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HISTOLOGICAL EFFECTS OF PESTICIDES ON INTERSTITIAL CELLS OF TESTIS IN ALBINO RATS

Anatomy

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ABSTRACT

INTRODUCTION: Pesticides, though present in the environment in small quantities as compared to other contaminants such as industrial wastes and fertilizers, account for public and scientific concern due to their high biological activity. Exposure of Pesticides not only reduced fertility but also embryo/fetal loss, birth defects, childhood cancer, and other postnatal or functional deficits. Carbaryl is one of the most important insecticides as it is widely produced and used which has prompted us to initiate this study .

MATERIAL AND METHODOLOGY: The present study was conducted on 40 male Wistar albino rats as experimental animals. The rats were procured from the Animals House of the Department of Pharmacology, Government Medical College, Jammu. The rats were divided in four groups as normal control group I, group II, group III and group IV. All the rats were group housed and were fed with standard pellet diet and water for two weeks. After two weeks, the rats of group I were left as such and rats of groups II, III and IV were given 50 mg, 100 mg and 200 mg/kg body weight/day of carbaryl drug in 0.2 ml of groundnut oil orally, 6 days/week for 60 days, respectively. After 60 days, all the rats were anaesthetized by keeping them in an inverted glass jar containing large piece of cotton soaked in anaesthetic ether. The testis were dissected out from each rat and were cut into smaller pieces. These pieces were immediately fixed in 10% formalin. The blocks were prepared for section cutting with a microtome by paraffin wax embedding method. The sections of 7 µ thickness were mounted on glass slides and were stained by H&E and Masson's trichome stain.

OBSERVATIONS: Interstitial spaces are showing the oedema & Leydig cells show degeneration.

CONCLUSION: These findings are highly conclusive of reproductive toxicity produced by an insecticide, Carbaryl. Moreover, intensity of toxic effects both in peripheral and central parts increases with increase in dosage of the carbaryl drug.

KEYWORDS

Albino rats; Testis; Carbaryl; Interstitial edema

INTRODUCTION

The key to man's health lies largely in his environment. Infact, much of man's ill health can be traced to adverse environmental factors, such as water pollution, soil pollution, air pollution etc. which pose a constant threat to man's health. Often man is responsible for the pollution of his environment through urbanization, industrialization and other human activities for example commercial agriculture and garden pest control etc by using pesticides.

Pesticides, though present in the environment in small quantities as compared to other contaminants such as industrial wastes and fertilizers, account for public and scientific concern due to their high biological activity.

Pesticidesis the general term for insecticides, acaricides, rodenticides, herbicides, fungicidesetc. They are widely used in industry, agriculture and for public health purposes. Unfortunately, pesticides are toxic to a greater or lesser extent towards non-target organisms, including humans (1).

The important contributions of pesticides to our heath and economy guarantee their continued use as a class and require the most complete knowledge of their toxicology that we can achieve in order to avoid hazards.

The contributions of pesticides to health and the economy are closely interrelated. They contribute directly to our health through control of certain vector-borne diseases and to the economy through increased production of food and fibre and through the protection of many materials during storage. However, improved health has sometimes permitted a more prosperous and stable economy. In some countries greater and more dependable production of food has eliminated famine and thus contributed as much to health as to the economy.

Nearly two-third of world population does not have sufficient food when more than 30% of our food crops continue to be destroyed by pests. The total crop losses have been estimated to be more than Rs. 5000 crores annually. The Indian farmer and national economy is not prepared to bear this colossal loss due to pests.

Therefore, it has become more important to control insect pests of crops to reduce the losses and meet the requirements of increasing human population. Secondly, there is a need to check insect pest population for attaining economic threshold levels. Thus, insecticides play a pivotal role in saving the cropping enterprise from devastations of pests and in increasing food production.

Roughly 55,000 tons of pesticides are annually applied in field crops. Of this 40,000 tons are insecticide, 8000 tons are fungicides and the rest herbicides. On an average the pesticide used in India is one tenth of what is applied per unit area of cropped field in the advanced countries. Pesticides hold a unique position among environmental contaminants, being present in the environment in such small quantities as compared to other contaminants such as industrial wastes and fertilizers. The major factors which account for public and scientific concern is their biological activity.

The Interstitial Tissue

The seminiferous tubules are basically cylindrical and when a number of cylinders are stacked together, this creates a series of three-sided spaces. These spaces, known as the interstitial tissue, contain the blood vessels, lymph vessels and nerves (none of which penetrate the tubules), as well as the interstitial cells or Leydig cells. These cells were described in a variety of mammals by (2), who considered them to be modified connective tissue. Their function of producing the male sex hormone was suggested by (3). It is now generally agreed that these are the cells which produce the majority of the steroid hormones formed de novo in the testis from cholesterol.

In the rat and mouse, the lymphatic endothelium surrounding the Leydig cells is discountinuous, exposing the Leydig cells directly to the lymph. In man, clusters of Leydig cells are scattered at random in an abundant loose connective tissue which appears to be rich in interstitial fluid, and which contains one or two discrete lymphatic vessels, and blood capillaries.

The outcome of such exposures have included not only reduced fertility but also embryo/fetal loss, birth defects, childhood cancer, and other postnatal or functional deficits.

A possible reason was hypothesized to be related to the increased amount of substances in the environment which do affect the endocrine system. A large number of chemicals that have been released into the environment are known to interfere with the endocrine system. Sexual

development during the prenatal and neonatal period is under hormonal control and is, therefore, sensitive to exogenous substances with an endocrine effect. Keeping in mind the pivotal role of testis in reproduction this experimental work was done.

Carbaryl is a broad-spectrum insecticide used to protect vegetables, cotton, fruits, cereals and other crops against a variety of insects and pests. Lot of work has been done on carbaryl indicating deleterious effects of chronic and subchronic administration of carbaryl on the male reproductive system.

In the recent years, use of carbamate insecticides has gained importance due to ban of the insecticides belonging to Organochlorine groups that is D.D.T., Aldrin, Lindane and Endosulfan. These pesticides have a tendency to persist and have potential to bioaccumulate in the body (4).

Carbaryl is one of the most important insecticides as it is widely produced and used Pesticides clearly have the potential to cause reproductive toxicity in animals and several compounds are known to affect human reproduction (5,6).

Epidemiological studies postulated that in the past 50 years the sperm number and sperm quality in human had been decreased (7,8).

Pathological effects of pesticides on the reproductive system of experimental animals were recorded by many authors (9-13).

Different studies have been done to see the effects of carbamate exposure on the testis of experimental animals.

The deleterious effects of carbaryl on reproductive system have prompted me to perform this study. The present study is aimed to evaluate the effect of carbaryl on the histomorphological characteristics of the Interstitial tissue of testis in a mammal, the albino rat.

MATERIAL AND METHODS

The present study is based on the findings carried out on 40 male Wistar albino rats as experimental animals.

COLLECTION OF MATERIAL

Healthy male Wistar albino rats weighing between 50-80 grams were obtained from the Animals House. Forty rats were included in this study.

GROUPING OF ANIMALS

The rats were divided into the following four groups and identification number was given to the rats of each group.

Identification No.

Group I	:	Normal control - 10 rats	N_1 (a to j)
Group II	:	Experimental group - 10 rats	N_2 (a to j)
Group III	:	Experimental group - 10 rats	N_3 (a to j)
Group IV	:	Experimental group - 10 rats	N_4 (a to j)

All the rats were group housed in small iron cages in a room, where temperature was maintained at $23^0 \pm 1^0$C. The rats were fed with standard pellet diet and water for two weeks.

After two weeks the rats of normal control group I were left as such.

ADMINISTRATION OF CARBARYL DRUG TO EXPERIMENTAL GROUPS

Experimental groups	Date of administration of 1st dose of carbaryl drug	Route of administration of carbaryl drug	Duration of carbaryl drug given	Dose of carbaryl drug administered to each group
Group II	28/12/2010	Oral	6 days/ week for 60 days	50 mg/kg body weight/ day in 0.2 ml of groundnut oil
Group III	28/12/2010	Oral	6 days/ week for 60 days	100 mg/kg body weight/ day in 0.2 ml of groundnut oil
Group IV	28/12/2010	Oral	6 days/ week for 60 days	200 mg/kg body weight/ day in 0.2 ml of groundnut oil

DISSECTION OF EXPERIMENTAL ANIMALS

After 60 days all the rats were sacrificed after anaesthetizing them in an inverted glass jar containing large piece of cotton soaked in anaesthetic ether and dissection of testis of albino rats was done on the same day

The scrotum was skinned and a midline incision was given with the help of scalpel and forceps. The testis were dissected out from the scrotum of each rat. The naked eye examination was done to see any external changes. The dissected out testis were cut into smaller pieces (5 mm) and were kept in tissue capsules along with a label indicating the serial groups I, II, III and IV. The dissected specimens were kept in 10% formalin solution for fixation.The fixation imparts firm consistency without excessive hardening and specimens were kept for optimum time 7 – 10 days for fixation.Paraffin wax embedding method was used for preparing the tissue for section cutting. Staining was done by :-

A) Harris Haematoxyline and Eosin Stain
B) Masson's Trichrome Stain.

OBSERVATIONS

OBSERVATIONS IN NORMAL CONTROL GROUP I

MICROSCOPIC CHANGES

Interstitial cells disposed in small groups in connective tissue stroma between the tubules.

INTERSTITIAL CELLS:-

The loose connective tissue between the seminiferous tubules contains several layers of contractile peritubularmyoid cells, clusters of steroid producing interstitial cells (Leydig cells), blood vessels, nerves and lymphatics. The interstitial cells (of Leydig): are large in size and round or polyhedral in shape. The cytoplasm of these cells takes a lighter stain with a foamy appearance. Their nuclei are eccentric. These interstitial cells secrete male sex hormones (testicular androgen).

OBSERVATIONS IN CARBARYL (50 mg/kg/day) TREATED RATS GROUP II

The rats fed with 50 mg/kg/day for 60 days show following features in the testis:-

MICROSCOPIC CHANGES –

CHANGES IN THE INTERSTITIUM:-

1. Interstitial cells of Leydig show mild to moderate degenerative changes in the interstitial spaces in between the seminiferous tubules.
2. Interstitial oedema is observed in the interstitial spaces which is more prominent in the peripheral region of testis.

OBSERVATIONS IN CARBARYL (100 mg/kg/day) TREATED RATS GROUP III

The group of rats fed with 100 mg/kg/day for 60 days show following observations:-

MICROSCOPIC CHANGES –

CHANGES IN INTERSTITIUM:-

1. In addition to above findings,there are changes in the interstitium like, there are moderate degenerative changes in the interstitial cells of Leydig.
2. There is also marked interstitial oedema especially in the peripheral region of the testis. The interstitial oedema is more marked as compared to the findings seen in rats given with (50 mg/kg/day).
3. Dilated blood capillaries are observed in the focal areas in the interstitium of the seminiferous tubules.

OBSERVATIONS IN CARBARYL (200 mg/kg/day) TREATED RATS GROUP IV

The following findings are seen in the testis of rats fed with 200 mg/kg/day for 60 days:-

MICROSCOPIC CHANGES –

CHANGES IN THE INTERSTITIUM:-

1. There is oedema of the interstitial spaces which is seen both in central and peripheral region but is more marked in the peripheral region. Interstitial oedema is also much significant in this group

compared with other groups.

2. Interstitial spaces exhibit much prominent degenerative changes in the Leydig cells compared to other groups with low doses of carbaryl.

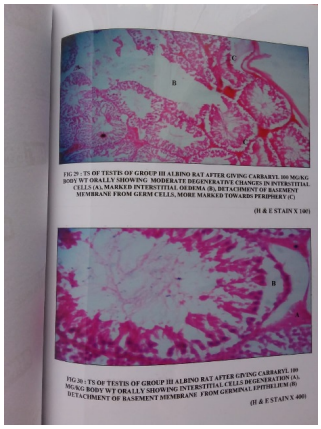
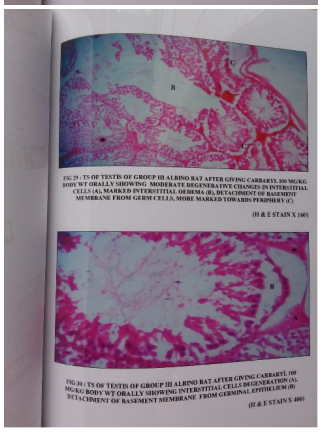


FIG 29 : TS OF TESTIS OF GROUP III ALBINO RAT AFTER GIVING CARBARYL 100 MG/KG BODY WT ORALLY SHOWING MODERATE DEGENERATIVE CHANGES IN INTERSTITIAL CELLS (A), MARKED INTERSTITIAL OEDEMA (B), DETACHMENT OF BASEMENT MEMBRANE FROM GERM CELLS, MORE MARKED TOWARDS PERIPHERY (C)

(H & E STAIN X 100)

FIG 30 : TS OF TESTIS OF GROUP III ALBINO RAT AFTER GIVING CARBARYL 100 MG/KG BODY WT ORALLY SHOWING INTERSTITIAL CELLS DEGENERATION (A), DETACHMENT OF BASEMENT MEMBRANE FROM GERMINAL EPITHELIUM (B)

(H & E STAIN X 400)



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(H & E STAIN X 100)

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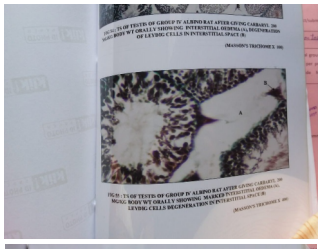
(H & E STAIN X 400)



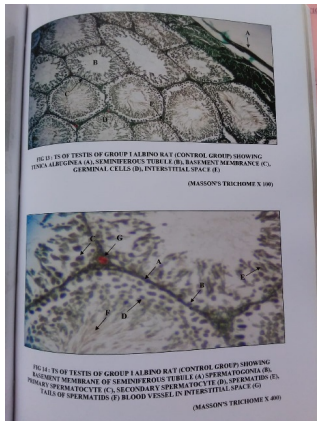


FIG 31 : TS OF TESTIS OF GROUP I ALBINO RAT (CONTROL GROUP) SHOWING TUNICA ALBUGINEA (A), SEMINIFEROUS TUBULE (B), BASEMENT MEMBRANCE (C), GERMINAL CELLS (D), INTERSTITIAL SPACE (E)

(MASSON'S TRICHOME X 100)

FIG 32 : TS OF TESTIS OF GROUP I ALBINO RAT (CONTROL GROUP) SHOWING BASEMENT MEMBRANE OF SEMINIFEROUS TUBULE (A) SPERMATOGONIA (B), PRIMARY SPERMATOCYTE (C), SECONDARY SPERMATOCYTE (D), SPERMATIDS (E), TAILS OF SPERMATIDS (F) BLOOD VESSEL IN INTERSTITIAL SPACE (G)

(MASSON'S TRICHOME X 400)

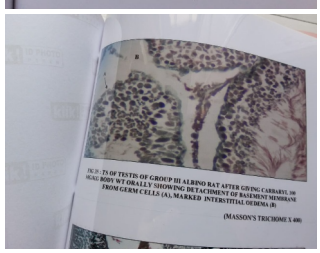


FIG 25 : TS OF TESTIS OF GROUP III ALBINO RAT AFTER GIVING CARBARYL 100 MG/KG BODY WT ORALLY SHOWING DETACHMENT OF BASEMENT MEMBRANE FROM GERM CELLS (A), MARKED INTERSTITIAL OEDEMA (B)

(MASSON'S TRICHOME X 400)

DISCUSSION

An extremely complex mechanism underlies the effects of various substances on reproductive components and functions. Various chemicals may interfere in different ways with components of reproductive system. They may affect directly by interference of the substance with reproductive components or indirectly by altering hormonal regulations.

The carbamate insecticides, one of which is Carbaryl, exert their insecticidal action by inhibiting cholinesterase enzymes. This inhibition is the primary mechanism by which these insecticides cause toxicity in mammals. The cholinesterase enzymes hydrolyze acetycholine and other choline esters; consequently, their inhibition leads to the accumulation of endogenous acetycholine and other choline esters. Probably most of the biologic effects of anticholinesterase agents, including carbaryl, are due to the inhibition of acetylcholinesterase which leads to the accumulation of endogenous acetycholine, the principal choline ester that has demonstrated physiologic significance in humans. The aim of present experiment is to study normal histological characteristics of the Interstitium of the testis of albino rats and also to study dose-related effects of carbaryl on the interstial tissue of testis of male albino rats with special reference to histomorphological changes.

The present study shows oedema of interstitial spaces of testis. Same findings were also reported by (14) in the testis of albino rats following administration of carbaryl in dose of 100 and 200 mg/kg body weight in 0.2 ml of groundnut oil orally/6 days week for 60 days. Similar findings were also reported by (15-17),who have reportedspermatotoxic effect of carbaryl in adult and young male rats given with 50 and 100 mg/kg body weight. Degraeveet al.(1976)(18) found that in mice injected intraperitoneally with 0.4 mg of carbaryl daily for one week, the incidence of sperm abnormalities were reportedly increased, but no degenerative changes in the testis were seen.

Kitagawa et al.(1977)(19) reported reduced numbers of spermatogonia and spermatozoa in rats given 3 mg of carbaryl per week orally for one year.

Rani et al.(2007)(14) found marked histopathological changes in testis of albino rats following oral administration of carbaryl in dose of 100 and 200 mg/kg body weight in 0.2 ml of groundnut oil orally, 6 days/week for 60 days.

The light microscopic observation demonstrated distorted shape of seminiferous tubules, disturbed spermatogenesis, accumulation of cellular mass in the lumen of tubules, oedema of interstitial spaces and loss of sperms of varying degrees and detachment of germ cells from the basement membrane. Nonetheless, carbamate use may pose a significant risk of poisoning if handled carelessly. Health professionals may need to assess the consequences of prior exposure and should understand the fate of these compounds after absorption by humans.

Male fed 5 days/week for 60 days, caused dose and age-dependent decline in epidydmal sperm count and sperm motility and an increase in number of sperms with abnormal morphology. Young animals in comparison to adults exhibited pronounced spermatotoxic effects. Some of these findings are in accordance with the present study in which the dose-related decreased spermatogenesis and loss of sperms of varying degrees have been found.

SUMMARY AND CONCLUSION

Carbaryl is being used extensively as a broad spectrum pesticide. It is known toxicant to the male reproductive system and, is therefore, under focus in the present study. The results of the present study have thrown some light on the toxic effects of carbaryl on testicular functions that are essential for reproductive success.

The present study was conducted on 40 male Wistar albino rats as experimental animals. The rats were divided in four groups as normal control group I, group II, group III and group IV. All the rats were group housed and were fed with standard pellet diet and water for two weeks. After two weeks, the rats of group I were left as such and rats of groups II, III and IV were given 50 mg, 100 mg and 200 mg/kg body weight/day of carbaryl drug in 0.2 ml of groundnut oil orally, 6 days/week for 60 days, respectively. After 60 days, all the rats were anaesthetized by keeping them in an inverted glass jar containing large

piece of cotton soaked in anaesthetic ether. The testis were dissected out from each rat and were cut into smaller pieces. These pieces were immediately fixed in 10% formalin. The blocks were prepared for section cutting with a microtome by paraffin wax embedding method. The sections of 7 thickness were mounted on glass slides and were stained by H&E and Masson's trichome stain.

The following findings are drawn from the study:-

(1) Interstitial spaces are showing the oedema.
(2) Degenerated Leydig cells are also seen.

However, since the study was conducted on experimental animals and results may not be exactly the same in humans, suffering from the carbaryl toxicity. But in no case it can be overlooked, while designing a therapy for pesticides, where it becomes necessary to take into consideration the effects of carbaryl on these tissues of vital importance.

It is concluded that the toxic effects are more pronounced in the peripheral parts of the sections of testis. Moreover, intensity of toxic effects both in peripheral and central parts increases with increase in dosage of the carbaryl drug.

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