First International Journal of Andrology
ANDROLOGIA

ORIGINAL ARTICLE

Rose oil inhalation protects against formaldehyde-induced testicular damage in rats

E. Köse[1], M. Sarsılmaz[2], U. Taş[3], A. Kavaklı[2], G. Türk[4], D. Özlem Dabak[5], H. Sapmaz[1] & M. Ögetürk[2]

1 Department of Anatomy, Faculty of Medicine, İnönü University, Malatya, Turkey;
2 Department of Anatomy, Faculty of Medicine, Fırat University, Elazığ, Turkey;
3 Department of Anatomy, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey;
4 Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Fırat University, Elazığ, Turkey;
5 Department of Histology-Embriology, Faculty of Medicine, Fırat University, Elazığ, Turkey

Keywords
Formaldehyde—rat—rose oil—sperm—testes

Correspondence
Asst. Prof. Dr Evren Köse, İnonu University Medical Faculty, Department of Anatomy, 44280 Malatya, Türkey
Tel.: +90 422 341 06 60 (1272);
Fax: +90 422 341 00 36;
E-mail: ekose@inonu.edu.tr

Accepted: January 24, 2011

doi: 10.1111/j.1439-0272.2011.01187.x

Summary

In this experimental study, harmful effects of formaldehyde (FA) inhalation on sperm concentration, sperm quality, serum testosterone levels and the rat testes were investigated. In addition, the possible protective effects of rose oil against to these harmful effects were evaluated. For this purpose, 21 albino-Wistar rats were used. The rats in Group I were used as control group. When the rats of Group II were exposed FA (10 ppm/1 h) for 35 days, the rats of Group III inhalated rose oil (1 ml/1 h) after FA. The epididymal tissues were taken for sperm analysing and the testes were removed for histological examination. In addition, testosterone levels were determined from the blood samples. Although the testosterone levels, the epididymal sperm concentration, and the progressive sperm motility significantly decreased, the abnormal sperm rate significantly increased in the Group II when compared to Group I. In the Group III, these damages were seen less. When the rats in the Group II compared with the control group, there were serious histological damages. In the Group III, it was determined that the histological changes were less than group II. It can be expressed that serious damages occurred via formaldehyde exposure in male reproductive system and that the rose oil had protective effects against these damages.

Introduction

Formaldehyde (FA) is a pungent, irritant, and colourless gas. It is a member of aldehyde family and found in nature in domestic air, cigarette smoke, and the polluted atmosphere of cities due to the incomplete combustion of organics, photochemical smog, and release from FA containing products. FA has a strong tendency to combine with protein, DNA, RNA which leads to harmful effects (Heck & Casanova, 1999; Usanmaz *et al.*, 2002). Use of FA in anatomy, histology, and pathology laboratories has been increasing. In addition, FA is used in the production of industrial and cosmetic products. Therefore, exposure to FA by inhalation is possible at any time (Khanzadeh *et al.*, 1994; Cohen *et al.*, 1998).

FA has been shown to have harmful effects on the central nervous, respiratory, and digestive systems (Kriebel *et al.*, 2001; Sarsilmaz *et al.*, 2007). It also affects the reproductive system and causes infertility (Taskinen *et al.*, 1994; Collins *et al.*, 2001). Various studies have shown that FA causes testicular damage and decreases the testosterone levels as well as sperm concentration and quality (Majumder & Kumar, 1995; Odeigah, 1997; Sarsilmaz & Ozen, 2000).

Aromatherapy is the therapeutic use of essential oils. Essential oils are defined as volatile parts of aromatic plants extracted by steam distillation or expression. Rose oil, which is often used in aromatherapy, is extracted from rose flowers by steam distillation. Nearly 4000 kg of rose are used to produce 1 kg of rose oil. Rose oil is a

limpid, light yellow, and volatile oil. Contents of five major constituents of the oil are: citronellol, geraniol, nerol, linalool, and phenylethyl alcohol (Ergen *et al.*, 2003; Kurkcuoglu & Baser, 2003).

In traditional medicine, rose oil is used for infections, anxiety, skin care, stomach and chest aches, and menstrual and digestive disorders (Basim & Basim, 2003; Boskabady *et al.*, 2006; Altıntaş, 2007). Experimental studies have revealed that rose oil has antibacterial (Basim & Basim, 2003), anti-HIV (Mahmood *et al.*, 1996), anxiolytic (Almeida *et al.*, 2004; Bradley *et al.*, 2007), anti-inflammatory, analgesic, hypnotic, anti-spasmodic, antitussive (Boskabady *et al.*, 2006), and antioxidant (Wei & Shibamoto, 2007) effects. In addition, rose oil improves learning and memory (Kose *et al.*, 2007).

Therefore, this study aimed to find the effects of rose oil inhalation on testicular damage caused by formaldehyde inhalation.

Materials and methods

Adult male Wistar rats (weighing 310–320 g, $n = 21$) were used for the study material. All procedures were approved by the Institutional Animal Care and Use Committee of the Medical School, Fırat University, Turkey. The animals were divided into three groups. The rats in Group I ($n = 7$) were used as the controls. While the rats in Group II were exposed to FA (10 ppm/1 h – formalin, Sigma-Aldrich Formaldehyde 37% solution, Deisenhofen, Germany) for 35 days, the rats in Group III inhaled rose oil (1 ml/1 h – Gülbirlik, Isparta, Turkey) after inhaled FA like in Group II. The rats in the Group II exposed the FA in a $100 \times 50 \times 20$ cm sized glass vase in the mornings for an hour. The FA dropped to cotton pad and concentration of the FA was measured by a formaldehydemeter (Enviromental Sensors Co., Boca Raton, FL, USA). The rats of Group III inhaled the rose oil in a different glass vase for an hour. The rose oil dropped a cotton pad. After this inhalation they exposed to FA like Group II. The dosage of rose oil was managed according to Komiya *et al.* (2006). Both the two inhalation were performed in a different room.

At the end of the experiment, the epididymis tissues were taken for sperm analysis and testes were removed for histological examination; testosterone levels were determined using the blood samples of the animals.

Histological studies

The testicular tissue specimens were fixed in Bouin's solution. Tissue specimens were embedded in paraffin wax and sectioned (5 μm). For light microscopic evaluation, paraffin sections were stained with haematoxylin–eosin (H&E) and Mason tricrom and examined with an Olympus BH2 light microscope.

In this investigation, the diameters of seminipherous tubule, the number of Leydig cells, and the number of Leydig cells with damaged nucleus (pyknosis, karyolysis, karyorrhexis) were determined. Large magnification ($40 \times$) was used and 100 interstitial spaces were calculated for Leydig cells count. The diameters of tubules were measured by ocular micrometer attached to the microscope.

Determination of testosterone levels

Plasma was stored at −20 °C for analysis. The plasma testosterone level was assayed using Coat-a-Count Radioimmunoassay kit (Active Testosterone RIA DSL-4000; Diagnostic System Laboratories Inc., Webster, TX, USA) and expressed as ng ml^{-1}.

Determination of epididymal sperm concentration

The epididymal sperm concentration was determined with a haemocytometer (Improved Neubauer, Weber, UK) using a modification of the haemoctometric method described by Turk *et al.* (2007) and Sonmez *et al.* (2007). Briefly, the right epididymis was finely minced using anatomical scissors in 1 ml of physiological saline (NaCl, 0.9%) in a Petri dish. It was completely squashed with tweezers for 2 min. Then, it was incubated at room temperature for 5 min to provide the migration of all spermatozoa from epididymal tissue to the fluid. After incubation, the epididymal tissue–fluid mixture was filtered via a strainer to separate the supernatant from tissue particles. The supernatant fluid was drawn into the capillary tube up to 0.5 line of the pipette designed for counting red blood cells. The solution containing 5 g sodium bicarbonate, 1mL formalin (35%, v/v) and 25 mg eosin per 100 ml distilled water were pulled up to 101 lines of the pipette. Approximately 10 ml of the diluted sperm suspension was transferred to counting chambers of hemocytometer and allowed to stand for 5 min. The sperm cells in both chambers were counted with the help of light microscope at the magnification of $200 \times$.

Determination of epididymal sperm motility

The percentage of progressive sperm motility was evaluated using a light microscope with heater table as described by Sonmez *et al.* (2005). For this process, a slide was placed on microscope and allowed to warm to a temperature of 35 °C on a heating table. Several droplets of Tris buffer solution [Tris (hydroxymethyl)

aminomethane 3.63 g, glucose 0.50 g, citric acid 1.99 g, and distilled water 100 ml] were dropped on the slide and a very small droplet of fluid obtained from the left cauda of epididymis with a pipette was added on this solution and mixed with a cover-slip. The percentage of progressive sperm motility was visually evaluated using a score ranging from 0% to 100% under magnification.

Determination of percentage of abnormal spermatozoa

Percentage of abnormal spermatozoa was determined by the method described by Turk *et al.* (2007). To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin-nigrosin (1.67 g eosin, 10 g nigrosin, and 2.9 g sodium citrate per 100 ml distilled water) were prepared. After preparation, the slides were viewed under a light microscope at 400× magnification. For each animal, 300 sperm cells were examined on each slide.

Statistical analysis

All the statistical analyses were undertaken with the statistical software package SPSS, version 12.00 (SPSS, Chicago, IL, USA). For all group evaluations, Kruskall–Wallis test was used. For intergroup comparisons, Mann–Whitney *U*-test was used. The level of significance was set at $P < 0.05$. Quantitative data are expressed as means ± standard deviations (SD) and shown in figures.

Results

Clinical findings

In the groups that were exposed to FA, after the tenth day of the study, the hair colour started to fade. This was more marked in the animals that were exposed to FA only than in the animals that were exposed to both rose oil and FA. In addition, the animals in the FA only groups displayed frequent blinking, dyspnoea, increased nasal cleaning, sneezing, and excessive licking associated with irritation due to FA exposure.

Biochemical findings

In the comparisons of the FA exposed rats with the controls, the serum testosterone levels of the FA exposed groups were significantly lower than those of the controls ($P < 0.05$). Moreover, in the group that inhaled FA and rose oil, the serum testosterone levels significantly increased compared to the levels of the rats exposed to FA only ($P < 0.05$) (Fig. 1).

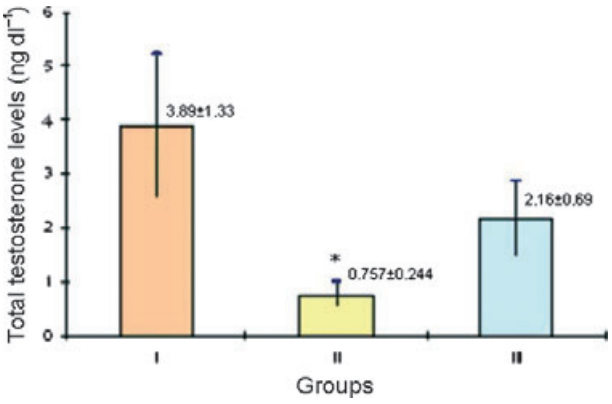

Fig. 1 Testosterone levels of all groups (*$P < 0.05$, compared with other groups).

Spermiogram results

The epididymal sperm counts and sperm motility of the rats that were exposed to FA significantly decreased compared to the control group ($P < 0.001$). In addition, the sperm counts of the rats in this group also increased ($P < 0.001$).

The epididymal sperm count of the group that was exposed to rose oil and FA significantly increased compared to that of the group that exposed to FA only and the abnormal sperm count improved. Moreover, there was an increase in the sperm motility of group that was exposed to rose oil and FA group. However, the difference between the rose oil and FA exposed group and only FA exposed group for sperm motility was not statistically significant ($P > 0.05$) (Table 1).

Histological results

In the light microscopic evaluation, the testes of the control group were normal. When the rats exposed to FA were compared with the control group, it was determined that the number of Leydig cells decreased in the FA only exposed group ($P < 0.001$). The count of Leydig cells in the group that was exposed to rose oil and FA increased

Table 1 Sperm concentration, sperm motility and rate of abnormal sperm of all groups. (mean ± SD, $n = 7$)

Groups	Sperm concentration (10^6 g^{-1})	Sperm motility (%)	Rate of abnormal sperm (%)
I	321.20 ± 35.06	83.83 ± 7.75	5.53 ± 1.23
II	223.15 ± 12.26[a]	72.14 ± 5.33[b]	15.10 ± 2.13[a]
III	326.57 ± 38.48	77.14 ± 4.33	7.77 ± 1.95

[a]$P < 0.001$, compared with other groups.
[b]$P < 0.001$, compared with Group I.

compared to that in only FA exposed group ($P < 0.001$) (Fig. 2).

The count of Leydig cells with damaged nucleus in the rats exposed to FA only decreased compared with that of the control group ($P < 0.001$). In the comparison of the rose oil and FA exposed and only FA exposed groups, it was determined that the count of the Leydig cells with damaged nucleus increased in only FA exposed group ($P < 0.001$) (Figs 3–6).

The diameters of tubules in the rats exposed to FA only decreased when compared with the control group ($P < 0.001$). In the group that was exposed to rose oil and FA, the diameters of the tubules increased compared with the rats exposed to FA only; however, the difference was not statistically significant ($P > 0.05$) (Fig. 7).

Discussion

Formaldehyde has been shown to present negative effects on the respiratory, digestive, and nervous systems, skin, and eyes and have mutagenic and carcinogenic characteristics (Kriebel *et al.*, 2001; Marsh *et al.*, 2007; Sarsilmaz *et al.*, 2007). In addition, it has negative effects on the reproductive system. In the experimental studies to date, FA applied systemically or externally was shown to inflict changes in the morphology of the testes as well as in the spermogenetic cells (Taskinen *et al.*, 1994; Majumder & Kumar, 1995; Odeigah, 1997; Sarsilmaz & Ozen, 2000; Collins *et al.*, 2001).

The testes are one of the organs affected by FA. Sarsilmaz & Ozen (2000) applied FA on rats and showed the damages caused by FA in the Leydig cells and their nuclei. Ozen *et al.* (2005) applied FA in the subchronic stage for 91 days at the doses of 5–10 ppm and determined significant reductions in the tubular diameters ($P < 0.001$). Similarly, Golalipour *et al.* (2007) used inhaled form of FA for 18 days in their subjects for 4 days a week, 2 and 4 h a day, and 2 days a week, 2 h

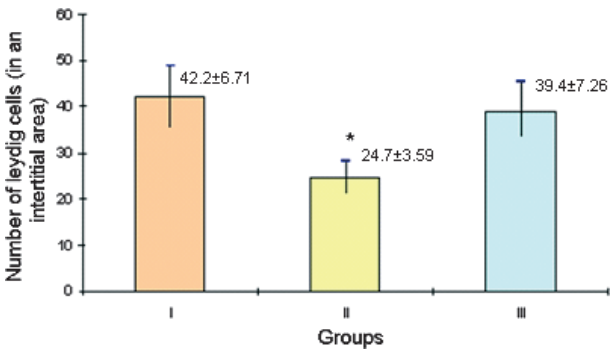

Fig. 2 Number of the Leydig cells of all groups (*$P < 0.001$, compared with other groups).

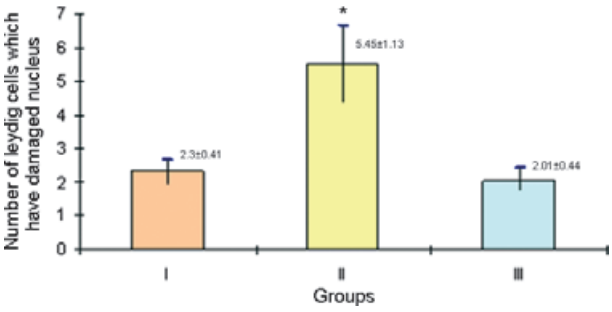

Fig. 3 Number of Leydig cells which have damaged nucleus (*$P < 0.001$, compared with other groups).

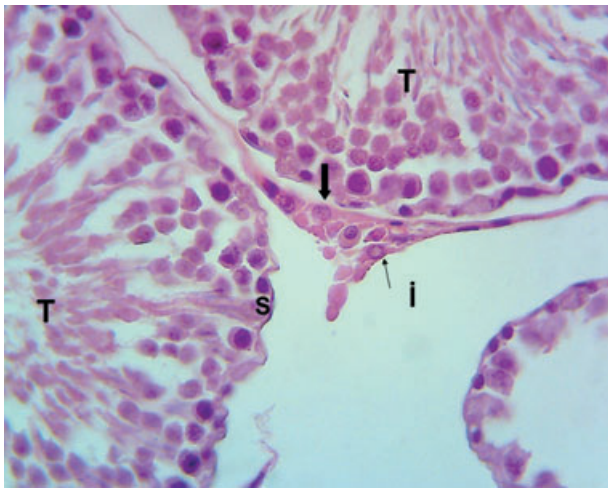

Fig. 4 The Leydig cells with damaged nucleus of Group II. Karyolytic cells (thick arrow), damaged Leydig cells (thin arrow), İ, Interstitial area; T, Seminiferous tubul; S, Sertoli cell (×40, H&E).

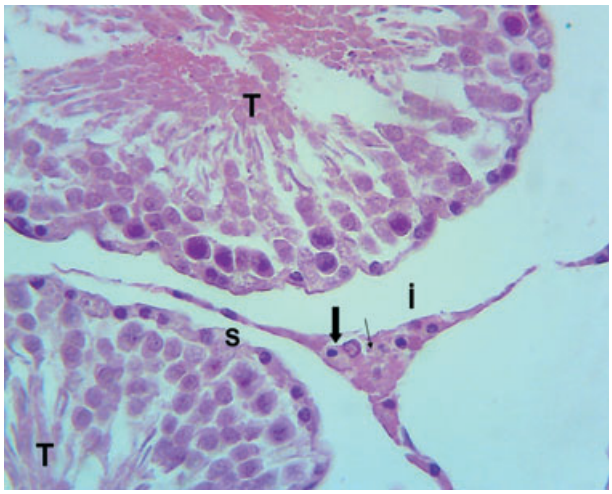

Fig. 5 The leydig cells with damaged nucleus of Group II. Pyknotic cells (thick arrow), damaged Leydig cells (thin arrow), İ, Interstitial area; T, Seminiferous tubul; S, Sertoli cell (×40, H&E).

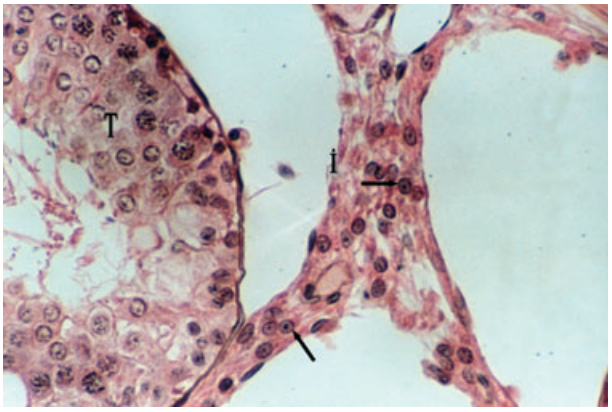

Fig. 6 The leydig cells with damaged nucleus (less than only FA exposed group). Pyknotic cells (arrow). I, Interstitial area; T, Seminiferous tubul (×40, H&E).

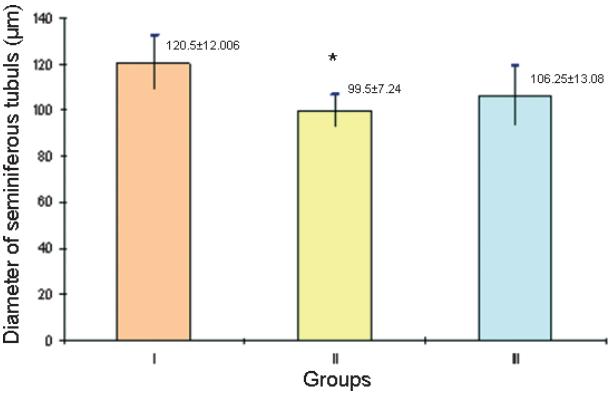

Fig. 7 Diameters of seminiferous tubuls of all groups (*$P < 0.001$, compare with Group I).

daily and they reported significant reduction in the tubule diameter and epithelial thickness. The findings of our study are compatible with the findings of the studies above. In our study, FA was applied for 35 days and 1 h a day at a dose of 10 ppm and it was found that the count of Leydig cells decreased and the rate of the damages in the nuclei of these cells increased, while the diameters of the seminipherous tubule reduced.

Sperm analyses of the epididymal tissues revealed decreased total sperm counts and sperm motility of the animals that were exposed to FA only. However, the count of abnormal spermatozoa increased. In other experimental studies, intraperitoneally applied FA was shown to have negative effects on sperm count and motility (Majumder & Kumar, 1995; Tang *et al.*, 2003; Zhou *et al.*, 2006). Damage to the seminiferous tubule, in which the spermatozoa develop, negatively affected the sperm count; i.e. the sperm count was reduced. Zhou *et al.* (2006) and Tang *et al.* (2003) suggested that intraperitoneal FA causes atrophy and degeneration in the seminiferous tubule, which lead to reductions in sperm counts. Likewise, significant reduction was reported in the sperm motility as a result of damage to the Leydig cells. Henkel *et al.* (2005) demonstrated a direct correlation between sperm motility and testosterone expressed by Leydig cells. In the study by Tang *et al.* (2003) on rats, increased abnormal sperm counts were reported. In the same vein, Odeigah (1997) determined anomalies of the sperm head in rats that had been given intraperitoneal FA. The findings of the sperm analyses in our study are compatible with the findings of earlier studies and supportive of histological findings as well.

The serum levels of testosterone, which has an important role in the reproductive functions, are also negatively affected by FA exposure. In the study by Chowdhury *et al.* (1992) and Zhou *et al.* (2006), intraperitoneally applied FA led to significant reductions in the serum testosterone levels. Similarly, with the inhalation form of FA, Ozen *et al.* (2005) reported a significant reduction in the testosterone levels. All these studies emphasised that damage in the Leydig cells caused decreases in the testosterone levels (Chowdhury *et al.*, 1992; Ozen *et al.*, 2005; Zhou *et al.*, 2006). In our study, the serum testosterone levels of the rats that were exposed to FA were only significantly reduced due to damage in the Leydig cells. Various studies have attributed these effects to the oxidative damage caused by FA (Zararsız *et al.*, 2004; Ozen *et al.*, 2008). Thus, the changes in the Leydig cells, their nuclei, tubule diameters, epididymal sperm analysis findings, and testosterone levels determined in our study may be explained by the oxidative damage caused by FA.

Rose oil, which is clear and light yellow in colour and characteristically fragrant, primarily contains acyclic terpenic substances and 40–50% of the oil consists of citronellol, and 20% geraniol. Geraniol produces the characteristic aroma of the rose (Ergen *et al.*, 2003; Kurkcuoglu & Baser, 2003). Rose oil is used in cosmetics as well as in aromatherapy as an alternative treatment. Its medical effects have also been shown in various experimental studies (Mahmood *et al.*, 1996; Basim & Basim, 2003; Wei & Shibamoto, 2007).

The experimental studies ***to date have determined the antioxidant effects of rose oil. Wei & Shibamoto (2007), based on the results of aldehyde/carboxylic acid test that is used in showing long-term antioxidant activity, have reported that rose oil presents an antioxidant activity almost at a 100% compared to the α-tokoferol, which was taken as a reference. In the same study, 1-diphenyl-2-picrylhydrazil (DPPH) free radical cleansing and malonaldehyde/gas chromatography (MA/GC) tests revealed 70% antioxidant activity of rose oil. α-Tokoferol used as a reference in these tests showed nearly 90% antioxidant activity

(Wei & Shibamoto, 2007). Likewise, in the studies using thiobarbituric acid (TBA) test, citronellol, nerol, and geraniol, the constituents of rose oil have been shown to inhibit MA formation at various levels (Roberto & Maratta, 2000).

The Leydig cell counts of the animals exposed to rose oil and FA increased compared to those of the animals exposed to FA only ($P < 0.001$) and the number of damaged nuclei of the Leydig cells in the rose oil and FA exposed group significantly reduced ($P < 0.001$). Seminipherous tubule diameters of this group also increased, but the difference was not significant ($P > 0.05$). In some of the earlier studies, the disorders of the testes due to oxidative damage were reported to improve with antioxidant therapy (Turk *et al.*, 2007; Ozen *et al.*, 2008). Turk *et al.* (2007), in their study evaluating the testes exposed to cycloporin A, used likopen to prevent oxidative damage and histological changes and reported successful findings. According to the results of these studies, prevention of oxidative damage in the testes by antioxidants have yielded improvements in histopathological changes. In our study, the significant improvement in the histopathological changes in the testes of the rats exposed to rose oil and FA compared to the rats exposed to FA only may be attributed to the antioxidant effect of rose oil.

The increase in the sperm count and motility and reduction in the abnormal sperm count may also be associated with the antioxidant effect of rose oil. This characteristic of rose oil may have indirectly led to improvements in the Leydig cells and seminipherous tubules, sperm counts, motility, and reductions in the abnormal sperm counts.

Total testosterone levels of the group exposed to rose oil and FA was significantly higher than those of the FA exposed group ($P < 0.05$). This might also be due to the antioxidant effect of rose oil. Recent studies have reported a direct correlation between Leydig cells and testosterone levels (Henkel *et al.*, 2005; Joensen *et al.*, 2008). In our study, increase in the testosterone releasing Leydig cell counts and reduction in the nucleic damage may be responsible for increased testosterone levels.

In conclusion, the decreases in the Leydig cell counts and tubular diameters increase in the nucleic damage, reduction in the sperm count and motility, and decreased serum testosterone levels indicate the negative effects of FA inhalation on the male reproductive system. Positive effects of the rose oil might be attributed to its antioxidative properties and rose oil can be used to improve some of these negative effects.

Acknowledgement

This work was supported by The Firat University Research Foundation (FÜBAP-1286).

References

Almeida R, Simone CM, Faturib CB, Catallanib B, Leite JR (2004) Anxiolytic-like effects of rose oil inhalation on the elevated plus-maze test in rats. *Pharmacol Biochem Behav* 77:361–364.

Altıntaş A (2007) Gül Gülsuyu Tarihte, Tedavide ve Gelenekteki Yeri (Rose, Rosewater, In the Past, Therapy and Traditional Application), 1st edn. Tempo Matbaacılık, İstanbul.

Basim E, Basim H (2003) Antibacterial activity of Rosa damascena essential oil. *Fitoterapia* 74:394–396.

Boskabady MH, Kaini S, Rakhshandah H (2006) Relaxant effects of Rosa damascena on guinea pig tracheal chains and its possible mechanism(s). *J Ethnopharmacol* 106:377–382.

Bradley BF, Starkey NJ, Brown SL, Lea RW (2007) The effects of prolonged rose odor inhalation in two animal models of anxiety. *Physiol Behav* 92:931–938.

Chowdhury AR, Gautam AK, Patel KG, Trivedi HS (1992) Steroidogenic inhibition in testicular tissue of formaldehyde exposed rats. *Indian J Physiol Pharmacol* 36:162–168.

Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS (1998) Formaldehyde evaluation from endodontic materials. *Oral Health* 88:37–39.

Collins JJ, Ness R, Tyl RW, Krivanek N, Esmen NA, Hall TA (2001) A review of adverse pregnancy outcomes and formaldehyde exposure in human and animal studies. *Regul Toxicol Pharmacol* 34:17–34.

Ergen A, Ozkan G, Bicer A (2003) The effect of pressure on the extraction of essential oil from flowers of lavender plants with supercritical CO_2. *Gazi Üniversitesi Journal of Science* 17:717–723.

Golalipour MJ, Azarhoush R, Ghafari S, Gharravi AM, Fazeli SA, Davarian A (2007) Formaldehyde exposure induces histopathological and morphometric changes in the rat testis. *Folia Morphol (Warsz)* 66:167–171.

Heck H, Casanova M (1999) Pharmacodynamics of formaldehyde: Applications of a model for the arrest of DNA replication by DNA-protein cross-links. *Toxicol Appl Pharmacol* 160:86–100.

Henkel R, Maass G, Schuppe HC, Jung A, Schubert J, Schill WB (2005) Molecular aspects of declining sperm motility in older men. *Fertil Steril* 84:1430–1437.

Joensen UN, Jørgensen N, Rajpert-De Meyts E, Skakkebaek NE (2008) Testicular dysgenesis syndrome and Leydig cell function. *Basic Clin Pharmacol Toxicol* 102:155–161.

Khanzadeh FA, Vaquerano MU, Khanzadeh MA, Bisesi MS (1994) Formaldehyde exposure, acute pulmoner response and exposure control options in a gross anatomy laboratory. *Am J Indust Med* 26:61–68.

Komiya M, Takeuchi T, Harada E (2006) Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. *Behav Brain Res* 172:240–249.

Kose E, Sarsilmaz M, Ogeturk M, Kus I, Kavakli A, Zararsiz I (2007) The role of rose essential oil aroma on the learning

behaviorus: an experimental study. *Fırat Medical Journal* 12:159–162.

Kriebel D, Myers D, Cheng M, Woskie S, Cocanour B. (2001) Short term effect of formaldehyde on peak expiratory flow and irritant symptoms. *Arch Environ Health* 56:11–18.

Kurkcuoglu M, Baser KHC (2003) Studies on Turkish rose concrete, ''absolute and hydrosol''. *Chem Nat Comp* 39:457–464.

Mahmood N, Piacente S, Pizza C, Burke A, Khan AI, Hay AJ (1996) The anti-HIV activity and mechanisms of action of pure compounds isolated from Rosa Damascena. *Biochem Biophys Res Commun* 229:73–79.

Majumder PK, Kumar VL (1995) Inhibitory effects of formaldehyde on the reproductive system of male rats. *Indian J Physiol Pharmacol* 39:80–82.

Marsh GM, Youk AO, Buchanich JM, Erdal S, Esmen NA (2007) Work in the metal industry and nasopharyngeal cancer mortality among formaldehyde-exposed workers. *Regul Toxicol Pharmacol* 48:308–319.

Odeigah PG (1997) Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. *Mutat Res* 389:141–148.

Ozen OA, Akpolat N, Songur A, Kus I, Zararsiz I, Ozaçmak VH, Sarsilmaz M (2005) Effect of formaldehyde inhalation on Hsp70 in seminiferous tubules of rat testes: an immunohistochemical study. *Toxicol Ind Health* 21:249–254.

Ozen OA, Kus MA, Kus I, Alkoc OA, Songur A (2008) Protective effects of melatonin against formaldehyde-induced oxidative damage and apoptosis in rat testes: an immunohistochemical and biochemical study. *Syst Biol Reprod Med* 54:169–176.

Roberto G, Maratta MT (2000) Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem* 69:167–174.

Sarsilmaz M, Ozen OA (2000) The histopathologic changes on leydig cells of the rats ınhaled formaldehyde along subchronic period. *Fırat Medical Journal* 2:1–5.

Sarsilmaz M, Kaplan S, Songur A, Colakoglu S, Aslan H, Tunc AT, Ozen OA, Turgut M, Bas O (2007) Effects of postnatal formaldehyde exposure on pyramidal cell number, volume of cell layer in hippocampus and hemisphere in the rat: a stereological study. *Brain Res* 1145:157–167.

Sonmez M, Turk G, Yuce A (2005) The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels in male wistar rats. *Theriogenology* 63:2063–2072.

Sonmez M, Yuce A, Turk G (2007) The protective effects of melatonin and vitamin E on antioxidant enzyme activities and epididymal sperm characteristics of homocysteine treated male rats. *Reprod Toxicol* 23:226–231.

Tang M, Xie Y, Yi Y, Wang W (2003) Effects of formaldehyde on germ cells of male mice. *Wei Sheng Yan Jiu* 32:544–548.

Taskinen H, Kyyronen P, Hemminki K, Hoikkala M, Lajunen K, Lindbohm ML (1994) Laboratory work and pregnancy outcome. *J Occup Med* 36:311–319.

Turk G, Atessahin A, Sonmez M, Yuce A, Ceribasi AO (2007) Lycopene protects against cyclosporine A-induced testicular toxicity in rats. *Theriogenology* 67:778–785.

Usanmaz SE, Akarsu ES, Vural N (2002) Neurotoxic effects of acute and subacute formaldehyde exposures in mice. *Environ Toxicol Pharmacol* 11:93–100.

Wei A, Shibamoto T (2007) Antioxidant activities and volatile constituents of various essential oils. *J Agric Food Chem* 55:1737–1742.

Zararsız İ, Kus İ, Colakoglu N, Pekmez H, Yılmaz HR, Sarsilmaz M (2004) Protective effects of melatonin hormone against exposure of formaldehyde-induced oxidative damage in lung of rats: light microscopic and biochemical study. *Van Med J* 11:105–112.

Zhou DX, Qiu SD, Zhang J, Wang ZY (2006) Reproductive toxicity of formaldehyde to adult male rats and the functional mechanism concerned. *Sichuan Da Xue Xue Bao Yi Xue Ban* 37:566–569.