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Effects of Intraperitoneally Administered Lipoic Acid, Vitamin E, and Linalool on the Level of Total Lipid and Fatty Acids in Guinea Pig Brain with Oxidative Stress Induced by H_2O_2

Sait Çelik* and Ahmet Özkaya
Department of Chemistry, Faculty of Science, Firat University, Elazig, Turkey

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The aim of our study was to investigate the protective effects of intraperitoneally-administrated vitamin E, dl-alpha lipoic acid, and linalool on the level of total lipid and fatty acid in guinea pig brains with oxidative stress that was induced by H_2O_2. The total brain lipid content in the H_2O_2 group decreased when compared to the H_2O_2 + vitamin E ($p<0.05$), H_2O_2 + linalool ($p<0.05$), ALA ($p<0.05$), control ($p<0.01$), linalool ($p<0.01$), and vitamin E ($p<0.01$) groups. While the proportion of total saturated fatty acid (ΣSFA) in the H_2O_2 group significantly increased ($p<0.005$) when compared to the vitamin E group, it only slightly increased ($p<0.01$) when compared to the control and H_2O_2 + vitamin E groups. The ratio of the total unsaturated fatty acid (ΣUSFA) in the H_2O_2 groups was lower ($p<0.05$) than the control, vitamin E, and H_2O_2 + vitamin E groups. The level of the total polyunsaturated fatty acid (ΣPUFA) in the H_2O_2 group decreased in when compared to the control, vitamin E, and H_2O_2+vitamin E groups. While the proportion of the total w3 (omega 3), w6 (omega 6), and PUFA were found to be lowest in the H_2O_2 group, they were slightly increased ($p<0.05$) in the lipoic acid group when compared to the control and H_2O_2 + lipoic acid groups. However, the level of ΣSFA in the H_2O_2 group was highest; the level of ΣUSFA in same group was lowest. As the proportion of ΣUSFA and ΣPUFA were found to be highest in the linalool group, they were decreased in the H_2O_2 group when compared to the control group. Our results show that linalool has antioxidant properties, much the same as vitamin E and lipoic acid, to prevent lipid peroxidation. Additionally, vitamin E, lipoic acid, and linalool could lead to therapeutic approaches for limiting damage from oxidation reaction in unsaturated fatty acids, as well as for complementing existing therapy for the treatment of complications of oxidative damage.

Keywords: Brain, Fatty acid, Guinea pig, Linalool, Lipoic acid, Vitamin E

Introduction

Reactive oxygen species (ROS) are known to play multiple roles in the physiological and pathological states, and are constantly produced in living organisms (Yar and Gilchrest, 1990; Darr and Fridovich, 1994). Endogenous sources of ROS in aerobic mammalian cells are the mitochondrial electron carriers and enzymes. Representative external sources include redox cycling drugs, radiation, and various lifestyle and environmental influences, such as smoking, drinking alcohol, excessive exercise, pollution, and overexposure to the sun (Machlin and Bendich, 1987; Dizdaroglu, 1993). The highly-reactive superoxide radical and hydrogen peroxide may be toxic to cells by a direct attack at the molecular level, or indirectly by generating secondary reactive species such as the hydroxyl radical (Brenneisen *et al*., 1997). These radicals may lead to oxidative damage of virtually any biomolecule (Kowoltowski and Vercesi, 1999). Lipids, especially polyunsaturated fatty acid, are preferential targets for such oxidative damage (Douillet *et al.*, 1993). If the reactions of unsaturated fatty acid with ROS occur in living cells, then the resulting defects in membrane functions may cause cell death (Gurr and Harwood, 1991). Despite the destructive potential of ROS, cells have developed defense mechanisms to prevent or limit oxidative injury. These mechanisms include several enzyme systems and antioxidants such as vitamin E and carotenoid, which prevent lipid peroxidation (Diplock, 1994; Sobajic *et al.*, 1998). Vitamin E is the name given to a number of structurally-related compounds, the most important of which is alpha-tocopherol. Vitamin E is needed for the

*To whom correspondence should be addressed.
Fax: 00904242330062
E-mail: scelik@firat.edu.tr

mitochondria electron transport function and prevents oxidation of various compounds, including unsaturated fatty acids (McCay, 1985). Vitamin E is important in biological systems. It is present in lipid bilayers of biological membranes and may play a structural role there. Vitamin E can act directly with a variety of oxygen radicals, including the peroxy radical ($ROO^{\cdot}$) and hydroxyl radical (·OH) (Burton *et al*., 1985), as well as with the superoxide radical ($O_2^{\cdot -}$) (Fukuzowa and Gebicki, 1983; Ozawa *et al*, 1983). Tocopherol can also react directly with singlet oxygen (Fahrenholtz *et al.*, 1974; Littarru *et al.*, 1984).

Lipoic acid is a naturally-occurring free radical scavenger that has been shown to regenerate endogenous antioxidants, like vitamin E, and increase formation of glutathione (Bienwenga *et al.*, 1997). It is a small molecule that is soluble in both water and lipid. This is significant because water-soluble antioxidant nutrients (vitamin C for example) are found in the cell, and fat soluble antioxidants (vitamin E for example) are found on the cell membrane. Because lipoic acid works both inside the cell and at the membrane level, it provides dual protection. Lipoic acid has long been known for its role in oxidative metabolism, the same as lipoamide, an essential cofactor in mitochondrial α-ketoacid dehydrogenase complexes (Packer *et al.*, 1997). Recent reports indicate that lipoate exerts its therapeutic efficacy in pathological conditions that involve free radicals (Cameron *et al.*, 1998; Kozlov *et al.*, 1999).

Linalool is a monoterpene compound that is reported to be a major component of essential oils in various aromatic species. Several linalool-producing species are used in traditional medicine. Among these is *Ueolanthus suaveolens G. Dom (Labiatae)*, which is used as an anticonvulsant in the Brazilian Amazon. Psychopharmacological *in vivo* evaluations of linalool showed that these compounds have dose-dependent marked sedative effects on the central nervous system, including hypnotic, anticonvulsant and hypothermic properties (Re *et al.*, 2000). However, we have no literature that reports about the protective effect of linalool against a decrease of unsaturated fatty acids.

Brain tissue has two unique characteristics. First, it is 60% lipid; second, it has a remarkably high energy consumption (Crowford, 1993). Also, this tissue is more susceptible to oxidative damage than other tissues (Foloyd *et al.*, 2001).

The object of our study was to investigate the protective effects of intraperitoneally (i.p.) administrated vitamin E, lipoic acid, and linalool on the level of total lipid and fatty acid in the guinea pig brain with oxidative stress that was induced by hydrogen peroxide.

Materials and Methods

Animals Ninety-seven guinea pigs were used during the experiment. At the start of the experiment, the guinea pigs weighed 460-640 g and were eleven months of age. All of the animals were divided into eight groups and kept at a room temperature of 20°C.

Table l. Diet composition

Ingredients	%
Wheat	10
Corn	22
Barley	15
Wheat bran	8
Soybean	26
Fish flour	8
Meat-bone flour	4
Pelted	5
Salt	0.8
Vitamin mineral mix[a]	0.2

[a]Vit. A, B_3, E, K_3, B_1, B_2, B_6, B_{12}, nicotinamide, folic acid, biotin, Mn, Fe, Zn, Cu, I, Co, Se, antioxidant, and Ca.

During the experiment, these animals were fed *ad libitum* a diet that included the ingredients that are shown in Table 1.

The first group was the control (9 animals), and it was subjected to i.p. injections of 25 µl corn oil. Group two was subjected to i.p. administered hydrogen peroxide (16 animals, 12 mg/kg) in water and 25 µl corn oil. Group three was subjected to i.p. administered with vitamin E (12 animals, dl-µ-tocopherol, 24 mg/kg) in corn oil. Group four was subjected to i.p. administered with hydrogen peroxide plus vitamin E (12 animals, 12 mg/kg H_2O_2+24 mg/kg vitamin E). Group five was subjected to i.p. administered with dl-α-lipoic acid (12 animals, 3 mg/kg) in corn oil. Group six was subjected to i.p. administered with H_2O_2 plus lipoic acid (12 animals, 12 mg/kg H_2O_2+3 mg/kg lipoic acid). Group seven was subjected to i.p. administered with linalool (12 animals, 120 mg/kg) in corn oil. Group eight was subjected to i.p. administered with H_2O_2 plus linalool (12 animals, 12 mg/kg H_2O_2+120 mg/kg linalool). We first injected H_2O_2 (in water, 25 µl) and 25 µl corn oil, followed by a second antioxidant (in corn oil, 25 µl) in the combination groups. This i.p. administration was done for 4 weeks every other day. The last dose was administrated 10 h before the operation.

The samples were prepared from animals at the end of the administration period after over-night fasting as follows. Each experimental guinea pig was anesthetized with ether and brain tissue samples were collected. These samples were kept at −25°C until lipid extraction and further analysis was performed.

Lipid extraction Total lipids were extracted with chloroform-methanol (2 : 1, v/v) by the method of Folch *et al.* (1957), as previously described (Christie, 1990). The tissue samples were homogenized. Three grams of the homogenized brain tissue samples were taken and mixed with chloroform-methanol (2 : 1, v/v) in a mixer. Non-lipid contaminants in lipid extracts were extracted into a 0.88% KCl solution. The extracts were evaporated in a rotary evaporator flask, then stored at −25°C.

Determination of total lipid This was determined according to the method of Frings *et al.* (1972). Twenty microliters of each solution from the extracted lipids was treated with 200 ml of the concentrated H_2SO_4 and heated in boiling water for 10 min. After

cooling, 10 ml phosphovanilin reagent was added. The mixture was incubated at 37°C for 15 min; the samples were then read at 540 nm. A good U.S. grade of olive oil (Sigma, St. Louis, USA) was used as a standard. The total lipid was calculated by reference to a standard curve.

Fatty acid analysis Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulfuric acid (v/v) in methanol (Christie, 1990). The fatty acid methyl esters were extracted three times with n-hexane. Then the methyl esters were separated and quantified by gas chromatography that was equipped with a flame-ionization detector (Unicam 610 gas chromatograph) that was attached to a Unicam 4815 computing recorder. Chromatography was performed with a capillary column (25 m in length and 0.22 mm in diameter, B 10×70) using hydrogen as a carrier gas (flow rate 0.5 ml/min). The temperature of the column, detector, and injection port were 185, 280, and 240°C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

Statistical analysis The collected values from the groups were reported as means ± SE. A statistical analysis was performed using SPSS 6.0 Software. The variance analysis (ANOVA) and LSD test was used for comparison between the groups.

Results and Discussion

Mortality and total lipid content At the end of the 4th week, after an overnight feeding, the mean body mass of each experimental group did not differ. Mortality in the control, vitamin E, and lipoic acid groups after 6 weeks were null. However, it reached 25% in the H_2O_2 and H_2O_2 + linalool groups, but approximately 17% in the linalool, H_2O_2 + vitamin E, and H_2O_2 + lipoic acid groups.

The total lipid amounts are reported in Table 2. While the amount of total lipid in the H_2O_2 + vitamin E and H_2O_2 + linalool groups slightly increased ($p<0.05$) when compared to the H_2O_2 group, it decreased ($p<0.01$) when compared to the vitamin E, linalool, and control groups. The level of total lipid in the lipoic acid group slightly increased ($p<0.05$) when compared to the H_2O_2 + lipoic acid group. It is concluded from these results that the total lipid amount decreased through the inhibition of lipid synthesis by the effect of H_2O_2 and/or its oxidation. Vitamin E, lipoic acid, and linalool also seem to modulate this degenerative effect.

Table 2. Content of total lipid in brain tissue in guinea pig

Groups	mg/gr, wet tissues	Groups	mg/gr, wet tissues
Control	64 ±4[d]	H_2O_2	48±3[a]
Vitamin E	66.±3[d]	H_2O_2+Vit. E	57±4[a]
Lipoic acid	63 ±4[b]	H_2O_2+Lipoic	49±4[a]
Linalool	71 ±4[d]	H_2O_2+Linalool	60±4[b]

[a] $p>0.05$, [b] $p<0.05$, [c] $p<0.01$, [d] $p<0.001$

Effect of vitamin E on fatty acid distribution Table 3 reports vitamin E effects on the level of fatty acid with oxidative stress-induced H_2O_2. The proportion of the myristic (14 : 0) and palmitic (16 : 0) acids within the total fatty acid were higher ($p<0.05$) in the control and H_2O_2 groups than in the vitamin E and H_2O_2 + vitamin E groups. The level of the stearic (18 : 0) and oleic (18 : 1) acids in the H_2O_2 and H_2O_2 + vitamin E groups increased ($p<0.05$) when compared to the control and vitamin E groups. The proportion of linoleic acid (18 : 2) in the control and vitamin E groups significantly increased ($p<0.005$) when compared to the H_2O_2 and H_2O_2 + vitamin E groups. While the level of arachidonic acid (20 : 4) did not differ between the control and H_2O_2 groups, it did increase slightly ($p<0.05$) in the vitamin E and H_2O_2 + vitamin E groups when compared to the same groups.

Table 3. The effects of vitamin E, lipoic acid and linalool against H_2O_2-induced oxidative stress on the level of Fatty Acids (%)

Fatty acids	Control	H_2O_2	Vit. E	H_2O_2+Vit. E	Lip.	H_2O_2+Lip.	Lin.	Lin+H_2O_2
14 : 0	1.9±0.4[c]	1.4±0.4[c]	1.0±0.1[b]	1.1±0.1[b]	0.7±0.1[a]	0.8±0.1[a]	0.6±0.1[a]	0.8±0.1[a]
16 : 0	38.6±1.4[c]	41.5±1.2[c]	35.9±1.4[b]	34.3±1.8[b]	26.2±1.3[a]	33.6±07[b]	28.9±2.2[a]	33.1±1.2[b]
18 : 0	13.2±1.3[a]	18.9±1.3[b]	11.3±1.0[a]	16.3±0.6[b]	18.1±2.8[b]	17.7±1.7[b]	21.7±0.9[b]	17.6±1.1[b]
18 : 1	23.7±1.0[a]	26.2±1.6[a]	24.1±1.1[a]	28.0±1.6[b]	21.5±2.8[a]	25.4±3.83[a]	21.9±3.3[a]	22.6±2.4[a]
18 : 2	9.2±1.0[d]	2.4±0.3[a]	8.1±0.8[d]	2.7±0.3[a]	8.0±0.9[d]	4.5±1.5[c]	9.0±1.0[d]	5.8±1.2[c]
18 : 3	1.3±0.3[d]	0.1±0.1[a]	0.7±0.1[c]	0.7±0.0[b]	0.7±0.1[c]	0.4±0.1[b]	0.9±0.1[c]	0.6±0.1[b]
20 : 4	5.7±0.9[a]	5.2±0.5[a]	8.3±0.5[b]	9.1±0.7[b]	10.5±0.5[b]	8.1±0.6[b]	8.7±0.7[b]	9.5±0.8[b]
22 : 6	7.3±0.4[c]	4.5±0.6[a]	10.7±0.9[d]	8.0±0.2[c]	12.8±1.7[d]	7.4±0.8[c]	11.5±1.2[d]	10.2±0.7[d]
ΣSFA	53.7±1.1[b]	61.7±1.5[d]	48.1±1.4[a]	51.6±1.9[b]	47.0±2.4[a]	52.2±2.4[b]	41.6±3.0[a]	51.6±2.1[b]
ΣUSFA	47.1±2.8[b]	38.4±1.8[a]	51.9±2.1[b]	48.4±1.9[b]	53.3±2.4[c]	47.8±1.0[b]	54.0±1.6[c]	48.6±1.5[b]
ΣW3	8.6±0.5[c]	4.7±1.0[a]	10.8±1.5[c]	8.6±1.7[c]	13.3±1.9[d]	7.8±1.6[c]	12.4±1.3[d]	10.8±1.0[d]
ΣW6	14.9±0.4[c]	7.6±2.3[a]	16.3±1.4[c]	11.8±1.4[b]	18.5±2.1[d]	14.7±1.6[c]	17.7±2.5[d]	15.2±1.2[c]
ΣPUFA	23.5±1.6[c]	12.3±0.7[a]	27.8±2.1[d]	20.4±1.5[c]	31.8±1.3[d]	22.5±2.4[c]	30.1±1.4[d]	26.0±1.5 [c]

[a] $p>0.05$, [b] $p<0.05$, [c] $p<0.01$, [d] $p<0.001$, Lin.=linalool, Lip.=lipoic acid, Vit. E=vitamin E

However, while the percentage of docosahexaenoic acid (22 : 6) was least in the H_2O_2 group, its level in the vitamin E group was the highest. The proportion of ΣSFA in the H_2O_2 group significantly increased ($p<0.005$) when compared to the vitamin E group, it increased ($p<0.01$) when compared to the control and H_2O_2 + vitamin E groups. The ratio of total ΣUSFA in the H_2O_2 group was lower ($p<0.05$) than in the control, vitamin E, and H_2O_2 + vitamin E groups. The level of ΣPUFA in the H_2O_2 group significantly decreased ($p<0.01$) in comparison to the control, vitamin E, and H_2O_2 + vitamin E groups. We previously showed that the levels of saturated fatty acid were slightly decreased, but the level of unsaturated fatty acid were slightly increased in rat and lamb tissues by dietary and intraperitoneally-administered vitamin E, without any other treatment (Dilsiz *et al.*, 1997; Yilmaz *et al.*, 1997a, 1997b; Çelik *et al.*, 1999). This vitamin E effect on fatty acids is clearer with hydrogen peroxide-induced oxidative stress. α-Tocopherol is an antioxidant that prevents biological membranes from undergoing oxidative damage. This effect is due to its ability to quench lipid peroxides, thereby protecting the cellular structures from an attack from free radicals (Traber and Sies, 1996). Both isolated PUFA and those incorporated into lipid are readily attacked by free radicals, which become oxidized into lipid peroxides. By contrast, both the monounsaturated and saturated fatty acids are more resistant to free radical attack; indeed, it has been suggested that the increased consumption of these in place of PUFA will render the circulating lipoproteins less sensitive to peroxidation (Reaven *et al.*, 1991). Hydrogen peroxide may be toxic to cells by direct attack at the molecular level, or indirectly by generating a secondary reactive species, such as hydroxyl radicals (Brenneisen *et al.*, 1997). Lipids, especially unsaturated fatty acids, are preferential targets for such oxidative damage (Douillet *et al.*, 1993). A significant reduction in PUFA in the hydrogen peroxide group that was observed in the present investigation supports the previous work. Chow *et al*. (1999) reported that vitamin E can directly regulate hydrogen peroxide production in mitochondria, and suggested that the overproduction of mitochondrial ROS is the first event that leads to tissue damage that is observed in Vitamin E-deficiency syndromes. H_2O_2 may accumulate, which would lead to a condition of mitochondial oxidative stress (Kowaltowski and Vercesi, 1999). Vitamin E can directly regulate H_2O_2 production (Chow *et al*., 1999). In our study, vitamin E supplementation restored fatty acid distribution near the control group.

Effect of lipoic acid on fatty acid distribution The proportion of ΣPUFA in the lipoic acid group significantly increased ($p<0.005$) when compared to the H_2O_2 group; it slightly increased ($p<0.05$) when compared to the control and H_2O_2 + the lipoic acid groups (Table 3). However, the level of ΣSFA in the H_2O_2 group was the highest; the level of ΣUSFA in same group was the least. While the ratio of 22 : 6 in the lipoic acid group significantly increased ($p<0.005$) when compared to the H_2O_2 group, it slightly increased ($p<0.05$) when compared to the control and H_2O_2 + lipoic acid groups. The levels of 20 : 4, 18 : 3, and 18 : 2 fatty acid in the lipoic acid groups were higher than the H_2O_2 and H_2O_2 + lipoic acid groups. The level of 14 : 0 and 16 : 0 fatty acid in the control and H_2O_2 groups were higher than the lipoic acid and H_2O_2 + lipoic acid groups. These results clearly demonstrate that unsaturated fatty acids peroxidation occurs *in vivo* in H_2O_2-induced-oxidative stress in the absence of lipoic acid supplementation. At the same time, intraperitoneal lipoic acid supplementation restored fatty acid distribution in total fatty acid in the H_2O_2 + lipoic acid groups that were near the control group. Magen *et al.* reported that feeding rats lipoic acid reduced the malondialdehyde levels, which is an indicator of lipid peroxidation (Magen *et al*., 1999). Lipid peroxidation is a free radical-induced process that leads to oxidative deterioration of polyunsaturated lipids. Under normal physiological condition, low concentrations of lipid peroxides are found in tissues. Free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation (Pryor, 1973; Girotti, 1985). Thiols may play a pivotal role in protecting cells against lipid peroxidation (Haenen *et al*., 1989). Lipoic acid effectively reduced the amount of hydroxyl radical that was generated by the Fenton-type reaction, and it also scavenges the peroxide and superoxide radical (Sumathi *et al*., 1993). Our present observations show that lipoic acid administration eventually resulted in decreases in 14 : 0, 16 : 0 and ΣSFA, and increased in 18 : 2, 18 : 3, 20 : 4, 22 : 6, ΣUSFA, ΣPUFA levels; therefore, substantiating the antioxidant property of lipoic acid.

Effect of linalool on fatty acid distribution The level of 18 : 1, ΣSFA, ΣUSFA, and ΣPUFA did not differ between the control and H_2O_2 + linalool groups (Table 3). The proportion of ΣPUFA in the linalool group was significantly increased ($p<0.005$) when compared to the H_2O_2 group; it was slightly increased ($p<0.05$) when compared to the control and H_2O_2 + linalool groups. The level of 22 : 6 fatty acid in the linalool group significantly increased ($p<0.005$) when compared to the H_2O_2 group, but slightly increased in the control group. The level of the same fatty acids in the linalool and H_2O_2 + linalool groups did not differ. The ratio of 18 : 2, 18 : 3 and 20 : 4 fatty acids in the linalool group were higher than the H_2O_2 group. While the level of 18 : 2, 18 : 3, 20 : 4, 22 : 6, ΣUSFA, and ΣPUFA in the H_2O_2 group were lower than the H_2O_2 + linalool groups; the level of 14 : 0, 16 : 0 and ΣSFA in the same group were higher than the H_2O_2 + linalool groups. These results clearly demonstrate that unsaturated fatty acid peroxidation occurs in brain tissues in H_2O_2-induced oxidative stress in the absence of linalool supplementation. Linalool supplementation inhibited the decrease in unsaturated fatty acids, and restored fatty acid distribution near the control group. Several linalool-producing species are used in traditional medical systems. Among these is

Ueolanthus suaveolens G. Dom (Labiatae), which is used as an anticonvulsant in the Brazilian Amazon. Psychopharmacological *in vivo* evaluation of linalool showed that these compounds have dose-dependent marked sedative effects on the central nervous system. These include hypnotic, anticonvulsant and hypothermic properties (Re *et al*., 2000). However, we have seen no literature reports on the protective effect of linalool against a decrease in unsaturated fatty acids.

Conclusions

Brain tissue has 60% lipid, and it has a remarkable high-energy consumption (Crowford, 1993). In addition, these tissues are more susceptible to oxidative damage than other tissues (Foloyd *et al*., 2001). Although, the vitamin E that was used was approximately 8 times higher than the lipoic acid dose, the present study illustrates that lipoic acid administration can be more effective than vitamin E in preventing lipid peroxidation in brain tissue. This may be attributed to the bioactivity of lipoic acid to directly react with various reactive oxygen species, as well as its ability to interfere with oxidation processes in the lipid and aqueous cellular compartment (Packer *et al*., 1997). Lipoic acid is a smaller molecule than vitamin E, and it is soluble in both water and fat. This is significant because water-soluble antioxidant nutrients (vitamin C for example) are found on the cell, and fat-soluble antioxidants (vitamin E for example) are found on the cell membrane. Because lipoic acid works both inside the cell and at the membrane level, you get dual protection. Our results indicate that linalool has considerable protective effects against the hydrogen peroxide-induced oxidative stress in brain tissue, but we used it in high concentrations. A dose-dependent study of the protective effect of linalool must be undertaken to determine its real efficiency. Linalool is a monoterpene compound that is reported to be a major component of essential oil in various aromatic species. If it is an effective antioxidant, then it can be used as a protector against oxidative damage in food and nourishment.

In conclusion, these observations suggest that lipoic acid seems to be a more effective antioxidant than vitamin E. These antioxidants could lead to therapeutic approaches for limiting damage from oxidation reactions in unsaturated fatty acids and for complementing existing therapy for treatment of the complications of oxidative damage. Our results show that linalool can be an antioxidant property, the same as vitamin E and lipoic acid.

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References

Bienwenga, G. P., Haenen, G. R. and Bast, A. (1997) The Pharmacology of the antioxidant lipoic acid. *Gen Pharmacol.* **29**, 315-331.

Brenneisen, P., Briviba, K., Wlaschek, M., Wenk, J. and Kochanek, K. S. (1997) Hydrogen peroxide increases the steady-state mRNA levels of collagenase/KKP-1 in human dermal fibroblasts. *Free Radic. Biol. Med.* **22**, 515-524.

Burton, G. W., Foster, D. O., Perly, B., Slater, T. F., Smith, I. C. P. and Ingold, K. U. (1985) Biological antioxidants. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **311**, 565-578.

Cameron, N. E., Cotter, M. A., Horrobin, D. H. and Tritschler, H. J. (1998) Effects of alpha lipoic acid on neurovascular function in diabetic rats: Interaction with essential fatty acid. *Diabetologia* **41**, 390-399.

Çelik, S., Yilmaz, Ö., Asan, T., Cay, M. and Aksakal, M. (1999) Influence of dietary selenium and vitamin E on the level of fatty acids of brain and liver tissues in lambs. *Cell Biochem. Funct.* **17**, 115-121.

Chow, K. C., Ibrahim, W., Wei, Z. and Chan, A. C. (1999) Vitamin E regulates mitochondrial hydrogen peroxide generation. *Free Radic. Biol. Med.* **27**, 580-587.

Christie, W. W. (1990) *Gas chromatography and lipids, The Oily Press*, Glasgow, UK.

Crowford, M. A. (1993) The role of essential fatty acids in neural development implications for prenatal nutrition. *Am. J. Clin. Nutr.* **57(Suppl)**, 703-710.

Darr, D. and Fridovich, I. I. (1994) Free radical in cutaneous biology. *J. Invest. Dermatol*. **102**, 671-675.

Dilsiz, N., Çelik, S., Yilmaz, Ö. and Digrak, M. (1997) The effects of selenium, vitamin E and their combination on the composition of fatty acids and proteins in Saccharomyces cerevisiae. *Cell Biochem. Funct.* **15**, 265-269.

Diplock, A. T. (1994) Antioxidants and free radical scavengers; in *Free Radical Damage and its Control*, Rice-Evans, C. A. and Burdon, R. H. (eds), pp. 113-130, Elsevier, Amsterdam, Netherlands.

Dizdaroglu, M. (1993) Chemistry of free radical damage to DNA and nucleoproteins; in *DNA and Free Radicals*, Halliwell, B. and Aruoma, O. I. (eds), pp. 20-39, Ellis Horwood, New York, New York.

Douillet, C., Chancerelle, Y., Chruz, C., Maroncles, C., Kergonou, J. F., Renaud, S. and Ciacatti, M. (1993) High dosage vitamin E effect on oxidative status and serum lipids distribution in streptozotocin-induced diabetic rats. *Biochem. Med. Metab. Biol.* **50**, 265-276.

Fahrenholtz, S. R., Doleiden, F. H., Trozzolo, A. M. and Lamola, A. A. (1974) On the quenching of singlet oxygen by alpha-tocopherol. *Photochem. Photobiol.* **20**, 505-509.

Folch, J., Lees, M. and Sladane-Stanley, G. H. A. (1957) Simple method for the isolation and purification of total lipids from animals tissues. *J. Biol. Chem.* **226**, 497-509.

Foloyd, R. A., West, M. and Hensley, K. (2001) Oxidative biochemical markers; clues to understanding aging in long-lived species. *Exp. Gerontol.* **36**, 619-640.

Frings, C. S., Frendley, T. W., Dunn, R. T. and Queen, C. R. (1972) Improved determination of total serum lipids the sulfo-phosphovanilin reaction. *Clin. Chem*. **18**, 673-674.

Fukuzowa, K. and Gebicki, J. M. (1983) Oxidation of alpha tocopherol in micelles and liposomes by the hydroxyl, perhydroxyl and superoxide free radicals. *Arch. Biochem. Biophys.* **226**, 242-251.

Girotti, M. W. (1985) Mechanism of lipid peroxidation. *Free*

Radic. Biol Med. **1**, 87-95.

Gurr, M. I. and Harwood, J. L. (1991) Fatty acid structure and metabolism; in *Lipid Biochemistry an Introduction*, Gurr, M. I. and Harwood, J. L. (eds), pp. 23-187, Chapman and Hall, London, UK.

Haenen, G. R. M. M., Vermeulen, N. P. E., Timmerman, H. and Bast, A. (1989) Effect of several thiols and lipid peroxidation in liver microsomes. *Chem. Biol. Interact.* **71**, 201-212.

Kagan, V. E., Shvedova, A. Serbinova, E., Khan, S., Swanson, C., Powell, R. and Packer, L. (1992) Dihydrolipoic acid-a universal antioxidant both in the membrane and in the aqueous phase, Reduction of peroxyl, ascorbyl and chromanoxyl radical. *Biochem. Pharmacol.* **44**, 1637-1649.

Kowaltowski, A. J. and Vercesi, A. E. (1999) Mitochondrial damage induced by conditions of oxidative stress. *Free Radic. Biol. Med.* **26**, 463-471.

Kozlov, A. V., Gille, L., Stanick, K. and Nohl, H. (1999) Dihydrolipoic acid maintains ubiquinone in the antioxidant active form, by two electron reduction of ubiquinone and one-electron reduction of ubisemiquinone. *Arch. Biochem. Biophys.* **363**, 148-154.

Littarru, G. P., Lippa, S., De Sole, P., Oradei, A., Dalla Torre, F. and Macri, M. (1984) Quenching of singlet oxygen by D-alpha-tocopherol in human granulocytes. *Biochem. Biophys. Res. Commun.* **119**, 1056-1061.

Machlin, L. J. and Bendich, A. (1987) Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J.* **1**, 441-445.

Magen, T. M., Ingersoll, R. S., Lykkesfeldt, J., Liu, J., Wehr, C. M., Vinarsky, V., Bartholomew, J. C. and Ames, B. N. (1999) Lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *FASEB J.* **13**, 411-418.

McCay, P. B. (1985) Vitamin E: Interactions with free radicals and ascorbate. *Annu. Rev. Nutr.* **5**, 323-340.

Ozawa, T., Hanaki, A. and Matsuo, M. (1983) Reactions of superoxide ion with tocopherol and model compounds: correlation between the physiological activities of tocopherols and the concentration of chromanoxyl radicals. *Biochem. Int.* **6**, 685-692.

Packer, L., Roy, S. and Sen, C. K. (1997) Alpha lipoic acid: a metabolic antioxidant and potential redox modulator of transcription. *Adv. Pharmacol.* **38**, 79-101.

Pryor, W. A. (1973) Free radical reaction and their importance in biological systems. *Fed. Proc.* **32**, 1862-1869.

Re, L., Barocci, S., Sonnio, S., Mencorelli, A., Vivani, C., Paolucci, G., Scarpantonio, A., Rinaldi, L. and Mosca, E. (2000) Linolool modifies the nicotinic receptor-ion channel kinetics at the mouse neuromuscular junction. *Pharmacol Res.* **42**, 177-182.

Reaven, P., Parthasarathy, S. and Grasse, B. J. (1991) Feasibility of using an oleate-rich diet to reduce the susceptibility of low density lipoprotein to oxidative modification in humans. *Am. J. Clin. Nutr.* **54**, 701-706.

Sobajic, S. S., Mihailovic, M. B. and Miric, M. O. (1998) The effects of selenium deficiency, dietary selenium and vitamin E supplementation on the oxidative status of pig liver. *J. Environ. Pathol. Toxicol. Oncol.* **17(3-4)**, 265-270.

Sumathi, R., Jayanthi, S. and Varalakshmi, P. l. (1993) DL-alpha-lipoic acid as a free radical scavenger in glyoxylate-induced lipid peroxidation. *Med. Sci. Res.* **21**, 135-137.

Traber, M. G. and Sies, H. (1996) Vitamin E in humans: demand and delivery. *Annu. Dev. Nutr.* **16**, 321-347.

Yaar, M. and Gilchrest, B. A. (1990) Cellular and molecular mechanisms of cutaneous aging. *J. Dermatol. Surg. Oncol.* **16**, 915-922.

Yilmaz, Ö., Çelik, S., Cay, M. and Dilsiz, N. (1997a) The Effects of dietary selenium and vitamin E on the fatty acids of erythrocytes, bone marrow and spleen tissues lipids of lambs. *Cell Biochem. Funct.* **15**, 1-7.

Yilmaz, Ö., Çelik, S. and Dilsiz, N. (1997b) Influence of Intraperitoneally and dietary administered vitamin E and selenium on the lipid composition in reproductive organs of male animals. *Biol. Chem.* **378**, 425-430.