EFFECT OF PROPOLIS AND OLIVE OIL ON LIPID PEROXIDATION AND PRODUCTION OF OH· RADICALS IN LIPOSOMES

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Abstract
Virgin olive oil, produced by filtering the hole fruit, is rich in phenolic and polyphenolic compounds, which are well known as good scavengers of free radicals, and good antioxidants. It is also known that these compounds contribute to lowering the risk of coronary diseases and cancer. Olive oil is very rich in unsaturated long-chain fatty acids, and poor in phytosterols at the same time, that means that olive oil shows plasm cholesterol lowering effect. Propolis is resinous substance that appears at the top of buds and in pollen seed cover. Because of the fact that propolis contains resins, ether oils, tannins, flavons, flavonoids, esters, organic acids and essential aminoacids, as well as substances which have the same effects as hormones, propolis is declared to be a real elixir. Propolis acts as scavenger of free radicals, because it contains propol, substance that exhibit higher antioxidative potential than vitamins C and E. Both propolis and olive oil are well known as medicines from ancient times and the earliest notes, and nowadays they are also used as remedies. So, the aim of this research was to investigate effects of propolis and olive oil on production of hydroxyl radicals - $OH^{\bullet}$ (main promotors of oxidative stress) and intensity of lipid peroxidation of liposomes (LP), trying to prove potential antioxidative activity of these secondary medicaments. Propolis inhibited lipid peroxidation at all concentrations, in all investigated systems. Propolis appeared to be an excellent antioxidant and a good scavenger of oxygen radicals. Propolis and olive oil inhibited the production of $OH^{\bullet}$ radicals. Olive oil increased the intensity of LP and showed high prooxidative effect.
Key words: Propolis, olive oil, lipid peroxidation, oxidative stress, hydroxyl radicals.

Introduction

Saved historical and cultural documents testify that plants have been used in treatment since the dawn of human civilization, so it could be concluded that the usage of plants in treatment is as old as humanity. Usages of olive oil and bees products (propolis) are well-known from the ancient times and the earliest notes.
Virgin olive oil, produced by filtering the hole fruit, is rich in phenolic compounds (hydroxytyrosol and europein) which are proved to be "scavengers" of free radicals(1), that is, good antioxidants(2), and beside these they protect organism from peroxidative damaging during aging(3). In combination with E vitamin, olive oil increases the level of Q coenzyme in liver and decreases the intensity of adriamycin-induced lipid peroxidation in rats(4). Polyphenolic compounds found in olive oil contribute to reducing risks of cardiovascular diseases and cancer(5). Beside that, olive oil is especially rich in unsaturated fatty acids, and poor in phytosterols, so it decreases the level of blood plasma cholesterol.

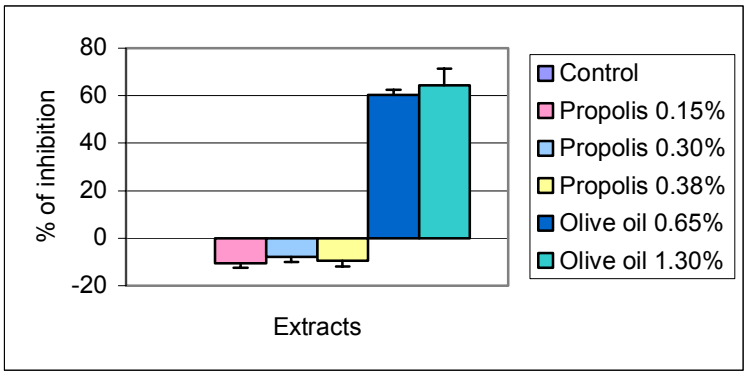
Propolis contains resins, essential oils and substances which have the same effects as hormones. Beside these, propolis contains tannins, flavons, flavonoids, esters, organic acids and essential aminoacids as well. It acts as strong preservative, and also exhibits antibacterial and antiviral effects(6). It is interesting that propolis is concerned to be a "scavenger" of free radicals. Namely, new compound, propol, is isolated, and exhibits higher antioxidative potential than vitamins C and E(7).
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.
Since there are only a few literature data on antioxidative properties of these, in folk medicine very often used remedies, the aim of our study was to investigate the effects of propolis and olive oil extracts on production of $OH^{\cdot}$ radicals (promotors of oxidative stress) and lipid peroxidation (LP) of liposomes, in order to establish possible antioxidative properties of these secondary medicaments.

Methods

In this paper, 0.15%, 0.30% and 0.38% propolis extracts obtained from 20% ethanol solution of propolis ("Medina", Kragujevac, SCG), as well as 0.65% and 1.30% olive oil extracts obtained from extra virgin olive oil (cold pressed, "Fraticello", Spoleto, Italy) were used in experiments.
The effects of extracts mentioned above on intensity of lipid peroxidation of liposomes were investigated according to Fukuzawa(8). 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(9).
The effects of these extracts on production of $OH^{\cdot}$ radicals were determined by monitoring the chemical degradation of deoxyribose(10). The reaction is initiated by hydroxyl radicals obtained in Fenton's reaction(11) 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(12).
Experiments with 5 samples of the same extract concentrations were performed in order to obtain statistical significance of the results.

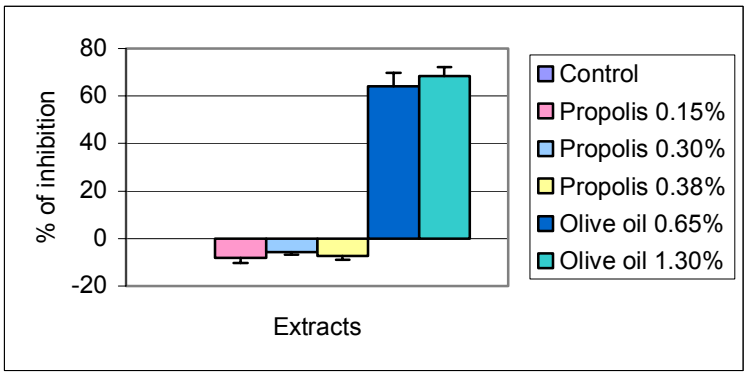
Results

Graph 1. represents the results obtained by the action of different concentrations (v/v) of propolis and olive oil extracts on lipid peroxidation of liposomes. Propolis extracts inhibited lipid peroxidation at each concentration for almost 60%. Since all results were obtained by measurement of 5 samples, they are certainly statistically significant.



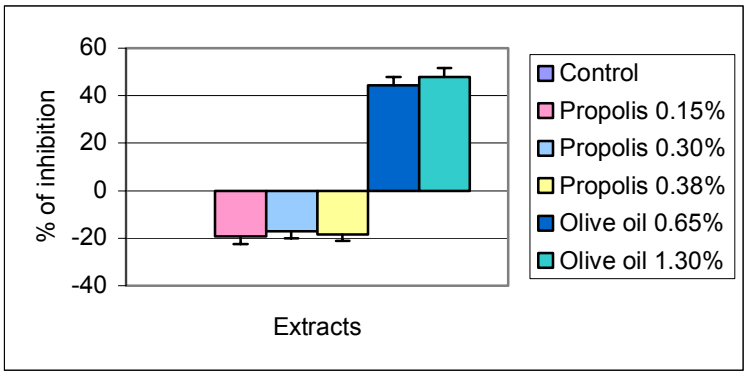
Graph 1. Effects of different concentrations of propolis and olive oil extracts on lipid peroxidation induced by Fe^{2+} ions.

In order to prove these results another series of experiments with the same solutions of propolis and olive oil were performed, but this time lipid peroxidation of liposomes was induced by Fe^{2+} ions and C vitamin. Results are shown on graph 2. Obtained results are very similar to the previous. Propolis decreased the intensity of lipid peroxidation induced by Fe^{2+} ions and C vitamin, while olive oil again showed prooxidative effect in a very high degree, with the increase percentage approx. 65%.



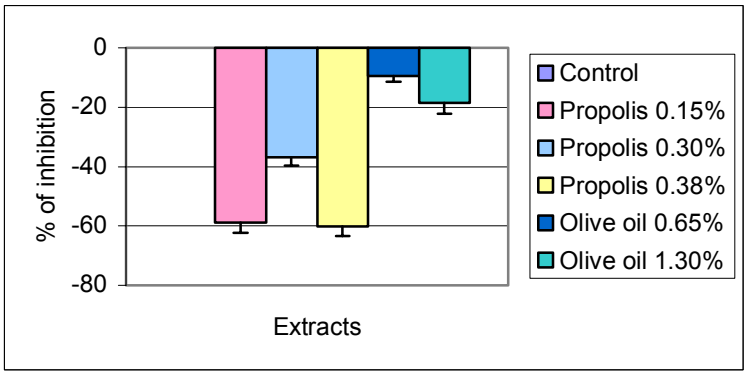
Graph 2. Effects of different concentrations of propolis and olive oil extracts on lipid peroxidation induced by Fe^{2+} ions and C vitamin.

Since our substances were dissolved in ethanol (shows effect on lipid peroxidation according to some authors), the third series of experiments were performed, in which lipid peroxidation of liposomes was induced by Fe^{2+} ions, C vitamin and ethanol (graph 3.). Obtained results confirmed previous effects of propolis and olive oil extracts.



Graph 3. Effects of different concentrations of propolis and olive oil extracts on lipid peroxidation induced by Fe^{2+} ions, C vitamin and ethanol.

Lipid peroxidation is just one of the parameters of oxidative stress, so in order to conclude more about effects of propolis and olive oil extracts on oxidative stress, the effect of different concentrations of propolis and olive oil extracts on production of $OH^{\cdot}$ radicals were also examined. Results are shown on graph 4. Both propolis and olive oil decreased the $OH^{\cdot}$ radicals production. Especially strong inhibition occured with propolis extracts (up to 60%). Concerning olive oil extracts, dosage dependence was obtained, that is, as concentration of olive oil extract increased, percentage of $OH^{\cdot}$ radicals inhibition also increased.



Graph 4. Effects of different concentrations of propolis and olive oil extracts on production of $OH^{\cdot}$ radicals.

Discussion

It is known that olive oil is very often used, and there are some literature data on it's antioxidative properties. In our experiments *in vitro*, olive oil exhibited prooxidative effect by increasing the intensity of lipid peroxidation. This might be due to the presence of high concentrations of polyunsaturated fatty acids in olive oil that can be easily oxidized to form products that can react with TBA.

On the other hand, olive oil decreased the production of $OH^{\cdot}$ radicals. Obtained results might look completely opposite in comparison to the effect of olive oil on lipid peroxidation, but this could be explained by the fact that olive oil is a "scavenger" of oxygen radicals.

Since propolis inhibited lipid peroxidation in each experiment, and inhibited the $OH^{\cdot}$ radicals production in a very high degree as well, it is concerned to be an excellent antioxidant.

These *in vitro* experiments may point to the reactions *in vivo*, but also they may be slightly different, due to the presence of a number of enzymatic and non-enzymatic systems that can influence the oxidative status of a living cell.

Conclusion

In this paper, effects of propolis and olive oil extracts on production of hydroxyl radicals (promotors of oxidative stress) and liposomal lipid peroxidation were analysed, trying to prove potential antioxidative properties of these secondary medicaments.

Propolis inhibited lipid peroxidation at each concentration in all three experimental systems.

Olive oil increased the intensity of liposomal lipid peroxidation approx. 60% in system in which process was induced by Fe^{2+} ions, and 65% in system in which peroxidation was induced by Fe^{2+} ions and C vitamin. In the third analysed system, olive oil exhibited strong prooxidative effect as well.

Both propolis and olive oil extracts inhibited the production of $OH^{\cdot}$ radicals.

Propolis appeared to be an excellent antioxidant and a good "scavenger" of oxygen radicals.

Olive oil exhibited opposite results at first sight. This might be due to the fact that these experiments were performed in *in vitro* systems. In *in vivo* systems, olive oil could be involved in protective effect through other metabolic pathways in comparison to propolis, that might be an idea for further investigations.

Acknowledgments

This work was financially supported by the Ministry of Science, Technology and Development of the Republic of Serbia (Project No. 1862).

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