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Intervention of Sambucus Nigra Polyphenolic Extract in Experimental Arterial Hypertension

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***Abstract*—**The research focuses on the effects of polyphenols extracted from *Sambucus nigra* fruit, using an experimental arterial hypertension pattern, as well as their influence on the oxidative stress. The results reveal the normalization of the reduced glutathion concentration, as well as a considerable reduction in the malondialdehide serum concentration by the polyphenolic protection. The rat blood pressure values were recorded using a CODA^{TM} system, which uses a non-invasive blood pressure measuring method. All the measured blood pressure components revealed a bio-statistically significant (p<0.05) blood pressure drop between the AHT and the AHT+P groups. The results prove that oxidative stress is considerably lower, statistically speaking, in rats with hypertension but also provided with natural polyphenolic protection from *Sambucus nigra* fruits than in the rats belonging to the control group. In addition to the demonstrated antioxidant effects, natural polyphenols also have other biological properties that might contribute to the cardioprotective effects**.**

***Keywords*—**Arterial hypertension, Oxidative stress, *Sambucus nigra*

I. Introduction

POLYPHENOLS are organic compounds synthesized by plants, including tannins, lignans and flavonoids. Isoflavones are flavonoid compounds with both antioxidant and estrogenic properties, such as the soybean isoflavones genistein and daidzein which can behave as estrogen mimics [1]. The effects of polyphenols, therapeutically relevant for the biological systems, are: they reduce the scavenger properties for oxygen free radicals [2], they reduce platelet aggregability, they have the capacity of interacting with the system, leading to the release of NO from the endothelium, they have an antiatherogenic effect [3] [4]. They show high affinity for different structures and may therefore be able to decrease oxidative damage mainly at such particular sites [5]. On the other hand, since polyphenols are redox active compounds they may also cause increased radical formation if they uncouple electron pathways in the body or if they chelate transition metals in such a way that they become more reactive like in the experimental Fenton oxidation systems. More evidence for a protective role of polyphenols against cardiovascular diseases arose from a number of clinical trials [6], experiments on animal models and mechanistic studies [7].

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Various epidemiological studies have shown an inverse association between the consumption of polyphenols or polyphenol-rich foods and the risk of cardiovascular diseases [8]. The purpose of the study was to emphasize the effects of the polyphenolic extract from the isolated and purified vegetable material represented by the mature fruit of the *Sambucus nigra* on biochemical parameters and blood pressure modifications. The experiment was performed on the arterial hypertension model.

II. Material And Methods

Ripe berries of *Sambucus nigra* Michx. (Elderberry) were shade-dried at room temperature for one week. Dried berries (100 g) were chopped into small pieces and extracted with 3 x 700 ml ethanol using a magnetic stirrer (FALC F30ST), each time for 3 h. The combined extracts were taken to dryness by evaporation under reduced pressure (BÜCHI R-210 rotavapor, BÜCHI V-850 vacuum controller, BÜCHI V-700 vacuum pump). Total phenolics quantification was performed by Folin-Ciocalteu method. The absorbance was measured at 765 nm after 2 h of incubation at room temperature. A calibration curve was plotted using gallic acid as standard. The total phenolic content was expressed as mg gallic acid equivalents/g extract. Sample was assayed in triplicate and the results were given as the mean ± standard deviation.

The research was performed on Wistar white rats, with an average weight of 250-280 g, which were divided into 4 groups of 12, namely: - Group *W* - control, normal animals, that didn't receive natural polyphenols; - Group *AHT* - animals which were administered L-NAME 40 mg/kg body/day, i.p., at every 2 days, for 8 weeks; - Group *P* – animals that were administered polyphenols under the form of solution, from the extract obtained from the *Sambucus nigra* fruit, with a dosage of 0.045 g/Kg body, p.o. (by tube feeding), at every 2 days, for 8 weeks; - Group *AHT+P* – animals which were administered polyphenols in the dosage mentioned p.o. at every 2 days, concomitantly with L-NAME, for 8 weeks.

The experimental study fulfils all the requirements of the guide regarding the use of laboratory animals and biological preparations issued by the International Society of Pain Study (IASP) and the European Council Committee (86/609/EEC).

Reduced glutathione (GSH) was also determined by the Beutler method [9], through the use of 5,5' ditio-bisnitro-benzoic acid (DTNB) and was expressed in μg GSH/mg protein or g Hb in erythrocyte. *The malondialdehide (MDA) concentration* – the index of lipid peroxidation – was determined by the Ohkawa method using the tiobarbituric acid (TBARS) [10].

The exploration of the *lipid profile* included the measurement by photocolorimetry, in the serum obtained after

separation, of the concentration of total cholesterol (Ch-T), of triglycerides (TG), of total lipids (LT), of high-density lipoproteins (HDL) of low-density lipoproteins (LDL) - according to the Friedewald formula - for all the animals included in the experiment.

The rat blood pressure values were recorded using a CODA^{TM} system purchased from Kent Scientific Corporation, which uses a non-invasive blood pressure measuring method. It records the blood volume-pressure by a band attached to the tail, homologated by Bland-Altman testing designed to reveal conformity with an invasive method (radiotelemetry), which enjoys proven accuracy, yet it is difficult to use in our study. The method is also recommended by the AHA in its blood pressure measuring guide for laboratory animals. The actual *experiment* consists of performing at least 6 blood pressure measurements in each laboratory animal, data collection by means of the CODA^{TM} software and subsequent data processing.

Statistical data interpretation **:** All the data are shown as mean value ± standard error of the mean (SEM). In order to assess the normal distribution of the groups, Shapiro-Wilk test was performed. Additionally, Levene test was performed to confirm the homoscedasticity of the groups, followed by ANOVA and paired or unpaired t-test to reveal the pairs of groups that differ biostatistical significantly in term of means. Statistical data interpretation considered the corresponding differences for a given significance threshold: $p>0.05$ statistically insignificant; $p<0.05$ statistically significant; $p<0.01$ strong statistical significance; $p<0.001$ very strong statistical significance.

III. Results And Discussion

Oxidative stress generates free radicals and oxidants that play a role in increasing lipid peroxidation, as confirmed by the high levels of MDA, of serum lipids and fractions of membrane lipids. MDA has been proposed as an indicator of lipid peroxidation because this molecule is one of the end products of this oxidative process [11].

There are highly significant values ($p<0.01$) for group P when compared with group W and for group AHT +P when compared with group AHT and extremely significant values ($p<0.001$) for group AHT when compared with group W, as shown by the statistical analysis of the MDA values (Table I).

Malondialdehide, the most abundant among the reactive aldehydes derived from lipid peroxidation, was significantly increased in blood as well as in peripheral mononuclear cells. These aldehydes have been implicated as causative agents in cytotoxic processes, and it is reasonable to suppose that releases from cell membranes may diffuse, interact, and induce oxidative modifications in other cells and in LDL molecules, thereby increasing the risk of cardiovascular damage [12].

The significant lipid peroxide diminution in the serum contained by the AHT+P group compared to the P group is a result of a considerable reduction in the MDA serum concentration.

Reduced levels of GSH have been related to an extensive number of metabolic and gene expression disturbances, since the tripeptide is not only an efficient antioxidant but also an important regulatory substance in biological systems. Whether the low GSH levels and activity of the antioxidant enzymes is the cause or the consequence of the increased oxidative status needs further evaluation, but the fact that the low activity included several systems points to the reduction being more a consequence than a cause.

The tripeptide γ-glutamyl-cysteinylglycine or GSH is the major nonenzymatic regulator of intracellular redox homeostasis, ubiquitously present in all cell types at millimolar concentration. This cysteine-containing tripeptide exists either in reduced (GSH) or oxidized (GSSG) form, better referred to as glutathione disulfide, and participates in redox reactions by the reversible oxidation of its active thiol. Reactive oxygen species oxidized GSH to GSSG, leading to a decrease in GSH and an increase in GSSG concentrations. Moreover, even though the increment in ROS may upregulate the antioxidant enzymes under higher amounts of pure oxygen or related species, consumption by ROS can overcome the increased production, leading to the low activity observed.

Since polyphenols may modulate eNOS via $O_2^{\cdot -}$ mediated activation of src kinase [13], it seems relevant to further investigate the source(s) and role(s) of $O_2^{\cdot -}$− and other ROS in soy isoflavone mediated activation of eNOS and antioxidant genes. Under conditions of oxidative stress, upregulation of Hsp90 expression [14] and increased intracellular Ca^{2+} will promote turnover and proteosomal degradation of proteins such as calmodulin and eNOS [15] and thereby affect NO bioavailability. The ability of dietary polyphenols to generate both NO and ROS in endothelial cells and activate ARE/ EpRE (Antioxidant response element/ Electrophile response element) mediated gene expression underlies their cardioprotective properties [16].

Dietary polyphenols may counteract oxidative stress in vascular and inflammatory diseases [17] by modulating key redox sensitive gene transcription via NF-κB and Nrf2/ARE [18] signaling pathways.

The balance between antioxidant and pro-oxidant characteristics of polyphenols have been attributed not only to their structural features, but also to the concentration, suggesting induction of antioxidant defence metabolism by low concentrations and ROS production at high concentrations [19]. Dietary polyphenols may offer an indirect protection by activating endogenous defense systems and by modulating cellular signalling processes such as NF-kB activation, glutathione biosynthesis, MAPK proteins, and PI3-kinase/Akt pathway.

The higher GSH levels in the heart of animals subjected to experimental arterial hypertension is an adaptive reaction triggered by the activation of the non-enzymatic antioxidant systems. GSH may be covalently bound to proteins through a process called glutathionylation and acts as a coenzyme of numerous enzymes involved in cell defense. The antioxidant capacity of the serum is significantly improved ($p<0.001$) in the AHT+P rats, as well as the GSH concentration being normalized (Table I).

TABLE I
GSH AND MDA MODIFICATIONS IN THE STUDIED GROUPS.VALUES ARE MEAN ± SEM. STATISTICAL ANALYSES *- P<0.05;**- P<0.01;***- P<0.001, VS. W GROUP. #- P<0.05; ## - P<0.01;###- P<0.001 VS. AHT GROUP

	W	P	AHT	AHT+P
GSH (μMOLI/ ML)	7.42±0.19	7.86±0.28*	4.91±0.58***	6.88±0.28##
MDA (nmol/ml)	0	0	8,73 x 10-2***	6,92 x 10-2##

It should also be noted that the total cholesterol and triglycerides-lowering activity of *Sambucus nigra* extracts was found in the case of rats fed with standard, non-hypercholesterolemic diet supplemented with high doses of chokeberry anthocyanins for 4 weeks [20]. When comparing total cholesterol and LDL-col levels, the results show that these are significantly higher in the AHT group than in the W group.

There are significant improvements taking place against the dislipidemia ocurring in arterial hypertention as a result of the administration of polyphenols extracted from *Sambucus nigra* fruit. The serum LDL levels in the AHT+P group were kept within normal limits by the polyphenolic protection (Table II). From the viewpoint of the variability coefficient (%), the mean values obtained are typical of the series considered. Research comparing the AHT+P and AHT groups shows that the HDL cholesterol is significantly higher in the first group.

TABLE II
LIPID PROFILE IN THE STUDIED GROUPS.VALUES ARE MEAN ± SEM. STATISTICAL ANALYSES *- P<0.05;**- P<0.01;***- P<0.001 VS. W GROUP. #- P<0.05; ## - P<0.01;###- P<0.001 VS. AHT GROUP

Experimental groups	W	P	AHT	AHT+P
Ch-T (mg/dL)	73.41±4.56	66.22±1.72	95.3±6.74***	70.42±3.77###
TG (mg/dL)	86.53±6.62	71.54±6.66*	144±15.38***	95.77±22.85###
HDL-col (mg/dL)	34.21±4.36	33.16±3.62	21.74±4.88***	28.27±3.42##
LDL-col (mg/dL)	23.41±5.32	20.88±2.79	42.83±5.41***	27.12±7.36###

The systolic and diastolic blood pressures, as well as their calculated mean, were measured. *The Shapiro-Wilk test* was positive, which supports sample normality, and the descriptive statistics and box-and-whisker plots are shown in Fig. 1 and 2.

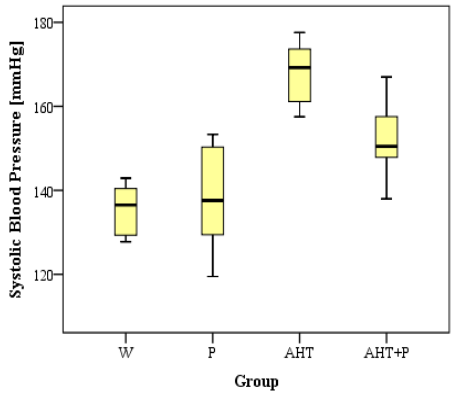

Fig. 1 The *box-and-whisker* plot of systolic blood pressure

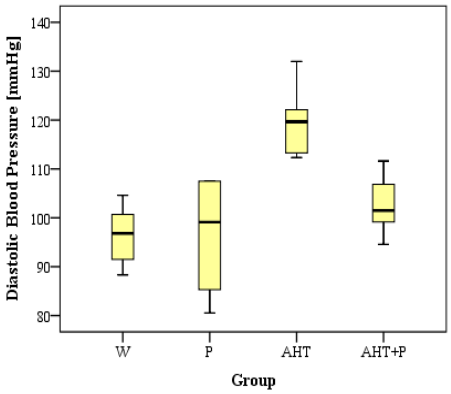

Fig. 2 The *box-and-whisker* plot of diastolic blood pressure

The Levene test confirmed group homoscedasticity, whereas the *ANOVA* test revealed a significant difference between the means of the 4 groups, as concerns systolic and diastolic blood pressure. All the measured blood pressure components revealed a bio-statistically significant (p<0.05) blood pressure drop between the AHT and the AHT+P groups (Table III).

TABLE III
ANOVA TEST

Blood pressure	*F value*	*p* value*
Sistolic	14.962	0.001
Diastolic	11.119	0.001
Mean	13.174	0.001

* $p<0.05$ indicates biostatistically significance

The understanding of endogenous mechanisms of hypertension by oxidative processes has advanced greatly in the last decade, yet the description of the molecular action of predisposing factors must be further elucidated to prevent and properly treat cardiovascular diseases.

IV. CONCLUSION

In the arterial hypertensive model the cardio-protective effects of the polyphenolic extract from *Sambucus nigra* are represented by the antioxidant, hypocolesterolemiant intervention. The results prove that oxidative stress is considerably lower, statistically speaking, in rats with hypertension but also provided with natural polyphenolic protection from *Sambucus nigra* fruits than in the rats belonging to the control group. In addition to the demonstrated antioxidant effects, natural polyphenols also have other biological properties that might contribute to the cardioprotective effects.

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