Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20160928012410

Original Research

Geraniol attenuates hydrogen peroxide-induced liver fatty acid alterations in male rats

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Received: June 24, 2016
Accepted: September 21, 2016
Published: October 04, 2016

ABSTRACT

Background: Hydrogen peroxide (H_2O_2) is an oxidant agent and this molecule naturally occurs in the body as a product of aerobic metabolism. Geraniol is a plant-derived natural antioxidant. The aim of this study was to determine the role of geraniol on hepatic fatty acids alterations following H_2O_2-induced oxidative stress in male rats. **Methods:** After randomization, male Wistar rats were divided into four groups ($n = 7$ each group). Geraniol (50 mg/kg, dissolved in corn oil) and H_2O_2 (16 mg/kg, dissolved in distilled water) were administered by an intraperitoneal injection. Administrations were performed during 30 days with 1-day interval. **Results:** Administration of H_2O_2 resulted with a significant increase in malondialdehyde (MDA) and a significant decrease in glutathione (GSH) peroxidase glutathione level; geraniol restored its effects on liver. However, hepatic catalase (CAT) activities were significantly higher in H_2O_2, geraniol, and geraniol+H_2O_2 groups than control group. The ratio of hepatic total saturated fatty acids increased in H_2O_2-treated animals compared with control. In addition, hepatic total unsaturated fatty acids reduced in H_2O_2 group compared with control. The percentages of both hepatic total saturated and unsaturated fatty acids were not different between geraniol+H_2O_2 and control groups. **Conclusions:** H_2O_2-induced oxidative stress may affect fatty acid composition in liver and body. Geraniol can partly restore oxidative hepatic damage because it cannot completely reverse the H_2O_2-induced increase in hepatic CAT activities. Moreover, this natural compound can regulate hepatic total saturated and unsaturated fatty acids percentages against H_2O_2-induced alterations.

KEY WORDS: Fatty acids, geraniol, hydrogen peroxide, liver, oxidative stress

INTRODUCTION

Reactive oxygen species (ROS) in particular hydroxyl and superoxide radicals, and non-radical oxidants such as hydrogen peroxide (H_2O_2), hypochlorous acid, and peroxynitrite are generated in organism, mainly as an outcome of aerobic metabolism [1,2]. However, the excessive productions of ROS can impair essential cellular components such as nucleic acids, proteins, and polyunsaturated fatty acids [3]. Even some other molecules such as peroxynitrite ($ONOO^-$) and H_2O_2 are not free radicals; they are accepted to generate free radicals through many biochemical reactions in various cases [4]. In general, all organisms are well protected against free radical damage by endogenous oxidative enzymes such as catalase (CAT)

and glutathione (GSH) peroxidase (GSH-Px). Whenever the balance between ROS generation and antioxidant defense is lost, "oxidative stress" results through a series of stages dysregulates the cellular functions leading to several pathophysiological conditions [5,6]. Several nonenzymatic antioxidant compounds such as phenolics, ascorbic acid, tocopherol, and other dietary compounds play an essential role in defending the body against free radical damage by scavenging or neutralizing oxidizing molecules and maintaining redox balance [7].

Recent studies have reported that the plant kingdom offers a wide range of natural antioxidant molecules including phenolic acids, flavonoids, and other secondary metabolites, and they can be useful for the treatment of various disorders [8,9]. Geraniol, a natural acyclic monoterpene, is the primary component of oils of rose and palmarosa [10] and several essential oils such as lemon, ginger, and orange [11]. This natural molecule possesses diverse biological effects, being an antioxidant [12], antibacterial [13], anti-inflammatory [14], and antiangiogenic [15] agent.

The mitochondrial respiratory chain is responsible for the primary source of ROS production in cells because this mechanism consumes about 80-90% of oxygen that a person utilizes and produces most of the ROS generated in the body. Another essential formation of ROS especially occurs in the liver [16]. This study aims to assess a possible protective role of geraniol on liver fatty acids composition in H_2O_2-induced oxidative stress. For this purpose, in our study biochemical analyses such as liver tissue and serum MDA, hepatic GSH-Px, GSH, and CAT concentrations besides liver and serum fatty acids percentages were evaluated.

MATERIAL AND METHODS

Animals and Experimental Procedure

A total of 28 adult Wistar albino male rats (230 ± 10 g body weight) were obtained from Experimental Research Unit of Firat University (Elazig, Turkey). The animals were housed under standard light/darkness cycle (lights on from 0700 to 1900 h), at a regular temperature (21 ± 1°C) and humidity (55 ± 5%) with free access to fresh water and food. The experimental applications were confirmed by Ethical Committee of Firat University (Document No: 146/2011-11), and the rats were treated in strict compliance with the international laws on the use and care of experimental animals.

Groups of animals were randomly divided into four groups as control, naringenin, lead acetate, and naringenin+lead acetate (n = 7 each group). Control group animals received vehicle solutions only. Geraniol (50 mg/kg, dissolved in corn oil) and H_2O_2 (16 mg/kg, dissolved in distilled water) were administered by intraperitoneal injection. Administrations were performed during 30 days with 1-day interval. The animals were sacrificed at the end of 30 days. Blood and hepatic tissue were obtained from animals. Serum and liver samples were stored at −20°C until the assays were performed.

H_2O_2, geraniol and other chemicals were obtained from Sigma (Dorset, UK) unless otherwise indicated.

Determination of Liver and Serum Oxidative Stress-related Parameters

Protein concentration was analyzed using Lowry method [17]. MDA level was measured at 532 nm and expressed as nmol g protein^{-1} [18]. CAT was measured by determining the decomposition of H_2O_2 at 240 nm and was expressed as kg protein^{-1} [19]. GSH-Px was analyzed according to Lawrence and Burk method [20] and was expressed as IU g protein^{-1}. GSH concentrations were determined using the method of Sedlak and Lindsay [21]. All analyzes were performed using a UV-visible spectrophotometer (Shimadzu-2R, Tokyo, Japan).

Lipid Extraction and Measurement of Fatty Acid Percentages

The liver tissue samples (3 g) were homogenized for analyzes. Nonlipid contaminants in the lipid solution were purified through the addition of 0.88% KCl solution. The total lipids were extracted by a mixture of hexane/isopropanol (3:2 v/v) according to the previous method [22]. Fatty acids were converted into methyl esters via adding of 2% sulfuric acid (v/v) in methanol [23]. These methyl esters were separated by a gas chromatography and measured via flame/ionization detection system (Shimadzu GC-17 Ver3, Japan). The chromatography process was performed via capillary column (Machery-Nagel, Germany) using nitrogen as a vehicle gas (flow rate 800 μl/min).

Statistical Analysis

Results were expressed as a mean±standard error of mean. Data were analyzed using one-way analysis of variance followed by *post-hoc* Tukey's honestly significant difference (HSD) test (SPSS 12.0 for Windows as a software program). For all analyzes, $P < 0.05$ was considered statistically significant.

RESULTS

Effects of Geraniol on Hepatic MDA, GSH, GSH-Px, and CAT Activity against H_2O_2-Treatment

Figure 1 represents the hepatic MDA concentration (nmol g protein^{-1}) of the groups. Administration of H_2O_2 caused significant increases ($P < 0.001$) MDA concentrations (23.5 ± 1.3) compared with control (16.3 ± 0.7), whereas its levels were lower in the liver of the geraniol+H_2O_2 (17.8 ± 1.4) group animals compared with H_2O_2-treated group. In H_2O_2-treated animals, liver GSH (Figure 2, nmol g protein^{-1}) and GSH-Px (Figure 3, IU g protein^{-1}) levels (2.9 ± 0.2 and 50.8 ± 6.5, respectively) were significantly lower ($P < 0.001$ and $P < 0.01$, respectively) than control group (3.5 ± 0.1 and 63.4 ± 7.7, respectively). These values were significantly

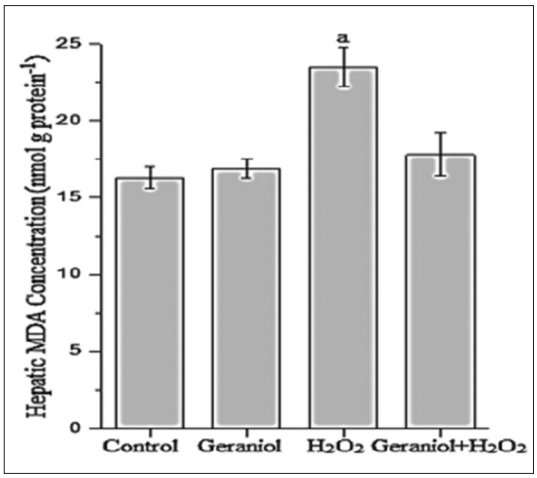

Figure 1: Hepatic malondialdehyde concentration of the groups. [a]*P*<0.001 vs. control group, (one-way analysis of variance followed by *post-hoc* Tukey's honestly significant difference test), *n* = 7 for each group

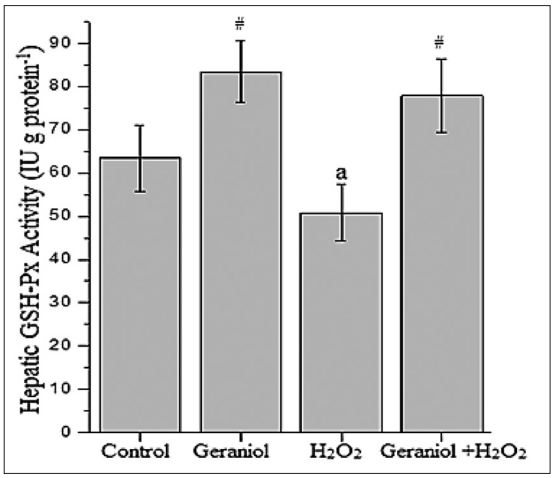

Figure 3: Hepatic glutathione-Px activity of the groups. [a]*P*<0.01 vs. control [#]*P*<0.001 vs. H_2O_2-treated group, (one-way analysis of variance followed by *post-hoc* Tukey's honestly significant difference test), *n*=7 for each group

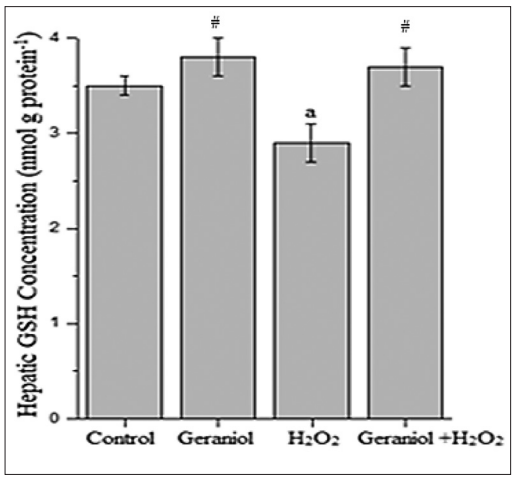

Figure 2: Hepatic glutathione concentration of the groups. [a]*P*<0.001 vs. control and [#]*P*<0.001 vs. H_2O_2-treated group, (one-way analysis of variance followed by *post-hoc* Tukey's honestly significant difference test), *n* = 7 for each group

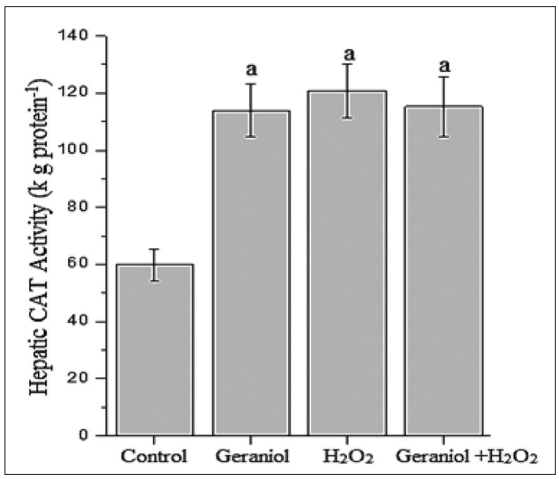

Figure 4: Hepatic catalase activity of the groups. [a]*P*<0.001 vs. control group, (one-way analysis of variance followed by *post-hoc* Tukey's honestly significant difference test), *n*=7 for each group

increased following coadministration of geraniol with H_2O_2 in geraniol+H_2O_2 group (3.7 ± 0.2 and 77.9 ± 8.6, respectively) rats compared with H_2O_2 alone-treated animals ($P < 0.001$). Liver CAT activity (Figure 4, kg protein^{-1}) increased in the geraniol (113.9 ± 9.2), H_2O_2 (120.9 ± 9.4) and geraniol plus H_2O_2 (115.2 ± 10.4) groups compared with control (59.9 ± 5.5) group ($P < 0.001$). In geraniol+H_2O_2 group animals, CAT levels were generally lower than the H_2O_2 but this effect did not differ between groups.

Effect of Geraniol on Serum MDA Concentration against H_2O_2-Treatment

We examined that effect of geraniol on the liver as well as body oxidant status in rats' serum after H_2O_2 treatment (Figure 5). There was no statistical difference between geraniol plus H_2O_2 group (8.5 ± 0.4) and control (7.2 ± 0.3) in MDA levels (nmol g protein^{-1}). We found that there was a significant increase in serum MDA level of H_2O_2-treated group (12.8 ± 0.5) when compared with control animals ($P < 0.001$). However, MDA concentrations were found to be lower in geraniol plus H_2O_2-treated animals compared with H_2O_2 group.

Effect of Geraniol on Hepatic Fatty Acid Percentages against H_2O_2-Treatment

The values regarding effects of geraniol and H_2O_2 on the total and individual fatty acids were summarized in Table 1. The differences in mean total saturated fatty acids (∑SFA) were detected between H_2O_2 and control groups ($P < 0.05$). There was no a significant alteration in ∑SFA percentage of control group compared to geraniol alone and geraniol plus H_2O_2 groups. The ratio of total unsaturated FA (∑USFA) was significantly lower in H_2O_2-treated animals compared to control group ($P < 0.05$). Although it was not statistically

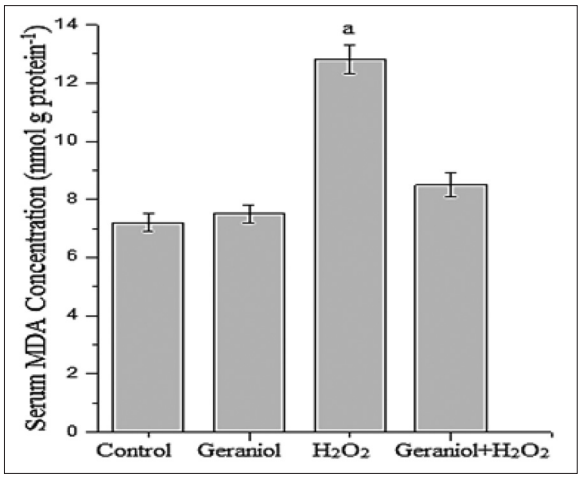

Figure 5: Serum malondialdehyde concentration of the groups. [a]*P*<0.001 vs. control group, (one-way analysis of variance followed by *post-hoc* Tukey's honestly significant difference test), *n*=7 for each group

Table 1: The percentages of fatty acids in liver

Fatty acids	Control	Geraniol	H_2O_2	Geraniol + H_2O_2
14:0	0.4±0.02	0.3±0.01	0.5±01	0.3±0.02
15:0	0.4±0.02	0.4±0.01	0.4±0.02	0.4±0.01
16:0	23.3±0.8	21.6±0.2	24.3±0.6	20.3±0.7[a]
16:1N7	2.6±0.7	1.5±0.1[a]	2.4±0.4	1.6±0.2[a]
17:0	0.7±0.1	0.9±0.04	0.7±0.1	0.9±0.03
18:0	15.1±0.1	16.9±0.2	15.7±1.03	17.9±1
18:1N9	7.1±0.6	4.8±0.2[b]	6.2±0.6	5±0.4[a]
18:1N7	3.5±0.2	3.6±0.1	3.6±0.1	3.8±0.2
18:2N6	16.9±0.7	16.3±0.4	17.8±0.6	16.5±0.2
18:3N6	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.02
18:3N3	0.2±0.01	0.1±0.01	0.2±0.05	0.1±0.01
20:1N9	0.4±0.02	0.2±0.01[a]	0.4±0.02	0.3±0.1
20:2N6	0.4±0.01	0.4±0.01	0.3±0.01	0.4±0.01[a]
20:3N6	1.4±0.1	1.4±0.03	1.1±0.1	1.4±0.1
20:3N9	0.2±0.01	0.3±0.01	0.2±0.02	0.3±0.02[a]
20:4N6	15.9±0.5	20.2±0.5[a]	15.3±1.5	19.8±1.1[a]
20:5N3	1.4±0.2	1.3±0.1	1.2±0.1	1.6±0.1
22:4N6	0.3±0.2	0.2±0.02	0.1±0.01	0.1±0.02
22:5N3	1.6±0.1	1.3±0.1	1.2±0.1[a]	1.3±0.1
22:5N6	0.3±0.01	0.3±0.02	0.3±0.01	0.3±0.01
22:6N3	7.4±0.2	7.4±0.2	7.3±0.7	7±0.2
24:0	0.3±0.01	0.2±0.1[a]	0.3±0.01	0.3±0.03
∑SFA	40.2±0.2	40.3±0.3	41.8±0.3[a]	40±0.3
∑USFA	59.7±0.4	59.7±0.4	58.1±0.3[a]	60±0.3
MUFA	13.5±1	9.3±0.2[c]	12.6±0.3	10.7±0.6[b]
PUFA	46.2±1.01	49.4±0.4[a]	45.5±2	49.2±1[a]
W3	10.6±0.4	10.2±0.2	10±0.8	10.1±0.3
W6	35.4±0.9	39±0.4[a]	35.3±1.2	38.8±1.1[a]

[a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001 versus control group, (Tukey's HSD test), *n*=7 for each group. MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

significant, the monounsaturated fatty acids (MUFA) levels of H_2O_2 and control groups, the liver MUFA concentration was found to be lower in the geraniol and geraniol plus H_2O_2 groups compared to control ($P < 0.001$ and $P < 0.01$, respectively).

The significant difference in the mean polyunsaturated fatty acids (PUFA) was detected in geraniol and geraniol+H_2O_2 groups compared with control group ($P < 0.05$). Although not statistically significant, the PUFA levels of H_2O_2 and control groups, its liver ratio was found to be higher in the geraniol and geraniol plus H_2O_2 groups compared with control group animals ($P < 0.05$). No overt differences in mean W3 percentages were observed among groups. There was no statistical difference between H_2O_2 and control group in the W6 levels. However, this FA percentage was found to be higher in geraniol and geraniol+H_2O_2 groups compared to control ($P < 0.05$).

Effect of Geraniol on Serum Fatty Acid Percentages against H_2O_2-Treatment

The differences in mean 18:0 percentages were detected in alone H_2O_2-treated and geraniol plus H_2O_2 groups compared to control rats ($P < 0.001$). No overt differences in this FA percentage were observed between control and geraniol-treated groups. There was a significant difference in H_2O_2 and geraniol+H_2O_2 groups compared to control animals in the mean 18:2n6 ratio ($P < 0.01$). The differences of these FA levels were not detected between geraniol and control groups.

In the rats administered geraniol, ∑SFA level was similar to control rats. However, the ∑SFA concentration was significantly affected by H_2O_2 and geraniol plus H_2O_2 treatment compared to control animals ($P < 0.01$). Serum ∑USFA level was significantly higher in the geraniol-treated animals compared to control rats ($P < 0.05$). ∑USFA concentration was found to be lower in the H_2O_2 and geraniol+H_2O_2 groups compared to control animals ($P < 0.01$). Although not statistically significant, MUFA level was determined to be higher in the geraniol-treated animals compared to control animals. However, the concentrations of MUFA were significantly lower in H_2O_2 and geraniol+H_2O_2 groups compared to control animals ($P < 0.05$). Serum PUFA level was significantly lower in H_2O_2 and geraniol+H_2O_2-treated groups compared with control rats ($P < 0.05$). Although not statistically significant, PUFA percentage was determined to be higher in the geraniol-treated animals compared to control group. Administration of H_2O_2 decreased serum w6 level compared to control rats ($P < 0.05$). However, the W6 percentages of the geraniol+H_2O_2 group were similar to the control group. The values regarding effects of geraniol and H_2O_2 on the total and individual fatty acids in the serum were summarized in Table 2.

DISCUSSION

The study provides an argument for the protective role of geraniol on changes of liver and serum oxidative stress related molecules, enzymes, and fatty acids in rats with H_2O_2-induced hepatic damage. Intraperitoneal administrations of both geraniol and H_2O_2 affected antioxidant status in the liver and serum of rats. While H_2O_2 increased MDA levels, geraniol restored its effects on the liver and serum lipid peroxidation in male rats. Treatment of geraniol to animals inhibited H_2O_2-induced decrease of GSH and GSH-Px concentrations in the liver. However, geraniol did not reverse the H_2O_2-induced increase in CAT activity completely.

Table 2: The percentages of fatty acids in serum

Fatty acids	Control	Geraniol	H_2O_2	Geraniol+H_2O_2
16:0	28.7±1	26.3±0.3[a]	28.1±0.2	27.6±0.1
18:0	17.8±1	18.9±0.6	20.8±0.3[c]	20.6±0.4[c]
18:1N9	8.2±1	9.2±0.5	7.04±0.2	6.9±0.2
18:1N7	3.7±0.1	3.5±0.1	3.4±0.2	3.9±0.2
18:2N6	24.6±1	24.1±0.6	22.1±0.4[b]	22.2±0.1[b]
20:4N6	10.6±0.4	12.1±0.2[a]	11.6±0.1	13.4±0.4[c]
22:6N3	6.3±1	5.8±0.2	6.9±0.4	5.3±0.2
∑SFA	46.5±0.3	45.2±0.3	48.9±0.4[b]	48.2±0.4[b]
∑USFA	53.5±0.3	54.8±0.3[a]	51.1±0.4[b]	51.7±0.4[b]
MUFA	12±1.1	12.8±0.2	10.4±0.1[a]	11±0.2
PUFA	41.5±0.2	42±0.2	40.7±0.2[a]	40.9±0.2[a]
W3	6.3±1	5.8±0.2	7±0.4	5.31±0.2
W6	35.2±0.2	36.2±0.2	33.7±0.4[a]	35.6±0.2

[a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ versus control group, (Tukey's HSD test), $n=7$ for each group. MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

Lipid peroxidation is an important toxic pathway because it involves the removal of hydrogen from fatty acid chains mediated by ROS [24,25] this way can lead to cell death in the body. The endogenous antioxidant enzyme includes GSH-Px that catalyzes the reduction of H_2O_2 to water through the oxidation of reduced GSH. CAT also participated in this conversion [26]. It was reported that oral administration of geraniol to 7,12-dimethylbenz(a)anthracene (DMBA)-treated mice significantly increased the activities of enzymatic antioxidants (CAT and GSH-Px) and non-enzymatic antioxidant (GSH) level in red blood cells, and skin tissues. Moreover, these parameters did not differ between geraniol alone treated and control animals [27]. Ibrahim *et al*. noticed [28] that 30 days administration of geraniol restores effects of the fructose-induced metabolic syndrome on hepatic and serum lipid peroxides related parameters in rats.

Hepatic nitric oxide (NO) content elevates in the fructose-induced model, possibly because of the increased synthesis of inducible NO-synthase activated by NF-κB [29,30]. Moreover, this mechanism is related with the increased lipid peroxidation and the decreased non-protein thiols (NPSH). Geraniol can suppress liver NO and lipid peroxides and enriches NPSH [28] via the activation of both GSH-Px and other reductase enzymes [31]. These reports that are related to hepatic lipid peroxidation and GSH-Px are consistent with our data. However, in this study, we observed that geraniol was not completely able to restore H_2O_2-induced increase in the hepatic CAT activity. Andrade *et al*. reported [32] that the inhalation of geraniol was increased serum alanine aminotransferase activity and hepatic lipid hydroperoxide in rats. Moreover, rats exposed to geraniol had higher CAT, SOD, and GSH-Px activities. The authors suggested that the lipoperoxide generation could be a result of ineffective antioxidant enzyme activities because these reactions were not sufficient to inhibit the ROS action and the lipoperoxide production in the liver of these animals. Koek *et al*. reported [33] that the activity of antioxidants enzymes increases early stage in the nonalcoholic steatohepatitis, but these alterations tend to decrease with progression of hepatic pathogenesis. The high concentration of H_2O_2 can be related with high activity of GSH-Px and CAT because these enzymes play an essential role in the elimination of H_2O_2. CAT is the most efficient enzyme in this interaction; also, it is so efficient that it cannot be saturated with H_2O_2 at any concentration [34]. Therefore, we can suggest that geraniol has an antioxidant role, but this effect is not full capacity on the H_2O_2-induced oxidative pathway in the liver of male rats.

Geraniol has anticancer efficacy that may be related to the inhibition of HMG-CoA reductase [35]. The latter role also confirms geraniol antiatherogenic effect, recently Jayachandran *et al*. suggested [14] that this natural monoterpene can ameliorate fructose-induced obesity and dyslipidemia in hamsters [28]. In 2011, it was reported [36] that geraniol activates *in vitro* several peroxisome proliferator-activated receptor (PPAR) subspecies. The interaction with geraniol and PPAR nuclear receptors that regulate the expression of target genes involved in lipid metabolism [37] is an important result because this data may provide a new treatment option for metabolic disorders such as hyperlipidemia, obesity, and diabetes. During the recent years, it was suggested that the antiatherogenic effect of geraniol is related to the activation of lipoprotein lipase to inhibit serum triglycerides, as well as lecithin cholesterol acyl transferase to elevate high-density lipoprotein cholesterol [14,28]. In this study, we first observed that the use of geraniol decreased high liver ∑SFA level caused by H_2O_2-treatment. Moreover, this monoterpene can protect the reduction of hepatic ∑USFA level induced by H_2O_2. Similar results were partly obtained for serum ∑SFA and ∑USFA levels.

As regards the individual fatty acids, the hepatic levels of palmitoleic acid, oleic acid, and eicosenoic acid, which are the member of MUFA, were affected by geraniol treatment. The percentages of hepatic oleic acid, palmitoleic acid, and eicosenoic acid decreased in the geraniol group and partly geraniol plus H_2O_2 groups. Therefore, the percentage of total MUFA decreased these groups. Interestingly, serum MUFA percentage did not differ between geraniol and control, while it decreased in H_2O_2 and geraniol plus H_2O_2 groups. Although not statistically significant, the percentage of hepatic arachidonic acid (20:4n6), which is a member of PUFA and is a partly essential fatty acid, was determined to be lower in the H_2O_2-treated rats, its level significantly increased in H_2O_2 and geraniol+H_2O_2 groups. In humans, levels of MUFA, such as oleic acid, are increased to the age of 18 years [38]. Several other PUFA particularly arachidonic acids are also decreased with age in the older persons [38]. Therefore, it can be said that these fatty acids change with metabolic and environmental factors over the years. Alterations in fatty acid composition of cells and its membrane are known to influence the activity of G proteins and protein kinase C (PKC) [39]. PKC and G proteins play a modulatory role in the regulating blood pressure [40]. Recently, it was reported that this natural compound can ameliorate the elevated systolic blood pressure against the fructose-induced metabolic syndrome [28]. Oleic acid is converted into nitrated-oleic acid in the presence of NO. The molecular mechanisms associated with physiologic roles of nitrated oleic acid remain unknown. It was suggested [41] that nitrated oleic exists in the blood and several organs, where it promotes vessel relaxation

and modulation of immune system cells. However, we did not determine whether geraniol and H_2O_2 affected blood pressure or NO-related metabolic parameters. Interestingly, although it was not statistically significant, oleic acid serum percentage was found to be higher in the geraniol-injected rats and lower in H_2O_2 and geraniol plus H_2O_2 groups. Otherwise, this fatty acid decreased in the liver of geraniol-treated group. Therefore, it can be said that the oleic acid and related fatty acids may have tissue dependent specific roles.

In this study, we observed that H_2O_2 causes oxidative stress in the liver and body; this effect can be restored partly through geraniol administration because it cannot reverse the H_2O_2-induced increase in CAT enzyme completely. In addition, this natural compound can be regulated in the liver several fatty acids percentage such as ∑SFA and ∑USFA levels against H_2O_2-induced alteration. However, geraniol has a distinct effect on other fatty acids like individual and total levels; this situation can be related to its other metabolic effects and interactions.

ACKNOWLEDGMENTS

We thank Dr. Z. Isik Solak Gormus for technical assistance and reviewing the use of English in the manuscript. This research was supported by the Scientific Research Projects Unit of Adiyaman University (ADYUBAP) project, number FEFBAP/2012-0001.

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Source of Support: Nil, Conflict of Interest: None declared.