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Original Article

Melatonin ameliorates oxidative DNA damage and protects against formaldehyde-induced oxidative stress in rats

Sezgin Aydemir[1,2], Sevcan Gul Akgun[1], Ayfer Beceren[1], Meral Yuksel[3], Meltem Kumas[4], Nusret Erdogan[2], Semra Sardas[1], Gulden Zehra Omurtag[5]

[1]Department of Toxicology, School of Pharmacy, Marmara University, Haydarpasa-Istanbul, Turkey; Departments of [2]Pathology Laboratory Technicianship, [3]Medical Laboratory Technicianship, [4]Medical Laboratory Techniques, Vocational School of Health Related Services, Marmara University, Haydarpasa-Istanbul, Turkey; [5]Department of Toxicology, School of Pharmacy, Istanbul Medipol University, Kavacık-Beykoz-Istanbul, Turkey

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Abstract: Formaldehyde (FA) is an organic chemical which is widely used all over the world and has hazardous effects for the environment. FA can react with many biomolecules in the biological systems and lead to toxic effects on humans. Melatonin (MEL), a neurohormone produced by pineal gland, has been shown to be an effective antioxidant with free radical scavenging properties. The present study aimed to evaluate the ameliorative effects of MEL on FA-induced toxicity by monitoring oxidant/antioxidant and histopathological changes in the lung, liver and kidney tissues of rats as well as DNA damage in the blood samples. FA was administered through inhalation at a rate of 6 ppm for 6 weeks and intraperitoneal injection at a rate of 10 mg/kg/day for 14 days. MEL was administered in related groups at a rate of 10 mg/kg/day. Upon the completion of the experimental protocol, tissues were dissected for processing biochemical assays and routine histological staining. Blood samples were collected to investigate DNA damage with the comet assay and ELISA kit for 8-hydroxydeoxyguanosine (8-OHdG). FA exposures increased the levels of DNA damage, malondialdehyde and myeloperoxidase activity and reduced glutathione levels. FA also significantly raised the level of tissue reactive oxygen species. FA-induced morphological changes in the tissues were also observed with the light microscope. These alterations were reversed by MEL treatment. In conclusion, the present study suggests that oxidative mechanisms play an important role in FA toxicity. MEL ameliorates oxidative tissue and DNA damage resulting from FA-induced toxicity by balancing oxidant-antioxidant status, inhibiting neutrophil infiltration and reducing 8-OHdG level, and might be beneficial in reducing FA-induced oxidative tissue and DNA damage.

Keywords: Formaldehyde, melatonin, oxidative stress, chemiluminescence, comet assay, DNA damage, histopathology

Introduction

Formaldehyde (FA), a colorless, water soluble and flammable organic compound, is widely used in various industrial facilities for the synthesis of numerous chemicals. These include urea and phenol resins, building materials (plywood, roofing felt etc.), and furniture manufacturing applications. FA is also widely used for preserving or fixing human and animal remains or tissues in spread anatomy and pathology laboratories of the hospitals and universities. FA is naturally found in fruits (apples, peals etc.) and is an endogenous metabolite in mammals, including humans [1-3]. Occupational exposure to FA occurs in a wide range of doses; for example it changes from 2 to 5 ppm in the furniture varnishing process, and it has been reported as greater than or equal to 3 ppm in short-term exposures for pathologists and embalmers in the pathology laboratories where embalming takes place. In both industrial areas and laboratories, human exposure to FA occurs mainly by inhalation [3].

Inhaled FA has toxic effects on central nervous system, respiratory system, reproductive system and bone marrow in addition to its well-known hepatotoxic effects [4, 5]. The main concern about its toxicity is that FA has a strong

mutagenic effect to living organisms and is classified as carcinogenic to humans in group 1 [3]. Additionally, FA causes irritation to the eye, nose and nasopharynx via inhalation, and is responsible for some adverse allergic reactions such as contact dermatitis and asthma [6]. The high solubility and reactivity of FA enable it to interact readily and reversibly with mucus or with other cellular macromolecules that have nucleophilic groups, including amino acids and DNA [7]. The ability of FA to interact with DNA leads to the formation of DNA cross-links and formaldehyde-derived adducts that are believed to be the responsible for the genotoxic and carcinogenic effects of FA [8]. Oxidative stress resulting from increased reactive oxygen species (ROS) levels in cells is thought to be related to the occurrence of various diseases including Alzheimer's, heart failure and cancer [9, 10]. The previous studies reported that FA exposure causes oxidative stress by effecting enzymes such as superoxide dismutase (SOD) and catalase (CAT) and disturbing the antioxidant defense system in the body [11].

Melatonin (MEL) is a neurohormone secreted at night, primarily by the pineal gland [20], but also by the skin, bone marrow, retinas and thymus [12]. Due to its ability to cross all biological membranes, MEL is secreted into the capillaries and distributed to most of the body tissues once formed. MEL plays a powerful role in the amelioration of cardiovascular complication, convulsion therapy and circadian rhythm sleep disorders like shift work sleep disorder and jet lag [13]. Along with the major physiological activity of MEL - the regulation of the sleep-wake cycle - several of its other properties have been also reported in the last two decades. These include the direct scavenging activity of free radicals and gene regulation of antioxidant enzymes such as SOD, CAT and glutathione peroxidase [14, 15]. The promising properties of MEL suggest that it might inhibit DNA-adducts upon reaching to its highest levels in the nucleus of the cell, protecting lipids, proteins and other biological macromolecules from both endogenous and exogenous free radical generated oxidative damage [16, 17].

The present study was carried out to determine the toxic effects of FA administered through inhalation and intraperitoneal (i.p.) injection, and to reveal the ameliorative effects of MEL on FA-induced oxidative stress and DNA damage in rats. For this purpose, Wistar albino rats were exposed to FA either by i.p. injection at a dose of 10 mg/kg/day for 14 days or by inhalation at a dose of 6 ppm for 8 hours/day, 5 consecutive days per week, for 6 weeks, to mimic occupational exposure. To evaluate the potentially protective role of MEL treatment on FA-induced toxicity in rats, certain biochemical assays and histopathological staining were conducted in the lung, liver and kidney tissues while genotoxicity assays were performed with the blood samples after completion of the experimental protocol.

Materials and methods

Chemicals

Melatonin, 10% neutral buffered formalin, 3-Carboxy-4-nitrophenyl disulfide (DTNB), dimethyl sulfoxide (DMSO), o-dianisidine, 2-thiobarbituric acid, trichloroacetic acid (TCA), low-melting agarose (LMA) and high-melting agarose (HMA) were purchased from Sigma-Aldrich, Inc. (St Louis, MO, USA); ELISA kit for 8-hydroxy-2'-deoxyguanosine (8-OHdG) was obtained from Wuhan USCN Business Co., Ltd. (Houston, USA). All other chemicals were used in analytically grade.

Animals

The experimental protocol used in the present study was approved by the Marmara University School of Medicine Animal Care and Use Committee (Approval ID and Date: 192015. mar, March 06, 2015). Female and male Wistar albino rats with a body weight of 250 to 300 g were used in this study. Animals were housed in polypropylene cages at room temperature (22±2°C) with 12 h light/12 h dark cycle. Animals fed with a standard rat pellet and water was available *ad libitum*.

Experimental design

Rats were divided randomly into 6 experimental groups (control, FA-inh, FA-ip, MEL, FA-inh+MEL and FA-ip+MEL) of 8 animals each, with an equal number of male and female rats. The FA-inh group was exposed to 6 ppm of FA for 8 hours/day and 5 consecutive days per week, for 6 weeks in a special inhalation chamber (18). The FA-ip group was injected i.p. 10 mg/

kg/day FA for 14 days. MEL treated groups (MEL, FA-inh+MEL and FA-ip+MEL) received i.p. injection of MEL (solute in saline; 1:10, ethyl alcohol and saline) at a dose of 10 mg/kg/day and control group was injected with same dose of saline. Upon completion of the experimental protocol, all animals were decapitated and the blood samples of each animal were collected separately into heparinized tubes and the comet assay was performed with the fresh blood samples. The rest of the blood samples were centrifuged at 3500 rpm for 10 min, after which the plasma was removed carefully and divided into aliquots kept at -20°C until 8-OHdG measurements were performed. The lung, liver and kidney of rats were carefully removed and washed with saline. For biochemical assays, tissue samples of each organ were separately kept at -20°C until the malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and luminol, lucigenin and nitric oxide (NO) chemiluminescence (CL) measurements were performed. For the histopathological examination, tissue samples of each organ were stored in 10% neutral buffered formalin at room temperature.

Inhalation chamber

The inhalation protocol of Valentine and Kennedy [18] was applied with minor modifications in a special inhalation chamber purchased from a local company named Zenon Diagnostic (Inhalation Exposure Unit, Cabinet 1000, Istanbul, Turkey). Gaseous FA was prepared from 10% formalin. The concentration during exposure was adjusted and monitored with a ToxiRAE Pro dedector (RAE Systems, San Jose, CA, USA). Air temperature, the relative humidity and airflow rate were maintained at 22±2°C, 45-55% and 1.65±0.15 m^3/h, respectively. A filter made of activated charcoal was used to inhibit the release of FA via the exhausted air from the cabin, in order to protect the environment and prevent undesired human exposure.

The single cell gel electrophoresis (comet assay)

The standard protocol for alkaline comet assay was operated for the lymphocytes of the animals with minor modifications of Singh *et al.* [19]. Cells were mixed with 0.65% LMA and placed on HMA-coated slides (two duplicates for each sample), with coverslip. The slides were kept at 4°C to solidify, after which the coverslip were removed and the slides were carefully immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10) for 1.5 h at 4°C. After the lysing process, the slides were placed in a tank and electrophoresed for 20 min at 300 mA and 15 V. Then the slides were gently removed from the tank and washed 3 times for 5 min each with a neutralizing solution (0.4 M Tris buffer, pH 7.5) to neutralize the residual alkali. Subsequently the slides were fixed with 50%, 75% and absolute ethyl alcohol respectively, and laid flat to dry at room temperature. Each slide was stained with 50 µL ethidium bromide (EtBr, 20 µl/ml) and then covered with a coverslip. Image analysis of slides was carried out at X40 magnification under a fluorescent microscope (Olympus, BX51, Tokyo, Japan) equipped with an 546 nm excitation filter and a 590 nm barrier filter. Based on the principle of releasing damaged DNA from the core of the nucleus during electrophoresis, comets are formed. 100 cells per sample (two duplicate sample slides, 50 randomly selected cells scored per slide) were scored to count the percentage DNA in tail (%DNA_T) using BAB Bs200Pro image analysis software (BAB LTD., Ankara, Turkey).

Enzyme-linked immunosorbent assay (ELISA) for 8-hydroxydeoxyguanosine (8-OHdG)

The plasma levels of 8-OHdG were measured with an ELISA Kit for 8-OHdG (Cat No: CEA660Ge, Wuhan USCN Business Co., Ltd., Houston, USA) according to the manufacturer's instructions. The principle of the assay is based on the competitive inhibition reaction between biotin labeled 8-OHdG and unlabeled 8-OHdG (standards or samples) with the pre-coated antibody specific to 8-OHdG. Briefly, standards (5 different concentrations) or samples were added into the appropriate wells in duplicate and then incubated at 37°C. Followed by the chromogenic reaction, the absorbance was measured at 450 nm using a microplate reader (EL×800, BioTek Instruments, Inc., Winooski, USA). The content of 8-OHdG in plasma samples was expressed as pg/ml.

Tissue MDA and GSH levels

Lipid peroxidation levels were investigated in terms of MDA equivalent in lung, liver and kidney tissues of rats. Tissues were homogenized

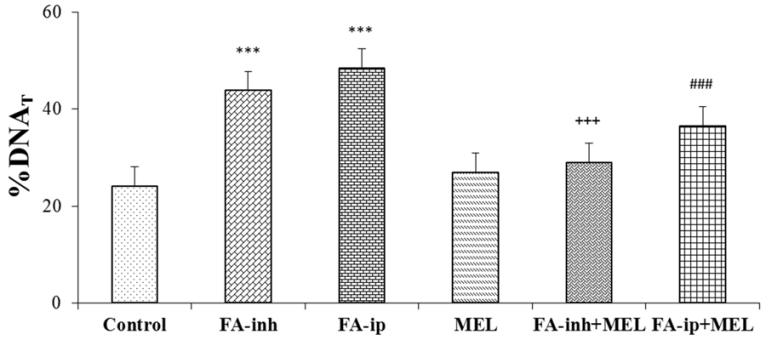

Figure 1. Measurements of the percentages DNA in tail (%DNA_T) in the lymphocytes. Data are represented as mean ± standard deviation. ***P<0.001 compared to the control group, +++P<0.001 compared to the FA-inh group and ###P<0.001 compared to the FA-ip group (n = 8).

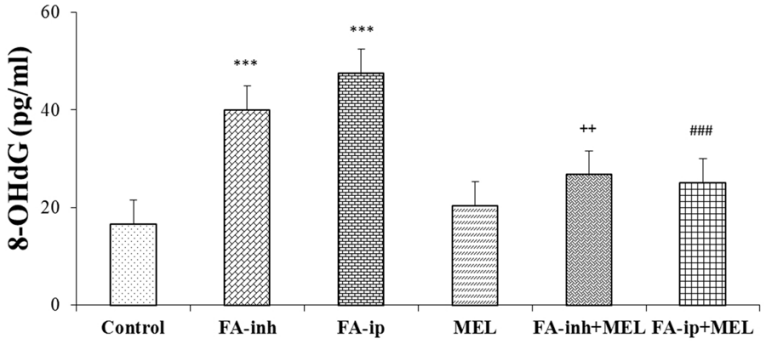

Figure 2. 8-Hydroxydeoxyguanosine (8-OHdG) levels in the plasma samples. Data are represented as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001 compared to the control group, ++P<0.01, +++P<0.001 compared to the FA-inh group and ###P<0.001 compared to the FA-ip group (n = 8).

(w/v 10%) in 10% trichloroacetic acid and then centrifuged at 3000 rpm for 15 min. Supernatant was aspirated and MDA levels were determined by monitoring the reaction between same volume of thiobarbituric acid and supernatant at 100°C based on the level of absorbance at 532 nm in a spectrophotometer (Beckman Coulter DU 73, Fullerton, California, USA). The results were presented as nmol MDA/g tissue [20]. A modified Ellman procedure was performed to measure GSH levels in tissues using a spectrophotometer at 412 nm [21].

Tissue myeloperoxidase activity

Tissue myeloperoxidase (MPO) activity assay was performed in a procedure similar to that described by Hillegas et al. [22]. Tissue samples were homogenized (w/v 10%) in an ice-cold 50 mM potassium phosphate buffer (PB, pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HETAB), and ethylenediaminetetraacetic acid (EDTA) and centrifuged at 12.000 rpm at 4°C for 10 min. Aliquots were added to 2.9 ml of reaction mixture consisting of 50 mM PB, o-dianisidine and 20 mM H_2O_2 solution in a 37°C water bath. The MPO activity was determined such that one unit of enzyme activity caused a change in the absorbance measured with spectrophotometer at 460 nm for 3 min. The results were expressed as U/g tissue.

Chemiluminescence measurements

Chemiluminescence (CL) is a noninvasive method typically used for the determination of ROS. The assay was carried out at room temperature using a luminometer (Junior LB 9509, EG & G Berthold, Germany). Luminol and lucigenin probes were used as enhancers to generate the light emissions of the ROS released from the tissues. Luminol is selective for hydroxyl radical, hydrogen peroxide, hydroperoxyl and hypochlorite, while lucigenin is selective for superoxide radical. Samples were put into vials containing 2.0 ml of 0.5 M phosphate buffered saline (PBS) solution and 0.02 M 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES) solution. ROS were quantified for an accounting period of 5 min with 1 min intervals after the addition of 0.2 mM of each enhancer. Nitric oxide (NO) levels were also measured in the tissues using luminol-sodium salt and H_2O_2. Results were given as relative light units per mg tissue (rlu/mg tissue) by calculating the area under the curve [23].

Histopathological analysis

For the light microscopic examinations, 10% neutral buffered formalin fixed tissues (lung,

Table 1. Malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) activity in the lung, liver and kidney tissues of rats

Tissues	Groups	MDA (nmol/g tissue)	GSH (μmol/g tissue)	MPO (U/g tissue)
Lung	Control	7.14±0.89	4.81±0.99	0.35±0.06
	FA-inh	13.33±1.49***	2.13±0.52***	0.79±0.22***
	FA-ip	8.8±0.67*	3.42±0.22***	0.52±0.13*
	MEL	6.91±0.73	4.91±0.35	0.29±0.07
	FA-inh+MEL	8.21±0.55+++	4.12±0.40+++	0.37±0.08+++
	FA-ip+MEL	7.39±0.50#	4.02±0.50	0.41±0.08
Liver	Control	11.31±1.01	6.57±0.60	0.22±0.07
	FA-inh	15.33±1.32***	2.42±0.90***	0.41±0.15**
	FA-ip	19.58±1.85***	3.17±0.48***	0.53±0.12***
	MEL	12.66±1.06	6.28±0.97	0.26±0.08
	FA-inh+MEL	12.83±0.89++	5.56±0.69+++	0.30±0.06+
	FA-ip+MEL	12.52±1.55###	5.4±1.05###	0.31±0.05##
Kidney	Control	15.90±3.20	4.70±0.39	0.18±0.03
	FA-inh	20.55±2.51*	3.16±0.40***	0.32±0.10***
	FA-ip	24.09±2.91***	3.35±0.30***	0.38±0.04***
	MEL	14.81±2.70	5.10±0.37	0.16±0.04
	FA-inh+MEL	16.94±2.48	4.26±0.33+++	0.25±0.06
	FA-ip+MEL	17.95±2.27##	4.02±0.57#	0.24±0.06##

Data are represented as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001 compared to the control group, +P<0.05, ++P<0.01, +++P<0.001 compared to the FA-inh group and #P<0.05, ##P<0.01, ###P<0.001 compared to the FA-ip group (n = 8).

liver and kidney) were embedded in paraffin, 3 μm sections were taken and stained with hematoxylin and eosin (H&E) [24].

Statistics

Statistical analysis was carried out using the GraphPad Instat (GraphPad Software, San Diego, CA) program. All results are given as mean ± standard deviation (SD); groups of data were compared with one-way analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison tests. Values of *P*<0.05 were considered as significant.

Results

The levels of %DNA$_T$

The effect of MEL on FA-induced DNA damage was evaluated by comet assay in lymphocytes and the results were given as %DNA$_T$. As a consequence of FA exposure through inhalation and i.p. injection, the %DNA$_T$ levels were significantly (P<0.001) higher than those of the control group (**Figure 1**). MEL treatment brought out a significant (P<0.001) reduction in DNA damage in the FA-inh+MEL and FA-ip+MEL groups compared to related FA exposure groups. Additionally, only MEL treated rats displayed levels of %DNA$_T$ similar to the control.

The plasma 8-OHdG levels

Increased levels of 8-OHdG were observed in FA-inh and FA-ip groups compared to the control, significantly (P<0.001). MEL treatment reduced the 8-OHdG levels in the FA-inh+MEL group (26.75±1.40; P<0.01) and FA-ip+MEL group (25.17±1.55; P<0.001) with respect to related FA exposure groups (**Figure 2**).

Tissue MDA and GSH levels

The results of MDA and GSH measurements are shown in **Table 1**. Significant differences in MDA and GSH levels between FA-exposed groups (FA-inh and FA-ip) and the control group were observed. In FA-inh group, FA exposure escalated the MDA levels of the lung, liver and kidney tissues (P<0.001, P<0.001, P<0.05, respectively) compared to the control. MDA levels of these tissues were also increased in the FA-ip group. Moreover, FA exposure induced the depletion of the GSH levels of the tissues. MEL treatment significantly reversed these effects and reduced the tissue MDA levels while increasing the GSH levels.

Tissue myeloperoxidase activity

Myeloperoxidase activity, a notable marker of neutrophil infiltration, was significantly higher in the lung, liver and kidney tissues of rats exposed to FA via inhalation (P<0.001, P<0.01 and P<0.001, respectively), and MEL treatment reduced this effect in the lung and liver tissues of FA-inh+MEL group, significantly (P<0.001 and P<0.05, respectively). The MPO activity of the lung, liver and kidney tissues in FA-ip group was determined to be statistically higher than that of the control group (P<0.05, P<0.001 and

Table 2. Luminol, lucigenin enhanced chemiluminescence (CL) measurements and nitric oxide (NO) levels in the lung, liver and kidney tissues of rats

Tissues	Groups	Luminol (rlu/mg)	Lucigenin (rlu/mg)	NO (rlu/mg)
Lung	Control	5.13±1.50	4.33±0.93	5.30±1.47
	FA-inh	13.44±2.76***	36.70±11.18***	234.50±82.22***
	FA-ip	7.48±3.02	15.69±4.70***	99.98±66.62**
	MEL	3.94±0.79	4.08±0.96	4.78±0.94
	FA-inh+MEL	5.04±0.64^{+++}	6.52±2.47^{+++}	69.25±17.57^{+++}
	FA-ip+MEL	5.40±1.72	4.50±1.01$^{###}$	38.80±10.50
Liver	Control	3.25±1.05	3.61±1.08	3.88±1.04
	FA-inh	5.96±1.48***	8.54±2.90***	26.71±12.33***
	FA-ip	5.60±1.05***	8.68±1.76***	35.28±15.90***
	MEL	2.64±0.70	2.70±0.43	2.69±1.10
	FA-inh+MEL	2.98±0.63^{+++}	4.60±1.78^{+++}	11.68±7.24^{+}
	FA-ip+MEL	2.93±0.56$^{###}$	3.88±1.48$^{###}$	14.37±4.52$^{##}$
Kidney	Control	4.01±1.29	3.81±1.14	4.44±1.47
	FA-inh	4.56±0.52	4.48±1.03	4.30±1.10
	FA-ip	5.63±2.04	15.47±8.16***	38.58±7.24***
	MEL	2.96±1.06	3.03±1.66	2.86±0.70
	FA-inh+MEL	5.36±2.26	3.53±1.33	3.75±0.88
	FA-ip+MEL	3.35±0.94	5.75±3.34$^{###}$	16.70±6.48$^{###}$

Data are represented as mean ± standard deviation. **P<0.01, ***P<0.001 compared to the control group, $^{+}$P<0.05, $^{+++}$P<0.001 compared to the FA-inh group and $^{##}$P<0.01, $^{###}$P<0.001 compared to the FA-ip group (n = 8).

P<0.001, respectively). MEL treatment in the FA-ip+MEL group significantly reduced the MPO activity of these tissues (**Table 1**).

Chemiluminescence measurements

Luminol enhanced CL measurements were significantly increased (P<0.001) in the liver tissues of the FA-inh and FA-ip groups (5.96± 1.48 rlu, 5.60±1.05 rlu, respectively) and in the lung tissue of FA-inh group (13.74±2.76 rlu) compared to the control group, however, no significant change in the kidney tissue was observed (**Table 2**). MEL treatment reduced the increased luminol levels in the lung and liver tissues of the FA-inh and FA-ip groups. Lucigenin enhanced CL measurements were significantly increased in the lung and liver tissues of the FA-inh (15.69±4.70 rlu, 8.54±2.9 rlu, respectively) and FA-ip (36.70±11.18 rlu, 8.68±1.76 rlu, respectively) groups compared to the control. In the kidney tissue, the lucigenin level of the FA-inh group remained unchanged (P>0.05), while only the FA-ip group showed increased lucigenin levels compared to the control (P< 0.001). MEL treatment reduced these effects in the kidney tissue significantly (P<0.001) and decreased the lucigenin levels in the FA-inh+MEL and FA-ip+ MEL groups (**Table 2**). The NO levels of the lung and liver tissues were significantly higher in FA-inh (P<0.001, P<0.001) and FA-ip groups (P<0.01, P<0.001). Treatment with MEL reduced NO levels of these tissues in the FA-inh+MEL and FA-ip+MEL groups. In the kidney tissue, only the FA-ip group showed a higher NO level than the control (P<0.001) and the MEL supplement decreased NO level in the FA-ip+MEL group, significantly (P<0.001).

Light microscopic findings

Formaldehyde inhalation induced alveolar damages as well as congestion, hemorrhage, edema and interstitial inflammatory cell infiltration in H&E stained sections of FA-exposed rat lung tissue (**Figure 3**). Epithelial desquamation, thickening of the alveolar septa and emphysematous changes were also observed in the lung samples. Similar findings were observed in FA-ip group with less severe inflammatory cell infiltration and emphysematous findings than in the FA-inh group. MEL therapy slightly reduced these effects as indicated by the less pronounced emphysematous changes and the thickening of the alveolar septa.

The normal structures of hepatic tissue were observed in the control group; no signs of inflammation, congestion and edema were detected and portal areas appeared normal (**Figure 4**). FA exposures (both via i.p. and inhalation) caused damages in the portal areas and mild edema along with Kupffer cell proliferation in portal areas. Mild congestions in sinusoidal areas and the hepatic vein were examined in H&E stained sections of the liver tissues of FA-exposed rats. MEL treatment slightly reduced the destructive effects of FA in hepatic tissue with reducing congestion in the sinusoidal and portal areas (**Figure 4**).

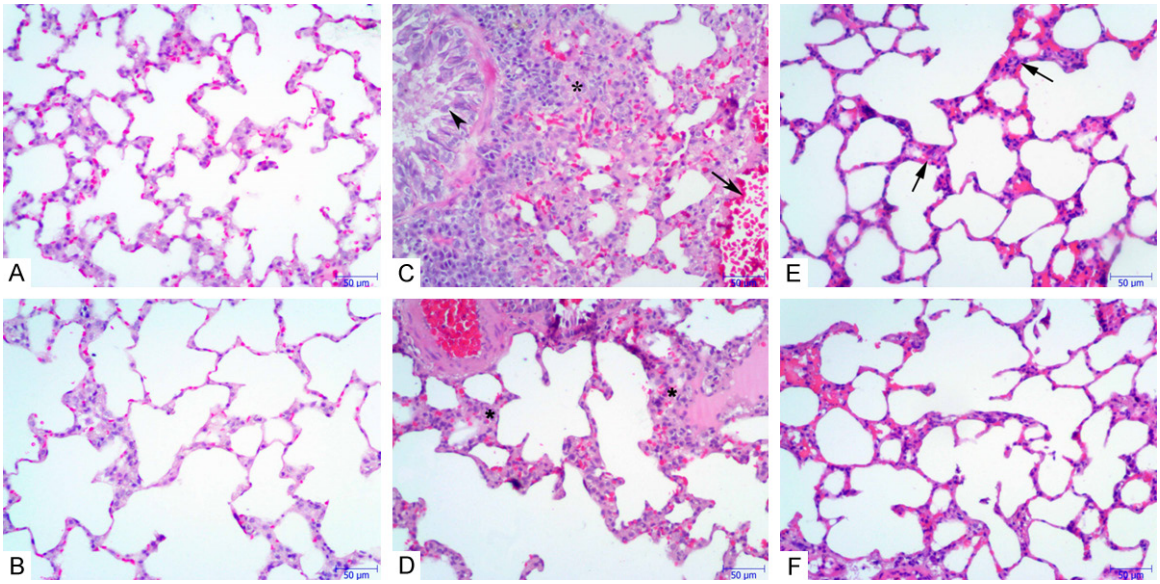

Figure 3. A: Alveoli structure of normal lung tissue in the control group. B: Alveolar shape and size were regular in the MEL group. C: Epithelial desquamation (arrowhead), inflammatory cell infiltration (*) and hemorrhaging (arrow) could be observed in the FA-inh group. D: The damages were less pronounced after MEL treatment in the FA-inh+MEL group. E: Slight alveolar septa thickening and congestion (arrow) in the FA-ip group. F: Alveolar septum was slightly regular after MEL treatment in the FA-ip+MEL group. (H&E, Bar = 100 μm).

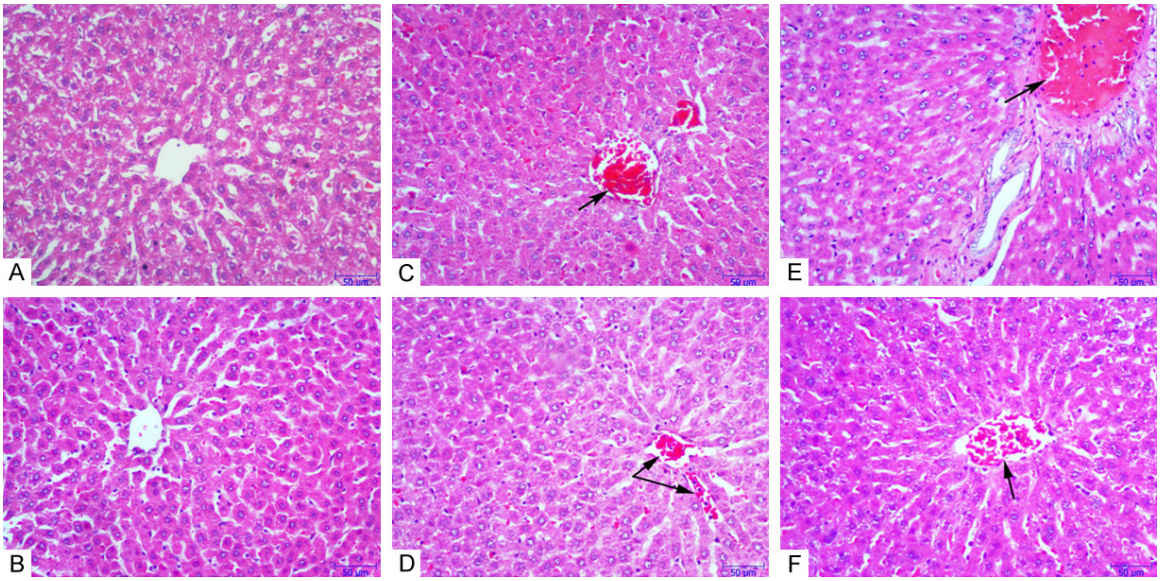

Figure 4. A: Normal structure of liver tissue in the control. B: MEL group; similar structures were observed as in control group. C: Congestion (arrow) in the FA-inh group. D: MEL treatment reduced the degeneration slightly (arrow). E: Congestion (arrow) in the FA-ip group. F: Congestion (arrow) was less pronounced in the FA-ip+MEL group. (H&E, Bar = 100 μm).

Histopathological examination of kidney tissue sections of the control rats revealed the normal structure of renal corpuscles and renal tubules. The amounts and shapes of glomerules were normal. Interstitial lymphoplasmocytes cell infiltration, glomerular enlargement and congestion were observed in the FA-exposed groups (i.p. and inhalation). MEL treatment did not reduce these effects adequately in FA-exposed rats (**Figure 5**).

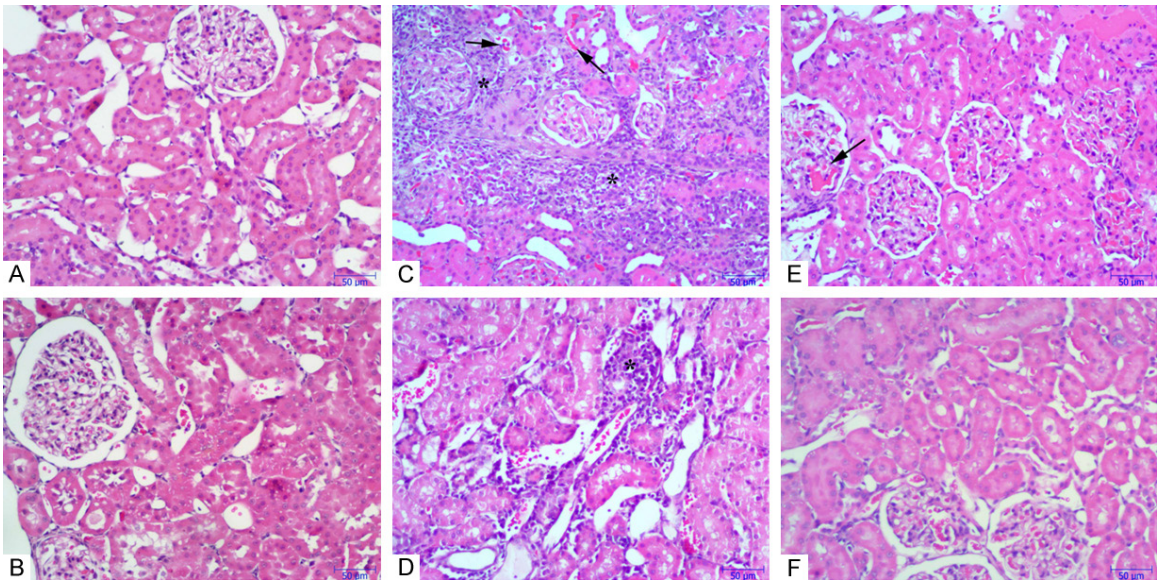

Figure 5. A: Section of normal kidney tissue in the control group. B: Similar observation was shown for MEL group as in the control group. C: Intense inflammatory cell infiltration (*) and congestion (arrow) in the FA-inh group. D: MEL treatment reduced inflammatory cell infiltration (*) in the FA-inh+MEL group. E: Degeneration and congestion in glomeruli and tubule (arrow) could be observed in the FA-ip group. F: MEL slightly reduced these effects in the FA-ip+MEL group. (H&E, Bar = 100 µm).

Discussion

The toxic effects of formaldehyde on lung, liver and kidney tissues have documented in previous studies. In addition to its detrimental effects on organ systems, FA has been found to be responsible for DNA damage due to the formation of strand breaks and adducts [3, 25-27]. In this study, we observed that FA exposure caused increases in the $\%DNA_T$, 8-OHdG level, MPO activity, MDA and ROS levels while reducing the GSH levels in rats; these were reversed by the MEL treatment.

The comet assay is used for biomonitoring exposure to genotoxic agents including formaldehyde and demonstrates the level of DNA damage in lymphocytes [28-30]. In this study, rats exposed to FA, either by inhalation or i.p. injection, showed enhanced levels of DNA damage as demonstrated by the comet assay along with the raised plasma levels of the oxidative DNA damage marker 8-OHdG. Similarly, in their experimental study on oxidative DNA damage, Ciftci *et al.* [31] observed that formaldehyde exposure increased the level of 8-OhdG. Additionally, the increased DNA damage in the peripheral blood lymphocytes and the relation of this damage to the levels of FA exposure was demonstrated in another study conducted with pathologic anatomy laboratory workers [32]. Moreover, the findings of the current study demonstrated that MEL treatment reduced the FA-induced DNA damage by decreasing 8-OHdG levels in the plasma and the $\%DNA_T$ in the lymphocytes.

Reactive oxygen species are a byproduct of intracellular metabolic pathways that form under natural conditions. The increased level of ROS may lead to oxidative damage on the tissues by altering the membrane lipids and structure of proteins as well as nucleic acids. Oxidative stress is considered to be one of the potential mechanisms behind the FA induced systemic toxicity; it has been reported to occur in multiple organs of rats exposed to FA [33, 34]. GSH is involved in the nonenzymatic part of anti-oxidative defense systems, and plays a vital role as a coenzyme in the detoxification of many chemicals including FA [35]. Therefore, increased FA exposure leads to the depletion of GSH storage in the cells. As a biomarker of lipid peroxidation, MDA levels may also rise following GSH depletion related to FA toxicity [2, 36]. A study by Ye et al. [26] revealed that FA exposure causes increased levels of MDA and decreased levels of GSH in the lung and liver tissues of mice, while Strubelt *et al.* [37] reported raised MDA levels in the liver of rats. The

present study demonstrates that FA exposure augments MDA levels and reduces GSH levels in the lung, liver and kidney tissues. However, it is observed that the MEL treatment alleviated these FA induced oxidative injury effects. MPO, which is an indicator of inflammatory response, is a heme enzyme and produces H_2O_2 from hypochloric acid at the site of inflammation [22]. Increased MPO activity could modulate production of oxidant molecules and ultimately, tissue injury under oxidative stress conditions. Although Sogut *et al.* [38] reported that the changes in the MPO activity was not significant following FA exposure, our study displays significant increases in the MPO activities in the lung, liver and kidney tissues, in accordance with the MDA levels; these effects were abolished by MEL treatment. Light microscopic investigation also illustrated the increased MPO activity by revealing moderate inflammation in the tissues.

The role of oxygen-derived free radicals in organ injury was previously documented [39] and scavenging free radicals were found to be useful in protecting the tissue from oxidative damage [36]. FA can induce tissue injury by creating an imbalance in the oxidant/antioxidant status of normal cells, triggering overproduction of ROS such as superoxide anions, hydroxyl radicals and as well as nitric oxide (NO) in the tissues [40, 41]. In the present study, the luminol and lucigenin enhanced CL measurements revealed that ROS-mediated FA-induced organ injury is relieved by MEL treatment, evincing the anti-oxidative effect of MEL. We also observed that FA exposure induced the production of NO in the lung, liver and kidney tissues, and that MEL therapy reduced these effects, significantly. On the other hand, FA can increase nitric oxide synthase (NOS) expression and NO level in the tissue, as well as interfering with antioxidant enzymes like CAT and SOD which are responsible for detoxifying the superoxide anion. The mechanism behind these oxidative injuries may be explained by the water soluble structure of FA, making easy for FA or its metabolite to circulate into membranes and directly react with biomolecules.

Campos *et al.* [42] reported an increase in the alveolar lumen area in mice as a result of cigarette smoke. In addition to increase in the alveolar lumen area, a decrease in the alveolar septa volume density and an increase in the inflammatory response were documented in 2016 by Murta et al. [43]. In our study, we observed increased inflammatory cell infiltration, thickening of the alveolar septa due to oxidative damage induced by FA-exposure. MEL treatment reduced the alveolar damages induced by FA slightly. These findings were supported by the changes in the oxidative stress parameters like increased MDA levels and depleted GSH stores in the lung tissue. The relationship between the inflammatory cells and oxidative stress was previously documented: the increased ROS levels induce an inflammatory response in the cells [43]. Thus, we suggest that FA might directly react with the lipids, proteins and DNA or indirectly interfere with the normal cell metabolism to induce the production of ROS. The antioxidant property of MEL was reported in a previous study demonstrating the ameliorative effects of MEL on oxidative tissue damage [16]. We assumed that MEL protects the pulmonary tissue from FA induced damage by supporting the antioxidant defense system.

The liver is a vital organ, being the center of metabolism for many toxic or nontoxic xenobiotics, including FA. Due to its high metabolic activity, the liver is the main target of many toxic substances. Various studies with experimental animals have reported that FA caused degenerations in hepatocytes [41], the portal area and central vein [36], the impairment of membrane integrity and vacuolization [37]; these observations correlated with the findings of the current study. The damage in the portal areas and hepatocytes, along with congestion in sinusoids were the results of the detrimental effects of FA in the liver observed by the light microscopic investigations. MEL treatment ameliorated the FA induced hepatic injury as distinguished by the less pronounced congestion in sinusoids and damage in hepatocytes. FA induced morphological changes in the kidney tissue were reported as epithelial damage in glomeruli, mononuclear cell infiltration and degeneration in the proximal and distal tubules [36, 44]. We observed that FA caused glomerular enlargement, interstitial cell infiltration, epithelial desquamation and mild congestion. Although the MEL treatment reduced the tissue damage induced by FA exposure, it did not restore the tissue back to its normal structure.

Once FA enters the body, it is metabolized to formic acid (which is later excreted in urine) by

formaldehyde dehydrogenase (FDH) which uses GSH as a cofactor, primarily in the liver. During FA exposure, plasma formic acid concentration increases and this may cause systemic acidosis in many organs including the lung, liver and kidney [45]. Furthermore, formic acid-induced acidosis may lead to an elevation in the cellular level of ROS including superoxide and hydroxyl radicals along with nitric oxide. Thus, formaldehyde can induce oxidative stress as a result of free radical production in the tissues in addition to causing DNA damage. The presence of an antioxidant like melatonin can reduce the harmful effect of FA.

In conclusion, the results of the present study indicate that melatonin considerably ameliorates the toxic effects of formaldehyde by inhibiting neutrophil infiltration, balancing oxidant/antioxidant status, reducing ROS levels of the lung, liver and kidney tissues and DNA damage in rats. Formaldehyde exists as a contaminant in the environment, everyday dietary consumption and work-place areas, and humans will unavoidably be exposed to this substance. Thus, we conclude that melatonin supplementation may be useful to guard against formaldehyde toxicity and further studies are required to adopt these results to human.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Sezgin Aydemir, Department of Pathology Laboratory Technicianship, Vocational School of Health Related Services, Marmara University Haydarpasa Yerleskesi, 49 Tıbbiye Cad., Uskudar-Istanbul 34668, Turkey. Tel: +90 5415050667; +90 2163382798-1139; Fax: +90 2165410075; E-mail: sezgin.aydemir@marmara.edu.tr; szgnaydemir@gmail.com

References

[1] Smith AE. Formaldehyde. Occup Med 1992; 42: 83-88.

[2] Chang CC and Gershwin ME. Perspectives on formaldehyde toxicity: separating fact from fantasy. Regul Toxicol Pharmacol 1992; 16: 150-160.

[3] International Agency for Research on Cancer (IARC): Formaldehyde. In: Chemical agents and related occupations Volume 100F a review of human carcinogens. IARC monographs on the evaluation of carcinogenic risks to humans, Lyon: 2012. pp. 401-430.

[4] Beall JR and Ulsamer AG. Formaldehyde and hepatotoxicity: a review. J Toxicol Environ Health 1984; 14: 1-21.

[5] Collins JJ, Ness R, Tyl RW, Krivanek N, Esmen NA and Hall TA. A review of adverse pregnancy outcomes and formaldehyde exposure in human and animal studies. Regulat Toxicol Pharmacol 2001; 34: 17-34.

[6] Air quality guidelines for Europe 2nd edition. Copenhagen, World Health Organization (WHO) Regional Office for Europe, WHO Regional Publications, European Series; 2000. pp. 88-97.

[7] Metz B, Kersten GF, Hoogerhout P, Brugghe HF, Timmermans HA, de Jong A, Meiring H, ten Hove J, Hennink WE, Crommelin DJ and Jiskoot W. Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J Biol Chem 2004; 279: 6235-6243.

[8] Cheng G, Shi Y, Sturla SJ, Jalas JR, McIntee EJ, Villalta PW, Wang M and Hecht SS. Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. Chem Res Toxicol 2003; 16: 145-152.

[9] Pohanka M. Alzheimer's disease and oxidative stress: a review. Curr Med Chem 2013; 21: 356-364.

[10] Halliwell B. Oxidative stress and cancer: have we moved forward? Biochem J 2007; 401: 1-11.

[11] Matsuoka T, Takaki A, Ohtaki H and Shioda S. Early changes to oxidative stress levels following exposure to formaldehyde in ICR mice. J Toxicol Sci 2010; 35: 721-30.

[12] Garfinkel D, Laudon M, Nof D and Zisapel N. Improvement of sleep quality in elderly people by controlled-release melatonin. Lancet 1995; 346: 541-44.

[13] Altun A and Ugur-Altun B. Melatonin: therapeutic and clinical utilization. Int J Clin Pract 2007; 61: 835-845.

[14] Pieri C, Marra M, Moroni F, Recchioni R and Marcheselli F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci 1994; 55: PL271-276.

[15] Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V and Reiter RJ. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004; 36: 1-9.

[16] Omurtag GZ, Tozan A, Sehirli AO and Sener G. Melatonin protects against endosulfan-indu-

ced oxidative tissue damage in rats. J Pineal Res 2008; 44: 432-438.
[17] Tan DX, Poeggeler B and Reiter RJ. The pineal hormone melatonin inhibits DNA adduct formation induced by the chemical carcinogen safrole in vivo. Cancer Lett 1993; 70: 65-71.
[18] Valentine R and Kennedy GL. Inhalation toxicology, Chapter 23. In: Principles and Methods of Toxicology, 4th edition. In: Hayes AW, editor. Philadelphia, PA: Taylor Francis Publishers; 2001. pp. 1085-1143.
[19] Singh NP, McCoy MT, Tice RR and Schneider EL. A simple technique for quantification of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175: 184-191.
[20] Casini A, Ferrali M, Pompella AS, Maellaro E and Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene intoxicated mice. Am J Pathol 1986; 123: 520-531.
[21] Aykac G, Uysal M, Yalcin AS, Kocak-Toker N, Sivas A and Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione peroxidase and glutathione transferase in rat. Toxicology 1985; 46: 71-76.
[22] Hillegas LM, Griswold DE, Brickson B and Albrightson-Winslow C. Assesment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 1990; 24: 285-295.
[23] Haklar U, Yuksel M, Velioglu A, Turkmen M, Haklar G and Yalcın AS. Oxygen radicals and nitric oxide in chondral and meniscal lesions or both. Clin Orthop Relat Res 2002; 403: 135-142.
[24] Emoto Y, Yoshizawa K, Kinoshita Y, Yuki M, Yuri T, Yoshikawa Y, Sayama K and Tsubura A. Green tea extract-induced acute hepatotoxicity in rats. J Toxicol Pathol 2014; 27: 163-174.
[25] Khamgaonkar MB and Fulare MB. Pulmonary effects of formaldehyde exposure: an environmental-epidemiological study. Indian J Chest Dis Allied Sci 1991; 33: 9-13.
[26] Ye X, Ji Z, Wei C, McHale CM, Ding S, Thomas R, Yang X and Zhang L. Inhaled formaldehyde induces DNA-protein crosslinks and oxidative stress in bone marrow and other distant organs of exposed mice. Environ Mol Mutagen 2013; 54: 705-18.
[27] Gerin F, Erman H, Erboga M, Sener U, Yilmaz A, Seyhan H and Gurel A. The effects of ferulic acid against oxidative stress and inflammation in formaldehyde-induced hepatotoxicity. Inflammation 2016; 39: 1377-1386.
[28] Collins AR. Comet assay for DNA damage and repair: principles, applications and limitations. Mol Biotechnol 2004; 26: 249-261.
[29] Sardas S, Omurtag GZ, Tozan A, Gul H and Beyoglu D. Evaluation of DNA damage in construction-site workers occupationally exposed to welding fumes and solvent-based paints in Turkey. Toxicol Ind Health 2010; 26: 601-608.
[30] Beceren A, Akdemir N, Omurtag GZ, Tatlıpınar ME and Sardas S. DNA damage in gasoline station workers caused by occupational exposure to petrol vapour in Turkey. Acta Pharm Sci 2016; 54: 53-62.
[31] Ciftci G, Aksoy A, Cenesiz S, Sogut MU, Yarim GF, Nisbet C, Guvenc D and Ertekin A. Therapeutic role of curcumin in oxidative DNA damage caused by formaldehyde. Microsc Res Tech 2015; 78: 391-395.
[32] Costa S, Coelho P, Costa C, Silva S, Mayan O, Santos LS, Gaspar J and Teixeira JP. Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. Toxicology 2008; 252: 40-48.
[33] Lino-dos-Santos-Franco A, Correa-Costa M, Durao AC, de Oliveira AP, Breithaupt-Faloppa AC, Bertoni Jde A, Oliveira-Filho RM, Camara NO, Marcourakis T and Tavares-de Lima W. Formaldehyde induces lung inflammation by an oxidant and antioxidant enzymes mediated mechanism in the lung tissue. Toxicol Lett 2011; 207: 278-285.
[34] Sener U, Uygur R, Kurt O, Caglar V, Gelincik I, Gurel A and Erdogan H. Protective effects of curcumin against formaldehyde-induced renal toxicity in rats. West Indian Med J 2015; [Epub ahead of print].
[35] MacAllister SL, Choi J, Dedina L and O'Brien PJ. Metabolic mechanisms of methanol/formaldehyde in isolated rat hepatocytes: carbonyl-metabolizing enzymes versus oxidative stress. Chem Biol Interact 2011; 191: 308-314.
[36] Bakar E, Ulucam E and Cerkezkayabekir A. Investigation of the protective effects of proanthocyanidin and Vitamin E against the toxic effect caused by formaldehyde on the liver tissue. Environ Toxicol 2015; 30: 1406-1415.
[37] Strubelt O, Younes M, Pentz R and Kuhnel W. Mechanistic study on formaldehyde-induced hepatotoxicity. J Toxicol Environ Health 1989; 27: 351-366.
[38] Sogut S, Songur A, Ozen OA, Ozyurt H and Sarsilmaz M. Does the subacute (4-week) exposure to formaldehyde inhalation lead to oxidant/antioxidant imbalance in rat liver? Eur J Gen Med 2004; 1: 26-32.
[39] Arthur MJ, Bentley IS, Tanner AR, Saunders PK, Millward-Sadler GH and Wright R. Oxygen-derived free radicals promote hepatic injury in the rat. Gastroenterology 1985; 89: 1114-1122.
[40] Gulec M, Gurel A and Armutcu F. Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. Mol Cell Biochem 2006; 290: 61-67.

[41] Ucmaklı E, Armutcu F and Ozturk A. The effects of formaldehyde intoxication on the inducible nitric oxide synthase expression and nitric oxide level in the liver tissue of rats. Turk J Med Sci 2013; 43: 52-56.
[42] Campos KK, Dourado VA, Diniz MF, Bezerra FS and Lima WG. Exposure to cigarette smoke during pregnancy causes redox imbalance and histological damage in lung tissue of neonatal mice. Exp Lung Res 2014; 40: 164-171.
[43] Murta GL, Campos KK, Bandeira AC, Diniz MF, Costa Gde P, Costa DC, Talvani A, Lima WG and Bezerra FS. Oxidative effects on lung inflammatory response in rats exposed to different concentrations of formaldehyde. Environ Pollut 2016; 211: 206-213.
[44] Faghani M, Kohestani Y, Nasiri E, Moladoust H and Mesbah M. Protective effect of Vitamin E on formaldehyde-induced injuries in the rat kidney. Gene Cell Tissue 2014; 1: e21539.
[45] Formic acid [MAK Value Documentation, 2003]. The MAK Collection for Occupational Health and Safety 2012; 19: 170-180.