ORIGINAL ARTICLE

Histological Changes Observed in the Mammalian Male Reproductive Organ Post-treated with Pesticide (Furadan): a light microscopic study

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ABSTRACT

Carbofuran (Furadan) is a broad spectrum carbamate pesticide that has a potential to cause damage to the reproductive system and to the health through prolong exposure. The widespread use of pesticide in public health and agriculture has caused severe environmental pollution and health hazards. This study describes the toxic effects of furadan on prostate gland of the albino rats. The animals (n=24) were grouped as A (control), B and C (experimental).The experimental animals of group B were administered with 2 mg/Kg body weight/ day carbofuran in saline for 60 days and then sacrificed. The animals of group C were kept without treatment for 30 days and then sacrificed to see the delayed effects. Prostate glands were fixed and processed to examine the histological changes by light microscope. The histlogical examination of prostate showed highly congested hyperplastic, fibromuscular stroma filled with inflammatory cells. The parenchyma showed degenerated glandular epithelial cells and empty lumen of the tubuloalveolar glands in animals treated for 60 days. These changes persisted to some extent even after stoppage of furadan treatment for 30 days.

Key words: Furadan, Prostate, Histological changes, Light microscopy

INTRODUCTION

The chemicals used to combat a variety of pests harmful to people, domestic and wild animals, crops and forests are generally called pesticides. They include insecticides, fungicides, herbicides, rodenticides, bactericides, miticides, nematocides and molluscicides, which can enter the body through the mouth, lungs, skin contact and wounds. Experts estimate that, in developing countries, thousands of people die from pesticide poisoning. A larger number of people are reported to be chronically exposed to pesticide residues on food and in water.

Carbofuran (Furadan) is a white crystalline solid with a slightly phenolic odor. This broad spectrum insecticide is sprayed directly onto soil and plants just after emergence to control beetles, nematodes and rootworm on contact or after ingestion. It is used against soil and foliar pests of field, fruit, vegetable, and forest crops. There are many examples of pesticide poisoning through food[1]. The greatest use of carbofuran is on alfalfa and rice, with turf and grapes making up most of the remainder. It is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. Death may result at high doses carbofuran exposure. The widespread use of pesticides in public health and agriculture has caused from respiratory system failure associated with severe environmental pollution and health hazards including cases of severe, sub- chronic and chronic human poisoning[2,3a,3b,4,5,6,7,8]. Chronic toxicity of furadan results in behavioral and neurochemical changes[9].

Gonadal toxicity has been reported in male after chronic exposure to various carbamates[10]. Toxic effects of carbofuran on semen characteristics has also been reported[11]. In a recent study, histo-toxicity of chronic use of furadan has also been reported on male reproductive organs like epididymis leading to infertility[12]. Prostate gland comprises of 50%-70% glandular tissue and 30%-50% fibromuscular tissue in mammals. It has duct-acini system. Acini are lined by columnar secretory cells and fibromuscular tissue comprises of smooth muscle fibers and collagen fibers mainly with scattered lymphocytes and other connective tissue cells. Angrogens from adrenal glands and testes control development, maintenance, growth and secretory functions of prostate[13]. It plays a vital role in the fertility of animals by providing 30-50% of the seminal fluid through its prostatic secretions which form early fraction of semen comprising of prostatic specific antigens, citrate, zinc, acid phosphatase and other enzymes.

This study was designed to see the acute and chronic toxic effects of Furadan on rat prostate gland which plays a vital role in the fertility of males

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because histo-toxicity of furadan on prostate gland has not been documented by previous studies.

MATERIALS AND METHODS

Adult albino male rats (n=24), taken from the Veterinary Institute, University Sains Malaysia were divided into three groups as A, B and C each comprising of eight animals. Each group was further subdivided into A1, A2, B1, B2, C1 and C2 respectively comprising of four animals in each sub-group. Animals of group A (control) were given normal saline orally for a period of two months. The rats were housed under standard laboratory conditions and free access to food and water ad libitum. Group B animals were given 2 mg/kg body weight/ day of furadan obtained from FMC Corporation (Philadelphia, USA) dissolved in normal saline. Each animal was given 1 ml / kg body weight furadan in saline daily for the same period as the control group. Group C was treated like group B for a period of two months and then left for another one month without treatment. The animals of group A1, B1, B2 were sacrificed by euthanasia with sodium phenobarbitol 100 mg / kg body weight on day 60. Laparotomy was performed, prostate glands were carefully dissected and were fixed in 10% formaline, embedded in paraffin and 5 um sections were cut and stained with haematoxylin and eosin stain. The tissue sections were observed under Nikon Eclipse E-600 Image Proplus V 4.5 Dual Dimension Microscope (Japan) for histological changes in prostate glands. The animals of group A2, C1 and C2 were sacrificed on day 90 and prostate glands were examined.

RESULTS

Group A (Control): A collection of tubuloalveolar glands. The parenchyma of the glands is formed by cuboidal or pseudostratified columnar epithelium. The stroma is formed by thick fibro-muscular tissue. It is surrounded by a fibroelastic capsule rich in smooth muscle fibers. The septas from its capsule penetrate the gland to divide it into lobes (Fig.1,2).

Group B (experimental)
Fibromuscular stroma is infiltrated with lymphocytes and other inflammatory cells.
The parenchyma is showing degenerated glandular cells. The lumen of the tubuloalveolar glands is empty (Fig. 3)

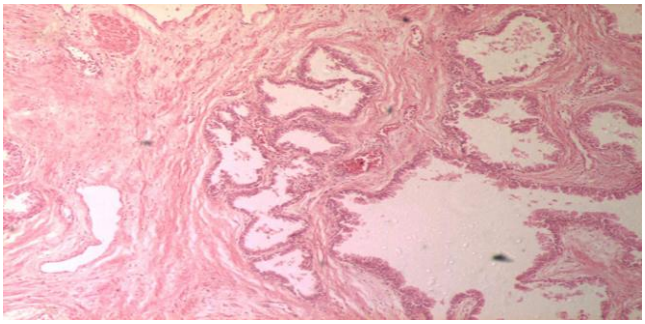

Fig. 1 Microphotograph of group A (control) rat prostate gland showing simple acini with complex intraluminal ridges, papillary infoldings and epithelial arches set in stroma of smooth muscle and collagen fibers H&E 40x

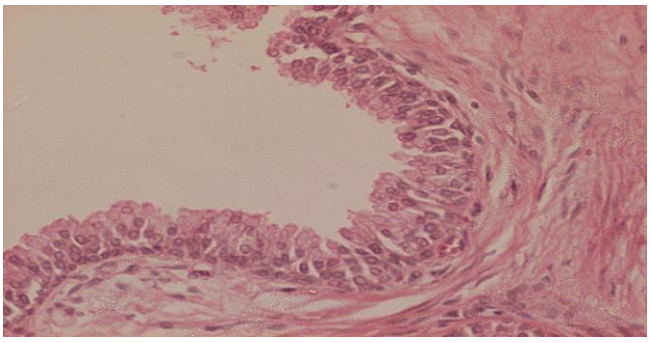

Fig. 2: Microphotograph of group A (control) rat prostate gland showing columnar epithelium with small round basal nuclei and a basal cell layer. H&E 200x

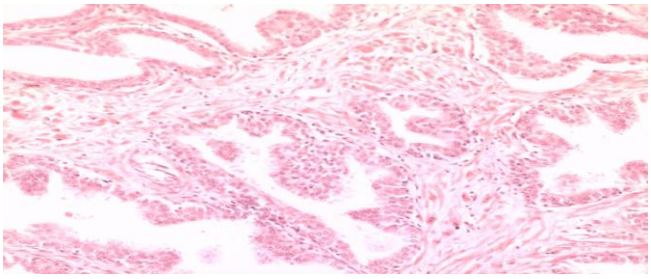

Fig. 3: Microphotograph of group B (experimental) rat prostate gland showing mild degeneration of glandular epithelium and cellular infiltration of stromal fibromuscular tissue. H&E 100x

Group C (experimental): Highly congested fibromuscular stroma filled with inflammatory cell. Degenerated and distorted glandular epithelium is prominent (Fig.4,5). Swelling of the cytoplasm and ruptured nuclear membrane shows the degeneration of the glandular epithelial cells. (Fig. 6**)**

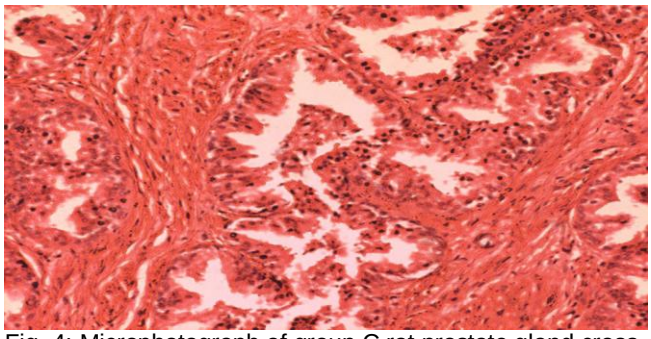

Fig. 4: Microphotograph of group C rat prostate gland cross section showing fibromuscular stroma and slight degeneration of glandular epithelial cells H&E 100x

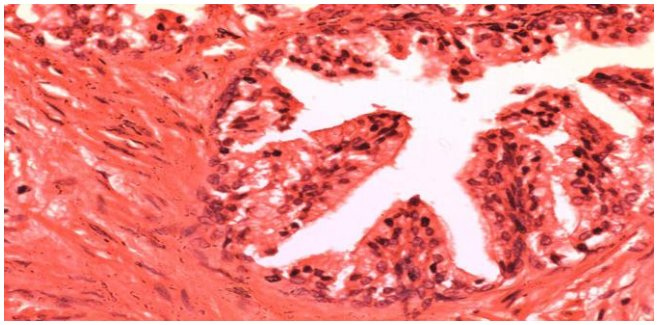

Fig. 5: Microphotograph of glandular epithelium and stroma of rat prostate gland showing moderate degeneration of glandular epithelium. H&E 200x

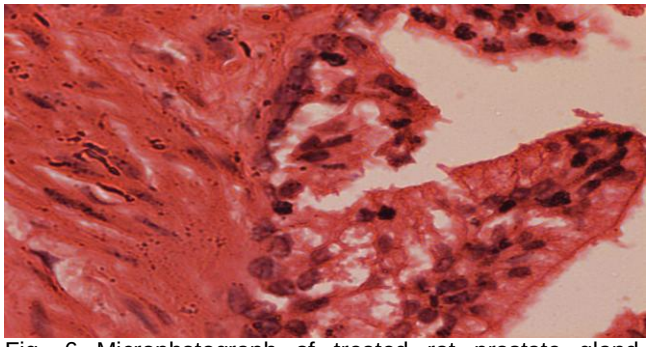

Fig. 6 Microphotograph of treated rat prostate gland showing distorted, degenerated secretory columnar epithelium, fibroblasts and smooth muscle cells. H&E 400x

DISCUSSION

Furadan has the potential to cause damage to the reproductive system at doses around 0.2 mg/kg bw in both rat and dog in the form of degeneration of seminiferous tubules, loss of spermatogenesis, degenerative changes in Sertoli cells, and depletion of a variety of cell types. The dramatic increase in the use of carbamate compounds substitutes for chlorinated hydrocarbon insecticides has resulted in a new dimension of occupational hazard for agriculture industry[13].

Chronic administration of carbofuran led to significant histological changes in male reproductive organs like testes, epididymis and vas deferens as documented by previous studies[12].

The spermatozoa travel through the epididymis for maturation under the influence of various enzymes and factors released by Sertoli and epididymal mucosal cells. Thence they pass to the vas deferens to get further maturation and motility under the influence of various enzymes secreted by the mucosal cells of vas deferens.These mature and active spermatozoa stay in the vas deferens till they reach to the prostatic urethra to receive prostatic secretions. Mature but inactive spermatozoa are incapable to fertilize the ovum in the absence of prostatic and seminal secretions which constitute the main bulk of semen. Cyto-toxicity of furadan to the glandular epithelium of the prostate gland affects the quality and quantity of prostatic secretions. Degeneration of prostatic glandular epithelium and empty tubulo-alveolar glands of prostatic parenchyma in this study confirms the acute toxicity of furadan. It may result into male infertility.The administration of methomyl carbamate (17 mg/Kg body weight in saline) daily for two months has also shown similar histological changes in male reproductive organs[14].

Normal functioning and growth of prostate gland is under control of male hormone, the testosterone. The spermatogenesis in mammals depends on testosterone production by Leydig cells in response to stimulation by FSH and LH. FSH increases Sertloi cell synthesis of an androgen binding protein needed to maintain high concentrations of testosterone. LH stimulates testosterone production by the interstitial cells of the testis[15].

The hormonal changes produced by carbofuran compounds favour direct toxic effect of insecticide or possibly through a change in the neuroendocrine environment resulting into acetyl cholin estrase inhibition[16].

The effect on germ cells might be due to the decrease in testosterone level resulting due to injury of the cells of Leydig[17]. Organophosphate insecticides cause disturbance of other metabolic processes apart from inhibiting acetylcholine esterase activity. Insecticides are capable of binding to the lipid component of mitochondrial membrane resulting into change in mitochondrial function[18].

Androgens from adrenal glands and testes control development, maintenance, growth and secretory functions of prostate[13]. Interaction of androgens with prostatic epithelium, stroma, neuroendocrine cells, other hormones and growth

factors form a complex system designed to regulate not only prostatic growth but also relative number of epithelial and non-epithelial cells[19]. Loss or decrease in the level of testosterone will lead to involution and apoptosis of prostate. The effects of estrogen and testosterone on sex accessory glands are antagonist[20]. So increase in estrogen as a result of decrease or absence of testosterone will increase the fibromuscular tissue of prostate. So degeneration of glandular epithelium and increase in fibromuscular tissue observed in present study is due to decrease in the level of testosterone as a result of direct toxic effects of furadan on Leydig cells and Sertoli cells in response to stimulation by LH and FSH respectively.

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