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 (Khazadheh 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...
lindip, 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; Altuntaş, 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, Firat 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 × 50 × 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 formaldehyde meter (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 ×) 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 haemocytometric 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, 1 mL 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 haemocytometer 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 ×.

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).](image)

Spermiogram results

The epidydimal 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 epidydimal 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>
<thead>
<tr>
<th>Groups</th>
<th>Sperm concentration (10^6 g⁻¹)</th>
<th>Sperm motility (%)</th>
<th>Rate of abnormal sperm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>321.20 ± 35.06</td>
<td>83.83 ± 7.75</td>
<td>5.53 ± 1.23</td>
</tr>
<tr>
<td>II</td>
<td>223.15 ± 12.26^a</td>
<td>72.14 ± 5.33^b</td>
<td>15.10 ± 2.13^b</td>
</tr>
<tr>
<td>III</td>
<td>326.57 ± 38.48</td>
<td>77.14 ± 4.33</td>
<td>7.77 ± 1.95</td>
</tr>
</tbody>
</table>

^aP < 0.001, compared with other groups.  
^bP < 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
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 seminiferous 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 ($P < 0.001$). Seminiferous 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 for 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 seminiferous 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

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References


