EFFECT OF BLACK RADISH (*Raphanus sativus* L.) JUICE ON LIPID PEROXIDATION AND PRODUCTION OF $OH^{\cdot}$ RADICALS IN LIPOSOMES

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Abstract
Black radish juice obtained from the whole root is rich in sulphur heterosides, cyanogenic glycosides, vitamins, phenolic substances which were proved to be excellent antioxidants (free radicals scavengers). Brown sugar contains, beside sucrose, organic acids, amines, pectins, reducing saccharides, aminoacids (proteins), vitamins of B complex, ions of potassium, calcium, sodium, magnesium, as well as phosphorus and sulphur oxides.
The aim of this research was to determine the effect of black radish juice and brown sugar solution on the production of $OH^{\cdot}$ radicals and intensity of lipid peroxidation in liposomes in order to establish potential antioxidative effect of these secondary medicaments.
Black radish extracts decreased the intensity of lipid peroxidation only at higher concentrations, while brown sugar decreased lipid peroxidation for about 50%, showing no dosage dependence. Synergistic action of black radish juice and brown sugar solution inhibited the intensity of lipid peroxidation more than the action of extracts alone. Production of $OH^{\cdot}$ radicals was inhibited by black radish extracts, while the brown sugar solution increased it nearly 100%. Negative synergistic effect was obtained in combination of black radish extract and brown sugar, indicated by the increase in $OH^{\cdot}$ radicals production for more than 100%.
Key words: Black radish (*Raphanus sativus* L.), brown sugar, lipid peroxidation, oxidative stress, hydroxyl radicals.

Introduction

Aerobic organisms use oxygen during the respiration process. Approximately 2-3% of respiratory oxygen form active oxygen species that may be toxic. Those species are radicals, ion radicals, as well as active forms of oxygen, formed by the absorption of energy.
Interacting with basic cell structures and biomolecules, toxic oxygen species may lead to a number of physiological and patophysiological disorders (Parkinson and Alzheimer disease, diabetes, cardiovascular diseases, carcinoma, rheumatism, etc.). Aerobic organisms developed different mechanisms of antioxidative protection that include antioxidative enzymes and antioxidative compounds. In cases of excessive production of free oxygen radicals, it is necessary to increase the intake of antioxidants – substances that exhibit the ability to inhibit the production of radicals and remove already formed oxygen radicals.
Oxidative stress is a result of increased production of reactive oxygen species in cells on one hand, and(or) insufficient antioxidative protection on the other.
Black radish (*Raphanus sativus* L.) is commonly used in diet, as an appetizer, and it is also well-known in folk medicine as a remedy. Today, black radish is most often used in treatment of liver and gallbladder function disorders. Black radish shows tonic effect on the

respiratory system, so it is useful in treatment of bronchitis, cough and asthma. It is also very efficient antiscorbutic, as well as stimulans (fatigue, anemia). Black radish juice exhibit diuretic properties, that is especially useful in treatment of arthritis, rheumatism and gout(1-3).

Brown sugar is the third crystal form of sugar beet extract, that contains physiologicaly significant compounds beside saccharose, so it is recomended to be used instead of refined sugar(1-3).

The aim of the study was to investigate the effect of black radish extracts and brown sugar solutions on production of $OH^{\cdot}$ radicals and intensity of lipid peroxidation (LP), in order to prove possible antioxidative properties of these, in folk medicine very often used remedies (secondary medicaments).

Methods

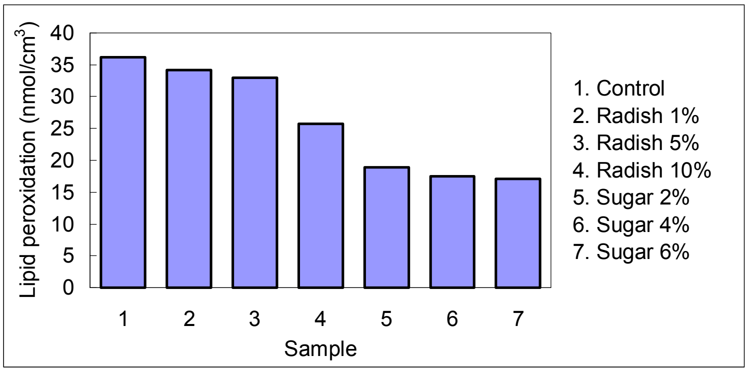
The effects of extracts mentioned above on intensity of lipid peroxidation of liposomes were investigated according to Fukuzawa(4). As a model system of biological membrane, a commercial preparation of proliposomes: "PRO-LIPO S" (Lucas Meyer) with 30% phosphatidylcholine of soybean, pH=5-7, was used in experiments. Lipid peroxidation was performed according to Afanas'ev(5).

The effects of these extracts on production of $OH^{\cdot}$ radicals were determined by monitoring the chemical degradation of deoxyribose(6). The reaction is initiated by hydroxyl radicals obtained in Fenton's reaction(3) which yields products that react with thiobarbituric acid (TBA test). Obtained products, among which malonyldialdehyde is the most important, are determined by spectrophotometric method according to Buege-Aust(7).

Results

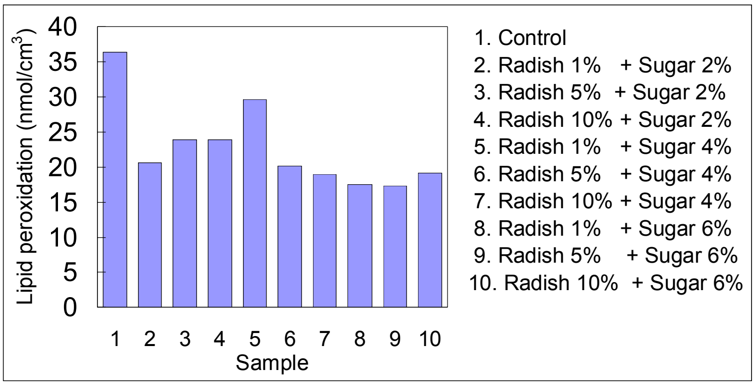
Raw extracts of black radish (1%, 5%, and 10% mass concentrations) and brown sugar solutions (2%, 4%, and 6% mass concentrations) at pH=7.4 were used in experiments.

Graph 1. shows the results obtained after measuring the effects of different concentrations of black radish juice and brown sugar extracts on the intensity of lipid peroxidation. Black radish extracts decreased the LP intensity only at higher concentrations (10% aqueous solution), since brown sugar inhibited the lipid peroxidation at each concentration for approx. 50% (without dosage dependence).



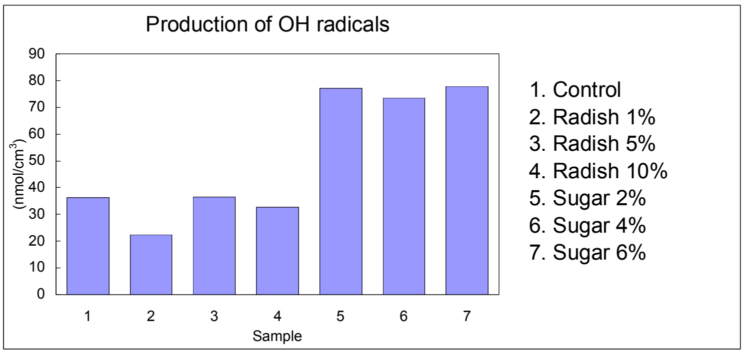
Graph 1. Effects of different concentrations of black radish extracts and brown sugar solutions on lipid peroxidation.

Graph 2. shows the results obtained after synergistic action of black radish juice and appropriate amounts of brown sugar solutions. Addition of brown sugar solutions exhibited positive synergistic effect, and inhibition of LP at increased concentrations of brown sugar added occured in a much higher degree.



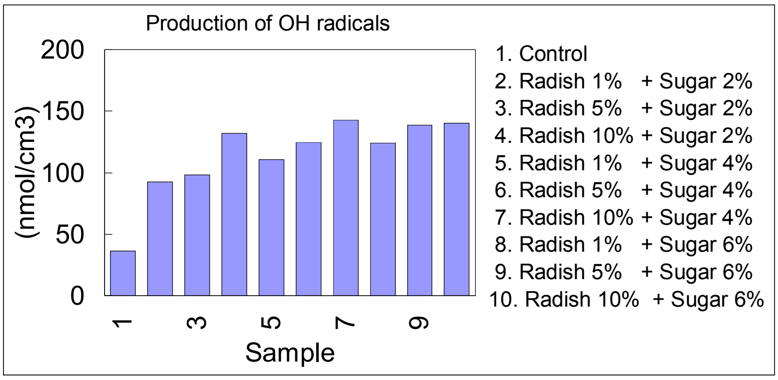
Graph 2. Synergistic effects of black radish extracts and brown sugar solutions on lipid peroxidation.

Graph 3. represents the effect of different concentrations of black radish extracts and brown sugar solutions on production of $OH^{\cdot}$ radicals (induced by Fe^{2+} ions and C vitamin). Black radish extracts decreased the $OH^{\cdot}$ radicals production only at 1% mass concentration, while other concentrations did not significantly change the production of $OH^{\cdot}$ radicals. Brown sugar solutions increased the $OH^{\cdot}$ radicals production twice (approx 100%) without dosage dependence.



Graph 3. Effects of different concentrations of black radish extracts and brown sugar solutions on production of $OH^{\cdot}$ radicals.

Graph 4. shows the results of synergistic effect of black radish extracts and appropriate amounts of brown sugar solutions on production of $OH^{\cdot}$ radicals. Addition of brown sugar solution caused negative synergistic effect, that means higher concentration of brown sugar added, the higher production of $OH^{\cdot}$ radicals occured.



Graph 4. Synergistic effects of black radish extracts and brown sugar solutions on production of $OH^{\cdot}$ radicals.

Discussion

Intensity of lipid peroxidation and production of hydroxyl radicals are important parameters of oxidative stress. In Serbia and Montenegro, black radish and brown sugar are widely used in folk medicine as remedies, so in this paper we wanted to investigate their potential antioxidative or prooxidative properties. For that purpose, we used liposomes as simple, easily accessible model of biological membrane in *in vitro* systems.

Intensity of lipid peroxidation was decreased by the action of black radish extracts as well as brown sugar solutions that points to their antioxidative properties. Synergistic effect of black radish extracts and brown sugar solutions shows opposite results toward lipid peroxidation and $OH^{\cdot}$ radicals production at first sight. Black radish extracts didn't significantly affect $OH^{\cdot}$ radicals production, since brown sugar solutions increased this process in a very high degree. These experiments show that black radish extracts exhibit antioxidative properties, that cannot be concluded for brown sugar solutions. This might be due to the fact that liposomal system still differs from the natural living cell system, because it is difficult to obtain microenviroment to which cell membrane is exposed. In order to confirm these results, *in vivo* experiments should be performed. Antioxidative properties of black radish extracts might be due to the presence of glycoflavonoids(8) and sulphuric heterosides(8). Decrease of LP intensity caused by brown sugar solutions could be explained by the presence of aminoacids, proteins and B vitamin complex. Increase of $OH^{\cdot}$ radicals production might be due to the presence of significant amounts of reducing saccharides in brown sugar solutions.

Conclusion

Black radish extracts decrease the intensity of lipid peroxidation and show concentration dependence, while brown sugar solutions decrease the LP intensity without concentration dependence. Concerning these results, black radish extracts and brown sugar solutions exhibit antioxidative properties.

Low concentration of black radish extract (1%) decrease the $OH^{\cdot}$ radicals production, and other concentrations don't significantly affect this process. Brown sugar solutions increase the $OH^{\cdot}$ radicals production approx. 100%, without concentration dependence.

Synergistic effect of black radish extracts and different brown sugar concentrations yields results that show that higher concentration of brown sugar added, the higher inhibition of lipid peroxidation (positive synergistic effect).

Negative synergistic effect of black radish extracts and brown sugar solutions occurs towards $OH^{\cdot}$ radicals production (the higher concentration of brown sugar added, the higher production of $OH^{\cdot}$ radicals).

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References

1. J., Tucakov, Healing with Herbs (Phytotherapy), Rad, Beograd, Yugoslavia, (1997).
2. M., Wichtl, Herbal Drugs and Phytopharmaceuticals, 446, Medpharm. Scientific Publisher, Stuttgart, Germany, (1994).
3. B., Halliwell, J.M.C., Gutteridge, Free radicals in biology and medicine, Clarendon Press, , Oxford, Great Britain, (1986).
4. K., Fukuzawa, H., Chida, A., Tokomura, H., Tsukatani, Antioxidative effect of α-Tocopherol Incorporation into Lecithin Liposomes on Absorbic Acid – Fe^{2+}-induced Lipid peroxidation, Arch. Biochem. Biophys., **206**, 173, (1981).
5. I.B., Afanas'ev, A.I., Dorozhko, A.V., Brodskii, V.A., Kostyuk, A.I., Potapovich, Chelating and free radicals scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation, Biochem. Pharmacol., **39/11**, 1763-1769, (1989).
6. K.H., Cheesman, In C.H. McBrien and T.F. Slater, eds., Free radicals, Lipid Peroxidation and Cancer, 197, Academic Press, London, Great Britain, (1982).
7. A.L., Buege, D.S., Aust, "Microsomal lipid peroxidation", Methods in Enzimology, Fleisher S., Parker L. (eds), 306-610, Academic Press, New York, USA, (1978).
8. M., Popović, B., Kaurinović, N., Mimica-Dukić, M., Vojinović-Miloradov, A., Đorđević, Combined effect of Plant Extracts and Xenobiotics on Liposomal Lipid Peroxidation, Part 2. Marigold Extract-CCl_4/fullerene, Oxid.Commun., **23/1**, 178, (2000).