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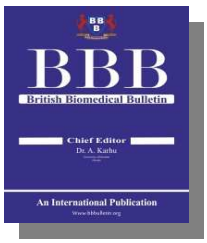

Original

Effect of Selenium on Carbendazim - Induced Ovarian Toxicity in Albino Rats: Histomorphometric and Histochemical Study

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A R T I C L E I N F O

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A B S T R A C T

Introduction: Carbendazim is a systemic benzimidazole fungicide used in plant disease control. Selenium is an essential micronutrient in the mammalian diet and showed many therapeutic effects. **Objective:** The present work evaluated the effect of carbendazim on ovary of albino rats and the ameliorative role of selenium. **Methods:** Animals were divided into four groups. G1 was considered as control. G2 was given sodium selenite (10 µg/kg b.w) for 3 days weekly for 6 weeks. G3 was orally given 100 mg/kg b.w. carbendazim for 3 days weekly for 6 weeks. G4 was orally administered carbendazim and sodium selenite 3 days/week for 6 weeks. Animals were sacrificed and ovaries were removed and stained with H&E for histological examinations and stained with PAS and bromophenol blue for polysaccharides and proteins, respectively. **Results:** Treating rats with carbendazim (100 mg/Kg b.w /3 days weekly for 6 weeks) causes histological alterations in the ovary compared with control group. The majority of the ovarian follicles were declined and diminished in number. The atretic follicles increased with increase of collagen fibers and the blood vessels were dilated and congested. Histochemical results revealed decrease in polysaccharides and DNA contents in the ovarian follicles and germinal epithelium. Treating animals with carbendazim and selenium (10 µg/kg b.w/3 days weekly for 6 weeks) caused an improvement in the histological structure as well as histochemical components of the ovarian tissue. **Conclusion:** It is recommended that the ameliorative impact of selenium against the histological and histochemical changes actuated via carbendazim may be because of its antioxidant activity.

Introduction

Pesticides exhibited a broad spectrum of activity against different arthropod pests of plants and animals. Owing to the extensive use of pesticides, there is a high risk of human exposure to these chemicals. Pesticides are viewed as exceptionally unsafe for their potential consequences for human wellbeing through residues in food and environment[1]. It was reported that workers exposed to pesticides may suffer from infertility[2]. Pesticides may cause conceptive lethality through a few distinctive mechanisms: immediate harm to the structure of cells, obstruction with biochemical courses of action essential for ordinary cell capacity, and biotransformation bringing about dangerous metabolites. Regenerative impacts that have been connected with pesticide introduction in women are decreased fertility, spontaneous abortions, stillbirth, premature birth, low birth weight, developmental abnormalities, ovarian disorders, and disruption of the hormonal function[3]. Fungicides are utilized to protect seeds, fruits and vegetables during storage or are applied directly to ornamental plants, trees, field crops, cereals and turf grasses. Carbendazime (methyl-2-benzimidazole carbamate), is a systemic benzimidazole fungicide utilized as a part of plant ailment control, as a preservative of fruits and is also used as a preservative in paint and papermaking[4]. The exposure of laboratory animals to carbendazim elicits a number of effects including hepatotoxicity and nephrotoxicity[5]. Long-term exposure to carbendazim brought about the diminished survival rate, body weight, and hematological, biochemical, and created histopathological changes in adrenal, thyroid, liver, and testis[6]. Additionally, carbendazim was linked to male genital deformaties[7].

Dietary strategies are likely to be of importance in prevention of different diseases.

Selenium is a fundamental micronutrient in the mammalian eating regimen and insufficiency of this follow component can result in a mixed bag of serious obsessive conditions[8]. Humans and animals require selenium for the function of a number of selenium-dependent enzymes, also known as selenoprotiens[9]. During selenoprotiens synthesis, it is incorporated into a very specific location in the amino acid sequence in order to form a functional protein. Two sorts of selenoproteins are fundamental for every animal cell, the first structure is the group of GSH-peroxidase and the second structure is the group of deiodinases. GSH-peroxidases are the most capable antioxidant enzyme, which shield the cell against oxidative harm prompted by oxidative stresses related diseases and disorders such as cardiovascular disease, malignancies, bacterial or viral diseases, muscle dystrophy and arthropathy[10]. Studies in laboratory animals prove that selenium can go about as an anticarcinogen and repress tumor inititation[11]. Lei *et al.*,[12] reported that selenium was successful in repressing AFB1 actuated hepatocarcinogenesis. Yu *et al.*[13] reported that selenium hindered oxidative stress , apoptosis and cell cycle changes incited by abundance fluoride in kidney of rats. Selenium has a defensive impact against rodent liver and kidney harm affected by mercury chloride[14]. Sakr *et al.*[15] reported that selenium improves carbimazole-instigated testicular harm in rats. There is no available work on the toxicity of carbendazim on the ovary. Therefore, the present work aims to study the effect of carbendazim on the ovary of albino rats and the possible ameliorative effect of selenium.

Materials and Methods

Animals and treatments

Sexually mature female Wistar rats weighing 140 ± 10 g were utilized. Animals were kept in the laboratory under constant temperature (24±2 °C) throughout the experimental work. They were maintained on a standard rodent diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch (Egyptian Company of Oils and Soap, Kafr-Elzayat, Egypt). Water was available ad libitum. This study and all procedures were approved by the Animal Care and Bioethics of the Egyptian Committee, and the animal work was done at Faculty of Science, Menoufia University. They were divided into 4 groups:

Group 1

These animals served as normal controls.

Group 2

These animals were orally given sodium selenite (10 µg/kg b.w) for 3 days weekly for 6 weeks. Na2 SeO3 obtained from British Drug Houses LTD, Laboratory Chemicals Division, England. It was dissolved in distilled water.

Group 3

Animals of this group were orally given 0.1 ml of corn oil comprising 100 mg/kg body weight carbendazim for 3 days weekly for 6 weeks.

Group 4

Animals of this group were orally administered carbendazim (100 mg/kg b.w) and sodium selenite (10 µg/kg b.w) 3 days/week for 6 weeks.

Histological and histochemical study

Ten animals from both the control and treated groups were sacrificed by cervical decapitation after 6 weeks of treatment. Immediately after decapitation animals were dissected, ovaries were removed and fixed in 10% formalin. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin wax. Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. For histopathological examination, sections were stained with Ehrlich's haematoxylin and counterstained with eosin. Sections of ovaries of control and experimental animals were examined histologically and used for quantitative analysis.

All serial sections of the ovary were counted for various stages of development of follicles as described by Bolon *et al*.[16] Follicles were classified into small (mean diameter<20 µm), medium (mean diameter 20-70µm) and large follicles (mean diameter>70 µm). Atretic follicles were classified into small and large. Masson trichrom method was used for staining collagen. For histochemical study specimens were fixed in Carnoy's fluid. Periodic acid Schiff's reaction was used for demonstration of polysaccharides, total proteins was demonstrated using the mercury bromophenol blue method and DNA was detected using Feulgen reaction[17].

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test. A value of $P<0.05$ was considered significant. SPSS17.0 for Windows, Chicago, Illinois, USA, was used to analyze the data.

Results

Histological observations

The histological observations of control group showed developing follicles

(primordial, primary and secondary follicles), corpus luteum and Graafian follicles in the cortex of ovary. Primordial follicles are composed of an oocyte surrounded by a small number of squamous granulosa cells (Fig.1a). Animals treated with selenium showed normal ovarian structure. Histological examination of ovaries of rats given carbendazim showed severely damaged blood vessels which appeared congested and dilated (Fig.1b). The follicles were degenerated, most of the oocytes became destructed and few granulosa cells remained (Fig.1c). Atretic follicles were increased and showed cellular debris in the antrum and pyknotic nuclei (Fig.2a). Animals treated with carbendazim and selenium showed an improvement in the ovarian structure with a decrease in the number of atretic follicles (Fig.2b). Sections of ovaries of control rats and stained with Masson trichrom method showed few collagen in the stroma (Fig.3a). An increase in collagen was observed in ovaries of animals treated with carbendazim (Fig.3b). Treating animals with carbendazim and selenium revealed a decrease of collagen (Fig.3c).

Quantitative results

Data in table 1 showed the change in the number of ovarian follicles during the experiment. A significant decrease in the number of medium, large and small follicles in ovaries of animals exposed to carbendazim. The number of these follicles was increased in rats treated with carbendazim and selenium. The number of atretic follicles increased after treatment with carbendazim and decreased after treatment with carbendazim and selenium.

Histochemical observations

Polysaccharides

Sections of ovaries of control rats stained with PAS showed that the germinal epithelium and theca follucli of the different follicles was strongly stained. The stromal cells stained moderately. The zona pellucida of the secondary follicles and Graffian follicles was markedly stained forming a clear layer encircling the oocytes (Fig.4a). Ovarian tissue of animals treated with carbendazim showed reduction in the polysaccharides content. The germinal epithelium, stromal cells and theca folliculi stained slightly. The cores of atretic follicles showed a strong stainability (Fig.4b). Treating animals with carbendazim and selenium caused an increase in polysaccharides content in the germinal epithelium and the ovarian follicles (Fig.4c).

Total proteins

Total proteins were demonstrated in the cells of the ovarian tissue in the form of small bluish irregularly shaped particles lying in a moderately to strongly stained cytoplasm. In control rats, germinal epithelium and stromal cells are stained strongly. The granulosa cells and theca folliculi exhibited a moderate stain (Fig.5a). Ovarian sections examined after treatment with carbendazim showed that proteins content of germinal epithelium and stromal cells decreased (Fig.5b). Animals treated with carbendazim and selenium showed an increase in protein contents comparable with rats given carbendazim (Fig.5c).

DNA

Examinations of ovaries of control rats revealed that the nuclei of the germinal epithelium, stromal and granulosa cells of all different follicles exhibited a marked positive Feulgen reactivity reflecting their richness in DNA content in red purple colour (Fig.6 a,b). Sections of ovaries examined following treatment with carbendazim showed a noticeable decrease in DNA content in the nuclei of germinal epithelium and granulosa cells of the

follicles (Fig.6 c,d). Treating animals with carbendazim and selenium leads to marked increase in DNA content in cells of germinal epithelia, stromal cells and granulosa cells (Fig.6 e,f).

Discussion

In the present study it has been observed that carbendazim affected the ovary structure in rats. The follicles were degenerated and decreased in number. This was associated with increase in number of atretic follicles and increase in collagen. In accordance with this result, Sakr *et al*.[18] who reported that topsin fungicide caused histopathological alterations in the ovary and decreased the number of follicles with an increase of the atretic follicles. They added that topsin significantly decreased serum levels of both LH and FSH and increased estradiol. Bolivar and Kaliwal[19], demonstrated that mancozeb fungicide treatment prompts a huge lessening in the quantity of healthy follicles with accompanying increment in the quantity of atretic follicles. Goldman *et al*.[20] reported that the fungicide sodium dimethyl dithiocarbamate caused a dose-related suppression of oocyte release in rats and this involves two separate mechanisms, one attributable to an alteration in ovarian hormonal feedback to the brain (or pituitary), inhibiting the LH surge, and the other associated with a direct, as yet undetermined, impact on nearby preovulatory occasions inside the ovary.

Concerning the histochemical results, carbendazim caused reduction in polysaccharides and total proteins in the ovarian tissue. Similarly, Mahadevaswami *et al*.[21] reported that mancozeb fungicide brought on a critical abatement in the levels of protein, glycogen, absolute lipid, phospholipids, and neutral lipid in the liver, uterus, and ovary. In addition to the decrease in the compensatory ovarian hypertrophy, mancozeb treatment reduced the number of healthy follicles with an associative increment in the number of atretic follicles. Sakr *et al*.[22] reported that lannate insecticide induced a decrease in glycogen and total proteins in ovarian components of rats. Pesticides may cause disruption in glucokinase activities and subsequently defectiveness in the process of glycogenesis, in addition to acceleration of both hexokinase and phosphorylase activities to promote glycolysis and glycogenolysis. In the same setting, Abdel-Raheem *et al*.[23] reported that the glycogen consumption in the liver of rodenticide-treated rats was parallel to a height in the action of glucose-6-phosphatase in the same organ, prompting a claimed condition of glycogenolysis in these cells. Downright proteins and DNA diminished in ovarian tissue of rats presented to carbendazim. This result was obtained in mammalian tissue exposed to different insecticides[24,25]. Such diminishing in DNA could be ascribed to disturbance of lysosomal membranes under the impact of different toxicants prompting liberating their hydrolytic catalysts (Dnase & Rnase) in the cytoplasm which brought about checked lysis and disintegration of the target materials, DNA and RNA. Awasthi *et al*.[26] expressed that height of lysosomal enzymatic movement was joined by a decline in protein and nucleic acids substance because of organophosphate insect poison with the arrival of nucleases and proteases influencing RNA, DNA and protein digestion system.

Oxidants and antioxidant agents have pulled in broad enthusiasm toward nourishment research, science and medication. The present results showed that selenium improved the histological and histochemical alterations induced by carbendazim in ovary of rats. These results are in agreement with Chattopadhyay *et al*.[27] who reported that sodium selenite

supplementation was able to prevent arsenic induced histopathological changes in the ovary and uterus. They added that selenium treatment increased the plasma levels of LH, FSH, and estradiol toward the control level. Sakr *et al*.[15] reported that selenium enhances histological and histochemical modifications instigated via carbimazole in testis of rats. Sodium selenite demonstrated a radioprotective impact and enhanced folliculogenesis in rats through expanding ovarian granulosa cells expansion, estradiol and FSH discharge, and Gpx movement, whilst diminishing lipid peroxidation and oxidative anxiety, prompting restraint of the apoptosis pathway through diminishing the statements of caspase 3 and cytochrome c[28]. Basini and Tamanini[29] demonstrated that selenium animated expansion of bovine granulosa cells and had stimulatory consequences for estradiol emission.

Reactive oxygen species produced by oxidative injuries have been involved in tissue injury. Oxidative damage occurs when the production of free radicals exceeds the antioxidant defense systems causing injury to macromolecules such as DNA, proteins and lipids[30]. There was a balance between ROS production and its scavenger system within the cells to prevent or minimize free radicals damage, including enzymatic and non-enzymatic mechanisms. Selenium is an antioxidant and trace element which is incorporated into the catalytic site of antioxidant enzymes, such as GPx, and is involved in protecting cells against the toxic and damaging effects of ROS[31]. In this worry, Uhm *et al*.[32] reported that the expansion of selenium to the culture media increased the blastocyst rate and cell number, diminished the apoptotic record and expanded the articulation of Gpx in porcine fetuses. Abedelahi *et al*.[33] reported that selenium enhances the *in vitro* follicular improvement by lessening the sensitive oxygen species level and increasing the total antioxidant capacity and glutathione peroxide activity. Other studies indicated the antioxidant effect of selenium against toxicity of different chemicals and drugs[34-36].

Conclusion

It is concluded from the present results that the preventive effect of selenium against carbendazim-induced histological and histochemical changes in the ovary of albino rats may be related to its antioxidant properties.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this work.

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Table 1. Effect of different treatments on the number of ovarian follicles

Treatment	Number of follicles			
	Small	Medium	Large	Atretic
Control	286 ± 12	66± 5	12± 2	18± 2
Selenium	267 ± 10	56± 5	10± 3	16± 2
Carbendazim	201 ±6*	32± 4*	5 ±2*	28 ±4*
Carbendazim+ selenium	230±10	41±4	6±1	19± 4

Results are expressed as mean ± SD
(*). Significant at P<0.05

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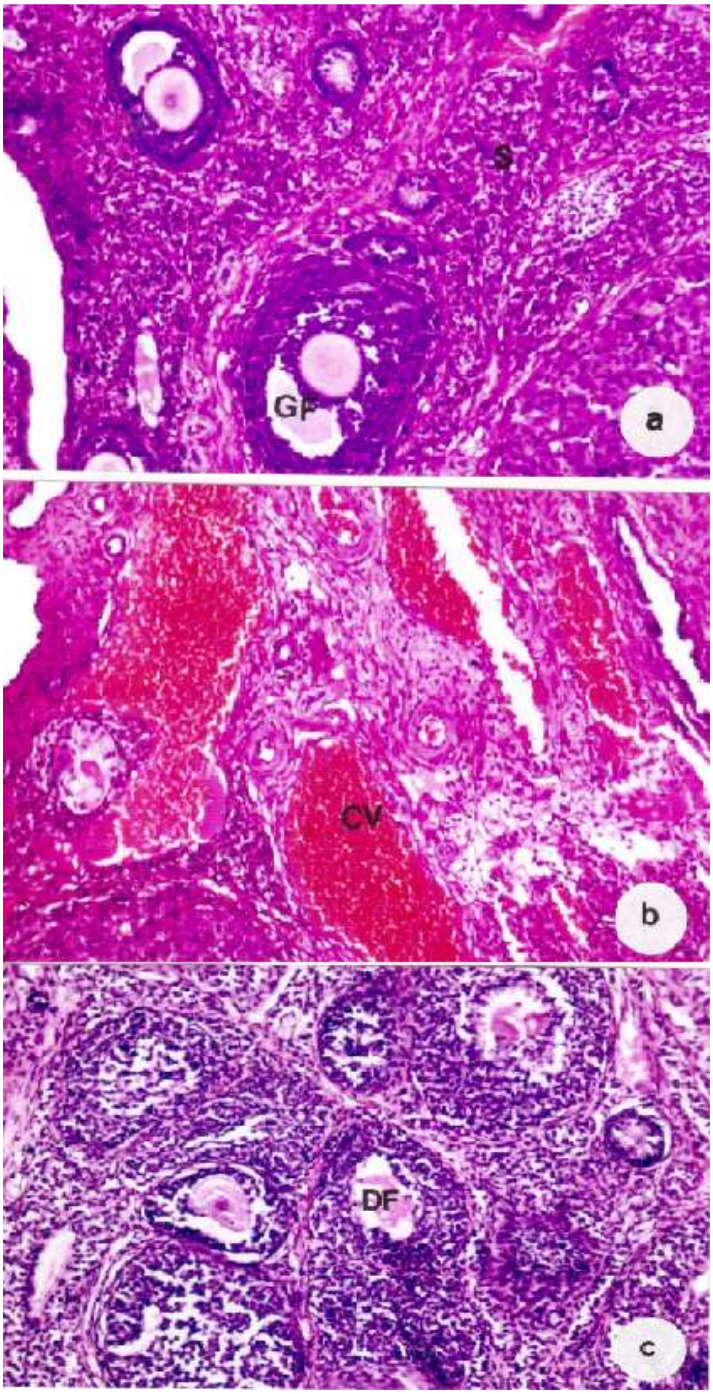

Figure 1. a. Section in ovary of a control rat showing different follicles, GF: Graffian follicle, S:stroma. **b**. Section of ovary of a rat treated with carbendazim showing enlarged and congested blood vessels (CV), **c**. Ovary of a treated rat showing degenerated follicles (DF),(H&E X400)

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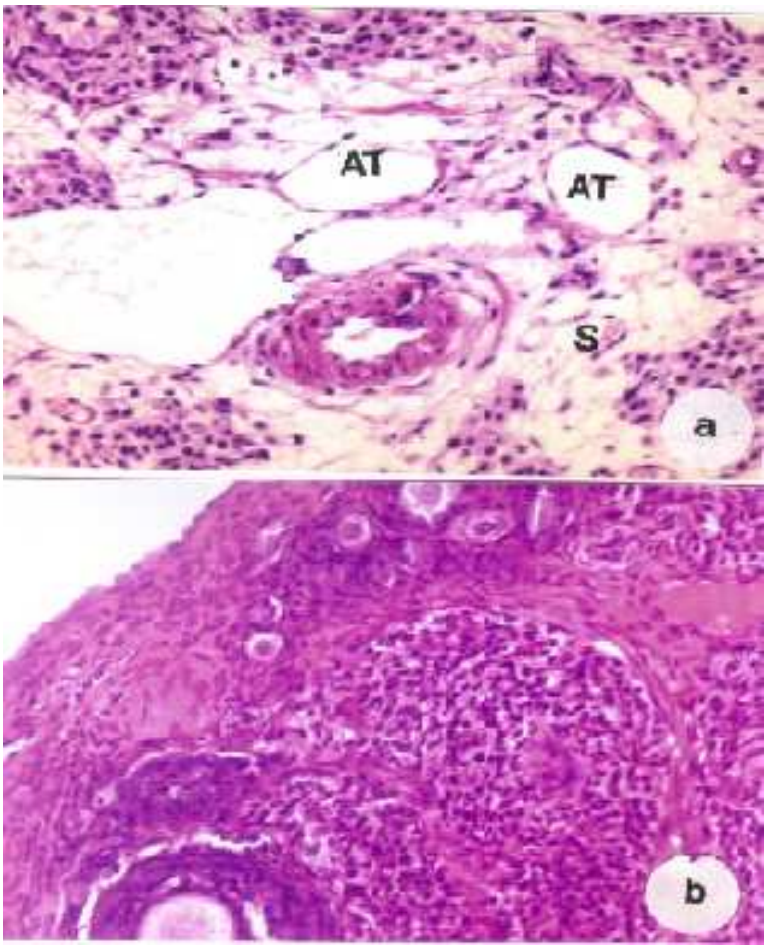

Figure 2. a. Ovary of a carbendazim-treated rat showing atretic follicles (AT), **b**. Ovary of a rat treated with carbendazim and selenium showing different types of follicles,(H&E, X 400)

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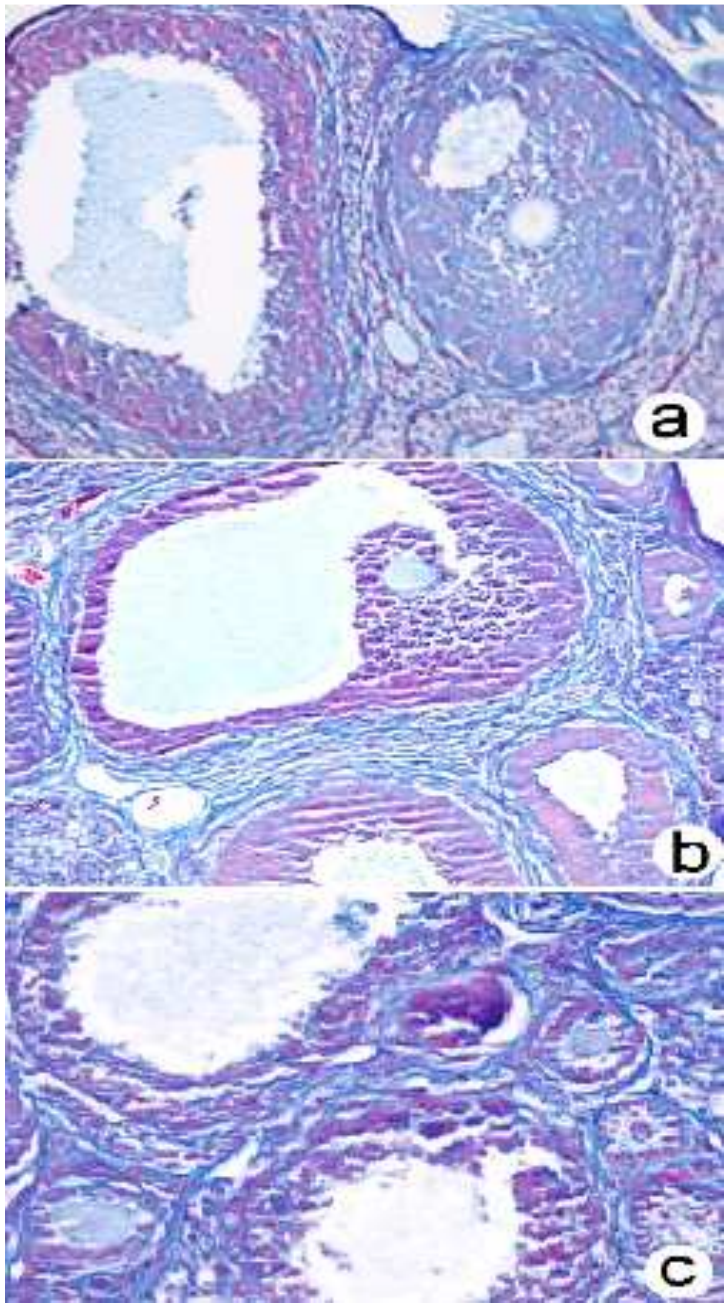

Figure 3. a. Section in ovary of a control rat showing few collagen in the stroma, **b**. Ovary of carbendazim-treated rat showing increase of collagen, **c**. Ovary of carbendazim+ selenium treated rat showing decrease of collagen (Masson trichrom, X400)

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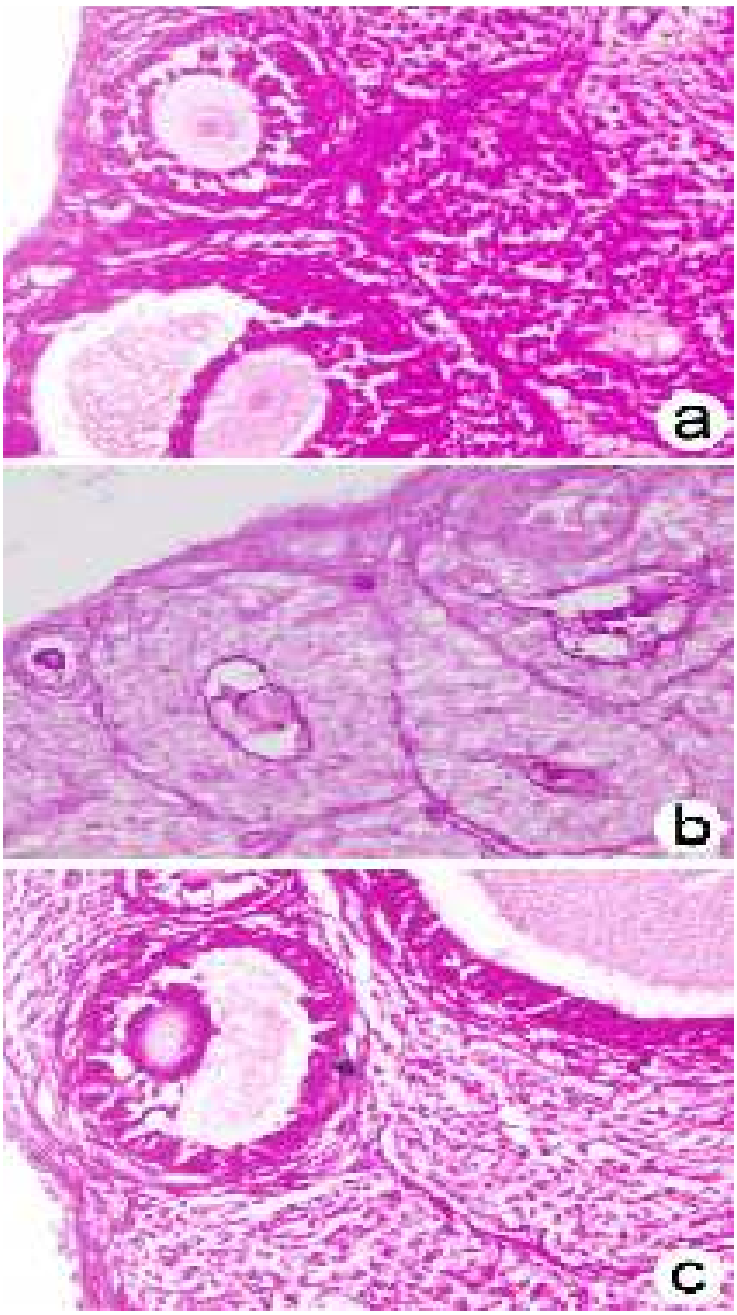

Figure 4. a. Section in ovary of a control rat showing polysaccharides in the follicles and stroma, **b**. A decrease in polysaccharides in ovarian tissue of a rat treated with carbendazim, **c**. An increase in polysaccharides in a rat treated with carbendazim+selenium, (PAS, X400)

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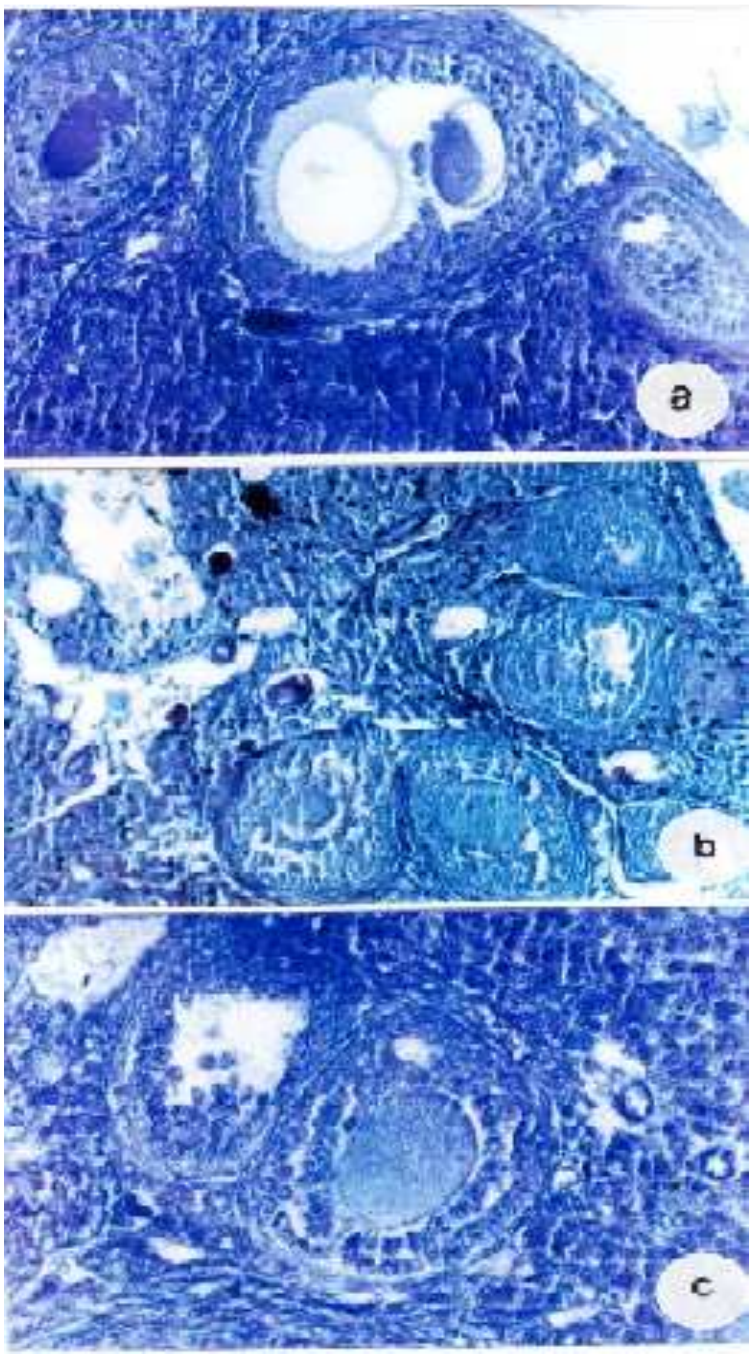

Figure 5. a. Section in the ovary of a control rat showing total proteins in stroma and ovarian follicles, **b.** Ovary of carbendazim-treated rat showing decrease of total proteins, **c.** An increase in total proteins in ovary of a rat treated with carbendazim+selenium,(Bromophenol blue X400)

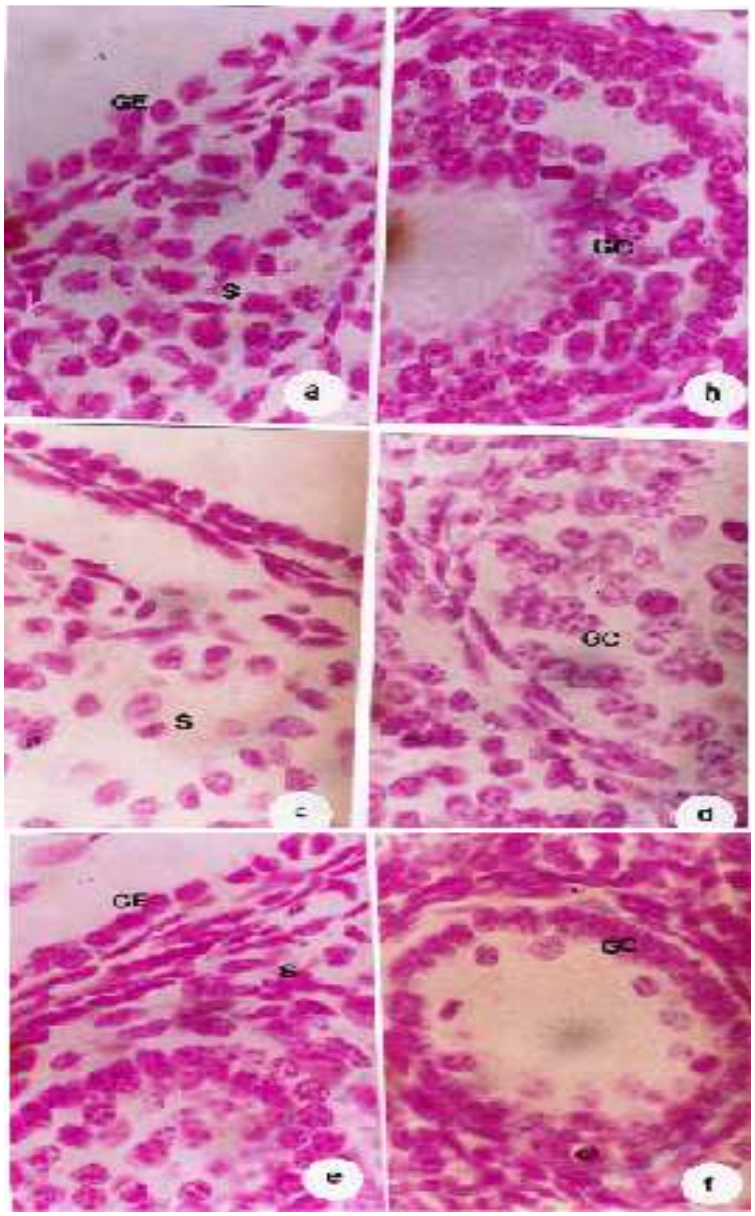

Figure 6. a,b. DNA in germinal epithelium (GE), stroma (S) and granulosa cells (GC) of ovary of a control rat, **c,d**. Decrease of DNA after treatment with carbendazim, **e.f.** an increase in DNA in ovary of rats treated with carbendazim+selenium, (Feulgen X400)