Journal of Engineering Science 10(2), 2019, 85-90

JES
an international Journal

STUDY ON THE EFFECT OF CARBOFURAN IN MALE REPRODUCTIVE ORGAN OF SWISS ALBINO MICE

P. Das[1], M. H. Morshed[2] and A. K. Roy*[1]

[1]Department of Genetic Engineering and Biotechnology, Rajshahi University, Rajshahi, Bangladesh
[2]Department of Chemistry, Khulna University of Engineering & Technology, Khulna-9203, Bangladesh

Received: 07 May 2019 Accepted: 12 November 2019

ABSTRACT

The carbamate pesticide carbofuran which is widely used in fruit, vegetable and crop fields in the world as well as in Bangladesh. The aim of this research work is to assess the toxicant consequences of carbofuran in male reproductive organ through histopathological observation of testis of Swiss albino mice. The experimental mice were grouped into T1, T2, T3 and T4 four groups and the different doses such as 0.5 ppm for T1 group, 1.0 ppm for T2, 1.5 ppm T3 and 2.0 ppm for last group T4 carbofuran were applied to each mice of the respective group. *Each of the mice of every group was subsequently sacrificed after 15 days, 25 days and 35 days. The histological slides of treated group of mice were compared with that of control mice and significant changes or abnormalities were observed in the treated mice like cytoplasmic vacuoles in the lethargic epithelium, declension of the cells in the testicular tissue with several cytoplasmic vacuoles, lumen with the loss of spermatid cells. Irregular seminiferous tubules separated from each other with complete degeneration, abnormal seminiferous tubules separated from each other with disintegration and degeneration of spermatogenic cells forming cytoplasmic vacuoles and lumen with loss of spermatid cells, widely separated irregular seminiferous tubules with complete degeneration of spermatogenic cells forming larger lumen with no germ cells. More visible abnormalities were found with the increase of dose and with long term exposure. The result of the study indicates a direct effect on male reproductive organ and may reduce fertility.*

Key words: *Pesticide; Reproductive; Histopathology; Fertility; Male Sterility*

1. INTRODUCTION

Generally, pesticides are extensively applied in agriculture sector to kill, dominate or resist pests like insect, fungi, weed and rodent. World Health Organization (WHO) defines Pesticides as chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (WHO/Pesticides, 2017). Due to the widespread using of agricultural chemicals or pesticides plants, animals and humans are being exposed to their toxic effect. Clary and Ritz (2003) studied on the activities of insecticides and reported that some of these contaminants cause chronic or persistent neurologic syndrome, immunosuppressive effects, malignant tumors, teratogenic action, abortion and reproductive failure for long time exposure. The vast use of carbamate insecticides in modern agriculture field has raised sincere public interest regarding the friendly environment and food security. The synthetic organic insecticides carbamates exhibit one of the main divisions of fungicides and introduced to the agrochemical market in the 1950s and are being used on a large scale worldwide (Tomlin, 1997). Amanullah and Hari (2011) have worked on the activities of carbamates except pesticide properties taking three carbamate insecticides such as carbaryl, baygon and carbofuran as anticancer chemotherapeutic agents on preliminary studies in vitro using trypsinized squamous cell carcinoma, since they inhibit cellular metabolism including energy, protein and nucleic acid metabolism, thereby, causing cell regression and death. The pesticide used for the present study is carbofuran which is one of the most toxic carbamate pesticides. The used insecticides carbofuran is highly powerful against a wide range of foliar-feeding and soil pests. Gupta(1994) has reported that the pesticide carbofuran is being used extensively to protect insects in a wide variety of field crops, including potatoes, corn and soybeans, alfalfa, peanuts, peppers, strawberries, tobacco, bananas, sorghum, cottonwood trees, sugar-cane and rice. The used carbamate is a systemic insecticide and has contact activity against pests. Baron (1991) studied on carbofuran and reported that it is a strong cholinesterase blockage and is highly hazard to humans and wildlife through the oral and inhalation routes of exposure. There lies the possibility that such genotoxic effects could also target the spermatozoas in human and cause deleterious effect in the long run (Roy *et al*., 2017). The applied pesticide carbofuran can strongly decrease libido and sperm number in rabbits (Yousef *et al*., 1995). Pant *et al.* (1995) has reported that the used pesticide carbamate carbofuran also disrupts testicular morphology and changes the activities of enzymes related with specific cell types of testes. Although various causative factors are intermingled in hampering the reproductive function in the selected subjects but long-term exposure to the given pesticides and insecticides could have a definite role in causing harm to the reproductive function of male individuals in this part of the country (Roy *et al*., 2017). The traces of this pesticide, in many ways, are being ingested by humans and animals. Nowadays, infertility and birth defects are commonly seen and there is a very possible chance that, pesticides like carbofuran are playing a

*Corresponding Author: akroygeb@ru.ac.bd
ISSN 2075-4914 (print); ISSN 2706-6835 (online)

vital role in such hazard. However, unlike many OP insecticides, carbamates do not require activation after absorption and onset of clinical features can be rapid (Vale *et al*., 2015). Carbamate insecticide self-poisoning to vary markedly according to the carbamate ingested although the case fatality varied according to the concentration and formulation of the insecticide (Lamba *et al.*, 2016). The industrialization of the agricultural sector has increased the chemical burden on natural ecosystems. Pesticides are agrochemicals used in agricultural lands, public health programs and urban green areas in order to protect plants and humans from various diseases (Stamati *et al.*, 2016). The purpose of the present work was to find out what this could lead to or how it may affect our health or the environment and to develop an animal model for evaluation of the developmental and reproductive effects of early pesticide exposures relevant to the human exposures.

2. MATERIALS AND METHODS

The analytical grade carbofuran was collected from alpha agro limited Bangladesh (Purity: 99.99%) and utilized for this experiment. The model animal of the experiments Swiss albino mice (male) collected from the animal rearing house of ICDDRB, Dhaka, Bangladesh at the age of 3 weeks with an average 95gm body weight. Steel cages (47×37×23 cm) were used for rearing and conducted the whole experiment. Suggested especial type of food from ICDDRB was supplied to mice with standard dose. Fresh water was provided with food wherever necessary. As histopathological tools, microtome machine, paraffin, beaker, blade, spirit lamp, alcohol (100, 95, 90, 70, and 50%), hematoxylin, eosin, Bouin's fluid, Canada balsam, mayer's albumin were utilized. The mice (16 nos) were grouped into 5 such as control (4 mice), Treatment T1 (3mice, dose 0.5 ppm), Treatment T2 (3 mice, dose 1.0 ppm), Treatment 3 (3 mice, dose 1.5 ppm) and Treatment 4 (3 mice, dose 2.0 ppm). They were kept in separate cages. Mice of control group were given only suggested standard food while treatment groups were given carbofuran supplement at different dose with standard food (Aziz *et al.,* 2007; Pant *et al.*, 1995). Each of the mice of every group was subsequently sacrificed after 15 days, 25 days and 35days. These mice were first anesthetized by diethyl ether and then sacrificed to collect the organs. The collected organs were immediately placed into saline water for washing the debris where they were sectioned into small pieces.

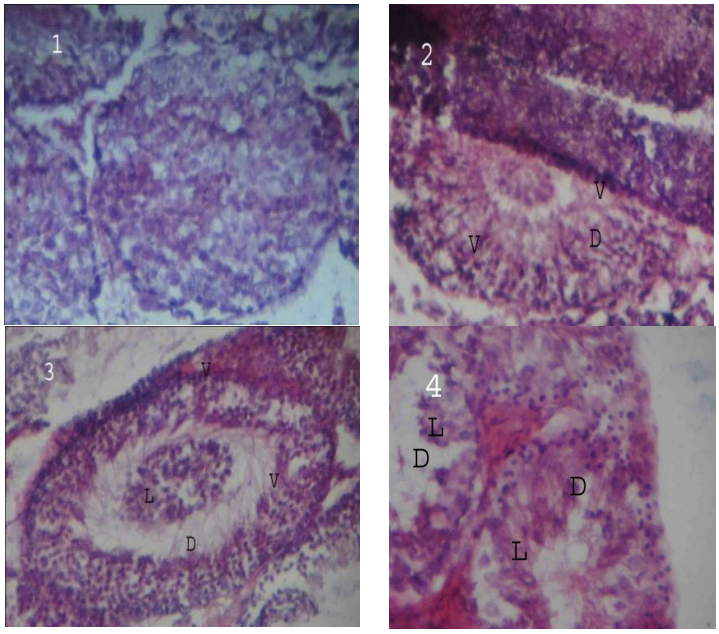

Figure 1: Histological slides of control and treated mice (dose 0.5 ppm/day)

Then these tissues were preserved into Bouin's fluid for 18 hours. After that period these tissues were washed in the running water to eliminate the trace amount of Bouin's fluid. Finally, these tissues were preserved into 70% alcohol. The preserved tissues were first taken from preserving jar. Dehydration is achieved in a series of gradually increasing concentrations of alcohol to reduce some of the shrinkage occurring in the tissue. Dealcoholization took place by xylene. Then the tissues were taken for infiltration which facilitates the penetration of wax to the available spaces inside the tissue. The tissues were then taken into paraffin bath for roasting. As soon as the tissue is thoroughly filtered with paraffin, it is ready for embedding or block making. After block making, trimming of the block were done. The cutting of ribbon was done by placing it in microtome machine. The ribbon was cut at 5 microns by microtome machine. Then these ribbons were taken into Mayer's albumin solution water. After this step the ribbon placed onto dry slide. These slides left for at

least 24hours to be dried. This slide then stained by hematoxylin and then washed by running water. After this process, staining with eosin took place (Gurr, 1962). Motic advanced system biological microscope was utilized to identify the disorder and abnormalities in cellular structure of replication organ tissue of mice with the help of Motic image J.01 software.

3. RESULTS AND DISCUSSION

The histological study of the testicular tissue of experimental animals of the control groups were in normal characteristics in comparison to the organs of the treated mice. In case of treated group of mice, histopathological changes occurred significantly. The testes from the mice of treated groups showed cytoplasmic vacuolization, disintegration and degeneration of spermatogenic cells, formation of lumen having decreased sperm cells in the seminiferous tubules, formation of irregular seminiferous tubules separated widely from each other, decreased number of pachytene spermatocytes and elongating spermatids. Highest dose provided for a long period of time created necrotic effects and complete degeneration of spermatogenic cells.

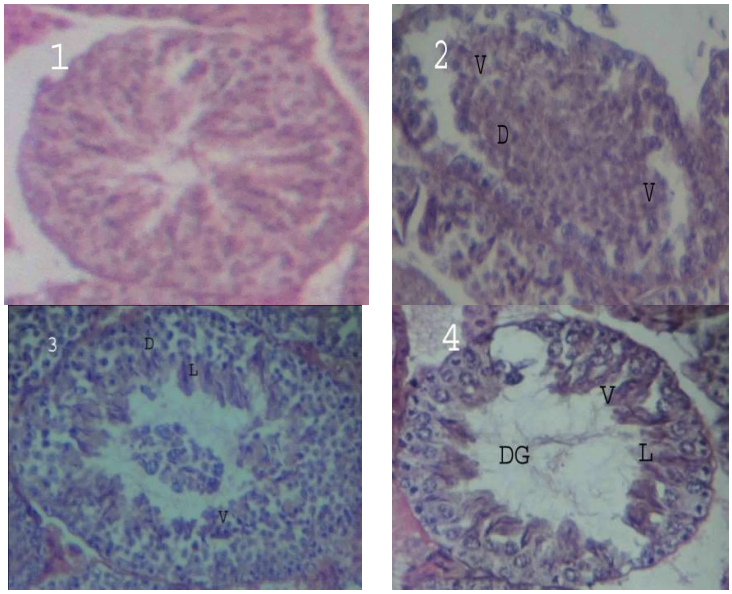

Figure 2: Histological slides of control and treated mice (dose 1.0 ppm/day)

Image (i) in Figure 1 shows the normal natural germ cells in seminiferous tubules of control testicular tissue of mice. Cellular disorder such as disintegration (D) of spermatogenic cells and formation of small cytoplasmic vacuoles (V) in the seminiferous epithelium were observed in mice organs of group T1 after 15 days of treatment (image ii). Slide (iii) reveals the disintegration (D) of cells resulting into degeneration of the cells in the testicular tissue with cytoplasmic vacuoles (V) and the formation of lumen with loss of sperm cells at the 25th day. A severe degeneration (D) of spermatogenic cells with loss of sperm cells (660x) was detected in testicular tissue of mice of the group T1 after 35 days of treatment (image iv). Slide (i) of Figure 2 presents the normal germ cells in seminiferous tubules of control testicular tissue of mice. Cellular disorder such as disintegration (D) of spermatogenic cells and formation of small cytoplasmic vacuoles (V) in the seminiferous epithelium were observed in mice organs of group T2 after 15 days of treatment (image ii of figure 2). Slide (iii) represents the disintegration (D) of spermatogenic cells with the formation of several vacuoles (V) and lumen (L) with the loss of spermatid cells after 25 days. Irregular seminiferous tubules separated from each other with complete degeneration (DG) of spermatogenic cells and a larger lumen with no germ cells (660x) were observed in testicular tissue of mice of the group T2 after 35 days of treatment (image iv of figure 2).

Slide (i) of Figure 3 shows the fresh natural germ cells in seminiferous tubules of control testicular tissue of mice. Cellular disorder such as disintegration (D) of spermatogenic cells and formation of small vacuoles (V) were observed in mice organs of group T3 after 15 days of treatment (image ii of Figure 3). Slide (iii) of figure 3reveals abnormal seminiferous tubules separated from each other with disintegration (D) and degeneration (DG) of spermatogenic cells forming cytoplasmic vacuoles (V) and lumen (L) with loss of spermatid cells at the 25th day. In slide (iv) of figure 3 irregular seminiferous tubules widely separated from each other with complete degeneration (DG) of spermatogenic cells and larger lumen with no germ cells (660 x) was observed in testicular tissue of mice of the group T3 after 35 days of treatment.

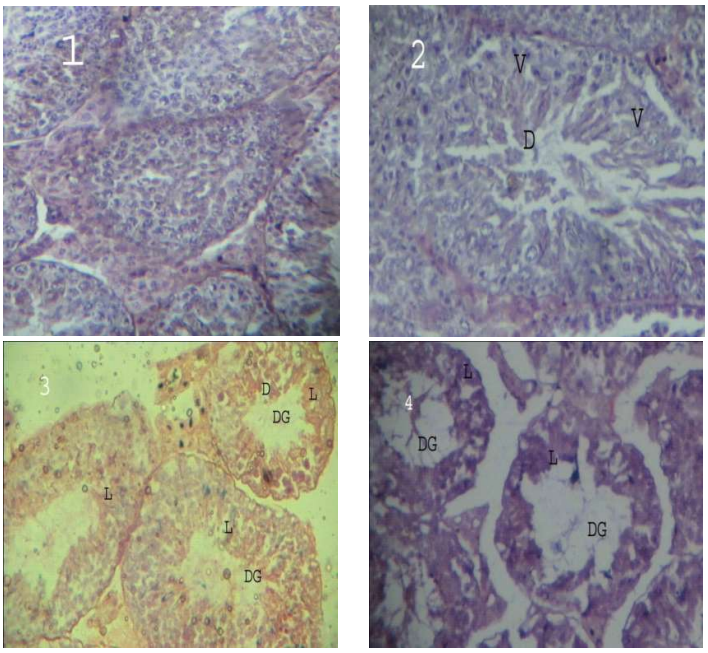

Figure 3: Histological slides of control and treated mice (dose 1.5 ppm/day)

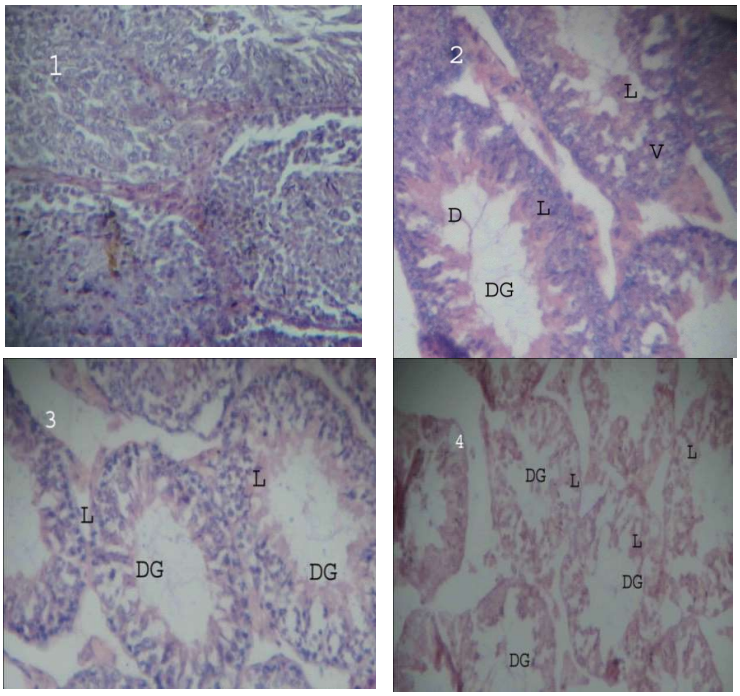

Figure 4: Histological slides of control and treated mice (dose 2.0 ppm/day)

Image (i) of Figure 4 shows the normal germ cells in seminiferous tubules of control testicular tissue of control mice. In slide (ii) of Figure 4, it is found that seminiferous tubules separated from each other, disintegration (D) of spermatogeniccells, formation of small cytoplasmic vacuoles (V) and Lumen (L) with the loss of germ cells in mice organs of group T4 after 15 days of treatment. Histological cross section (iii) presents widely separated seminiferous tubules with degeneration (DG) of spermatogeniccells forming lumen (L) with damage of spermatogenic cells at the 25th day in mice organs of group T4. In slide (iv) of Figure 4, widely separated irregular seminiferous tubules with complete degeneration (DG) of spermatogenic cells forming larger lumen with no germ cells (660x) was observed in testicular tissue of mice of the group T4 after 35 days of treatment. It is clear from the present work that carbofuran caused harmful effects in testes of albino mice. The carbamate pesticide carbofuran shows high potential to create disorder or damage to the reproductive system for long time exposure.

Tejada *et al*. (1988) studied on activities of carbofuran in rate and found the disrupters in testicular texture and change in functions of enzymes related with definite cell types of testes. On the other hand, exposure of carbofuran to the utero or lactationalin male rats produced testicular and spermatotoxicity (Pant *et al.*, 1998). Some important reports were publishedby Uzunhisarcikli *et al.* (2007) on pesticides which can createdifferent types of histopathological variations in replication process of male mammals. Continual application of common pesticide carbofuran induced to remarkable histological modifications in male reproductive organs like testes, epididymis and vas deferens as documented by previous and present study. Farag *et al.* (2000) reported that generally organophosphate insecticide acephate decrease the spermatogenic cells in the testes. Gradual sperm production was observed in male rats when they are discovered to phoxim, along with fenvalerate (Xu *et al.*, 2004). A scientific report on insecticides was published by Uzunhisarcikli *et al.* (2007) and mentioned that these are decreasing sperm counts with reductions of sperm movement. All the above studies support our present work.

4. CONCLUSIONS

Remarkable changes were observed in seminiferous tubules of the treated mice organs with tested systemic pesticides, especially with highest dose T4 in the present study. Degenerations of spermatogenic cells or sertoli cell fibrosis results in the loss of sperms. The effect also includes necrosis and disintegration of spermatogenic cells, formation of cytoplasmic vacuoles and lumen having no spermatids in the seminiferous tubiles. These condition leads to decreased number of sperm counts and abnormal sperms. In summary, carbofuran has adversely affected the reproduction organ of the test animal. The present results indicate that exposure to carbofuran has direct effects on mice testis and the decrease of spermatids may reduce fertility. Carbofuran residue remains inside the food which is being ingested by humans. The laboratory animal utilized in this research protocol showed the strong lethal effect of the used carbamate pesticide carbofuran on the male gamete generating organ. The effect results into decreased reproductively by having a direct effect on germ cell production.

REFERENCES

Amanullah, M. and Hari B.Y., 2011. Evaluation of carbamate insecticides as chemotherapeutic agents for cancer*, Indian J. Cancer,* 48, 74-79.

Aziz, N., Parveen R., Shah S. W. and Nasir R., 2007. Optical microscopic changes in male reproductive organs post-treated with Carbofuran – II. *Biosciences, Biotechnology Research Asia.,* 4 (1), 5-9.

Baron, R. L., 1991. Carbamate insecticides*, In: Handbook of pesticide toxicology*. Eds. by Hayes, W. J. and Laws, E. R., Academic Press, New York., pp.3–8.

Clary, T. and Ritz, B., 2003. Pancreatic cancer mortality and organochlorine pesticide exposure in California 1989-1996. *Am. J. Ind. Med.,* 43, 306-313.

Farag, A. T., Eweidah M. H. and El-Okazy A. M., 2000. Reproductive toxicology of acephate in male mice. *Reprod. Toxicol.,* 14(5), 457-62.

Gupta, R.C., 1994. Carbofurantoxicity, *J. Toxicol. Environ. Health*, 43(4), 383-418.

Gurr, E., 1962. Staining animal tissues, Leonard Hill Limited, London, pp.301.

Lamba T., Selvarajaha L. R., Mohamed F., Jayamanneb S., Gawarammanab I., Mostafae A., Nicholas A. Buckleyb, Robertse M. S. and Eddlestona M., 2016. High lethality and minimal variation after acute self-poisoning with carbamate insecticides in Sri Lanka – implications for global suicide prevention, *Clinical Toxicology*, 54 (8), 624–631.

Pant, N., Prasad A. K., Srivastava S. C., Shankar R., Srivastava S. P., 1995 . Effect of oral administration of carbofuran on male reproductive system of rat, *Human Experim. Toxicology*, 14(11), 889-94.

Pant, N., Shankar R. and Srivastava S. P., 1997. In utero and lactational exposure of carbofuran to rats: Effect on testes and sperm. *Human. Experim. Toxicol.*, 16, 267-272.

Roy, P., Phukan P. K., Changmai D., Boruah S., 2017. Pesticides, insecticides and male infertility, *Int. J. Reprod. Contracept Obstet Gynecol.,* 6, 3387-91.

Stamati P. N., Maipas S., Kotampasi C., Stamatis P. and Hens, 2016. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture, Review Article, *Public Health*, https://doi.org/ 10.3389/fpubh, 00148.

Tejada, A.W., Samaniego N.A, Sanchez T. F., and Magallona F. D., 1988. Fate of C1-carbofuran in lactating goats, *Phillip Agriculture*, 71, 149-155.

Tomlin, C. D. S. eds., 1997. The Pesticide Manual, 11th ed. Surrey, UK: British Crop Protection Council.

Uzunhisarcikli, M., Kalender Y., Dirican K., Kalender S., Ogutcu A., and Buyukkomurcu F., 2007. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E, *Pestic. Biochem. Phys.*, 87, 115–122.

Vale, A., and Lotti M., 2015. Organophosphorus and carbamate insecticide poisoning. *Handb. Clin. Neurol.,* 131, 149–168.

Xu, L., Zhan N., Liu R., Song L., and Wang X., 2004. Joint action of phoxim and fenvalerate on reproduction in male rats, *Asian J. Androl.*, 6, 337–341.

Yousef, M.I., Salem M.H., Ibrahim H.Z., Helmi S., Seehy M.A., and Bertheussen K., 1995. Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. J. *Environ. Sci. Health B.,* 30 (4), 513-34.

WHO, 2017. Pesticide Evaluation Scheme (WHOPES). WHO/Pesticides, www.who.int. 2017.