Effects of camphor on histomorphometric and histochemical parameters of testicular tissue in mice

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Abstract:

BACKGROUND: In traditional medicine of some Asian countries it is believed that camphor could act as a sexual depressant. However, limited studies have been published on this issue. **OBJECTIVES:** In the current study, effects of camphor on testes, sperm and serum factors, and roles of vitamin E as antioxidant in treatment of toxicity of camphor for testes were studied. **METHODS:** Fifty adult male mice (20-25 g) were categorized into five groups. Control group, two control sham groups received olive oil and combined vitamin E and olive oil respectively, and two treatment groups received camphor and combined camphor and vitamin E, respectively. Camphor with doses of 30 mg/kg/day and vitamin E with doses of 100 mg/kg/day were prepared. All substances were administered using gavage. After 35 days, blood was collected from the animal heart for serology and testosterone assessment. Sperms were collected and tissue samples were removed and fixed in Bouin and liquid nitrogen. Paraffin embedded and freezing sections were stained with H&E and specific stain and studied. **RESULTS:** Results showed a significant decrease in sperm count, average proportions of live and mature sperms and major testicular morphometric parameters ($p > 0.05$). Although histochemical changes were seen, no changes were observed in serum testosterone in groups that received camphor. Vitamin E moderated toxicity of camphor in immature sperms, diameter of lumen and TDI index. **CONCLUSIONS:** It can be concluded that camphor includes adverse effects on parameters of testes and sperm quality. Furthermore, vitamin E, as an antioxidant, can moderate toxicity of camphor.

Introduction

Camphor is a solid, greasy and clear white crystal with sharp odor. It is well known in Asian nations. Camphor is derived from Cinnamonom camphor. Nowadays, the industrial type of camphor is synthesized and extensively used in medicine, health and various industries (Yu et al., 2003; Anczewski et al., 2003; Lattanzi et al., 2003). This substance is used as anticipative, odorant, cosmetics, stimulator of blood circulation and respiration, psychological stimulant, libido modulator, anti-conceptive and abortive (Libelt and Shannon, 1993; Reynolds, 1996; Gerald et al., 2002; Liu et al., 2006). Camphor is synthesized from tur-

pentine oil in ointment, lotion and gel forms and used as antibacterial, anti-itching, local analgesic and anti-sunlight (Yu et al., 2003; Anczewski et al., 2003; Lattanzi et al., 2003). Furthermore, it is used in UV-filter oils, sanitation materials, gums, cigarettes, prevention of insect biting and mummification (Chatterjie and Alexander, 1986; Liu et al., 2006).

Evidence indicates adverse effects of camphor on the body. However, there is limited information on effects of camphor on body organs such as the genital system. Camphor can be absorbed through skin, digestive system (5-90 min after consumption) and respiratory system. Overdose of camphor causes toxicity with signs of vision obscurity, vomiting, colitis, vertigo, delirium, heart muscle spasm, hard breathing, paroxysm and death. In one of the recent investigations on the role of camphor in the reproductive system of male mice, camphor (30 mg/kg/day) was shown to make histological changes in testes and induce immature seminiferous tubules (Nikravesh and Jalali, 2004). Studies indicate that organic compounds such as camphor may decrease P450 B1 cytochrome activity. This enzyme interferes with one of the key enzymes in testosterone synthesis, hydroxilase-17 (Barzegari and Mirhosseini, 2012; Mokhtari et al., 2007). Studies have shown that camphor content of UV-filter oil affects gonadotropines and sex hormones and causes impotency in teenagers and atrophy of copulatory organs in both sexes (Durrer et al., 2007; Schlumpf et al., 2004). In one study, researchers have shown that use of camphor ointment includes no effects on gonadotropines (LH and FSH) and testosterone (Janjua et al., 2004). Furthermore, they have reported that camphor content of UV-filter oil causes inhibition of 17b-hydroxysteroid dehydrogenase Type 3 which catalyses the last step of testosterone synthesis (Nashev et al., 2010). Effects of camphor on gene expression of estrogen and estrogen receptor activity have been studied (Maerkel et al., 2007; Heneweer et al.,

2005). In contrast, compounds have been studied with protective effects on genital system. One of these compounds, vitamin E (tocopherol), is a powerful non-enzymatic antioxidant that is involved in lipid peroxidation in cell membrane by limiting action of free radicals; thereby, inhibiting protected cell membranes from induced damage. Vitamin E protects testes and sperms via antioxidant defense system (Ganesh et al., 2012). In traditional medicine in Iran, camphor is used as libido suppressor. A small study has been carried out on the effects of camphor on sperm and testis parameters. Therefore, the aim of the current study was to investigate these effects of camphor on reproductive parameters and the role of vitamin E in treatment of sexual disorders caused by camphor.

Materials and Methods

Fifty adult male mice (balb/c) weighing 20-25 g were randomly selected and equally divided into five groups including one control group, two control sham groups and two treatment groups. They were housed under standard conditions with proper food and water available. Before the trial, animals were accustomed to the environment with 12-h day/night illuminating cycles at 23-25 °C. Since olive oil is reported as the most appropriate solvent for camphor, doses of 30 mg/kg/day of camphor (Henan Xingfa, China) were dissolved in olive oil and prescribed using gavage for 35 days (Budavari et al., 1996). Vitamin E (α-tocopherol) (Zahravi, Iran) was prescribed daily with doses of 100 mg/kg/day via gavage one hour after receiving camphor (Ganesh et al., 2012). Animal groups were categorized as follows: Control group received normal saline (0.3 ml); sham Group 1 received equal volume of olive oil (0.3 ml); sham Group 2 received olive oil (0.3 ml) with vitamin E (100 mg/kg/day); treatment Group 1 received camphor (30 mg/kg/day) dissolved in olive oil (0.3 ml); and

treatment Group 2 received camphor (30 mg/kg/day) dissolved in olive oil (0.3 ml) with vitamin E (100 mg/kg/day) (Nikravesh and Jalali M., 2004; Ganesh et al., 2012).

After 35 days of experiment, animals were weighed and then sacrificed using chloroform. Blood samples were collected from the heart and centrifuged at 4,000 rpm for 10 min to separate serum. Serum samples were assessed for antioxidant activity (AOA) using ferric reducing ability of plasma (FRAP) method, and lipid malonaldehyde (MAD) peroxidation using reaction with thiobarbituric acid method (Kheradmand et al., 2013; Koracevic et al., 2001). Serum testosterone level was assessed using testosterone measurement kit (DRG Instruments, Germany). Volume of testes was quantified using graduated tubule containing water. Left testes were used for histochemical study including alkaline phosphatase (ALP), lipid and periodic acid Schiff (PAS) staining. Right testes were used for histological and morphometric studies. Assessment of sperms included sperm count and motility using hemocytometer slide and sperm viability using eosin-nigrosin staining. Rate of DNA fracture was calculated using acridine-orange staining and nucleus maturation using aniline-blue staining (Rezvanfar et al., 2008; Rezvanfar et al., 2013). Testes were fixed for histological studies using dehydration, clearing, paraffin embedding, blocking, sectioning to 5-7 μm thickness, mounting on slides, and staining processes. Histological studies included capsule, interstitial tissue and seminiferous tubules for appearance, attachment and abnormal features. Histometrical studies of testes included thickness of capsule, height of germinal epithelium of seminiferous tubules, number of Sertoli cells (in each seminiferous tubule), number of Leydig cells, and seminiferous tubule and lumen diameters. Spermatogenesis and spermiogenesis studies included tubular differentiation indices (TDI), spermiogenesis indices (SI) and repopulation indices

(RI) (Kalantari Hesari et al., 2015). Histomorphometrical studies were carried out using Dino-Lite lens digital camera and Dino-capture 2 Software. Frozen specimens in liquid nitrogen were sectioned to 15-20 μm using cryostat at -45 °C. SPSS V.19 Software was used for data analysis. Distribution of data was controlled by K-S test and since distribution of all data was normal, parametric tests were used for data analyses. One way ANOVA test was used to compare two groups and t-test to compare several groups with each other. When necessary, Tukey test was used followed by ANOVA test. Results were shown as average ±SD (standard deviation) with a minimum significance $p > 0.05$.

Results

Body weight and testes volume: No significant difference was observed in average body weight between control, control sham and treatment groups (p=0.61). The maximum body weight (34.75 ±2.46 g) was seen in olive oil receiving group while the minimum body weight was seen in group receiving a combination of camphor and vitamin E (29.25 ±2.84 g). No significant difference was observed in average testis volume between groups (p= 0.66) (Table 1).

Quality of sperm: The average percentage of motile sperms and sperms with intact (normal) DNA included no significant difference (p= 0.22 and p= 0.84, respectively). However, changes were seen in other parameters as follows: total number of sperms showed a significant decline in camphor group (7,250,000 ±11.64) compared to that in control, control sham and treatment groups. A significant difference was observed in control, olive oil and combined camphor and vitamin E groups. No significant difference was seen in combined camphor and vitamin E group (p= 0.6). Other groups showed no significant difference (Table 2). Camphor group showed a significant
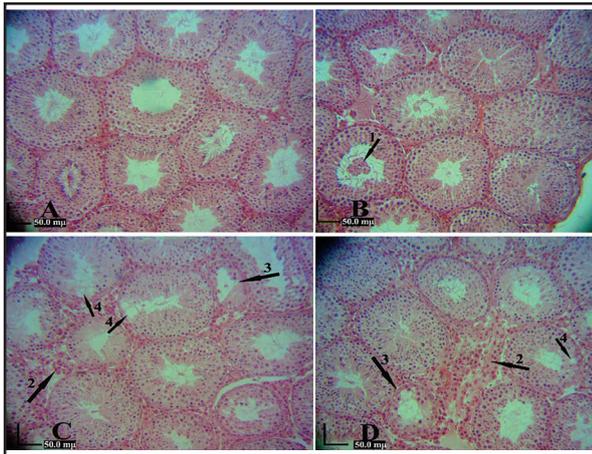
Figure 1. Histology of testes stained by H&E (100×). A, control group; B, olive oil group; C, Camphor group; D, Camphor + Vitamin E group. No. 1, indicates spermatogenesis line cells were separated and poured into the lumen of seminiferous; No. 2, increased of Leydig cells; No. 3, degenerative tubules; No. 4, Vacuoles appearing on spermatogenesis cell lines.
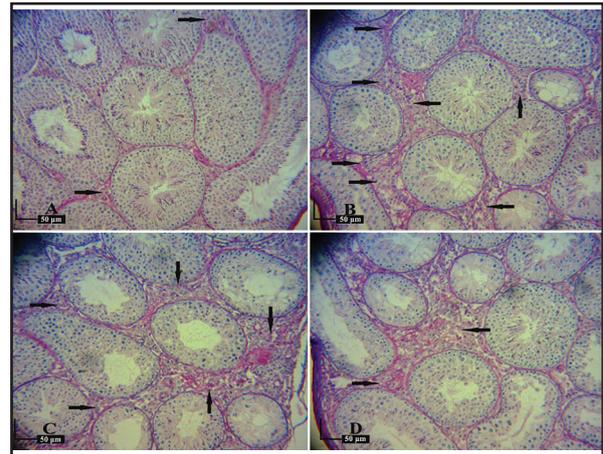

Figure 2. Excessive increase the number of Leydig cells in the groups receiving olive oil (PAS staining) (100×). A: In control group, Leydig cells are normal; B: olive oil group; C and D: Respectively group that received of camphor solution in olive oil and group that received of camphor solution in olive oil + vitamin E, increased the number of Leyding cells, especially near the capsule, in both groups was observed.
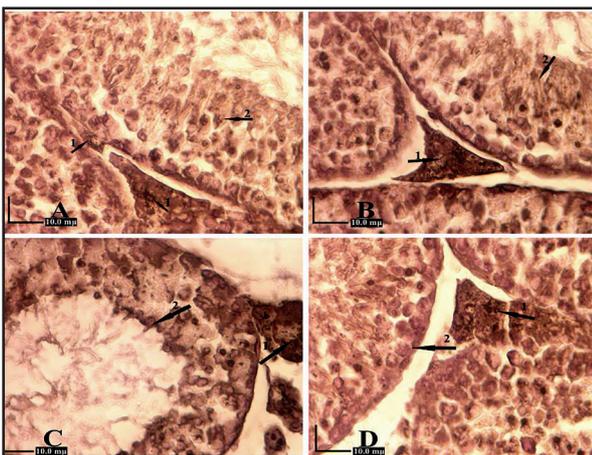

Figure 3. Alkaline phosphatase staining in control, control sham and treatment mice (400×). A, control group slightly reacted with ALP stain; B, olive oil group with a significant increase in the intensity of stain compared to control group; C, camphor group with maximum reaction with ALP stain compared to other groups; D, combined camphor and vitamin E group. Arrows no. 1 indicate ALP particles in Leydig cells; arrows no. 2 indicate ALP particles in spermatogenesis cells.
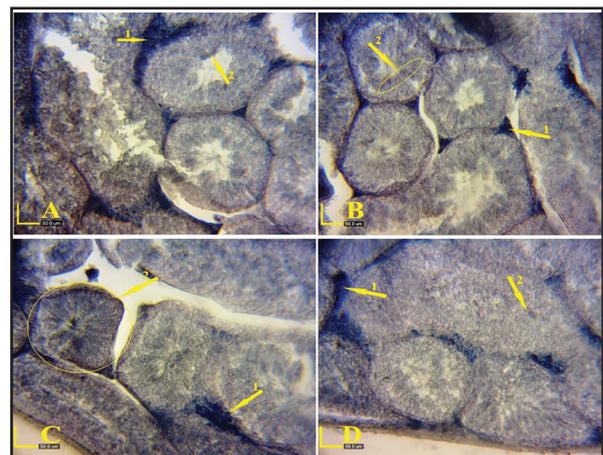

Figure 4. Sudan-black staining in control, control sham and treatment mice (100×). A, control group; B, olive oil group; C, camphor group (the highest accumulation of lipids was observed in cells of seminiferous tubules. Leydig cells were similar to those in sham groups); D, combined camphor and vitamin E group. Arrows no. 1 indicate Sudan black stain in Leydig cells; arrows no. 2 indicate Sudan black stain in spermatogenesis cells.

decrease in motile sperms (34.75% ±2.81). A significant difference was seen in control and olive oil groups. No significant difference was observed in the other groups. (Table 2). A majority of immature sperms were reported in camphor group (28.25% ±2.50), compared to that in control, olive oil, and combined olive oil and vitamin E groups (p<0.05). However, immature sperms showed an insignifi-

cant increase in camphor group, compared to that in combined camphor and vitamin E (p≥ 0.05). Immature sperms showed a significant increase in combined camphor and vitamin E group, compared to that in control, and combined olive oil and vitamin E groups (p< 0.05) (Table 2).

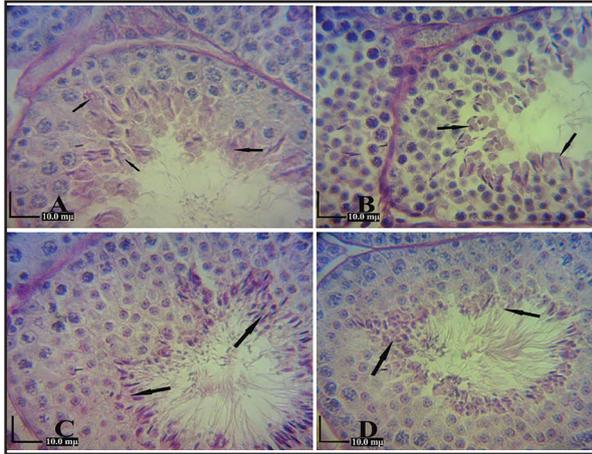**Histological results:** Histological study of testes showed normal structures in control

Figure 5. PAS staining in control, control sham and treatment mice (400×). A, control group; B, olive oil group; C, camphor group; D, combined camphor and vitamin E group.

group, including normal capsule and lack of congestion edema. No degenerated tubules or detached spermatogenic cells were observed (Fig. 1.A). Histological study in olive oil group demonstrated abnormal features including detached spermatogenic cells (spermatocytes and spermatids) inside lumen of seminiferous tubules and significantly increased Leydig cells in interstitial tissue. This increased number of Leydig cells was more obvious near the capsule in all groups, except control group (Fig. 2). Testis capsule was normal; however, degrees of edema and congestion were seen (Fig. 1.B). Furthermore, numerous degenerated tubules were seen in testis tissue sections. In group receiving combined olive oil and vitamin E, tissue sections showed that capsule and other histological structures including spermatogenic cells were normal (Fig. 1.C). Histological study in camphor group showed changes including increased number of degenerated tubules, wider epithelium and therefore a smaller lumen of seminiferous tubules, excessively increased number of Leydig cells in interstitial tissue, and presence of vacuoles in spermatogenic cells. No changes, edema and congestion were observed in tissue of testes (Fig. 1.D). Histological study in combined camphor and vitamin E group showed similar findings but with a lower rate. However, cap-

sule was normal and no edema or congestion was observed (Fig. 1.E).

Morphometric results: In morphometric study, capsule thickness of testes showed a significant increase in combined camphor and vitamin E group, compared to that in control group ($p < 0.05$). Minimum and maximum thicknesses were seen in control group (8.82 ±1.05 µm) and combined camphor and vitamin E (12.7 ±1.21 µm), respectively. No significant difference was seen in capsule thickness of testes in other groups ($p \geq 0.05$) (Table 3). Diameter of seminiferous tubules revealed a significant decrease in olive oil group, compared to that in other groups except control group ($p < 0.05$). The smallest diameter of seminiferous tubules was seen in olive oil group (161.65 ±9.29 µm). No significant difference was observed in diameter of seminiferous tubules in other groups ($p \geq 0.05$) (Table 3). Germinal epithelium thickness showed a significant increase in camphor group, compared to that in olive oil group ($p < 0.05$). No significant difference was seen in germinal epithelium thickness in other groups ($p \geq 0.05$) (Table 3). Internal lumen diameter of seminiferous tubules showed a significant decrease in camphor group, compared to that in olive oil, and combined camphor and vitamin E groups ($p < 0.05$). Maximum internal lumen diameter of seminiferous tubules was seen in control group (12.24 ±7.32 µm) (Table 3). No significant difference was seen in internal lumen diameter of seminiferous tubules in other groups ($p \geq 0.05$) (Table 3). No significant difference was seen in number of Sertoli cells in study groups ($p = 0.06$) (Table 3). Minimum and maximum numbers of Leydig cells were observed in control (14.375 ±1.51 µm), and combined camphor and vitamin E groups (25 ±3.55 µm), respectively, ($p = 0.064$) (Table 3). TDI index showed a significant decrease in camphor group, compared to that in combined olive oil and vitamin E, and combined camphor and vitamin E groups ($p < 0.05$). The lowest TDI index was seen in camphor group

Table 1. Average weight of body and testis volume in control and treatment mice. No significant difference was seen between the groups.

	Control	Olive oil	Olive oil + vitamin E	Camphor	Camphor + vitamin E
Body weight (g)	31 ±2.61	34.75 ±2.46	32 ±0.85	32 ±2.94	29.25 ±2.84
Testis volume	0.07 ±0.01	0.09 ±0.01	0.09 ±0.01	0.08 ±0.01	0.10 ±0.03

Table 2. Sperm parameters in control and treatment groups. Dissimilar letters indicate significant differences between the groups (p< 0.05).

	Control	Olive oil	Olive oil + vitamin E	Camphor	Camphor + vitamin E
Total count of sperm	54375000 ±117.26[a]	52750000 ±151.90[a]	60375000 ±14.77[a]	7250000 ±11.64[b]	20375000 ±10.08[ab]
Sperm viability (%)	53 ±2.74[a]	39.75 ±3.71[a]	51.25 ±4.42[ab]	34.75 ±2.81[b]	47.25 ±6.44[ab]
Sperm motility (%)	84.5 ±3.62	82.25 ±2.25	76.50 ±1.76	67.25 ±5.50	77.75 ±7.61
Sperm with intact DNA (%)	99.25 ±0.48	99 ±0.58	98.50 ±0.65	99 ±0.71	99.5 ±0.29
Immature sperm (%)	9.5 ±1.55[a]	13.5 ±1.19[ac]	11.75 ±1.80[a]	28.25 ±2.50[b]	19.50 ±1.32[c]

(79% ±071), compared to that in other groups (Table 3). RI index showed a significant decrease in olive oil group (64% ±1.25), compared to that in other groups except control group (p< 0.05). No significant difference was seen in RI index in other groups (p≥ 0.05) (Table 3). SI index showed a significant decrease in olive oil group, compared to that in camphor group (p< 0.05). Maximum SI index was seen in camphor group (89.25% ±0.73). No significant difference was observed in SI index in other groups (p≥ 0.05) (Table 3). Assessment of degenerated tubules showed a significant increase in camphor, and combined camphor and vitamin E groups, compared to that in control group (p< 0.05). Minimum and maximum numbers of degenerated tubules were seen in control group (1% ±0.27), and combined camphor and vitamin E (15.5% ±3.51), respectively. No significant difference was seen in number of degenerated tubules in other groups (p≥ 0.05) (Table 3).

Histochemical results: Results of testicular tissue section staining using ALP have revealed the highest rate of small brown particles in cytoplasm of Leydig and spermatogenic cells in camphor group, compared to that in other groups. Furthermore, ALP staining results showed a similar finding in olive oil group but with a lower rate. A higher density was seen in combined camphor and vitamin E group than control group (Fig. 3, Table 4). Cytoplasm of testicular cells included black particles with lipid contents using Sudan-black staining. These black particles were mostly detected in cells closed to lumen. Furthermore, Sudan-black staining showed a significant increase in lipids in olive oil, combined olive oil and vitamin E, combined camphor and vitamin E, and camphor groups, compared to that in control group. A majority of black particles was seen in spermatogenic cells in camphor group and then in combined olive oil and camphor, and vitamin E groups, respectively (Fig. 4, Table 4). PAS staining findings have revealed normal in all groups capsule. PAS positive particles were mostly detected in cytoplasm of spermatogenic cells, specifically in spermatids. PAS positive particles were mostly observed in groups with the thickest epithelium, compared to other groups. No other significant differences were reported (Fig. 5, Table 4).

Serum tests: In the present study, antiox-

Table 3. histomorphometrical and serum parameters in control and treatment groups. Dissimilar letters indicate significant differences between the groups (p< 0.05).

	Control	Olive oil	Olive oil + vitamin E	Camphor	Camphor + vitamin E
Testicular capsule thickness (μ)	8.82 ±1.04[a]	12.70 ±1.20[ab]	18.37 ±3.51[ab]	14.14 ±2.16[ab]	21.22 ±11.00[b]
Diameter of Seminiferous tubules (μ)	194.92 ±4.86[ab]	161.65 ±9.28[b]	219.81 ±9.07[a]	221.08 ±7.93[a]	215.93 ±10.19[a]
Thickness of the seminiferous tubular epithelium (μ)	65.77 ±4.55[ab]	64.19 ±4.32a	66.67 ±4.06[ab]	75.73 ±3.35[b]	72.09 ±3.48[ab]
Diameter of seminiferous tubules lumen (μ)	112.24 ±7.30[ab]	84.09 ±6.51[a]	98.54 ±5.26[ab]	115.85 ±11.13[b]	83.69 ±4.17[a]
Number of Sertoli (n)	18.12 ±1.09	15.25 ±0.67	15.00 ±1.10	15.50 ±1.22	18.87 ±1.46
Number of Leydig cells (n)	14.37 ±1.51	16.12 ±3.02	15.25 ±1.38	20.37 ±3.76	25.00 ±3.54
TDI (%)	82.50 ±1.61[ab]	81.25 ±1.39[a]	86.00 ±1.03[a]	79.00 ±0.70[b]	84.00 ±1.16[a]
RI (%)	69.50 ±0.82[ab]	64.00 ±1.25[b]	74.50 ±1.89[a]	73.75 ±1.39[a]	73.25 ±3.03[a]
SI (%)	79.50 ±1.93[ab]	80.50 ±1.61[a]	86.00 ±1.10[ab]	89.25 ±0.73[b]	82.00 ±3.02[ab]
Degenerative tubules (%)	1.00 ±0.26[a]	12.05 ±4.01[ab]	6.00 ±0.88[ab]	13.25 ±3.34[b]	15.50 ±3.51[b]
Antioxidant activity of serum or AOA (μmol/l)	0.813 ±0.018[a]	0.809 ±0.027[a]	0.931 ±0.013[b]	0.791 ±0.005[a]	0.837 ±0.014[a]
Malondialdehyde concentration of serum or MDA (TBARS μmol/ml)	0.295 ±0.003	0.275 ±0.007	0.299 ±0.002	0.318 ±0.004	0.314 ±0.010
Testosterone level of serum (ng/ml)	4.52 ±1.14	7.62 ±3.61	5.87 ±0.31	3.01 ±0.99	2.75 ±1.10

Table 4. Qualitative assessment of alkaline phosphatase and Sudan-black stained testicular tissues in control, control sham and treatment mice. Nos. 1-5 were used to show minimum and maximum levels of staining for alkaline phosphatase and Sudan-black staining, respectively. Other groups are shown by the stain levels 1-5.

	Control	Olive oil	Olive oil + vitamin E	Camphor	Camphor + vitamin E
Alkaline phosphatase staining	1+	3+	2+	5+	4+
Sudan-black staining	1+	4+	2+	5+	3+

idant activity of plasma was assessed using AOA method. The highest antioxidant activity was seen in combined olive oil and vitamin E group (0.931 ±0.01 mmol/l). AOA showed a significant increase in combined olive oil and vitamin E group, compared to that in other groups (p< 0.05). No significant difference was seen in other groups (p≥ 0.05) (Table 3). No significant difference was seen in lipid peroxidation in groups (p= 0.06). Minimum and maximum values of MDA were seen in control (0.295 ±0.01 μmol/l) and camphor groups (0.308 ±0.01 μmol/l), respectively (Table 3). The highest and smallest values of testosterone were seen in olive oil (7.6250 ±3.693 ng/ml) and combined camphor and vitamin E groups (2.7500 ±1.108 ng/ml), respectively. No significance was observed in serum testosterone in groups (p= 0.30) (Table 3).

Discussion

In Asian and Islamic traditional medicine, it is believed that camphor can suppress sexual excitation, especially in males. Different and sometimes contradictory reports have been published on effects of camphor on genital system (Jamshidzadeh et al., 2006; Nikravesh and Jalali, 2004; Shahabi et al., 2013). Since little information has been published on the effects of camphor, the current study included quality of sperms, histological and histochemical studies, biochemistry tests and assessment of serum testosterone in mice exposed to camphor. Furthermore, protective role of vitamin E in camphor toxicity for testis was investigated. Hydroxylation of carbons nos. 5 and 8 (or 9) results in camphor hydroxy which con-

sequently produces ketones and carbon dioxide. This 7-carbon dioxide can conjugate glucoronic acid (Koppel et al., 1982). Glucoronic acid plays three important roles in the body: 1) Detoxification through conjugation and elimination of toxicants, 2) Transportation of hormones and other important substances by combining with and releasing materials into target tissues, and 3) Synthesis of ascorbic acid (except in primates and guinea pigs). Camphor can prevent glucoronic acid activity by connecting to it (Rabl et al., 1997). Toxic effects of camphor reported in the current study can be associated to decreased glucoronic acid and consequently reduced detoxification capacity of the body (Rabl et al., 1997). For example, increased number of degenerated seminiferous tubules can be a consequence of released toxicants produced through camphor metabolism or due to the failure of body in detoxification of other toxicants (Rabl et al., 1997). Moreover, 4-Methyl-benzylidene camphor (4-MBC), an indicator of estrogenic activity, acts as a preferential ligand of estrogen receptor and includes a direct role in differentiation of sex organs and brain in both sexes (Durrer et al., 2007). The 4-MBC affects endocrine glands which are the locations for synthesis of various hormones including sex hormones (Saleha, 2009). Camphor is suggested as a disorganizer of endocrine glands and an agonist for estrogen hormone (Caserta et al., 2008).

Spermatogenesis is a complicated process and depends on function of endocrine and paracrine hormones and interaction between spermatogenic and Sertoli cells. Studies have shown that in addition to principal regulatory hormones of spermatogenesis such as testosterone, LH and FSH, 17-β-estradiol includes an important role in regulation of reproduction in males, as absence of estrogenic receptors in mice causes termination of spermatogenesis and induces infertility (Hess, 2003). Therefore, some changes following exposure to camphor such as decreased sperm number, increased im-

mature sperms, increased epithelial thickness of seminiferous tubules and failure to release espermatids from germinal epithelium in treatment groups seem to occur due to disordered hormone balance in the body. Decreased inner diameter of seminiferous tubules has been observed in studies on animals receiving camphor; therefore, spermatogenesis cells are suggested to be poorly differentiated (Goel et al., 1985; Leuschner, 1997; Nikravesh and Jalali, 2004). This makes two reactions occur; first, wall thickness of seminiferous tubules increases and hence their inner diameter decreases and second, released cells into lumen decrease (Wing and Christensen, 1982). Results of the current study have revealed increased seminiferous epithelium thickness, in contrast to reports by other researchers. SI index showed an increased number of tubules containing spermatids in groups that received camphor. This might be due to the failure of seminiferous tubules to release spermatids from its germinal epithelium, as shown in previous reports (Jadhav et al., 2010; Nikravesh and Jalali, 2004). In a study by Jadhav et al. in 2010, effects of camphor on motility and viability of human sperms were investigated (Jadhav et al., 2010). Results showed that camphor could play a role in prevention of pregnancy through decreasing motility and viability of sperms. In the current study, sperms decreased in groups receiving camphor; similar to those in a previous study (Jadhav et al., 2010). Furthermore, vitamin E was found to prevent camphor negative effects on sperms, to some extent. The proportion of live sperms was also decreased. Decrease in sperms can be explained by increased degenerated tubules and decreased TDI. Release of immature germ cells into lumen was seen in the current study, as reported in another study (Pereira et al., 2012).

Effects of camphor on sexual parameters may be associated to mediators affecting sympathetic system. Camphor has been shown to inhibit secretion of catecholamines through

blocking nicotin-acetylcholin receptors (Park et al., 2001). Role of sympathetic and parasympathetic systems on sexual parameters in males and inhibitory effect of camphor on secretion of catecholamines support the hypothesis that camphor can change sexual parameters including quality and quantity of sperms (Janjua et al., 2004). In a study by Shahabi et al. (2013) on the effects of camphor on sexual hormones, no changes in testosterone level were seen. However, an increase in LH and a decrease in FSH were observed in groups receiving camphor (Shahabi et al., 2013). In the current study, no significant differences were seen in serum testosterone within the groups. Furthermore, camphor has been shown to include no mutant effects (Gomes-Carneiro et al., 1998; Knezevic-Vukcevic et al., 2006). No significant differences were seen in number of sperms with defective DNA.

Olive oil is rich in phytoestrogens which contain phenol compounds (Owen et al., 2000; Carrion, 2010). Several studies have shown that these compounds can decrease serum testosterone, number and motility of sperms and structural changes in main and accessory genital glands (Weber et al., 2001; Roberts et al., 2000; Najafizadeh et al., 2013). In the current study, obvious changes were seen in morphometric, histologic and histochemical characteristics in groups that received olive oil. Major changes indicated unfavorable effects of olive oil on testis and sperm parameters. In histological studies, detached spermatogenic cells inside luminal space of seminiferous tubules, increased degenerated tubules, changes in diameter, decreased RI, increased reaction to alkaline phosphatase and Sudan black staining were observed in groups which used olive oil. In summary, although the precise mechanism of camphor is not well known, continuous administration of camphor has been shown to cause histological changes in testis and spermatogenesis. Furthermore, vitamin E as an antioxidant, can slightly moderate toxicity of camphor in immature sperms, diameter of seminiferous tubule lumen and TDI indices. Further studies are needed to describe the effects of camphor on reproductive system.

Acknowledgments

References

1. Anczewski, W., Dodzuik, H., Ejchart, A. (2003) Manifestation of chiral recognition of camphor enantiomers by alphacyclodextrin in longitudinal and transverse relaxation rates of the corresponding 1:2 complexes anddetermination of the orientation of the guest inside the host capsule. Chirality. 15: 654-659.
2. Barzegari, F., Mirhosseini, M. (2012) Effect of persian hogweed (*Heracleum persicum*) on the morphological changes in mice testis and the level of hormone testostrone. Razi J Med Sci. 19: 18-24.
3. Budavari, S., O'Neil, M.J., Smith, A., Heckelman, P. E., Kinneary, J.F. (1996) The Merck index, an encyclopedia of chemicals, drugs, and biologicals. (12th ed.) Merck & Co. New Jersey, USA.
4. Carrion, Y., Ntinou, M., Badal, E., Olea europaea, L. (2010) In the north mediterranean basin during the pleniglacial and the early-middle Holocene. Quat Sci Rev. 29: 952-968.
5. Caserta, D., Maranghi, L., Mantovani, A., Marci, R., Maranghi, F., Moscarini, M. (2008) Impact of endocrine disruptor chemicals in gynaecology. Hum Reprod Update. 14: 59-72.
6. Chatterjie, N., Alexander, G.J. (1986) Anticonvulsant properties of spirohydantoins derived from optical isomers of camphor. Neurochem Res. 11: 1669-1676.
7. Durrer, S., Ehnes, C., Fuetsch, M., Maerkel, K., Schlumpf, M., Lichtensteiger, W. (2007) Estrogen sensitivity of target genes and expression of nuclear receptor co-regulators in rat prostate after pre-and postnatal exposure to the ultraviolet filter 4-methylbenzylidene camphor. Environ Health Perspect. 115: 42-50.

8. Ganesh, E., Chowdhury, A., Malarvani, T., Ashok-vardhan, N. (2012) Hepatoprotective effect of Vitamin- E & C in Albino rats. Int J Adv Lif Sci. 3: 21-26.

9. Gerald, G.B., Roger, K.F., Sumner, J.Y. (2002) Drugs in Pregnancy and Lactation. (6th ed.) Lippincott Williams and Wilkins. Philadelphia, USA.

10. Goel, H.C., Singh, S., Adhikari, J.S., Rao, A.R. (1985) Radiomodifying effect of camphor on the spermatogonia of mice. Jpn J Exp Med. 55: 219-223.

11. Gomes-Carneiro, M.R., Elzenszwalb, I.F., Paumgartten, F.J. (1998) Mutagenicity testing (+/-)-camphor, 1, 8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay. Mutat Res Genet Toxicol Environ Mutagen. 416: 129-136.

12. Heneweer, M., Muusse, M., van-den-Berg, M., Sanderson, J.T. (2005) Additive estrogenic effects of mixtures of frequently used UV filters on pS2-gene transcription in MCF-7 cells. Toxicol Appl Pharm. 208: 170-177.

13. Hess, RA. (2003) Estrogen in the adult male reproductive tract: a review. Reprod Biol Endocrinol. 1: 1-14.

14. Jadhav, M.V., Sharma, R.C., Rathore, M., Gangawane, A.K. (2010) Effect of *Cinnamomum camphora* on human sperm motility and sperm viability. J Clin Res lett. 1: 1-10.

15. Jamshidzadeh, A., Sajedianfardb, J., Nekooeianc A.K., avakolia, F., Omranid, G.H. (2006) Effects of Camphor on Sexual Behaviors in Male Rats. Iran Journal of Pharmaceutical Sciences. 2: 209-214.

16. Janjua, N.R., Mogensen, B., Andersson, A.M., Petersen, J.H., Henriksen, M., Skakkebaek, N.E., Wulf, H.C. (2004) Systemic absorption of the sunscreens benzophenone-3, octyl-methoxycinnamate, and 3-(4-methyl-benzylidene) camphor after whole-body topical application and reproductive hormone levels in humans. J Invest Dermatol. 123: 57-61.

17. Kalantari Hesari, A., Shahrooz, R., Ahmadi, A., Malekinejad, H., Saboory, E. (2015) Cro-

cin prevention of anemia-induced changes in structural and functional parameters of mice testes. J Appl Biomed. 53: 213-223.

18. Kheradmand, A., Alirezaei, M., Dezfoulian, O. (2013) Cadmium-Induced Oxidative Stress in the Rat Testes: Protective Effects of Betaine. Int J Pept Res Ther. 19: 337-344.

19. Koracevic, D., Koracevic, G., Djordjevic, V, Andrejevic, S., Cosic, V. (2001) Method for the measurement of anti oxidant activity in human fluids. J Clin Pathol. 54: 356-361.

20. Knezevic-Vukcevic, J., Vukovic-Gacic, B., Stevic, T., Stanojevic, J., Nikolic, B. Simic, D. (2006) Antimutagenic effect of essential oil of sage (*Salvia officinalis* L.) and its fractions against uv-induced mutations in bacterial and yeast cells. Arch Biol Sci. 57: 163-172.

21. Koppel, C., Tenczer, J., Schirop, T., Ibe, K. (1982) Camphor poisoning - abuse of camphor as a stimulant. Arch Toxicol. 51: 101-106.

22. Lattanzi, A., Iannece, P., Vicinanza, A., Scettri, A. (2003) Renewable camphor-derived hydroperoxide: synthesis and use in the asymmetric epoxidation of allylic alcohols. Chem Commun. 12: 1440-1441.

23. Leuschner, J. (1997) Reproduction toxicity studies of D-camphor in rats and rabbits. Arzneimittel- Forschung. 47: 124-128.

24. Libelt, E.L., Shannon, M.W. (1993) Small doses, big problems: a selected review of highly toxic common medications. Pediatr Emerg Care. 9: 292-297.

25. Liu, C.H., Mishra, A.K., Tan, R.X., Tang, C., Yang, H., Shen, Y.F. (2006) Repellent and insecticidal activities of essential oils from Artemisia princeps and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean. Bioresour Technol. 97: 1969-1973.

26. Maerkel, K., Durrer, S., Henseler, M., Schlumpf, M., Lichtensteiger, W. (2007) Sexually dimorphic gene regulation in brain as a target for endocrine disrupters: Developmental exposure of rats to 4-methylbenzylidene camphor. Toxicol Appl Pharm. 218: 152-165.

27. Mokhtari, M., Sharifi, E., Moghadamnia, D.

(2007) Effect of alcoholic extract of phoenix dactylifera spathe on histological change in testis and concentrations of LH, FSH and testosterone in male rat. IJBMS. 9: 265-271.

28. Najafizadeh, P., Dehghani, F., Panjeh-Shahin, M.R., Hamzei-Taj, S. (2013) The effect of a hydro-alcoholic extract of olive fruit on reproductive argons in male sprague-dawley rat. Iran J Reprod Med. 11: 293-300.

29. Nashev, L.G., Schuster, D., Laggner, C., Sodha, S., Langer, T., Wolber, G., Odermatt, A. (2010) The UV-filter benzophenone-1 inhibits 17beta-hydroxysteroid dehydrogenase type 3: Virtual screening as a strategy to identify potential endocrine disrupting chemicals. Biochem Pharmacol. 79: 1189-1199.

30. Nikravesh, M.R., Jalali, M. (2004) The effect of camphor on the male mice reproductive system. Urol J. 4: 268-272.

31. Owen, R., Mier, W., Giacosa, A., Hull, W., Spiegelhalder, B., Bartsch, H. (2000) Identification of lignans as major components in the phenolic fraction of olive oil. Clin Chem. 46: 976-988.

32. Park, T.J., Seo, H.K., Kang, B.J., Kim, K.T. (2001) Noncompetitive inhibition by camphor of nicotinic acetylcholine receptors. Biochem Pharmacol. 61: 787-793.

33. Rabl, W., Katzgraber, F., Steinlechner, M. (1997) Camphor ingestion for abortion (case report). Forensic Sci Int. 89: 137-140.

34. Pereira, M.D.L., Rodrigues, N.V., Costa, F.G. (2012) Histomorphological evaluation of mice testis after co-exposure to lead and cadmium. Asian Pacific J Reprod. 1: 34-37.

35. Reynolds, J.E.F. (1996) Martindale, the extra pharmacopeia. (31st ed.) Royal pharmaceutical society. London, Uk.

36. Rezvanfar, M., Sadrkhanlou, R., Ahmadi, A., Shojaei-Sadee, H., Rezvanfar, M., Mohammadirad, A., Salehnia, A., Abdollahi, M. (2008) Protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source; evidence for role of free-radical

toxic stress. Hum Exp Toxicol. 27: 901-910.

37. Rezvanfar, M., Shahverdi, A.R., Ahmadi, A., Baeeri, M., Mohammadirad, A., Abdollahi, M. (2013) Protection of cisplatin-induced spermatotoxicity, DNA damage and chromatin abnormality by selenium nano-particles. Toxicol Appl Pharmacol. 266: 356-365.

38. Roberts, D., Veeramachaneni, D.R., Schlaff, W.D., Awoniyi, C.A. (2000) Effects of chronic dietary exposure to genistein, a phytoestrogen, during various stages of development on reproductive hormones and spermatogenesis in rats. Endocrine. 13: 281-286.

39. Saleha, Y.M.A. (2009) Evaluation of camphor mutagenicity in somatic cells of pregnant rats. Asian J Biotechnol. 1: 111-117.

40. Schlumpf. M., Schmid, P., Durrer, S., Conscience, M., Maerkel, K., Henseler, M., Gruetter, M., Herzog, I., Reolon, S., Ceccatelli, R., Faass, O., Stutz, E., Jarry, H., Wuttke, W., Lichtensteiger, W. (2004) Endocrine activity and developmental toxicity of cosmetic UV filters--an update. Toxicology. 205: 113-122.

41. Shahabi, S., Jorsaraei, S.G., Moghadamnia, A., Barghi, E., Zabihi, E., Golsorkhtabar-Amiri, M., Maliji, G., Sohan-Faraji, A., Abdi-Boora, M., Ghazinejad, N., Shamsai, H. (2013) The effect of camphor on sex hormones levels in rats. Cell J (Yakhteh). 16: 231-234.

42. Weber, K., Setchell, K., Stocco, D., Lephart, E. (2001) Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. J Endocrinol. 170: 591-599.

43. Wing, T.Y., Christensen, A.K. (1982) Morphometric studies on rat seminiferous tubules. Am J Anat. 165: 13-25.

44. Yu, S.C., Bochot, A., Bas, G.L., Chéron, M., Mahuteau, J., Grossiord, J.L., Seiller, M., Duchêne, D. (2003) Effect of camphor/cyclodextrin complexation on the stability of O/W/O multiple emulsions. Int J Pharm. 261: 1-8.

مطالعه هیستومورفومتری و هیستوشیمی تغییرات بیضه موش متعاقب تجویز کافور و بررسی نقش محافظتی ویتامین E

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چکیده

زمینه مطالعه: در طب سنتی برخی از کشورهای آسیایی این تصور وجود دارد که کافور می‌تواند در کاهش میل جنسی افراد نقش داشته باشد. با این حال مطالعات در این زمینه نیز بسیار محدود می‌باشد. **هدف:** از این رو در تحقیق حاضر علاوه بر بررسی دقیق‌تر اثرات کافور بر پارامترهای بیضه، اسپرم و فاکتورهای سرمی، نقش ویتامین E به عنوان یک آنتی‌اکسیدانت در درمان عوارض ناشی از کافور بررسی شد. **روش کار:** در این تحقیق از ۵۰ قطعه موش سوری نر بالغ (balb/c) ۲۰ تا ۲۵ گرمی در ۵ گروه استفاده شد. گروه اول کنترل، دریافت کننده سرم فیزیولوژی (CO)، دو گروه کنترل شم، به ترتیب دریافت کننده روغن زیتون تنها (OL) و گروه دریافت کننده ترکیبی از ویتامین E و روغن زیتون (OL+E)، و نهایتاً دو گروه تجربی دریافت کننده کافور تنها (CA) و گروه دریافت کننده کافور به همراه ویتامین E (CA+E). کافور با دوز ۳۰ mg/kg و ویتامین E نیز با دوز ۱۰۰mg/kg تهیه گردید. تمام مواد به صورت خوراکی (گاواژ) و دوره ۳۵ روزه تجویز شدند. پس از اتمام دوره درمان از طریق قلب خونگیری انجام و نمونه‌های سرمی جهت آزمایشات سرمی و تعیین سطح تستوسترون، نمونه‌های اسپرم برای بررسی اسپرم و نمونه‌های بافتی نیز پس از جدا سازی در داخل فرمالین و ازت مایع فیکس شده و پس از انجام برش‌های پارافینی و انجمادی در نهایت با رنگ آمیزی‌های معمولی و اختصاصی مورد بررسی قرار گرفتند. **نتایج:** نتایج نشان دهنده کاهش معنی‌دار تعداد اسپرم، درصد اسپرم‌های زنده و بالغ، کاهش اکثر پارامترهای مورفومتریک بیضه، تغییرات هیستوشیمی و عدم تغییر سطح هورمون تستوسترون سرمی در گروه دریافت کننده کافور بود در حالی که ویتامین E به عنوان یک آنتی اکسیدانت بصورت خفیفی توانسته بود اثرات کافور را کاهش دهد (۰/۰۵>p). **نتیجه گیری نهایی:** می‌توان نتیجه گرفت که کافور بر پارامترهای کیفیت اسپرم و بیضه در موش سوری تأثیر داشته و ویتامین E به عنوان یک آنتی اکسیدانت، قادر به تعدیل عوارض ناشی از دریافت کافور در رابطه با پارامترهای مذکور می‌باشد.

واژه‌های کلیدی: کافور، هیستوشیمی، موش سوری، بیضه، ویتامین E

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