

The Protective Effects of Carrot Seed Extract on Spermatogenesis and Cauda Epididymal Sperm Reserves in Gentamicin Treated Rats

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Abstract

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Objective: Carrot (*Daucus carota* L.) is known to possess antifertility properties in female. However, according to Iranian traditional medicine, it can increase the potency in men. The aim of this study was to investigate the influence of carrot seed extract (CSE) on spermatogenesis, number and motility of sperms in cauda epididyme in male rats.

Materials and Methods: Forty adult male rats were randomly divided into 5 groups: control group, groups receiving low- and high doses of CSE, animals that received high-dose of CSE with gentamicin, and a gentamicin only group. After 4 weeks treatment, fasting serum samples were obtained for the sex hormone analysis. Under anesthesia, testis, cauda epididymides and sperm ducts were dissected and sperm count, motility and cauda epididymis sperm reserves (CESR) were determined. Histopathological changes of testis were also studied to assess spermatogenesis. Data analysis was performed using one-way ANOVA followed by Tukey HSD tests.

Results: Administration of CSE caused a significant increase in CESR compared with the control (28.2 ± 1.8 vs. $45.1 \pm 2.0, \times 10^6$). The extract could also protect testis from the gentamicin-induced necrosis. The CSE administration caused about 3.5-times increase in the LH levels even in spite of receiving 5 mg/kg/day gentamicin with no significant effect on FSH levels. The testosterone concentrations in the group received 400 mg/kg CSE were 30% and 83% higher than its levels in the control and the gentamicin treated group, respectively.

Conclusion: CSE can overcome reproductive toxicity of gentamicin and induces spermatogenesis probably mainly through the elevation of testosterone levels. It appears that this extract has opposite effects on male and female reproductive systems.

Keywords: Carrot Seed Extract, Spermatogenesis, Gentamicin, Gonadotrophin

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Introduction

Daucus carota commonly known as "Carrot" belongs to the family *Apiaceae* and is cultivated throughout the world as a useful vegetable. Apart from its wide usage as a vegetable, different parts of this plant have been used in folk medicine for the treatment of a broad spectrum of ailments including kidney dysfunction, asthma, worm infections, inflammation and leprosy (1-3). According to some studies, carrot extract has also some protective effects on myocardial infarction (4) and lindane-induced hepatotoxicity (5). The plant has been extensively studied for its chemical composition and a large number of active ingredients such as volatile oils, steroids, tannins, flavonoids, and caroten have been isolated (6-8). Recently, pharmacological studies have shown that *Daucus carota* seeds exhibit antifertility properties in females (9, 10). Majumder et al. have shown that

the petroleum ether extract and fatty acids of carrot seeds are able to arrest the normal estrus cycle of the adult mouse and reduce the weight of ovaries significantly (9). They also found that treatment with carrot seed extract (CSE) can lead to a significant inhibition of delta 5,3-beta-hydroxy steroid dehydrogenase and glucose-6-phosphate dehydrogenase, the two key enzymes involved in ovarian steroidogenesis, in mouse ovaries. In another in vitro study carried out using an isolated rabbit ovarian perfusion system, it was found that progesterone and human chorionic gonadotropin-induced progesterone secretions are significantly diminished after the acute feeding of carrots (10).

The effects of carrot on human fertility have been also mentioned in Iranian traditional medicine. In the Zakhireh Kharazmshahi (Kharazmshahi's Treasure), a book written by Seyyed-Esmail Jorjani born

in the 11th Century, one chapter was allocated to the properties of carrots (11). Interestingly, according to Jorjani, the effect of carrot on fertility is gender dependent. He claimed that carrot can increase the potency in men, while in women it stimulates menstruation, with its seeds being more effective than other parts of the plant. There is also a direct relation between potency in men and the levels of testosterone which could be affected by gonadotrophins. It is also well-known that testosterone is able to increase spermatogenesis. Therefore, the present study was designed to investigate the effects of CSE on the male rat reproductive system through assessment of possible changes in testicular morphology, cauda epididymal sperm reserves, count and motility, and also through effects on gonadotrophin and testosterone levels.

Materials and Methods

Plant Material

The seeds of *Daucus carota* used for the extraction were obtained from the local market in Tabriz (Iran).

Preparation of the CSE

The finely ground carrot seeds (88 g) were macerated in 2 liters of ethanol (95.5%) for 24 hours. This procedure was repeated three times. After filtration, the filtrate was concentrated in vacuo in a rotary evaporator (Heidolph, Laborta 4003) at 50°C yielding a 24.31% ethanolic extract of carrot seeds. The required concentrations of CSE were prepared by dissolving the ethanolic extract in a mixture of normal saline and DMSO (4:1) and used for administration to the animals.

Experimental animals

Forty adult male Wistar rats weighing 130-180 g (Tabriz University of Medical Sciences, Iran) were used in this study. They were fed with standard diet pellets and allowed food and water ad libitum for an acclimation period of two weeks. The animals were maintained in a strictly controlled temperature ($18 \pm 1^\circ\text{C}$). Humidity was kept at 50% and the lighting cycle was 07.00-19.00 hours light and 19.00-07.00 h dark with adequate ventilation. Animals were handled with human care in accordance with the National Institutes of Health guidelines and the recommendations of the local and national ethics committees. The rats were randomly divided into 5 groups each consisting of eight animals (Table 1). At the end of 4 weeks of treatment, testis, cauda epididymides and sperm ducts were dissected from each rat under anesthesia exactly 24 hours after the last administration. Before anesthesia, a 5 ml blood sample was collected from each rat in heparinized tubes and allowed to settle for 5 minutes at room

temperature. The sample was centrifuged at 750 g for 10 minutes and the plasma was separated and kept at -70°C for hormonal assays.

Table 1: Summary of the study design and drug administration protocol*

Group	Treatment
1: normal control	Daily administration of the vehicle, a mixture of normal saline and DMSO (4:1), for 24 days via intraperitoneal injection (i.p.)
2: low-dose group	Daily administration of 200 mg/kg of CSE (i.p.) per rat for 24 days
3: high-dose group	Daily administration of 400 mg/kg of CSE (i.p.) per rat for 24 days
4: high-dose with gentamicin group	Daily administration of 400 mg/kg of CSE and 5 mg/kg gentamicin (i.p.) per rat for 24 days
5: gentamicin treated group	Daily administration of 5 mg/kg gentamicin (i.p.) per rat for 24 days

* Each group consists of eight adult male wistar rats.

Assessment of sperm count and motility

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin (12). After 5 min incubation at 37°C (with 5% CO₂), the cauda epididymis sperm reserves were determined using the standard hemocytometric method (13) and sperm motility was analyzed using a microscope (Olympus IX70) at 10 successive field for each sample and reported as the percentage mean of motile sperm of each sample according to the World Health Organization (WHO) method.

Histological preparations

The samples for histological studies were prepared as described by Obidike et al. (14) with minor modifications as follows: Specimens of the dissected testes of rats in the control and test groups were weighed (Shimadzu Libror AEU-210 balance) and fixed by immersion in formalin-buffer 20% for 48 hours. Later, they were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. 5 µm thick sections were cut, mounted on glass slides, and stained with hematoxylin and eosin for light microscopy. Cross sections of 100 tubules per specimen were assessed and the mean number of tubules demonstrating atrophy and regeneration were calculated.

Hormone Assays

Plasma concentration of luteinizing hormone (LH) was assayed using the Biocode Rat LH RIA AHR002- radioimmunoassay Kits. Briefly, dupli-

cate aliquots of 25 μ L of plasma were incubated at 37°C with 100 μ L of Ab Rat LH. Three hours later, approximately 40,000 cpm of labeled rat LH was added to each tube and allowed to react overnight. The precipitating antibody was then added. After one hour incubation, the tubes were centrifuged at 1,500 \times g for 20 minutes, and the radioactivity of the pellets was measured in a gamma counter (Cap-Ria 16). The level of the hormone was estimated from the standard curve and expressed as nanograms per milliliter. The intra-assay and interassay coefficients of variation were 10% and 12%, respectively. Follicle-stimulating hormone (FSH) concentrations in plasma were measured by Bicode Rat FSH IRMA CT AHROO4 Kits. Anti-rat FSH was used as the primary antibody for the radioimmunoassay. The hormonal content of the samples, as estimated from the standard curves, was expressed as nanograms per milliliter. The assay had a sensitivity of 0.2 ng/mL and the average intra-assay and interassay coefficients of variation were 2.9% and 7.8%, respectively. Plasma concentrations of testosterone (T) were measured using specific Spectria RIA kits (Ori Diagnostic) according to the manufacturer's instructions. The intra-assay and interassay coefficients of variation for T were found to be 5.5% and 7.0%, respectively. All of the samples were assayed in a single assay.

Statistical analysis

Assessment of the results was performed using one-way ANOVA followed by Tukey HSD as Post-ANOVA test. The 0.05 level of probability was used as the criterion for significance. All data are presented as Mean \pm SD.

Results

Cauda epididymal sperm reserves and relative weights of testes

In table 2, the effects of CSE administration on cauda epididymal sperm reserves (CESR) and motility

of the sperms in rats have been tabulated. Compared to the control group, the CESR increased following administration of either 200 or 400 mg/kg CSE, but this elevation was statistically significant ($p < 0.05$) only in the later case. Administration of 5 mg/kg/day gentamicin, a destructive reactive oxygen species generating aminoglycoside (15), caused a significant reduction in the CESR. When this dose of gentamicin was administered together with 400 mg/kg CSE, the CESR level was significantly ($p < 0.05$) elevated from 10.5 ± 1.8 to 22.5 ± 1.4 ($\times 10^6$) indicating the protective effect of CSE against gentamicin-induced necrosis.

There were no significant differences ($p > 0.05$) between the motility of sperm in any group apart from the group receiving gentamicin only.

The motility value of this group was different from all other four groups. This indicates the effectiveness of CSE in the maintenance of sperm motility in rats in the face of gentamicin administration.

Hormones. No significant differences ($p > 0.05$) were found in the FSH levels between the control and any treated group (Table 3).

However, CSE administration caused a marked increase in LH levels even in the face of 5 mg/kg/day gentamicin, although the level was relatively low in the group receiving high-dose of CSE with gentamicin compared with the low-dose and high-dose groups.

Radioimmunoassay of serum samples revealed significant increases in the testosterone concentrations of the high-dose animals compared with the control and gentamicin treated groups (Table 3).

Histopathology of testis. The results obtained from histopathological study showed that the cycle of spermatogenesis was regular in the control group (Fig 1A). However, in all animals exposed to 5 mg/kg gentamicin, a depletion of germ cells, germinal cell necrosis (especially in spermatogonia), cell debris, and the presence of lymphocytes and plasmocytes in the lumen were observed.

Table 2: Cauda epididymal sperm reserves (CESR) and motility of sperm in male rats exposed to carrot seed extract (CSE)*

Parameter	control	CSE (200 mg/kg)	CSE (400 mg/kg)	Gentamicin (5 mg/kg) + CSE (400 mg/kg)	Gentamicin (5 mg/kg)	p Level**
CESR ($\times 10^6$)	28.2 \pm 1.8 ^a	32.1 \pm 2.2 ^b	45.1 \pm 2.0 ^c	22.5 \pm 1.4 ^d	10.5 \pm 1.8 ^e	ac: $p < 0.05$ ae: $p < 0.01$ bc: $p < 0.05$ be: $p < 0.01$ cd: $p < 0.05$ ce: $p < 0.001$ de: $p < 0.05$
Motility (%)	41.8 \pm 2.6 ^a	45.7 \pm 2.8 ^b	46.4 \pm 3.1 ^c	31.0 \pm 2.8 ^d	15.2 \pm 1.6 ^e	ae and de: $p < 0.05$ be and ce: $p < 0.01$

* The number of animals per group was eight rats. Data are presented as Means \pm SE.

** Letters indicate comparison of the differences between the specified groups.

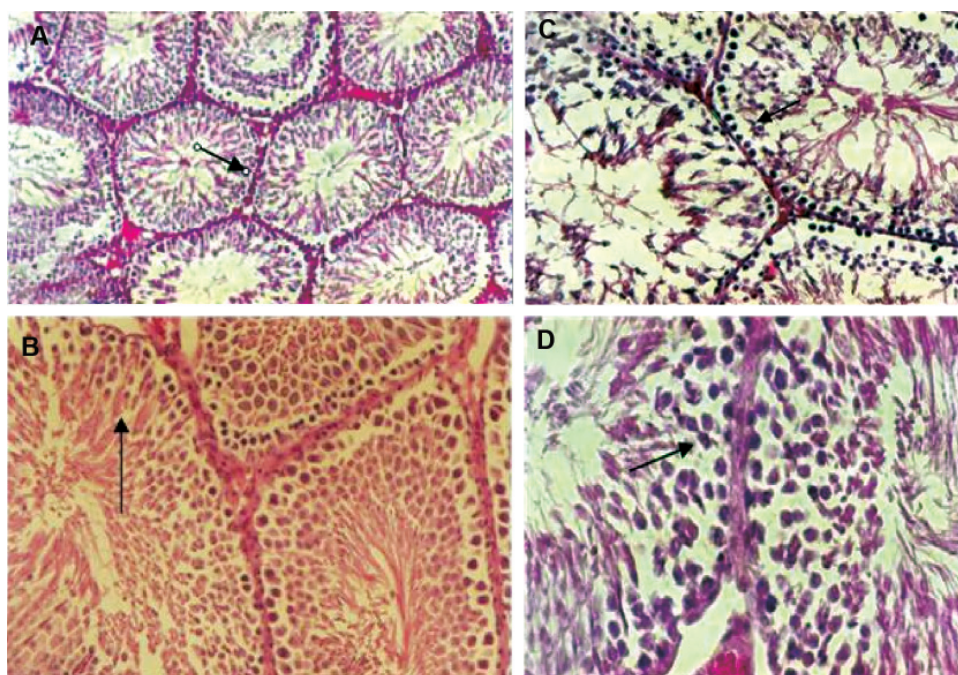


Fig 1: Histological appearance of testis samples. *A:* Regular seminiferous tubule with normal germinal epithelium morphology in the control group (arrow). *B:* Photomicrograph of testis in gentamicin group show reduced of epithelium seminiferous layers (arrow). *C:* Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence in the lumen of groups that received 400 mg/kg carrot seed extract (arrow). *D:* Regeneration and tubular healing of seminiferous tubules in the gentamicin group after exposure to 400 mg/kg carrot seed extract. All graphs are viewed at x640 magnification.

Table 3: The effect of different doses of carrot seed extract (CSE) on plasma levels of FSH, LH and testosterone in male rats*

Parameter	control	CSE (200 mg/kg)	CSE (400 mg/kg)	Gentamicin (5 mg/kg) + CSE (400 mg/kg)	Gentamicin (5 mg/kg)	p Level**
LH (ng/mL)	0.41 ± 0.19 ^a	1.92 ± 0.60 ^b	2.56 ± 0.64 ^c	1.43 ± 0.22 ^d	0.32 ± 0.15 ^e	ab: p<0.05 be: p<0.05 ac: p<0.05 cd: p<0.05 ce: p<0.05 ad: p<0.05 de: p<0.05 ae: p<0.05
FSH (ng/mL)	20.80 ± 1.67	20.22 ± 1.84	22.44 ± 1.21	19.11 ± 2.15	17.68 ± 3.40	
Testosterone (ng/mL)	2.11 ± 0.38 ^a	1.64 ± 0.15 ^b	2.84 ± 0.34 ^c	1.21 ± 0.13 ^d	1.04 ± 0.10 ^e	cd: p<0.05 ce: p<0.05 cd: p<0.05 ae: p<0.05

* The number of animals per group was eight rats. Data are presented as Mean ± SE.

Each dose of the extract and gentamicin were administered by intraperitoneal injection once a day for 28 consecutive days and the animals were killed 24 h after the last dose. The control group received a mixture of vehicle consisting of normal saline and DMSO (1:4).

** Letters indicate comparison of the differences between the specified groups.

Expansion of interstitial and intertubular spaces and congestion in the veins were also seen relative to the control group (Fig 1B). There were no differences in the cycle of spermatogenesis between the control animals and those that received either 200 or 400 mg/kg CSE; however, an accumulation of sperm in the lumen of the seminiferous tubules of rats that received a high-dose of CSE was observed (Fig 1C). This dose of CSE was also able to regenerate and repair the damage

to the seminiferous tubules caused by gentamicin administration (Fig 1D).

In Table 4, the percentages seminiferous tubules demonstrating tubular atrophy and regeneration in have been summarized.

Discussion

As all the stages of spermatogenesis occur in the seminiferous tubules of the testis, it is possible to evaluate the extent of spermatogenesis by determi-

Table 4: Percentage of seminiferous tubules demonstrating atrophy and generation in male rats exposed to carrot seed extract (CSE)*

Parameter	Control	CSE (200 mg/kg)	CSE (400 mg/kg)	Gentamicin (5 mg/kg) + CSE (400 mg/kg)	Gentamicin (5 mg/kg)	p Level**
Tubular atrophy	1.2 ± 0.1 ^a	1.3 ± 0.2 ^b	1.1 ± 0.1 ^c	11.5 ± 1.7 ^d	22.5 ± 1.4 ^e	ad: p<0.01 ae: p<0.01 bd: p<0.01 be: p<0.01 cd: p<0.01 ce: p<0.01 de: p<0.05
Tubular degeneration	1.0 ± 0.1 ^a	1.1 ± 0.1 ^b	1.4 ± 0.1 ^c	15.6 ± 1.4 ^d	33.0 ± 2.1 ^e	ad: p<0.01 ae: p<0.01 bd: p<0.01 be: p<0.01 cd: p<0.01 ce: p<0.01 de: p<0.05

* The number of animals per group was eight rats. Data are presented as Means ± SE.

** Letters indicate the comparison of the differences between the specified groups.

nation of the number of spermatozoa produced per one gram of testicular parenchyma (14).

The sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis (16,17). It has also been reported that there is a direct correlation between both the epididymal sperm count and motility and fertility in animals (18).

Oxidative damage, elevated lipid peroxidation and the alteration of membrane properties can lead to germ cell death at different stages of development and a decrease in the sperm count (19, 20). Accordingly, it is expected that antioxidant therapy will act as a protective defense against oxidative stress and improve fertility parameters. The ability of antioxidants such as ascorbic acid, in semen to protect spermatozoa from oxidative damage has been shown by some authors (21). Dawson et al. have been able to improve sperm quality in smokers and infertile men by the administration of ascorbic acid (22). In the present study, administration of carrot seed extract led to a significant elevation in CESR. This increase in the sperm count could have arisen from an increase in testicular spermatogenesis which in turn may be indicative of an elevation in the number of differentiated germ cells. As carrot seeds are rich of antioxidants (23), the elevation in CESR observed in this study could be attributed to the antioxidant properties of carrot seed extract, although the role of other factors such as male hormones cannot be ruled out. In addition, co-administration of this extract with gentamicin was able to counterbalance the negative effect of gentamicin on the sperm count demonstrated in this study and others (24).

Gentamicin is able to generate destructive reactive oxygen species including superoxide, hydrogen peroxide and hydroxyl radical (15) and frequently used to produce oxidative and necrotic damage (25, 26).

The role of gentamicin in the induction of apoptosis and oxidative damage has also been reported (27). Accordingly, the administration of compounds with antioxidant properties and reactive oxygen species scavengers can ameliorate the severity of gentamicin-induced tissue damage (26, 28). The histological results obtained in the present study indicated severe necrosis and degeneration of spermatogenic cells accompanied by a marked decrease in spermatocytes, spermatids and spermatosoides following gentamicin administration. In addition, there was a relative increase in the number of spermatogonia. This indicates that gentamicin has disrupted the maturation of spermatogonia in the meiotic stage, a finding that has been reported by others (24). The damage generated by gentamicin was ameliorated by co-administration of carrot seed extract, demonstrating the effectiveness of this extract in the prevention of cell necrosis and apoptosis. This could be indicative of the free radical scavenging properties of carrot seeds that have been reported previously (23). The results of this study also showed the ability of carrot seed extract to enhance the cauda epididymal sperm reserves of rats resulting from increased testicular spermatogenesis. Spermatogenic activity and the number of developing germ cells were also increased after exposure to carrot seed extract indicating the positive effect of the extract on meiosis.

It was also observed that the administration of a high dose of carrot seed extract can significantly elevate LH levels in plasma. This shows that the extract exerts some of its effects on the male reproductive system through sex hormones. Testosterone levels increased after carrot seed extract administration indicating the enhancement effect of the extract on the hormones involved in spermatogenesis (29). On the other hand, levels of testosterone reduced significantly following exposure to gentamicin. This is probably an indica-

tion that interstitial cell necrosis arises from a reduction in the level of this hormone.

Conclusion

It was demonstrated that the administration of carrot seed extract can overcome the reproductive toxicity of gentamicin. This natural extract was also able to induce spermatogenesis and cauda epididymal sperm reserves, probably mainly through the elevation of testosterone levels. These results are very interesting if we bear in mind the suppressive effect of carrot seed extract on the female reproductive system reported by others (8, 9). Therefore, it appears that this extract has opposite effects on male and female reproductive systems. As carrot is an easily accessible and popular vegetable, it is likely that its consumption would be an effective and safe way to reduce the toxic effects of chemicals on the reproductive system and infertility in males; a subject requiring further studies.

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