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Effects of α lipoic acid on noise induced oxidative stress in rats

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ABSTRACT

This study was conducted to identify how exposure to ambient noise that is over 75 dB affects the oxidant-antioxidant profile using hematological and biochemical indicators, and to investigate the effects of a strong and current antioxidant, α lipoic acid, on rats that were subjected to noise stress. For this purpose, five groups of eight rats were formed as follows: Control (K), Noise Exposure (GK), Lipoic Acid (LA), Noise Pollution + αLipoic Acid (GK + LA) and Oil (Y). The blood samples collected from rats were analyzed and MDA (malondialdehyde), GSH (glutathione), SOD (superoxide dismutase), CAT (catalase), NO (nitrit oxide), GPx (glutathione peroxidase), leukocytes, monocytes, lymphocytes, erythrocytes, hemoglobin, hematocrit, glucose, cholesterol, total protein, triglycerides, HDL (high density lipoprotein), LDL (low density lipoprotein), and urea-N levels were measured. The physical factory environment in a textile factory was preferred to simulate the noise exposure. Ambient noise was measured to be 75 dB. Exposure to physical ambient noise was sustained for 30 days. MDA level was measured at the lowest level in the LA and GKLA groups while it was statistically significantly higher in other groups than it was in the control group. It was observed that GSH reached its lowest level in the group that was exposed to noisy environment, the 100 mg/kg/day αlipoic acid administered on the experimental model increased this level to that of the control group and this change was statistically significant ($p < 0.05$). Considering the urea levels, the increases in GK and GKLA groups and the decreases in LA and Y groups were observed to be statistically significant. When glucose levels were compared to the control group, they were found to be statistically significantly lower in all groups. As a result, it was observed that exposure to noise for 30 days was likely to lead to leukocyte-based immune deficiency and using α lipoic acid as an antioxidant might provide a significant protection against the noise stress.

1. Introduction

The noise levels at home and in the work environment should not exceed a certain level in order for a peaceful and healthy life. Increased noise levels are suggested to underlie many adverse conditions such as loss of hearing and balance, difficulties in concentration and failures, various psychological and psychiatric disorders ranging from minor problems to major complications, infertility, and tendency to get involved in criminal events (Agarwall, 2009). Therefore, ambient sound that reaches to the level where it is classified as noise poses a significant health risk on the people exposed to the noise. Noise is an auditory energy level, especially one that is loud, unpleasant, and causes disturbance. Environments with such high levels of noise have a negative impact on the psychology and the health of people, animals, and even other environmental stakeholders. World Health Organization (WHO) research indicated that there are nearly 10 million adults and 5 million children who are suffering from the irreversible noise induced hearing impairment in the United States, and also found out that environment noise is at the dangerous levels for humans; impacting 250 million population worldwide (Seidman and Standring, 2010). Although the tolerable noise levels in the workplace range between 70 and 90 dB depending on the country and the standards in place, WHO has published the optimum noise levels as 45 dB during the day and 35 dB at night and categorized noise levels around 80 dB as risky (WHO, 1999). As a result of increasing industrialization and urbanization, noise has become a significant environmental polluter. Although it has been discovered to negatively affect cellular and organismic processes, many people consider noise as an ordinary phenomenon and do

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not classify it as a risk (Güvercin and Aybek, 2003). Table 1 shows common sources of sound and their corresponding noise levels. Ambient noise can impair productivity, auditory health, and perceptions of employees and result in negative physiological and psychological consequences (Güvercin and Aybek, 2003). As the noise levels increase and the exposure to noise is lengthened, it becomes possible to observe the negative effects on the cardiovascular, gastrointestinal, endocrine, and nervous systems (Akyıldız, 1980; Ersoy et al., 2014). In most businesses, noise levels can reach the upper noise limits that may impair the employees' health. On the other hand, it is a fact that employers and employees do not pay as much attention to this as necessary and do not take the necessary precautions (Güvercin and Aybek, 2003). Table 2 shows noise levels around weaving looms in textile factories. The loss of balance between the free radicals and the antioxidant defense system as a result of an increase of free radicals or a decrease of antioxidants lead to oxidative stress and oxidative damage. Oxidative stress and oxidative damage have been proven to play a role in the pathogenesis and the progression of many diseases (Kargın and Fidancı, 2001).

Lipoic acid is a naturally occurring compound that acts as a cofactor in mitochondrial enzymes involved in energy production in the metabolism (Cadenas, 2001; Azizi et al., 2015). The effects of lipoic acid can be listed as removal of free radicals, metal chelation, antioxidant regeneration, repair of molecular damages, reduction of aging from glycation with lipoic acid, and a natural source of energy (Biewenga et al., 1997; Sumathi et al., 1996; Packer et al., 1997; Kok and Berkel, 1996; Packer, 1998; Shih, 1983). Among the antioxidant molecules, αlipoic acid is unique because it has protective effects both in reduced and oxidized forms (Kramer, 2001). Moreover, treatment with lipoic acid increases the in vivo and in vitro glutathione (GSH) levels (Busse et al., 1992; Podda et al., 1994; Han et al., 1997; Sen et al., 1997; Sen et al., 1999).

This study was conducted to investigate the influence in rats exposed to an ambient noise of 75 dB (A) in the physical working environment and the effects of α lipoic acid on various biochemical and hematological parameters as well as the oxidative stress.

Table 1
Common sources of sound and their corresponding noise levels (Agarwall, 2009).

Sound source	Decibel (dB)
Rocket sound	180
Jet aircraft take off	150
Thunder, concert	120
Horn sound (from 1 m)	110
Metro station, truck, drilling compressor	100
Kitchen blender	90
Hair dryer	80
Traffic on the highway	70
Normal conversation	60
Living room	40
Library environment	20–30
Rustling leaves, wind turbine	10
Lower limit of hearing	0

Table 2
Noise levels around weaving looms in textile factories.

Noise level dB (A)	Literature
105	Yıldırım et al. (2007)
77–101	www.yildiz.edu.tr/~gonul/bildiriler/b81.pdf
85	Babalık (2003)
96.5–101.5	Toröz (2009)
75	Karafakıoğlu et al. (2011)

2. Material and method

2.1. Material

The operations on the experimental animals were performed using the appropriate procedures in line with the reference no 08-07, decision number 064 of Afyon Kocatepe University, Animal Ethics Board. In the study, 3–4 months-old, 40 male "*Wistar-albino*" rats weighing 175–250 g were used.

2.2. Method

The animals were accepted to the care of the research team 10 days prior to the study and hence, they were adapted to the experimental environment. Subjects were exposed to 12 h' dark, 12 h' light cycle. Eight rats of the same group (K, GK, LA, GKLA, Y) were kept inside the same cage. The experimental set up and applications were performed in Usak whereas the analysis of blood samples collected from the animals were conducted in Afyon Kocatepe University, Faculty of Veterinary Medicine. Five groups of eight rats were formed in the study as follows: Control group (K), noise pollution group (GK), α lipoic acid group (LA), noise pollution + α lipoic acid group (GKLA) and oil group (Y). In terms of exposure to noise, the research team preferred the physical factory environment in textile factories that provide continuous exposure to noise. The animals were kept in this environment for one month where they were exposed to approximately 75 dB noise. The level of noise in the environment was measured by the Provincial Directorate of Environment and Urban Planning using a Svantek svan-957 branded equipment.

2.3. Feeding and additions to the ration

Control group (K) was fed on standard rat chow. Lipoic acid group (LA) was fed on standard rat chow and given 100 mg/kg/day αlipoic acid dissolved in 2 ml olive oil by means of oral gavage. Rats in the noise group (GK) were kept in factory environment and fed on standard rat chow. The rats in the noise pollution and α lipoic acid group (GKLA) were fed on standard rat chow and 100 mg/kg/day α lipoic acid (dissolved in 2 ml olive oil and administered through oral gavage). Oil group (Y) was fed on standard rat chow and given 2 ml/day olive oil through oral gavage.

2.4. Collecting blood samples

Rats in the experiment group were fasted till morning and anesthetized with 10 mg/kg Xylazine HCl and 50 mg/kg Ketamine HCl injection at the end of the 30-day-long study period. Then the rib cages of the animals were opened in accordance with the necessary technique. An average of 6–9 ml blood was taken from the running heart using injectors with 5 ml heparin. Then, the blood samples were transferred into vials with heparin and transported to the laboratory within the cold chain. 5 ml of the blood samples were set aside to determine MDA, GSH, and SOD whereas the remaining blood sample was centrifuged in a cooled environment at 3500 rpm for 10 min to separate the plasma. Plasmas were placed inside 1.5 ml Eppendorf (microcentrifuge) tubes and kept in the deep freezer at −30 °C for a week to measure the nitric oxide metabolites.

2.5. Measurements

The MDA, which is one of the products of peroxidation reaction occurring due to free radicals, was determined using the double boiling method of Draper and Hadley (1990) whereas GSH analysis

was done in line with the method reported by Beuter et al (1975). The superoxide radical forms a red farmazon compound when it is treated with INT (inositol). The SOD activity is measured by the degree of inhibition of this reaction (Flohe and Otting;1984). The amount of nitric oxide was determined according to Miranda et al.'s (2001) "Vanadium-3-chloride-Gries reaction" method (Bulbul, 2003). The method reported by Beutler (1984) was used to determine the level of catalase enzyme. Complete blood analysis (leukocytes, monocytes, lymphocytes, erythrocytes, hemoglobin, hematocrit, glucose, cholesterol, total protein, triglycerides, HDL, LDL, and Urea-N) was done in Afyon Kocatepe University Hospital.

2.6. Statistics

Findings were reported using averages ± standard errors after testing the data for normality. ANOVA test was used to assess the differences between groups statistically, followed by the Duncan test as the post-test. Three p-values ($p < 0.05$, 0.01, and 0.001) were used to test the statistical significance (Özdamar, 2003).

3. Results

MDA as per the Draper and Hadley (1990) method, GSH as per the method reported by Beuter et al (1975), SOD as per the method of Flohe and Otting (1984), CAT as per the method reported by Beutler (1984), NO as per the method used by Miranda et al.'s (2001), GPx, leukocytes, monocytes, erythrocytes, hemoglobin, hematocrit, glucose, cholesterol, total protein, triglycerides, HDL, LDL, and urea-N levels performed in Afyon Kocatepe University Hospital laboratories on rats exposed to noisy environment and administered with αlipoic acid are presented in Tables 3–5. Table 3 shows arithmetic average, standard error, and significance levels of biochemical parameters. In terms of NO, the highest level is in the GK group and the lowest level is in the control (K) group ($p > 0.05$). The level of NO became the indicator of the fact that addition of oil to the animals' diet and exposure to noisy environment increased oxidative stress in the experiment subjects. Although not statistically significant, the serum catalase level was observed to be the lowest in the noise +α lipoic acid group and the highest in the lipoic acid group and the oil group. Erythrocyte package SOD levels slightly increased in the group that was exposed to noise whereas the highest level was observed in the αlipoic acid group. When the erythrocyte package is investigated in terms of GSH levels, it was observed that GSH reached its lowest level in the group that was exposed to noisy environment, the 100 mg/kg/day α lipoic acid administered on the experimental model increased this level to that of the control group and this change was statistically significant ($p < 0.05$). A common and reliable indicator of oxidant status, MDA, was at its lowest level in the α lipoic acid group (LA) and the noise +α lipoic acid group (GKLA) whereas it was statistically significantly higher in other groups than the control group ($p < 0.05$). Table 4 shows blood biochemical values. When compared to the control group (K), the Tp increases in the GK and the LA groups and the Tp decreases in the GKLA and the Y groups were not found to be statistically significant. Considering the urea levels, the increases in GK and GKLA groups and the decreases in LA and Y groups were observed to be statistically significant. Cholesterol levels were the highest in the oil (Y) group and the lowest in the GK group ($p > 0.05$). When glucose levels were compared to the control group, they were found to be statistically significantly lower in all groups. The changes in LDL, HDL, and triglycerides were not found statistically significant. Table 5 shows arithmetic average, standard error, and significance levels of hematologic parameters. Although not statistically significant, exposure to noisy environment reduced the leukocyte levels. Irrespective of its interpretation, this is an unwanted result. It is noteworthy that α lipoic acid administration increased the leukocyte levels in

Table 3
Arithmetic average, standard error, and significance levels of biochemical parameters.

	K	GK	GKLA	LA	Y	p
NO µmol/l	24.95 ± 1.01	33.20 ± 2.24	32.20 ± 3.09	26.78 ± 2.09	32.82 ± 3.01	0.06
Catalase U/ml blood	3.53 ± 1.73	2.20 ± 0.31	1.87 ± 0.51	5.93 ± 1.91	6.87 ± 3.04	0.220
SOD U/ml blood	11.03 ± 3.87	16.52 ± 5.03	9.91 ± .1.70	34.23 ± 13.41	13.92 ± 7.06	0.125
GSH nmol/ml	26.43 ± 4.13 ^{a,b}	13.71 ± 2.05^{b}	37.15 ± 8.42^{a}	31.45 ± 5.17 ^{a}	32.25 ± 3.89 ^{a}	**0.032**
MDA nmol/ml	6.98 ± 0.45 ^{a}	7.47 ± 0.42^{a}	5.09 ± 0.45^{b}	5.47 ± 0.34^{b}	7.23 ± 044 ^{a}	**0.001**

$p < 0.05$, 0.01, and 0.001.

Table 4
Blood biochemical values.

	K	GK	GKLA	LA	Y	p
Tp g/dl	6.17 ± 0.14	6.25 ± 0.26	5.63 ± 0.29	6.28 ± 0.32	5.75 ± 0.18	0.271
Urea mg/dl	22.77 ± 1.77^{a,b}	23. 73 ± 1.30^{a}	24.30 ± 2.05^{a}	21.02 ± 1.10^{a,b}	17.96 ± 1.27^{b}	**0.043**
Chol mg/dl	46.75 ± 3.17	43.33 ± 2.44	50.16 ± 4.27	44.80 ± 3.12	51.16 ± 3.43	0.407
Glu mg/dl	135.25 ± 6.98^{a}	103.16 ± 8.90^{b}	90.50 ± 9.20^{b}	104.40 ± 4.92^{b}	131.83 ± 8.13^{a}	**0.003**
LDL mg/dl	10.20 ± 1.77	11.25 ± 0.62	11.71 ± 1.25	10.12 ± 1.53	13.06 ± 1.36	0.502
HDL mg/dl	35.27 ± 1.54	29.58 ± 2.27	36.26 ± 3.08	32.72 ± 1.90	36.83 ± 2.40	0.205
Trigl Mg/dl	48.50 ± 13.55	37.83 ± 6.26	35.66 ± 11.49	29.00 ± 4.38	30.16 ± 2.78	0.566

$p < 0.05$, 0.01, and 0.001.

Table 5
Arithmetic average, standard error, and significance levels of hematologic parameters.

	K	GK	GKLA	LA	Y	*p*
Wbc $10^3/\mu L$	12.21 ± 2.14	7.96 ± 1.48	9.02 ± 1.21	13.54 ± 2.06	9.72 ± 0.83	0.119
Lym$10^3/\mu L$	62.08 ± 2.05	66.87 ± 4.36	65.64 ± 5.60	61.85 ± 4.40	66.21 ± 2.94	0.850
Mon $10^3/\mu L$	13.47 ± 0.59	11.30 ± 1.19	10.87 ± 0.54	14.35 ± 1.2	12.31 ± 1.03	0.083
Erythrocyte $10^3/\mu L$	9.1 ± 1.2	9.25 ± 0.09	9.17 ± 0.23	90.95 ± 0.53	9.20 ± 0.44	0.895
Hemoglobin g/dl	18.15 ± 1.00	16.05 ± 0.12	16.21 ± 0.49	17.48 ± 098	16.11 ± 0.52	0.131
Hematocrit %	44.90 ± 4.48	43.11 ± 0.39	44.68 ± 1.08	45.10 ± 2.51	44.40 ± 1.57	0.981

$p < 0.05$, 0.01, and 0.001.

control group animals that were kept under noise-free environment. However, α lipoic acid was not able to increase the leukocyte levels of the animals in the LA group to the control group levels. This suggests that higher levels of α lipoic acid administration options might be more effective.

When the lymphocyte levels are compared to the control group, they were observed to have increased in the GK, GKLA, and Y groups but decreased in the LA group ($p > 0.05$). The highest level of monocytes was observed in the LA group and the lowest level was observed in the GKLA group ($p > 0.05$). The changes of erythrocyte levels were observed to be the highest in the GK group and the lowest in the LA group, although not statistically significant. Similarly, hemoglobin levels decreased in all groups compared to the control group but this decrease was not statistically significant. Hematocrit levels decreased in the GK group and increased in all other groups; however, none of the changes were statistically significant.

4. Discussion

Many factory workers, primarily those in the printing and textile sectors (Yıldırım et al., 2007), professionals working in noisy environments (Yazıcı, 2007), have psychological and physiological problems due to workplace noise and they even face the risk of hearing loss (Agarwall, 2009).

Sound types and frequencies that are affecting psychological and physiological processes negatively, disturbing the living organism, undesired, and damaging the natural acoustic characteristics, harmony. Balance of the environment can impair physiological and psychological processes in living organisms with their biophysical and biochemical effects, and reduce the productivity performance (Güler and Çobanoglu, 1994). Unwanted and disturbing noises might eventually lead to metabolic, psychological, and psychiatric disorders (Dalgıc, 1992). In the study, the rats were exposed in a textile factory as the ambient noise environment. This study was conducted to identify how exposure to ambient noise that is over 75 dB affects the oxidant-antioxidant profile using hematological and biochemical indicators, and to investigate the effects of a strong and current antioxidant, αlipoic acid, on rats that were subjected to noise stress. MDA, GSH, SOD, CAT, NO, GPx, leukocytes, monocytes, erythrocytes, hemoglobin, hematocrit, glucose, cholesterol, total protein, triglycerides, HDL, LDL, and urea-N levels on rats were observed. Not only the noise, but also the ambient pH, breathing air, moisture, and the aura of the workplace were included in the study. However, the assessment was conducted only on the noise and the antioxidant materials. Exposure to noisy environment reduced the leukocyte levels, which are among our important defense mechanisms. Although αlipoic acid administration increased the leukocyte levels in the control group, it could not increase the leukocyte levels in the groups that were exposed to noise to the control group levels. The 100 mg/kg/day αlipoic acid amount was not sufficient to repair the noise-based leukopenia profile. The most well-known indicator of oxidative status, MDA, was found to be at its lowest levels in the noise + α lipoic acid groups and was observed to be statistically significantly higher in other groups than the control group ($p < 0.05$). Another indicator of oxidative stress induced under exposure to noisy environment was the fact that the NO level increased in these experimental animals. Erythrocyte package SOD levels slightly increased in the group exposed to noise; however, the highest level was observed in the α lipoic acid group. Erythrocyte package GSH levels reached their lowest levels under exposure to noise and application of α lipoic acid as antioxidant raised these levels up to the levels of the control group ($p < 0.05$). Demirel et al. exposed the rats for 20 days/4 h to 100 dB noise, and observed the MDA and NO levels and GSH-Px activities. MDA and NO levels and GSH-Px activities were found to be increased significantly at the end of experiment in the group exposed to noise (Demirel et al., 2009).

In Karaca's PhD study conducted in 2007, rats were injected 150 mg/kg ip. DEN to induce oxidative stress and hepatotoxicity; and it was investigated whether administering α lipoic acid at a dose of 100 mg/kg/day for 7–14 days was protective against such stresses. Although the changes in the catalase levels are not statistically significant, the catalase level reached its lowest level in the noise +α lipoic acid group and increased to high levels in the lipoic acid and oil groups. Its fat- and water- solubility in makes lipoic acid an attractive antioxidant in cellular and extracellular environments and allows it to be rapidly consumed in intracellular and extracellular spaces (Kramer, 2001). The GSH level statistically significantly decreased under exposure to noise. The decrease in the GSH level might suggest that enzyme activities of GSH-peroxidase and GSH-transferase that used GSH as a substrate and that reduced glutathione (GSH) might have decreased during the oxidative process (Dündar and Aslan, 2000). In Karaca's PhD study conducted in 2007, rats were given 150 mg/kg ip. DEN to induce oxidative stress and hepatotoxicity; and it was investigated whether administering α lipoic acid at a dose of 100 mg/kg/day for 7–14 days was protective against such stresses. As a result of the study, it was observed that αlipoic acid improved the impaired liver enzymes, reduced oxidative stress, and increased antioxidant capacity following both 7 and 14 days of treatment. α lipoic acid was reported to decrease the oxidative stress and increase the antioxidant capacity (Karaca, 2007). In Diao's study the results are; noise exposure causes a decrease in serum TAC (total antioxidative capacity) and an increase in NO in cochlea. alpha-Lipoid acid exerts a protective effect against hearing loss in acoustic trauma through its antioxidant effects (Diao et al., 2003). α lipoic acid at the levels we suggest and administer might be protective against the exposure to 75 dB ambient noise. In our study, blood glucose levels decreased in rats that were exposed to a noisy environment. This result might originate from the increased glucose use owing to the effect of a possible metabolic stress. The noisy environment affects glucose metabolism and supportsα lipoic acid's effect of decreasing blood glucose levels (Ziegler and Gries, 1997). Turkyilmaz et al. (2011) investigated the effect of acute

noise on fear and various stress indicators in broilers at noise levels of 55, 80, 100, and 120 dB. The researchers reported increased levels of cholesterol in all experiment groups and determined that the glucose level increased at 120 dB noise level. In our study, GK group has the lowest level of HDL. An increase of more than 10% was observed between the control and the GK groups and this suggested that noise stress had a negative impact on HDL, which is one of the blood cholesterols. In the study conducted by Derekoy et al. (2004), six rabbits, which were not treated with any drugs or given any antioxidant support, were exposed to broadband noise at 100 dB for an hour. They measured oxidative parameters, plasma protein sulfhydryl content, protein carbonyl content, malondialdehyde levels, glutathione levels in the erythrocytes, and dismutase and catalase enzyme activities before and after exposure to noise. In order to determine the changes in hearing thresholds, the effect of the noise was assessed using temporarily stimulated otoacoustic emission test before and after the application. They performed the same operations on the six rabbits in the second group, which was given ascorbic acid. Consequently, they reported that the noise-induced free radical formation was kept at lower levels by using ascorbic acid as an antioxidant material; ascorbic acid acutely protected erythrocytes and strengthened the antioxidant defense wall (Derekoy et al., 2004).

Although it was not included within the main research question in our study, a noteworthy finding is related to the data collected from the group treated with olive oil. Administration of 2 ml/day olive oil via oral gavage for 30 days increased the blood cholesterol level. α lipoic acid retracted the levels to the control group's levels. The amount of urea was reduced in olive oil administered rats. Again, a noteworthy result is that when olive oil and αlipoic acid were administered together, no changes were observed in urea levels. Administration of α lipoic acid dissolved in olive oil should be monitored in the following studies. Although its mechanism is unknown, the amount of urea decreased in the group that was given the additional ration of olive oil. This may be a joint effect of vitamins A and E (tocopherols) which are antioxidants in the olive oil, oleic acid, hydrocarbon squalene, and phenolic compounds. Oleic acid that is found at a ratio of 71–91% in the olive oil composition decreases the LDL cholesterol which causes formation of vascular plaques and increases the HDL cholesterol. Oleic acid at the same time is resistant against reactions that destruct the nutritive and sensory quality of the oil (Duru and Bozdogan, 2014). The hydrocarbon squalene that is found at high levels such as 0.8–12 g/kg as well has antioxidant and cell regenerative properties. Vitamin E and tocopherols are fat-soluble strong antioxidants that protect our bodies against aging. The daily reference intake of Vitamin E is 8 mg for women and 10 mg for men. A tablespoon of olive oil includes 1.94 mg of Vitamin E on average. This amount corresponds to 20–25% of daily Vitamin E requirement (Duru and Bozdogan, 2014; Demirci et al., 2008). Therefore, it was concluded in our experimental model that 75 dB ambient noise is a stressor. In animals exposed to an ambient noise at this level for 30 days, leukocytes caused weaknesses in the defense mechanism. The fact that one of the important lipid profile indicators, the HDL concentration, decreased under the environment at these noise levels shows that 75 dB ambient noise negatively affects blood lipid profile and this picture may result in a predisposition to cardiovascular problems.

5. Conclusions

It was observed that α lipoic acid as an antioxidant provided a significant protection against these stressors and improved the indicators in question. Olive oil as well can be used as an antioxidant. However, considering that the major issue in protection against oxidative processes is "antioxidant safety and determination of specific antioxidant supports", it would be appropriate to investigate the effects of administering both α lipoic acid and olive oil, and other antioxidants at varying levels in order to determine the most effective antioxidant material and its dosage.

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