Evaluation of the Effects of a *Pinus Brutia* Bark Extract on Biochemical Parameters and Blood Pressure in an Experimental Arterial Hypertension

CATRINEL FLORENTINA PADURARU (GIURESCU BEDREAG)[1,2], NINA FILIP[3], ADRIANA TRIFAN[1], SORIN DAN MIRON[4], CODRUTA BADESCU[5]*, IRINA IULIANA COSTACHE[6]*, RAZAN AL NAMAT[6], BOGDAN DIACONESCU[7], NICOLETA DUMITRESCU[7], MANUELA CIOCOIU[7]

[1]Grigore T. Popa University of Medicine and Pharmacy, Faculty of Pharmacy, Discipline of Pharmacognosy, 16 Universitatii Str., 700115, Iasi, Romania

[2]Commercial Society for Medicinal Plant Research and Processing Plantavorel - Piatra Neamt, 46 Cuza Voda Str., 610019, Piatra Neamt, Romania

[3]Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, Discipline of Biochemistry, 16 Universitatii Str., 700115, Iasi, Romania,

[4]Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, Discipline of Biophysics and Medical Physics 16 Universitatii Str., 700115, Iasi, Romania

[5]Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, 3rd Medical Department, 16 Universitatii Str., 700115, Iasi, Romania

[6]Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, 1st Medical Department, 16 Universitatii Str., 700115, Iasi, Romania

[7]Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, Department of Pathophysiology. 16 Universitatii Str., 700115, Iasi, Romania

The new hypertension therapies which are thought to improve the mechanisms impairing the target organs in arterial hypertension (AHT) would have great practical value. The aim of our study was to investigate the effects of Pinus brutia bark extract (EPb) on serum lipid profiles and oxidative stress in N(G)-Nitro-L-arginine-methyl ester (L-NAME)-induced hypertension. The experiment demonstrated that PbE improved lipid profile and reduced pro-oxidative effects of L-NAME, thus suggesting a possible role of the extract in the management of AHT. Systolic and diastolic blood pressure decrease was significant in the group undergoing simultaneous EPb extract and L-NAME therapy, as compared to the group that was administered only L-NAME. Due to its effects, the Pinus brutia bark extract may be used for the prophylaxis and as adjuvant therapy of cardiovascular conditions.

Keywords: Pinus Brutia bark extract, oxidative stress, experimental arterial hypertension

There are five species of the genus *Pinus*; *P. brutia Tenore* (Turkish pine), *P. halepensis Miller* (Aleppo pine), *P. nigra J.F. Arnold* (European black pine), *P. pinea L. (*stone pine, umbrella pine), and *P. sylvestris L.* (Scots pine). The bark of *Pinus brutia* consisted of 15 compounds: gallic acid, gentisic acid, protocatechuic acid, 4-hydroxy benzoic acid, catechin hydrate, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, myricetin, resveratrol, luteolin, naringenin, kaempferol. Major compound detected was catechin hydrate [1, 2]. The human body protects itself from the harmful effects of oxidative stress by means of a series of enzymatic (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase) and non-enzymatic (albumin, ceruloplasmin, ferritin, reduced glutathione, uric acid, lipoic acid, bilirubin, ascorbic acid, α-tocopherol, β-carotene) antioxidants. In physiological conditions, there is a balance between reactive oxygen species (ROS) production and the activity of the enzymatic and non-enzymatic antioxidant systems [3].

The determination of the level of oxidative stress and the activity of antioxidant systems is of particular importance since plasma markers of oxidative stress play a role in predicting coronary heart diseases. Many studies assess the total antioxidant status of plasma by determining the inhibitory capacity of an oxidative process at plasma level, whereas others determine the lipid peroxidation products [4, 5]. Intracellular and extracellular antioxidants, as well as cell membrane antioxidants, are designed to neutralize excess ROS and their formation. Total antioxidant status in plasma consists of the net effect of different antioxidants and of the interactions between them, and may be determined by spectrophotometry. There was a connection found between the initial antioxidant status in plasma and the independent risk factors for coronary heart diseases, and between the antioxidant status in plasma and specific oxidative stress markers, respectively [6, 7].

Our research emphasizes the effects of the polyphenolic extract from *Pinus brutia* bark extract (**EPb**) on biochemical parameters and blood pressure modifications. The experiment was performed on the arterial hypertension model.

Experimental model

Material and methods

The research was performed on Wistar white rats, with an average weight of 250-280 g, which were kept in individual cages, in a room maintained at 22 C with an alternating 12 h light-dark cycle and were divided into 4 groups of 12, namely: - Group **W** (martor) - control, normal

* email; codruta.badescu@gmail.com; irinaiulianacostache@yahoo.com

animals, that did not receive natural polyphenols; - Group **EPb**, animals received solution of *Pinus brutia* extract (PbE) dosage of 22.85 mg/kbw/day), p.o. (by tube feeding), at every 2 days, for 8 weeks; - Group **L-NAME**, treated with *N*(G)-nitro-l-arginine-methyl ester (L-NAME) 40 mg/kbw/day, i.p., at every 2 days, for 8 weeks; - Group **EPb + L-NAME**, animals received simultaneously PbE+L-NAME in the dosage mentioned p.o, at every 2 days, for 8 weeks.

The DL_{50} value of the **EPb** extract obtained from *Pinus brutia* bark was 228.51 mg/kg body weight, and the efficient dose used for later determinations was 1/10 of DL_{50} (22.85 mg/kgc). Serum total cholesterol, HDL-cholesterol and triglycerides were measured by enzymatic colorimetric methods on a TECAN micro plate reader by commercially available kits (Audit Diagnostics Ireland). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. The total antioxidant capacity was expressed in Trolox equivalents of testing animal serum using the colorimetric method developed by Rice-Evans and Miller. Blood pressure and heart rate were determined by non-invasive means, using a CODA4 device.

Results and discussions

According to our findings, the **EPb** extract increased the value of the total antioxidant status compared to the control (1.389 ± 0.04 mmol / L vs. 1.296 ± 0.1 mmol / L), while L-NAME caused its significant decrease (1.133 mmol / L). When the extract was delivered in combination with L-NAME, the decrease in the total antioxidant status was less significant (1.236 mmol / L). This proves the ability of the **EPb** extract to reduce the pro-oxidant effects of L-NAME, which confirms the remarkable antioxidant effects evidenced for this extract by *in vitro* studies [8, 9] (fig. 1).

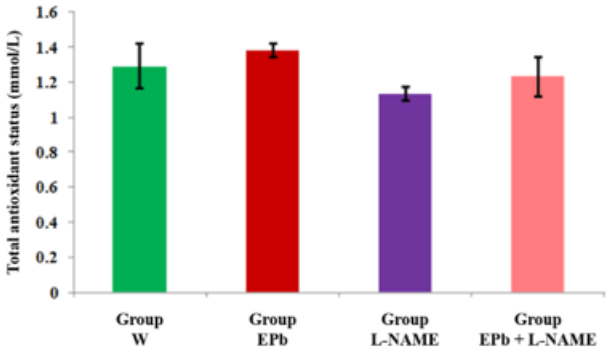

Fig. 1. Influence of EPb on the total antioxidant status

Hypercholesterolemia is a key factor in the triggering and progress of inflammatory cardiovascular diseases such as atherosclerosis. LDL-cholesterol therapy leads to a decrease in the incidence of major coronary and vascular events, thereby attenuating the evolution of the atherosclerotic process [10, 11]. After 8 weeks of treatment, the **EPb** extract caused the serum cholesterol to decrease by 9.21% compared to the control group, while L-NAME produced a 26.22% increase. The **EPb** extract administered together with L-NAME annihilated its hypercholesterolemia-decreasing effect, showing a 23.53% decrease in total cholesterol in this group compared to the group receiving only L-NAME.

L-NAME has a strong influence on the lipid profile. The findings of our research are in line with the findings of other studies, which have shown that L-NAME increases total cholesterol and lowers HDL levels in plasma; moreover, it reduces plasma fibrinogen, shortens prothrombin times, increases arterial blood pressure and plasma levels of certain cardiac necrosis markers (creatine kinase, troponin C) [9, 12]. The cholesterol level was significantly lower in the **EPb** extract and L-NAME group compared to the L-NAME group. The ability of the **EPb** extract to significantly reduce L-NAME-induced hypercholesterolemia is evident. It should be noted that the level of cholesterol in the group treated with **EPb** extract and L-NAME (69.20 ± 5.88 mg / dL) was close to that of the control group (71.7 ± 3.77 mg / dL) (table 1).

An elevated LDL-cholesterol lipoprotein fraction level is an important risk factor in the occurrence of atherosclerosis [13]. The LDL-cholesterol level in the L-NAME group was higher than that of the group treated with **EPb** extract and L-NAME. In contrast, the LDL-cholesterol levels increased significantly in the group treated with **EPb** extract and L-NAME compared to the control (table 1). The findings showed that after 8 weeks, LDL-cholesterol decreased by 3.54% in the group receiving **EPb** compared to the control group, while L-NAME produced a 102.52% increase. Administration of the extract concomitantly with L-NAME resulted in a decrease in the LDL-cholesterol level of about 11% as compared to the L-NAME group.

The HDL-cholesterol lipoprotein fraction has antioxidant properties and, therefore, it prevents LDL oxidation. The low blood levels of HDL-cholesterol significantly increase the risk of atherosclerosis and coronary heart disease [13]. The HDL-cholesterol level decreased significantly in the L-NAME group compared to the **EPb** and L-NAME group. A comparative analysis, with clear findings, may also be made between the control group (30.30 ± 1.12 mg / dL) and the group treated with **EPb** extract and L-NAME (24.20 ± 3.36 mg / dL). After 8 weeks of treatment, the group treated with **EPb** extract showed an increase in the HDL-cholesterol level of 10.23% compared to the control group, while the administration of L-NAME produced a decrease of 38.29%. In the group treated with **EPb** extract and L-NAME, the increase in HDL-cholesterol was 29.41% (table 1).

The hypotriglyceridemic effect is significant in the group treated with EPb extract and L-NAME as compared to the group treated only with L-NAME. However, the triglyceride level was higher in the EPb extract and L-NAME group compared to the control group. After 8 weeks of EPb extract treatment, triglycerides increased by 1.36% compared to the control group, while L-NAME produced an increase of 75.12% over the control group. When EPb extract was associated, the decrease was about 29%.

Group	Total cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)
W	71.70 ± 3.77	19.80 ± 3.04	30.30 ± 4.98	80.40 ± 6.05
EPb	65.10 ± 5.08	19.10 ± 3.54	33.40 ± 4.52	81.50 ± 8.01
L-NAME	90.5 ± 5.1	40.10 ± 3.31	18.70 ± 4.39	140.80 ± 17.66
EPb+L-NAME	69.20 ± 5.88	35.70 ± 7.88	24.20 ± 3.35	100.20 ± 7.25

Table 1
INFLUENCE OF EPb EXTRACT ON THE LIPID PROFILE

Group	Diastolic blood pressure (mmHg)	Systolic blood pressure (mmHg)	Heart rate (beats/min)
W	88.6 ± 6.8	121.05 ± 4.97	311.4 ± 36.51
EPb	87.8 ± 5.6	123.75 ± 6.75	315.9 ± 34.1
L-NAME	105.75 ± 7.86	152.2 ± 8.9	338.65 ± 43.39
EPb+L-NAME	93.10 ± 7.96	135.5 ± 8.2	324.75 ± 48.68

Table 2
INFLUENCE OF EPb EXTRACT ON ARTERIAL BLOOD PRESSURE AND HEART RATE

Arterial hypertension is the most important risk factor in the occurrence of cardiovascular diseases. It is well known that oxidative stress leads to endothelial dysfunction (imbalance between vasodilator and vasoconstrictor factors) [7, 11], and vascular remodeling, both processes being involved in the occurrence of arterial hypertension [14]. There were no statistically significant differences as concerns systolic and diastolic blood pressure between the EPb extract group and the control group.

While L-NAME increased diastolic blood pressure by 19.35% compared to the control, when administered together with L-NAME, the EPb extract significantly decreased its hypertensive effect, lowering diastolic blood pressure by about 12%. Similarly, with regard to systolic blood pressure, as one may notice, when administered together with L-NAME, the EPb extract significantly reduced its hypertensive effect and systolic blood pressure by approximately 11%. The EPb extract did not reduce arterial blood pressure in normotensive animals, but only in animals with L-NAME-induced hypertension. This suggests that the antihypertensive action of the EPb extract could be due, at least in part, to its antioxidant properties (table 2). As concerns heart rate, the EPb extract did not produce significant changes (table 2).

The Pearson correlation proves an indirect correlation between total antioxidant status and total cholesterol levels. Thus, in 70% of the animals, the low total antioxidant status values are correlated with elevated total cholesterol values ($r = - 0.703$, $R^2 = 0.494$, $p = 0.001$). A direct correlation is undeniable between total antioxidant status and HDL-cholesterol values, in which 53.8% of animals with elevated total antioxidant status values have elevated HDL-cholesterol values ($r = + 0.538$, $R^2 = 0.2897$, $p = 0.014$). Another indirect correlation between total antioxidant status and LDL-cholesterol values occurs in 63.2% of animals, where the low total antioxidant status values are correlated with elevated LDL-cholesterol values ($r = -0.632$, $R^2 = 0.399$, $p = 0.003$) (fig. 2). We found an indirect correlation between total antioxidant capacity and diastolic blood pressure values in 58.4% of the testing animals ($r = - 0.584$, $R^2 = 0.3406$; $p = 0.007$) and systolic blood pressure values, respectively, in 59.7% of the testing animals ($r = - 0.597$, $R^2 = 0.3568$, $p = 0.005$). There was no correlation between total antioxidant status and heart rate ($r = - 0.044$, $R^2 = 0.002$, $p = 0.852$) (fig. 3).

L-NAME is a potent inhibitor of NO synthase and implicitly of $NO^{\bullet}$ production. At the same time, L-NAME causes an increase in oxidative stress through various mechanisms: decrease of catalase and superoxide dismutase activity, intensification of lipid peroxidation processes [9]. The antihypertensive effects of polyphenols are mainly due to their antioxidant properties [15, 16].

Polyphenols induce endothelial nitric oxide synthase expression, increase intracellular glutathione levels and inhibit the activity of certain pro-oxidant enzymes (NADPH oxidase, xanthine oxidase) [17-19]. Many epidemiological studies have analyzed the correlation between the incidence of morbidity and mortality due to cardiovascular diseases and the high level of total cholesterol, LDL-cholesterol and lipoproteins (a) [10, 13]. The measurement of the cholesterol level and HDL-cholesterol and LDL-cholesterol levels allows specialists to estimate the risk of cardiovascular conditions. Hypertriglyceridemia is also a risk factor for coronary heart disease by highlighting the presence of certain triglyceride-rich lipoproteins that undergo partial degradation on very low density lipoproteins, VLDL, which are also atherogenic.

That is precisely the reason why the most important research directions are related to the investigation of protective capacity against ischemia-reperfusion injuries, as well as to the influence of polyphenolic extracts on the tone of the arterial wall; when a vasorelaxant action is

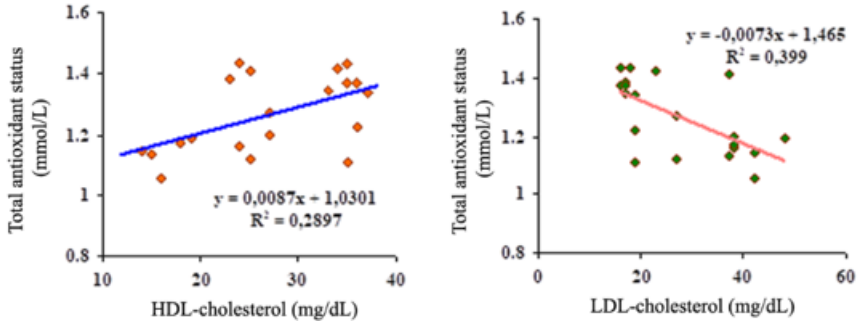

Fig. 2. Total antioxidant status in relation with lipid metabolism markers

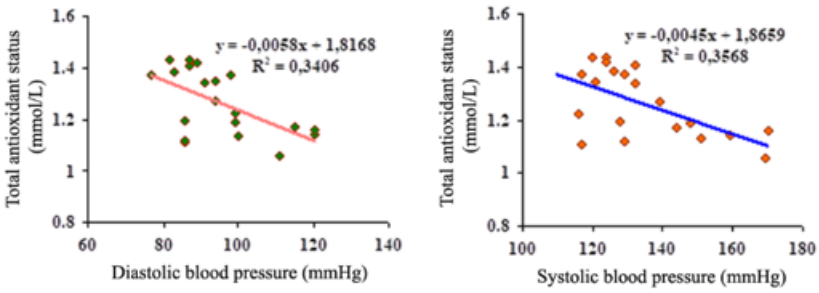

Fig. 3. Total antioxidant status in relation to blood pressure

detected, it would be useful to determine its type (endothelium-dependent or endothelium-independent relaxation).

Conclusions

Our experiment demonstrated that **EPb** improved lipid profile and reduced pro-oxidative effects of L-NAME, thus suggesting a possible role of the extract in the management of AHT. The *in vivo* study of the **EPb** extract revealed important antihypertensive effects in animals with induced hypertension. The decrease of systolic and diastolic blood pressure was significant in the group treated with **EPb** extract and L-NAME, as compared to the group that was administered only L-NAME. The findings justify the continuation of research in this direction, namely on the possible uses of extracts obtained in the prophylaxis and adjuvant therapy of cardiovascular diseases.

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Manuscript received: 13.06.2018