

Protective Effect of Nigella Sativa, Linseed and Celery Oils against Testicular Toxicity Induced by Sodium Valproate in Male Rats

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Abstract: The protective effect of nigella sativa, linseed and celery oils against testicular toxicity induced by sodium valproate (SVP) in male rats was studied. The experiment was carried out along 4 weeks on fifty male rats divided into 5 equal groups. Group (1) was kept as normal control (given vehicle), while rats of group (2) were given orally SVP in a dose of 500 mg/kg /day during the last week of experimental period (positive control). The other protected three groups were pretreated by oral administration of nigella sativa, linseed and celery oils each in a dose of 250 mg/kg/day and received SVP in the last week. At the end of experiment, sex organs were obtained for semen analysis, changes in sex organs weight and for lipid peroxidation in testicular tissue and histopathology. Also blood samples were collected for serum testosterone level. Results showed that pretreatment with nigella sativa, linseed and celery oil in SVP-intoxicated rats induced significant increase in the weight of testis, sperm count and motility with a decrease in abnormal sperms. An increase in serum testosterone levels and a decrease in testicular lipid peroxides (MDA) with increase in reduced glutathione were reported in the protected rats. Histopathological examination of the testes of protected rats revealed that these oils caused alleviation of testicular degenerative lesions which seen in valporate-treated rats. In conclusion, nigella sativa, linseed and celery oils produced protective effects against testicular damage induced by SVP. This study recommends that consumption of nigella sativa, linseed or celery oils may be useful as protective agents for patients who suffer from sexual impotency.

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1. Introduction:

It is well known that insufficient vitamins intake can cause deleterious effects on spermatogenesis and production of normal sperm (Mosher and Pratt, 1991). On the other side, sufficient consumption of vitamins and natural antioxidants can protect sperm DNA from oxidative stress and improve male fertility (Jedlinska *et al.*, 2006). Vitamin E was found to exhibit a protective effect on the testis of rats with cadmium induced-toxicity (Yang *et al.* 2006) and on the testis of rabbits with cyhalothrin insecticide induced - testicular damage (Youssef, 2010).

Vegetable oils contain several natural antioxidant constituents. Some of them such as pumpkin oil (Al- Zuhair *et al.*, 1997); celery oil (Momin and Nair, 2002); nigella sativa oil (Shalaby and khater, 2007) and sunflower oil (Di-Benedetto *et al.*, 2010) were reported to possess marked antioxidant activity. In addition, celery oil was found to produce a protective effect against testicular injury induced by sodium valproate in male rats (Hamza and Amin, 2007). Marjoram and grape seed oils were reported to minimize the hazard effects of ethanol toxicity on male fertility in rats (El-Ashrawy *et al.*, 2007). Ginger oil has a protective effect against DNA damage induced by H₂O₂ in the testis and improves

sperm quality and quantity in rats (Khaki *et al.*, 2009).

The present study was done to investigate the protective effect of nigella sativa, linseed and celery oils against testicular injury induced by sodium valproate in rats and their effect on lipid peroxidation in testicular tissues.

2. Materials and Methods:

Vegetable oils:

Nigella sativa, linseed and celery oils were obtained from local market of Agricultural Herbs and Medicinal plants, Cairo, Egypt. These oils were packed in dark brown bottles of 100 ml capacity each. Watery suspension of each tested oils was prepared by suspending 20 ml oil in 100 ml water gradually with the aid of 2 ml Tween 80 (suspending agent) to obtain 20% suspension.

Sodium valproate (Depakin ®):

It is a product of Sanofi-Synthelabo Company, Paris, France. It is dispensed as oral solution packed in brown bottles each containing 40 ml; each 1 ml contains 200 mg of sodium valproate. It is commonly used as an antiepileptic drug. The dose given to the rat was calculated from the human

therapeutic dose according to conversion table of Paget and Barnes, (1964).

Preparation of basal diet:

Basal diet for rats was prepared using AIN 93 according to Reeves *et al.* (1993). It was consisted of 15 % casein, 10% sucrose, 5 % fat, 2% choline chloride, 1% vitamin mixture, 4 % salt mixture and 5% cellulose (fibers). The remainder is corn starch.

Animals:

Mature male rats of Sprague Dawley strain weighing 160 -165 g b.wt. each and 12-14 week old were obtained from the Laboratory Animal Colony, Helwan, Egypt. The rats were kept under controlled hygienic conditions in plastic cages and fed on basal diet for one week before starting the experiment for acclimatization. Water was provided *ad libitum*.

Experiment and grouping of rats:

Fifty mature male rats were randomly allocated into 5 groups, each of 10 animals. Group (1) was given 1 ml distilled water/day (vehicle) and kept as control normal. Rats of the other four groups were given sodium valproate in a dose of 500 mg/kg b.wt./ day during the last week of experiment period (4 weeks) to induce testicular damage according to Hamza and Amin (2007). Rats of group (2) was kept as control positive (without pretreatment), while rats of groups (3), (4) and (5) were pretreated orally with aqueous suspension of nigella sativa, linseed, and celery oils in a dose of 250mg/kg b.wt./day for 4 weeks, respectively. At end of the experiment, blood samples were collected from orbital plexus of eye for estimation of testosterone level in the serum. Semen samples were collected from the cauda epididymis of sacrificed rats and semen was used for estimating the sperm characters. The testes and accessory glands (seminal vesicles and prostate) were carefully dissected out and weighed. The index weight (I.W.) of the organ was calculated by: $I.W. = \text{organ weight} / \text{body weight} \times 100$. These organs were then kept in 10% formalin solution pending for histopathological examination.

Determination of serum testosterone:

Testosterone levels were determined using analog method using radioimmunoassay (RIA) kits (Gamma Trade Company, Cairo, Egypt). The method is based upon the competitive binding principal. The unknown or standard samples were incubated with radioactive iodine¹²⁵ labeled testosterone in antibody-coated tubes. After incubation, the liquid contents in the tubes were withdrawn and the bound radioactivity was determined using gamma counter according to the method of Wilke and Utley (1987).

Semen analysis:

The progressive motility of sperms was evaluated microscopically within 2–5 minutes of their isolation from the cauda epididymis as described by Bearden and Fluquary (1980). Microscopic examination of seminal smears stained with Eosin Nigrosin stain was carried out to determine the percentages of sperm viability (ratio of alive/dead) and sperm abnormality according to the method described by Yokoi *et al.* (2003).

Estimation of lipid peroxidation:

Lipid peroxides as malondialdehyde (MDA) were measured in the testis spectrophotometrically after the reaction with thiobarbituric acid-reactive substances. Briefly, after ice-water washing of the testis a part was weighed and homogenized in 9 volumes buffered 0.9% saline solution in a homogenizer (Type PT 45/80, Littau, Switzerland) and kept at -70°C until analyzed for MDA content as nmol /g tissue, according to the method of Placer *et al.* (1966).

Measurement of glutathione:

Reduced glutathione (GSH) content in the testis was measured chemically with specific kits (Biodiagnostic Company, Dokki, Egypt) according to the method described by Sedlack and Lindsay (1968) using Ellman's reagent. This method is based on the reactive cleavage of 5, 5-dithiobis-(2-nitrobenzoic acid) by sulfhydryl group to yield a yellow colour with maximum absorbance at 412 nm. The content of reduced GSH was calculated as $\mu\text{mol} / \text{g}$ tissue.

Histopathological examination:

The testes and accessory glands of rats were taken and fixed in neutral formalin 10 % solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were then stained with Hematoxylen and Eosin and examined microscopically according to Bancroft and Stevens (1990).

Statistical analysis:

All data are expressed as means \pm S.E. Statistical analysis was carried using Student't' test according to Snedecor and Cochran (1986).

3. Results

As shown in table (1); oral administration of sodium valproate (500 mg/kg b.wt.) to male rats for 7 days significantly ($P < 0.01$) decreased the weight of testes as compared to the normal control group. Male rats pretreated with nigella sativa oil, linseed oil and celery oil for 4 weeks had a significant ($P < 0.01$) increase in the weight of testes as compared to the positive (sodium valproate-treated) control rats.

Data in table (2) show that sodium valproate when given orally to male rats for 7 days decreased the sperm count, percents of sperm motility and viability and increased the percent of sperm cell abnormalities. The most frequently seen sperm cell abnormalities in rats treated with sodium valproate were detached, double and large size heads and coiled tail as illustrated in Fig. (1). Smears obtained from normal control rats showed normal sperms (Fig. 2) Pretreatment with nigella sativa oil, linseed oil and celery oil to male rats significantly ($P < 0.01$) increased sperm cell count, motility and viability as compared to the control positive group.

As shown in table (3), serum testosterone level in rats given orally sodium valproate for 7 days was 3.30 ± 0.03 ng/dL versus to 7.73 ± 0.04 ng/dL in the normal control rats. Pretreatment with nigella sativa, linseed oil and celery oils to male rats for 4 weeks significantly ($P < 0.01$) increased serum testosterone level as compared to the positive control group.

As shown in table (4), oral administration of sodium valproate to male rats for 7 days significantly

($P < 0.01$) increased the level of malondialdehyde (MDA) and decreased the content of reduced glutathione (GSH) in testicular tissue as compared to the normal control group. In rats pretreated with nigella sativa oil, linseed oil or celery oil, the level of MDA was decreased and content of GSH was increased in the testis as compared to the positive control rats.

Histopathological examination of the testes of normal rats showed normal histological structure of active mature functioning seminiferous tubules associated with complete spermatogenic series as demonstrated in Fig. (3). Examination of the testes of rats given orally sodium valproate for 7 days revealed marked degeneration and atrophy of most seminiferous tubules with absence of spermatogenic series in tubular lumen as shown in Fig. (4). Microscopic examination of the testes of rats pretreated with nigella sativa, linseed or celery oil for 4 weeks revealed normal histological structure of most seminiferous tubules with normal spermatogenic series (Fig. 5 and 6).

Table (1): Effect of some vegetable oils (250 mg/kg/day) on the weight of sexual organs in rats with testicular damage induced by sodium valproate (SVP)

Groups and treatments	Weight of sexual organs (g/100 g b.wt.)		
	Testes	Seminal vesicles	Prostate glands
Normal control (Vehicle)	2.80 ± 0.01	1.82 ± 0.03	0.56 ± 0.01
Positive control (SVP)	$1.85 \pm 0.02^{**}$	1.70 ± 0.02	0.48 ± 0.01
Nigella sativa oil	$2.77 \pm 0.03^{**}$	1.60 ± 0.04	0.45 ± 0.03
Linseed oil	$2.74 \pm 0.06^{**}$	1.65 ± 0.02	0.53 ± 0.02
Celery oil	$2.78 \pm 0.01^{**}$	1.70 ± 0.04	0.49 ± 0.01

Data represent Means \pm S.E.

All pretreated groups were compared to the positive control group using Student't' test

** Significant at $P < 0.01$ n=10 rats.

Table (2): Effect of some vegetable oils (250 mg/kg/day) on sperm cell characters in rats with testicular damage induced by sodium valproate (SVP)

Groups and treatments	Sperm cell characters			
	Count (10^6 /ml)	Motility (%)	Viability (%)	Abnormality (%)
Normal control (Vehicle)	71.67 ± 0.48	80.0 ± 1.6	88.0 ± 0.12	3.67 ± 0.2
Positive control (SVP)	$52.20 \pm 0.23^{***}$	$50.0 \pm 1.4^{**}$	$46.0 \pm 0.16^{**}$	$7.76 \pm 0.2^{**}$
Nigella sativa oil	$66.67 \pm 0.43^{***}$	$60.0 \pm 2.3^{**}$	$55.0 \pm 0.10^{**}$	5.13 ± 0.4
Linseed oil	$70.67 \pm 0.28^{***}$	$65.0 \pm 1.2^{**}$	$60.0 \pm 0.13^{**}$	5.96 ± 0.3
Celery oil	$65.00 \pm 0.30^{**}$	$70.0 \pm 2.2^{**}$	$77.0 \pm 0.13^{**}$	5.24 ± 0.4

Data represent Means \pm S.E.

All pretreated groups were compared to the positive control group using Student't' test ** Significant at $P < 0.01$

** Significant at $P < 0.001$ n=10 rats.

Table (3): Effect of some vegetable oils (250 mg/kg/day) on serum testosterone level in rats with testicular damage induced by sodium valproate (SVP)

Groups and treatments	Testosterone level (ng/dL)
Normal control (Vehicle)	7.73 ± 0.04
Positive control (SVP)	3.30 ± 0.03**
Nigella sativa oil	5.08 ± 0.01**
Linseed oil	7.13 ± 0.04**
Celery oil	6.64 ± 0.08**

Data represent Means ± S.E.

All pretreated groups were compared to the positive control group using Student't' test

** Significant at P < 0. 01 n=10 rats.

Table (4): Effect of some vegetable oils (250 mg/kg/day) on the levels of malondialdehyde (MDA) and reduced glutathione (GSH) in rats with testicular damage induced by sodium valproate (SVP).

Groups and treatments	MDA (nmol /g tissue)	GSH (µmol /g tissue)
Normal control (Vehicle)	5.2 ± 0.3	14.6 ± 0.8
Positive control (SVP)	8.6 ± 0.2**	10.0 ± 0.6**
Nigella sativa oil	4.8 ± 0.1**	13.7 ± 0.2**
Linseed oil	4.9 ± 0.4**	13.5 ± 0.4**
Celery oil	4.4 ± 0.1**	13.2 ± 0.2**

Data represent Means ± S.E.

All pretreated groups were compared to the positive control group using Student't' test

** Significant at P < 0. 01 n=10 rats.

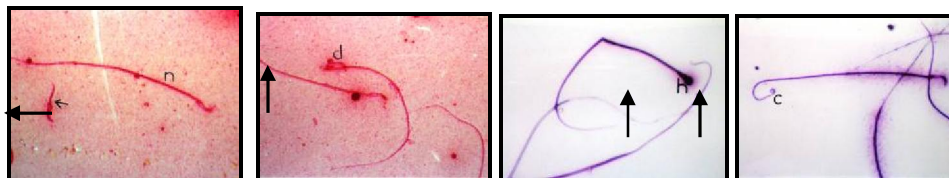


Fig. (1): Seminal smears of rats given sodium valproate showing detached head (A), double head (B), large size head (C) and coiled tail (D) of sperms. (Eosin Nigrosin stain X 160).

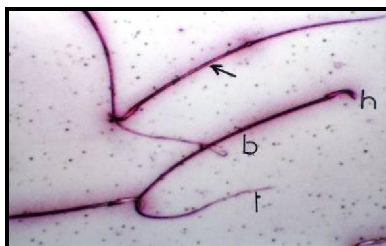


Fig. (2): Seminal smear of a normal control rat showing normal sperm with head (h), body (b) and tail (t). (Eosin Nigrosin stain X 160).

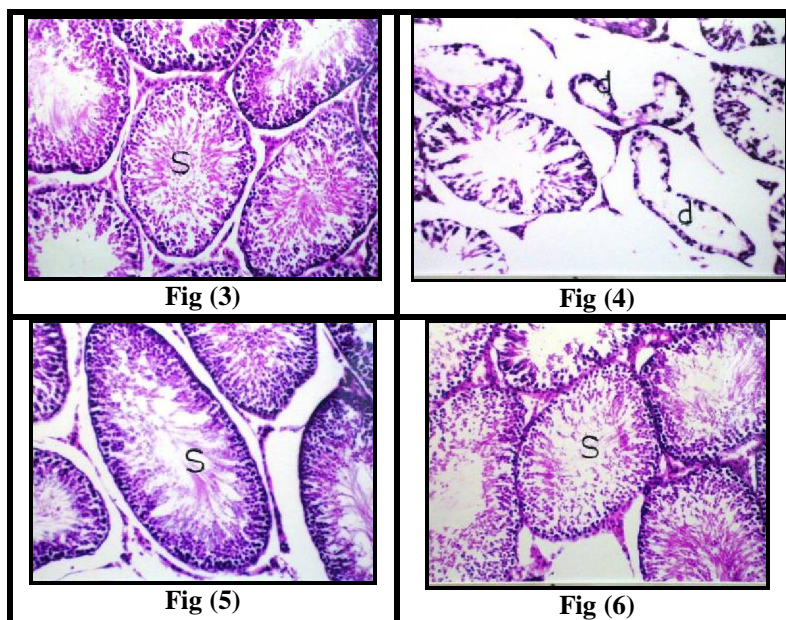


Fig. (3): Testis of normal control rat showing normal histological structure of active mature functioning seminiferous tubules(S) associated with complete spermatogenic series.

Fig. (4): Testis of a rat given sodium valproate showing marked degeneration (d) and atrophy of most seminiferous tubules with absence of spermatogenic series in tubular lumen.

Fig. (5): Testis of a rat pretreated by nigella sativa oil at 250 mg/kg/ day for 4 weeks showing normal histological structure of most seminiferous tubules(S).

Fig. (6): Testis of a rat pretreated by celery oil at 250 mg/ kg/ day for 4 weeks showing normal histological structure of most seminiferous tubules(S). (H & EX 200)

4. Discussion:

The protective effect of some vegetable oils against testicular injury induced by sodium valproate in male rats was investigated. The results revealed that oral administration of sodium valproate to male rats caused testicular injury characterized by decreased weight of the testis, lowered semen quantity and quality, decreased serum testosterone level. It also caused degenerative changes of most seminiferous tubules with absence of spermatogenic series in tubular lumen. In addition, sodium valproate increased lipid peroxidation and reduced glutathione content in testicular tissue. These effects were similar to those reported by Soliman and Abd Elmguid (1999) and Sveberg et al. (2001) who found that there was a highly significant decrease in testicular weight in rats treated by high dose of sodium valproate. A decrease in plasma testosterone, FSH and LH hormones and a widespread testicular atrophy with histological verified spermatogenic arrest were reported in the treated rats. Hamza and Amin (2007) reported that sodium valproate decreased the relative weight of testes and

epididymis and reduced the sperm quantity and viability in the treated rats. Serum testosterone level was decreased and severe degenerative changes were also seen in the testes following administration of sodium valproate to rats. Vijay *et al.* (2008) found that the intratesticular testosterone level was significantly reduced by administration of sodium valproate to rats. The results of this study correlate to those obtained by Bairy *et al.* (2010) who reported that sodium valproate significantly decreased sperm count and motility and increased, in a dose dependant manner, the percent of abnormal sperms of testes in rats.

Pretreatment of male rats with nigella sativa, linseed and celery oils for 4 weeks produced a protective effect against testicular injury induced by sodium valproate. This effect was manifested by increased weight of the testis, improved semen quality and quantity, elevated serum testosterone level, decreased lipid peroxidation in the testis as well as alleviation of degenerative changes in testes of rats given sodium valproate. The protective effect nigella sativa oil on rat testis, reported herein, was

partially similar to that reported by Ali and Blunden (2003) who concluded that nigella sativa essential oil contains thymoquinone and carvacrol which may be related to its cytoprotective and antioxidant actions in rats. The antioxidant effect of nigella sativa oil was also proved by Abdel-Wahhab and Ali (2005) in aflatoxins- intoxicated rats. Butt and Sultan (2010) concluded that nigella sativa oil contains thymoquinone which has potent antioxidant effect. The mechanism(s) underlying the protective effect of nigella sativa oil on the testis might be due to its direct cytoprotective effect and/ or indirect antioxidant and androgen like activities that reported in this study. Thymoquinone probably has an important role in causing these effects. The antioxidant activity of nigella sativa oil that reported in this study agree with that revealed by Badary *et al.*(2003) who concluded that thymoquinone acts mainly as potent superoxide anion scavenger.

The protective effect of linseed oil against testicular injury in rats given sodium valproate that reported herein was partially similar to that reported by Palmer *et al.* (1980) who isolated two glycosides (linustatin and neolinustatin) from linseed oil meal and suggested that they provide a probable explanation for its protective activity against selenium toxicity in rats. Moreover, Comhaire and Mahmoud (2003) concluded that linseed oil contains alfa-linolenic acid (ALA) that corrects the deficient intake of omega-3 essential fatty acids, which is correlated with impaired sperm motility among subfertile men. In addition, Prasad (2009) concluded that linseed oil is rich in alfa-linolenic acid and suppresses oxygen radical production by white blood cells so induces potent antioxidant activity in rats.

The protective effect of celery oil against testicular damage in rats given sodium valproate ,reported in this study, agree with that obtained by Hamza and Amin(2007) who concluded that pretreatment with *Apium graveolens* (celery) plant extract has effectively alleviated most of the sodium valproate - induced injury on the testis, suggesting a protective role of celery plant. The mechanism(s) underlying the protective effect of celery oil against testicular injury induced by valproate in male rats might be due to the antioxidant effect (Momin and Nair, 2002) and /or androgenic activity (Hamza and Amin, 2007). Because of the antioxidant activity of celery oil it may decrease the oxidative stress in testicular tissue and the damaging effect of free radicals on sperm allowing normal spermatogenesis. Piomboni *et al.* (2008) reported that the natural antioxidants could protect sperm during maturation and migration, leading to improved sperm function. The increased serum testosterone after pretreatment

of male rats with celery oil, reported in this study, might be the reason for its protective effect.

In conclusion, pretreatment with nigella sativa, linseed and celery oil exhibits a protective effect against testicular injury induced by sodium valproate in male rats, so intake of these oils may be beneficial as protective agents for patients who suffer from sexual impotency.

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