mg in control mice. The mean weight of the adrenals with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 43.23, 41.66, 39.97 and 39.31 mg respectively. There is no significant change in the weight of the adrenals of all the carbofuran treated mice when compared to that of the control mice.
Spleen

The mean weight of the spleen is 438.66 mg in control mice. The mean weight of the spleen with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 439.66, 438.33, 437.33 and 434.99 mg respectively. There is no significant change in the weight of the spleen of all the carbofuran treated mice when compared to that of the control mice.

Liver

The mean weight of the liver is 51.18 g in control mice. The mean weight of the liver with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 4.09, 4.99, 4.84 and 4.66 g respectively. There is no significant change in the weight of the liver of all the carbofuran treated mice when compared to that of the control mice.

Thymus

The mean weight of the thymus is 83.69 mg in control mice. The mean weight of the thymus in 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 85.19, 84.95, 82.42 and 79.99 mg respectively. There is no significant change in the weight of the thymus of all the carbofuran treated mice when compared to that of the control mice.

Thyroid

The mean weight of the thyroid is 13.86 mg in control mice. The mean weight of the thyroids with 1.3 mg carbofuran treatment for 5, 10, 20 and 30 days is 13.79, 14.73, 14.99 and 15.00 mg respectively. There is no significant change in the weight of the thyroid of all the carbofuran treated mice when compared to that of the control mice.
The results of the present study indicate that the treatment with 1.3 mg carbofuran for 30 days causes a significant decrease in the body weight. However, there is no significant change in the weight of the kidneys, adrenals, spleen, liver, thymus and thyroid of all the carbofuran treated mice.

**Biochemical constituents**

**Testis (Table 4.12; Graph 4.16)**

The levels of the testis protein, glycogen and total lipids are 89.64, 7.02 and 37.84 µg respectively in the control mice. The levels of the testis protein, glycogen and total lipids are 90.00, 7.06 and 37.90 µg with 1.3 mg carbofuran treatment for 5 days respectively. There is no significant change in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the testis protein, glycogen and total lipids are 89.12, 7.02 and 40.80 µg with 1.3 mg carbofuran treatment for 10 days respectively. There is no significant change in the levels of protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the testis protein, glycogen and total lipids are 88.08, 6.60 and 43.14 µg respectively in mice treated with 1.3 mg carbofuran treatment for 20 days. There is no significant change in the levels of the protein and glycogen. However, there is significant increase in the levels of the total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the testis protein, glycogen and total lipids are 80.14, 4.26 and 46.68 µg respectively in mice treated with 1.3 mg carbofuran treatment for 30 days.
There is significant decrease in the levels of protein and glycogen but, there is a significant increase in the levels of total lipids when compared to those of the corresponding parameters of the control mice.

The results indicate that treatment with 1.3 mg carbofuran for 30 days causes a significant decrease in the levels of the testis protein and glycogen with a significant increase in the total lipids. Treatment with 1.3 mg carbofuran for 20 days causes a significant increase in the levels of the testis total lipids, but the levels of the protein and glycogen are not changed significantly. However, treatment with 1.3 mg carbofuran for 5 and 10 days causes no significant change in the levels of the testis protein, glycogen and total lipids. It is duration dependent (Table 4.12; Graph 4.16).

Liver (Table 4.13; Graph 4.17)

The levels of the liver protein, glycogen and total lipids are 180.40, 4.48 and 40.76 μg respectively in control mice. The levels of the liver protein, glycogen and total lipids are 177.40, 4.68 and 41.08 μg respectively with 1.3 mg carbofuran treatment for 5 days. There is no significant change in the levels of protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the liver protein, glycogen and total lipids are 176.80, 4.56 and 40.18 μg respectively with 1.3 mg carbofuran treatment for 10 days. There is no significant change in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.
The levels of the liver protein, glycogen and total lipids are 176.20, 4.06 and 37.76 µg respectively with 1.3 mg carbofuran treatment for 20 days. There is no significant change in the levels of the protein and glycogen, but there is a significant decrease in the levels of the total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the liver protein, glycogen and total lipids are 171.20, 2.96 and 32.90 µg in respectively with 1.3 mg carbofuran treatment for 30 days. There is a significant decrease in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The results indicate that the treatment with 1.3 mg carbofuran for 30 days causes a significant decrease in the levels of the liver protein, glycogen and total lipid. Treatment with 1.3 mg carbofuran for 20 days causes a significant decrease in the total lipids, but no significant change in the levels of protein and glycogen. However, treatment with 1.3 mg carbofuran for 5 and 10 days causes no significant change in the levels of the protein, glycogen and total lipids. It is duration dependent (Table 4.13; Graph 4.17).

**Kidney (Table 4.14; Graph 4.18)**

The levels of the kidney protein, glycogen and total lipids are 92.36, 4.56 and 33.86 µg respectively in control mice. The levels of the kidney protein, glycogen and total lipids are 93.80, 4.66 and 33.56 µg respectively with 1.3 mg carbofuran treatment for 5 days. There is no significant change in the levels of protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.
The levels of the kidney protein, glycogen and total lipids are 92.40, 4.16 and 32.08 μg respectively with 1.3 mg carbofuran treatment for 10 days. There is no significant change in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the kidney protein, glycogen and total lipids are 90.92, 4.08 and 32.06 μg respectively with 1.3 mg carbofuran treatment for 20 days. There is no significant change in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The levels of the kidney protein, glycogen and total lipids are 84.86, 2.42 and 46.80 μg respectively with 1.3 mg carbofuran treatment for 30 days. There is a significant decrease in the levels of the protein, glycogen and total lipids when compared to those of the corresponding parameters of the control mice.

The results indicate that treatment with 1.3 mg carbofuran for 30 days causes a significant decrease in the levels of the kidney protein, glycogen and total lipids. However, treatment with 1.3 mg carbofuran for 5, 10 and 20 days causes no significant change in the levels of the kidney protein, glycogen and total lipids. It is duration dependent (Table 4.14; Graph 4.18).
DISCUSSION

Effect of carbofuran on the testes and accessory reproductive organs in albino mice

The testis is a complex organ containing three important cell types (germ cells, Sertoli cells and Leydig cells) in close proximity with unique autonomic and vascular features, regulated by endocrine, paracrine and autocrine mechanisms. It has two well established functions namely spermatogenesis and steroidogenesis. The anterior pituitary participates in the control of both of these functions through the secretion of gonadotropins, Follicle Stimulating Hormone (FSH) and Lutenizing Hormone (LH) (Steinberger and Steinberger, 1975; Sharpe, 1987). Luteinizing hormone (LH), secreted by the anterior pituitary gland, under stimulation from the hypothalamus, acts on the Leydig cells within the testis to induce the synthesis and secretion of testosterone which allows normal spermatogenic development. In the testes, testosterone is synthesized almost exclusively within the Leydig cells (Ewing and Zirkin, 1983; Neaves, 1977). A block in the production and release of testosterone can occur either at the site of the Leydig cell or via an effect on the pituitary or hypothalamus by inhibiting the release of LH (Martin et al., 1998).

The testis of humans and other mammals are highly susceptible to damage caused by genetic disorders, environmental or occupational exposure to chemicals or by other means. Specific causes of testicular damage have been catalogued by several workers (Jackson and Ericsson, 1970; Lucier et al., 1977), although these listings are by no means complete. Quality of sperm production has been adversely affected due to the exposure of certain drugs and chemicals, particularly mutagens.
and teratogens. Pesticides can be translocated, bioconcentrated or converted into more dangerous chemicals (Matsumura et al., 1972). There are some reports of varying degrees of testicular dysfunction in pesticide factory workers such as oligospermia, azospermia, degeneration of the germinal epithelium in testicular biopsies and elevated serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Whorton et al. 1979; Patashnik et al., 1978). There is a clear correlation between the degrees, duration of exposure to pesticides, the extent of spermatogenic arrest and hormonal imbalance.

The primary function of the Leydig cell is the biosynthesis and secretion of testosterone, the principal circulating androgen. The synthesis and secretion of testosterone from the Leydig cell is under the control of the pituitary, luteinizing hormone (LH), which in turn is regulated by luteinizing hormone releasing hormone (LHRH). The biosynthesis of testosterone involves numerous reactions and requires several enzymes and cofactors that carry out the process in the mitochondria, as well as in the microsomes. Testosterone or its metabolites play an important role in the support of sexual behaviour and in maintaining the reproductive tract, including the maintenance of spermatogenesis (Sundaram and Witorsch, 1995). Estrogens also have anti-androgenic properties in the males causing azoospermia and reduction of plasma testosterone levels (Verjans et al., 1974; Hunt et al., 1979), another mechanism of action of estrogenic substances is inhibiting the testicular steroidogenesis (Samuel et al., 1964, 1967; Oshima et al., 1967). In certain cases higher doses of estrogens are known to inhibit the male reproductive function (Kalra and Prasad, 1967; Samuel et al., 1964, 1967). The target organs for estrogens of any
origin in the male are the testis, epididymis, vas deferens, seminal vesicle and prostate gland (Stupf et al., 1971; Van Beurdan-Lamers et al., 1974). Epididymidis are important because they provide suitable environment for morphological and biochemical changes in spermatozoa (Orgebin-Crist, 1969). The spermatozoa attain their maturity and fertility capacity during their passage in the efferent ducts and highly convoluted single tubular epididymis.

It has been stated that the long epididymis provides suitable environment for the spermatozoa to undergo fertilization. The epididymis provides a favourable milieu for the acquisition of fertilizing ability, motility, storage and survival of the spermatozoa (Jehan et al., 1973; Brooks 1981). Physiological and biochemical integrity of epididymal canal is dependent on androgens (Setty et al., 1977; Brooks, 1974). It has been reported that epididymis performs both secretory and absorptive functions; sperm maturation takes place because of the proteins synthesized and secreted by epididymal tissue (Klinefelter and Hamilton, 1985). Brooks (1981) has reported that androgen deficiency causes a marked reduction in the tubular diameter, a general regression of epididymal epithelium, a rapid decline in the number of spermatozoa within the cauda epididymis and changes in the composition of the epididymal plasma.

The data obtained in the present study revealed that the mice treated with 1 and 1.3 mg carbofuran showed a significant decrease in the weight of the testis. Treatment with 0.7, 1 and 1.3 mg carbofuran showed a significant decrease in the number of spermatogonia, spermatocytes and spermatids. Mice treated with 1.3 mg carbofuran showed a significant decrease in the diameter of the spermatogonia,
spermatocytes and spermatids. There is no significant change in the diameter of the testis and seminiferous tubules in all the carbofuran treated mice. There is also a significant decrease in the weight of epididymis, seminal vesicles, Prostate glands, Coagulatory glands and Cowper’s glands in 1.3 mg carbofuran treatment. Treatment with 1 mg carbofuran caused a significant decrease in the weight of the prostate gland. However, there is no significant change in the weight of all the accessory reproductive organs with 0.4 and 0.7 mg carbofuran treatment.

In the temporal study, mice treated with 1.3 mg carbofuran for 20 and 30 days caused a significant decrease in the weight of the testis. Treatment with 1.3 mg carbofuran for 10, 20 and 30 days caused a significant decrease in the number of the spermatogonia. There was a significant decrease in the number of the spermatocytes and spermatids with 1.3 mg carbofuran treatment for 20 and 30 days. Treatment with 1.3 mg carbofuran for 20 and 30 days causes a significant decrease in the diameter of the spermatogonia. Treatment with 1.3 mg carbofuran for 30 days caused a significant decrease in the diameters of the spermatocytes and spermatids. There was also a significant decrease in the weight of the epididymis with 1.3 mg carbofuran treatment for 20 and 30 days. There was a significant decrease in the weight of seminal vesicles, prostate glands, coagulatory glands and Cowper’s glands with 1.3 mg carbofuran treatment for 30 days.

In the study of Pant et al., (1995), male rats administered with carbofuran by gavage caused decrease in the sperm motility, reduced epididymal sperm count and increase in the morphological abnormalities of the sperms. An organophosphorus pesticide, quinolphos administered rats show decreased testicular mass and AChE
activity in central as well as in peripheral organs increased serum LH, FSH, prolactin and testosterone concentrations; decreased pituitary or increased ACE activity; severe disruption of spermatogenesis with increasing doses of pesticide and no significant effects on dopamine, noradrenaline concentrations in the hypothalamus or pituitary. The histologic data indicates that the organophosphate treatment can focally inhibit spermatogenesis and in certain areas of seminiferous tubules, destroy all the cells of the seminiferous epithelium (Sarkar et al., 2000). Similar effects on the seminiferous epithelium were observed after treatment with LH alone or in combination with LHRH agonist (Sharpe et al., 1983; Kerr and Sharpe, 1986). Carbofuran has been listed as a potential endocrine disrupter by the German Federal Environment Agency (ENDS, 1999). Degeneration of Sertoli cells and germ cells and the presence of dense aggregation of extracellular material may be due to high concentrations of circulating LH (Kerr and Sharpe, 1986) as LH has a central role in initiating and sustaining variable spermatogenic disruption (Rivier et al., 1979). Investigation of ACE activity in organophosphate treated rats revealed that ACE has two opposite actions on the pituitary and testis. Inhibition of pituitary ACE activity may cause inhibition of pituitary angiotensin II, which in turn increases the secretion of prolactin from the anterior pituitary, as administration of angiotensin II results in the decrease in secretion of growth hormone and prolactin by the direct action of angiotensin II at the anterior pituitary (Steel et al., 1981). Alternatively, increased testicular ACE activity may be due to the increased production of the testosterone, which is also essential for the development and maintenance of testicular ACE (Velletri et al., 1985). Organophosphates have a
direct effect on acetylcholinesterase (AChE), resulting in alterations in the pituitary
gonadotropins and could influence testicular function directly through its effect on
the pituitary AChE (Sarkar et al., 2000). The anticholinesterase properties of the
three major carbofuran metabolites were evaluated; particularly those with intact
carbamyl-moieties. The moieties, 3-hydroxycarbofuran, 3-ketocarbofuran, and 3-
hydroxy-N-hydroxy-methylcarbofuran were found to have less anticholinesterase
activity than the carbofuran itself (Dorough, 1968, 1983). Thus, the carbofuran may
have direct effect on acetylcholinesterase, resulting in the alteration in the pituitary
gonadotropins as suggested by Sarkar et al., (2000). It has been reported that
carbaryl induces sperm abnormalities but no degenerative changes in the testis and
reduces the number of spermatogonia and spermatozoa in mice and rats (Degrave et
al., 1976; Kitagawa et al., 1977). It has been shown that in the treatment with
carbamate insecticide, carbaryl affects the spermatogenic cells, degenerates the
Leydig cells and alters the testosterone, gonadotropin levels in blood serum,
testicular total lipids and the activity of alkaline acid phosphatase (Shrivastava and
Shrivastava, 1998).

Effects on the sperm quality and on the amount of ejaculate due to
carbofuran exposure in rabbits were studied by Yousef et al., (1995). In this study,
the authors reported on overall decline in the body weight and a decrease in the
amount of the sperms released following the treatment. The latter effect was most
evident with higher doses. Carbofuran was administered orally to adult male rats at
levels of 0.1, 0.2, 0.4 or 0.8 mg/kg for five days per week for 60 days showed a dose
dependent decrease in the body weight of rats, decreased weight of the organs
including the epididymidis, seminal vesicles, ventral prostate and coagulation
glands at dose levels of 0.2 mg/kg and above, decreased the sperm motility, reduced
epididymal sperm count and increased the morphological abnormalities of the
sperms and a significant change in the various testicular enzymes including sorbitol
dehydrogenase (SDH), glucose 6-P-dehydrogenase, lactate dehydrogenase (LDH)
and γ glutamyl transpeptidase (Pant et al., 1995).

Pant et al., (1997) also investigated the effects on male offsprings of
carbofuran treated female rats. In this study, the female Druckery rats were mated,
and after assuring that pregnancy had occurred, the female rats were divided into
two groups and given either peanut oil or peanut oil with 0.2 or 0.4 mg/kg
carbofuran daily. Treatment was stopped at parturition. After attaining 90 days, pups
were sacrificed. The testis, epididymidis, seminal vesicles, ventral prostate and
coagulating glands were quickly removed and weighed. No general pathological
effects were observed with either of the treatment. Significant variations in
enzymatic activities were noted with SDH, LDH and γ-glutamyl dehydrogenase
(γ-GT). Decreases in the sperm motility and sperm count, along with an increase in
the abnormal sperms were also noted. Histopathological examination revealed
atrophied seminiferous tubules and degenerative changes of Sertoli cells at 0.4
mg/kg/day.

Recently it has been reported that dicofol treated rats showed a significant
decrease in the number and diameter of spermatogenic cells and a significant
decrease in the weight of the epididymidis and testes (Jadaramkunti and Kaliwal,
2001). Similar results have also been reported with endosulfan, mancozeb and
dimethoate treated mice (Hiremath, 2000; Ksheersagar, 2001; Mahadevaswami, 2002).

The present investigation is comparable to other organophosphorus pesticides on account of the decrease in the number and diameter of the spermatogenic cells arrest of spermatogenesis, atrophy of Leydig cells, decrease in the number of the sperms in the tubules of the testis and epididymis and a significant decrease in the weight of the epididymidis, prostate, coagulatory glands and Cowper's glands due to hormonal imbalances as suggested by earlier finding mentioned above. The present study on the dose and duration effect of carbofuran on the histologic structures of testis and epididymis also revealed two principal impacts on the male reproductive system of mice namely, the anti-spermatogenic and anti-androgenic effects. The anti-spermatogenic adverse effect is reflected in the arrest of spermatogenesis as seen on the diameter and number of the spermatogenic cells in the seminiferous tubules. The anti-androgenic action of carbofuran in the present study possibly reflected the atrophy of Leydig cells and decrease in the number of sperms in the tubules of epididymis and decrease in the weights of the epididymis, prostate, coagulatory and Cowper's glands.

There are several possible mechanisms for the antigonadal actions of organophosphates. They may exert a direct inhibitory action on the testis, they may affect the pituitary, causing changes in the gonadotropin concentrations and thus subsequent spermatogenic impairment or they may change the concentrations of the neurotransmitters (Sarkar et al., 2000). Pesticides induce inhibition of the acetylcholenesterase (AChE) which in turn might increase the concentrations of
acetylcholine (ACh) in the pituitary and hypothalamus, which in the complex circuitry of neuroendocrine regulation can invariably affect a secondary transmitter, especially dopamine or 5-HT (Corrodi et al., 1967; Butcher, 1979; Robinson, 1983; Bradford, 1986).

The high dose and long term exposure of carbofuran causes a complete inhibition of spermatogenesis which may be due to an imbalance in the androgens which are essential for normal spermatogenesis (Greep et al., 1936; Steinberger and Steinberger, 1975; Sharpe, 1987) and there was also a possibility that the inhibition of spermatogenesis may be due to defective gonadotropin secretion, via the mechanism of central nervous system, as it was observed in the rats and mice, following the administration of the carbamates (Degrave et al., 1976; Kitagawa et al., 1977; Goldman et al., 1994, 1997; Shrivastava and Shrivastava, 1998). Goldman et al., (1990) have reported that the insecticide, chloridimeform may destroy the endocrinologic homeostasis by suppressing the release of GnRH. It has also been reported that the toxic agents may act directly on the gonadotropins to alter the synthesis of gonadotropins and their secretion or indirectly by altering the responsiveness of the pituitary cells to the GnRH or to the gonadal steroids. Both of the actions will result in the alterations in the serum levels of FSH and LH (Dickerson and Safe, 1992).

The present investigation gives a clue that a high dose and long term exposure of carbofuran affect the spermatogenesis showing antispermaticogenic and antiandrogenic property, either directly or indirectly in dose and duration dependent fashion. Carbofuran having a direct effect on the testis or an indirect effect through
the hypothalamo-hypophysial testicular axis cannot be concluded from this study. Hence, it is suggested that with a high dose and long term exposure of carbofuran affects the process of spermatogenesis, which may be through the deprived levels of the androgens mediated through the gonadotropins of the pituitary. The author is fully aware that the conclusions of this study, which are mainly based on gravimetric, histologic and histometric observations, are not adequate to understand the clear mechanism of action of carbofuran on testis, epididymis and accessory reproductive organs. Therefore, further investigation is essential to support the findings.

Effect of carbofuran on the weight of the body and organs in albino mice

In the present study, administration of 1 and 1.3 mg carbofuran showed a significant decrease in the gain of the body weight. However, there is no significant change in the weights of the kidneys, adrenals, spleen, liver, thymus and thyroid of all the carbofuran treated mice. In the present temporal study, the treatment with 1.3 mg carbofuran for 30 days caused a significant decrease in the weight of the body. However, there is no significant change in the weights of the kidneys, adrenals, spleen, thymus and thyroid of all the carbofuran treated mice.

Nutrition is an important factor for the findings of the toxicity, because nutritional deficiencies have shown to alter the reproductive function (Piacsek and Mites, 1967; Howland, 1971; Knuth and Friesen, 1983). Although intake of food has not been measured in this study, this may be one of the reasons for low gain in the weight. This supports the findings of Lu and Kennedy (1986) and Hayes and Laws (1991) on the study of mancozeb exposed to male rats. Recently, similar
findings have been reported on the decreased weight of the body and organs in rats and mice treated with organophosphorus pesticides namely monocrotophos and dimethoate (Adilaxmamma et al., 1994; Ratnasooriya et al., 1995; Radhika and Kaliwal, 2001; Sanderson and Edson, 1964; Mahadevaswami, 2002).

Vos et al., (1989) reported that environmental pollutants such as pesticides and metals caused changes in the weights of lymphoid organs such as thymus, spleen and lymph nodes in mice and rats. It has been reported that there was a significant decrease in the weight of the body and spleen in melathion treated male mice (Barlas, 1996). The inhibition of aminotransferase might explain, in part the observed loss in body weight in chronic studies in toxicity of the insecticides (Sadek et al., 1989). The present study is also comparable to high doses and long term exposure of carbamate fungicide mancozeb resulting in a significant decrease in the weight of body, kidney, spleen, liver, thymus and thyroid in male mice (Ksheersagar, 2001). Similar findings have been suggested that the administration of mancozeb with high dose and chronic exposure shows signs of poisoning, loss in the body weight, decrease in the weights of the kidney and pathomorphological changes in liver, brain and kidneys in male rats (Kacker et al., 1999). Hore et al., (1997) have reported that exposure of mancozeb causes pathologic changes in the liver and kidney and also the heart showed congestion and hemorrhage.

Effect of carbofuran on biochemical constituents of testis, liver and kidney in albino mice

Proteins, carbohydrates and lipids are constituents of the food, which are essential for animals. Proteins are the building blocks, carbohydrates are the
Immediate source of energy and lipids are the reservoirs of energy. Proteins function in three different ways in the cells as enzymes, structural proteins some as antibodies. Enzymes catalyse all cellular reactions, which make them extremely important in the cell activity. Structural proteins are the major constituents of skeletal and muscular tissues and also all membranous structures contain a structural protein component (Nelson et al., 1978). Glycogen is the main metabolic fuel in the polymer of glucose and is known as an animal starch in the muscle (Wittenberger, 1996). The fat that is deposited in various tissues may be derived from either dietary fat or de novo synthesis. Both synthesis and mobilization are processes that are self-regulating to some extent but many hormones influence the rate of lipid metabolism and thus there is a balance between lipogenesis and lipolysis.

The present biochemical study revealed that there is a significant decrease in the glycogen of the kidney and liver total lipids with 0.7 mg carbofuran treatment. In mice treated with 1 and 1.3 mg carbofuran showed a significant decrease in the testis and kidney protein and glycogen with significant increase in the testis total lipids and a significant decrease in the kidney total lipids. However, there was a significant decrease in the liver glycogen and total lipids in 1 mg carbofuran treatment and a significant decrease in the liver protein, glycogen and total lipids in 1.3 mg carbofuran treated mice.

The data obtained in the temporal study showed a significant increase in the total lipids of the testis with 1.3 mg carbofuran treatment for 20 and 30 days. There was a significant decrease in the protein, glycogen of the testis and also significant
decrease in the protein, glycogen and total lipids of the liver and kidney with 1.3 mg carbofuran treatment for 30 days.

Stott, (1997) has reported that dimethyl dithiocarbamate inhibits hepatic cyt P-450 dependent enzyme activity in rats. It has been suggested that there was a significant decrease in the microsomal protein and cyt P-450 content of the liver, lungs, brain and kidneys of rats treated with pesticide vapacid (Mohd., 1993). Rapid loss in the proteins of the brain during pesticide toxicity was reported (Richardson, 1981). The decrease in total protein and soluble proteins indicate their metabolic utilization. The increase in the activity of proteases correlated with the decrease of soluble and total protein (Swamy et al., 1992). It was reported that impaired energy supply leads to the break down of tissue proteins making them susceptible to the action of tissue proteolytic enzymes and leading to their consequent degradation by proteases (Berger et al. 1983). Quantitative changes were noted in the contents of the glycogen, protein and lipids activity of acetylcholinesterase (AChE) in dichlorvos intoxication in the fresh water prawn (Geraldine et al., 1999). It has been reported that mancozeb treatment altered the levels of protein, glycogen and total lipids in the ovary, uterus and liver in intact and hemicastrated rats (Baligar and Kaliwal, 2001; Mahadevaswami et al., 2000). Recently it has been reported that endosulfan, dimethoate and mancozeb treated mice caused a significant change in the protein, glycogen and total lipids content in the testis, liver and kidneys (Hiremath, 2000; Mahadevaswami, 2002; Ksheerasagar, 2001). Subramaniam et al., (1991) have reported that increase in the levels of phosphoinositides and phosphotidic acid in the liver suggests the likely involvement of phospholipase in
the toxicity of mancozeb in different tissues at varying levels. In the present study, changes in the levels of the protein, glycogen and total lipids with carbofuran treatment suggests either an increased catabolism of the biomolecules to meet the enhanced energy demand of the animals under stress or their reduced synthesis due to the impaired tissue function at the various biochemical enzymes (Ivanova-Chemishanska, 1982). Pant et al., (1995) have reported that the treatment with carbofuran caused significant changes in various testicular enzymes including sorbitol dehydrogenase (SDH), glucose 6-P-dehydrogenase, lactate dehydrogenase (LDH) and γ glutamyl transpeptidase in rats.

Therefore, in the present study, the decrease in the protein, glycogen and lipid contents of testis, liver and kidney may be due to the inhibition of enzyme activity or decrease in the androgen production or in turn depress the oxidative metabolism or carbohydrate metabolism mediated by insulin or suppression in the production of sex steroid hormones in mice as suggested by the earlier finding as mentioned above. At present, it is not possible to say whether these changes in the biochemical parameters in the tissues of the mice discussed herein are reversible or permanent. Nevertheless they provide new information to identify the sites of acute organ damage. These biochemical changes caused by carbofuran are indicative of a compensation reaction-taking place at the organ level in response to the cell damage or death. In an environment where there is a continual low-level exposure to carbamate insecticides, the biochemical effects reported here gain the importance.

It is important to note here that the response of the male and female animal is related to the dose of the test chemical, which interacts with molecular/receptor site
in the organism, i.e. the initiation and perpetuation of the toxic response in the organism has a relationship with the bioavailability and the concentration of the test chemical at the reactive site (Brown, 1980) and also with the duration it remains (Klaassen, 1980). A toxic insult of one element influences the function of the other element if not in the short term, certainly in the long term. Hence, the primary site of a toxicant action is difficult to identify. In vitro techniques have been used in identifying the type of impairment in a particular type of the cell or structure, but the in-vivo studies point to different or multiple toxic responses. Hence, further study is necessary to state the primary site of the action of the carbofuran.
SUMMARY

The present investigation was aimed to elucidate the graded doses and temporal effect of carbofuran on testes, accessory reproductive organs and biochemical constituents such as protein, glycogen and total lipids of testis, liver and kidney in albino mice. Carbofuran was administered orally in the graded doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d for 30 days to the male mice. Olive oil was given to the control mice.

1. Mice treated with 1 and 1.3 mg carbofuran treatment showed a significant decrease in the weight of the testis. However, there was no significant change in the weight of the testis with 0.4 and 0.7 mg carbofuran treatment.

2. Mice treated with 0.7, 1 and 1.3 mg carbofuran showed a significant decrease in the number of spermatogonia, spermatocytes and spermatids. However, there is no significant change in the number of spermatogonia, spermatocytes and spermatids with 0.4 mg carbofuran treatment.

3. Mice treated with 1.3 mg carbofuran caused a significant decrease in the diameter of spermatogonia, spermatocytes and spermatids. However, there is no significant change in the diameter of the spermatogonia, spermatocytes and spermatids with 0.4, 0.7 and 1 mg carbofuran treated mice. There was no significant change in the diameter of the testis and seminiferous tubules of all the carbofuran treated mice.

4. There was a significant decrease in the weights of epididymidis, seminal vesicles, prostate glands, coagulatory glands and Cowper's glands with 1.3 mg carbofuran treatment. Treatment with 1 mg carbofuran caused a significant
decrease in the weight of the prostate gland. However, there is no significant 
change in the weight of all the accessory reproductive organs with 0.4 and 0.7 
mg carbofuran treatment. Mice treated with 1 and 1.3 mg carbofuran caused a 
significant decrease in the body weight. However, there is no significant 
change in the weight of the kidneys, adrenals, liver, spleen, thymus and thyroid 
of all the carbofuran treated mice.

5. Mice treated with 0.7 mg carbofuran showed a significant decrease in the 
glycogen of the kidney and total lipids of the liver. In mice treated with 1 and 
1.3 mg carbofuran showed a significant decrease in the protein of the testis and 
glycogen of the kidney with significant increase in the total lipids of the testis 
and significant decrease in the total lipids of the kidney. However, there was a 
significant decrease in the glycogen of the liver and total lipids with 1 mg 
carbofuran treatment and a significant decrease in the liver protein, glycogen 
and total lipids with 1.3 mg carbofuran treatment.

6. Based on the dose experiment 1.3 mg/kg body weight/d carbofuran was an 
effective dose, administered orally for 5, 10, 20 and 30 days to male mice. 
Olive oil was administered to the control mice.

7. Mice treated with 1.3 mg carbofuran for 20 and 30 days showed a significant 
decrease in the weight of the testis. However, there was no significant change 
in the weight of the testis with 1.3 mg carbofuran treatment for 5 and 10 days.

8. The mice treated with 1.3 mg carbofuran for 10, 20 and 30 days caused a 
significant decrease in the number of the spermatogonia. There was a 
significant decrease in the number of spermatogonia, spermatocytes and
spermatids with 1.3 mg carbofuran treatment for 20 and 30 days. However, there is no significant change in the number of the spermatogonia and spermatids with 1.3 mg carbofuran treatment for 5 and 10 days.

9. There was a significant decrease in the diameter of the spermatogonia with 1.3 mg carbofuran treatment for 20 and 30 days. Mice treated with 1.3 mg carbofuran for 30 days caused a significant decrease in the diameter of the spermatocytes and spermatids. However, there was no significant change in the diameter of the spermatocytes and spermatids with 1.3 mg carbofuran treatment for 5, 10 and 20 days. There was no significant change in the diameter of the testis and seminiferous tubules of all the durational treatment of carbofuran.

10. There was a significant decrease in the weight of the epididymidis with 1.3 mg carbofuran treatment for 20 and 30 days. Mice treated with 1.3 mg carbofuran treatment for 30 days caused a significant decrease in the weight of the seminal vesicles, prostate glands, coagulatory glands and Cowper's glands. However, there is no significant change in the vas differentia of all the durational treatment of carbofuran. Mice treated with 1.3 mg carbofuran for 30 days caused a significant decrease in the body weight. However, there is no significant change in the weight of the kidney, adrenals, liver, spleen, thymus and thyroid of all the durational treatment of carbofuran.

11. There was a significant increase in the testis total lipids and significant decrease in the total lipids of the liver with 1.3 mg carbofuran treatment for 20 and 30 days. There was a significant decrease in the protein, glycogen of the
testis and protein, glycogen and total lipids of the liver and kidney with 1.3 mg carbofuran treatment for 30 days.

The above results suggest that carbofuran has adverse effects on the testis. These effects on the testis may be either direct or indirect through the hypothalamo-hypophysial-testicular axis. However, this finding cannot be conducted from the above study. The dose and durational treatment of carbofuran affects the process of spermatogenesis, which may be by the deprived level of androgen mediated through the pituitary gonadotropins. The author is fully aware that the conclusions of this study, which are mainly based on the gravimetric, histologic, histometric and biochemical data are not adequate to understand the clear mechanism of action of carbofuran on testis and epididymidis. Hence, further investigation is necessary to support these findings.
EXPLANATION TO PHOTOMICROGRAPHS

Effect of carbofuran on testis in albino mice

Fig. 1. T.S. of the testis of the control mouse showing the presence of normal tubular structure with spermatogenic cells at different stages of development. Seminiferous tubules are packed closely. The tubular spaces are packed with interstitial tissue, containing clusters of Leydig cells. Testis weight / 100 g body weight = 720.06 mg (Mean weight = 738.96 mg).

Fig. 2. T.S. of the testis of the mouse treated with 0.4 mg/kg body weight / d carbofuran for 30 days showing normal spermatogenesis. The Seminiferous tubules are closely packed. Sperms concentrated at the centre of the lumen. The reduced tubular spaces are packed with interstitial tissue, containing clusters of Leydig cells. Testis weight / 100 g body weight = 701.55 mg (Mean weight = 739.99 mg).

Fig. 3. T.S. of the testis of the mouse treated with 0.7 mg/kg body weight / d carbofuran for 30 days showing symptoms of arrest of spermatogenesis. The Seminiferous tubules are loosely packed. The spermatogonia, spermatocytes and spermatids are closely arranged and less in number. Leydig cells are in deformed condition. Testis weight / 100 g body weight = 740.02 mg (Mean weight = 736.99 mg).

Fig. 4. T.S. of the testis of the mouse treated with 1 mg / kg body weight / d carbofuran for 30 days showing symptoms of arrest of spermatogenesis. The Seminiferous tubules are loosely packed. The epithelium is ruptured. Spermatogonia, spermatocytes and spermatids are loosely arranged and very less in number. Leydig cells are highly compact and in deformed condition. Testis weight / 100 g body weight = 700.54 mg (Mean weight = 711.02 mg).

Fig. 5. T.S. of the testis of the mouse treated with 1.3 mg / kg body weight/d carbofuran for 30 days showing complete arrest of spermatogenesis. The Seminiferous tubules are loosely packed. The epithelium seen ruptured. Spermatogonia, spermatocytes and spermatids are loosely arranged and less in number. Leydig cells are highly compact on one side and leading to disruption of other side. Testis weight/100 g body weight=710.26 mg (Mean weight =704.44 mg).

Photographs original exposure at X 200

ST – Seminiferous tubules
EP – Epithelium
LU – Lumen
IN – Inter tubular tissue
SG – Spermatogonia
PS – Primary spermatocytes
SS – Secondary spermatocytes
SP – Spermatids
SM – Sperm
EXPLANATION TO PHOTOMICROGRAPHS

Effect of carbofuran on epididymis in albino mice

Fig. 6. T.S. of the epididymis of the control mouse showing the sperms fully packed in the lumen. Epididymis weight / 100 g body weight = 315.56 mg (Mean weight = 312.63 mg).

Fig. 7. T.S. of the epididymis of the mouse treated with 0.4 mg/kg body weight/d carbofuran for 30 days showing the sperms less in concentration. Epididymis weight / 100 g body weight = 310.43 mg (Mean weight = 316.33 mg).

Fig. 8. T.S. of the epididymis of the mouse treated with 0.7 mg/kg body weight/ d carbofuran for 30 days showing less concentration of sperms and disordered tubules. Epididymis weight / 100 g body weight = 315.74 mg (Mean weight = 313.30 mg).

Fig. 9. T.S. of the epididymis of the mouse treated with 1 mg/kg body weight/ d carbofuran for 30 days showing very less sperm storage and disordered tubules. Epididymis weight / 100 g body weight = 310.68 mg (Mean weight = 307.37 mg).

Fig. 10. T.S. of the epididymis of the mouse treated with 1.3 mg/kg body weight/ d carbofuran for 30 days showing the absence of sperm storage and disordered tubules. Epididymis weight / 100 g body weight = 290.48 mg (Mean weight = 298.99 mg).

Photograph original exposure at X 200

EP – Epithelium
LU – Lumen
IN – Inter tubular tissue
SM – Sperm
EXPLANATION TO PHOTOMICROGRAPHS

Temporal effect of carbofuran on testis in albino mice

Fig. 11. T.S. of the testis of the control mouse showing the presence of normal tubular structure with spermatogenic cells at different stages of development. Seminiferous tubules are packed closely. The tubular spaces are packed with interstitial tissue, containing clusters of Leydig cells. Testis weight / 100 g body weight = 720.06 mg (Mean weight = 738.96 mg).

Fig. 12. T.S. of the testis of the mouse treated with 1.3 mg / kg body weight/d carbofuran for 5 days showing normal spermatogenesis. The seminiferous tubules are closely packed. The tubular spaces are packed with interstitial tissue, containing clusters of Leydig cells. Testis weight / 100 g body weight = 715.25 mg (Mean weight = 735.66 mg).

Fig. 13. T.S. of the testis of the mouse treated with 1.3 mg / kg body weight/d carbofuran for 10 days showing normal spermatogenesis. The seminiferous tubules closely packed. However, the inter tubular spaces are highly increased. Testis weight / 100 g body weight = 725.25 mg (Mean weight = 733.99 mg).

Fig. 14. T.S. of the testis of the mouse treated with 1.3 mg/ kg body weight/d carbofuran for 20 days showing symptoms of arrest of spermatogenesis. The seminiferous tubules are closely packed. The epithelium seen ruptured. Spermatogonia, spermatocytes and spermatids are seen loosely arranged and significantly less in number. Testis weight / 100 g body weight = 718.68 mg (Mean weight = 730.33 mg).

Fig.15. T.S. of testis of the mouse treated with 1.3 mg / kg body weight / d carbofuran showing complete arrest of spermatogenesis. The Seminiferous tubules are loosely packed. The epithelium is ruptured. Spermatogonia, spermatocytes and spermatids are loosely arranged and less in number. Leydig cells are highly compact on one side and leading to disruption of other side. Testis weight / 100 g body weight = 710.26 mg (Mean weight = 704.44 mg).

Photographs original exposure at X 200

ST – Seminiferous tubules
EP – Epithelium
LU – Lumen
IN – Inter tubular tissue
SG – Spermatogonia

PS – Primary spermatocytes
SS – Secondary spermatocytes
SP – Spermatids
SM – Sperm
Temporal effect of carbofuran on epididymis in albino mice

Fig. 16. T.S. of the epididymis of the control mouse showing the sperms fully packed in the lumen. Epididymis weight / 100 g body weight = 315.56 mg (Mean weight = 312.63 mg).

Fig. 17. T.S. of the epididymis of the mouse treated with 1.3 mg / kg body weight /d carbofuran for 5 days showing the sperms moderate in concentration. Epididymis weight / 100 g body weight = 305.32 mg (Mean weight = 311.70 mg).

Fig. 18. T.S. of the epididymis of the mouse treated with 1.3 mg / kg body weight /d carbofuran for 10 days showing less concentration of sperms. Epididymis weight / 100 g body weight = 308.55 mg (Mean weight = 306.66 mg).

Fig. 19. T.S. of the epididymis of the mouse treated with 1.3 mg / kg body weight /d carbofuran for 20 days showing the drastic decrease in the sperm concentration and disordered tubules. Epididymis weight / 100 g body weight = 298.22 mg (Mean weight = 301.66 mg).

Fig. 20. T.S. of the epididymis of the mouse treated with 1.3 mg/kg body weight/ d carbofuran showing the absence of sperm storage and disordered tubules. Epididymis weight / 100 g body weight = 290.48 mg (Mean weight = 298.99 mg).

Photograph original exposure at X 200

EP - Epithelium
LU - Lumen
IN - Inter tubular tissue
SM - Sperm
Table 4.1.  Effect of carbofuran on weight of testes, number of spermotogonia, spermatocytes and spermatids in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Testes weight (mg/100g body wt.)</th>
<th>Number of Spermatogenic cells; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spermatogonia</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>738.96 ± 1.95</td>
<td>78.80 ± 1.70</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>10</td>
<td>739.99 ± 2.28</td>
<td>77.95 ± 2.05</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>10</td>
<td>736.99 ± 1.75</td>
<td>69.70 ± 1.21*</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>711.02 ± 1.41*</td>
<td>61.95 ± 1.16*</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>704.44 ± 1.14*</td>
<td>54.55 ± 0.79*</td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control
Table 4.2. Effect of carbofuran on diameter of the testes, seminiferous tubule, spermatogonia, spermatocytes and spermatids in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Diameter of testes (mm)</th>
<th>Diameter (μm) of seminiferous tubule and spermatogenic cells; (mean ± SEM)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Seminiferous tubules</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>3.45 ± 0.15</td>
<td>221.48 ± 1.24</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>10</td>
<td>3.42 ± 0.17</td>
<td>218.56 ± 1.50</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>10</td>
<td>3.46 ± 0.15</td>
<td>221.12 ± 1.49</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>3.40 ± 0.24</td>
<td>217.99 ± 1.00</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>3.35 ± 0.14</td>
<td>216.99 ± 1.17</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.3. Effect of carbofuran on accessory reproductive organs weight in albino mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Relative weight mg/100g body weight; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>Cowper's glands: 26.66 ± 1.31</td>
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<tr>
<td></td>
<td></td>
<td>Coagulatory glands: 26.66 ± 1.31</td>
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<tr>
<td></td>
<td></td>
<td>Prostate glands: 83.66 ± 1.26</td>
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<tr>
<td></td>
<td></td>
<td>Seminal vesicles: 623.17 ± 1.67</td>
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<tr>
<td></td>
<td></td>
<td>Vas deferens: 100.66 ± 0.97</td>
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<tr>
<td></td>
<td></td>
<td>Epididymides: 312.63 ± 4.35</td>
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<tr>
<td></td>
<td></td>
<td>No. of mice: 10</td>
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</tbody>
</table>

- Significant P <0.05 compared to control
Table 4.4. Effect of carbofuran on body and organ weights in male albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Body weight gain (g)</th>
<th>Relative organ weight/100g body weight; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kidney (g)</td>
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<td></td>
<td></td>
<td></td>
<td>Adrenal (mg)</td>
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<td></td>
<td>Liver (g)</td>
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<td></td>
<td></td>
<td></td>
<td>Spleen (mg)</td>
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<td></td>
<td></td>
<td>Thymus (mg)</td>
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<td></td>
<td></td>
<td></td>
<td>Thyroid (mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>3.40 ± 0.22</td>
<td>1.64 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>42.33 ± 1.79</td>
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<td>5.18 ± 0.13</td>
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<td>438.66 ± 0.55</td>
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<td>83.69 ± 0.90</td>
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<td>13.86 ± 0.64</td>
</tr>
<tr>
<td>II</td>
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<td>10</td>
<td>3.20 ± 0.20</td>
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<tr>
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<td>41.73 ± 1.59</td>
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<td>4.99 ± 0.12</td>
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<td>446.66 ± 1.11</td>
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<td>85.00 ± 1.48</td>
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<td>13.07 ± 0.61</td>
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<tr>
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<td>3.10 ± 0.18</td>
<td>1.51 ± 0.02</td>
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<td>81.33 ± 0.74</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>14.07 ± 0.75</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>2.20 ± 0.13*</td>
<td>1.38 ± 0.04</td>
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<td>40.31 ± 1.48</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>5.12 ± 0.20</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>439.99 ± 1.22</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>80.33 ± 1.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.09 ± 0.78</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>1.50 ± 0.17*</td>
<td>1.47 ± 0.04</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>39.31 ± 1.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.66 ± 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>434.99 ± 2.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79.99 ± 0.99</td>
</tr>
<tr>
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<td></td>
<td>15.00 ± 0.73</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Protein (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Glycogen (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Total lipids (µg / mg wet weight of tissue; mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>89.64 ± 0.79</td>
<td>7.02 ± 0.18</td>
<td>37.84 ± 1.13</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>10</td>
<td>87.92 ± 0.82</td>
<td>6.62 ± 0.25</td>
<td>36.4 ± 1.42</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>10</td>
<td>85.66 ± 1.54</td>
<td>6.50 ± 0.15</td>
<td>40.92 ± 1.38</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>82.54 ± 1.57*</td>
<td>5.60 ± 0.13*</td>
<td>44.28 ± 1.43*</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>80.14 ± 1.20*</td>
<td>4.26 ± 0.20*</td>
<td>46.68 ± 1.23*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.6. Effect of carbofuran on biochemical constituents of the liver in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Protein (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Glycogen (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Total lipids (µg / mg wet weight of tissue; mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>180.40 ± 1.72</td>
<td>4.48 ± 0.25</td>
<td>40.76 ± 0.92</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>5</td>
<td>182.20 ± 0.97</td>
<td>4.74 ± 0.20</td>
<td>40.10 ± 0.86</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>5</td>
<td>178.80 ± 0.97</td>
<td>4.32 ± 0.27</td>
<td>37.94 ± 0.70*</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>5</td>
<td>175.50 ± 1.29</td>
<td>3.20 ± 0.14*</td>
<td>35.46 ± 0.84*</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>5</td>
<td>171.20 ± 1.07*</td>
<td>2.96 ± 0.16*</td>
<td>32.90 ± 0.86*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.7. Effect of carbofuran on biochemical constituents of the kidney in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Protein (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Glycogen (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Total lipids (µg / mg wet weight of tissue; mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>92.36 ± 0.98</td>
<td>4.56 ± 0.15</td>
<td>33.86 ± 1.17</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>5</td>
<td>92.54 ± 0.98</td>
<td>4.28 ± 0.18</td>
<td>33.56 ± 0.49</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>5</td>
<td>89.50 ± 0.65</td>
<td>3.78 ± 0.13*</td>
<td>31.90 ± 0.99</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>5</td>
<td>87.72 ± 0.80*</td>
<td>3.40 ± 0.17*</td>
<td>29.08 ± 0.64*</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>5</td>
<td>84.86 ± 1.12*</td>
<td>2.42 ± 0.20*</td>
<td>26.80 ± 1.09*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.8. Temporal effect of carbofuran on weight of testes, number of spermatogonia, spermatocytes and spermatids in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Testes weight (mg / 100 g body wt.)</th>
<th>Number of Spermatogenic cells; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spermatogonia</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>738.96 ± 1.95</td>
<td>78.80 ± 1.70</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>10</td>
<td>735.66 ± 1.99</td>
<td>75.25 ± 2.05</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>10</td>
<td>733.99 ± 1.71</td>
<td>70.25 ± 1.57*</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>10</td>
<td>730.33 ± 2.13*</td>
<td>63.57 ± 1.33*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>10</td>
<td>704.45 ± 1.14*</td>
<td>54.55 ± 0.79*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.9. Temporal effect of carbofuran on diameter of the testes, seminiferous tubule, spermatogonia, spermatocytes and spermatids in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Testes (mm)</th>
<th>Diameter (µm) of seminiferous tubules and spermatogenic cells; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Seminiferous tubules</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>3.45 ± 0.15</td>
<td>221.48 ± 1.24</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>10</td>
<td>3.43 ± 0.14</td>
<td>220.98 ± 1.26</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>10</td>
<td>3.38 ± 0.15</td>
<td>221.81 ± 1.29</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>10</td>
<td>3.38 ± 0.12</td>
<td>218.46 ± 1.85</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>10</td>
<td>3.35 ± 0.14</td>
<td>216.99 ± 1.17</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.10. Temporal effect of carbofuran on accessory reproductive organ weights in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Relative weight mg/100g body weight ; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epididymides</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>312.63 ± 4.35</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>10</td>
<td>311.70 ± 2.22</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>10</td>
<td>306.66 ± 1.72</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>10</td>
<td>301.66 ± 1.94*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>10</td>
<td>298.99 ± 2.44*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.11. Temporal effect of carbofuran on body and organ weights in male albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Body weight gain (g)</th>
<th>Relative organ weight/100g body weight; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kidney (g)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>3.40 ± 0.22</td>
<td>1.64 ± 0.03</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>10</td>
<td>3.20 ± 0.13</td>
<td>1.62 ± 0.03</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>10</td>
<td>2.90 ± 0.23</td>
<td>1.61 ± 0.04</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>10</td>
<td>2.80 ± 0.24</td>
<td>1.55 ± 0.02</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>10</td>
<td>1.50 ± 0.17*</td>
<td>1.47 ± 0.04</td>
</tr>
</tbody>
</table>

*Significant P <0.05 compared to control
Table 4.12. Temporal effect of carbofuran on biochemical constituents of the testes in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Protein (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Glycogen (µg / mg wet weight of tissue; mean ± SEM)</th>
<th>Total lipids (µg / mg wet weight of tissue; mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>89.64 ± 0.79</td>
<td>7.02 ± 0.18</td>
<td>37.84 ± 1.13</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>5</td>
<td>90.00 ± 0.43</td>
<td>7.06 ± 0.20</td>
<td>37.90 ± 0.17</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>5</td>
<td>89.12 ± 0.36</td>
<td>7.02 ± 0.17</td>
<td>40.80 ± 0.28</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>5</td>
<td>88.08 ± 0.18</td>
<td>6.06 ± 0.22</td>
<td>43.14 ± 0.22*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>5</td>
<td>80.14 ± 1.20*</td>
<td>4.26 ± 0.20*</td>
<td>46.68 ± 1.23*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Table 4.13. Temporal effect of carbofuran on biochemical constituents of the liver in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Protein (μg / mg wet weight of tissue; mean ± SEM)</th>
<th>Glycogen (μg / mg wet weight of tissue; mean ± SEM)</th>
<th>Total lipids (μg / mg wet weight of tissue; mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>180.40 ± 1.72</td>
<td>4.48 ± 0.25</td>
<td>40.76 ± 0.92</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>5</td>
<td>177.40 ± 1.21</td>
<td>4.68 ± 0.13</td>
<td>41.08 ± 0.84</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>5</td>
<td>176.80 ± 1.39</td>
<td>4.56 ± 0.15</td>
<td>40.18 ± 0.33</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>5</td>
<td>176.20 ± 1.85</td>
<td>4.06 ± 0.09</td>
<td>37.76 ± 0.18*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>5</td>
<td>171.20 ± 1.07*</td>
<td>2.96 ± 0.16*</td>
<td>32.90 ± 0.86*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 Compared to control
Table 4.14. Temporal effect of carbofuran on biochemical constituents of the kidney in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Protein (μg / mg wet weight of tissue; mean ± SEM)</th>
<th>Glycogen (μg / mg wet weight of tissue; mean ± SEM)</th>
<th>Total lipids (μg / mg wet weight of tissue; mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>92.36 ± 0.98</td>
<td>4.56 ± 0.15</td>
<td>33.86 ± 1.17</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>5</td>
<td>93.80 ± 0.15</td>
<td>4.66 ± 0.13</td>
<td>33.56 ± 0.28</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>5</td>
<td>92.40 ± 0.29</td>
<td>4.16 ± 0.15</td>
<td>32.08 ± 0.25</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>5</td>
<td>90.92 ± 0.21</td>
<td>4.08 ± 0.22</td>
<td>32.06 ± 0.21</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>5</td>
<td>84.86 ± 1.12*</td>
<td>2.42 ± 0.20*</td>
<td>26.80 ± 1.09*</td>
</tr>
</tbody>
</table>

* Significant P <0.05 compared to control
Graph 4.1. Effect of carbofuran on weight of the testes and diameter of seminiferous tubules in albino mice

Graph 4.2. Effect of carbofuran on number of spermatogonia, spermatocytes and spermatids in albino mice
Graph 4.3. Effect of carbofuran on the diameter of the spermatogonia, spermatocytes and spermatids in albino mice

Graph 4.4. Effect of carbofuran on the accessory reproductive organs weight in albino mice
Graph 4.5. Effect of carbofuran on body, kidney and liver weight in albino mice

Graph 4.6. Effect of carbofuran on the weights of adrenal, spleen, thymus and thyroid in albino mice
Graph 4.7. Effect of carbofuran on biochemical constituents of the testis in albino mice

Graph 4.8. Effect of carbofuran on biochemical constituents of the liver in albino mice
Graph 4.9. Effect of carbofuran on biochemical constituents of the kidney in albino mice

Graph 4.10. Temporal effect of carbofuran on weight of the testes and diameter of the seminiferous tubules in albino mice
Graph 4.11. Temporal effect of carbofuran on number of spermatogonia, spermatocytes and spermatids in albino mice

Graph 4.12. Temporal effect of carbofuran on the diameter of spermatogonia, spermatocytes and spermatids in albino mice
Graph 4.13. Temporal effect of carbofuran on accessory reproductive organs weight in albino mice

Graph 4.14. Temporal effect of carbofuran on body, kidney and liver weight in albino mice
Graph 4.15. Temporal effect of carbofuran on the weight of adrenal, spleen, thymus and thyroid in albino mice

Graph 4.16. Temporal effect of carbofuran on biochemical constituents of the testis in albino mice
Graph 4.17. Temporal effect of carbofuran on biochemical constituents of the liver in albino mice

Graph 4.18. Temporal effect of carbofuran on biochemical constituents of the kidney in albino mice