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Effect of fenugreek seed extract on carbendazim-inhibited spermatogenesis in albino rats

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ABSTRACT

Carbendazim is a carbamate fungicide used in control of various fungal pathogens. Fenugreek (*Trigonella foenum-graecum* Linn.) is a leguminous plant cultivated in several Asian and African countries and its seeds are used as herbal medicine. In the present work the effect of aqueous extract of fenugreek seeds on carbendazim-induced testicular toxicity in albino rats was studied. Treating rats with carbendazim induced significant decrease in testis weights, diameters and germinal epithelial heights of the seminiferous tubules. Histological results revealed degeneration of seminiferous tubules and reduction of spermatogenic cells. Moreover, carbendazim caused elevation of testicular malondialdehyde (MDA), and reduced the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT). Animals treated with carbendazim and fenugreek showed improvement in the histomorphological and histopathological changes observed in animals treated with carbendazim. In addition fenugreek treatment leads to a significant decrease in the level of MDA and increase in the activity of SOD and CAT. It is concluded that fenugreek extract can improve the testicular toxicity of carbendazim and this effect may be attributed to its antioxidant properties.

Keywords: Carbendazim, Fenugreek, Testis, Histology, Antioxidants

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INTRODUCTION

Exposure to pesticides) elicits a number of effects, including embryotoxicity, genotoxicity and cytotoxicity changes in developing and adult animals (Fahmy and Abdollahi, 2001). Carbendazim is a member of the benzimidazole group with broad- spectrum nature. It is widely used as an agricultural and horticultural fungicide/pesticide around the world (Dreisbach, 1983). On the other hand, carbendazim was found to cause adverse effects in different mammalian systems including male reproduction such as sloughing of germ cells (Gray *et al.*, 1990), inhibition of germ cell division (Nakai *et al.*, 1998), seminiferous tubular atrophy (Gray *et al.*, 1990), and alterations in hormone concentrations (Lu *et al.*, 2004). The used of plants and their extracts in medicinal proposes has been rapidly increasing worldwide. Fenugreek (*Trigonella foenum graecum*) is a herb belongs to family leguminosa.

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It has used as medicinal herb (Kaviarasan *et al.*, 2004). Its seeds are used in many oriented countries as a spice in food preparations due to their strong flavor and aroma (Kaviarasan *et al.*, 2007). It is also used as herbal medicine for their carminative, tonic and aphrodisiac effects (Xue *et al.*, 2007). Fenugreek seeds exhibit hypoglycemic, hypolipidaemic, antifertilitic, antiandrogenic, antinociceptive and wound healing properties and are good source of dietary fibers (Abou El-Soud *et al.*, 2007). Recently, Sakr *et al.* (2012) reported that fenugreek seeds prevent adriamycin-induced cytogenetic and testicular damage in albino rats. The present study was undertaken to demonstrate the effect of fenugreek seeds extract on testicular abnormalities induced by carbendazim in rats.

MATERIALS AND METHODS

Carbendazim

Carbendazim (Methyl benzimidazol-2-yl carbamate) obtained from Saudi Delta Company for Industrial Chemicals, Riyadh, Saudi Arabia, was used.

Fenugreek aqueous extract

Dried and fresh batches of fenugreek seeds were purchased from local market, Makkah, Saudi Arabia. Seeds were washed with distilled water to get rid of extraneous matter, air-dried and ground into a fine powder in a mixer. The powder was mixed with distilled water (1 g of seed powder per 100 ml) in a vortex cyclomixer for 10 minutes and then centrifuged at 10000 rpm and the supernatant was collected. The supernatant was used as the aqueous extract for feeding the animals and was freshly prepared. In this study, each animal was orally given 1 ml of the final aqueous extract containing 0.4g/kg body weight fenugreek seeds (Sakr *et al.*, 2012).

Animals

Male Wistar rats weighting 150 ± 10 g were obtained from King Fahd Center for Medical Research, Jeddah, Saudi Arabia. They were kept in the laboratory under constant temperature (24 ± 2 °C) throughout the experimental work. They were maintained on a standard rodent pellets and water was available *ad libitum*. Animals were divided into 4 groups:

Group I

These animals (10 rats) served as controls.

Group II

Animals in this group were orally given 1 ml of final aqueous extract of 0.4 gm/kg fenugreek 3 times weekly by gastric intubation for 6 weeks.

Group III

Animals of this group (25 rats) have been orally given 0.1 ml of corn oil contains 100mg/kg body weight carbendazim, 3 days weekly for 6 weeks (Metwally *et al.*, 2011).

Group IV

Animals of this group (30 rats) have been orally given carbendazim (100mg/kg body weight), followed by fenugreek extract 3 days weekly for 6 weeks.

Histological Study

Immediately after decapitation animals were dissected, testis were removed from treated and control animals and fixed in Bouin's solution. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. For histological examination, sections were stained with Ehrlich's haematoxylin and counterstained with eosin. Seminiferous tubules diameter and germinal epithelial height were measured from the spermatogenic cells on the inner surface of the basement membrane through the most advanced cell types lining the lumen of the tubules.

Oxidative stress and antioxidant enzymes assays

For determination of oxidative stress and antioxidant enzymes in the testes, tissues were homogenized in 0.1 M phosphate buffer (pH 7.4) using a polytron homogenizer, the homogenate was centrifuged at $18,000 \times g$ for 30 min and the supernatant was utilized for biochemical analysis. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialdehyde) according to Ohkawa *et al.*, (1979). Superoxide dismutase (SOD) activity was measured using the methods of Rest and Spitznagel (1977). Catalase activity was determined from the rate of decomposition of H_2O_2 (Aebi *et al.*, 1974).

Statistical analysis

All values were presented as means \pm S.E.M. Differences between group means were calculated by a one-way analysis of variance (ANOVA) using the SPSS/PC computer program (version 12.0). Results were considered statistically significant when $p < 0.05$.

RESULTS

Biochemical results

Data exist in table1 revealed non-significant ($P < 0.05$) difference in the level of MDA in testis of animals treated with fenugreek in comparison with control. Rats treated with carbendazim showed significant increase in the level of MDA. On the other hand, animals treated with carbendazim and fenugreek showed decrease in MDA level compared with carbendazim group.

Treating rats with carbendazim showed decrease in the activity of SOD and CAT compared with control group. Animals treated with carbendazim and fenugreek showed significant increase in the activity of SOD and CAT compared with carbendazim group. No significant change was obtained in SOD and CAT activity in rats treated with fenugreek.

Table. 1: Effect of different treatments on malondialdehyde, superoxide dismutase and catalase in testes of rats.

Animal group	MDA (n mol/ml)	SOD (μ /ml)	CAT (μ mol/sec/ml)
Control	30.8 \pm 1.5	141.3 \pm 6.8	5.7 \pm 1.2
Fenugreek	32.6 \pm 2.0	145.6 \pm 5.5	5.6 \pm 1.3
Carbendazim	53.7 \pm 3.4*	100.6 \pm 4.3*	3.3 \pm 0.6*
Carbendazim+ fenugreek	33.2 \pm 3.8	125.3 \pm 2.8	4.1 \pm 1.2

(*). Significant at $P < 0.05$ in comparison with controls.

Morphometric results

Results in table 2 revealed that treatment with carbendazim caused atrophy of the seminiferous tubules. The diameter of seminiferous tubules was significantly decreased in carbendazim treated animals. A decrease in germ cell height of seminiferous tubules is appeared in compare with normal ones. Animals treated with carbendazim and fenugreek seed extract showed marked improvement in the mean tubular diameter and in germ cell height in comparison with carbendazim treated animals.

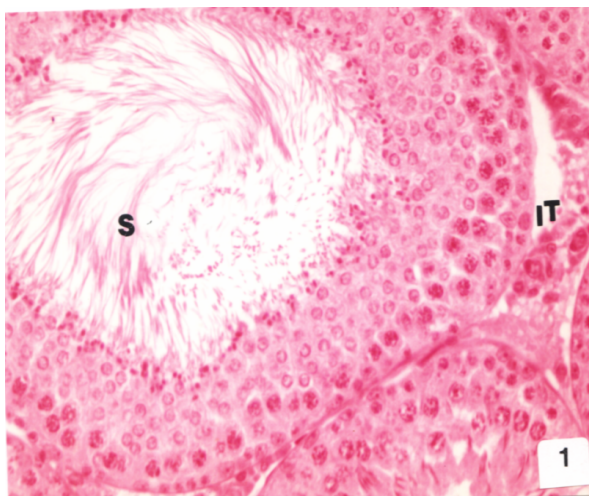
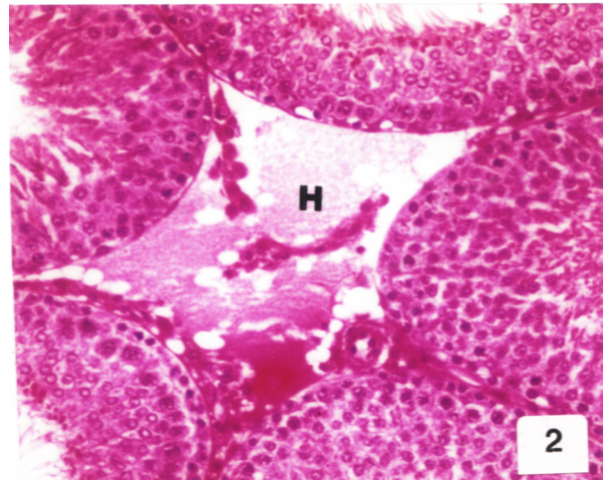
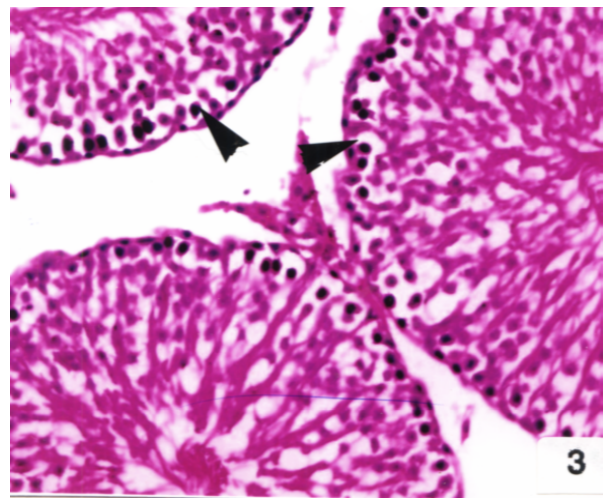
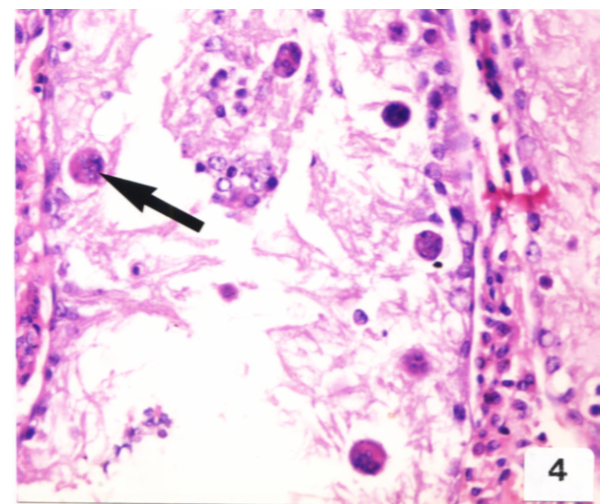
Table. 2: Mean value of the diameter and epithelial height of seminiferous tubules in rats treated with carbendazim and fenugreek seeds.

Animal group	Diameter of tubules	Germinal epithelial height
Control	234 \pm 6.8	108 \pm 5.19
Fenugreek	240 \pm 5.32	112 \pm 7.3
Carbendazim	165 \pm 10.22*	60 \pm 4.32*
Carbendazim+ fenugreek	205 \pm 4.06	82 \pm 6.42

(*). Significant at $P < 0.05$ in comparison with controls.

Histological results

Examination of testis of control rats showed typical features of normal seminiferous tubules, spermatogenic cells, intertubular connective tissue and spermatozoa (Fig.1). Animals administered fenugreek seeds extract weeks showed normal histological structure. Animals given carbendazim for 6 weeks exhibited a distinct histological differences. Haemorrhage was seen in the intertubular connective tissue (Fig.2). The spermatogonia appeared vacuolated with pyknotic nuclei (Fig.3).

**Fig.1:** Section in testis of a control rat showing normal seminiferous tubules, S: Sperm, IT: interstitial tissue, X 300.**Fig.2:** Section in testis of a rat treated with carbendazim showing intertubular hemorrhage (H), X 300.**Fig.3:** Section in testis of a rat treated with carbendazim showing cytoplasmic vacuolation of germ cells (arrow heads), X 300.**Fig.4:** Section in testis of a rat treated with carbendazim showing multinucleated giant cells (arrow), X 300.

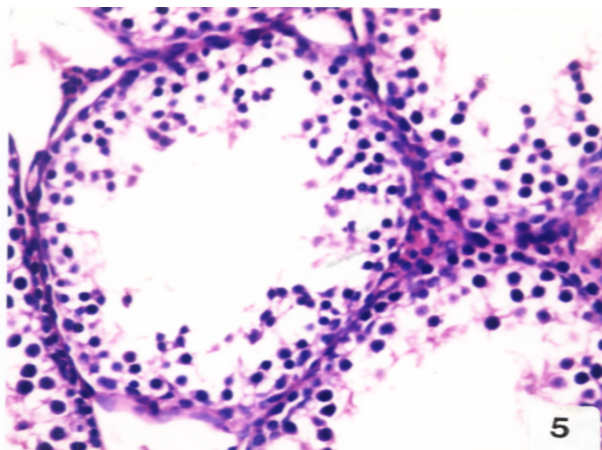


Fig.5: Section in testis of a rat treated with carbendazim showing inhibition of spermatogenesis, X 300.

Multinucleate giant cells, mostly of early spermatids origin, were observed (Fig.4). Marked degeneration of spermatogenic cells and intertubular connective tissue was appeared in large number of seminiferous tubules and sperm bundles were less abundant or completely absent (Fig.5), Sections of testis of animals administered carbendazim and fenugreek seeds revealed less prominent histopathological alterations when compared with animals given carbendazim. Most of the seminiferous tubules were compact with each other and the spermatogenic layers increased (Fig.6).

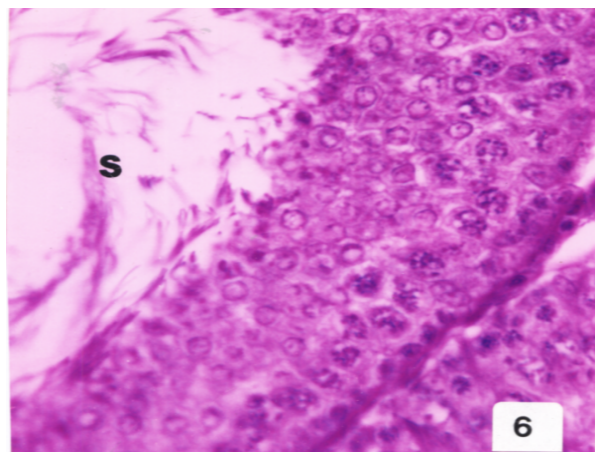


Fig.6: Section in testis of a rat treated with carbendazim and fenugreek showing increase of spermatogenic cells, S: Sperm X 300.

DISCUSSION

Administration of carbendazim resulted in significant decrease in seminiferous tubule diameter, and epithelial height. Histopathological results showed degeneration in decrease of spermatogenic cells. Nakai *et al.*, (2002) reported that these alterations occur with carbendazim treatment from disruption of the Sertoli cell cytoskeleton, propagating loss of germ cell adhesion). The effect of carbendazim on male reproduction was studied by many investigators. Yu *et al.*(2009) reported that treating rats with carbendazim showed atrophic testis and

epididymides, marked histopathological abnormality of the testis, reduced weight of the right testis and epididymis, and decreased sperm motility and counts in the left cauda epididymis. Gawande *et al.*, (2009) reported that carbendazim disrupt the development of sperm and damage testicular development in rats. Long term exposure of male animals with carbendazim revealed the decreased testicular, epididymal weights, altered sperm morphology, testicular atrophy and thus infertility (Nakai *et al.*, 1992; Lim and Miller, 1997; Moffit, 2007; Gawande *et al.*, 2009). Chronic low dose treatment of carbendazim is capable of inducing reproductive and endocrine toxicity (Rajeshwary *et al.*, 2007).

Results obtained in this work showed that carbendazim caused an increase in lipid peroxidation and marked decrease in testicular CAT and SOD activity. Similarly, Metwally *et al.*, (2011) reported that carbendazim induced significant increase in malondialdehyde and significant decrease in the activity of SOD and glutathione peroxidase in testis of rat. Also results of Hamdy *et al.*, (2010) showed that carbendazim administration caused testicular dysfunction with an increase of malondialdehyde and reduced of SOD and glutathione peroxidase activity. Rajeshwary *et al.*, (2007) found that in carbendazim – treated rats, Leydig cellular activities of antioxidant enzymes SOD, CAT, GPx, GR, GST, gamma-GT, G-6-PDH and non-enzymatic antioxidants such as GSH, vitamins E, C and A were significantly diminished, whereas LPO and ROS were markedly elevated. Results of the present work together with the above mentioned authors indicated that carbendazim induced oxidative stress to rats which in turn lead to testicular toxicity.

Fenugreek (*Trigonella foenumgraecum* L., Leguminosae), is one of the oldest medicinal plants, its aqueous extracts of seeds and leaves of fenugreek have been shown to possess many beneficial effects in therapy of diseases. Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30% proteins high in lysine and tryptophan; 5-10% fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2-0.36%), choline (0.5%), gentianine and carpaine; the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin; free amino acids, such as 4- hydroxyisoleucine (0.09%); arginine, histidine and lysine; calcium and iron; saponins (0.6-1.7%); glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin); cholesterol and sitosterol; vitamins A, B1,C and nicotinic acid; coumarin compounds and 0.015% volatile oils (nalkanes and sesquiterpenes)(Blumenthal *et al.*, 1988).

The present results showed that oral administration of fenugreek seeds extract improved the histological changes induced by carbendazim and suppress the oxidative stress as indicated by decrease of lipid peroxidation and increase activity of SOD and CAT. In agreement with this results, Sakr *et al.*, (2012) reported that fenugreek seeds extract ameliorate adriamycin induced testicular toxicity and oxidative stress in mice. Fenugreek seeds have been documented for their multiple pharmacological activities including antioxidation (Ahmadiani *et al.*, 2001), fenugreek seed polyphenols prevented oxidative hemolysis and lipid peroxidation

induced by H₂O₂ *in vitro* in human erythrocyte (Kaviarsan *et al.*, 2004). Moreover, it was demonstrated that the supplementation of fenugreek seed powder in the diet leads to a reduction in biomarkers of oxidative damage in alloxan-diabetic rats (Ravikumar and Anuradha, 1999). It was also showed that the polyphenolic extract of fenugreek seeds has an antioxidation activity *in vitro* (Kaviarasan *et al.*, 2007). The antioxidant activity of fenugreek was attributed to the presence of flavonoids and polyphenols. Thus the effect of fenugreek against testicular toxicity of carbendazim may be due to the antioxidant activity of these constituents.

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