Asian Pacific Journal of Tropical Biomedicine

Journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.245970

Protective efficacy of *Nigella sativa* oil against the harmful effects of formaldehyde on rat testicular tissue

Hilal Irmak Sapmaz[1]✉, Azibe Yıldız[2], Alaadin Polat[3], Nigar Vardı[2], Evren Köse[4], Kevser Tanbek[3], Songül Çuğlan[4]

[1]*Tokat Gaziosmanpaşa University, Medical Faculty, Department of Anatomy, Tokat, Turkey*

[2]*İnönü University, Medical Faculty, Department of Histology and Embryology, Malatya, Turkey*

[3]*İnönü University, Medical Faculty, Department of Physiology, Malatya, Turkey*

[4]*İnönü University, Medical Faculty, Department of Anatomy, Malatya, Turkey*

ARTICLE INFO

Article history:
Received 11 October 2018
Revision 1 November 2018
Accepted 21 November 2018
Available online 27 November 2018

Keywords:
Formaldehyde
Testis
Nigella sativa oil

ABSTRACT

Objective: To explore the effects of *Nigella sativa* oil (NSO) on the histopathological and biochemical changes that inhaled formaldehyde (FA) induces on the testicular tissue of rats. **Methods:** Thirty three adult male rats were separated into five groups as follows: C, the control group; 4FA group which received FA for 4 weeks; 13FA group which was given FA for 13 weeks; 4FA+NSO group which was administered FA plus NSO for 4 weeks; 13FA+NSO group which was treated with FA plus NSO for 13 weeks. FA was administered through inhalation for 8 h 5 days a week at a dose of 5 ppm in a special glass cage, and NSO was administered orally 1 mL/kg once daily. Rats were decapitated at the end of the experiment and testicular tissue specimens were harvested for histopathologic and biochemical assessment. **Results:** Compared to the C group, reduction was observed in the number of intact tubules and in the mean germinative epithelium thickness of the FA groups. Significant increase was observed in the number of intact tubules with the long-term (13 weeks) administration of NSO together with FA. Reduced glutathione peroxidase activity was found and oxidative stress index values were measured higher in the 4FA and 13FA groups versus the C group ($P<0.05$). Moreover, total antioxidant status levels decreased only in the 4FA group ($P<0.05$) while only the 13FA group significantly increased malondialdehyde levels and reduced catalase activities in comparison with the C group. In the 13FA+NSO group, malondialdehyde levels decreased however glutathione peroxidase and catalase activities increased compared to the 13FA group. Differences measured in total antioxidant status levels were found to be statistically significant only between the 4FA and the 4FA+NSO groups. **Conclusions:** NSO as an antioxidant should be used for a longer term to achieve protective efficacy both histopathologically and biochemically in the testicular tissue.

1. Introduction

Formaldehyde (FA) is a ubiquitous chemical compound which is released as a result of the burning process from vehicle exhausts, power plants, refineries and wood stoves. Building materials such as chipboard, paint and varnish also contain FA. While the compound naturally occurs in some fruits, it forms endogenously in mammals, including humans, as part of the oxidative metabolism[1]. FA is

✉First and corresponding author: Hilal Irmak Sapmaz, Tokat Gaziosmanpaşa University, Medical Faculty, Department of Anatomy, Tokat, Turkey.
Tel: + 90 533 424 9601
Fax: +90 356 213 31 79
E-mail: hisapmaz@yahoo.com

This study was presented as a poster at 15th National Anatomy Congress, 5-8 September 2013, Samsun, Turkey.

How to cite this article: Irmak Sapmaz H, Yıldız A, Polat A, Vardı N, Köse E, Tanbek K, et al. Protective efficacy of *Nigella sativa* oil against the harmful effects of formaldehyde on rat testicular tissue. Asian Pac J Trop Biomed 2018; 8(11): 548-553.

usually used for embalming cadavers in anatomy laboratories and preserving tissues in pathology laboratories. Besides, the compound is used as preservative, sterilizer and stabilizer in the production of cosmetics, shampoos, some personal care products, textiles, and furniture, as well as in many foods[2,3]. FA is mostly taken to the body through digestion, respiration, and *via* the skin[3]. It induces carcinogenicity and mutagenicity by strongly binding to proteins, DNA and RNA[4].

Inhaled FA has been identified to induce atrophy in seminiferous tubules, increase in spaces between germinative epithelial cells, degeneration in Leydig cells, disintegration of seminiferous epithelial cells and decrease in sperm motility[5]. Also decrease in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and increase in malondialdehyde (MDA) levels have been identified in the testicular tissues of rats[6].

Nigella sativa (black cumin) is a member of the Ranunculaceae family and native to Anatolia. The seeds have diuretic and digestive effects[7,8]. It has been shown to have many favorable effects, especially antioxidant and antidiabetic[9,10]. Oral administration of the *Nigella sativa* oil (NSO) has been observed to increase sperm count and sperm motility in the epididymis while reducing serum MDA levels and increasing glutathione (GSH), GSH-Px and catalase (CAT) activities[11].

The present study was conducted to investigate whether NSO has protective efficacy against the harmful effects FA induces on the male reproductive system.

2. Materials and methods

2.1. The subjects and the study protocol

Thirty three adult male Sprague-Dawley rats weighing 270 to 300 g were used in the study. This study was carried out after obtaining the approval (2011/A-29) of Local Ethics Committee for Animal Research, and all of the rats which were used in the experiment were given humane care in accordance with the European Convention on Animal Care. The rats were separated into five groups as follows: C, the control group (n=5); the 4FA group (n=7) which received FA (subacute) for 4 weeks; the 4FA+NSO group (n=7) which received FA together with NSO for 4 weeks; the 13FA group (n=7) which received FA (subchronic) for 13 weeks; and the 13FA+NSO group (n=7) which received FA together with NSO for 13 weeks. Special glass cages of 20 cm × 50 cm × 100 cm with two air ventilation holes each were manufactured. Air pumps (Optima-Ein Weltweit-Product Holm bei Hamburg, Art. Nr. A-10007) were used to ensure air flow in and out of the cages. Paraformaldehyde (Merck KGaA, 64271 Darmstadt, Germany) was heated at 35-40 ℃ and depolymerized to obtain gaseous FA. FA concentration inside the cages was measured using a formaldehyde monitor (Environmental Sensors Co. Boca Raton FL 33432 USA-Catalog No:ZDL-300) recommended by Occupational Safety and Health Administration. The rats were placed in the glass cages and exposed to FA through inhalation for 8 h 5 days a week at a dose of 5 ppm (1 ppm=1.25 mg/m^3). NSO (Origo, Gaziantep, Turkey) was administered orally by gavage and 1 mL/kg once daily on the same days as FA exposure.

The rats were decapitated after the experimental period, and testicular tissue specimens were harvested. The specimens were measured biochemically for MDA, SOD, GSH-Px, CAT, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) values and histopathologically for tubular atrophy, germinative epithelium height and seminiferous tubule diameter.

2.2. Histological procedure and evaluation

Testis tissues were fixed in 10% formalin, dehydrated through 80%, 95% and absolute ethyl alcohol, cleared in xylene and embedded in paraffin. Sections were cut at 4 μm, mounted on slides and stained with hematoxylin and eosin (H&E) for general structure.

Seminiferous tubules were classified as intact and atrophic. Tubules lined by multi-layered Sertoli and spermatogenic cells sitting on the basal membrane were evaluated as intact, and tubules with impaired cellular organization and decreased or no spermatogenic cell count were evaluated as atrophic. Atrophic tubules were scored based on the severity of the atrophy. Tubular atrophy was scored zero if absent, 1 if involved 1%-25% of the tubule, 2 if involved 26%-50% of the tubule, and 3 if involved 51%-100% of the tubule. Tubule diameter and germinative epithelium thickness were measured on each slide under 20× magnification. All assessments were performed with a Leica DFC 280 optical microscope and the Leica Q Win analysis system.

2.3. Biochemical analyses

The MDA contents of the homogenates were determined spectrophotometrically by evaluating the presence of thiobarbituric acid-reactive substances[12]. The results were shown as nmol/g according to a prepared standard graph. The method of Sun *et al.* was used to measure total SOD activity[13]. The principle of the method is the inhibition of nitroblue tetrazolium reduction by the xanthine-xanthine oxidase system as a superoxide generator. SOD activity was shown as U/mg protein. Aebi's method was used to measure CAT activity[14]. The principle of the assay is based on the determination of the rate constant (k, s-1) or the H_2O_2 decomposition rate at 240 nm. Results were shown as K/g protein. The method which was developed by Paglia and Valentine was used for calculating GSH-Px

activity[15]. An enzymatic reaction in a tube containing nicotinemide adenine dinucleotide phosphate, GSH, sodium azide, and glutathione reductase was initiated by adding H_2O_2; the change in absorbance at 340 nm was observed using a spectrophotometer. Activity was shown as U/mg protein. A novel automatic measurement method developed by Erel was used to measure the levels of TAS in testicular tissue[16]; this assay is perfect from a sensitive standpoint, with sensitive values lower than 3%. The results were shown as μmol Trolox equivalent/L. Testicular tissue TOS levels were calculated with a new automated measurement method developed by Erel[17]. The assay was calibrated H_2O_2 and the results were shown as μmol H_2O_2 Trolox equivalent/L. The TOS:TAS ratio was used to measure OSI, and the results were denoted as arbitrary units[18].

2.4. Statistical analysis

Statistical analyses were performed using the SPSS program (SPSS for Windows version 17) and the MedCalc (2007, Belgium) statistical software package. Tubule diameter and germinative epithelium thickness were analyzed with ANOVA and Tamhane's T2 tests; atrophic tubule counts of the groups were compared with the *Chi*-square test; atrophic tubule severity was assessed with Kruskal-Wallis variance analysis and Conover's test. Tubule diameter and germinative epithelium thickness data were displayed as arithmetic average±SD, and atrophic tubule severity data as median (min-max). Biochemical parameters were assessed with the Kruskal-Wallis variance analysis, the Mann Whitney *U* and the ANOVA tests. $P<0.05$ was considered as significant difference.

3. Results

3.1. Histopathological results

Seminiferous tubules and germinative epithelium in control group were observed to be histologically normal. In this group, mean germinative epithelium thickness was (39.2±9.1) μm and mean tubule diameter was (249.2±20.1) μm. A total of 71.1% of tubules in the examined slides were identified as intact in control group.

Statistical comparison with the control group showed that the numbers of intact tubules were significantly less in the 4FA group (42.2%) and the 13FA group (17.2%) ($P=0.004$). Germinative epithelium thicknesses of these two groups were (26.0±5.8) μm and (15.0±5.4) μm, respectively. Besides, tubule diameters of the 4FA and 13FA groups were (236.3±27.8) μm and (243.7±35.5) μm, respectively. Severity of tubular atrophy was statistically significantly increased in the 13FA group compared to the 4FA group ($P<0.000\,1$). While in some of the tubules in the 4FA group, underdeveloped spermatogenic cells were found to have accumulated in the lumen (Figure 1B), no spermatogenic cells were seen to have translocated to the lumen in the sections of the 13FA group.

Atrophic tubules that were distinctly seen in the groups exposed to FA only were also observed in the FA+NSO groups (Figure 1B, C, D, E). While 40.6% of the tubules in the 4FA+NSO group

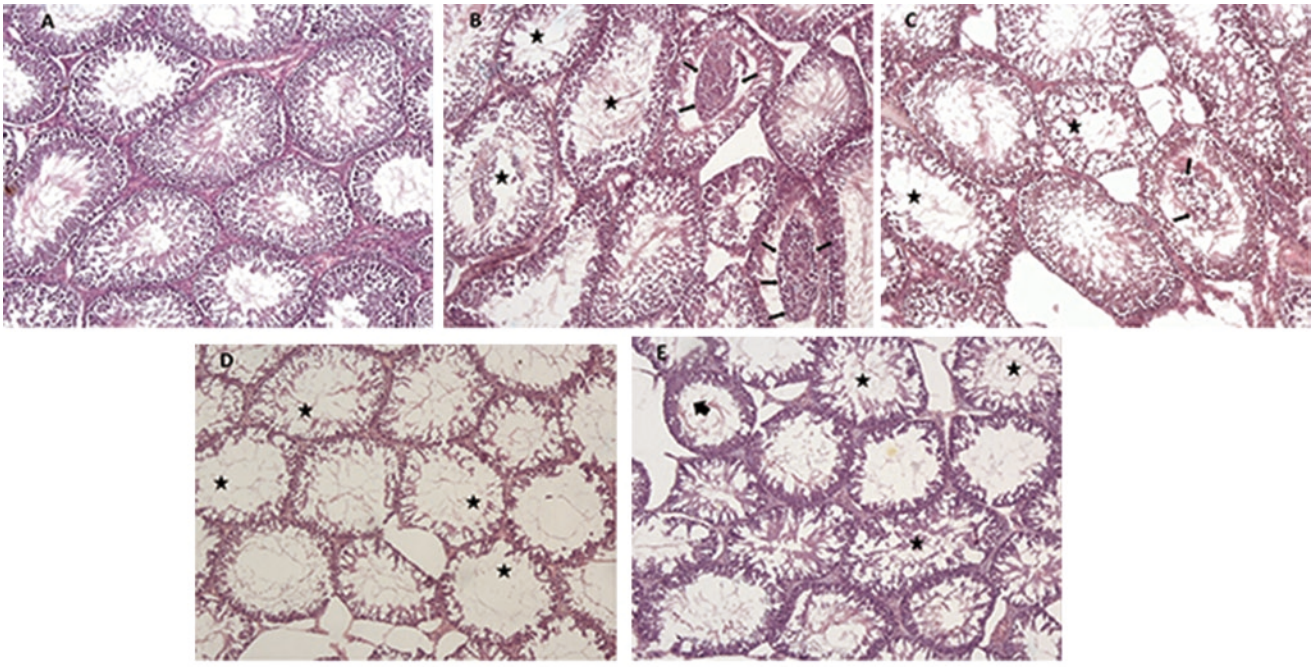

Figure 1. Testicular tissue of all groups.

C group (A) appeared normal; 4FA group (B) showed underdeveloped spermatogenic cells (thin arrows) in the lumen and atrophic tubules (stars); 4FA+NSO group (C) showed a small amount of underdeveloped spermatogenic cells (thin arrows) in the lumen and atrophic tubules (stars); 13FA group (D) showed atrophic tubules (stars); 13FA+NSO group (E) showed atrophic tubules (stars) and almost preserved intact epithelium of the seminiferous tubule (thick arrow) of H & E. × 20.

were observed to remain intact, the percentage of intact tabules was 30.6% in the 13FA+NSO group (Figure 1E). Germinative epithelium thicknesses of 4FA+NSO and 13FA+NSO groups were (24.6±8.4) μm and (16.4±6.0) μm, respectively, and tubule diameters of these two groups were (253.2±28.9) μm and (240.5±33.3) μm, respectively. Tubule diameters in the 4FA+NSO group were found statistically significantly larger than those in the 4FA group (P=0.000), and germinative epithelium thicknesses were identified to be unaffected (P>0.05). Whereas only 17.2% of the tubules were found to be intact in the 13FA group, this rate was found higher (30.6%) in the 13FA+NSO group. Likewise, the counts of atrophic tubules that presented a severity score of 3 were 18.3% and 6.1%, respectively in these two groups.

3.2. Biochemical results

In the 4FA and 13FA groups, GSH-Px activities were found to be lower and OSI values were measured higher than those of the control group (P<0.05). TAS levels were reduced significantly versus the control group only in the 4FA group (P<0.05). When compared to the control group, significantly increased MDA levels and significantly reduced CAT activities were seen only in the 13FA group (P<0.05). The differences identified in these parameters (MDA and CAT) between the 4FA group and the control group were found insignificant (P>0.05). NSO administration for 13 weeks increased GSH-Px activities but not affected OSI levels. In the 13FA+NSO group, decreased MDA levels and increased CAT activities were found compared to the 13FA group (P<0.05). No significant differences were found between the 4FA and 4FA+NSO groups in terms of these parameters. Differences measured in TAS levels were found to be statistically significant only between the 4FA and the 4FA+NSO groups (P<0.05) (Table 1).

4. Discussion

FA, a compound which is ubiquitously found in our environment, naturally occurs in the lower layer of the atmosphere through hydrocarbon oxidation. Exhaust gases from motor vehicles are also a major source of environmental FA emission. The compound is also emitted by cigarette smoke, and in the heating and cooking processes[3]. Harmful effects of FA on the male reproductive system have been demonstrated in a number of experimental studies[5,6,19]. Both long- and short-term inhalations of FA are reported to cause atrophy in seminiferous tubules, degeneration in Leydig cells, disintegration of seminiferous epithelial cells and decrease in sperm motility[5,6,20,21]. Likewise, in our study, FA inhalation was seen to have caused atrophy in the seminiferous tubules and decrease in germinative epithelium thickness. Zhou *et al.* reported reduced SOD and GSH-Px activity and increased MDA levels in the testicular tissue associated with FA inhalation[6]. Similar to the reports in the literature, this study identified a decrease in GSH-Px activities and an increase in MDA levels. Histopathological and biochemical changes due to FA administration have been reported in several studies. When FA was administered together with antioxidants such as proanthocyanidin or manganese chloride, there was less histopathological damage and also less increase in MDA levels[19,22]. The antioxidant we used in our study was found to have affected MDA levels and histopathologic findings were similar to those reported in the literature.

In another study, administration of NSO for 6 weeks at a dose of 0.4 mL/day *via* a gastric tube was found to increase the sperm count and sperm motility in the epididymis. A decrease in serum MDA levels was also observed while GSH, GSH-Px and CAT activities increased[11]. Similarly, after administering 5 mL/kg of NSO for 60 d to rabbits, Umar *et al.* reported significant increases in the spermatogenic epithelium, the seminiferous tubule diameter and area, the seminiferous tubule lumen diameter, and the count of spermatogenic layers[23]. Furthermore, many studies have demonstrated NSO reduced the damage caused by toxic agents in the testicles. Insecticides such as acetamiprid and chlorpyrifos have been shown to reduce the weights of the testicles and the epididymis in rats, to decrease sperm count, to increase levels of serum thiobarbituric acid reactive substances which indicate

Table 1

Biochemical marker levels in testicular tissues of groups.

Groups	MDA (nmol/g)	SOD (U/mg protein)	GSH-Px (U/mg protein)	CAT (K/g protein)	TAS (μmol Trolox equivalent/L)	TOS (μmol H_2O_2 Trolox equivalent/L)	OSI (Arbitrary unit)
C (n=5)	171.46 (103.5–188.9)	0.28 (0.27–0.29)	8.26 (4.6–9.4)	2.13±0.34	0.92±0.06	5.81 (4.16–7.59)	6.20±0.35
4FA (n=7)	160.39 (118.9–314.9)	0.26 (0.24–0.28)	5.25 (3.1–7.7)[a]	1.89±0.10	0.52±0.05[a]	7.64 (4.83–10.51)	14.70±1.75[a]
4FA+NSO (n=7)	302.61 (240.2–334.7)	0.26 (0.24–0.31)	4.58 (2.9–8.1)	1.64±0.17	0.89±0.04[c]	9.67 (5.44–14.17)	10.64±1.26
13FA (n=7)	348.52 (240.0–743.3)[a]	0.26 (0.24–0.33)	3.39 (1.8–5.6)[a]	1.62±0.14[a]	0.86±0.04	9.57 (6.13–12.25)	11.20±1.29[a]
13FA+NSO (n=7)	299.95 (125.2–386.8)[b]	0.26 (0.24–0.29)	5.29 (2.6–7.7)	2.28±0.16[b]	0.79±0.10	7.89 (4.79–11.41)	10.20±0.70

[a]: P<0.05 compared to the C group; [b]: P<0.05 compared to the 13FA group; [c]: P<0.05 compared to the 4FA group.

oxidative cell injury, and to reduce plasma and tissue GSH-Px, CAT and SOD activities. Administering NSO together with insecticides was reported to have reduced these injuries[24,25]. Likewise, in our study, we both histologically and biochemically demonstrated that NSO reduced the toxic effects of inhaled FA. However, a statistically significant increase in CAT activity was seen only in the group to which NSO was administered for 13 weeks, the longest time, which supports the fact that the effect of NSO on CAT activity does not occur in the short term.

There are studies in the literature that likewise report that changes were identified in both histopathologic and biochemical parameters after testicular injury was induced with various chemicals or by torsion/detorsion, and these were corrected by various antioxidant agents[26–30].

Lipids are one of the major targets of oxidative stress. MDA is a highly toxic substance and a secondary product of lipid peroxidation. Its interaction with DNA and proteins can give rise to severe outcomes[31]. Measuring TOS, as well as TAS for antioxidants such as GSH-Px, CAT and SOD, can be useful in determining MDA levels[30]. Changes in TAS, TOS and OSI levels are widely used for assessing oxidative stress[28]. Compared with measuring each antioxidant individually, TAS provides more information about the total antioxidant capacity. OSI, calculated by dividing total oxidants into total antioxidants, is a significant marker showing the increase in oxidants or the decrease in antioxidants. OSI presents both cost and time savings compared to individually calculating each of the oxidants and antioxidants[29]. Depending on the chemical agents, reduction in antioxidant enzyme activities such as SOD, CAT, GSH-Px unfavorably affects the male reproductive system[24,25]. NSO, on the other hand, makes a favorable impact on the male reproductive system by reducing the formation of free radicals[24].

Antioxidants play a protective role against oxidative damage in the testicles. Prevention of oxidative damage will lead to improvement in histopathologic changes[20]. Similar to the reports in the literature, in our study, administration of FA for a long-term (13 weeks) indicated an increase in oxidative stress and tissue injury more than a short-term (4 weeks) administration[32]. In summary, NSO as an antioxidant should be used for a longer term to achieve protective efficacy both histopathologically and biochemically in the testicular tissue.

Conflict of interest statement

We declare that there is no conflict of interest.

References

[1] Baan R, Benbrahim-Tallaa L, Bouvard V, Carel R, Cogliano V, El Ghissassi F, et al. Chemical agents and related occupations. A review of human carcinogens. [Online]. Available from: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100F-29.pdf [Accessed on 16 August 2018].

[2] Tang X, Bai Y, Duong A, Smith MT, Li L, Zhang L. Formaldehyde in China: Production, consumption, exposure levels, and health effects. *Environ Int* 2009; **35**(8): 1210-1224.

[3] Danzon MA, Rolaf van Leeuwen FX, Krzyzanowski M. Air quality guidelines for Europe. [Online]. Available from: http://www.euro.who.int/__data/assets/pdf_file/0014/123062/AQG2ndEd_5_8Formaldehyde.pdf [Accessed on 16 August 2018].

[4] Heck H, Casanova M. Pharmacodynamics of formaldehyde: Applications of a model for the arrest of DNA replication by DNA-protein cross-links. *Toxicol Appl Pharmacol* 1999; **160**(1): 86-100.

[5] Vosoughi S, Khavanin A, Salehnia M, Asilian Mahabadi H, Shahverdi A, Esmaeili V. Adverse effects of formaldehyde vapor on mouse sperm parameters and testicular tissue. *Int J Fertil Steril* 2013; **6**(4): 250-267.

[6] Zhou D, Zhang J, Wang H. Assessment of the potential reproductive toxicity of long-term exposure of adult male rats to low-dose formaldehyde. *Toxicol Ind Health* 2011; **27**(7): 591-598.

[7] Akman Y. *Angiospermae*. 1st edition. İstanbul, Turkey: Palme Yayıncılık; 2007, p. 36-46.

[8] Toptaş A. *Black cumin, healthful from top to bottom*. 1st edition. İstanbul, Turkey: Sade Ofset; 2008, p. 37-52.

[9] Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylenetetrazol-induced kindling in mice. *Neuropharmacology* 2005; **49**(4): 456-464.

[10] Benhaddou-Andaloussi A, Martineau LC, Spoor D, Vuong T, Leduc C, Joly E, et al. Antidiabetic activity of *Nigella sativa* seed extract in cultured pancreatic beta-cells, skeletal muscle cells, and adipocytes. *Pharm Biol* 2008; **46**: 96-104.

[11] Mansour SW, Sangi S, Harsha S, Khaleel MA, Ibrahim AR. Sensibility of male rats fertility against olive oil, *Nigella sativa* oil and pomegranate extract. *Asian Pac J Trop Biomed* 2013; **3**: 563-568.

[12] Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* 1978; **86**: 271-278.

[13] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988; **34**: 497-500.

[14] Aebi H. Catalase. In: Hu B (ed.) *Methods of enzymatic analysis*. New York: Academic Press; 1974.

[15] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70**(1): 158-169.

[16] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004; **37**(4): 277-285.

[17] Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005; **38**(12): 1103-1111.

[18] Aycicek A, Erel O, Kocyigit A. Decreased total antioxidant capacity and

increased oxidative stress in passive smoker infants and their mothers. *Pediatr Int* 2005; **47**(6): 635-639.

[19]Ulucam E, Bakar E. The effect of proanthocyanidin on formaldehyde-induced toxicity in rat testes. *Turk J Med Sci* 2016; **46**(1): 185-193.

[20]Kose E, Sarsilmaz M, Tas U, Kavakli A, Turk G, Ozlem Dabak D, et al. Rose oil inhalation protects against formaldehyde-induced testicular damage in rats. *Andrologia* 2012; **44**: 342-348.

[21]Han SP, Zhou DX, Lin P, Qin Z, An L, Zheng LR, et al. Formaldehyde exposure induces autophagy in testicular tissues of adult male rats. *Environ Toxicol* 2015; **30**(3): 323-331.

[22]Tajaddini S, Ebrahimi S, Behnam B, Bakhtiyari M, Joghataei MT, Abbasi M, et al. Antioxidant effect of manganese on the testis structure and sperm parameters of formalin-treated mice. *Andrologia* 2014; **46**(3): 246-253.

[23]Umar Z, Qureshi AS, Rehan S, Ijaz M, Faisal T, Umar S. Effects of oral administration of black seed (*Nigella sativa*) oil on histomorphometric dynamics of testes and testosterone profile in rabbits. *Pak J Pharm Sci* 2017; **30**: 531-536.

[24]Mosbah R, Djerrou Z, Mantovani A. Protective effect of *Nigella sativa* oil against acetamiprid induced reproductive toxicity in male rats. *Drug Chem Toxicol* 2018; **41**(2): 206-212.

[25]Mosbah R, Yousef MI, Maranghi F, Mantovani A. Protective role of *Nigella sativa* oil against reproductive toxicity, hormonal alterations, and oxidative damage induced by chlorpyrifos in male rats. *Toxicol Ind Health* 2016; **32**(7): 1266-1277.

[26]Pinar N, Cakirca G, Ozgur T, Kaplan M. The protective effects of alpha lipoic acid on methotrexate induced testis injury in rats. *Biomed Pharmacother* 2018; **97**: 1486-1492.

[27]Daggulli M, Dede O, Utangac MM, Bodakci MN, Hatipoglu NK, Penbegul N, et al. Protective effects of carvacrol against methotrexate-induced testicular toxicity in rats. *Int J Clin Exp Med* 2014; **7**(12): 5511-5516.

[28]Tusat M, Mentese A, Demir S, Alver A, Imamoglu M. Medical ozone therapy reduces oxidative stress and testicular damage in an experimental model of testicular torsion in rats. *Int Braz J Urol* 2017; **43**(6): 1160-1166.

[29]Yuvanc E, Tuglu D, Ozan T, Kisa U, Balci M, Batislam E, et al. Investigation of the antioxidant effects of pheniramine maleate and nebivolol on testicular damage in rats with experimentally induced testis torsion. *Acta Cir Bras* 2018; **33**(2): 125-133.

[30]Jahromi AR, Rasooli R, Kamali Y, Ahmadi N, Sattari E. Short-term effects of date palm extract (*Phoenix dactylifera*) on ischemia/reperfusion injury induced by testicular torsion/detorsion in rats. *Pharmacognosy Res* 2017; **9**(1): 69-73.

[31]Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* 2005; **15**(4): 316-328.

[32]Sapmaz E, Sapmaz HI, Vardi N, Tas U, Sarsilmaz M, Toplu Y, et al. Harmful effects of formaldehyde and possible protective effect of *Nigella sativa* on the trachea of rats. *Niger J Clin Pract* 2017; **20**(5): 523-529.