Introduction

Geraniol, the main component of rose and palmarosa oils, is a natural molecule belonging to the class of monoterpens. It is found in the composition of essential oils of some plants such as ginger, lemon, lime, lavender, coconut, and orange in small quantities (1). Terpenes, including geraniol, have biological functions to protect plants against microorganisms and insects as well as to generate aromatic flavor and fragrance (2). Nowadays, natural or synthetic products of these molecules are used as taste and fragrance components in foods as well as in perfumery and cosmetic products (3). In addition, geraniol has a potential as the pharmacological agent. It was reported that geraniol has an anticarcinogenic effect because it could conduct the cell cycle to reduce uncontrolled proliferation in the human pancreatic adenocarcinoma cells (4). In our previous study, we observed that this monoterpenic has antioxidant activity and, it improves liver fatty acid changes due to hydrogen peroxide (H$_2$O$_2$)-induced oxidative stress in rats (5).

Approximately 23% of total cholesterol in the body is present in the central nervous system (CNS) structures. Therefore, the brain is one of the richest organs in terms of cholesterol content (6). As long as...
the blood-brain barrier is intact, the transition of the 
cholesterol from the plasma to the brain is unlikely. 
This indicates that the high-cholesterol content of 
CNS is completely synthesized by its own cells 
(7). Interestingly, there is a continuous cholesterol 
transition from the brain to the general circulation. 
This is accomplished as a result of the conversion of 
the cholesterol to the 24S-hydroxycholesterol, which 
can pass blood-brain barrier, via hydroxylation with 
the 24-hydroxylase (Cyp46a1), a member of the 
neuron-specific cytochrome P450, in the CNS (8,9).

The brain cholesterol dysregulation may occur 
in several neurodegenerative disorders, including 
Alzheimer’s disease, Huntington’s disease, 
Parkinson’s disease, and stroke (10). Although the 
brain constitutes only 2% of body weight, it accounts 
for 20% of total oxygen consumption (11). Therefore, 
the brain is highly sensitive to oxidative stress due to 
limited antioxidant capacity, high-energy requirement 
and high lipid and iron content (12). The ROS formed 
in the brain is eliminated with the antioxidant molecules 
and vitamins as well as the enzymatic inactivation. 
Vitamins A, C and E are essential antioxidant vitamins 
in the body (13).

In recent years, the roles of oxidative stress, 
antioxidant vitamins and changes in brain 
cholesterol metabolism in the pathophysiology of 
neurodegenerative diseases are discussed (14). Both 
vitamin A and vitamin E are important antioxidant 
vitamins for the brain because they are soluble in 
fat. Effects of the geraniol on brain cholesterol and 
fat-soluble vitamins are unknown. Therefore, in this 
study, we aimed to determine the effect of geraniol 
on brain cholesterol, vitamin A, and E levels in rats 
treated with H$_2$O$_2$, which is a strong oxidizing agent.

**Materials and Methods**

In this study, Wistar albino adult male rats obtained 
from the Experimental Research Center of Firat 
University (FÜDAM) were used. The experimental 
groups were selected randomly, with seven animals 
in each group. The groups were designed as; 
control, geraniol, H$_2$O$_2$, and geraniol+H$_2$O$_2$. Geraniol 
(50 mg/kg, dissolved in corn oil) and H$_2$O$_2$ (16 mg/
kg, dissolved in distilled water) were administered 
by an intraperitoneal injection for 30 days with one-
day interval (5). The control group rats received both 
vehicle solutions in same ways. During the experiment, 
the animals were kept at constant temperature (21 
± 1 °C) and 12 hours night/12 hours daytime (lights 
turned on 07:00). Food and water are given as ad 
libitum. This study has been conducted in accordance 
with the ethical standards and according to the 
Declaration of Helsinki and according to international 
and national guidelines and has been approved by 
the Ethics Committee of Elazig Firat University.
Lipid extraction

Brain samples were extracted with isopropanol-hexane- (2/3, v/v) by the method of Hara and Radin (15). For this purpose, frozen brain samples were weighed and homogenized (500 mg tissue) with a 5 ml isopropanol/hexane mixture.

Determination of Cholesterol

Cholesterol was analyzed from lipid extracts according to previous methods with minor modifications (16,17). Briefly, a mixture of isopropyl alcohol / n-hexane (5 ml) was treated with potassium hydroxide solution (5 ml of 0.5M in methanol) that was then vortexed for 30 seconds. Samples tubes were placed in a water bath for 15 minutes (80 ºC). After cooling, 5 ml of hexane and 1 ml of distilled water were added, and then centrifuged for 5 minutes at 5000 rpm. The residue was redissolved with the mobile phase (1 ml of methanol:68/acetonitrile:28/ distilled water:4, v: v: v). Finally, a 20 µl of sample was injected into the HPLC column. Detection was operated using a UV spectrophotometer 208 nm for cholesterol. Quantification was carried out by external standardization using Class-VP chromatography software (Shimadzu, Japan). The results of measurement were expressed as μmol/g.

Statistical analysis

All data were presented as mean±standard error mean (SEM). Results were analyzed using one-way analysis of variance (ANOVA) followed by a post hoc Tukey’s test (SPSS 17). A p value less than 0.05 was accepted as statistically significant.

RESULTS

Brain Cholesterol Levels

Brain cholesterol levels are shown in Figure 1. There was a significant increase in brain cholesterol level in the H₂O₂ group (17.17±0.63 µmol/g tissue) compared to control group (12.88±0.73 µmol/g tissue, p <0.01). The differences in mean cholesterol concentrations were not detected between geraniol (15.13±0.50 µmol/g tissue) and geraniol + H₂O₂ (14.68±1.74 µmol/g tissue) groups.

Brain α-tocopherol, α-tocopherol acetate, and retinol levels

The brain α-tocopherol levels of the geraniol (63.91±1.65 µg/g tissue), H₂O₂ (66.57±1.27 µg/g tissue), and geraniol + H₂O₂ (65.52±8.75 µg/g tissue) groups were not different from the control group (57.21±3.79 µg/g tissue, Figure 2). The differences in α-tocopherol acetate levels were not detected between geraniol-treated (14.6±0.16 µg/g tissue) and control group (14.08±0.35 µg/g tissue), while H₂O₂ (15.62±0.2 µg/g tissue) and geraniol + H₂O₂ (15.4±0.16 µg/g tissue) groups had higher α-tocopherol acetate levels than control (p <0.05, Figure 3). The brain retinol levels of the geraniol (0.24±0.03 µg/g tissue), H₂O₂ (0.31±0.06 µg/g tissue), and geraniol + H₂O₂ (0.24±0.06 µg/g tissue) groups were not different from the control group (0.3±0.02 µg/g tissue, Figure 4).

DISCUSSION

More than half of the body cholesterol is obtained by synthesis, and the rest is taken with nutrients. On the other hand, the brain has higher cholesterol content than any other organ, and this is entirely achieved by endogenous cholesterol synthesis (7). Therefore, understanding the cholesterol metabolism and the changes in the brain may help to understand the pathophysiology of neurodegenerative diseases. In our study, the effects of geraniols on brain cholesterol levels, vitamin E and vitamin A levels in rats treated with H₂O₂ were evaluated.
brain cholesterol was significantly increased in the H$_2$O$_2$-treated group. In the geraniol+H$_2$O$_2$ group, it can be said that cholesterol level is same as the control group. Although H$_2$O$_2$ is not a free radical, it can form hydroxyl radical, a very strong ROS, as well as superoxide radical due to reaction with transition metal ions (18). Several researchers reported that the oxidative stress can impair cholesterol metabolism in the brain, and this effect may result in a cholesterol deposition in the brain tissue (19,20). Thus, our results indicate that the increase in brain cholesterol level of the H$_2$O$_2$ group is a reflection of the oxidative stress.

Recently, Kreilaus et al. (21) reported that 24-hydroxycholesterol decreased by 60%, cholesterol increased by 30%, and cholesterol oxidation products due to oxidative stress increased by 50-70% in postmortem brain tissue of suffering Huntington disease. Thus, it can be said that the increase in cholesterol level of the H$_2$O$_2$ group is probably related to the dysregulation of 24-hydroxycholesterol, a cholesterol carrier, and brain cholesterol synthesis. H$_2$O$_2$ is an oxidant agent, and its negative effect on lipid peroxidation and cholesterol regulation should be taken into consideration because the in vivo administration of this molecule induces oxidative stress in the body (5,22). Moreover, geraniol has antioxidant activity, and it exerts an ameliorative role in the H$_2$O$_2$-induced oxidative stress (5). In the geraniol + H$_2$O$_2$ group, brain cholesterol level was same as the control group. This result may be related to an antioxidant effect of the geraniol. Geraniol has the ability to cross the blood-brain barrier (23). Although the effect of geraniol on brain cholesterol regulation is unknown, it has been suggested that this monoterpene has an inhibitory effect on mevalonate pathway and lipid metabolism (24). Mevalonate is an important step for cholesterol synthesis and cell proliferation in the body. This process is completed by firstly conversion of acetyl-CoA to 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA), and then by conversion to cholesterol from mevalonate with a series of biochemical reactions (25). In the recently reported that geraniol has a cholesterol-lowering effect in the rat serum (26) and mice (27). Regarding brain cholesterol level, in accordance with the above reports, we observed that the level of cholesterol increased in the H$_2$O$_2$ group but not in the geraniol + H$_2$O$_2$ group.

In the present study, there were no significant changes in the brain α-tocopherol level. However, α-tocopherol acetate concentrations increased in the H$_2$O$_2$ and geraniol + H$_2$O$_2$ groups. The α-tocopherol is the most biologically active member of the vitamin E family (28). The members of vitamin E family are metabolized in the body via cytochrome P450 enzymes (29). ROS production occurs in the cell membrane and microsomal areas except for mitochondria due to changes in cytochrome p450 activity in the physiological or especially pathophysiological conditions (30). The H$_2$O$_2$ is a microsomal ROS product that occurs in reactions involving cytochrome P450 (31). An activity of the cytochrome p450 enzymes may be affected by oxidative stress in the brain and other organs because H$_2$O$_2$ can inhibit cytochrome p450 enzymes (32,33). In the present study, changes in the α-tocopherol acetate level of the H$_2$O$_2$ group may be a result of this interaction. However, high levels of α-tocopherol acetate in the H$_2$O$_2$ group as well as geraniol + H$_2$O$_2$ group also indicate that, apart from its antioxidants role, geraniol can interact with cytochrome p450 enzymes. It is known that there is a relationship between cytochrome p450 activity and geraniol metabolism in the cells (34,35). In a recent study reported that geraniol treatment in rats with non-alcoholic steatohepatitis models reduce oxidative stress and regulate cytochrome p450 activity (36).

In conclusion, H$_2$O$_2$ administration caused an alteration in the brain cholesterol and α-tocopherol acetate levels. We suggest that geraniol may exert a modulating role on the effects of H$_2$O$_2$-induced oxidative stress in the brain of male rats.

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