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Protective Effect of Celery Oil, Vitamin E and Their Combination Against Testicular Toxicity in Male Rats

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Abstract: The protective effect of celery oil, vitamin E and their combination against testicular toxicity induced by sodium valporate (SVP) in male rats was studied. The experiment was carried out on 49 mature male rats distributed into 7 equal groups. The 1st group was kept as a normal control, while rats of the 2nd group were given orally SVP in a dose of 500 mg/kg /day in the last week of experiment period. The other protected groups were pretreated by oral administration of celery oil (100 and 200 mg/kg/day) and vitamin E (200 mg/kg/day), alone and in combination, respectively, for 4 weeks and received SVP as in the 2nd group. The pretreatment by celery oil, vitamin E and their combination in rats with testicular toxicity increased the weight of testes and accessory genital glands; sperm motility, count and viability; serum testosterone level; and alleviated the degenerative changes seen in the testes of rats with testicular injury. Conclusively, pretreatment by celery oil, vitamin E and their combination produces a protective effect against reproductive toxicity-induced by SVP in male rats. This study recommends that intake of celery oil with vitamin E may be beneficial for male patients who suffer from sexual impotency.

Key words: Celery oil · Vitamin E · Male fertility · Sperm · Rat

INTRODUCTION

Infertility is one of the major health problems in life and approximately about 30 % of this problem is due to male factors [1]. Several factors can interfere with spermatogenesis; reduce sperm quantity and quality and lower the male fertility. Many diseases and conditions such as coronary heart diseases, diabetes mellitus chronic liver diseases, chronic smoking, alcohol intake, prolonged exposure to industrial and insecticide contaminants, air pollutants and insufficient vitamins intake have deleterious effects on spermatogenesis and production of normal sperm [2-5]. On the other hand, some previous studies reported that intake of antioxidants and vitamins E and C can protect sperm DNA from oxidative stress of free radicals n rats [6] and antagonized testicular toxicity caused by a pyrethroid Lambda - cyhalothrin insecticide [7].

Celery oil is a valuable essential oil obtained from celery (*Apium graveolens* L. Family *Apiaceae*) seeds. Different extracts of celery seeds were previously reported to produce hypocholesterolemic and hepatoprotective [8]; and antioxidant [9] activities and protective effect against reproductive toxicity in male rats [10]. It is mentioned in Wikipedia, [11] that celery is thought to be an aphrodisiac (increase sexual desire of males) by some people because it contains androsterone, a metabolic product of testosterone hormone.

Vitamin E (α- tocopherol) is a fat soluble vitamin which regulates oxidation processes in the body as it acts as a powerful antioxidant. Previous studies showed that high levels of free radicals found in 40% of infertile men's semen. This could lead to low sperm count and abnormality of sperm cells. By taking vitamin E, it could neutralize the damaging effects of free radicals [12-14]. Supplementation of vitamin E and C to the diet could protect sperm DNA from oxidative stress of free radicals and improve the fertility [6] in male rats. Vitamin E was found to exhibit a protective effect on the testis of rats with cadmium induced - toxicity [15].

The current study was designed to explore the protective effect of pretreatments by oral administration of celery oil and vitamin E, when given alone and in combination, to male rats with experimental testicular damage induced by sodium valporate.

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MATERIALS AND METHODS

Material

Celery Oil: It is the essential oil of celery (*Apium graveolens* L. Family *Apiaceae*) seeds. It was purchased from local market of Herbs and Medicinal Plants, Cairo, Egypt, packed in brown bottles each containing 30 ml of celery oil.

Vitamin E: It was obtained from Pharco Company for Pharmaceuticals; Alexandria, Egypt. It is dispensed in form of soft gelatin capsules each containing 400 g/dL-alpha tocopherol acetate.

Sodium Valporate: It is one of products of Sanofi-Synthelabo Company, Paris, France, dispensed as an oral solution packed in dark brown bottles each containing 40 ml. Sodium valporate is sold commercially under trade name of Depakin ® 200 mg/ml solution.

Animals: Forty nine mature male rats of Sprague Dawley strain weighing 180 ± 5 g and 14-16 weeks old were purchased from Laboratory Animal Colony Helwan Egypt. Animals were fed on locally manufactured rat pellets and water was provided *ad libitum*. Rats were allowed to acclimatize to the laboratory environment for 7 days before start of the experiment.

Experiment: Forty nine mature male rats were used in this study and allocated into 7 equal groups. The 1st group was given 1 ml distilled water/day (vehicle) and kept as negative control (normal rats). Rats of the other five groups were given sodium valporate in a dose of 500 mg/kg during the last week of experimental period (28 days) for induction of testicular damage [10]. The 2nd group was kept as positive control (without pretreatment), while rats of the 3rd and 4th groups were pretreated with celery oil in doses of 100 and 200 mg/kg b.wt. for 28 days, respectively. The 5th group was pretreated with vitamin E in a dose of 200 mg/kg b.wt. for the same period. Rats of the 6th and 7th groups were pretreated with combination of celery oil (100 and 200 mg/kg b.wt.) and vitamin E. (200 mg/kg b.wt.) At end of the experiment, blood samples were collected from orbital plexus of veins for separating serum to estimate the serum testosterone level. Semen samples were collected from cuda epididymis of the sacrificed rats by cutting the tail of epididymis and squeezing it gently on a clean slide. The semen was used for evaluating epididymal sperm characteristics. The testes, seminal vesicles and prostate glands of the sacrificed rats were dissected out and weighed. The testes were persevered in 10% formalin solution till processed for histopathological examination.

Semen Analysis: The epididymal contents of treated rats were obtained after cutting the tail of epididymis, squeezing it gently on clean slide and the sperm progressive motility and cell count were determined [16]. Microscopic examinations of the seminal smears stained with Eosin Nigrosin stain were also carried out to determine the percentages of sperm viability (ratio of alive/dead) and abnormality [17].

Testosterone Determination: Serum testosterone concentration was determined using RIA method (kit catalog #1119) which is intended for the quantitative determination of total testosterone in the serum [18]. RIA method to measure testosterone concentration in the serum of male rats was formalized, tested and physiologically validated. The procedure measured testosterone equally well, whether or not estimation of recovery and chromatographic purification preceded the RIA. The results were equivalent to those achieved by the method of competitive protein binding.

Histopathological Examination: The testes of treated rats were dissected out and fixed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (HandE) then examined microscopically [19].

Statistical Analysis: Data were expressed as means ± S.E. and statistical analysis was carried using a computerized SPSS program (Version14). Significance was performed using the least significant difference and paired Student's t- test [20].

RESULTS

Oral administration of sodium valporate to male rats in a dose of 500 mg/kg for 7 days induced decreases ($P < 0.001$) in the weight of testes, seminal vesicles and prostate glands as compared to the normal control group. Male rats pretreated by oral administration of celery oil at 100 and 200mg/kg for 28 days showed increased ($P < 0.01$) weight of testes, seminal vesicles and prostate glands as compared to the positive control group.

Table 1: Effect of oral administration of celery oil and vitamin E, alone and in combination, on the weight of male sexual organs of rats with testicular damage induced by sodium valporate. (n= 7 rats)

Groups and Treatments	Mean ± SE of weights of sexual organs (g)		
	Testes	SVs	PGs
Negative control (1 ml Distilled water)	2.90 ± 0.21	1.82 ± 0.03	0.56 ± 0.01
Positive control (500 mg/kg Sodium valporate)	1.85*** ± 0.22	0.99*** ± 0.02	0.23***± 0.01
Celery oil (100mg/kg b.wt.)	2.15** ± 0.33	1.11** ± 0.04	0.45** ± 0.03
Celery oil (200mg/kg b.wt.)	2.30** ± 0.26	1.15** ± 0.02	0.49** ± 0.02
Vitamin E (200mg/kg b.wt.)	2.55** ± 0.13	1.37** ± 0.01	0.52** ± 0.04
Celery oil (100mg/kg b.wt.) + Vitamin E (200mg/kg b.wt.)	2.62*** ± 0.22	1.52**± 0.02	0.53** ± 0.01
Celery oil (200mg/kg b.wt.) + Vitamin E (200mg/kg b.wt.)	2.75*** ± 0.12	1.62*** ± 0.03	0.54** ± 0.01

Svs = Seminal vesicles PGs = Prostate glands

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

** Significant at P < 0. 01 *** Significant at P < 0. 001

Table 2: Effect of oral administration of celery oil and vitamin E, alone and in combination, on sperm cell characteristics in rats with testicular damage induced by sodium valporate. (n= 7 rats)

Groups and Treatments	Epididymal sperm cell characteristics			
	Motility(%)	Count (10^6/epididymis)	Viability (%)	Abnormality (%)
Normal control (1 ml Distilled water)	90.00 ± 1.0	73.67 ± 0.48	88.0 ± 0.12	3.67 ± 0.18
Positive control (500 mg/kg Sodium valporate)	50.00*** ± 1.03	51.00*** ± 0.33	46.0± 0.16	7.76 ± 0.08
Celery oil (100mg/kg b.wt.)	64.00*** ± 2.30	64.67*** ± 0.43	55.0 ± 0.10	4.33 ± 0.18
Celery oil (200mg/kg b.wt.)	90.0***± 1.22	73.67*** ± 0.28	60.0 ± 0.13	3.76 ± 0.18
Vitamin E (200mg/kg b.wt.)	70.00*** ± 0.12	65.00*** ± 0.35	70.0 ± 0.15	4.43 ± 0.18
Celery oil (100mg/kg b.wt.) + Vitamin E (200mg/kg b.wt.)	78.00*** ± 0.20	69.00*** ± 0.30	77.0 ± 0.13	3.24 ± 0.24
Celery oil (200mg/kg b.wt.) + Vitamin E (200mg/kg b.wt.)	85.00*** ± 0.10	71.00*** ± 0.20	85.0 ± 0.10	3.00 ± 0.15

All pretreated groups were compared to the positive control group using Student't' test *** Significant at P < 0.001

Pretreatment with vitamin E at 200 mg/kg b.wt. for the same period caused increases (P < 0. 01) in the weight of testes, seminal vesicles and prostate glands. Combination of celery oil and vitamin E produced increases (P < 0. 01) in the weight of sexual organs as compared to the positive control group (Table 1).

As shown in table 2 oral administration of sodium valporate to male rats for 7 days caused decreases (P < 0. 01) in sperm motility and count as compared to the normal control group. Percentage of sperm viability and abnormality were 46.0 and 7.76 % in rats given sodium valporate versus to 88.0 and 3.67 % of the normal control rats, respectively. The most frequently seen sperm cell abnormalities were detached head and coiled tails (Fig. 1). Pretreatment by celery oil (100 and 200 mg/kg b.wt.) or vitamin E (200 mg/kg b.wt.) for 28 days increased (P < 0. 05) sperm motility, count and viability but decreased sperm cell abnormality. Combination of celery oil and vitamin E induced increases (P < 0. 01) in sperm motility, count and viability associated with a decrease in the percentage of sperm abnormality.

Oral administration of sodium valporate in a dose of 500 mg/kg/ day for 7 days to rats decreased (P < 0. 01) serum testosterone level to 3.30 ± 0.03 ng/dL versus 7.73 ± 0.04 ng/dL in the normal control rats. Pretreatments with celery oil at both tested doses (100 and 200 mg/kg b.wt.) or vitamin E (200 mg/kg b.wt.) or their combination for 28 days significantly increased serum testosterone levels (Table 3).

Table 3: Effect of oral administration of celery oil and vitamin E, alone and in combination, on serum testosterone level in rats with testicular damage induced by sodium valporate. (n= 7 rats)

Groups and Treatments	Mean ± SE of serum testosterone level (ng/dL)
Normal control (1 ml Distilled water)	7.73 ± 0.04
Positive control (500 mg/kg Sodium valporate)	3.30*** ± 0.03
Celery oil (100mg/kg b.wt.)	4.18** ± 0.01
Celery oil (200mg/kg b.wt.)	4.46** ± 0.04
Vitamin E (200mg/kg b.wt.)	5.46** ± 0.03
Celery oil (100mg/kg b.wt.) + Vitamin E (200mg/kg b.wt.)	5.64*** ± 0.05
Celery oil (200mg/kg b.wt.) + Vitamin E (200mg/kg b.wt.)	5.70*** ± 0.02

All pretreated groups were compared to the positive control using Student't' test ** Significant at P < 0. 01 *** Significant at P < 0.001

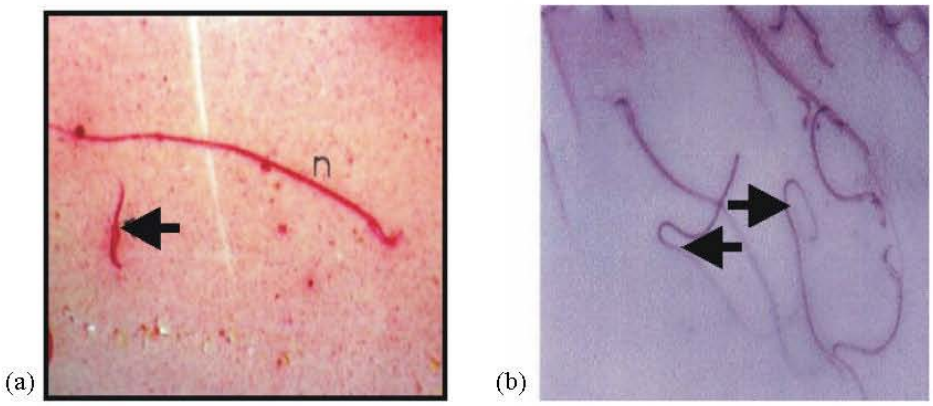

Fig. 1: Seminal smear obtained from a rat given orally sodium valporate for 7 days (Positive control) showing detached head (A) and coiled tails (B). (Eosin Nigrosin stain X 60)

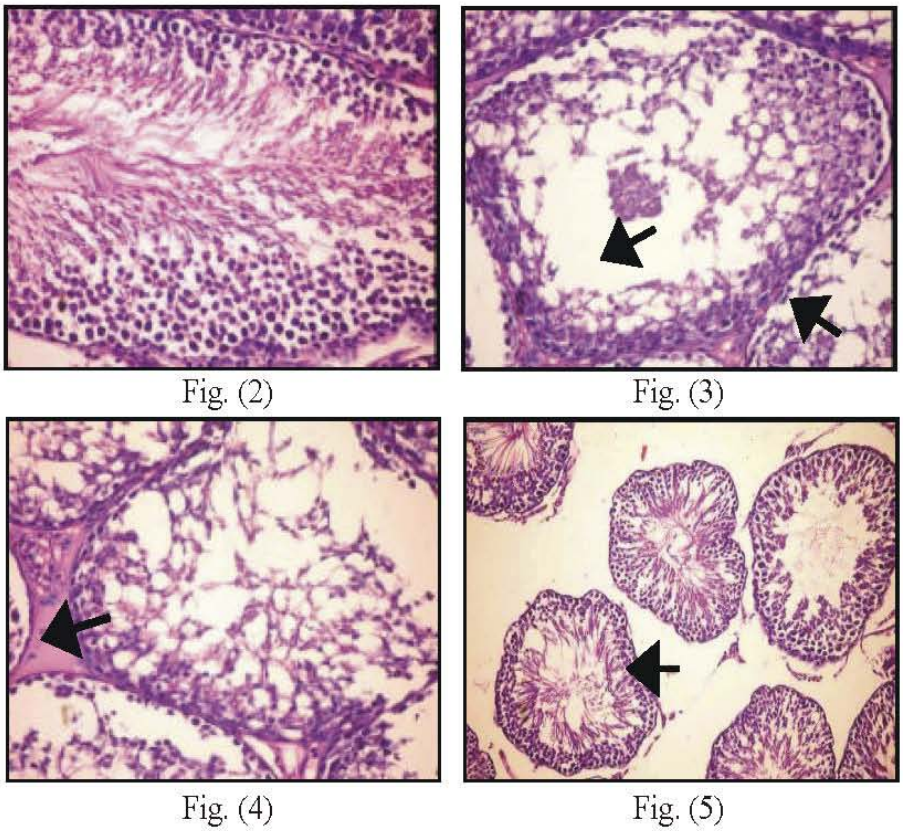

Fig. 2: Testis of a control rat showing normal histological structure of mature functioning seminiferous tubules associated with complete spermatogenic series. (HandE X 200)

Fig. 3: Testis of a rat given sodium valporate showing large vacoulations (Arrow), degeneration and necrosis (Arrow) of germ cell lining seminiferous tubules.(HandE X 200)

Fig. 4: Testis of a rat given sodium valporate showing large vacoulations and interstitial diffuse edema (Arrow). (HandE X 200)

Fig. 5: Testis of a rat given celery oil at 200 mg/ kg showing only decreased diameter (atrophy) of seminiferous tubules (Arrow). (HandE X 200)

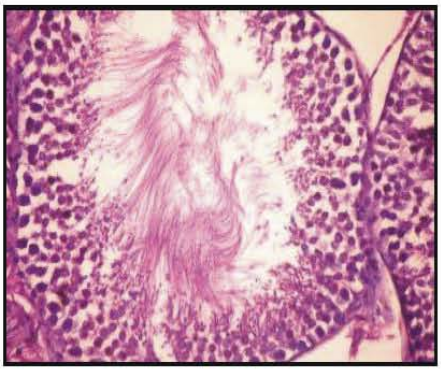

Fig. (6)

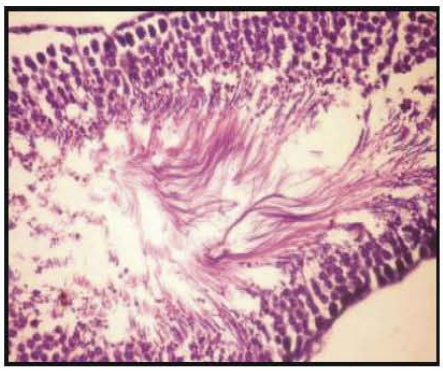

Fig. (7)

Fig. 6: Testis of a rat given vitamin E at 200 mg/kg showing normal active seminiferous tubules and complete spermatogenic series(lumen was filled with spermatozoa). (HandE X 200)

Fig. 7: Testis of a rat given combination of celery oil (200 mg/ kg) and vitamin E (200 mg/ kg) showing normal active seminiferous tubules with complete spermatogenic series. (HandE X 200)

Histopathological examination of the testes of normal control rats showed normal histological structure of mature functioning seminiferous tubules associated with complete spermatogenic series (Fig. 2). The testes of rats given sodium valporate in a dose of 500 mg/kg for 7 days showed large vacoulations, degeneration and necrosis of germ cell lining seminiferous tubules (Fig. 3) associated with diffuse interstitial edema (Fig. 4). Microscopic examination of the testes of rats pretreated by celery oil in a dose of 200 mg/kg for 28 days revealed only decreased diameter (atrophy) of seminiferous tubules (Fig. 5). The testes of rats pretreated by vitamin E showed no histopathological lesions and the lumen of seminiferous tubules was filled with mature normal spermatozoa (Fig. 6). Pretreatment by combination of celery oil (200 mg/kg) and vitamin E (200 mg/kg) caused alleviation of the histopathological lesions induced by sodium valporate and revealed normal histological architecture of the testis with normal spermatogenic series (Fig. 7).

DISCUSSION

The results of this study revealed that oral administration of sodium valporate to male rats in a dose of 500 mg/kg b.wt. for 7 days produced reproductive toxicity. The toxic effect of sodium valporate characterized by decreased weights of the testes and accessory genital glands, lowered semen quantity and quality, decreased serum testosterone level as well as testicular damage. These effects were similar to those reported by [10] who found that the relative weights of testes and epididymes and the sperm numbers and viability were all decreased following sodium valproate administration to rats. Testosterone levels were also dropped and severe histopathological lesions were seen. The authors attributed the toxic effect of sodium valporate to its direct cytotoxic effect on the testis and / or indirectly by decreasing the serum testosterone level. However, [21] reported that sodium and calcium valporate (anticonvulsant drugs) caused testicular atrophy when given to male dogs and rats at 400 mg/kg for 13 weeks.

Oral administration of celery oil from celery (*Apium graveolens* L.) seeds to male rats, as reported herein, produced a protective effect against reproductive toxicity induced by sodium valporate. This effect was manifested by significant increases in the weight of male sexual organs; in percentages of sperm concentration (count), motility and viability; in serum testosterone level and by alleviating the testicular degenerative changes that induced by sodium valporate administration. The protective effect of celery oil against testicular injury induced by sodium valporate in rats was found to be a dose dependant since its large dose (200 mg/kg) exhibited more protection and improvement than the small (100 mg/kg) dose. Our findings were nearly similar to the results obtained by [10] who concluded that pretreatment with *Apium graveolens* (celery) extract has effectively alleviated most of the sodium valporate - induced effects in male rats, suggesting a protective role of celery extract against experimental testicular toxicity induced by sodium valporate.

The mechanism(s) underlying the protective effect of celery oil on male reproductive system of the rat might be due to the antioxidant effects of celery that reported by [9]

and /or its androgenic activity reported by Hamza and Amin. [10]. Because of the antioxidant activity of celery, it may decrease the oxidative stress in testicular tissue and the damaging effect of free radicals on sperm allowing normal spermatogenesis. In this concern, Piomboni *et al.* [22] reported that natural antioxidants (beta-glucan, lactoferrin and vitamins C and E) could protect sperm during maturation and migration, leading to improved sperm function. However, it was mentioned in Wikipedia, [11] that celery is thought to be an aphrodisiac by some people because it contains androsterone, a metabolic product of testosterone hormone. The increased level of serum testosterone following pretreatment of male rats by celery oil, reported in this study, might be the reason for the protective effect of celery.

Concerning vitamin E, results of the current study showed that it effectively alleviated most of the sodium valporate - induced effects, suggesting a protective role of vitamin E against experimental sodium valporate induced reproductive toxicity. Previous studies [12 - 14] revealed that vitamin E could neutralize the damaging effects of free radicals due to its antioxidant activity. Moreover, Jedlinska *et al.* [6] concluded that vitamin E can protect sperm DNA from oxidative stress of free radicals and improve the fertility. Yang *et al.* [15] also found that vitamin E exhibited a protective effect on the testis of rats with cadmium induced - testicular injury. However, Ogli *et al.*[23] concluded that the sperm motility is significantly improved with the separate oral supplementation of vitamin C and E as compared with the combined supplement of both vitamins in rats.

Combination of celery oil and vitamin E, as reported herein, exhibited a more powerful protective effect against male reproductive toxicity induced by sodium valporate in rats. This finding could be explained by the additive effect of combined administration of celery oil and vitamin E.

In conclusion, sodium valporate induced male reproductive toxicity which is manifested by decreased weights of the testes and accessory genital glands, lowered semen quantity and quality, decreased serum testosterone level as well as testicular morphological degenerative damage. Pretreatments with celery oil, vitamin E and their combination exhibit a protective effect against reproductive toxicity induced by sodium valporate in rats. This study recommends that intake of celery oil combined with vitamin E may be beneficial for patients who suffer from sexual impotency.

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