

Carbimazole-induced histopathological and histochemical changes in epididymis of albino rats: Ameliorative effect of selenium

S.A.Sakr*, H.A.Mahran, A.E.Nofal

Zoology Department, Faculty of Science, Menoufia University, Shebin El-kom, (EGYPT)

E-mail: sabsak@yahoo.com

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ABSTRACT

Carbimazole is an antithyroid drug used in treatment of hyperthyroidism. The present investigation studied the effect of carbimazole on epididymis of albino rats and the ameliorative role of selenium. Treating rats with carbimazole (1.35 mg/Kg b.w) daily for 8 weeks caused distinct histological alterations in the epididymis compared with control group. The ductus epididymis showed hyperplasia, congestion of the interductular blood vessels, marked vacuolization, apoptosis and decrease of characteristic stereocilia. Also, inflammatory infiltrations were observed in the stroma. The morphometrical results showed that the ductual diameter and epithelial height of epididymal duct were significantly reduced. Histochemical examination of the epididymis of treated rats showed noticeable decrease in the polysaccharide, protein and nucleic acids contents. The observed results were time-dependent. Treating animals with carbimazole and sodium selenite (10 µg/Kg b.w) showed an improvement in the histological structure as well as histochemical components of the epididymis. It is suggested that the curative effect of selenium against damage induced by carbimazole may be due to its antioxidant properties.

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KEYWORDS

Carbimazole;
Selenium;
Epididymis;
Rat.

INTRODUCTION

Carbimazole is a thionamide drug used in treatment of hyperthyroidism and reduce thyroid function before surgery. Carbimazole is a pro-drug as after absorption it is converted to the active form, methimazole. Methimazole prevents the thyroid peroxidase enzyme from coupling and iodinating the tyrosine residues on thyroglobulin, hence reducing the production of the thyroid hormones T3 and T4^[1]. On the other hand, many authors reported that the use of carbimazole was accom-

panied by deleterious effects including hepatotoxicity, nephrotoxicity and pancreatitis^[2-4]. Zaidi et al.^[5] reported that carbimazole administration even in therapeutic dose during pregnancy and lactation resulted into alteration of the thyroid microstructure of newborn. After carbimazole treatment, the risk of thyroid carcinoma increases with the time and appears to be considerable higher than after radioiodine treatment^[6]. Sakr et al.^[7] reported that carbimazole caused reproductive toxicity in male albino rats.

Selenium is an essential important element in many

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biochemical and physiological processes including the biosynthesis of coenzyme Q (a component of mitochondrial electron transport systems), regulation of ion fluxes across membranes, maintenance of the integrity of keratins, stimulation of antibody synthesis, and activation of glutathione peroxidase^[8]. Gärtner^[9] reported that the plasma selenium levels indicate the amount of circulating selenoproteins and selenoenzymes. These are important for modulating the immune system and also for thyroid hormone metabolism. Moreover, sodium selenite is commonly used as a direct supplement for the treatment of selenium deficiency. Selenium had antioxidative effect and capacity to prevent cancer^[10]. Liao et al.^[11] reported that selenium played a beneficial role for prevention of cisplatin hepatotoxicity in mice. Jihen *et al.*^[12] found that selenium has a cooperative effect in the protection against cadmium-induced structural damage in the liver. El-Shenawy and Hassan^[13] reported that selenium has a protective effect against liver and kidney damage induced by mercury chloride in rats. The results of several studies indicate that selenium may play a part in the male reproductive system^[14,15]. An accumulation of ⁷⁵Se was found in the testis of mice^[16] and rats^[17] after the administration of ⁷⁵Se. The authors added that ⁷⁵Se moved with the spermatozoa from testis to epididymis. The present work studied the effect of selenium on carbimazole-induced histological and histochemical alterations in epididymis of albino rats.

MATERIAL AND METHODS

Chemicals

Carbimazole (Neomercazole) is an antithyroid drug obtained from Chemical Industries Development Co. Giza, Egypt. Sodium selenite was supplied British Drug Houses LTD Laboratory Chemicals Division, England. All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

Animals and treatments

Sexually mature male albino rats (*Rattus norvegicus*) weighing 115 ± 5 g and aged 15 weeks were purchased from the breeding center of experimental animals at Helwan University, Helwan, Egypt. The animals were kept in the laboratory under constant temperature ($22 \pm 1^\circ\text{C}$) for at least one week before and

along the period of the experimental work. They were maintained on a standard rodent diet composed of 55% corn starch, 20% casein, 15% corn oil, 5% salt mixture and 5% vitaminized starch (Egyptian Company of Oils and Soap Kafr-Elzayat, Egypt). Water available *ad libitum*. Animals were divided into 4 groups:

Group 1: animals of this group (20 rats) were served as normal control.

Group 2: animals of this group (30 rats) were orally given carbimazole (1.35 mg/Kg b.w)(equivalent to the therapeutic dose for human^[18]) dissolved in water, daily for 8 weeks.

Group 3: animals of this group (30 rats) were orally given sodium selenite (10 $\mu\text{g/Kg b.w}$) dissolved in water, daily for 8 weeks.

Group 4: animals of this group (30 rats) were orally administered carbimazole and sodium selenite^[14] daily for 8 weeks. All the experiments were done in compliance with the Guide for the Care and Use of Laboratory animals. The treated animals were sacrificed by cervical decapitation after 4 and 8 weeks of treatment.

Histological and histochemical examination:

Ten rats were weighted and sacrificed from treated and control groups after 4 and 8 weeks. Their epididymis were excised. For histological study epididymis were fixed in alcoholic Bouin's fluid, dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of five micrometers thickness were cut and stained with haematoxylin and eosin for histological examination. For histochemical study specimens were fixed in Carnoy's fluid. Periodic acid Schiff's reaction^[19] was used for demonstration of polysaccharides. Total proteins were detected using the mercury bromophenol blue method^[20]. Nucleic acids (DNA&RNA) were determined using Schiff-methylene blue method^[21]. The ductal diameter and epithelial height of epididymal duct were measured in sections of the acinus stained with HE. All data were obtained from 10 random microscopic fields per animal at X 100 objective.

RESULTS

Morphometrical results

Data in figure (1a&b) showed that treating animals with carbimazole for 8 weeks caused significant decrease ($P < 0.05$) in the ductal diameter and epithelial

height of epididymal duct. On the other hand, animals treated with carbimazole and selenium for the same period showed significant increase in these parameters in comparison with carbimazole group. No significant changes were recorded in ductual diameter or epithelial height of epididymal duct in selenium-treated rats compared with controls.

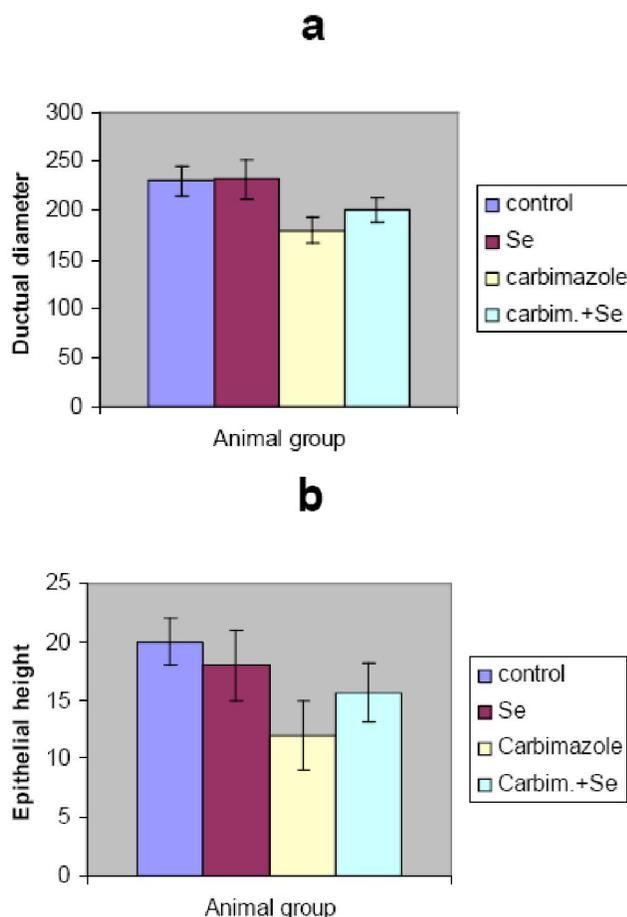


Figure 1 : Change in ductual diameter (a) and epithelial height of epididymal duct (b) in different animal groups (mean± SD µm).

Histopathological results

Figure 2 showed epididymis of control rat. Sections of caput epididymis consisted of numerous ductus epididymis surrounded by a myoconnective tissue sheath. The duct had a wide lumen in which sperms were stored. The entire ductus epididymis was lined with a pseudostratified stereociliated columnar epitheliumk. There are four cell types: principal, basal, apical and migratory cells. Epididymis of rats administered with carbimazole for 4 weeks showed many histopathological changes. These changes included hyperplasia and congestion of the interductular blood vessels (Figure 3a).

The epithelial cells appeared with marked vacuolization and decrease of characteristic stereocilia (Figure 3b). Examination of epididymis of rats after 8 weeks of the same treatment revealed that the ductus epididymis were deformed and lost their normal shape. Most of them showed marked hyperplasia and inflammatory infiltrations were observed in the stroma (Figures 4a & b). Some of the epithelial cells were exhibited apoptosis, others appeared with marked cytoplasmic vacuolization and few stereocilia (Figures 5a& b). The connective tissue stroma showed thick smooth muscle fibers and congested interductular blood vessels (Figure 5c). The sperm bundles were degenerated in some of the ductus and completely absent in the others. Examination of epididymis of rats treated with carbimazole and selenium for 8 weeks revealed less prominent histopathological changes when compared with the same period of carbimazole group. In these specimens, The ductus epididymis showed normal epithelial cells with increase in stereocilia and there was an obvious increase in the sperm bundles in their lumens. The interductular blood vessels appeared less congested (Figures 6a&b).

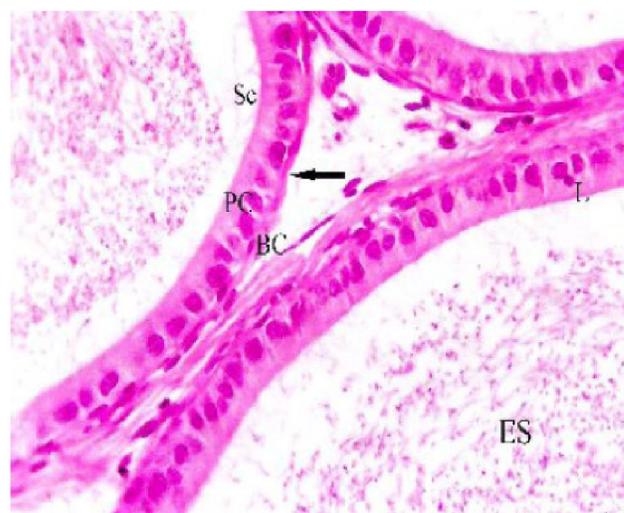


Figure 2 : Enlarged portion of ductus epididymis of a control rat showing normal ductus structure. The ductus epithelium containing lymphocytes (L), principle cell (PC) and basal cell (BC). Basal membrane (thin arrow), stroma (Sm), stereocilia (Sc) and elongated sperm (ES) are seen, (H&E. X400).

Histochemical results

Polysaccharides

Epididymis of control rat showed a moderate PAS-positive reaction in the cytoplasm of principal, basal and apical cells. The nuclei of these cells showed nega-

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tive stain. The stereocilia showed a moderately to strongly PAS-positive reaction. Muscle fibers and head of sperms appeared with strong PAS-positive reaction (Figure 7a). Epididymis of carbimazole-treated animals revealed gradual decrease of PAS-positive mate-

rials. This decrease started after 4 weeks of treatment and reached its maximum after 8 weeks (Figure 7b). Examination of epididymis of rats treated with carbimazole followed by selenium showed a gradual increase of the polysaccharide content (Figure 7c).

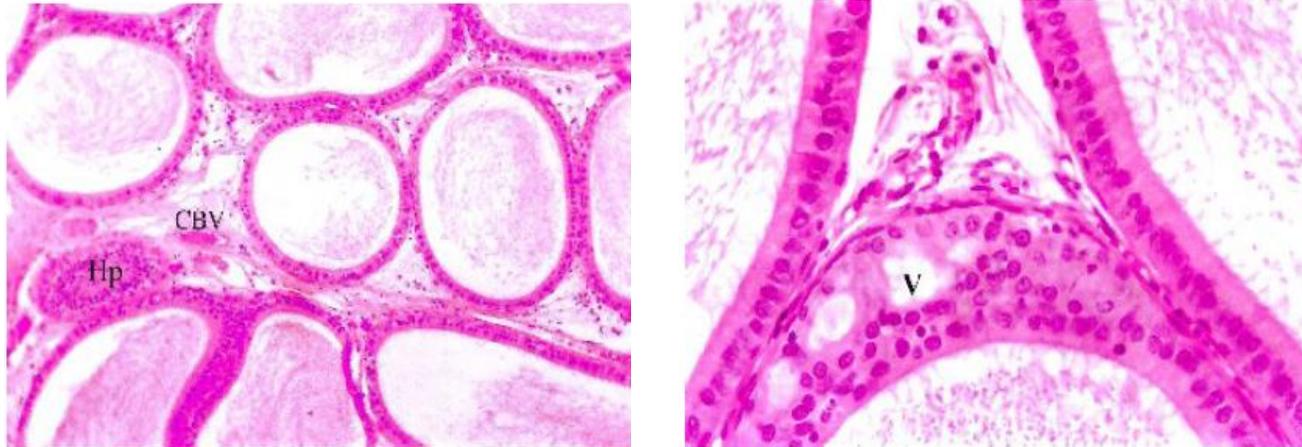


Figure 3 : Sections in epididymis of a rat treated with carbimazole for 4 weeks showing (a) hyperplasia (Hp) and congested interductular blood vessels (CBV), (H&E., X100) and (b) degeneration and marked vacuolization of ductus (V), (H&E., X400).

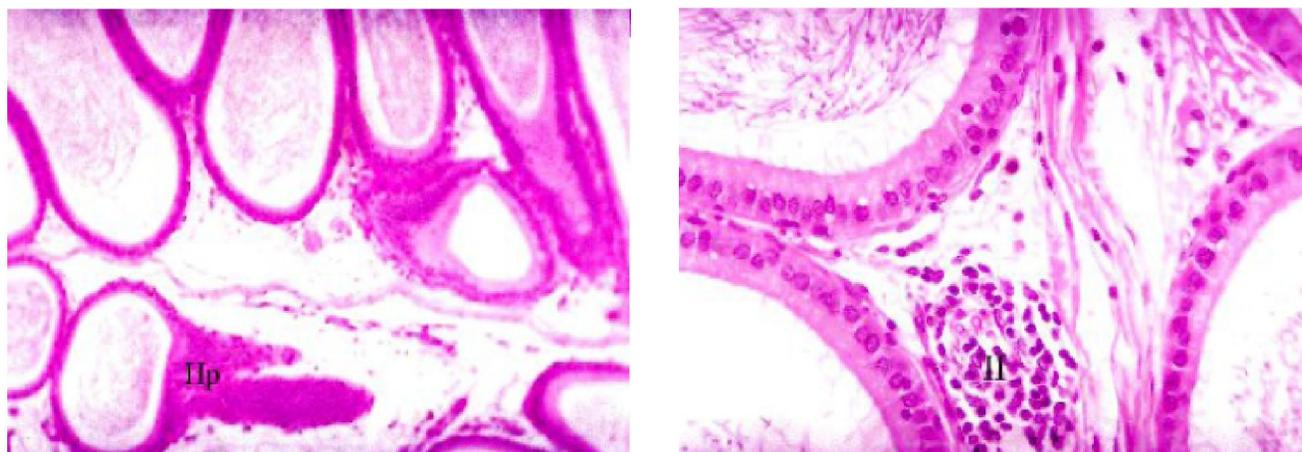


Figure 4 : Sections in epididymis of a rat treated with carbimazole for 8 weeks showing (a) marked hyperplasia (Hp), (H&E., X100) and (b) inflammatory infiltration (II), (H&E., X400).

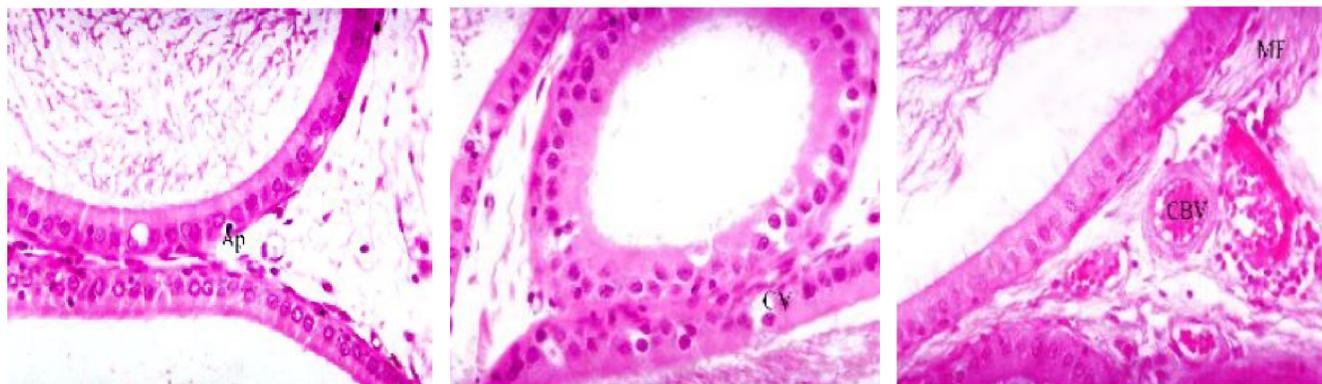


Figure 5 : Sections in epididymis of a rat treated with carbimazole for 8 weeks showing (a) apoptotic cell (AP), (b) marked cytoplasmic vacuolization (CV) and decrease in stereocilia, (H&E., X400) and (c) marked increase in muscle fibers (MF) and congested interductular blood vessel (CBV), (H&E., X400).

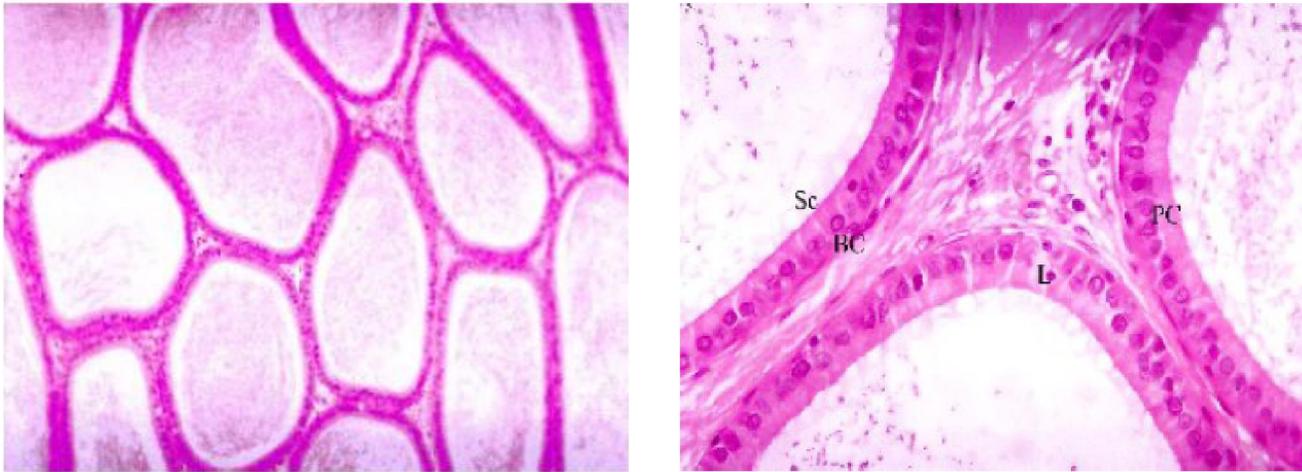


Figure 6 : Sections in epididymis of a rat treated with carbimazole and selenium showing (a) normal structure of ductus epididymis, (H&E, X 100) and (b) improvement in the ductus epithelium containing lymphocytes (L), principle cell (PC), basal cell (BC) and increased stereocilia (Sc), (H&E., X 400).

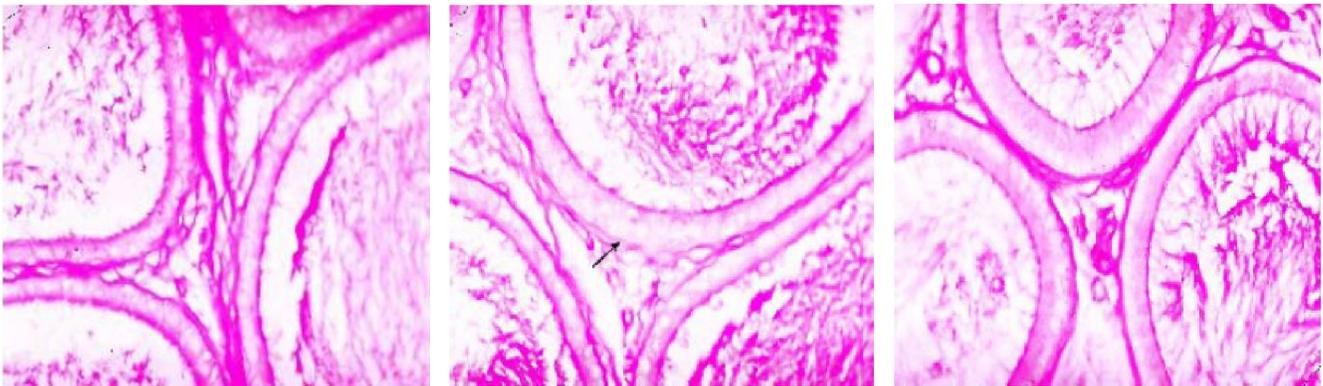


Figure 7 : Enlarged ductus epididymis of (a) a control rat showing moderate PAS-positive reaction in the epithelial cells and strong reaction in muscle fibers, (b) a rat daily treated with carbimazole for 8 weeks showing a decrease of PAS-positive reaction in the epithelial cells (arrow) and (c) a rat daily treated with carbimazole and selenium for 8 weeks showing improvement PAS-positive materials, (PAS, X400).

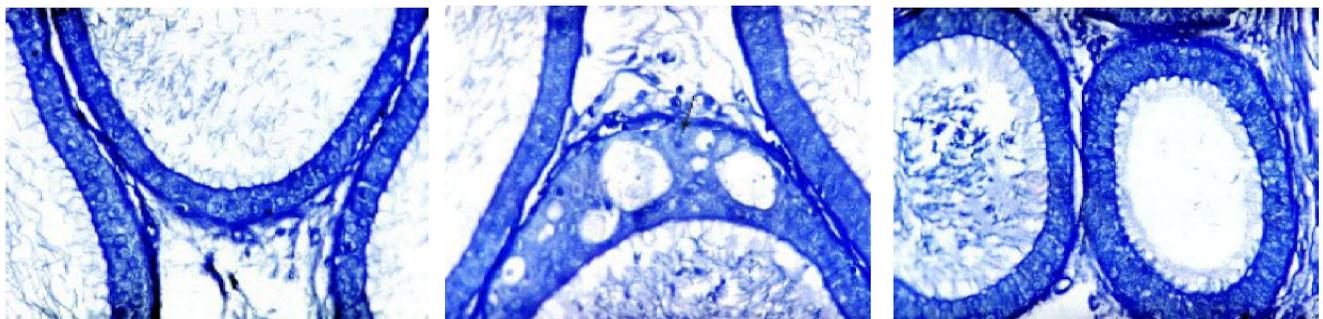


Figure 8 : Sections in epididymis of (a) a control rat showing normal protein content in the epithelial cells, (b) a rat daily treated with carbimazole for 8 weeks showing marked decrease in protein materials (arrow) and (c) a rat daily treated with carbimazole and selenium for 8 weeks showing marked increase in protein materials in the epithelial cells, (Bromophenol blue, X400).

Total proteins

The total proteins appeared in the epididymis of control rats as deeply stained granules inside the nuclei and cytoplasm of all the epithelial cells, also the stereocilia and sperm showed a moderate to strong

reaction with the mercury bromophenol blue (Figure 8a). Animals treated with carbimazole showed a noticeable decrease in the protein content (Figure 8b). Treating animals with carbimazole and selenium revealed that the epithelial cells, stereocilia and sperm

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contained a somewhat normal content of total protein (Figure 8c).

Nucleic acids (DNA, RNA)

DNA present in the epididymis of control rat as granules of magenta colour in the nuclei of all the epithelial cells. RNA was shown as blue fine granules in the cytoplasm of all these cells (Figure 9a). Marked

loss of both RNA and DNA-containing particles was appeared after 8 weeks of the treatment with carbimazole (Figure 9b). Animals treated with carbimazole and selenium showed an increase in RNA content in the cytoplasm and the nuclei appeared with marked DNA-stainability and symptoms of regaining of a normal-like picture of nuclear structure was observed (Figure 9c).

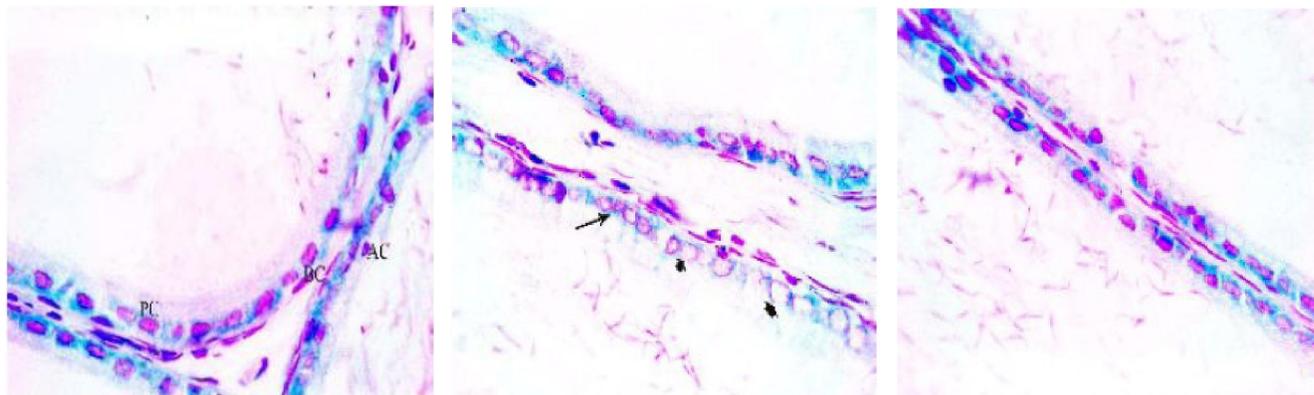


Figure 9 : Sections in epididymis showing (a) normal content of RNA and DNA-containing particles in all the epithelial cells of epididymis of a control animal; principle cell (PC), basal cell (BC) and pical cell (AC), (b) Marked reduction in RNA - containing particles of an animal treated with carbimazole for 8 weeks (arrow) and marked decrease of DNA can be seen most of the nuclei (arrows head) and (c) Marked increase in RNA and DNA-containing particles in all epithelial cells of an animal daily treated with carbimazole and selenium for 8 weeks, (Schiff-methylene reaction, X1000).

DISCUSSION

Our results indicated that carbimazole affected the structure of the epididymis in albino rats. The epididymis of treated animals showed degeneration of epithelial cells, cytoplasmic vacuolization, hyperplasia and congestion of blood vessels. The effect of antithyroid drugs including carbimazole on reproductive system was studied in different animals. Sahoo et al.^[22] reported that feeding lactating mothers and adult rats with 0.05% 6-n-propyl thiouracil in drinking water for 30 days or for 90 days from birth resulted in a decrease in body weight, the testicular germ cell counts and the number of sperms in epididymis. Anguiano et al.^[23] reported that the enzymatic activity in epididymis, semen and prostate was completely inhibited by 1 mm 6-n-propyl-2-thiouracil suggesting that local generation of T₃ could be associated with the development and function of epididymis and spermatozoa maturation. Marty et al.^[24] found that absolute testes, epididymal, prostate and seminal vesicle weights were decreased by 6-propylthiouracil. Also, it significantly decreased serum T₄ level. Maran and Aruldas^[25] reported that daily ad-

ministration of 0.05% methimazole to the nursing mothers induced many changes in newborn male rats; significantly reduced seminiferous tubules diameter, the proliferation and differentiation of germ cells were arrested and their number were decreased. Also, the absolute weight of testes, plasma testosterone, estradiol and sex hormone binding globulin levels were significantly decreased.

Results obtained in the present work revealed that treating rat with carbimazole caused reduction in polysaccharides, total proteins and nucleic acids contents in tissue of epididymis. Similarly, Sakr et al.^[26] reported that carbimazole treatment caused reduction in polysaccharids and total proteins in prostate of albino rats. Mori^[27] reported that carbimazole caused a significant decrease of the proliferation, nucleic acids and protein synthesis of the thyroid follicular and adrenocortical cells. Palmero et al.^[28] reported that oral administration of methimazole from the day of birth of rats was characterized by a net inhibition of the increase in Sertoli cell gamma-glutamyl transpeptidase activity as well as in androgen-binding protein and lactate production. These results are consistent with the impairment of protein synthesis in Sertoli cells from hypothy-

roid rats compared with control. Depletion of hepatic microsomal p⁴⁵⁰ reductase activity and protein was recorded in both male and female rats treated with mewthimazole^[29]. Propylthiouracil treatment decreased both hepatic receptor-related protein and low-density-lipoprotein receptor expression in rats^[30]. Meisami et al.^[31] reported that testicular weight and DNA content were markedly reduced in rat pups administered by propylthiouracil.

It was reported that one of the most established mechanisms of carbimazole toxicity is its ability to induce oxidative stress, through generating reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂) and superoxide anion (O₂⁻)^[22]. In agreement with this result, Sakr et al.^[7] reported that a high lipid peroxidation with a concomitant decrease in the enzymatic antioxidant status, superoxide dismutase and catalase were recorded in testis of rats treated with carbimazole. Thus the observed alterations in the epididymis may be due to oxidative stress generated by carbimazole.

The present study indicated that selenium had an ameliorative effect against the toxicity of carbimazole. Treating rats with carbimazole and selenium revealed a histological and histochemical improvement in the structure of epididymis. The protective effect of selenium against toxicity of different drugs and chemicals was studied by several investigators. Seema et al.^[15] investigated the effect of exogenous selenium on the testicular toxicity induced by nicotine in rats. Administration of nicotine caused reduction in sperm count and sperm motility. Activities of testicular enzymes 3beta hydroxysteroid dehydrogenase and 17beta hydroxysteroid dehydrogenase were decreased. Levels of testosterone in the serum were also reduced. However, the extent of these alterations was lesser in the group administered with nicotine along with selenium. Swathy et al.^[14] reported that selenium had a protective effect against methanol-induced testicular toxicity in rats. Selenium is essential for the production of normal spermatozoa and thus plays a critical role in reproduction^[32].

Selenium has shown to be a powerful antioxidant through inhibiting generation of reactive oxygen species (ROS). In this concern, Jana et al.^[33] reported that sodium selenite supplementation significantly protected against exercise-induced testicular gametogenic and spermatogenic disorders, prevented testicular oxidative stress and increased antioxidant status. El-Maraghy and

Nassar^[34] Selenium reduced the cadmium induced histopathological changes in testes of rat, oxidative stress, endocrine disorder and apoptosis. Sakr et al.^[7] revealed that selenium ameliorates testicular toxicity of carbimazole in rats. They added that it enhanced the activities of the antioxidant enzymes (catalase and superoxide dismutase) and reduced lipid peroxidation. In conclusion, the present study demonstrated that selenium is effective in reducing carbimazole epididymal toxicity. This may be take place by it antioxidant activity.

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