Chapter 6

Possibilities to Limit the Values of Clinical and Biochemical Parameters in Experimental Arterial Hypertension

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Additional information is available at the end of the chapter

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Abstract

The aim of this study is to estimate the influence of polyphenolic compounds, renin inhibitors (Aliskiren) and their association on clinical and biochemical parameters, on an experimental model of arterial hypertension (AHT). The combination of Aliskiren and polyphenolic extract has the effect of reducing systolic and diastolic blood pressure. Experimental data highlight the hypocholesterolemic, antiatheromatous, hypolipidemic and cardioprotective effects of polyphenolic extracts. The results demonstrate a significant decrease in the measured biochemical parameters of the oxidative stress (unspecific - ceruloplasmin, uric acid and enzyme - GSH-Px and SOD) of the groups treated with polyphenolic extracts. In the polyphenolically protected AHT group there are statistically significant differences compared to the AHT group, regarding the platelet adhesion index. Aliskiren has more evident vascular protective effects when associated with polyphenols in the experimental AHT compared to unprotected hypertensive group. The antioxidant properties of anthocyanins, combined with the vascular properties of these substances, recommend them as promising therapeutic agents in the prevention/therapy of cardiovascular disorders in general and of AHT in particular. The characterization of polyphenolic extracts, as well as the studies on biocompatibility, will constitute the baseline for understanding the mechanisms, by which phytopreparations can be used for preventive or adjuvant therapeutic purposes.

Keywords: systolic and diastolic blood pressure, aliskiren, polyphenolic extract, oxidative stress, lipid profile

1. Introduction

Hypertension, the most common cardiovascular disease, is the primary cause of stroke, coronary artery disease and sudden cardiac death. Hypertension, a major public health issue, is a

multifactorial disease dependent on complex interactions between genetic and environmental factors, yet many of these causes are not completely understood. Although there is a wide range of hypertension drugs available, a number of new antihypertensive drugs have been introduced during the last two decades. [1].

The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in the homeostatic regulation of blood pressure, fluid electrolytic balance, tissue perfusion and vascular growth. Pharmacologic blockade of the RAAS has proven to be an effective therapeutic strategy in the treatment of several cardiovascular disorders, including hypertension [2]. ACE inhibitors (ACEIs), which block the conversion of Ang I to Ang II, angiotensin receptor blockers (ARBs), which interfere with the Ang II binding to its type 1 receptors [3] and aldosterone antagonists, which inhibit the aldosterone action via the mineralocorticoid receptor (MR) receptor are considered RAAS-inhibiting drugs in clinical practice. Nonetheless, these agents do not allow the complete RAAS suppression since the negative feedback effect of Ang II on renin release is disrupted and consequently, the plasma renin activity and reactive activation of the RAAS increase.

Renin-like enzymes, such as cathepsin D or tonins, which occur in the vascular wall and release angiotensin I from angiotensinogen, are not blocked by renin inhibitors [4].

Aliskiren is the first direct orally active renin inhibitor [5, 6]. It is an effective hypertension drug with specific characteristics, among which we should mention its good renin-angiotensin system blocking ability, its long-lasting action, its pharmacological effects that outlive drug discontinuation and its positive tolerability as compared to placebo [7, 8]. The antihypertensive effect of aliskiren administered alone is similar or even better than that of other first-line hypertension agents. Moreover, its effect is considerably enhanced in combination with various other antihypertensive drugs and there are no adverse drug interactions [9–12]. Aliskiren is an extremely potent competitive inhibitor of renin. It has a high specificity for primate renin. This high specificity for renin makes it unlikely to produce adverse effects through interaction with other enzymes [13, 14].

Treatment of hypertension depends on the etiology of the disease and includes diet alterations, weight loss, exercise and pharmacological interventions. Pharmacological therapies (e.g., renin inhibitors, ACEIs, diuretics) that exist to treat hypertension are successful but they may be associated with negative side effects such as persistent cough, dry throat, allergic reactions, dizziness, angioedema and kidney failure. The popularity of nutraceuticals, which is another name for dietary supplements, has increased in hypertension treatment and prevention [15].

In addition to their high vitamin and fiber content, edible plants are also valuable since they are rich in polyphenols, which are antioxidant compounds responsible for some of the plants' color, flavor and healing qualities [16].

Phytodrugs having a complex composition develop superior qualitative effects compared to the drugs which specify the synthetic and semisynthetic chemicals. Some natural antioxidants, such as alkaloids, lycopene, phenolics, vitamin A/C/E, lipoic acid, and so on, are known

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to provide oxidation protection to biological components (e.g., DNA, proteins, lipids, etc.). In cardiovascular disease, neurodegeneration, inflammation, aging, metabolic syndrome and others, the level of oxidative stress is increased [17].

Natural polyphenols, obtained from many plants, have been shown to exert important actions on the cardiovascular system and may be a potential source of new compounds to treat cardiovascular diseases [18–21]. Observational evidence to date indicates that polyphenol-rich foods, in particular berries and dark chocolates, may influence cardiovascular disease risk factors [22–24]. The *Sambucus nigra* L. (elderberry) fruit extract is considered to be rich in primary polyphenols, leading to its high biological value [25].

2. The biochemical modifications of *S. nigra* extract on experimental arterial hypertension model

The French paradox undoubtedly emphasizes the effects of polyphenolics [17, 26], known as antioxidants, AMPK activator, ACE inhibitor and bioactive phytochemicals as they have many other biological benefits in addition to cardioprotection.

Scientists researching the medicinal benefits of the black elderberry plant have focused their work on the European species, *S. nigra.* The dark purple-black berries produced by this plant are rich in phytonutrients. It contains nearly four times the anthocyanins as compared to other commonly consumed berries. Elderberries are reported to include several bioactive compounds, both phenolic compounds like anthocyanin derivatives, including cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-sambubioside-5-glucoside and cyanidin 3,5-diglucoside [27–29] and triterpenic compounds such as ursolic and oleanolic acids and sterols such as β-sitosterol [30, 31].

2.1. The study of vascular reactivity by preliminary tests on the effects of a polyphenolic extract on *in vitro* isolated arterial fragments

Anthocyanins and some flavone (apigenin) and flavan-3-ol compounds may contribute to the prevention of hypertension. These vasodilatory properties may result from specific structural similarities (including the B-ring hydroxylation and methoxylation pattern) [32].

Polyphenol-rich extracts from fruits of *S. nigra* attenuate endothelial dysfunction induced by oxidative stress in mesenteric resistance arteries of rats.

Endothelial dysfunction, defined as the impairment of the endothelial-dependent relaxation by decreasing the nitric oxide (NO) bioavailability of endothelial origin, occurs through various mechanisms and is present in many pathological situations, and oxidative stress is often a major component of the pathogen mechanism, especially due to the fact that NO is inactivated by a reaction with various highly reactive molecular species containing oxygen (free radicals). The vasodilatory and antioxidant effects of polyphenols from various plant sources are well-known but there are relatively few *in vitro* studies

concerning their influence on vascular reactivity and endothelial dysfunction induced by oxidative stress [33–35].

Anthocyanins are powerful antioxidants that are found in significant amounts in the extracts that we investigate and may be responsible for the protective action observed *in vitro* [36–38].

Dried and powdered elder fruits (50 g) were extracted with 2 × 250 ml acidulated methanol (0.5% HCl) using a magnetic stirrer, each time for 1 h. The total phenolic content in elder fruit extract was determined by the **Singleton and Rossi method** [39]. The amount of total phenolic content was expressed as g gallic acid equivalents (GAE)/100 g extract. The result is the mean of triplicates ± standard deviation. The absorbance levels of all the solutions were determined by means of a UV–VIS Able Jasco V-550 spectrophotometer.

We used isometric myography to study the fragments of the first-order branches of the mesenteric artery from the rat. Endothelial dysfunction was induced by incubation for 15 minutes with 0.4 mM pyrogallol with or without the concomitant presence of the polyphenol-rich extract. All substances, including extracts obtained from the *S. nigra* fruit (SAMB), were administered in the organ bath (5 ml) as small volumes of stock solutions (50 μl). For the evaluation of the antioxidant effect, we used the primary stock solution (50 mg extract per ml DMSO), administered in 1/100 dilution in the organ bath; this is also the maximum dose used in other tests related to vascular reactivity.

We examined the protector potential of the studied extracts in terms of endothelial dysfunction induced through acute oxidative stress in isolated resistance arteries from rats (**Figure 1**).

The obtained results highlight the effects of the studied extracts on vascular reactivity, specifically following antioxidant protection in vitro and assessing according to its ability to attenuate the endothelial dysfunction induced by pyrogallol.

An immunohistochemical study performed by Kawa et al. [40] revealed that quercetin-3-O-glucuronide, one of the main quercetin metabolites in the circulatory system, accumulates in macrophage-derived foam cells of human atherosclerotic lesions but not in the normal aorta. It is still difficult to say whether some polyphenols accumulate in specific target organs; probably, the endothelium is likely to be one of the primary sites of flavonoid action.

2.2. Effects of polyphenolic extract, renin inhibitors and their association in arterial hypertension induced by deoxycorticosterone acetate (DOCA)-salt

S. nigra extract contains 6.9 ± 0.3% g polyphenols and 290.72 ± 4.02% mg anthocyanins besides other compounds. The dry polyphenol extract was diluted in 100 ml polyphenolic solution containing 840 mg natural polyphenols, 95 ml distilled water and 5 ml DMSO. The experiment used active therapeutic doses, well-determined fractions of DL50 on an experimental model of arterial hypertension.

Current preclinical studies were done on arterial hypertension models induced by DOCA-salt (deoxycorticosterone acetate-salt).

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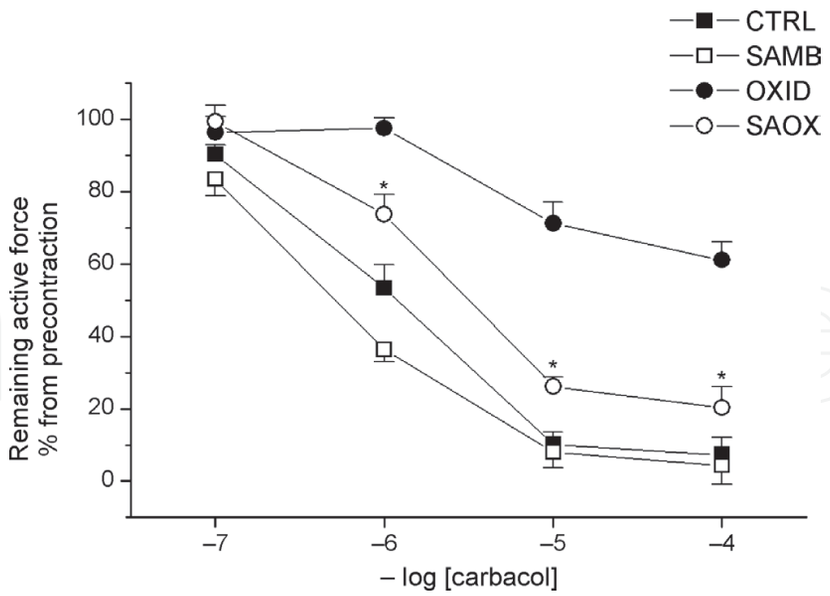

Figure 1. The carbazole-induced endothelium-dependent relaxant effect (CTRL) in the first-order branches from the mesenteric artery of the rat is inhibited by pretreatment with 0.4 mM pyrogallol (OXID) and is not altered by pretreatment with 0.5 mg/ml extract of *S. nigra* fruit (SAMB, $p > 0.05$). This extract attenuates the pyrogallol-induced endothelial dysfunction (SAOX; * $p < 0.01$ vs. OXID). N = 4 in all series; student test for grouped values.

2.2.1. The monitoring of heart rate, systolic and diastolic blood pressure

The monitoring of heart rate, systolic and diastolic blood pressure in the arterial hypertension experimental model was carried out with the **CODA™ noninvasive blood pressure system** [41] on white Wistar rats. American Heart Association (AHA) also recommends this method in its blood pressure measuring guide for laboratory animals. The actual experiment consists of carrying out at least six blood pressure measurements in each laboratory animal. The data collected should then be stored and processed using the CODA™ software.

The experiment was performed on the arterial hypertension model. The study was conducted on white Wistar rats with an average weight of 250–280 g, which were grouped in 6 groups of 12 (**Table 1**).

According to the provisions of the Federation for Laboratory Animal Science Associations on working with laboratory animals, all the rats were kept in 12 h light/12 h dark conditions with free access to water and food.

In the first stage, the *Shapiro-Wilk test* was done to confirm the normality of the specimens (groups); this was considered positive on exceeding the materiality threshold of 0.05 (if $p > 0.05$). Then *the descriptive statistics* were done for each group, including the *box-and-whisker* plots.

Aliskiren, which acts in limited steps against the RAAS, is a logical component in the mixed therapy because it increases the suppression of the RAAS and alleviates the reactive increase of the plasmatic renin activity when it is added to other antihypertensive agent classes. The mixed inhibition of RAAS might allow the usage of smaller doses of each component for obtaining a

Group **W**	Control group, contained normal animals, that did not receive natural polyphenols
Group **PS**	Animals that were administered polyphenols under the form of solution, from the extract obtained from the *S. nigra* fruit, with a dosage of 0.045 g/Kg bw, p.o. (by tube feeding), at every 2 days for 4 weeks;
Group **AHT**	Animals that were given s.c. DOCA-salt 20 mg/kg twice a week and NaCl (1%) added to the drinking water for 4 weeks
Group **AHT + PS**	Animals that were given polyphenols PS in the mentioned dosage at every 2 days p.o., together with DOCA-salt for 4 weeks
Group **AHT + Alisk**	Animals with AHT DOCA-induced that were given s.c. aliskiren 30 mg/Kg bw/day for 4 weeks
Group **AHT + Alisk + PS**	Animals with hypertension (AHT) that were given DOCA-salt and PS polyphenols in the dosage mentioned for 4 weeks

Table 1. Groups used in experimental model.

more efficient and more sustainable suppression of RAAS and probably with less side effects [16, 42].

Using the direct renin suppressor in the mixed therapy with polyphenolic *S. nigra* extract assures an enhanced protection and improves the results compared to monotherapy. The electrocardiographic aspect is significantly improved in the hypertensive group protected with polyphenols compared to the unprotected hypertensive group.

Experimental data indicate the fact that the polyphenol-rich extract is capable not only to effectively slow down the evolution of hypertension in the AHT experimental model but also to normalize the blood pressure levels in the group which received *S. nigra* extract, respectively, AHT + PS and AHT + Alisk + PS. The separate association of renin inhibitor and polyphenols in the hypertensive group reveals an improvement of the medium systolic blood pressure compared to the values obtained in the group which only received aliskiren. Similar data have been obtained for the diastolic component of blood pressure as well (**Figure 2**).

2.2.2. Biochemical plasma determinations (lipid profile, ceruloplasmin, uric acid and fibrinogen)

Polyphenol-induced AMPK activation suppresses lipogenic transcription factors (e.g., SREBP1/2, C/REBP, etc.) and enzymes (e.g., HMG-CoA reductase, acetyl-CoA carboxylase, etc.) for de novo biosyntheses of cholesterol and fatty acids and TG formation [43, 44].

In our study, the data underlined the fact that the lowest medium values of total cholesterol were recorded in hypertensive groups which received polyphenols (*S. nigra*) in addition to the renin inhibitor (aliskiren) (**Figure 3a**). Moreover, in order to support the lipid lowering effect of polyphenols, the mean triglyceride values detected were also lower in the groups which received *S. nigra*.

Due to the protection provided by polyphenols to the rats in the group AHT + PS, the LDL serum level is reduced, approaching the normal limits. Polyphenols have an important

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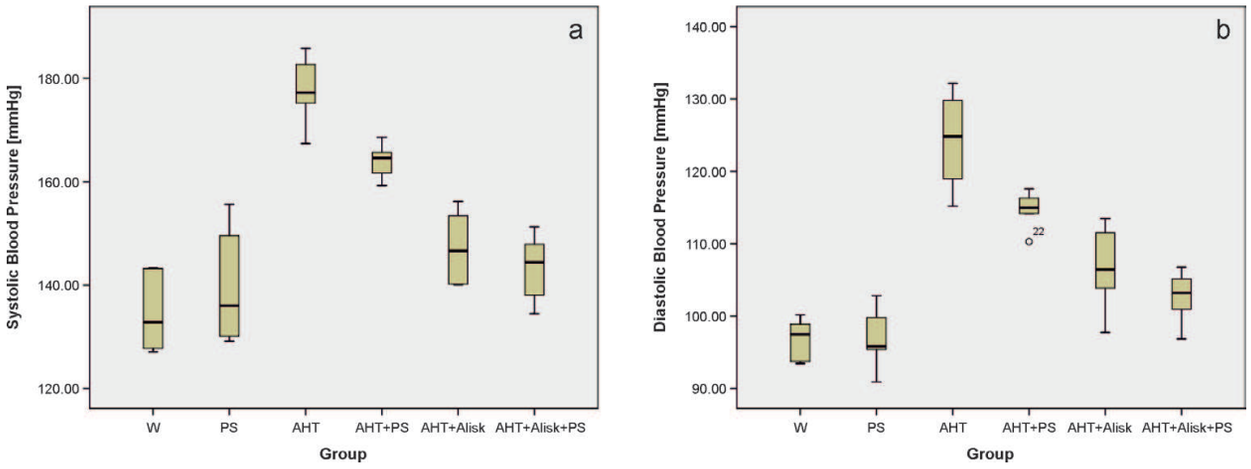

Figure 2. (a) The box-and-whisker plot of systolic blood pressure and (b) the box-and-whisker plot of diastolic blood pressure.

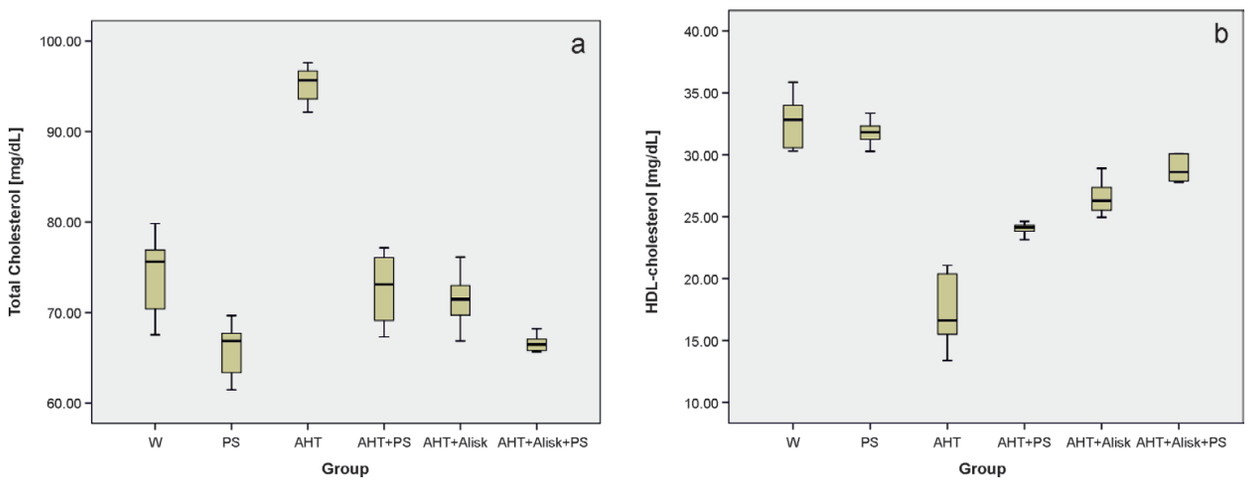

Figure 3. (a) The box-and-whisker plot of total cholesterol and (b) the box-and-whisker plot of HDL-cholesterol.

antiatherogenic role by their lipid lowering effect, especially by decreasing the synthesis and secretion of LDL and VLDL. In our study, HDL has significantly low values in the AHT group of rats compared to the rats in the W group and in the AHT + Alisk + PS group (**Figure 3b**).

Plasma proteins with high-molecular weight, such as ceruloplasmin, fibrinogen and C-reactive protein, have a powerful effect on red cell aggregation, a phenomenon found in hypertensive animals. In the experimental model we studied, the fact that the medium values of ceruloplasmin are higher in the other groups compared to the AHT group is to be noted, which suggests the beneficial antiradical effect of ceruloplasmin. *S. nigra* extract administration proved to be the most efficient, which determines the mean ceruloplasmin values close to those recorded with the control. By stimulating the ceruloplasmin activity, the effect of the polyphenols is favorable, proving their additional antioxidant role [45, 46].

The increase in the fibrinogen level generates modifications of the rheologic properties of the blood such as increased plasma viscosity, red cell aggregation, platelet thrombosis, alterations of the vascular reactivity and compromised endothelial integrity. The increase of fibrinogen concentration determines an increase of blood viscosity, which generates emphasized shear stress that activates endothelial cells and platelets. Thus, in our study, the highest mean values of fibrinogen are recorded in the AHT group, significantly higher than the other groups as well as fibrinogen-associated higher lipidic profile and heart rate.

Uric acid acts like a free radical scavenger due to the covalent bonds it can establish with the singlet oxygen. In animal models, hyperuricemia predisposes to high blood pressure by different mechanisms such as endothelial dysfunction, inflammation and vascular modifications at the renal microcirculation level, activation of the system renin-angiotensin-aldosterone. The data in our study is in line with that provided by the study [47], thus, the highest serum levels of the uric acid being registered in the hypertensive group. The lowest mean values of the uric acid are recorded in the groups treated with *S. nigra* and in the control group, significantly lower compared to those recorded in the other investigated groups.

2.2.3. Enzymatic determinations (SOD, CAT, GSH-Px) and nonenzymatic ones (GSH) regarding the antioxidant capacity

Antioxidative stress is mainly achieved by the classical antioxidation, which is also ensured by ACE inhibition interrupting AT-II-induced ROS generation and by the anti-inflammatory actions blocking inflammation-oxidation axis. The mechanisms involved in the antioxidant capacity of polyphenols include suppression of ROS formation by either inhibition of enzymes involved in their production, scavenging of ROS or upregulation or protection of antioxidant defenses [25, 48].

Polyphenol administration may offer indirect antioxidant protection by activating the endogen defensive systems and by modulating the cellular signaling processes such as NF-kB activation, glutathione biosynthesis and MAPK proteins [49]. The mechanism of polyphenols on vascular function depends on the ability of nitric oxide synthase (eNOS) and its bioavailability to the endothelium. This vascular nitric oxide regularity mechanism is believed to have involvement of polyphenols with kinase molecular signaling like PI3-kinase/Akt pathway and intracellular Ca^{2+} on eNOS phosphorylation which ultimately results in NO production [50].

Reduced glutathione (GSH) is an intracellular thiol antioxidant; lower level of this GSH causes higher ROS production, which results in imbalanced immune response, inflammation and susceptibility to infection [51].

GSH regeneration is provided by the enzymes in the pentose-phosphoric shunt, generator of NADPH (Zn-enzyme). GSH reacts with a wide variety of free radicals, having a free radical "scavenger" function and it contributes to the repair of the biologic disturbances mediated by radicals [52] (**Figure 4**). We did not record significant modifications regarding serum values of CAT in the AHT + PS, AHT + Alisk and AHT + Alisk + PS groups, compared to the control group.

The more the effects represent the total of their combined action, the more efficient is the activity of the antioxidants, each of them functioning according to different mechanisms and at

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various levels of the free radical evolution link in the organism. As a result, polyphenols have the possibility of adjusting and limiting the free radical or reactive species (peroxide) excess [53].

The results obtained following statistical analysis prove a significant modification of the measured biochemical parameters of the oxidative stress (nonspecific—ceruloplasmin and uric acid and enzymatic—GSH-Px and SOD) in the sense of decreasing oxidative stress in the one which was administered *S. nigra* extract.

When the AHT group was under polyphenol and aliskiren protection (the AHT + Alisk + PS group), the serum activity of SOD returned to normal values. As a result of the oxidative stress in the AHT group, serum activity of SOD has significantly lower values ($p < 0.001$) compared to those recorded with the W, AHT + PS and AHT + Alisk groups. We noticed that due to protection, the serum activity in the AHT + Alisk + PS group rats is more intense than in the case of the nonprotected AHT group (**Figure 5**). It should also be noted that, in the AHT group, low SOD values were noticed in association with low levels of ceruloplasmin and elevated levels of uric acid and fibrinogen.

Regardless of their antioxidant effect, polyphenols increase the production of vasodilator factors (NO, EDHF, prostacyclin) and inhibit the endothelin-1 synthesis with vasoconstrictor effect in the endothelial cells. Furthermore, it inhibits the expression of two major proangiogenic factors such as vascular endothelial growth factor (VEGF) and MMP-2 in smooth muscle cells.

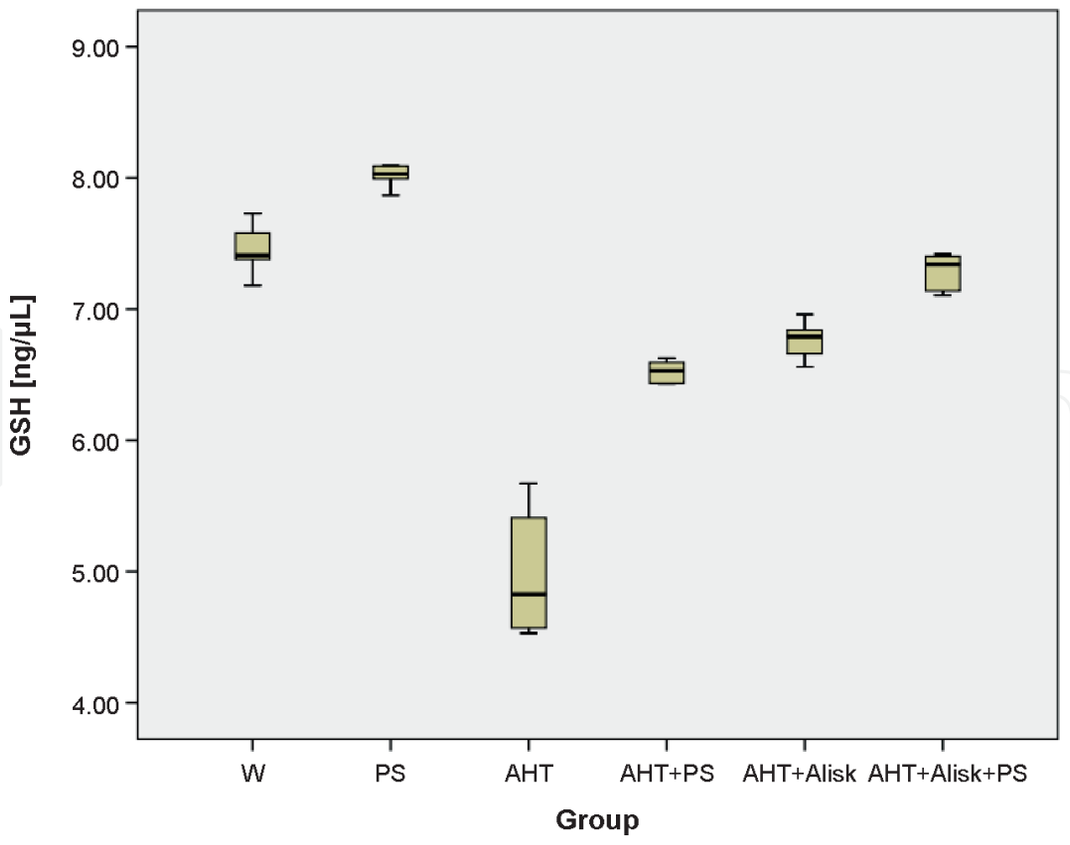

Figure 4. The box-and-whisker plot of GSH.

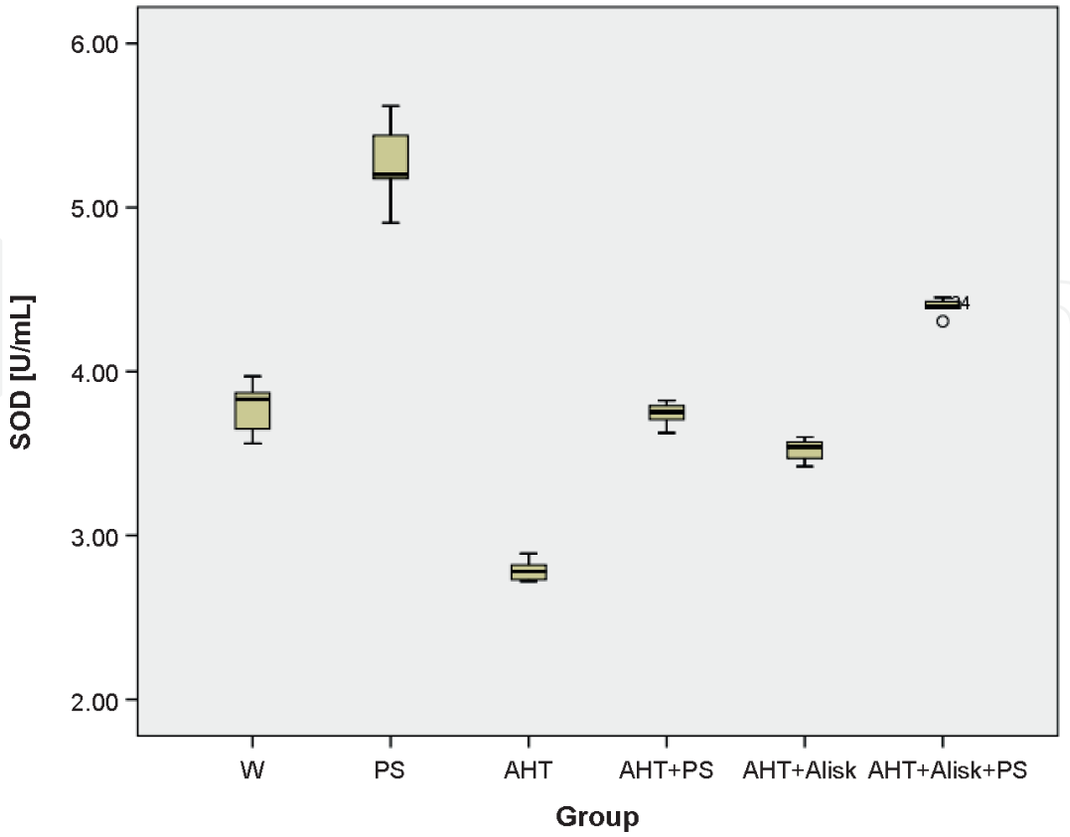

Figure 5. The box-and-whisker plot of SOD.

The changes in the redox status of the cell are determined by the endothelial dysfunction. Starting from previous studies that have discussed the potential antioxidant extract of *S. nigra* is proved that incorporation of anthocyanins from these fruits into endothelial cells causes protective effect against oxidative stress [54].

2.2.4. Platelet adhesion index determination

Activation of platelets adhering to the vascular endothelium induces the formation of lipid peroxidation and oxygen free radicals, which will inhibit synthesis of endothelial prostacyclins and NO. The effect of polyphenols in decreasing platelet activity has a strong impact on cardiovascular disease and can explain the epidemiological data on polyphenol function in cardiovascular disease [55, 56].

The main mechanisms by which flavonoids inhibit the platelet aggregation are: inhibition of phosphodiesterase with intracellular cAMP increase, cytoplasmic calcium reduction, cyclooxygenase inhibition, the enzyme involved in the transformation of arachidonic acid into TxA2, which is an aggregator and powerful vasoconstrictor [55]. In the AHT + PS group, there are statistically significant differences compared to the AHT group as well as to the control group (**Figure 6**). Platelet adhesion index (PAI) has been consistently correlated with the MDA level from the erythrocyte. Thus, there is a significant correlation between the intensity of the oxidative stress and the supplementation with the polyphenolic extract.

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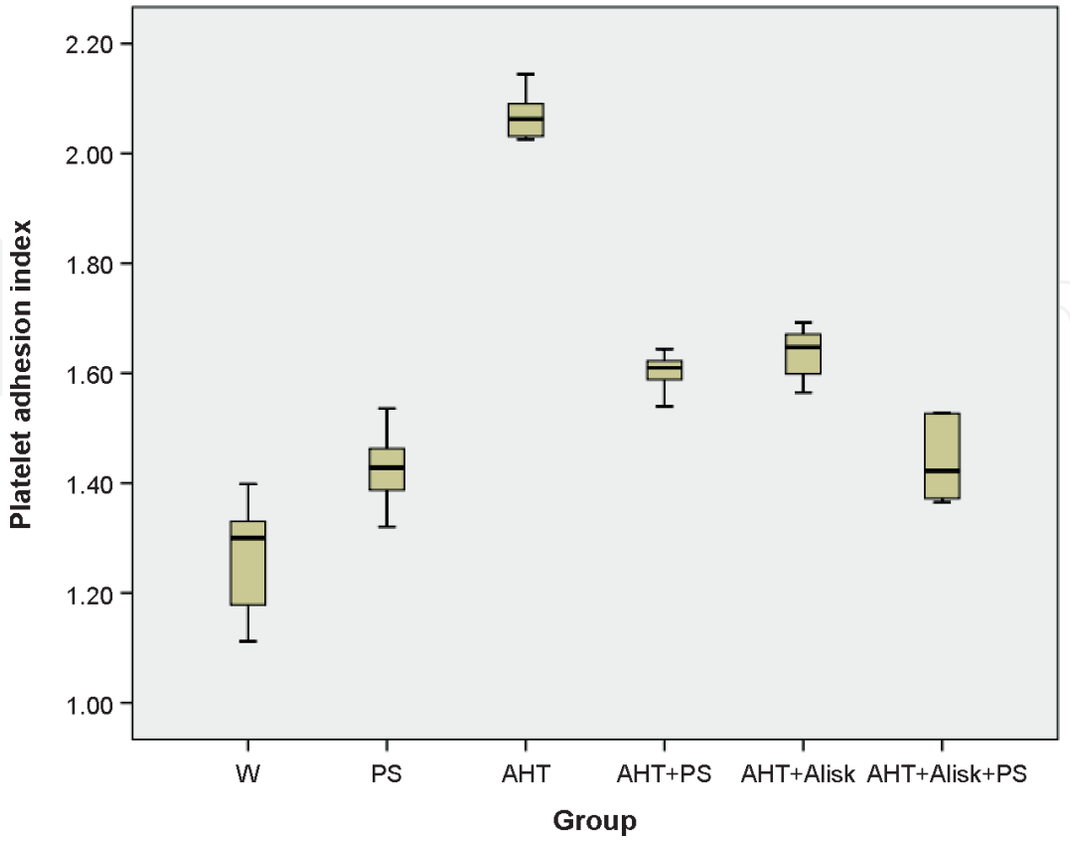

Figure 6. The box-and-whisker plot of platelet adhesion index.

The antioxidant properties of anthocyanins, combined with the vascular properties, characteristic of these substances, recommend it as promising therapeutic agents in the prevention/therapy of cardiovascular diseases in general and of AHT in particular.

Natural polyphenols and aliskiren may influence enzymatic and nonenzymatic changes in experimental arterial hypertension, in the sense of their favorable evolution, by improving lipid alterations and oxidative stress.

The potential cardioprotective properties of *S. nigra* extract include mainly antihypertensive, antiatherogenic and anti-inflammatory activities as well as inhibition of the platelet activation and aggregation, and attenuation of endothelial dysfunction. The main antihypertensive effects are the increase in NO bioactivity, the reduction of endothelin-1 and the decrease in ACE.

The obtained results show that antioxidant activity is all the more intense, as the polyphenolic preparation is administered for a long time during the course of the disease. Ensuring a diet rich in anthocyanins has beneficial effects on the whole body, the assumption being supported by the experimental data that highlight the hypocholesterolemic, antiatheromatosus, hypolipidemic and cardioprotective effects of polyphenolic extracts.

Mechanistically, it has been suggested that dietary polyphenols can alleviate hypertension through anti-inflammatory and antioxidant effects and increased oxide nitric (NO) production [16]. The anti-inflammatory effect is associated with a reduced expression of the redox-sensitive

nuclear factor-kB (NF-kB), while that the antioxidant effect of polyphenols is related to improved enzymatic activities of superoxide dismutase, catalase and glutathione peroxidase. In addition, polyphenols participate in the activation of the redox-sensitive phosphoinositide3 (PI3)-kinase/Akt pathway, leading to increased formation of NO [16]. Taken together, all these pathways help to reduce blood pressure in hypertensive conditions.

The dynamics of international scientific research on hypertension and on its complications materialized in the creation of preparations with a wide range of action and variable side effects, which require further investigations. These preparations are designed to have the widest possible action range, both as concerns their main action and as concerns their pleiotropic effects.

3. Conclusions

Subsequent studies will explore the potential of the direct renin inhibitors/(pro)renin blockers both in monotherapy and in combination with antihypertensive drugs of other classes, as well as their use not only to reduce arterial pressure but also for their renal and cardioprotective effects. In this respect, the characterization of polyphenolic extracts, as well as the studies on their bioavailability and biocompatibility, will constitute the baseline for understanding the mechanisms by which phytopreparations can be used for preventive or adjuvant therapeutic purposes.

By carefully extrapolating the experimental findings on animals to humans, the study could contribute to the increase of the life expectancy of hypertension patients and to the improvement of the life quality of the patients suffering from cardiovascular conditions.

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