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# Protective effects of safranal against subchronic thinner inhalation induced oxidative stress in rats

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In phytotherapy, research on the effects of components in plant extracts on various diseases gets increasing attention. . Saffron is an herb with antioxidant effects, known to have an increasing significance due to its therapeutic effects. Safranal is one of the components of saffron extract. Here, we explored the effects of subchronic thinner inhalation on the oxidant-antioxidant balance, the relation between toxicity and oxidative stress, and a possible protective effect of safranal against thinner toxication in rats. Sprague−Dawley rats were divided into four groups as follows: control (Gr. I), safranal (Gr. II), thinner (Gr. III) and thinner+safranal Gr. IV). Each group consisted of 10 rats, and the study lasted for 8 weeks. After completing the animal studies malondialdehyde (MDA), reduced glutathione (GSH), toluol, 8OHdG (8-hydroxy-2-deoxyguanosine), protein oxidation, nitric oxide metabolites (NOx), total antioxidant capacity (TAC), total oxidant capacity (TOC), glucose, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, WBC, VLDL, RBC, HCT, Hb, aspartate amino transferase (AST), and alanine amino transferase (ALT) levels were determined from blood specimens of the rats. Brain and lung tissues were also examined histopathologically. The data obtained from the study were statistically analyzed using SPSS, and both ANCOVA and Bonferroni tests were performed. $P$ <0.05 was accepted as statistically significant. The results indicated that safranal had a protective and balancing effect against thinner inhalation oriented complications in rats.

**Keywords**: Brain, *Crocus sativus*, Lungs, Saffron, Volatile organic solvents

Occupational diseases and problems arising from the nature of work and stress have become an important issue today[1]. Exposure to chemicals and other hazardious atmosphere leads to such diseases. Several studies have been carried out on this subject which reveal that many chemicals cause non-repearable damages[2]. Volatile organic solvents (VOC) play a role among these chemicals which can cause serious health problems and deaths[3]. Most commonly used organic solvent among them is the thinner and it is widely used in our daily life and in many industries. It is reported that these and similar substances cause many damages in the living organism. Moreover, they are synergistic with each other and can even increase the toxic effects of each other, even if they are in very low concentrations when they are mixed[4]. Widespread use of VOCs as drugs makes this issue even more important[5].

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Few medicinal plants are widely used in the food industry as spices and have high antioxidant capacity. Most of the plants especially used as spices or food protectors or utilized in cosmetics or perfumery products belong to labiatae family[6]. One of and the most important plants that grows in our country and belongs to the Iridaceae family is saffron[7]. The main metabolites of saffron obtained by drying the stigmas of *Crocus sativus* L. flower are crocin, crocetin, picrocrocin and safranal. Saffron is one of the plants that are used in the ethnopharmacological treatment of many diseases including depression and tumors[8]. In this study, we have investigated the effectiveness of safranal in ameliorating the negative effects of increased oxidative stress caused by subchronic thinner inhalation and in preventing or alleviating cell damage that may occur.

## Materials and Methods

Forty, average of 3-month old, Wistar albino male rats were used in the study. Rats were separated into

ventilated, 12 h in dark\light cages as 3-4 rats in each cage with 20-22ºC. The rats fed with standard rat food and *ad libitum* water during the study. All interventions to animals throughout the study were carried out in Afyon Kocatepe University Animal Husbandry Application and Research Center in accordance with the approval of the ethical committee numbered AKUHEDYAK-453-15 dated 07th May 2015 (49533702/46) obtained from Afyon Kocatepe University Experimental Animals Local Ethics Committee. The experiments lasted for 8 wk and test subjects were divided into four groups each comprising 10 rats. Gr. I, control group; Gr. II, safranal group; GR. III, subchronic thinner inhalation group; and Gr. IV, subchronic thinner inhalation+ safranal group. All the groups were fed with standard rat food for 8 weeks and their weights were recorded regularly during the experiments. Subchronic thinner inhalation group (Gr. III) was treated with 5 mL of thinner and embedded in cotton wool in an air-proof isolated condition including NaOH tablets. This was repeated twice daily and treatment was finalized when 50% of the rats' standing reflex disappeared. Sub-chronic thinner inhalation+safranal group (Gr. IV) was treated as Gr. III and additionally administrated 100 mg/kg/day safranal. The safranal was introduced by gastric gavage. Group II was administered 100 mg/kg/day safranal by gastric gavage[9]. All other chemical reagents were of analytical grade obtained from Sigma Aldrich Co. (St. Louis,MO,USA).

**Experimental design and preparation of samples for analysis**

At the end of the experimental period of 8 wk, the rats were anesthetized and sacrificed by cervical dislocation. Rats were anesthetized with 10 mg/kg of xylazine HCl and 50 mg/kg ketamine HCl injection. All rats were sacrificed on the 56th day (8 wk) of the experiment and tissue samples (brain and lung) were collcted for examination of clinical biochemical analysis. Tissue samples (0,5 g) were placed in homogenizer and were added 5 mL of phosphate buffer solution (PBS) (pH:7.4) and were homogenized by means of the homogenizer and the sonicator. The homogenates were centrifuged at 15000 rpm, cooled at 4°C for 10 min, and the supernatants were stored at –80°C in deep freeze pending analysis. Haematologic analyses in whole blood sample from the rats (Erythrocyte-WBC, Lymphocyte-RBC, Hematocrit-HCT and Haemoglobin-Hb), malondialdehyde (MDA) and reduced glutathione (GSH) analyses were performed. In addition, whole blood sample obtained from the experimental animals was centrifuged for 10 min at 3500 rpm and their plasma was separated. The analyzes of 8-OhdG (8-hydroxy-2-deoxy-guanosine), PO (protein oxidation), TAC (total anti-oxidant capacity), TOC (total oxidant capacity), NOx (nitric oxide metabolites), glucose, triglyceride, cholesterol, HDL-cholesterol, LDL-cholesterol, aspartate amino transferase (AST) and alanine amino transferase (ALT) in plasma samples were performed.

**Determination of MDA**

Malondialdehyde is a peroxidation product of free radicals. Draper & Hadley's[10] double boiling method was used for MDA determination. This method is based on the interaction of thiobarbituric acid (TBA) and MDA and their maximum absorbance at 532 nm, spectrophotometrically (UV 1601, Shimadzu, Tokyo, Japan).

**Determination of protein oxidation**

Protein oxidation was measured using both Levine *et al.*[11] and Ceylan *et al.*[12]. This method is based on the colour of hydrozone compounds which occur in there actions of carbonyl groups of oxidized proteins and 2,4-dinitrophenylhydrazine.

**Determination of reduced glutathione (GSH)**

Blood samples were hemolyzed using distilled water, and proteins containing no –SH group were precipitated using a solution containing 1.67 g metaphosphoric acid, 0.2 g ethylenediaminetetraacetic acid (EDTA) and 30 g NaCl in 100 mL of water. GSH was measured using the absorbance (412 nm) of the colour yellow occurring in the supernatant by reacting –SH groups with DTNP [5,5'-dithiobis (2-nitro-benzoic acid)][13].

**Total antioxidant capacity and total oxidant capacity**

Total antioxidant capacity and TOC levels, which are among the oxidative stress parameters, were measured using kits (RelAssay, Gaziantep, Turkey) that work with spectrophotometric methods[14,15]. The TAC method is calibrated using a standard anti-oxidant solution, which is a vitamin E analog called Trolox Equivalent. Measured TAC levels are expressed as mmol Trolox Equivalent/L. However, the TOC assay is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange.

**Determination of 8-OHdG and NOx**

In order to measure the amount of 8-OHdG in the plasma which is a biomarker of an oxidative DNA

damage, a specific commercial ELISA kit (OXIS, BIOXYTEC, Portland, USA) was applied to the rat. NOx levels in the plasma samples were determined using the methods of Miranda *et al*.[16] which was based on the vanadium chloride (III)-Griess reaction.

**Determination of biochemical parameters**

Glucose, total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol levels were determined spectrophotometrically (Shimadzu UV1601) and conventional methods were by commercial kits. Aspartate amino transferase and ALT levels were determined by Cobas Integra 400 autoanalyzer (Roche). Toluol levels were determined at Acıbadem Labmed Central Lab by GC-MS.

**Statistical analyses**

In order to determine the protective effect of safranal against the oxidative stress by thinner, ANCOVA analysis was applied to the parameters measured in four different groups and whether there was a difference between the groups or not was determined by Bonferroni test. Live weight avarages of the groups in zeroth and eighth weeks were selected as covariates[17,18]. Statistical calculations of the obtained findings were carried out using SPSS 24 package programme and the data used in the study were stated as "average ± standard deviation" (X±SD).

## Results

**Variation in biochemical parameters**

Glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL, triglycerides, WBC, RBC, ALT, AST, Hb and HCT from the 8-wk period and from all analyzed groups are shown in Table 1. As seen in the table, glucose levels of the treated groups (Gr. II-IV) were lower when compared to the control group (Gr. I). Triglycerides and VLDL levels increased in the control group. LDL-cholesterol levels and HDL levels increased in the Gr. III. Total cholesterol levels of the treated groups were higher compared to the control group. Similarly, AST and ALT levels in the treated groups were higher than in the control group. WBC levels increased in the Gr. IV compared to all other groups. RBC and HCT levels increased in the Gr. II compared to the rest of the groups. Hb levels increased in the Gr, III compared to all other groups.

**Toluol level measurement in thinner inhaled groups**

Whole blood levels were determined at Acıbadem Labmed Central Laboratory (Istanbul, Turkey) by GC-MS. Obtained blood toluol levels were identified in ng/ml as in Table 2. When the plasma toluene levels were analyzed, no statistically significant difference was found in the inhaled toluol levels in the Gr. III & IV.

**Changes in biochemical parameters**

Malondialdehyde, GSH, TAS, TOS, NO, 8-OHdG and PO levels were determined in blood and plasma samples taken from four experimental groups established in our study. Statistical values and comparisons of the findings belonging to the blood levels of these indicators at the end of 8 wk of research period are presented in Table 3.

When the MDA levels were observed, they increased in Gr. III significantly when compared to the Gr. II (safranal) group ($P$ <0.005). GSH levels were higher in the Gr. II compared to the rest of the groups.Total antioxidant capacity levels were lower in the Gr. III (thinner) compared to all othergroups

Table 2 — Toluol Level in Thinner Inhaled Groups

| Groups | Control (n=10) | Safranal (n=10) | Thinner (n=10) | Thinner +Safranal | P |
|---|---|---|---|---|---|
| Toluol (ng/mL) | | | 1.525± 0.071 | 1.481± 0.071 | 0.730 |

[X ± SE: mean ± standard error; $P$ <0.05]

Table 1 — Plasma ALT, AST, HDL, LDL, VLDL, triglyceride, total cholesterol and glucose levels of the experimental groups

| | Control | Safranal | Thinner | Thinner+Safranal | P |
|---|---|---|---|---|---|
| ALT (U/L) | 47.241±8.636 | 61.600±6.203 | 56.700±5.777 | 53.626±7.566 | 0.485 |
| AST (U/L) | 63.881±37.747 | 150.655±31.175 | 149.567±28.163 | 171.930±37.351 | 0.236 |
| Total cholesterol (mg/dL) | 61.582±6.503 | 62.843±4.237 | 72.445±3.946 | 73.528±5.234 | 0.096 |
| TRG (mg/dL) | 50.346±11.921 | 41.861±8.562 | 46.046±7.974 | 46.525±10.444 | 0.938 |
| HDL (mg/dL) | 59.166±5.329 | 54.878±3.828 | 66.178±3.565 | 64.067±4.669 | 0.098 |
| LDL (mg/dL) | 13.912±2.518 | 13.589±1.809 | 18.145±1.684 | 15.966±2.206 | 0.184 |
| VLDL (mg/dL) | 10.072±2.385 | 8.372±1.713 | 9.209±1.595 | 9.305±2.089 | 0.938 |
| Glucose (mg/dL) | 268.105±18.761 | 234.651±14.492 | 266.537±13.824 | 231.507±17.110 | 0.470 |
| WBC ($10^9$/L) | 5.088 ±0.728 | 5.748±0.563 | 5.080±0.537 | 6.405±0.664 | 0.578 |
| RBC ($10^{12}$/L) | 8.303±0.180 | 8.481±0.139 | 8.313±0.133 | 8.446±0.164 | 0.875 |
| HCT (%) | 45.813±0.928 | 47.456±0.717 | 46.625±0.684 | 47.226±0.847 | 0.690 |
| Hb (g/dL) | 15.189±0.285 | 15.708±0.220 | 15.752±0.210 | 15.751±0.260 | 0.366 |

[X ± SE: mean ± standard error; $P$ <0.05]

Table 3 — Aritmetic Mean, Standard Error and significance levels of the findings belonging to MDA, GSH, TAS, TOS, NO, 8OHdG, PC parameters measured in the plasma

| | Control(n=10) | Safranal (n=10) | Thinner (n=10) | Thinner+Safranal (n=10) | P |
|---|---|---|---|---|---|
| MDA( mmol/mL) | 13.048±0.383$^{ab}$ | 12.346±0.296$^{a}$ | 13.952±0.282$^{b}$ | 12.731±0.350$^{ab}$ | 0.004 |
| GSH (mg/dL) | 99.959±19.440 | 103.130±9.612 | 75.105±8.295 | 80.806±13.471 | 0.073 |
| TAS (mmolTrolox Equiv./L) | 1.250±0.336 | 1.380±0.256 | 0.923±0.199 | 1.309±0.306 | 0.455 |
| TOS µmol$H_2O_2$Equiv./g protein ) | 9.056±2.077$^{a}$ | 12.853±1.441$^{ab}$ | 14.517±1.217$^{b}$ | 10.149±1.764$^{ab}$ | 0.018 |
| NO (µmol/L) | 48.946±3.640$^{ab}$ | 47.213±1.920$^{a}$ | 49.381±1.826$^{a}$ | 39.311±2.499$^{b}$ | 0.010 |
| 8-OHdG (ng/mL) | 3.279±0.620 | 2.543±0.246 | 3.143±0.228 | 2.885±0.339 | 0.319 |
| PC (ng/mL) | 40.851±6.006 | 44.949±4.494 | 48.391±4.525 | 41.718±5.772 | 0.604 |

[$P$ <0.05. There is statistical significance between groups shown in different letters on the same line]

including control group. Total oxidant capacity levels were higher in the Gr. III compared to the oher three groups ($P$ <0.05). NO levels were significantly higher in Gr. III compared to Gr. IV. ($P$ <0.01). Protein oxidation levels increased in the Gr. III, compared to the other three groups. Levels of 8-OHdG increased in the control group compared to the treated groups.

**Histopathological examinations**

Brain and lung tissue samples taken from the animals in the groups in accordance with the technique for histopathological examination were fixed at 10% of formol. The tissues were fixed in buffered formalin solution for histopathological examination. After routine processing, tissues were embedded in parafin wax. Fine 5 mm thick sections were cut with a rotary microtome and mounted on to glassslides. The sections were stained with hematoxylin and eosin (H&E) and analyzed with an Axio Cam ICc camera attached to a ZeissAxio Lab. A1 light microscope. (BX 51, Olympus, Tokyo, Japan). In H&E-stained sections, (1) inflammation, hemorrhage, and connective tissue proliferation in interstitial areas;(2) epithelial cell degeneration and/or desquamation, cystic dilatation, and intraluminal debris and/or hyaline cylinders in tubules, and (3) glomerular lesions were screened. The findings belonging to the changes in lung and brain tissues are as follows.

**Analysis of Lung tissues**

Findings in the form of mononuclear cell infiltration, interstitial tissue thickening, emphysema and bleeding around the bronchi and bronchioles in the lungs were dominant in the groups. Belonging to the severity, these findings were evaluated semi quantitatively with the scores as no lesions (0), minimal severity (1), moderate severity (2), very severe (3) (Table 4).

The data obtained by the statistics performed using the scores of the groups are presented as average ± standard deviation (n=6). Statistical differences between experimental groups were referred as

Table 4 — Statistical examination of the data obtained from lung and brain tissues

| Groups | Pathology score of lung tissue (Arbitrary unit) | Pathology score of brain tissue (Arbitrary unit) |
|---|---|---|
| I | 0.67±0.52$^{b}$ | 0.33±0.52$^{b}$ |
| II | 0.83±0.41$^{b}$ | 0.33±0.52$^{b}$ |
| III | 2.17±0.75$^{a,c}$ | 2.00±0.63$^{a,c}$ |
| IV | 1.67±0.82$^{a,c}$ | 1.83±0.75$^{a}$ |
| P | 0.005 | 0.001 |

[$^{a}P$ <0.05, $^{b}P$ <0.05 and $^{c}P$ <0.05; refers to groups that are statistically different from the control, thinner and safranal group, respectively]

subscripts ($^{a,b,c}$) in letters. Whether there was no statistical difference among the groups or not was determined by Kruskal-Wallis test from non-parametric tests because the data did not indicate normal distribution. Man-Whitney U test was applied to the data in order to determine among which groups the differences were.

When the pathology data of the lung tissue was analyzed, it was determined that tissue damage occurred in the lungs as a result of thinner inhalation. It was observed that safranal application had no negative effect on lungs alone. When we analyze the effect of safranal occurring as a result of thinner inhalation, we can see that safranal decreases the occurring damage level. However, it was observed that safranal had no positive effect on the damage in lung as a result of thinner inhalation because this decrease did not lead to a statistical difference between thinner group and thinner-safranal group (Fig. 1).

**Analysis of Brain tissues**

Significant neuronal pathological changes were observed in the CA1 region of the hippocampus at the end of histopathological examination. There were necrobiotic changes in many neurons in various amounts in the region according to the groups. The cytoplasms of these cells were dark blue, shrunken in angular morphology. Nucleus of the neurons were shrunk and hyperchromatic and their nuclei could not be selected. These cells, which had degenerative-

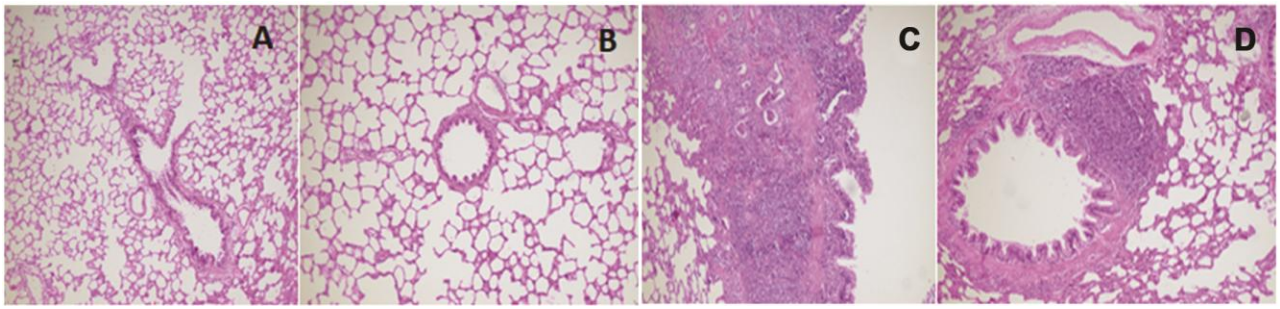


Fig. 1 — Microscope images of lung tissues from the experimental groups stained with the HE-staining technique. (A) Control group's lung tissue; alveolar and bronchiolar structures in normal view; (B) Safranal group's lung tissue; alveolar and bronchiolar structures in normal view; (C) Thinner group's lung tissue; severe peribronchiolar mononuclear cell in filtration; and (D) Thinner+Safranalgroup's lung tissue; mild peribronchiolar mononuclear cell in filtration.

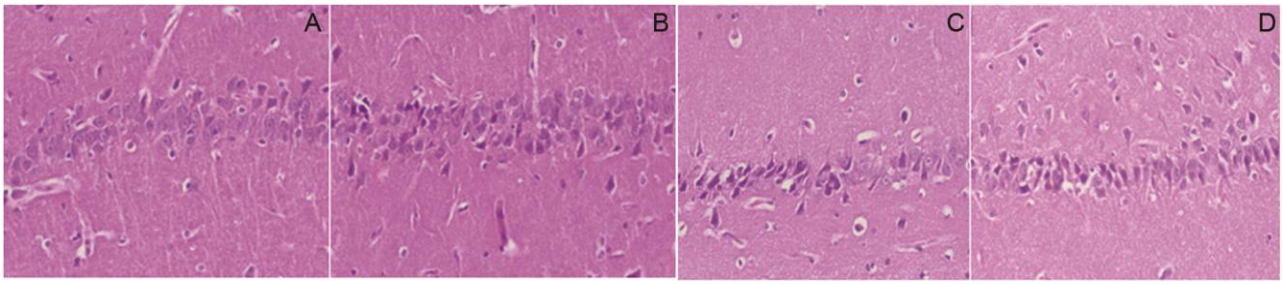


Fig. 2 — Microscope images of brain tissues from the experimental groups stained with the HE-staining technique. (A) Conrol group's brain tissue; neurons in normal view; (B) Safranal group's brain tissue; neurons in normal view; (C) Thinner group's brain tissue; severe degenerative necrotic primidal neurons; and (D) Thinner+Safranal group's brain tissue; moderatede generative necrotic primidal neurons.

necrotic changes in the region, were scored according to their percentage as (3) if they were more than 50%, as (2) if they were between 25-50% and as (1), if they were between 5-25% and as (0) if they were less than 5% and the results were indicated in Table 4. When the pathology data of the brain tissue was analyzed, it was determined that tissue damage occurred in the brain as a result of thinner inhalation. When the data in Table 4 were analyzed, control group (Gr. I) was $0.33\pm0{,}52^{b}$; however, Gr. III was found as $2.00\pm0.63^{a,c}$. When we analyze the effect of safranal on the damage as a result of thinner inhalation, it is seen that safranal decreases the damage level. It was observed that safranal application had no effect on brain alone (Fig. 2).

## Discussion

Volatile compounds caused generation and activity losses in the structure of biological membranes. In the study, the rats inhaled the thinner twice a day without physical contact. During the inhalation rats' activities slowed, they had difficulty in walking, their inhalation was impaired and they became aggressive. It was reported that organic solvents took their toxic effect on cell injury through free radical formation[12,19]. It is also reported that saffron and its compounds indicate high radical scavenging activity and this high radical scavenging activity of those compounds is probably due to their ability to bind a hydrogen atom to the DPPH radical[20]. Karabulut *et al*.[21] reported that toluene in human remarkably increased both *in vivo* and *in vitro* oxidative stress parameters and at the same time led to a significant decrease in antioxidant enzyme activities.

Ulakoğlu *et al*.[22] observed significant increase in lipid peroxidation products in thinner inhaled rats. Additionally, the positive relation between MDA and toluene levels may be due to the increase in lipid peroxidation resulting from an increase of toluene in the blood. The increment in lipid peroxidation in the Gr. III (thinner) is almost the same[23]. In addition, the decrease in MDA levels in Gr. IV relatively with Gr. III suggests that safranal has both a protective effect and antioxidant capacity against harmful oxidation products. Hosseinzadeh *et al*.[24] stated that safranal had hydroxyl radical scavenging activity. In the researches carried out with safranal used as an antioxidant molecule in our study Hosseinzadeh & Sadeghnia[25] indicate that safranal decreases the MDA level increasing as a result of ischemia-reperfusion occurring in various tissues and organs due to its antioxidative properties. Mehdizadeh *et al*.[26] reported

that both saffron extract and safranal, main compound of the saffron, decrease MDA levels which increase as a result of myocard infarctus created through isoproterenol in rats depending on the dose and reduce the oxidative stress. In our study here as well, we investigated the protective effect of subchronical thinner inhalation on rats and safranal against those effects while it is observed that MDA level in thinner inhaled rats throughout the 8 weeks significantly decreased more in safranal group (Gr. II) as compared to control group, it significantly increased in thinner group (Gr. III) as compared to control group ($P$ <0.05). It is seen that MDA level in Gr. IV has a non-significant decrease compared to Gr. III and converges to control group data. The fact that MDA levels in the Gr. IV converges to control group data suggests that safranal has antioxidant potential in preventing lipid peroxidation. These findings are compatible with the previous studies.

GSH, which has more intracellular concentration, is highly important as an antioxidant molecule in the structural and functional protection of the integrity of cell, tissue and organ systems against oxidative stress[27]. Ilgazlı *et al*.[28] who analyzed the changes in blood of rats which inhaled thinner for one hour twice a day throughout 12 weeks, observed that though, there was an increase in SOD activities and a decrease in glutathione levels, there was no significant change. Dillioglugil *et al*.[29] in their study reported significantly decreased GSH levels in rat lungs exposed to thinner inhalation for 8 weeks. While it was observed in our study that blood GSH level increased in Gr. II compared to Gr. I (control), there was a decrease in Gr. III compared to control group. There was a decrease in Gr. IV compared to control group; however, there was an increase as compared to Gr. III. Although there is no statistically significant difference between the groups in the level of GSH obtained in our study, the study results are compatible with the literature information. GSH levels, which especially have an effect on detoxification, seem to decrease with thinner injection, but GSH levels increase with safranal treatment. This indicates that safranal contributes to the detoxification of thinner in plasma and reduces oxidative stress.

TAC represents the total effect of all antioxidants in the plasma and body fluids and TOC represents the total effect of oxidants[15]. Bayil *et al*.[30] in their study determined the effects of volatile organic solvents commonly used in textile dye industry on free radical levels and antioxidant enzyme system of textile workers. They observed significantly higher MDA and SOD values compared to controls in textile workers. However, it was reported that there was no significant difference between them for TAC. Konuk *et al*.[31] stated that the decrease in TAC levels due to subchronical thinner inhalation was the result of oxidative stress. In our study, there was an increase in plasma TAC level in Gr. II compared to the conyrol group, while in the Gr. III there was a decrease compared to the control. No statistically significant difference occurred between groups in plasma TAC level. We could see a significant increase in plasma TOC level in Gr. III compared to the other three groups and an increase in Gr. IV compared to the control group (Gr. I), but for a significant decrease compared to Gr. III ($P$ <0.05). The findings about TAC and TOC in our study corroborate with the literature. Considering plasma TAC and TOC levels in our study, we observed an increase in TAC levels in Gr. IV compared to Gr. III, but a decrease in TOC levels. When the data of safranal group (Gr. II) is compared with control group (Gr. I), we could see that the expected TAC-TOC negative correlation was not observed and an antioxidant matter, safranal may create thinner inhalation in body and disturb the oxidant-antioxidant balance in the absence of oxidative stress and therefore more scientific studies should be carried out in order to adjust the dose of this matter in medical applications.

NO may harm the proteins, lipids and DNA directly or after reacting with superoxide and this causes the formation of a highly reactive peroxynitrite anion[32-34]. Maniscalco *et al*.[35] measured NO level in the blood of shoe and leather sector workers who inhaled organic solvents, such as toluene, xylene and methyl-ethyl ketone. NO concentration was higher in both workers groups compared to the control group. Bukhari *et al*.[36] in their study estimated the antioxidant potential of safranal and crocin, among the basic compounds of *Crocus sativus*, in bronchial epithelium cells and monitored the anti-inflamatory potential of safranal, the active compound, in asthma rat model. They indicated that there was a decrease in NO, iNOS levels and in the formation of peroxynitrite ion and therefore those matters prevented cytochrome c release as a result of safranal and crocin application, among the saffron and its compound. Hazman & Bozkurt[37] report that safranal treatment that they

applied for the diabetic nephropathy in rats decreases NO levels of both HFD (high fatty diet) treatment group (HFD-Saf) and diabetes treatment group (DYB-Saf). In our study while there is a significant decrease in plasma NO levels of safranal (Gr. II) and thinner+safranal groups compared to Control group, there is a significant increase in thinner group (Gr. III) compared to control group ($P$ <0.05). This increase is compatible with the previous studies. It may indicate that increased NO activity in the thinner groups compared to the control is activated against the lipid peroxidation of the antioxidant system. When NO levels of Thinner+safranal group (Gr. IV) are compared with the levels of thinner group (Gr. III), there is a statistically significant decrease.This finding is compatible with the literature and calls to mind that safranal may have reduced NO concentration by fighting toxic agents created by thinner inhalation.

Base damage analyses are often carried out as an indicator of oxidative DNA damage. Guanine is the base which is exposed to oxidative damage most because $Cu^{+2}$ ions are highly included in DNA in G-C rich spaces. For that reason, most commonly measured base damage is 8-OHdG and it is regarded as the indicator of oxidative DNA damage[38]. Alfaro *et al*.[39] in their study on the effects of thinner inhalation on DNA identified that DNA damage increased depending on thinner inhalation and also there was a correlation among DNA damage, increased MDA level and decreased glutathione levels. Konuk *et al*.[31] in 8-wk experimental study formed five groups as control group (C), oliveoil (Z), α- lipoic acid (LA), thinner (T) and thinner + α-lipoic acid (LAT) and observed that there was a significant increase in T and LAT groups compared to the control group and a significant decrease in the LAT group compared to the control group. In our study, it is observed that the plasma level of 8-OHdG, which is regarded as the indicator of DNA damage, decreases in the safranal (Gr. II) and thinner+safranal groups (Gr. IV) compared to the control group, while the 8-OHdG level in Gr. III is close to the control group. However, there was no statistically significant difference among the groups in 8-OHdG level. The fact that there is a positive relationship between increased oxidative stress and DNA damage in consideration of literature data suggests that DNA damage may be a result of increased oxidative stress due to thinner inhalation. There duction of DNA damage levels in Gr. IV compared to Gr. III reveals that safranal has a strong antioxidant potential in preventing DNA damage and can be used as a protective in suppressing DNA damage due to thinner inhalation.

Protein oxidation occurs as a result of covalent modification of proteins with reactive oxygen derivatives or oxidative stress products[40]. It was observed in the study that thinner inhalation increased the protein oxidation and the protein oxidation increased in thinner group compared to control group. It was also observed that safranal application decreased this protein oxidation increase in thinner inhalation. During our study, we also evaluated the routine biochemical parameters as well as the oxidative stress table. As one of these parameters, the effect of safranal on glucose homeostasis in thinner toxicity was also handled by analyzing serum glucose levels. As a result of the study, it was observed that the glucose levels indicated a non-significant decrease in the groups II & IV compared to the control group, whereas in Gr. III, the glucose level was close to the control group. In this context, low levels of analyzed glucose levels in safranal applied groups (Gr. II & IV) compared to the control group can be considered as an indicator of the antidiabetic efficacy of safranal in the literature information[41].

In Kocyigit *et al*.[42] where the workers were exposed to organic solvents by their profession, the levels of total cholesterol, triglyceride, LDL and HDL were statistically significantly higher compared to the control group, and there was no significant difference between the triglyceride, HDL and LDL levels reported that total cholesterol, LDL, VLDL and triglyceride levels were significantly higher in non-obese individuals and HDL levels were statistically lower in obese individuals exposed to solvents. While Şahin *et al*.[31] in their 8 wk of experimental study in which they made rats inhale thinner observed a significant increase in LDL-cholesterol level in all experimental groups compared to control group, they reported that they could not find any difference among groups in HDL and total cholesterol levels. In our study it was observed that there was an in significant increase in all groups compared to control groups in total cholesterol level; however there was an in significant decrease in all groups in TRG level. Again, HDL level decreased in the Gr. II compared to the control, while it in significantly increased in groups III & IV. There is an in significant increase in these two groups (III & IV) compared to control

group, but a decrease in Safranal group in LDL level. However, VLDL level in significantly decreased in all groups compared to control group. Our study results differ from the literature and it is thought that this difference may be due to the dose and it can be supported by further studies.

Guzelian *et al*.[43] in a study of 289 print workers revealed that 8 h of less than 200 ppm of toluene per day caused an increase in ALT and AST levels, which are indicators of liver damage. Fornazzari *et al*.[44] reported that exposure to toluene caused liver damage and again Boewer *et al*.[45] there was a positive correlation between γ-glutamyltransferase (GGT) and AST, ALT level, which are the indicators of toluene exposure and liver damage. In our study, there was an in significant increase in ALT and AST levels in all groups compared to the control group. The findings in our study are compatible with the overall literature.

Hosseinzadeh *et al*.[46] in their study that they estimated the acute and subacute toxicity in different doses of safranal did not find a change in blood WBC values between control (Gr. I) and safranal groups (G-II). They found that RBC decreased along with the increasing dose of safranal, Hb value increased significantly only in 0.5 mL/kg safranal dose, HCT decreased at the same rate in all doses of safranal compared to control group. Konuk *et al*.[31] in their study that they analyzed the toxic effect of thinner appreciated methemoglobin (MetHb) which occured as a result of the oxidation of ferrous during the binding and release of hemoglobin to oxygen and concluded that MetHb was significantly high in thinner applied group. In our study, an increase in blood WBC, RBC and HCT levels was observed in Gr. II and IV compared to the control group, but this increase statistically significant. It was observed that Hb level remained at the same level in all groups. Our study results differ from the literature and it is thought that this difference may be due to the dose and it can be supported by further studies. From the histopathology findings of this study, it is clear that tissue damage occurred in the lungs and brain as a result of thinner inhalation, while safranal application reduced the tissue damage caused by the thinner in the lungs and brain. Öztek *et al*.[47] who studied thinner inhalation also observed mild edema in the brain, rare focal vacuolar degeneration spaces, gliosis and perivascular demyelination, an increase in chronic inflammatory changes in the mucous epithelium of the lungs, trachea and main bronchial mucosa.

## Conclusion

Thinners, though serve as an important agent in many industries, it adversely affect health both in occupational exposure and in drug use. In this study, we observed that after inhalation, rats' activities slowed down, had difficulty in walking, their inhalation impaired, and they turned aggressive. Those observations indicate that thinner acts as a neurotoxic agent and favour the intoxication findings that the thinner inhalation creates on central nervous system. All the data obtained from the analysis in the study revealed that the safranal used against the thinner toxification in rats exposed to subchronic thinner inhalation showed positive effects by reducing the oxidative stress especially through supporting the weakening antioxidant system, decreased the damage in the brain and lung cells and can be protective in buffering the negative effects of increased oxidative stress and preventing or all eviating complications. However, it is thought that dose-dependent further studies on the antioxidant activity of safranal are required.

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## Conflicts of interest

Authors declare no competing interests.

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