

The effect of lavender oil on serum testosterone levels and epididymal sperm characteristics of formaldehyde treated male rats

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Abstract. – Background and Objectives: In this study, the harmful effects of formaldehyde (FA) on serum testosterone levels and epididymal sperm characteristics were investigated. In addition, possible protective effect of lavender oil was evaluated.

Materials and Methods: For this purpose, 21 adult male Wistar-Albino rats were used. The rats of group I was used as control group. The rats of group II were exposed FA (10 ppm/1 hour) for 35 days. The rats of group III inhaled lavender oil (1 ml/1 hour) with FA.

Results: While the testosterone levels, the epididymal sperm concentration and the progressive sperm motility were significantly decreased, the abnormal sperm rate was significantly increased in FA treated group when compared to control group. However, in group III, the epididymal sperm concentration and the progressive sperm motility were significantly increased, the abnormal sperm rate was significantly decreased in comparison with the FA treated group.

Conclusion: It can be expressed that serious damages occurred via formaldehyde exposure in reproductive system and that the lavender oil had protective effects against these damages.

Key Words:

Formaldehyde, Epididymis, Lavender oil, Testosterone, Rat.

Introduction

Formaldehyde (FA) is an opaque and pungent substance. FA, (CH₂O) a member of aldehyde family, is released into the ambience together

with cigarette smoke and exhaust gas and also can be naturally found in atmosphere. FA, when inhaled, causes harmful effects by joining into the structure of macromolecules like protein, DNA and RNA¹⁻³.

Formaldehyde is intensely used in anatomy, histology and pathology laboratories. Along with this, in process of producing various materials like paint, plastic, wallpapers, woodworks, glues, cleaning materials used in houses, toothpaste and shampoo, FA is made use of⁴⁻⁶. So in almost any kind of ambience exposure to FA is possible through inhalation.

On various experimental researches, it has been reported that formaldehyde has harmful effects on various systems like respiratory system, nervous system and digestion system⁷⁻⁹. This substance which also has negative effects on reproductive system, causes infertility¹⁰⁻¹². On researches made on experiment animals, it has been reported that FA exposure damages the morphological structure of testicles and causes a fall in serum testosterone levels. Besides, it has been mentioned in these research reports that formaldehyde decreases sperm amount and motility and increases abnormal sperm amounts¹³⁻¹⁶.

Usage of pure essential oils for treatment purposes which are derived from aromatic plants is called aromatherapy. One of the oils that are often used in aromatherapy applications is lavender oil. This oil which is obtained by means of steam distillation contains up to 30-40% linalool, and 35-55% linalyl acetate. Additionally this oil contains tanins, caffeic acid and terpenic substances like geraniol, borneol, and ocaliptol¹⁷⁻¹⁹.

Lavender oil which is used by public physicians in treatments of disorders like various infections, anxiety, stomach aches and kidney problems also has an aphrodisiacal effect¹⁹⁻²¹.

On recently made researches, it has been informed that lavender volatile oil creates sedative, hypnotic and hypotensive effects on humans²²⁻²⁴. Along with this, it has been also informed that lavender volatile oil is used for infertility treatment²⁰. However, in literature scans we have made, no experimental work on lavender oil's effects on reproductive system has been encountered.

And, on the research we made on rats, FA toxicity on reproductive system has been examined. In addition, possible protective effects of lavender oil against to FA toxicity have also been appreciated.

Materials and Methods

Adult male Wistar rats (weighing 310-320 g, n=21) comprised 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 hour – formalin, Sigma-Aldrich formaldehyde 37% solution, Deisenhofen, Germany) for 35 days, the rats in Group III inhaled lavender oil (1 ml/1 hour – Mecitefendi, İzmir, Turkey) along with FA. At the end of the experiment, all the animals were killed by means of decapitation. The blood samples taken from the rats were used to determine the serum testosterone levels. And the epididymis tissue samples were utilized for sperm count, sperm motility and abnormal sperm rate.

Serum Testosterone Analysis

Serum was stored at -20°C for analysis. The serum testosterone level was assayed using Coat-a-Count Radioimmunoassay kit (Active Testosterone RIA DSL-4000, Diagnostic System Laboratories Inc, Texas, USA) and expressed as ng/mL.

Determination of Epididymal Sperm Concentration

The epididymal sperm concentration was determined with a hemocytometer (Improved

Neubauer, Weber, UK) using a modification of the hemocytometric method described by Turk et al²⁵ and Sönmez et al²⁶. Briefly, the right epididymis was finely minced using anatomical scissors in 1mL 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 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 200X.

Determination of Epididymal Sperm Motility

The percentage of progressive sperm motility was evaluated using a light microscope with heater table as described by Sönmez et al²⁷. 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

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 slide²⁷.

Statistical Analysis

All the statistical analyses were undertaken with the statistical software package SPSS, version 12.00 (SPSS Inc., Chicago, IL, USA). For all group evaluations, Kruskal 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 \pm standard deviations (SD) and shown in tables.

Results

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 lavender oil, the serum testosterone levels increased compared to the rats exposed to FA. However, this increase was not seen statistically significant (Table I).

Spermiogram

After the right epididymis of each rat was prepared by a special hemocytometric method, it was evaluated under a microscope with Neubauer glass, and thus, the total sperm counts were determined. Sperm motility and abnormal sperm counts were determined using the samples from the left cauda of epididymis, which were evaluated via a microscope with a heating plate.

The epididymal sperm counts and sperm motility of the rats that were exposed to FA significantly decreased compared to the control group ($p < 0.05$). In addition, the sperm counts of the rats in this group also increased ($p < 0.05$).

The epididymal sperm counts and sperm motility of the rats that were exposed to lavender oil and FA significantly increased compared to that of the group that exposed to FA only and the abnormal sperm count improved ($p < 0.05$) (Table II).

Table I. Serum testosterone levels of all groups (ng/dl) (means \pm SD, n = 7).

Group	Testosterone levels (ng/dl)
Group I	3.89 \pm 1.33
Group II	0.75 \pm 0.24 ^a
Group III	1.28 \pm 0.52

^a $p < 0.05$, compared with group I.

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^{7-9,28-30}. Additionally FA has negative effects on reproductive system. It has been shown in the experimental researches that, both systemic and external FA applied was shown to inflict changes on testicular morphology and spermatogenic cells¹⁰⁻¹⁶.

In our study, there were decreases in the total spermatozoa counts and sperm motility of the animals that were exposed to FA only. Similarly, Zhou et al³¹, in their study where FA inhaled by the animals (8 ppm for two weeks), reported reduced sperm counts and motility but increased abnormal sperm counts. In other experimental studies, intraperitoneally-applied FA was shown to have negative effects on the sperm count and motility^{13,32,33}. Damage to the seminiferous tubule, in which the sperms develop, negatively affected the sperm count; i.e. the sperm count reduced. Tang et al³² and Zhou et al³³ have suggested that intraperitoneal FA causes atrophy and degeneration in the seminiferous tubule, which leads to reductions in the sperm counts. The findings of the sperm analyses in our study are compatible with the findings of earlier studies.

The serum levels of testosterone, which has an important role in the reproductive functions, are also negatively affected by FA exposure. In the

Table II. Sperm concentration, sperm motility and rate of abnormal sperm of all groups. (mean \pm SD, n = 7).

Groups	Sperm concentration (10 ⁶ /g)	Sperm motility (%)	Rate of abnormal sperm (%)
Group I	321.20 \pm 35.06	83.83 \pm 7.75	5.53 \pm 1.23
Group II	223.15 \pm 12.26 ^a	72.14 \pm 5.33 ^a	15.10 \pm 2.13 ^a
Group III	272.04 \pm 19.83 ^b	78.29 \pm 3.25 ^b	9.25 \pm 1.25 ^b

^a $p < 0.05$, compared with other groups; ^b $p < 0.05$, compared with group II.

work by Chowdhury et al³⁴ and Zhou et al³³, intraperitoneally applied FA led to significant reductions in the serum testosterone levels. Similarly, with the inhalation form of FA, Özen et al³⁵ reported a significant reduction in the testosterone levels. All of these studies emphasized that the damage in the Leydig cells caused the decreases in the testosterone levels. In our investigation, the serum testosterone levels of the rats that were exposed to FA only significantly reduced due to the damage in the Leydig cells. In our previously study³⁶ with FA toxicity on testes, we showed that FA had harmful effects on seminiferous tubule and Leydig cells.

Lavender oil has sedative, anxiolytic, anti-convulsive, antimicrobial, spasmolytic, and antioxidant due to its contents as linalool and linalyl acetate^{17,20-24,37}. In traditional medicine, it has been reported that, this oil which shows specialities as making menstruation easier and aphrodisiac, has positive effects on reproductive functionalities^{18,20}. In a clinical investigation which perfected on males³⁷, it was shown that inhaled lavender oil increased the penil blood flow at the rate of around 40%. This effect of lavender oil's is explained in two mechanisms. The first one is to help the erection by maintaining relaxation and decreasing anxiety. And the second possible mechanism is made by means of direct anatomical connection between olfactory tract and hypothalamus³⁸.

Along with this, it has been pointed out that lavender oil which applied through inhalation on female rats experimentally menopausal, causes changes in gonadotropin levels³⁹. In our study, positive effects of lavender oil might have occurred thanks to the anatomical connections between olfactory tract and hypothalamus.

The experimental researches to date have determined the antioxidant effects of lavender oil. In a study, the 2,20-diphenylpicrylhydrazil free radical cleaning test of lavender oil, showed high antioxidant activity. Moreover in this work, the lipid peroxidation test, which is made with linoleic acid that is one of the crucial components of lavender oil, it has been shown that at the percentage of 58% peroxidation is blocked⁴⁰. In another research, 1,1-diphenyl-2-picrylhydrazil free radical cleaning test was applied on the saliva excretions of the humans inhaled lavender oil. In this study, free radical cleaning activation was found higher. Lavender oil's this effect is linked to its ability to impact over nervous system⁴¹. And in this work we have made, a

regeneration has been spotted on epididymis sperm characteristics of rats that have been implemented lavender oil along with FA formaldehyde exposure. We think that this regeneration is a result of lavender oil's antioxidant effect as explained above.

In conclusion, it has been shown in this work that the negative effects that occur as a result of FA exposure in the parameters belonging to reproductive functionalities, is prevented by lavender oil application. Two mechanisms take part in appearance of this protective effect. The first one has shown up after lavender oil has affected hypothalamus and septal nuclei by means of olfactory tract. And the second one is that antioxidant activity that lavender oil holds has made a protective effect against the oxidative effect that formaldehyde has created.

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