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Investigation of Lipid Peroxidation and Antioxidant Capacity of Rats Fed with Various Oils

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In this study, the effects of the oils such as butter, olive oil, sunflower oil and margarine on the serum lipid peroxidation, oxidized low density lipoprotein (LDL) and serum total antioxidant activity were investigated. Except control group, rats of all groups have been nourished by a special fodder embracing 15 % oil addition for a period of two months and were measured malondialdehyde (MDA), ox-LDL and antioxidant activity levels of samples. No significant difference was found in serum MDA and ox-LDL levels of the groups subjected to oil. However, when the serum total antioxidant activity levels were compared, it was noted that the measurements of olive oil group (1.08 ± 0.23 nmol/mL) were statistically higher ($p < 0.05$) than those of sunflower oil group (0,83 ± 0.12 nmol/mL). In addition to this, serum total antioxidant activity values of butter group (1.17 ± 0.14 nmol/mL) were statistically higher ($p < 0.001$) than those of sunflower group (0.83 ± 0.12 nmol/mL). It was found that MDA results decreased as: butter > margarine > olive oil > sunflower oil > control group and the antioxidant activity results decreased as: butter > control > olive oil > margarine > sunflower oil group. Butter gives the highest oxidized LDL and total antioxidant activity results and margarine gives the lowest oxidized low density, sunflower oil gives the lowest total antioxidant activity results.

Key Words: Serum lipid peroxidation, Oxidized low density lipoprotein, Antioxidant capacity, Oil.

INTRODUCTION

The resistances of unsaturated fats against free radicals are less, more oxidized form of low density lipoproteins (LDL) occured by oxidation by the way it becomes more risky factor from the point of view of coroner heart disease (CHD). Olive oil is accepted as the healthiest fat because of unsaturated fats ratio and the portion of vitamin E[1]. There are saturated, unsaturated fatty acids and at the same time styrols, hydrocarbons and antioxidants of less quantity in sunflower oil. Butter is rich in terms of vitamins which are melted in fat, it contains saturated fatty acids and cholesterol (240 mg/100 g) in large quantity. Margarine is a heterogenic mixture which is composed of fat and water phases and produced from refined fats which

are obtained from the partly hydrogenation of various fats. There are liquid and hard fats at at the phase of fat and there are salt, ferments and antioxidants at the phase of water. Double bonds of unsaturated fats form peroxidation products after reacting with free radicals easily. Biological membranes are rich in terms of unsaturated fatty acids so they are susceptible to damage[2-4].

Human plasma lipoproteins are very sensitive against peroxidation. Low density lipoprotein (LDL) oxidation is a free radical reaction in which a lot of aldehydes and other peroxidation products are formed after LDL demolished by the lipid peroxidation of polyunsaturated fatty acids in the structure of it[5]. The role of LDL at atherogenesis, is definitely proved. It is determined that the oxide of LDL that is isolated from atherosclerotic lesions is different from natural LDL in terms of structure and characteristics[6].

The evidences, which support that oxidative modifications of lipoproteins have an important role at atherogenesis, are increased gradually[7]. The increase of malonedialdehydes (MDA), the lipid peroxidation production, is evaluated as the marker of unstable cardiovascular diseases.

Lipid peroxidation is either ended by scavenger reactions or is continued by autocatalytic separation reactions. Vitamin E, vitamin C and β-carotene like antioxidants are oxidized and used in the treatment of free radicals and prevent the oxidation of LDL, cholesterol in blood, membrane and unsaturated fatty acids[8-10].

EXPERIMENTAL

The examinations were occured with 60 female Sprague-Dawley rats and classified for 5 groups and each group has 12 rats.

Freezer, colding centrifuge Hettich Universal 30 RF, spectrophotometer Shimadzu UV-1601 were used.

10 % TCA and 0.675 % TBA solution were prepared with distilled water. Ox-LDL cit: Mercodia (Cat. No. 10-1143-01) and AOA cit: Randox (Cat.No. NX 2332) were supplied from.

A diet were prepared according to their daily needs of rats. The oil of each group was added to the standard fodder of rats, prepared in the special fodder fabrics, in the ratio of 15 %. These was no addition to the fodder of rats in the control groups.

Rats have been stayed at 18-20 °C and 70-80 % moistured medium for 2 months. 12 h dark/light siclus was supplied. Each groups, contains 12 rats, were feeded with fodder, added olive oil, sunflower oil, butter and margarine in the ratio of 15 %, respectiely for 2 months.

Butter and margarine have been melted and added. Every oil have been made a homogeneous mixture. Diet have been prepared for a week and kept in dark jar. After 2 months, the blood was taken by cardiac puncture. Blood have been took to EDTA tubes to prevent coagulating. Blood samples keeped in -80 °C freezer and before 1 week they were used to study.

Determination of malonaldehyde: Malonaldehyde, a product of oil acid peroxidization, gives a reaction with TBA at hot and acidic medium and forms a colour complex which gives maximum absorbance at 532 nm. 2.5 mL 10 % (w/v) TCA solution and 0.5 mL serum were mixed with vortex in a tube. Close the tube and kept at 90 °C for 15 min. Cold by cold water and centrifuge it for 10 min in the speed of 3000 turn/min. 2 mL supernatant was taken to another tube and added 1 mL 0.675 % (w/v) TBA and waited for 15 min at 90 ºC water bath and treated with cold water. Their absorbance was read at 532 nm. Water was added as samples volume to blind tube.

Ox-LDL measurement: ox-LDL measured by cit and ELISA method. Principle is oxidized apolipoprotein-1 against determinants two monochlonal ELISA method. Cerum ox-LDL gives reaction by solid phases anti-ox-LDL anticor (Ig-ox-LDL). After incubation non-reactive plasma components have been removed. Ox-LDL gives reaction by peroxidase signed apolipoprotein B anticor solid phases ox-LDL. After second incubation non-bonded signed anticor removed. 3,3′,5,5′-Tetramethylbenzidine (TMB) which gives reaction with solid phases bonded conjugate was added. The reaction was completed by the addition of acid and the colour, occured, was read at 450 nm. The concrentrations were calculated by semi-paper drawing standards ox-LDL absorbance graphic.

Determination of antioxidant activity: Antioxidant activity sized Randox trademark kit was used and carried out. The principle of the test is based on the decrease of blue-green colour, formed by $ABTS^+$ radical with added antioxidants[11]. In 600 nm $ABTS^+$ radical is partially stable blue-green. This colour is protected by the quantity of antioxidants, added to the sample. The antioxidant activity is calculated according to the protection. 20 µL is mixed with 1 mL cromogene (6.1 µmol/L metmioglobine + 610 µmol/L ABTS) and initial absorbance at 600 nm was monitored. Then 200 µmol/L H_2O_2 is added and after 3 min absorbance monitored again. The antioxidant activity is calculated by the following formula:

$$\text{Antioxidant activity (mmol/L)} = \frac{\text{Abs}_{\text{kor}} - \text{Abs}_{\text{numune}}}{\text{Abs}_{\text{kor}} - \text{Abs}_{\text{standard}}} \times \text{Standard konsantrasyonu}$$

As a standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid was used, but as a control bidistilled water was used.

RESULTS AND DISCUSSION

In this study, lipid peroxidizations and antioxidants capacity of diet solid and liquid oils consumed by human being were investigated and it can be said that unsaturated oils are more sensitive for peroxidization. Table-1 and Fig. 1 shows that highest serum MDA results are butter and the lowest results are sunflower oil. Serum MDA are decreasing in this order: butter > margarine > olive oil > sunflower oil > control.

TABLE-1
OIL GROUPS SERUM MDA RESULTS

Oil groups	Average value (X) (nmol/mL)	Standard deviation (± SD) (nmol/mL)
Control	2.39	0.27
Sunflower	2.55	0.34
Olive oil	2.67	0.49
Margarine	2.68	0.53
Butter	2.72	0.58

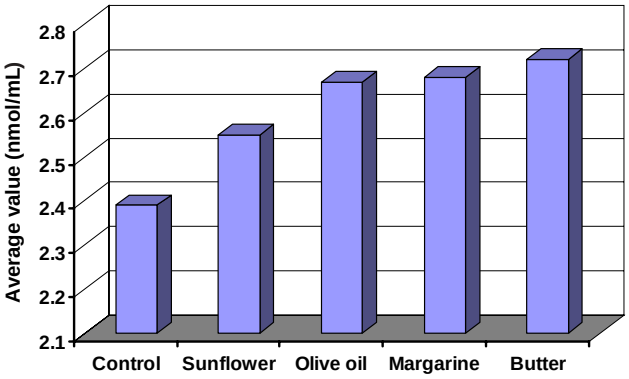

Fig. 1. Oil groups serum MDA results

It is known that antioxidants are added to liquid oils. Olive and sunflower oil have vitamin E as an antioxidant. It was claimed that, olive oil diets decrease the coroner heart disease risk than others[12]. Margarines which produced by liquid oils double-bond hydrogenation, include the *trans*- oil acids, that has an effect. In this study liquid oil groups MDA levels infects by antioxidants. For this reason, it can be suggested that the oils which has no antioxidant, can be used.

The oxidation of LDL is affected from the amount of antioxidant in blood and the oil acid consumption of LDL particules. PUFA amount which is taken by diet increases LDL oxidization[13].

It was thought that butters ox-LDL level should be low than others because of its saturated oil acids. But butters high cholosterol level increases free radical production and this increases oxidative modification[14]. Previous studies[15,16] show that olive oils polyphenols saves LDL against oxidization in plasma. Sunflower oil includes antioxidants against LDL oxidization. Margarines *trans*- oil acids are resistant to oxidization. Table-2 and Fig. 2 shows that highest ox-LDL levels are control group, lowest is margarine. Levels are decreasing by that: control > butter > olive oil > sunflower oil > margarine.

TABLE-2
OIL GROUPS SERUM ox-LDL RESULTS

Oil groups	Average value (X) (nmol/mL)	Standard deviation (± SD) (nmol/mL)
Control	7.78	0.96
Sunflower oil	6.34	2.43
Olive oil	6.44	1.60
Margarine	5.93	1.17
Butter	7.08	1.78

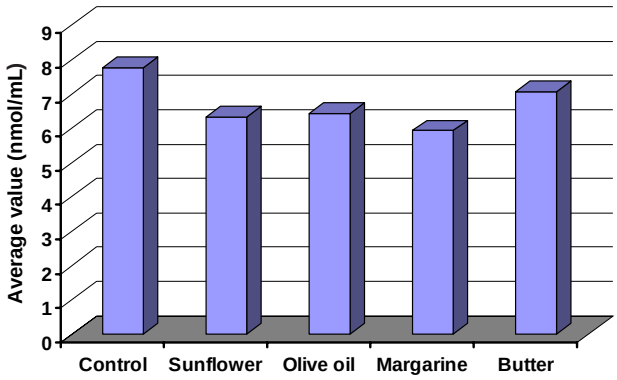

Fig. 2. Oil groups serum ox-LDL results

Though many investigations have been carried out about the protection effect of antioxidants, obtained by diet, against several diseases, there are fewer researches about the effect of diet oils on antioxidation condition in human body. The oils, obtained by diet, influence on antioxidation system, which is important for human health.

The II level of angiotence, which is increased by hypercholesterol, increases the oxydase activity of NADH major deposit of superoxide[17]. In the work the highest antioxidant activity level was seen in butter group. Together with saturated fatty acids and cholesterol butter contains vitamin A. The height of antioxidant activity values of butter is explained by the vitamin A. Especially in people, who uses butter, antioxidants are not spend like PUFA, because butter is rich with saturated fatty acids. Table-3 and Fig. 3 shows that highest serum antioxidant activity results are butter and the lowest results are sunflower oil. It is determined that antioxidant activity results decreased like that: butter > control >olive oil > margarine > sun-flower oil group. In addition to this, serum total antioxidant activity values of butter group (1.17 ± 0.14 nmol/mL) were statistically higher ($p < 0.001$) than those of sunflower group (0.83 ± 0.12 nmol/mL).

TABLE-3
OIL GROUPS SERUM TOTAL AOA RESULTS

Oil groups	Average value (X) (mmol/mL)	Standard deviation (± SD) (mmol/mL)
Control	1.13	0.12
Sunflower	0.83	0.12
Olive oil	1.08	0.23
Margarine	0.96	0.15
Butter	1.17	0.14

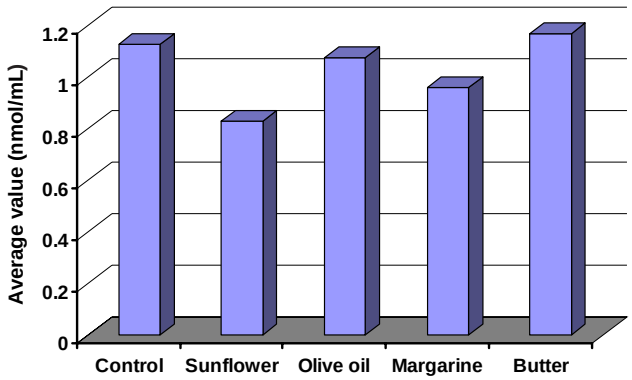

Fig. 3. Oil groups serum total antioxidant activity results

One of the known mechanisms for antioxidant capacity of olive oil tyrosole and hydroxytyrosol are phenol compounds, which have high antioxidant activity[18]. Antioxidant activity of polyphenols is explained by complex with metal ions, free radical collecting, breaking lipid peroxidation chain, regeneration of antioxidant vitamins and stimulation of free radical adoption and other functions[19]. In the work it was determined by statistic calculations, that antioxidant activity values of olive oil are more than the values of sunflower oil ($p < 0.05$).

Especially, in comparison with oxidation olive oil is richer than the stable MUFA. Stable MUFA protects lipoproteins against oxidation. It was determined, that in tomato antioxidant licopen is useful for health. In this research it was shown, that olive oil increases the antioxidant effect of licopen, but sunflower oil decreases antioxidant effect of licopen[20]. During the nourishment with oils, which have high PUFA concentration, it was defined, that quantity of vitamin E is not enough in oil by the capacity of antioxidant[21].

High quantity of PUFA in sunflower oil increases the use of antioxidant and that decreases antioxidant capacity. In the research of vitamin C and α-tocopherol in brain, plasma and livers of hamsters, nourished with some oils, it was found out, that the values of group, which were enriched with margarine, is less[22]. This result shows that in present research margarine group is equal with the low antioxidant activity values.

Conclusion

In this study it is thought that oils antioxidants can effect malonaldehyde and antioxidant activity levels of oils. For this reason, it can be offered that in the studies like that the oils which hasn't get antioxidants, can be used. The soruce of highest ox-LDL level can be explained by the high cholosterol level of butter.

Butter gives the highest total antioxidant activity results and sunflower oil gives the lowest total antioxidant activity results. It is determined that antioxidant activity results decreased as: butter > control > olive oil > margarine > sunflower oil group. As a result, butter gives the highest oxidized low density lipoprotein and total antioxidant activity results and margarine gives the lowest oxidized low density lipoprotein, sunflower oil gives the lowest total antioxidant activity results.

REFERENCES

1. M. Nardini, C. Scaccini, M. D'Aquino and P.C. Beneddetti, *J. Nutr. Biochem.*, **4**, 39 (1993).
2. S. Nas, H. Gokalp and M. Unsal, Bitkisel Yag Teknolojisi, Ataturk University Press, Erzurum, p. 723 (1992).
3. C. Kolanci, Biokimya ve Klinik Biokimya, Nobel Press, Istanbul, p. 143 (2003).
4. B.A. Freeman, J. Eiserich and V.O. O'Donnell, Free Radicals, Oxidative Stress and Antioxidants, Plenum Press, New York, p. 65 (1998).
5. E. Cadenas, Free Radicals, Oxidative Stress and Antioxidants, Plenum Press, New York, p. 237 (1998).
6. P.C. Champe and R.A. Harvey, Biochemistry-Lippincott's Illustrated Reviews, Nobel Press, Istanbul, p. 205 (1997).
7. J.L. Witztum and D. Steinberg, *J. Clin. Invest.*, **88**, 1785 (1991).
8. D. Steinberg, *Circulation*, **84**, 1420 (1991).
9. R.W. Browne and D. Armstrong, Free Radical and Antioxidant Protocols, Humana Press, New Jersey, p. 269 (1998).
10. I. Akkus, I. Serbest radikaller ve fizyopatolojik etkileri, Mimoza Press, Konya, 1995.
11. N. Miller and C. Rice-Evans, *Free Rad. Res.*, **26**, 195 (1997).
12. H.F. Wayne, A.J. Sylvia, J.W. Robert, J.A. Michael, S. Murray, D. Ashley and H. Michelle, *Atherosclerosis*, **160**, 195 (2002).
13. R. Korpela, L. Seppo, J. Laakso, J. Lilja, K. Karjala, T.Lahteenmaki, E. Solatunturi, H. Vapaatalo and M.J. Tikkanen, *Eur. J. Clin. Nutr.*, **52**, 802 (1999).
14. N.A. Flavahan, *Circulation*, **85**, 9 (1992).
15. M. Massaro, M.A. Carluccio and R. DeCaterina, *Cardiologia*, **44**, 507 (1999).
16. R.J. Nicolosi, T.A. Wilson, G. Handelman, T. Foxall, J.F. Keaney and J.A. Vita, *J. Nutr. Biochem.*, **13**, 392 (2002).
17. H. Zhang, A. Schmeisser, C.D. Garlichs, K. Plotze, U. Damme, A. Mugge and W.G. Daniel, *Cardiovasc Res.*, **44**, 215 (1999).
18. K.L. Tuck and P.J. Hayball, *J. Nutr. Biochem.*, **13**, 636 (2002).
19. C. Rice-Evans, N.J. Miller and G. Paganga, *Free Rad. Biol. Med.*, **20**, 933 (1996).
20. L. Alison, I.T. David and C. Mridula, *Free Rad. Biol. Med.*, **29**, 1051 (2000).
21. R. Koop and I. Elmadfa, *Z. Ernahrungswiss*, **22**, 271 (1983).
22. C. Sanchez-Moreno, S.E. Dorfman, A.H. Lichtenstein and A. Martin, *J. Nutr.*, **134**, 655 (2004).

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