Charles University in Prague

First Faculty of Medicine

Autoreferát disertační práce

Summary of Ph.D. Thesis

Biochemie účinků polyfenolických látek v léčbě vaskulárních onemocnění.

Biochemistry of polyphenols effects in the treatment of vascular disease

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

Contents

Souhrn

Tato disertační práce se zabývá analýzou učinků polyfenolických látek ve vztahu k oxidativnímu stresu a kardiovaskulárním onemocněním. Práce obsahuje řadu výsledků studia účinků přírodních biologicky aktivních látek červených vín a brambor, a jejich aplikace na modelech spontánní hypertenzních a normotezních experimentálních zvířat.

Metody a přístroje použité v experimentech nám umožnily formulovat několik nových výroků o univerzálním charakteru vztahů mezi antioxidační kapacitou a obsahem polyfenolických látek bez ohledu na zdroj potravin.

Strava je zdrojem minerálních látek, které rovněž přispívají k celkové antioxidační kapacitě, a proto také mohou mít vliv na endogenní antioxidační enzymatický systém tím, že poskytuje základní kofaktory. Experimentálně bylo zjištěno, že koncentrace hořčíku ve víně a extraktu červeného vína známého pro jeho léčebné účinky jsou srovnatelné s minerálními vodami doporučenými pro léčbu a prevenci kardiovaskulárních a metabolických onemocnění. Kromě toho v řadě našich experimentů byly potvrzeny synergické vztahy mezi vybranými minerály.

Klíčová otázka biologické dostupnosti polyfenolů na podporu myšlenky prospěšnosti stravy bohaté na ovoce a zeleninu v prevenci cévních onemocnění byla ověřena na modelech laboratorních zvířat. Polyfenolické sloučeniny byly detekovány v plazmě v koncentraci několikanásobně vyšší u pokusných zvířat léčených polyfenoly ve srovnání s kontrolními skupinami. To vše nám umožňuje dospět k závěru, že tyto látky cirkulují v krvi pokusných potkanů a mohou způsobit pozitivní účinky. Experimentální výsledky potvrdily, že aktivita jednoho z hlavních antioxidačních enzymů superoxid dismutázy a NO syntázy, klíčového enzymu pro udržení normálního cévního tonu, byly zvýšeny ve skupině pokusných zvířat se spontánní hypertenzí, léčených extraktem s vysokým obsahem polyfenolů.

Klíčová slova: polyfenoly, ateroskleróza, červené víno, brambory, NOS, SOD, obsah polyfenolů, antioxidační aktivita, stopové prvky.

Summary

This Ph.D. thesis deals with the deep analysis of polyphenols effects toward vascular disorders. This work provides a number of experimental results of studying both the effects of natural bioactive compounds in red wines and potatoes, and their application to the experiments which includes experimental animal models with spontaneous hypertension.

Methods and equipment used in experimental studies allowed us to make several new statements regarding the universal nature of the relationships between the antioxidant capacity and the polyphenolic content in examined foodstuffs.

Besides this it is also recognized that food is a source of minerals which also contribute to the total antioxidant capacity and therefore may have influence the endogenous antioxidant enzyme system by providing the essential cofactors. Experimentally we have found that the concentrations of magnesium known for its therapeutic action in wine and red wine extract are comparable to the mineral waters recommended for the treatment and prevention of cardiovascular and metabolic diseases. Moreover, synergistic interactions between selected minerals have been found.

The key issue of bioavailability of polyphenols for supporting the idea of the beneficial effects of diet rich in fruits and vegetables toward vascular disease prevention was verified in laboratory animal models. Polyphenolic compounds were detected in plasma in concentrations of several times higher in experimental animals treated with the polyphenols compared to control groups. This enables us to conclude, that these compounds circulate in the blood of experimental rats and may exert positive effects toward vessels. Moreover, our experimental findings have confirmed that activity of one of the main antioxidant enzyme superoxide dismutase and the nitric oxide synthase, an enzyme crucial for the maintaining of normal vascular tonus were increased in the group of experimental animals with spontaneous hypertension treated with the polyphenols rich extract.

Key words: polyphenols, atherosclerosis, red wine, potato, NOS, SOD, phenolic content, antioxidant activity, minerals.

1. Introduction

Epidemiological and experimental studies support the hypothesis that diets with high contents of plant foods are beneficial in the prevention of chronic diseases and cancer in humans, although the benefits for individuals may depend on their genetic profile.

Throughout the history of medicine there have been efforts in searching for drugs that can effectively treat the diseases. Most of the drugs used in modern medicine are initially plant origin. Many biologically active substances have been synthesized from the plant sources.

A typical example of such drugs is aspirin (acetylsalicylic acid). This drug has more than 100 years of successful use in medicine. Initially the compound was found and isolated from the willow bark extract. During past decades a lot have been done in studying of the factors and mechanisms promoting the development of cardiovascular disease. Nowadays oxidative stress is recognized as a principal factor underlying in the pathogenesis of a number of diseases such as cardiovascular and metabolic disorders. This thesis deals with the deep analysis of oxidative stress and it's relationships with the vascular disorders. This work provides a number of experimental results of studying both the effects of natural bioactive compounds in red wines and potatoes, and their application to the experiments which includes experimental animal models.

This thesis provides evidence about the effects of plant polyphenols, with the emphasis on grape, red wine and potato polyphenols. This is due to the fact that grapes and potatoes are the richest and commonly consumed sources of dietary polyphenols in European type of diet.

Biochemical analysis of wine and potatoes was performed, including selected mineral and vitamin content, and significant relationships were described. Finally, the effects of red wine phenolics on superoxide dismutase (SOD) activity as well as endothelial nitric oxide synthase (NOS) activity, and blood pressure trends were tested on the experimental animal models, such as Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). The relevant information regarding the vascular parameters and experimental proof of efficacy of bioactive compounds, vitamins and minerals are summarized.

2. Hypotheses and aims of the Ph.D. thesis

This thesis covers two main areas. The first part of experiments is devoted to the isolation, assessment and finding the relationships between the bioactive compounds content and the antioxidant properties in the most consumed foodstuffs in western populations (red wine and potatoes).
The second part was focused on the application of red wine extract on the models of experimental animals with the spontaneous hypertension and their comparison with the normotensive animals.
Here analysis of physiological parameters such as blood pressure development, activity of endogenous antioxidant enzymes (SOD) and nitric oxide release was performed.

The specific aims of this study were following:

A. To find the appropriate methods and techniques that allow to test the hypothesis that antioxidant capacity has a direct relationships with the total polyphenolic content, and to investigate whether these relationships occur in different dietary sources.

B. To evaluate synergistic reactions among bioactive compounds presenting in red wine

C. Using of 2 models of experimental animals to verify hypothesis that polyphenols and other bioactive compounds from the red wine extract are able to affect the physiological parameters as blood pressure, superoxide dismutase activity (SOD) and the nitric oxide synthase activity, the producer of nitric oxide, a factor of vasodilatation.

3. Materials and methods

3.1 Chemicals

Gallic acid, chlorogenic acid, catechin, epicatechin, caffeic acid, gallic acid, quercetin, 4-hydroxybenzoic acid, ABTS radical and Trolox were purchased from Sigma-Aldrich, SOD assay kit (Fluka, Germany), Folin-Ciocalteau reagent, 2,4,6–Tri(2- pyridyl)-s-triazine (TPTZ) was from (Fluka, Germany), commercial set Randox TAS (radical ABTS, Randox Laboratories, Antrim, United Kingdom) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, All the other reagents were of analytical grade.
The examined Cabernet Sauvignon and Merlot wines were purchased in several supermarkets and wine shops in Prague. Samples were selected to be representative of the most consumed foreign wines in the Czech Republic. The selecting criteria for the samples were to find monovarietal wines that are widespread in different parts of the world but not planted in the Czech Republic. Cabernet Sauvignon

and Merlot wines fit this criteria and comparative evaluation of their constituents was performed. The alcohol content ranged from 11.5% to 13.5% and 0% in Carl Jung dealcoholised Merlot sample.
The wines examined for the treatment of experimental animals were obtained from the Slovak State Institute of Viniculture (Modra, Slovakia). Samples of Alibernet red wine variety were subjected to the process of dealcoholization, and concentration. Finally, an alcohol free ten times concentrated Alibernet red wine extract was prepared.
Twelve potato samples of different varieties with diverse colored pulps such as there of yellow, three of red and six of blue tuber flesh color were evaluated from two agriculture areas in the Czech Republic.

3.2 Methods employed in the thesis

Measurement of Total Antioxidant Capacity
Measurement of Total Phenolic Content
Determination of minerals
Measurements of Riboflavin (B_2) and Pyridoxine (B_6) content
HPLC analysis of single phenolic compounds in red wines and potatoes
HPLC analysis of catechins in animal plasma
SOD activity determination
NOS activity determination
Measurement of superoxide level.

3.3 Animals and treatment

The entire procedures and experimental protocols were approved by the Ethical Committee of the Institute of Normal and Pathological Physiology Slovak Academy of Sciences, and conform to the European Convention on Animal Protection and Guidelines on Research Animal Use. Experimental animals were 6 weeks males old from following groups: normotensive Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). These animals were treated with red wine extract (24,2 mg/kg/day) for 3 weeks. The extract was given in tap water. To ensure that each animal received the complete dose of polyphenolic substances from the Alibernet extract, the calculated amount of liquid extract was given to each cage in the appropriate volume of water and adjusted to the animal's water consumption. Daily water consumption was estimated individually for every animal 1 week before the experiment. During the experiment, water consumption was controlled. All animals were housed as four in one cage at a temperature of 22–24ºC and fed with a regular pellet diet ad libitum.
Systolic blood pressure (SBP) was measured by a noninvasive method of tail-cuff-plethysmography twice a week.

After 3 weeks of treatment, the animals sacrificed and the body weight (BW), heart weight (HW), left ventricle weight (LVW) and right ventricle weight (RVW) were determined. Samples of the heart (LV), aorta and kidney were used to determine NO synthase activity and superoxide dismutase activity (SOD). The plasma samples were immediately frozen and taken for analysis.

4. Results and discussion

Evidence suggests that a diet high in fruits and vegetables may decrease the risk of chronic disease, such as cardiovascular disease, metabolic disease, and to slowdown aging process. This is due to the plant bioactive compounds such as polyphenols and minerals that may play a key role in the reducing chronic disease risk and development of pathological alterations.

Being one of the most consumed foodstuffs in Western populations red wine and potatoes nowadays have attracted considerable attention from scientific community due to possible protective effects toward cardiovascular system. In several large scale epidemiologic studies, there were proven that positive effects in reducing oxidative stress, prevention and even treatment of cardiovascular and metabolic disorders are attributed to the polyphenols, minerals and vitamins found in dietary sources [1-6].

Thesis provides numerous data from the experiments on the determination of phenolic and mineral content in red wines and potatoes. The application of red wine extract on two models of experimental animals of WKY and spontaneously hypertensive rats (SHR) allowed us to confirm the positive effects toward NOS activity, a producer of NO, that mediates the vasodilatation, and the activity of the SOD, an enzyme, which plays an important role in the functions of endogenous antioxidant system.

The structure of the results and discussion reflects the specific aims of the thesis.

1. The relationships between the antioxidant capacity, phenolic and mineral content in red wines, red wine extract and potatoes.

At the beginning of study there were two questions to test, whether the antioxidant capacity has a direct relationship with the total phenolic content in red wines, and whether this relationship occurs in different food sources.

The results of the study with the red wines of Cabernet-Sauvignon and Merlot grape variety that also included the dealcoholised wine suggested, that polyphenols are crucial for the antioxidant capacity. It was found that Cabernet Sauvignon red wine samples had the highest concentrations of the total polyphenols simultaneously with the highest TAC among the all wines subjected to the study. The total phenolic content of the wine samples determined by using the Folin-Ciocalteau colorimetric method varied from 1447 mg/l in Merlot wines to 2912 mg/l of gallic acid equivalents (GAE) in Cabernet Sauvignon wine samples.

The results are visualized in the fig.1.

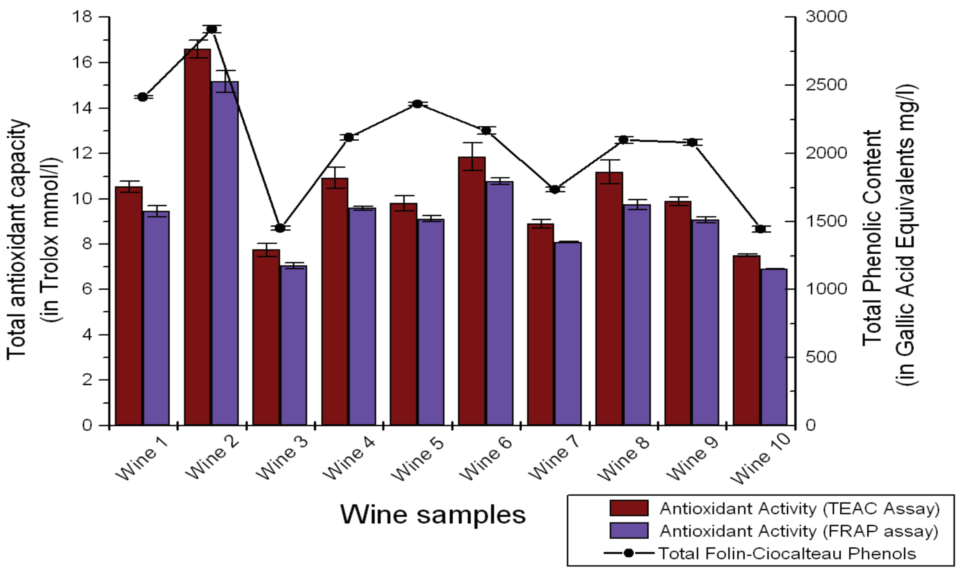

Fig. 1. Relationship among total antioxidant capacity and total phenolic content of examined red wines. Antioxidant capacity determined by two methods, the TEAC assay and FRAP assay. Both assays expressed antioxidant power in Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (mmol/l). Total phenolic content was determined by Folin-Ciocalteau colorimetric assay [results expressed as mg/l gallic acid equivalents (GAE)]. All data are expressed as mean values $\pm$ SD.

Another decisive role also play variety of grapes, this became evident from our experiments, where Cabernet Sauvignon wines possessed higher total antioxidant capacity together with the higher TPC, potassium, magnesium, phosphorus, copper, zinc and vitamin B_6 content compared to Merlot red wines.

Despite considerable variation, the data from the other experimental studies showed that wine and dealcoholized wine enhances absorption of calcium (Ca), phosphorus (P), and magnesium (Mg) and zinc (Zn). This is due to the multiple compounds presented in wine and also due to the natural acidity of wine which may play a role in creating a more favorable intraluminal environment for absorption [7-9].

This enable us to conclude that minerals presented in wine are able to reach blood circulation in required concentrations to exert their effects.

Red wine contains a number of trace elements essential for the proper function of endogenous antioxidant system.

Moreover, copper (Cu), manganese (Mn), selenium (Se) and zinc (Zn) act as co-factors of antioxidant enzymes. Despite the requirement to maintain the balance for the redox trace element such as copper (Cu), which can initiate free radical reactions at the same time it acts as a co-factor for the Cu/Zn- SOD [10].

Despite the fact that the deficiencies are uncommon, the transition to a modern Western diet has led to a substantial decline in potassium (K) intake. A high K intake has the protect effect against insulin resistance, cardiovascular disease and the development of bone pathologies though the maintaining Ca homeostasis [11, 12]. There is close relationship between potassium and magnesium.

This tight connection is illustrated in the study of Humphries et al. [13] and Huerta et al. [14] which focused on a possible protective role of dietary Mg in insulin resistance; their data also demonstrated a high degree of correlation of dietary K with insulin sensitivity. In fact, it was proposed that abnormalities in cellular ion homeostasis may be a major link between cardiovascular and metabolic diseases [15].

Interestingly, magnesium (Mg) in red wines and extracts were presented in the amounts relevant to exert the physiological effects. For instant, Mg in red wines reached concentration up to 146 mg/l, and in Alibernet red wine extract has reached concentrations up to 1337 mg/l.

These findings are in correspondence with the data in the literature published for the mineral content of wines [16-18].

These concentrations of magnesium (Mg) in the red wines and extract are the same level as in mineral waters as Magnesia and Donat Mg respectively, well known for their effects in the treatment of vascular and metabolic disorders [19, 20].

Zinc (Zn) was found as a multipurpose trace element involved besides the prevention of free radicals formation in immune responses, vascular disorders and aging.

Zn is required for structural and functional integrity of more than 2000 transcription factors [21] and 300 enzymes [22]; therefore, almost every signalling and metabolic pathway is in some way dependent on at least one, but often several, Zn-requiring proteins. Recent studies have shown that Zn plays a crucial role in endothelial NO synthase function and in NO signalling. NO synthases are catalytically active only as a dimer of two subunits, the association of which is stabilised by the tetrahedral binding co-ordination of Zn with thiol ligands at the dimer interface [23].

The activity of NO synthase is strongly inhibited by the formation of peroxynitrite, a product of superoxide radical reaction with NO, and is directly related to peroxynitrite-induced release of NO synthase-bound Zn.

Since NO synthase expression is dependent on NF-kB activation, it is also possible that Zn deficiency could influence NO synthase by this mechanism [24].

Furthermore, studies by Reiterer et al. [25] in an atherogenic mouse models confirmed in vivo evidence that zinc deficiency induced pro-inflammatory events. This could be an explanation of the effects that trace elements may have influence the NO synthase activity we observed in our experiments.

Magnesium and zinc plays a crucial role in ageing processes. As far as in elderly the deficit of these minerals are the most common case of the progression of insulin resistance, cardiovascular disease and the neurodegenerative alterations in brain function, supplementation of these element seems to a optimal solution in many cases [26-29].

On the base of these examples it could be assumed that minerals in red wines could affect the action of the endogenous antioxidant system, primarily the antioxidant enzyme activities, as well as tissue metabolism and cardiovascular alteration development.

This hypothesis was proved in our experimental treatment of animals with the red wine extract.

Despite the strong antioxidant activities and one of the highest content of polyphenols and other micronutrients in red wines, several studies shows that to achieve the desired concentrations of these compounds it is need to consume the big amounts of wine or grape juices. The feasible solution of this lies in the concentrates that is possible to prepare from the red wines. In our study, to settle this problem, the extract with ten times higher concentration of polyphenols and minerals was used for the treatment of experimental animals of following models: normotensive and spontaneously hypertensive animals.

Further details will be discussed in the part 3 of results and discussion.

Potatoes

The similar strong relationships between the antioxidant capacity and phenolic content were found in potatoes as for the red wines.

Total phenolic content in 12 potato cultivars subjected to the study were following, the lowest in potatoes with a yellow flesh: from 80 to 100 mg/100 g, compared to 110-150 mg/100 g in red flesh varieties. The highest phenolics content up to 180 mg /100 g was detected in potatoes with blue flash.

Greater content of total phenols in the varieties with of colored flesh was associated with a high proportion of oenocyanin, which does not occur in potatoes with white or yellow colored flesh.

Strong positive relationships between the total antioxidant capacity (TAC) and the phenolic content were found in all potato cultivars. The significance of these relationships was for DPPH assay (r = 0.87, $p < 0.0002$) and for ABTS assay (r = 0.71, $p < 0.0097$).

Similarly, like in red wines results for total antioxidant capacities and total phenolic content in twelve potato samples proved the strong relationships between phenolics and antioxidant properties. It was found that TAC measured by the ABTS and DPPH assays resembled trends as in total phenolics in all potato samples. At the same time it is need to mention that the antioxidant capacities as well as total phenolc content of red wines are much higher compared to potatoes. Differences also occur in the principal phenolic compounds. The HPLC analysis has revealed that among the most abundant phenolics in red wines are anthocyanins and catechins whereas a chlorogenic acid in potatoes. These are consistent with results published for red wines and potatoes by other investigators [30-33].

These results allow us to conclude that total polyphenols contribute in the great extent to the antioxidant capacities of examined foods and beverages. Moreover, this strong positive correlation between TAC and TPC seems to be universal and appears regardless of the type of the foodstuff.

2. Synergy

The synergistic interaction among the polyphenols, minerals and vitamins become the matter of concern in some recent publications. Authors compared the antioxidant activities of the single bioactive compounds with the TAC of foodstuff and beverages and revealed that the total antioxidant capacity measured in the fruits and beverage was higher than the sum of antioxidant activities of single compounds. An assumption was that this occurs due to synergistic interactions among bioactive compounds.

Boyer et al. [34] published relevant information regarding the antioxidant activities of apples. The total antioxidant activity of apples with the peel was approximately 83 μmol vitamin C equivalents, which means that the antioxidant activity of 100 g apples (about one serving of apple) is equivalent to about 1500 mg of vitamin C. However, the amount of vitamin C in 100 g of apples was only about 5.7 mg [35]. Vitamin C is a powerful antioxidant, but these authors show that nearly all of the antioxidant activity from apples comes from a variety of other compounds. Vitamin C in apples contributed less than 0.4% of total antioxidant activity.

This example was consistent with the findings by the other authors [36, 37]. In our experiments with the red wines and the antioxidant capacities, we found that besides polyphenols certain minerals may contribute to the TAC. The results from the Cabernet Sauvignon and Merlot red wine analysis show that wine with lower phenolic content has higher TAC if it simultaneously has the highest values of

Mg, Cu and Zn. However, a sample high in phenolics did not demonstrate the highest TAC when having low mineral content.

Additionally, a positive correlation ($r = 0.63$, $p < 0.05$) between the magnesium (Mg) and zinc content (Zn) was found This correlation was also confirmed in the case of red wine extract.

There are some evidences relating to the synergistic interactions among polyphenols. Mertens-Talcott et al. [38] experimentally verified an enhanced effect of quercetin and the ellagic acid in soft fruits, and particularly in Muscadine grape variety. Other investigators are also considering the possible interactions among polyphenols, and other bioactive compounds. In a very recent study of Peinado et al. [39] it was confirmed that polyphenols and sugars presented in musts act synergistically. It was demonstrated that polyphenols from musts are active in inhibiting radicals, while sugars were highly effective in inhibiting assays mediated by hydroxyl radical formation. So, different molecules as DNA, lipids, and sugars were protected from the oxidation by phenolics. In addition, the deleterious effects of sugars on proteins could be counteracted by phenolics.

Another important aspect of possible synergy are vitamin and minerals interactions. In our study with Cabernet Sauvignon and Merlot wines riboflavin (B_2) and pyridoxine (B_6) were determined. These two vitamins despite the low concentrations of the B vitamins found in red wines, they potentially may affect the metabolism in experimental models by their participation in the reactions of oxidation and reduction in the case of Riboflavin (B_2) and transamination and decarboxylation of amino acids in the case of Pyridoxine (B_6) [40].

These interrelationships between vitamins and minerals could be quite important and became the research topic in several studies.

The most significant example of vitamin action on mineral metabolism is the role played by vitamin D in calcium and phosphorus metabolism. The interrelationship of vitamin C and some minerals is also discussed, with emphasis on its relationship with iron [41, 42]. Vannucci et al. [41] found the possible interactions between pyridoxine and zinc. Our experiments with the red wines also confirmed the positive relationship between pyridoxine and zinc content in Cabernet Sauvignon wines.

Nowadays it is difficult to evaluate all synergistic interactions, however in recent years new analytical technologies will achieve the levels of sophistication. New scientific approaches called metabolomics (the intensive study of very small, previously undetectable metabolites), nutrigenomics and related strategies will allow to elucidate phytochemical complexes and reveal mechanisms by which bioactive compound exert their effects.

3. Animal studies

The question of bioavalability of polyphenols remains to answer in the upcoming decade. In our study catechins were measured in plasma of animals subjected to the experiment (WKY, SHR).

New analytical HPLC techniques and methods enabled us for the first time to determine the concentration of catechins in the plasma of rats with spontaneous hypertension after three weeks of experimental feeding with the red wine extract and to compare these results with the model of normotensive rats. Catechins are one of the major groups of phenolics presented in wine [4, 43].

In our several experiments it was found that catechins were detected in the plasma of rats of both models SHR and WKY treated with Alibernet red wine extract, while in rats of the control groups these substances were found in the concentration of several times lower or even have not been identified. The results are visualized in the Table 1.

Table 1. Catechin concentration in plasma of WKY and SHR animals.

sample	WKY mg/l of plasma	WKY extract mg/l of plasma	SHR mg/l of plasma	SHR extract mg/l of plasma
1	ND	1,36	0,57	1,85
2	ND	4,77	2,88	4,33
3	ND		ND	4,57
4	0,52	1,52	0,41	0,9
5	0,37	1,51	0,13	1,55
6	0,36	2,2	0,24	0,6

These data on bioavailability of catechins in our two experimental models allow us to better understand and explain the benefits that are ascribed for polyphenols for the vascular health and the functions of endogenous antioxidant system.

Some increase in catechins concentration was found after the red wine extract treatment. We assume that from polyphenols catechins maybe the main ones which affect both NOS and SOD activities.

It is possible that also another polyphenols like anthocyanins and phytoalexins have similar effects. In the relation to this statement publication of Mazza have described the impact of anthocyanins on stability and total antioxidant capacity of wines [44].

Data on the total and single phenolic content together with the catechins concentration in plasma were given. That allows us to conclude, that polyphenols were circulated in the blood and were able to increase activities of NOS in the examined tissues.

Total NOS activity increased significantly (P<0.05) after the Alibernet wine extract (AWE) treatment of rats with spontaneous hypertension (SHR). This increase of NOS activity was observed in the left ventricle, aorta and kidney of SHRs compared to untreated SHR (Fig. 2). The Alibernet wine extract did not change NOS activity in WKY rats.

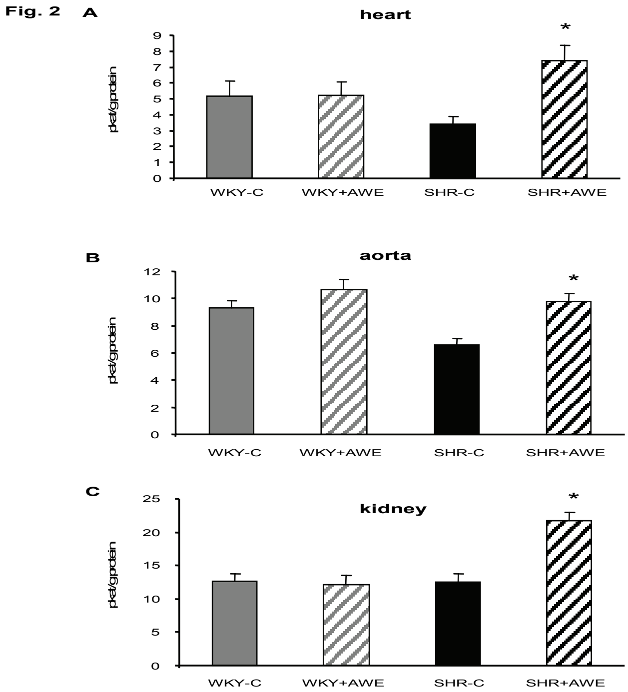

Fig. 2. NOS activity in different organs.

Legend: effect of treatment with Alibernet red wine extract, WKY and SHRs on nitric oxid synthase (NOS) in the left ventricle (LV) (a), aorta (b) and kidney (c). P< 0.05 as compared with control.

Activities of SOD were also increased by 45% in plasma and by 54% in the left ventricle in the SHRs treated with Alibernet red wine extract. This may indicate that plant bioactive compounds together with the minerals, such as magnesium (Mg) and zinc (Zn) are able to change activities of SOD. At the same time after treatment with the Alibernet red wine extract of WKY group of animals were weren't any significant changes in SOD activity in any of the abovementioned tissues and in plasma either.

This new finding enable us to suggest that the positive effects of fruits and vegetables are related to the increase in activity of the endogenous antioxidant enzymes and enhancing the antioxidant effects. This occurs together with the increase of NO production, important for the vasodilatation and the blood flow improvement. This is particularly important in the case of hypertension, which is known with the elevated free radical and cytokine production, chronic inflammation and the suppression with the NO production.

Alibernet red wine extract increased both NOS and SOD activities only in SHRs, where the abovementioned harmful effects are described. That means that polyphenols may affect only pathological mechanisms while they have no actions in normal conditions. Similar effects of red wine polyphenols were described in L-NAME induced hypertension [45].
This enables us to suggest that polyphenols and micronutrient exert their effects only in models with the developed cardiovascular alterations.

5. Conclusions

The results of the experiments proposed in the Ph.D. thesis, allow us to make a number of important new findings on the content of plant bioactive compounds (polyphenols) in foodstuffs and their role in the antioxidant activity. In this work, a comprehensive biochemical analysis of the most consumed foodstuffs in Western diet was performed. In addition to polyphenols other micronutrients such as minerals were evaluated. Techniques and methods of integrated assessment of wines and red wine extract, enabled us to succeed in experiments with two models of animals WKY and SHR.
The conclusions are:

1. According to our results antioxidant activity is directly related not only to polyphenols but also to micronutrients, such as minerals. This phenomenon appears to be universal, since it was confirmed in wines and potatoes.

2. The content of certain trace elements in wine, for example, magnesium (Mg) is comparable to the mineral waters, known for their therapeutic action. Moreover, contemporary clinical studies found magnesium (Mg) and zinc (Zn) as key micronutrients in the treatment of cardiovascular and metabolic diseases. We found, that Alibernet red wine extract has up to ten folder higher mineral content compared to red wine and to mineral waters as well. These results confirm that wine could be a rich source of essential minerals together with polyphenols.

3. Total antioxidant capacity of fruits, wine, juices and vegetables is higher than the sum of the potentials of individual substances. This occurs due to synergistic interactions among the bioactive substances and micronutrients. This fact was confirmed by our analysis of wine Cabernet Sauvignon, where it was found that in spite of the polyphenols, wine samples with high content of Mg and Zn showed a higher TAC.

4. For the first time catechins were determined in plasma of WKY and SHR after treatment with the red wine extract. Certain concentrations of catechins enable us to conclude, that these compounds circulate in the blood of experimental animals and therefore, the effects that we observed on the activity of NOS and SOD may be attributed to the action of polyphenols.

5. The activity of NOS and SOD were increased in the group of experimental animals with spontaneous hypertension after taking the extract of wine, whereas there were no changes in normotensive rats. It means that Alibernet red wine extract affects only the vasodilatation and antioxidant system of SHR.

6. Our results demonstrate that modification of SOD activity was not due to increases in superoxide. This was proven by direct measurement of superoxide radical in heart (LV) and aorta of the both the SHR and WKY groups, where no significant changes were found. The measurement results show that the increase in SOD activity was not induced by the superoxide radical, but could be caused mainly by the action of substances present in the extract such as minerals and polyphenols.

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