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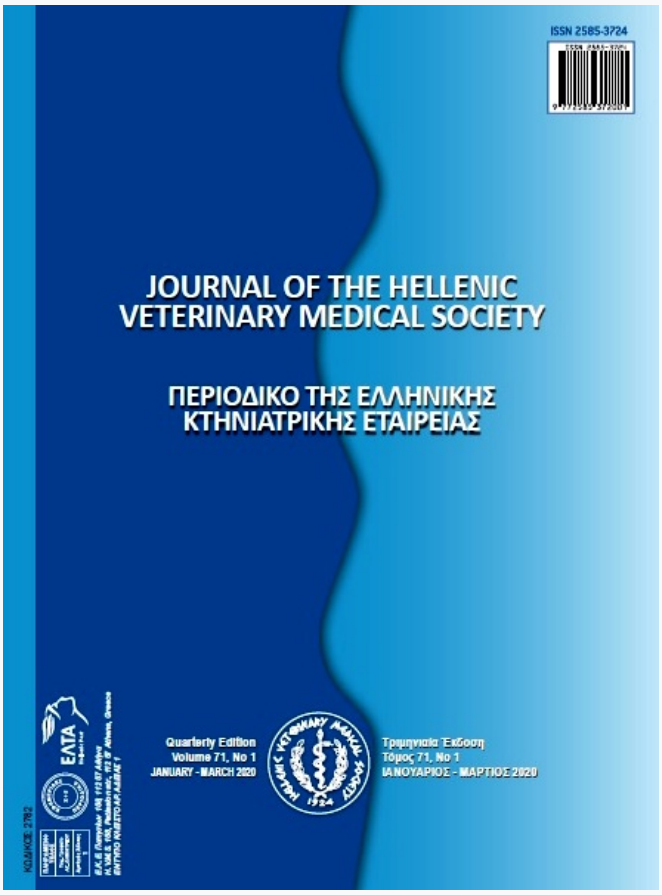

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Effects of Safranal on Tissue Oxidative Stress in Sub-Chronic Thinner-Addicted Rats

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ABSTRACT. The present study was carried out to determine the effects of sub-chronic thinner addiction on the oxidant-antioxidant balance and oxidative stress on certain tissues and the possible protective effect of safranal against thinner toxication in rats. Adult male Wistar albino rats were randomly divided into four groups of 10 animals each as follows: control (C), safranal (S), thinner (T) and thinner+safranal (T+S). The control group received 1cc saline by gastric gavage. Safranal was administered to S and T+S groups by using gastric gavage at a dose of 100 mg/kg/day and volume of 0.1 mL/kg/day. Thinner inhalation was applied to T and T+S groups in a container with NaOH tablets twice a day. Levels of malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO_x) metabolites, total antioxidant capacity (TAS) and total oxidant capacity (TOS) were determined in liver, lung, brain, kidney and testis tissues of the rats. In the T+S group, it was observed that the MDA levels significantly decreased in all tissues, except the kidney, in comparison to the thinner inhalation group ($p = 0.000$). When the NOx levels of the T+S group were compared with the levels of the T group, it was concluded that there existed a statistically significant decrease in the NOx levels in all tissues ($p = 0.000$).

In T+S group, it was observed that safranal either eliminated or mitigated oxidative stress that developed in tissues through decreasing MDA and TOS levels and increasing GSH and TAS levels and caused significant decreases in NO_X levels in all tissues. As a result, it was determined that safranal, although not uniform for all tissue types, had a protective potential against the damaging effects of oxidative stress caused by sub-chronic thinner inhalation.

KeyWords: Thinner inhalation, oxidative stress, safranal, tissue

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INTRODUCTION

Thinner is an organic solvent which is commonly used for industrial applications and currently has an increasing improper use as a pleasure inducing and narcotic substance. Thinner is a mixture of toluene, benzene, acetone, methanol, hexane and other substances (Nedzvetskii et al., 2012). It was indicated that the thinner used in Turkey was composed of 63% of toluene, 13% of acetone, 10% of isobutyl acetate, 7.5% of isobutane and 6.5% of butylene glycol and its harmful effects were mostly associated with toluene, where the remaining substances activated these harmful effects (Cobanoglu et al., 2007).

Reactive oxygen (ROS) and nitrogen (RNS) derivatives occur in the organism due to exposure to environmental and chemical agents and the interaction of such derivatives with macromolecules leads to oxidative stress (Mattia et al., 2003). Similar to various internal and external toxic agents, the toxic effects of thinner also emerge through free radicals (Mattia et al. 1991, Martínez-Alfaro et al., 2011).

Long-term use of volatile organic solvents has been shown to cause permanent damage to the lung and liver (Marjot and McLeod, 1989; Al-Alousi, 1989). Mattia et al. (1991; 1993a,b) reported in vivo and in vitro studies that ROS formation is accelerated and lipid peroxidation is increased in brain, liver, kidney and lung tissues with the effect of intraperitoneal administration of toluene. Al-Alousi (1989) emphasized that pulmonary edema is the cause of death due to volatile substances. When the results of many studies are considered, it has been observed that there are many damages as a result of using thinner in industrial area and for narcotic use. Studies show that MDA levels increase and GSH levels decrease due to degradation of lipid peroxidation due to oxidative stress of organic solvents (Ulakoglu et al. 1998b, Dillioglugil et al. 2005, El-Nabi Kamel and Shehata 2008).

In recent years, research in phytotherapy focused on the effects of the components in plant extracts on various diseases, rather than the effect of plant extracts. Safranal is one of the components of saffron extract. Saffron is an herb with antioxidant effects, included in medical literature and is acknowledged to have an increasing significance due to its therapeutic effects (Moghaddasi, 2010; Assimopoulou et al., 2015).The radical scavenging activity of safranal results with antidiabetic, antioxidant, anticancer and hypotensive properties, thus various studies concluded that safranal could be used in industries such as food, pharmaceuticals and cosmetics (Assimopoulou et al., 2005, Imenshahidi et al., 2010, Hazman ve Bozkurt, 2015). Karafakioglu et al. (2017) reported that safranal (200 mg / kg) treatment may have a protective effect against cisplatin-induced nephrotoxicity and oxidative stress in rats. Alayunt et al.(2019) observed that particularly high-dose (100 mg/kg) administration of safranal to rats promoted the antioxidant system and be inflammatory against TNF-α and IL-6 cytokines on damage induced by CCl_4.

The main aim of our study is to investigate to what extent thinner, which is a mixture of organic solvent, affects brain, lung, liver, kidney, testis via respiratory system and to investigate the effect of safranal on this condition. The results of the study may contribute to the development of new approaches both in the protection of worker health on an industrial scale and in the treatment of addicts using volatile substances.

MATERIALS AND METHODS

Chemicals

Safranal (Cat no:Sigma-W338907), Total oxidant (TOS) and antioxidant status (TAS) commercial kits (Rell Assay:RL0024, RL0017) and the other chemical used in the study were purchased from Sigma -Aldrich (Sigma Aldrich Chemical Co.
St. Louis, Missouri, ABD).

Animals

In the present study , 40 Wistar albino male rats (weighing ~250-300 g) were used. The animals were housed under standard conditions of temperature (23±2°C), humidity, and dark-light cycle (lights on from 6:00 am to 6:00 pm).. Throughout the study, the rats were maintained on standard rat feed and tap water *ad libitum*. They were fed twice daily at 0900 and 1900. Before starting the thinner experiments, rats were subjected to preexperiments in order to prevent possible toxication severity complications. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the University of Afyon Kocatepe Animal Ethics Committee The permission was gained on 7 May 2015 with the number 49533702/46.

Experimental procedure

A total of 40 rats were included in the study. They were divided into 4 groups as follows: Control Group (C) (n=10), Sub-chronic Thinner Inhalation Group (T) (n=10), Sub-chronic Thinner Inhalation + Sa-

franal Group (*T+S*) (n=10) and Safranal Group (S) (n=10). Control group (C); comprised 10 healthy rats. The rats in the control group were administered 1 cc of physiological saline daily by gastric gavage for 8 weeks. Rats were fed with standard rat feed for 8 weeks and their weights were recorded regularly during the experiments. Sub-chronic Thinner Inhalation Group (T); rats were placed in a specially designed cage size 100x60x40 cm with air ventilation that allowed inhalation of controlled amounts of thinner (5ml). This application was repeated twice a day for 8 weeks and was terminated when 50% of the rats lost their reflexes to stand (About 5-6 min) (Konuk et al., 2012). Sub-chronic Thinner Inhalation+Safranal Group (*T+S*); treated as T group and additionally a dose of 100 mg/kg of safranal dissolved in 1 cc of physiological saline solution was administered via gastric gavage for 8 weeks. Commercial safranal (Sigma-w338907) was used in the study. Safranal Group (S); a dose of 100 mg/kg of safranal dissolved in 1 cc of physiological saline solution was administered via gastric gavage for 8 weeks. The dose of safranal used in this study was based on previous studies (Hosseinzadeh et al., 2013; Hazman ve Bozkurt, 2015; Hazman and Ovalı, 2015).

Sample preparation

At the end of the experimental period, the rats were anesthetized and sacrificed by cervical dislocation. Rats were anesthetized with 10 mg/kg-im xylazine and 50 mg/kg-im ketamine injection. All rats were sacrificed on the 56th day (8 weeks) of the experiment and tissue samples (brain, lung, liver, kidney, testis) were collected 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 15000xrpm and +4˚C for 10 minutes, and the supernatants were stored at -80 °C in deep freeze pending analysis.

Measurement of parameters

In the obtained supernatants, the Malondialdehyde (MDA) levels were determined according to Ohkawa et al. (1979), the reduced glutathione (GSH) analyzes were conducted based on Beutler (1963) and the amounts of Nitric oxide (NOx) were determined via the "Vanadium chloride (III) - Gries Reaction" method of Miranda et al. (2001). Total oxidant (TOS) and antioxidant status (TAS) measurements were performed via Eliza Reader (Biotek, ELx800) using commercial kits based on the method developed by Erel (2004, 2005). The amount of toluene in the blood was measured and the level of inhalation in T and T + S groups were determined. The toluene levels in the rats' blood were determined using the GS-MS in Acıbadem Labmed Central Laboratory (Istanbul, Turkey).

Statistical analysis

ANCOVA method was used for statistical analysis and the difference between the groups was tested using the Bonferroni test. The live weight averages of the groups at the zeroth and eighth week were chosen as the covariate. Data were analyzed using the SPSS® 24 package program. The results were expressed as "mean±standard error" (X±SE) and $p < 0.05$ was considered as the significance value.

RESULTS

Blood toluene levels

Blood toluene levels of rats that inhaled sub-chronic thinner are presented in Table 1. No statistically significant difference was determined for inhaled toluene levels in T and T+S groups.

MDA levels measured in tissue samples

The changes in MDA, which is an indicator of from lipid peroxidation, in the liver, lung, kidney, brain and testis tissue samples retrieved from the four experiment groups constituted in the present study, are presented in Table 2. Comparing the MDA levels of the T group with that of the control group levels it could be observed that a significant increase of MDA levels were determined in liver, lung, testis and kidney tissues (p=0.000).

GSH levels measured in tissue samples

The GSH levels in groups are presented in Table 3. It was observed that GSH levels in liver, kidney brain, and testis tissues was a significant decreased between T group and that of the control groups (Respectively p=0.013; p=0.000; p= 0.015; p= 0.007)

TAS and TOS levels measured in tissue samples

Changes of TAS and TOS levels in all tissues in groups are presented in Table 4 and Table 5, respectively. Comparing the TAS levels of the T group with that of the control group levels it was determined that TAS levels only significantly decreased in liver and kidney tissues (p=0.000). It was identified that liv-

er and brain TOS levels only significantly increased between the T group and that of the control group (p=0.000; p=0.001). It was observed that the liver and kidney tissues TAS levels significantly increased between T+S group and that the T group (p =0.000). It was concluded that liver tissue TOS levels significantly decreased between the T+S group and the T group (p =0.000).

NO_x levels measured in tissue samples

The changes in the NO_x levels obtained from all tissues of the experiment groups are presented in Table 6. It was observed that the NO_x level significantly increased only in the brain tissue between T group and the control group (p=0.000). When the NO_x levels of the T+S group were compared with the levels of the T group, it was concluded that there existed a statistically significant decrease in the NO_x levels in all tissues (p =0.000).

Table 1. Blood toluene levels of rats in T and T+S groups

Groups	C **x̄±SE**	S **x̄±SE**	T **x̄±SE**	T+S **x̄±SE**	p
Toluene (ng/ml)	-	-	1,525±0,071	1,481±0,071	0,730

C: Control Group; T : Sub-chronic Thinner Inhalation Group; T+S: Sub-chronic Thinner Inhalation + Safranal Group; S: Safranal Group; X ± SE: mean ± standard error; p<0.05

Table 2. MDA levels measured in tissue samples

MDA **(nmol/g tissue)**	C **x̄±SE**	S **x̄±SE**	T **x̄±SE**	T+S **x̄±SE**	p
LIVER	34,82±1,60^{bc}	36,71±1,24^{ab}	41,81±1,18^{a}	29,38±1,46^{c}	0,000
LUNG	19,95±0,94^{b}	19,83±0,73^{b}	24,27±0,69^{a}	18,32±0,86^{b}	0,000
KIDNEY	24,18±1,81^{b}	27,32±1,39^{ab}	32,83±1,33^{a}	27,82±1,65^{ab}	0,000
BRAIN	27,32±0,88^{a}	22,72±0,68^{b}	28,35±0,65^{a}	17,09±0,79^{c}	0,000
TESTIS	20,28±2,05^{b}	19,58±1,58^{b}	29,54±1,51^{a}	17,97±1,87^{b}	0,000

MDA: Malondialdehyde; C: Control Group; T : Sub-chronic Thinner Inhalation Group; T+S: Sub-chronic Thinner Inhalation + Safranal Group; S: Safranal Group; X ± SE: mean ± standard error; a,b,c: different letters on the same line indicate a significant difference p<0.05

Table 3. GSH levels measured in tissue samples

GSH **(nmol/g tissue)**	C **x̄±SE**	S **x̄±SE**	T **x̄±SE**	T+S **x̄±SE**	p
LIVER	19,93±1,05^{a}	19,25±0,81^{ab}	16,47±0,78^{b}	18,69±0,96^{ab}	0,013
LUNG	16,46±0,70^{ab}	16,71±0,54^{a}	17,37±0,52^{a}	13,64±0,64^{b}	0,000
KIDNEY	15,99±0,98^{ab}	17,39±0,76^{a}	12,91±0,72^{c}	14,09±0,89^{bc}	0,000
BRAIN	14,70±0,75^{a}	14,36±0,58^{a}	12,41±0,55^{b}	13,14±0,69^{ab}	0,015
TESTIS	18,8±1,6^{a}	18,71±1,22^{ab}	13,76±1,16^{b}	18,60±1,44^{ab}	0,007

GSH: reduced glutathione**;** C: Control Group; T : Sub-chronic Thinner Inhalation Group; T+S: Sub-chronic Thinner Inhalation + Safranal Group; S: Safranal Group; X ± SE: mean ± standard error; a,b,c: different letters on the same line indicate a significant difference p<0.05

Table 4. TAS levels measured in tissue samples

TAS **(mmol Trolox Equiv./L)**	C **x̄±SE**	S **x̄±SE**	T **x̄±SE**	T+S **x̄±SE**	p
LIVER	0,89±0,07^{a}	0,39±0,05^{b}	0,38±0,05^{b}	0,80±0,06^{a}	0,000
LUNG	0,28±0,09	0,29±0,07	0,32±0,07	0,49±0,09	0,213
KIDNEY	0,56±0,07^{a}	0,45±0,06^{a}	0,15±0,06^{b}	0,49±0,07^{a}	0,000
BRAIN	0,35±0,07	0,22±0,05	0,32±0,05	0,35±0,06	0,158
TESTIS	0,43±0,05	0,33±0,04	0,30±0,03	0,38±0,04	0,060

TAS: Total antioxidant status; C: Control Group; T : Sub-chronic Thinner Inhalation Group; T+S:Sub-chronic Thinner Inhalation + Safranal Group; S: Safranal Group; X ± SE: mean ± standard error; a,b,c: different letters on the same line indicate a significant difference p<0.05

Table 5. TOS levels measured in tissue samples

TOS (μmol H_2O_2Equiv./L)	C x̄±SE	S x̄±SE	T x̄±SE	T+S x̄±SE	p
LIVER	18,24±2,03[b]	28,82±1,56[a]	35,32±1,49[a]	22,51±1,85[b]	0,000
LUNG	13,24±1,89	16,17±1,46	16,75±1,39	13,26±1,73	0,129
KIDNEY	12,64±2,31	12,62±1,78	16,10±1,70	12,56±2,11	0,361
BRAIN	7,46±1,98[b]	13,14±1,53[ab]	17,26±1,46[a]	15,44±1,81[ab]	0,001
TESTIS	11,74±1,25	11,74±0,97	13,77±0,92	10,91±1,14	0,193

TOS: Total oxidant status; C: Control Group; T : Sub-chronic Thinner Inhalation Group; T+S: Sub-chronic Thinner Inhalation + Safranal Group; S: Safranal Group; X ± SE: mean ± standard error; a,b,c: different letters on the same line indicate a significant difference $p<0.05$

Table 6. NO_x levels measured in tissue samples

NO_x (μmol/g tissue)	C x̄±SE	S x̄±SE	T x̄±SE	T+S x̄±SE	p
LIVER	7,23±1,314[ab]	12,09±1,02[a]	9,32±0,97[a]	3,00±1,19[b]	0,000
LUNG	4,32±0,89[ab]	6,86±0,69[a]	6,54±0,66[a]	2,85±0,82[b]	0,000
KIDNEY	5,09±0,90[ab]	8,63±0,69[a]	6,99±0,67[a]	2,29±0,82[b]	0,000
BRAIN	3,32±1,01[b]	7,71±0,78[a]	6,98±0,74[a]	3,62±0,92[b]	0,000
TESTIS	5,64±0,95[ab]	5,87±0,73[a]	6,69±0,69[a]	2,81±0,86[b]	0,006

NOx**:** Nitric oxide; C: Control Group; T : Sub-chronic Thinner Inhalation Group; T+S: Sub-chronic Thinner Inhalation + Safranal Group; S: Safranal Group; X ± SE: mean ± standard error; a,b,c: different letters on the same line indicate a significant difference $p<0.05$

DISCUSSION

In the present study, it was observed that rats did not exhibit any escape reflexes during the application in the first two weeks of the inhalation, however, rats became more aggressive and exhibited escaping movements after the third week, their urination and excretion increased, and the uncontrolled rotational movements and standing reflexes deteriorated. Such observations obtained during the present study supported the previous findings reported in the literature suggesting that thinner acted as a neurotoxic agent and there existed evidence of intoxication in the central nervous system due to thinner inhalation (Bölükbaşı, 2005). It was also observed that the rats in the T+S group exhibited calmer behaviors contrary to the aggressive behaviors detected in the thinner inhalation group. Such finding could be interpreted as the mitigating or suppressing effect of safranal in case of the intoxication due to thinner inhalation. However, such finding should be supported through research conducted at molecular level. The difference between the blood toluene levels of the rats in the T and T + S groups indicates that the groups inhaled the same level of thinner.

Hosseinzadeh et al. (2013) suggest that the oral administration of safranal, in subacute toxicity test did not induce any toxic effects in many organs. Safranal at doses of 100 mg/kg by oral rout for 8 weeks did not cause any death in rats. However, an increase in the movement of animals was observed in the first 5 minutes after the application. This might be related to the irritant nature of most essential oils.

Higher doses and longer durations of thinner inhalation increases the monooxygenases based on CYP-450 and therefore results in ROS (Backes, 1993).. It is acknowledged that the toxic effects of organic solvents on cellular damage occurs due to the initiation of lipid peroxidation by free oxygen radicals such as superoxide anion ferryl and hydroxyl ions or more generally by the ROS (Mattia et al., 1991, 1993; Ahmed-Choudhury et al.,1998). Konuk et al. (2012) reported that sub-chronic thinner inhalation in rats increased free radical production. Additionally, exposure to sub-chronic toluene was reported not only to increase ROS production measures, but also to significantly affect various antioxidants (Kodavanti et al. 2015).

Given that the membrane phospholipids are the main target of free radicals, concentrations of MDA, one of the end products of lipid peroxidation (LP), were reported to be directly related to the severity of damage (Aleksandrovskii et al., 1988). In the pres-

ent study, once the MDA levels of the thinner group were compared with the MDA levels of the control group, it was observed that the MDA levels significantly increased in liver, lung, testis and kidney tissues, however, no significant increase was detected for the brain tissues despite the relative increase. Such findings were found to be consistent with the previous studies (Ulakoglu et al., 1998a, 1998b; Karaözler et al., 2002; Halifeoğlu et al., 2000). It was reported that in vivo and in vitro exposure to thinner or toluene accelerated the ROS formation in the brain, liver, kidney and lungs of rats and increased lipid (Mattia et al., 1991;1993; Halifeoğlu et al., 2000; Baydas et al., 2005). In parallel with these results, it is possible to state that sub-chronic thinner inhalation increased lipid peroxidation in tissues in the present study. It was reported that saffron and its components exhibited hydroxyl and DPPH radical scavenging activity (Hosseinzadeh et al., 2009; Assimopoulou et al., 2013). The literature review yielded no studies that focused on investigating the effects of safranal on thinner toxicity. In studies conducted through the use of safranal as an antioxidant molecule, it was stated that the increased MDA levels were decreased due to the antioxidative properties of safranal (Hosseinzadeh and Sadeghnia 2005; Mehdizadeh et al., 2013; Sadeghnia et al., 2013; Samarghandian et al., 2014, 2015). In the present study, the tissue MDA levels in T+S groups were found to be close to the levels of the control group and such finding indicated that safranal had a protective effect against the damaging effects of oxidation reactions in the organs and had an antioxidant potential in preventing lipid peroxidation, however such potentials did not exhibit uniform intensities for all tissue types.

GSH protects cells from oxidative damage through a direct interaction with the free radicals of the sulfhydryl group or through acting as a cofactor (Valko et al., 2006). In the present study, once the GSH levels of the tissue samples of the five organs of the T group were compared to those of the control group, it was determined that the GSH levels in the liver, testis, brain and kidney tissues were statistically significantly lower. On the other hand, there existed a statistically insignificant increase in the lung tissues, contrary to the findings in literature. Similar studies that focused on the effects of thinner on GSH levels reported that GSH levels decreased due to thinner inhalation (Baydas et al., 2003,2005; Ulakoğlu et al.,1998a; El-Nabı Kamel and Shehata 2008; Dillioglugil et al., 2005; Ahmadizadeh et al., 2014). In conclusion, the decreasing GSH levels in all tissues except the lung were considered to be due to the free oxygen radicals such as superoxide anions, ferryl and hydroxyl ions formed by the catabolism of thinner metabolites such as benzyl alcohol and benzaldehyde, which increased due to thinner inhalation. Hazman and Bozkurt (2015) and Samarghandian et al. (2014) reported that safranal increased the GSH levels in diabetic rats and suppressed the oxidative stress based on diabetes. In the present study, it was observed that the GSH levels decreased in the liver, testis, brain and kidney tissues of the T group were back to the control levels in the T+S group. Therefore, the authors considered that the excessive amounts of free radicals due to thinner inhalation were neutralized by safranal and the oxidative stress levels were decreased or safranal increased the biosynthesis GSH.

It was reported that the antioxidant/oxidant status in the body was more simply evaluated by TAS and TOS measurements and gave an insight about the sensitive balance between the in vivo oxidants and antioxidants as a result of synergistic interactions (Ghiselli et al., 2000; Erel, 2004; Erel, 2005; Kayar et al., 2015). However, once the antioxidant/oxidant balance was deteriorated in favor of oxidants and oxidative stress occurred, a significant negative correlation could be observed between the TAS and TOS levels (Erel, 2005). Considering the TAS and TOS levels for all tissues, TAS levels were determined to numerically increase in the T+S group when compared to the T group, and the TOS levels were determined to decrease. Once the data from the Safranal group were compared to the data of the control group, the expected TAS-TOS negative correlation could not be observed. On the other hand, similar studies that focused on the effects of thinner on TAS and TOS levels indicated that the decrease in total antioxidant capacity levels due to thinner inhalation was a result of the oxidative stress (Bayil et al., 2008; Konuk et al.,2012 Hazman and Bozkurt (2015) suggested that safranal increased the TAS levels and decreased the TOS levels especially in diabetic rats, hence, acted exactly similar to an antioxidant. The present study also states that safranal has a protective effect against the oxidative damage due to thinner inhalation.

NO is a free radical that is defined as a biological signal molecule and plays an important role in both physiological and pathophysiological processes (Velayutham. and Zweier, 2013). In the presence of stressors, it could be produced by the catalytic ac-

tion of inducible NO-synthase (iNOS) and could be at higher concentrations. NO could lead to damage to proteins, lipids and the DNA through direct or superoxide reaction and thus lead to the formation of a highly reactive peroxide anion (Rahal et al., 2014). In the present study, the increased NO_x levels in the T groups in comparison to the control group indicated the presence of thinner metabolites and their activated release. While Konuk et al. (2012) determined that nitric oxide metabolites in rats were higher in T groups when compared to the control, Maniscalco et al. (2004) concluded that NO_x levels increased in the blood of shoe and leather workers, breathing organic solvents such as toluene, xylene and methyl-ethyl ketone. Once the NO_x levels of the S group were compared to those of the control group, a numeric increase was observed for all tissues. Farahmand et al. (2013) stated that safranal could be effective as a hormetin and in cases of mild oxidative damage that causes the activation of the antioxidative enzymes. Several studies that investigated the anti-inflammatory and antioxidant potentials of safranal reported a decrease in NO_x level due to safranal administration (Samarghandian et al., 2014; Bukhari et al., 2015; Hazman and Bozkurt 2015). In the present study, decreased NO_x levels in the T+S group indicated that safranal decreased the thinner-based stressors.

CONCLUSIONS

In conclusion, it was determined that the oxidative stress in liver, lung, brain, testis and kidney tissues developed due to thinner inhalation was eliminated or mitigated due to safranal administration, either by decreasing the NO_x, MDA and TOS levels or by increasing the GSH and TAS levels. Safranal was considered to have important antioxidant potentials in protecting the studied tissues against the harmful effects of oxidative stress. However, it was evident that safranal did not provide a uniform effect for every tissue. Although safranal appears to be a biological antioxidant, there exists scarce evidence of its effect as a direct chemical antioxidant. However, in the present study, the comparison between the data of the safranal group and the control group indicated close values for NO_x and TOS levels of the T group and the expected TAS-TOS correlation was not achieved. Such results could indicate the significance of safranal dose in medical applications. Therefore, it was considered that safranal, effective as a hormetin, could also act indirectly as an antioxidant. Within the scope of these outcomes, it becomes essential to consider not to exceed the specific limit values of solvent inhalation in several industries. It is as well considered significant that applying treatment methods that include safranal for the rehabilitation of volatile substance addicts could contribute to the development of new treatment modulations.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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