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Effects of high dose olive leaf extract on the hemodynamic and oxidative stress parameters in normotensive and spontaneously hypertensive rats

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Abstract: The antihypertensive activity of olive leaf extract (OLE), a natural antioxidant is recognized, but its influence on the cardiovascular system when administered in a high dose has not yet been investigated. The aim of the present study was to determine the acute effects of excessive intake of standardized OLE on blood pressure, heart rate and oxidative status in both spontaneously hypertensive rats and normotensive Wistar rats. The systolic arterial pressure and heart rate were measured using a tail-cuff and pneumatic pulse detector before and 60 and 120 min after intragastric OLE administration. The activities of catalase, glutathione peroxidase, superoxide dismutase (SOD) and glutathione reductase in erythrocytes, as well as lipid peroxidation in plasma (pTBARS) were measured spectrophotometrically at the same time points. A high-dose of OLE did not influence blood pressure, heart rate or pTBARS in normotensive rats, while the SOD, catalase and glutathione reductase activities were significantly increased. The same dose significantly decreased blood pressure in hypertensive rats, but increased the pTBARS and SOD activity. Excessive oral intake of OLE induced moderate hypotensive effects only in spontaneously hypertensive rats, suggesting the absence of harmful hemodynamic effects after an oral overdose in both rat strains. However, its pro-oxidative role when given in a high dose in hypertensive organisms should not be neglected.

Keywords: *Olea europaea* L.; hypertension; acute oral toxicity; spontaneously hypertensive rat; oxidative stress.

INTRODUCTION

Hypertension is the most important cardiovascular risk factor worldwide, contributing nearly 50 % to prevalent coronary heart disease and approximately

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70 % of prevalent cerebrovascular disease burdens.[1] It is widely known that dietary changes play an important role in managing blood pressure. A number of studies showed that the Mediterranean food pattern plays a significant role in the prevention of cardiovascular disease.[2] It was reported that olive oil represents a key healthy component of Mediterranean diet and that dietary intake of virgin olive oil, thanks to its minor constituents, exhibits cardio-protective effects.[3] Moreover, there is evidence that daily doses of blood pressure medication could be reduced during olive oil diet. This finding could be partly attributed to the polyphenols, which enhance nitric oxide (NO) concentrations and may help vasodilatation, which reduces blood pressure.[4]

The results of recent investigations suggested beneficial effects of phenolic-rich olive leaf extracts (OLE) as dietary supplements in modifying cardiovascular risk biomarkers, such as blood pressure, hyperglycemia, oxidative stress and inflammation, as well as in improving vascular function and lipid profiles.[5] Several preclinical studies confirmed the antihypertensive activity of OLE or its active constituents on different experimental models,[6,7] and the mechanisms of this action are continuously being studied. Moreover, the dual effect of OLE in both reducing blood pressure and improving lipid profile was presented as the result of clinical trials.[8,9] OLE contains large amounts of potentially useful phytochemicals, many of the same phenolics as in olive oil, but in much higher concentrations.[10] Its chemical content makes olive leaf one of the most potent natural antioxidants. The main constituent is oleuropein, an iridoide monoterpene. This compound, obtained by decoction of olive leaves, has been recognized as one of the constituents responsible for vasodilating activity on isolated rat aorta.[11] Moreover, as a constituent of aqueous OLE, it showed high angiotensin-converting-enzyme (ACE) inhibitor activity.[12] Furthermore, olive leaf contains triterpenes (oleanolic, ursolic and maslinic acid) with confirmed antihypertensive, anti-atherosclerotic and antioxidant activity on a Dahl salt-sensitive, insulin-resistant genetic model of hypertension.[13] Flavonoids, including luteolin, apigenin and quercetin, are also important antihypertensive components of OLE.[14,15] The beneficial properties of olive leaf are further enhanced by the good absorption of its phenolic constituents and their bioavailability, which is a necessary pre-condition for its bioactivity.[16]

Herbal medicines are classified as dietary supplements or foods rather than drugs and do not require approval by regulatory drug agencies to be marketed. Hence, their efficacy and potential toxicity is not evaluated thoroughly. Due to traditional use, there is general opinion that these products are safe and harmless. However, many of them previously used in other forms, a diluted tea for example, may now be available as concentrated and potent extract formulations and thus potentially harmful.[17] Based on the available safety/toxicity studies of total olive leaf extract and its constituents, and the history of the use of the components of

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the extract through table olives, olive products and olive oil, the consumption of OLE could be considered as safe.[18] A recent investigation showed that no report has indicated the negative effects of recommended, moderate doses of OLE over longer periods. However, there is an issue with many different commercial extracts of olive leaf since the composition of OLE in these products varies. There is data available that concerns the correct dosage and the side effects from long-term intake of mega doses of OLE. Recently, findings regarding the effects of different concentrations of OLE on the function of mice liver over the course of 14 weeks revealed that alanine aminotransferase and alkaline phosphatase serum enzyme activities increased significantly, and hepatic fibrosis was observed in the groups in which higher (0.5 and 0.75 %) OLE concentrations were used. All the groups exposed to OLE exhibited hyperplasia of the bile ducts, cholestasis, and hepatocyte necrosis.[19] It was also shown that feeding doses of 0.2–0.9 % of OLE to Wistar rats for a period of 6 weeks may induce hematological, biochemical as well as hepatocellular and renal abnormalities of experimental animals.[20] Hitherto, the effects of acute excessive intake of concentrated, oleuropein-rich OLE have not been seriously investigated, in particular effects on the cardiovascular system. Hence, the safety of high-dose intake of this "cardiovascular friendly" natural product has been quite neglected.

The aim of the present study was to investigate the acute *in vivo* blood pressure effects of high doses of this natural antihypertensive agent administered to spontaneously hypertensive and to normotensive rats. In addition, the effects of high-dose of this natural antioxidant on the plasma lipid peroxidation and antioxidant defense system in erythrocytes of the both strains of experimental rats were investigated.

EXPERIMENTAL

Chemicals

Olive leaf extract EFLA® 943, standardized to 18–26 % of oleuropein, was purchased from Frutarom Switzerland Ltd. (Wadenswil, Switzerland). The extract was manufactured from the dried leaves of *Olea europaea* L., applying an ethanol (80 vol. %) extraction procedure. After a patented filtration process (EFLA® Hyperpure), the crude extract was dried. The stability and microbiological purity of the extract were confirmed by the manufacturer. A comprehensive phytochemical analysis was performed previously.[21] In this study, the same batch of EFLA® 943 was used. It was kept in sealed microtubes, stored at room temperature and protected from light until use. All other chemicals used for biochemical analyses were obtained from Sigma (St Louis, MO, USA).

Animals

Male, adult, age matched spontaneously hypertensive rats (SHR) and Wistar (normotensive) rats, weighing about 280–330 g, were bred in the Institute for Medical Research, University of Belgrade. The rats were housed 4 in a cage under constant environmental conditions (20–24 °C; 12h light-dark cycle), and fed *ad libitum* with a standard chow for laboratory rats (Veterinary Institute, Subotica, Serbia).

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The experimental protocol was approved by the Ethical Committee of the Institute for Medical Research, University of Belgrade, Serbia (No. 0316-1/11) according to the National Law on Animal Welfare ("Official Gazette of RS" No. 6/10, in Serbian) that is consistent with guidelines for animal research and principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Official Daily N. L 358/1-358/6, 18, December 1986) and EU Directive on the protection of animals used for scientific purposes (Directive 2010/63).

Acute oral toxicity study

Acute oral toxicity test was run strictly in accordance with OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects: Test No. 423: Acute Oral toxicity – Acute Toxic Class Method, which was described in details in a recent publication.[22] Briefly, three male and three female young adult Wistar rats (ten weeks old, weight 190 to 230 g) were fasted 16 h before the experiment. A single dose of 2000 mg kg^{-1} b.w. of OLE was administered intragastrically (*i.g.*) through a metal gavage tube. Three hours after treatment, diet was available *ad libitum*. The rats were observed for abnormal behavioral signs, somnolence, dizziness, restlessness, neurological signs, respiratory distress or mortality, and weighed daily after administration of OLE. At the end of the test (day 14), the rats were sacrificed by cervical dislocation, and gross pathological changes in the main organs (brain, liver, kidney, spleen, gastric and intestinal mucosa) were evaluated.

Blood pressure and heart rate measurements

The systolic blood pressure and heart rate were indirectly measured using a tail-cuff, pneumatic pulse detector and a direct recorder (Physiograph Four, Narco Bio-System, Houston, TX, USA). Before the experiment, two separate baseline determinations of body weight and systolic blood pressure were made for all experimental animals over a span of 4 days and the rats were divided into six groups, three groups for each rat species (8 rats per group).

The cardiovascular effect of a high dose (2000 mg kg^{-1}, dissolved in 0.5 mL of tap water) of *i.g.*-administered OLE was investigated. The study was performed on six experimental groups according to strains and treatment they received as follows: W Control – Wistar rats received tap water (0.5 mL *i.g.*); WOLE60′ and WOLE120′ – Wistar rats in which the measurements of blood pressure, heart rate and oxidative stress parameters were performed 60 or 120 min after OLE administration, respectively; SH Control – spontaneously hypertensive rats (SHR) received tap water (0.5 mL *i.g.*); SHOLE60′ and SHOLE120′ – SHR received OLE and the measurement of hemodynamic and oxidative stress parameters were performed in 60 or 120 min after OLE administration, respectively. After cardiovascular measurement, animals were anaesthetized (35 mg kg^{-1} sodium pentobarbital; *i.p.*) and blood samples were collected immediately. At the end of the experiment, the animals were sacrificed by a pentobarbital overdose injection.

Biochemical measurements

Blood samples were centrifuged at 4 °C at 3000 rpm for 15 minutes and erythrocytes were separated. Hemoglobin (Hb) content was estimated by the method of Drabkin and Austin.[23]

All spectrophotometric analyses of the activities of the antioxidant enzymes in the plasma or erythrocytes were performed using an Ultrospec 3300 pro UV/Vis spectrophotometer (Amersham Biosciences Corp., USA).

The plasma thiobarbituric acid reactive substances (pTBARS), as a marker of plasma lipid peroxidation, were measured using 2-thiobarbituric acid (2,6-dihydrooxypyrimidine-2-

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-thiol; TBA). An extinction coefficient of 156,000 M^{-1} cm^{-1} was used for the calculation[24] and level of pTBARS is expressed as nmol mL^{-1} plasma.

The endogenous antioxidant status of the red blood cell was investigated in all experimental groups. The activity of catalase (CAT) was determined according to the procedure of Beutler by following the absorbance of hydrogen peroxide at 230 nm.[25] The activity of catalase is expressed as U g^{-1} of hemoglobin where one unit of CAT activity is defined as mmol H_2O_2 min^{-1}.

Glutathione reductase (GR) activity was estimated according to the method of Glatzle.[26] The GR activity is expressed as U g^{-1} hemoglobin, where one unit of GR activity is defined as (mmol oxidized NADPH) min^{-1} g^{-1} hemoglobin.

Glutathione peroxidase (GSH-Px) activity was determined according to the previously described method suggested by Paglia and Valentine.[27] One unit of GSH-Px activity is defined as (μmol oxidized NADPH) min^{-1}.

Activity of superoxide dismutase (SOD) was measured spectrophotometrically using a previously described method of epinephrine auto-oxidation[28] and is expressed as U g^{-1} Hb.

Statistical analysis

The data are given as mean ± *SEM*. One-way analysis of variance (ANOVA) was used for comparison between the experimental groups of the same rat strain, while the Fisher LSD test was performed for *post hoc* multiple comparisons. Comparison between normotensive Wistar control rats and control SHRs was made using Student's *t*-tests. *P* values less than 0.05 were considered as significant (Statistica 8.0 for Windows).

RESULTS

Acute oral toxicity

For the evaluation of acute oral toxicity of the OLE, a single dose (2000 mg kg^{-1}) was orally administered to Wistar rats. During the study period of two weeks, no death occurred in the treated animals. The body weight did not vary after drug administration, and the autoptic analysis failed to show appreciable macroscopic alterations of the internal organs. The absence of adverse effects at concentrations as high as 2000 mg kg^{-1} b.w. did not allow the calculation of the median lethal dose (LD_{50}) value. In principle, the method used is not intended to allow the calculation of a precise LD_{50} value. Exceptionally, and only when justified by specific regulatory needs, may the use of an additional upper dose level of 5000 mg kg^{-1} b.w. be considered. In accordance with OECD Guidelines and for concern of animal welfare, the testing of animals in GHS Category 5 ranges (2000–5000 mg kg^{-1}) is discouraged. Thus, the standardized OLE EFLA® 943 used in this study belongs to the so-called Category 5 or unclassified.

Hemodynamic and oxidative stress parameters after excessive oral intake of OLE

As expected, a significant difference ($P < 0.001$) was recorded in the systolic blood pressure and heart rate values between W Control and SHR Control (Fig. 1). Orally ingested OLE, even in high doses, did not influence the blood pressure of normotensive experimental animals. The same dose had reduced the blood pressure in SHR 60 and 120 min after the treatment by 20 and 13 %, respectively

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(Fig. 1A). The heart rate remained unaffected in the Wistar strain of rats. In the hypertensive rats, the heart rate was significantly higher in the SHOLE120′ than in the SHOLE60′ group, but there was no significant difference in comparison to the SH control group (Fig. 1B).

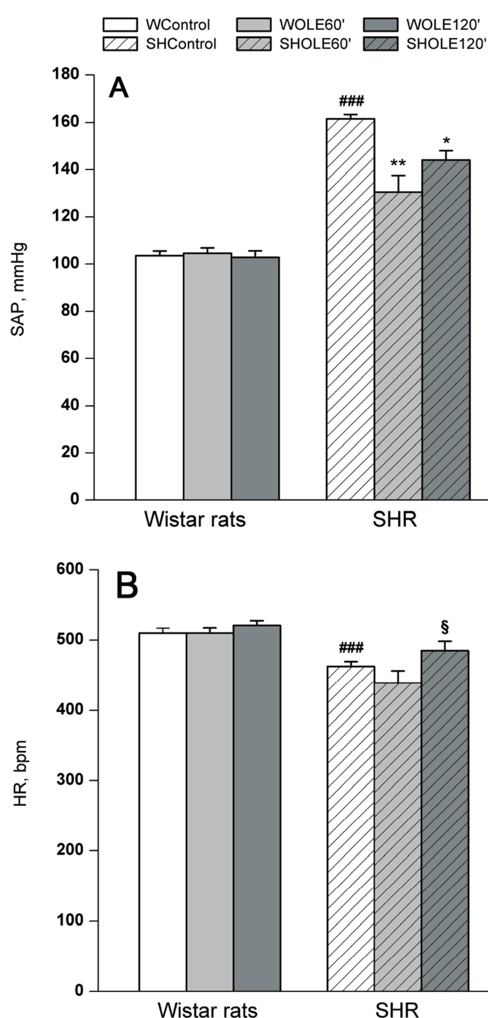

Fig. 1. Effect of *Olea europaea* L. leaf extract (OLE, 2000 mg kg^{-1}; *i.g.*) on: A) systolic arterial pressure (SAP, 1 mm Hg = = 133.3 Pa) and B) heart rate (HR) of normotensive Wistar (W) rats and spontaneously hypertensive rats (SHR). WControl – W rats treated with water; WOLE60′ – W rats, 60 min after OLE treatment; WOLE120′ – W rats, 120 min after OLE treatment; SHControl – SHR treated with water; SHOLE60′ – SHR, 60 min. after OLE treatment; SHOLE120′ – SHR, 120 min. after OLE treatment; *$P < 0.05$ and **$P < 0.01$, indicates statistical significance difference compared to the respective control; $^{###}P < 0.001$, the difference between SHControl and WControl groups; § indicates the difference ($P < 0.05$) between SHOLE120′ and SHOLE60′.

In normotensive rats, pTBARS levels were unchanged in both the OLE treated groups compared to the control. However, the level of lipid peroxidation in the plasma of hypertensive animals increased significantly ($P < 0.05$) after excessive oral intake of OLE at both the examined time points (Fig. 2).

The results of the determination of the activity of four anti-oxidative enzymes at three time points, before, 60 and 120 min after OLE administration, are pre-

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sented in Fig. 3. It is evident that activities of CAT and GR were significantly higher in the SH Control in comparison to the W Control group. After OLE administration to normotensive Wistar rats, the activities of CAT, SOD and GR (Fig. 3A, C and D) were significantly higher in comparison to the W Control group, while the activity of GSH-Px (Fig. 3B) was significantly lower. Administration of OLE did not influence the activities of CAT, GSH-Px and GR (Fig. 3A, B and D) in SHR, but the SOD activity was markedly increased 120 min after OLE treatment (Fig. 3C).

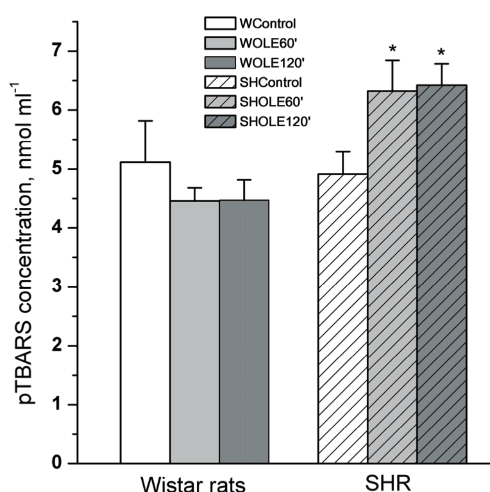

Fig. 2. Effects of *Olea europaea* L. leaf extract (OLE, 2000 mg kg^{-1}; *i.g.*) on the plasma concentration of thiobarbituric acid reactive substances (pTBARS) of normotensive Wistar (W) rats and spontaneously hypertensive rats (SHR). WControl – W rats treated with water; WOLE60′ – W rats, 60 min after OLE treatment; WOLE120′ – W rats 120 min after OLE treatment; SHControl – SHR treated with water; SHOLE60′ – SHR, 60 min after OLE treatment; SHOLE120′ – SHR, 120 min after OLE treatment; * indicates statistical significance ($P < 0.05$) of the difference compared to the respective control.

DISCUSSION

The purpose of this study was to evaluate the effects of excessive intake of standardized, oleuropein-rich olive leaf extract on blood pressure, heart rate and oxidative stress parameters in an established experimental model of genetically induced hypertension and in normotensive animals. To the best of our knowledge, this is the first *in vivo* study on the effects of a single high-dose OLE intake on lipid peroxidation and the activities of anti-oxidative enzymes in experimental hypertension. Thus, the results of this study represent a contribution to the overall safety assessment of *Olea europaea*-derived constituents as dietary supplements.

As a basic step in this study, OLE was assessed for acute toxicity using the OECD Test Guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method). The limit test was applied since data from the literature indicated that OLE is likely to be non-toxic. There are only few preclinical safety data for various olive leaf extracts and some incomplete toxicological data concerning the toxicity of oleuropein. The LD_{50} of an extract of olive leaf was not precise when it was given intraperitoneally (*i.p.*) as a single dose of 1300 mg kg^{-1} or as dose of 3000 mg kg^{-1}

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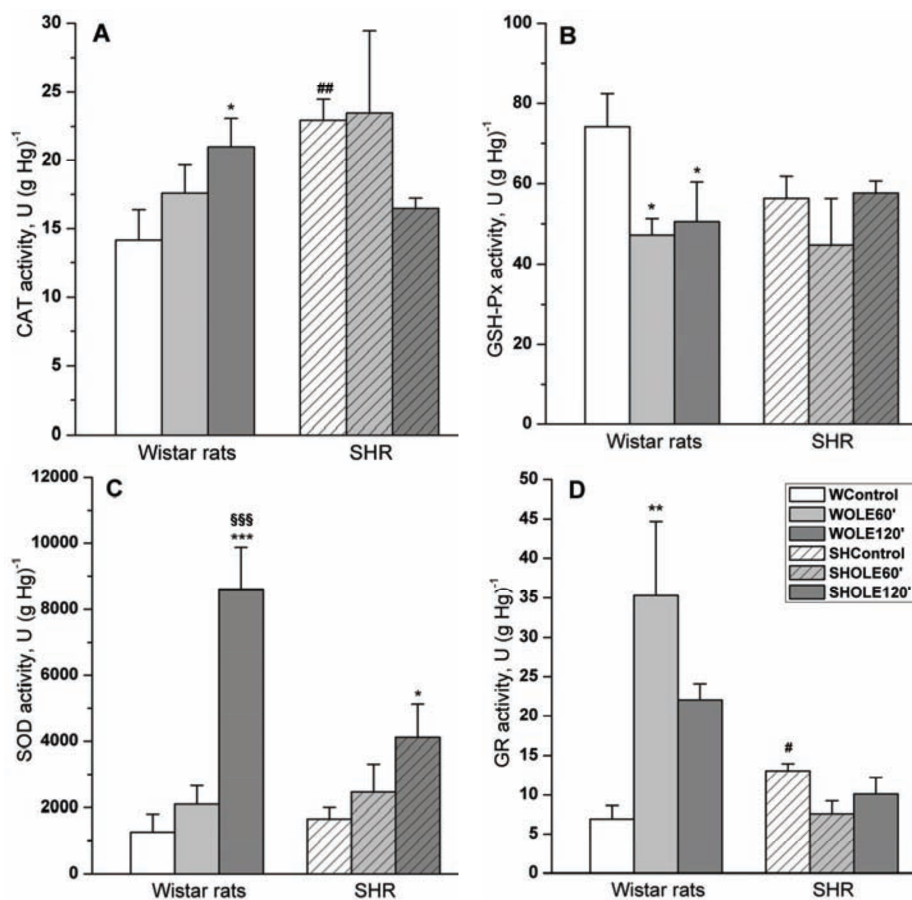

Fig. 3. Effect of *Olea europaea* L. leaf extract (OLE, 2000 mg kg^{-1}; *i.g.*) on: A) catalase (CAT), B) glutathione peroxidase (GSH-Px), C) superoxide dismutase (SOD) and D) glutathione reductase (GR) activity of normotensive Wistar (W) rats and spontaneously hypertensive rats (SHR). WControl – W rats treated with water; WOLE60′ – W rats, 60 min after OLE treatment; WOLE120′ – W rats, 120 min after OLE treatment; SHControl – SHR treated with water; SHOLE60′ – SHR, 60 min after OLE treatment; SHOLE120′ – SHR, 120 min after OLE treatment; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ indicates statistical significance difference in the enzyme activity compared to the respective control. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ represent the difference between SHControl and WControl groups; §§§ indicates the difference ($P < 0.001$) between WOLE120′ and WOLE60′.

orally in mouse.[29] The LD_{50} of a standardized aqueous olive pulp extract with hydroxytyrosol as the major constituent of biological significance was also reported to be greater than 2000 mg kg^{-1}. Moreover, in a subchronic study, the no-observed-adverse-effect-level (NOAEL) of the same extract in rats was found to be 2000 mg kg^{-1} day^{-1}. In developmental and reproductive toxicity studies, the studied extract did not cause toxicity at levels up to 2000 mg kg^{-1} daily.[18]

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The results of the present acute oral toxicity study are consistent with previous findings, and confirmed that OLE EFLA® 943 is a safe material when administered *via* oral gavage to rats in a single dose of 2000 mg kg^{-1}. Clinical signs and gross findings of treatment-related adverse effects were not observed in the experimental rats.

Different experimental models were used with the intention of investigating the hypotensive effect of total olive leaf extract. In a study by Khayyal *et al*., oral administration of OLE EFLA® 943 showed a dose dependent prophylactic effect against the rise in blood pressure induced by L-NAME.[6] Commercial *Olea europaea* L. leaf extract caused a concentration-dependent decrease in systolic left ventricular pressure and heart rate on isolated rabbit hearts.[7] Nevertheless, no information is available regarding its high dose activity in normotensive conditions and on an experimental model of genetically induced hypertension, such as SHR. Hence, the cardiovascular effect of excessive oral intake of OLE under hypertensive and normotensive conditions was the next step in the present study. It is known that oleuropein, the main component of OLE, is rapidly absorbed after oral administration, with maximum plasma concentration occurring after two hours.[30] According to this data, the tested time intervals 60 and 120 min after OLE oral administration were chosen to obtain more detailed information on the dynamic changes in the cardiovascular parameters and the oxidative stress rate in blood. The results obtained revealed that orally ingested OLE, even at the high dose of 2000 mg kg^{-1}, did not influence blood pressure and heart rate in normotensive experimental animals. The same dose reduced blood pressure by approximately 20 and 13 %, 60 and 120 min after the treatment, respectively. These findings could be explained by the dissimilar oxidative status in the normotensive and hypertensive strains of the studied rats. Namely, while multiple diverse factors likely contribute to the development of hypertension, the pathogenesis of this disease appears related, at least in part, to the development of a state of excessive oxidative stress. Local excessive superoxide production in the kidney, CNS, and vasculature, along with inflammatory activation, are central findings in hypertension models, including spontaneous hypertension in rats.[31,32] It was previously reported that either excess production of oxidants or a deficiency of antioxidant systems may contribute to high blood pressure and the endothelium-dependent impairment of vascular relaxation in SHR.[33] The results of the same study suggested that different antioxidants (ascorbic acid, aminotriazole and glutathione), administered *in vivo* as a single dose, significantly decreased blood pressure in SHR, but not in Wistar Kyoto rats. In a previous study, the high anti-oxidative potential of OLE EFLA® 943 was confirmed *in vitro* using 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$).[34] Additional findings suggested its strong anti-oxidative potential *in vivo*, in global cerebral ischemia and the reperfusion experimental model and in cold restraint stress-induced oxidative

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changes in rat liver.[34,35] According to these results and to the results from the present study, antihypertensive activity of OLE, at least partly, could be attributed to its anti-oxidative potential.

It was recently reported that extracts of *Olea europaea* L. leaves induced an increase in protein oxidation products in a concentration dependent manner.[36] The pro-oxidant activity of these plant extracts could be attributed to the unstable state of their phenoxyl radicals. Therefore, it was reasonable to expect pro-oxidative effects of the high-dose OLE intake in the present study. It is obvious from the obtained results that OLE did not cause changes in plasma lipid peroxidation of normotensive animals. This could be explained by effective anti-oxidative protection in normotensive rats. Namely, increased CAT activity in response to enhanced H_2O_2 production (due to intensive SOD activity) probably could be responsible for the unchanged pTBARS. The antioxidant status in the SH Control group was compromised in the present study, as in the study by Yuan *et al*.[37] The activities of catalase, which converts hydrogen peroxide into water and molecular oxygen, and glutathione reductase, which reduces the antioxidant glutathione from its oxidized to its reduced form, were significantly higher in SHR in comparison to the activities of these enzymes in normotensive Wistar rats before OLE administration. It is important to note that anti-oxidative enzymes have a bimodal behavior. In the short term, as was the case in some other experimental models,[34,35] their activity decreases after acute oxidative damage, while in the long term, as in the case of genetically induced hypertension,[13] it increases; in both cases, this may be an indicator of increased oxidative damage. Good oral bioavailability, good safety profile with limited side effects, efficacy in hypertension originating from disparate etiologies, as well as limited potential for their pro-oxidative role are the recognized conditions for an optimal profile of natural antioxidant agent for anti-hypertensive therapy.[38] Excessive oral intake of OLE reduced high blood pressure, but induced the lipid peroxidation process in the plasma of SHR, one and two hours after administration. In parallel, the activities of anti-oxidative enzymes, except SOD, remained unaffected in SHR.

According to the results obtained in this study, it could be stated that OLE, like other dietary phenols, may be beneficial in the correct dosage. It could be considered for antihypertensive therapy in appropriate doses only, and its pro-oxidative role when given in high dose (which could not be effectively buffered by increased SOD activity) in hypertensive organism should not be neglected.

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ИЗВОД

ЕФЕКТИ ВИСОКЕ ДОЗЕ ЕКСТРАКТА ЛИСТА МАСЛИНЕ НА ХЕМОДИНАМСКЕ И ПАРАМЕТРЕ ОКСИДАТИВНОГ СТРЕСА КОД НОРМОТЕНЗИВНИХ И СПОНТАНО ХИПЕРТЕНЗИВНИХ ПАЦОВА

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Антихипертензивно дејство природног антиоксиданса, екстракта листа маслине (OLE) је познато, али до сада није испитиван његов утицај на кардиоваскуларни систем када се примењује у високој дози. Наш циљ је био да одредимо акутне ефекте прекомерног уноса стандардизованог екстракта на крвни притисак, срчану фреквенцију и оксидативни статус како код пацова са урођеном хипертензијом, тако и код Вистар пацова са нормалним артеријским притиском. Систолни притисак и фреквенција рада срца мерени су помоћу уређаја за индиректно регистровање крвног притиска у репној артерији, пре интрагастричног апликовања OLE, 60 и 120 min након давања. У истим временским тачкама, спектрофотометријски су мерене активности каталазе, глутатион-пероксидазе, супероксид дисмутазе (SOD) и глутатион редуктазе у еритроцитима, као и липидна пероксидација у плазми (pTBARS). OLE у дози од 2000 mg kg^{-1} није утицао на крвни притисак, срчану фреквенцију и pTBARS код нормотензивних пацова, док су се активности SOD, каталазе и глутатион-редуктазе значајно повећале. Иста доза довела је до значајног смањења крвног притиска код хипертензивних животиња, али је повећала вредности pTBARS и активност SOD. Унос OLE у дози од 2000 mg kg^{-1} изазвао је умерен хипотензивни ефекат само код пацова са спонтаном хипертензијом, указујући на одсуство штетних хемодинамских дејстава након предозирања код оба соја пацова, али не би требало занемарити прооксидативно дејство његових високих доза у хипертензивном организму.

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