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Effect of Geraniol on Macro and Micro Elements Levels in Lung Tissue of Rats That are Subjected to Oxidative Stress

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ABSTRACT
In this study, protective effect of geraniol administered intraperitoneally on levels of Fe, Zn, Cu, Ca, Mn and Mg elements in lung tissue of the rats exposed to oxidative stress with H_2O_2 were investigated. In our study, 24 male rats in total were used. Four groups with equal number of male rats in each were created and designated as control (K), H_2O_2 (H), geraniol (G), H_2O_2+geraniol (H+G). 50 mg/kg of geraniol and 20 mg/kg of H_2O_2 were injected intraperitoneally every other day for a period of 30 days. In this study, levels of Fe and Ca were higher in H group when compared with others groups ($p<0.01$). Levels of Fe and Ca compared with the H group decreased in H+G group, they were equal level with K group. Levels of Mn and Zn in H group decreased when compared with to the other groups ($p<0.01$). Levels of Mn and Zn in H+G group were found to be near to K group. The difference of Cu and Mg levels among the groups were found as not significant ($p>0.05$). According to our results, it can be concluded that hydrogen peroxide administered to rats as a source of oxidative stress caused a negative impact on macro and micro elements in lung tissue that geraniol eliminated negative impact of hydrogen peroxide on element levels.

Keywords: Geraniol, H_2O_2, lung, rat, trace element

1. INTRODUCTION
Recent studies, geraniol, an acyclic monoterpene alcohol found in lemongrass and aromatic herb oils, has been shown to exert antitumor efficiency against murine leukemia, hepatoma, and melanoma cells[1-4]. Camphene and geraniol were found to significantly decrease lipid peroxidation[5]. In a study by Burke et al., geraniol and farnesol were administered to rats with pancreatic cancer tumor for 20 days. It was reported that geraniol and farnesol were effective on pancreas tumor and reduced tumor radius[6].

It is reported that any change in normal physiological limits of transition metals result in antioxidant system and reactive oxygen species (ROS) production being affected[7-10]. Trace metals, especially iron are implicated as causative agents in excessive generation of free radical which are capable of causing oxidative damage to erythrocytes[11,12]. It is stated that some elements have a significant role during biological processes covering activation or inhibition of enzymatic reactions. The association of trace elements with oxidative stress and cancer diseases has known[13]. The most important damage caused by hydroxyl radical is free radical chain reaction known as lipid peroxidation. Since cell membrane doesn't contain water, the primary target of OH is fatty acids. Peroxidation of membrane lipids decomposes the membrane structure and increases permeability leading to cell death[14]. Some metals combining with excess hydrogen peroxide, such as Fe and Cu, increases hydroxyl radical formation by Haber-Weiss reactions. Hydroxyl radical is also an over oxidizing reactive radical and can be involved in DNA hydroxylation, protein aggregation, and can interaction with some biomolecules[7,15].

2. MATERIALS AND METHODS

2.1 *Chemicals*
HNO_3 (65%), $HClO_4$ (60%) and of H_2O_2 (30%) were obtained from Merck Chemical Company. Geraniol (98%) was obtained from Sigma Aldrich.

2.2 *Laboratory animals*
Male Wistar albino rats used in the experimental study were supplied from Firat University Medical School Experimental Research Centre (FÜTDAM) and experimental study was performed in the same place. The temperature of the environment where laboratory animals were kept was held stable in the range 22-25^{0}C and the animals were monitored for 12 hours under light and 12 hours in dark. During experimental studies, a total of 24 four -months-old wistar albino male rats weighing 260 gr (260±40 gr) in average were used. Criteria specified by NIH (National Institutes of Health) with respect to animal rights were strictly followed during the experiment. Four groups, namely control group, H_2O_2 group, geraniol group and H_2O_2+geraniol group, were created in the experiment. Corn oil was given to control group. 50 mg/kg of geraniol and 20 mg/kg of H_2O_2 were administered to the rats. H_2O_2 solution was administered following its preparation in distilled water. Geraniol was administered after it was prepared in corn oil. Corn oil was also given to H_2O_2 group as standard. Abovementioned compounds were intraperitoneally injected to rats every other day for a period of 30 days. At the end of 30 days, the rats were decapitated and their lung tissues were removed by surgical procedure and allowed to stand at -20^{0}C until analysis.

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2.3 *Solubilizing lung tissue samples*

1.5 mL of HNO_3 and 1.5 mL of $HClO_4$ were added to 0. 4-1.0 grams of lung tissue samples and they were allowed to stand for 3 hours; then, following administration of 2 mL of H_2O_2, solubilization procedures were carried out in closed system PTFE (polytetrafluorethylene) containers in a microwave oven. Digestion program in microwave oven was applied as 5 minutes at 250 W, 10 minutes at 800 W, and 5 minutes at 450 W. The resultant clear mixture was completed to 10 mL with a solution of 0.1 M HNO_3. Same methods were applied also for blank solutions not containing any tissue[16].

2.4 *Analyzing lung tissue samples*

Sample solutions prepared for analysis were analyzed for Zn, Cu, Fe, Ca and Mg by atomic absorption spectrophotometer via calibration curve method provided that each group is tested separately, and dilution was performed for some elements and direct reading was performed for others. Mn analysis was carried out by Inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.5 *Statistical analysis*

Statistical evaluation was performed by SPSS:10 programs. Comparison between the groups was performed by Variance analysis (ANOVA) and Least Significant Difference (LSD) test was performed to compare the results from different groups. P-value of less than 0.05 was accepted as statistically significant.

3. RESULTS AND DISCUSSION

The levels of some elements are directly associated with various factors (such as nutrition, age, disease, environment, etc). Zn, Cu and Mn in particular, besides being an immunity factor in infection diseases, show protective feature against oxidative stress and cancer. While levels of trace elements in tissues lower than normal cause some diseases, higher levels create toxic reactions[17]. Some elements level in analyzed lung tissue is given in Table-1.

Table-1: Lung tissue levels of some elements (ppm) (n=6).

Groups	Fe	Cu	Zn	Ca	Mn	Mg
K	61.13±3.02^a	0.95±0.02^a	15.76±1.26^a	79.16±4.43^a	0.18±0.01^a	35.45±2.47^a
H	77.27±3.11^c	1.02±0.05^a	9.05±0.35^d	93.70±4.50^b	0.15±0.01^d	32.10±1.34^a
G	58.44±4.12^a	0.90±0.04^a	16.28±1.45^a	75.88±4.10^a	0.19±0.01^a	36.02±3.27^a
H+G	70.46±4.83^a	1.01±0.07^a	15.43±1.19^a	82.43±3.28^a	0.18±0.01^a	34.49±1.79^a

a,b,c,d: Different letters in the same columns indicate statistical differences (P<0.01)

As a result of the analyses performed, we observed that Fe level was higher in group H_2O_2 ($p<0.01$). In the case of group H+G, we observed that the amount of iron which was initially high decreased and approached to the amount in control group as a result of the effect of geraniol. From these results, we believe that geraniol eliminated negative radical effect of H_2O_2 at Fe level. Excess H_2O_2 is believed to increase oxidative stress even more, increase iron intake and storage (i.e. by increasing iron in the cell) and increase oxidative damage, fenton reaction[18, 19]. It was reported that superoxide anions produce by oxidative stress and reducing agents increase releasing of iron from ferrite. "Iron hypothesis" claimed to take part in pathogenesis of atherosclerosis is the affect of endothelial damage and vascular cell proliferation by iron as a result of it increasing formation of free radicals, oxidant stress and lipid peroxidation [20]. Ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) form hydroxyl radicals by Fenton and Haber-Weiss reactions and entail cellular damage[21]. In skin tumorogenesis samples formed with (DMBA) 7, 12-dimethylbenz-[a]anthracene, significantly high amounts of iron were determined[22,16]. In was reported that in the case of thalassemia patients exposed to frequent transfusion, oxidative stress was generated in the medium as a result of excess iron load, and SOD enzyme activity was significantly higher in them than in control group[23, 24].

In analysis of tissue, level of Zn in H group was lower than in other groups ($p<0.01$). We observed that Zn levels compared with the H group increased in H+G group, it was equal level with K group. As in our study, there are studies in the literature investigating the association of Zn with oxidative stress. In a study, Zn was reported to act as a protector against radicals causing harm in the cell, such as reactive oxygen types, hydrogen peroxide (H_2O_2), hydroxyl radicals and singlet oxygen[25]. In a study by Çiftçi et al., it was stated that Zn level was lower in (7, 12-dimethylbenz-[a]anthracene) DMBA administered subjects than in the control group and oxidative stress of lipoic acid caused a positive effect on Ca level by increasing it[16].

In our study, it was determined that Ca level was higher in groups that received H_2O_2 than in control group ($p<0.01$). In the case of group H+G, high Ca level was shown to decrease and approach to Ca level of control group due to the effect of geraniol. We believe that geraniol eliminates high Ca level that has increased as a result of oxidative stress. In several studies by Orrenius et al. (1992), it was reported that calcium related defense system enzymes become activated by increasing level of free calcium in the cell and calcium level increases in the cell in conjunction with free radical production[26]. Increase in Ca level has been reported in multiple myeloma patients [27]. As a result of lung tissues analysis, level of Mn in H group was lower than in other groups ($p<0.01$). From our results, it

was determined that Mn level in H+G group was near K group owing to effect of geraniol. Manganese also serves as a free oxidation radical remover in the cases of low oxidation[28]. Singh and colleagues [29] observed on the effectiveness of Mn as an intravenous superoxide scavenger in mammals, suggesting a antioxidant property of Mn. Manganese (Mn), an essential trace element, plays an important role in antioxidant defenses and is found in many enzymes, including mitochondrial superoxide dismutases, glutamine synthetase, alkaline phosphatase, and arginase[30,31]. The difference of Cu and Mg levels among the groups were found as not significant ($p>0.05$).

4. DISCUSSION

As a result, the substances of geraniol in the lung tissue of the rats in which we induced oxidative stress by giving hydrogen peroxide were seen to suppress the oxidative stress through exhibiting favorable effect at macro and micro elements level. In conclusion, it can be concluded that geraniol may a potential against oxidative stress.

5. REFERENCES

1. Shoff, S. M., Grummer, M., Yatvin, M. B., and Elson, C. E., Cancer Res, **(1991)**, 51:37–42.
2. Yu, S. G., Hildebrandt, L. A., and Elson C. E., J Nutr, **(1995)**, 125:2763–2767.
3. Crowell, P. L., and Gould, M. N., Crit Rev, **(1994)**, Oncog, 5:1–22, http://dx.doi.org/10.1615/CritRevOncog.v5.i1.10.
4. Carnesecchi, S., Schneider, Y., Ceraline, J., Duranton, B., Gosse, F., Seiler, N., Raul, F., J. Pharm. Exp., **(2001)**, 298(1),197-200.
5. Tiwari, M., Kakar, P., Toxicology in Vitr., **(2009)**, 23, 295-301, http://dx.doi.org/10.1016/j.tiv.2008.12.014.
6. Burke, Y. D., Stark, M. J., Roach, S. L., Sen, S. E., Crowell, P. L., Lipids., **(1997)**, 32(2),151-156, http://dx.doi.org/10.1007/s11745-997-0019-y.
7. Aydemir, B., Kızıler, A. R., Onaran, I., Alıcı, B., Ozkara, H., Akyolcu, M. C., Biol. Trace Elem. Res., **(2006)**, 112(3),193-203, http://dx.doi.org/10.1385/BTER:112:3:193.
8. O'Dell, B. L., Nutr. Rev., **(1992)**, 50(2),48-50, http://dx.doi.org/10.1111/j.1753-4887.1992.tb02513.x.
9. Nasr, M. R., Ali, S., Shaker, M., Elgabry, E., East Mediterr Health J., **(2002)**, 8(4-5), 490-495.
10. Steinkühler, C., Pedersen, J. Z., Weser, U., Rotilio G., Biochem. Pharmacol. **(1991)**, 42(9), 1821-1827, http://dx.doi.org/10.1016/0006-2952(91)90521-6.
11. Widad, N. M., Al-Naama, L., Meaad, Haem, **(2003)**, 6(3) : 376-83.
12. Ghone, R. A., Kumbar, K. M., Suryakar, A. N., Katkam, R. V., Joshi, N. G., IJCB, **(2008)**, 23 (4) 337-340.
13. Sattar, N., Scott, H. R., McMillan, D. C., Talwar, D., O'Reilly, D. S., Fell G. S., Nutr. Cancer, **(1997)**, 28(3), 308-312, http://dx.doi.org/10.1080/01635589709514592.
14. Nishiyama, Y., Ikeda, H., Haramaki, N., Am. Heart J., **(1998)**, 135(1),115-120, http://dx.doi.org/10.1016/S0002-8703(98)70351-5.
15. Gutteridge, J. M. C., FEBS Lett., **(1986)**, 201(2), 291-295, http://dx.doi.org/10.1016/0014-5793(86)80626-3.
16. Çiftçi, H., Özkaya, A., Dayangaç, A., Ölçücü, A., Çelik, S., Şahin, Z., Ateş, S., Kafkas, Univ. Vet. Fak. Derg., **(2009)**, 15(4), 569-573
17. Williams, R. J., Endeaouvr New Series, **(1984)**, 8(2), 65-70, http://dx.doi.org/10.1016/0160-9327(84)90040-1.
18. Ponka, P., Beaumont, C., Richardson, D. R., Hematol, (**1998)**, 35(1), 35-54.
19. McCord, J. M., Semin. Hematol, (**1998)**, 35(1), 5–12.
20. Klipstein-Grobusch, K., Koster, J. F., Grobbee, D. E., Lindemans, J., Boeing, H., Hofman, A., Witteman, J. C., Am. J. Clin. Nutr., (**1999)**, 69(6), 1231–1236.
21. Braughler, J. M., Duncan, L. A., Chase, R. L., J. Biol. Chem., **(1986)**, 261(22), 10282-10289.
22. Razazadeh, H., Athar, M., Redox Rep., **(1997)**, 3(5-6),303-9.
23. Naıthani, R., Chandra, J., Bhattacharjee, J., Verma, P., Narayan, S., Pediatr. Blood Cancer, **(2006)**, 46 (7), 780-785, http://dx.doi.org/10.1002/pbc.20669.
24. Meral, A., Tuncel, P., Sürmen-Gür, E., Ozbek, R., Oztürk, E., Günay, U., Pediatr. Hematol. Oncol., **(2000)**, 17(8), 687-693, http://dx.doi.org/10.1080/08880010050211402.
25. Coudray, C., Rachidi, S., Favier, A., Biol. Trace Elem. Res., **(1993)**, 38, 273-287, http://dx.doi.org/10.1007/BF02785311.
26. Orrenius, S., McCabe, M. J., Toxicol Lett., **(1992)**, 64-65, 357-364, http://dx.doi.org/10.1016/0378-4274(92)90208-2.
27. Oyajobi, B. O., Arthritis Res. Ther., **(2007)**, 9 (1), 1-6, http://dx.doi.org/10.1186/ar2104.
28. Donaldson, J., McGregor, D., LaBella, F., Can. J. Physiol. Pharmacol., **(1982)**, 60(11), 1398-1405, http://dx.doi.org/10.1139/y82-208.
29. Singh, R. K., Kooreman, K. M., Babbs, C. F., Fessler, J. F, Salaris, S. C., Pham J., Am. J. Vet. Res., **(1992)**, 53, 1822−1829.
30. Gerber, G. B., Le´onard, A., Hantson, P., Crit. Rev. Oncol/Hematol., **(2002)**, 42, 25–34.
31. Zhang, S. R., Zhou, Z. C. and Fu, J. L., Environ. Res. **(2003)**, 93(2):149-157.