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ARTICLE

Protectivity of Blue Honeysuckle Extract against Oxidative Human Endothelial Cells and Rat Hepatocyte Damage

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The effect of *Lonicera caerulea* L. (blue honeysuckle) phenolic fraction (18.5% anthocyanins) on cell viability and against oxidative damage in low density lipoproteins (oxLDL), in rat microsomes and in primary cultures of rat hepatocytes and human umbilical vein endothelial cells (HUVEC), was tested. The phenolic fraction was nontoxic to rat hepatocytes and HUVEC at tested concentrations (1−1000 μg/mL) and time intervals up to 24 h inclusive. Phenolic fraction inhibited rat liver microsome peroxidation, induced by *tert*-butyl hydroperoxide (*t*BH), with IC_{50} values of 160 ± 20 μg/mL. The fraction at 0.5, 1.0, and 2.0 μg/mL delayed LDL oxidation, induced by Cu^{2+}, by 130 ± 20%, 200 ± 30%, and 400 ± 10%, respectively. The treatment of HUVEC with oxidatively modified LDL induced an increase in lactate dehydrogenase (LDH) leakage and thiobarbituric acid reactive substances (TBARS) formation, and resulted in lower formazan formation from 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) uptake, most pronounced for 200 μg/mL (24 h oxidation) after 2 h of incubation. The protective effect of the phenolic fraction against cell damage caused by oxLDL was noted at 0.1 μg/mL for HUVEC and against *t*BH at 1000 μg/mL for both HUVEC and hepatocytes. The observed protective effects were probably due to the antioxidant properties of *L. caerulea* constituents, mainly anthocyanins. Microsome peroxidation and LDL oxidation inhibition results provide promising perspectives into the prevention of some oxidative stress-associated diseases. Other data are important in in vitro systems but seem to be accidental in vivo.

KEYWORDS: *Lonicera caerulea*; cytotoxicity; LDL; HUVEC; rat hepatocytes; microsomes; phenolic fraction; anthocyanins

INTRODUCTION

In recent years, a large number of studies have investigated the therapeutic effects of fruits in the prevention of a range of diseases, and there is increasing interest in herbal medicine products. Berries constitute one of the most important sources of potential health supporting phytochemicals in the human diet. They are a rich source of ascorbic acid and phenolic compounds, in particular. Their biological activities include antitumorigenic (*1*), antimicrobial (*2*), antiinflammatory-allergic (*3*), and antimutagenic (*4*).

Lonicera caerulea L. (blue honeysuckle) is a member of the Caprifoliaceae family and is also known as blue honeysuckle, honeyberry, edible honeysuckle, or sweet berry honeysuckle. *Lonicera* is native to Russia (Kamchatka Peninsula, Siberia), North Eastern Asia, and Japan but is unknown as an edible berry in Europe and North America (*5*). The berries contain dry matter, saccharides, lipids, proteins, organic acids, and polyphenols as major components and ascorbic acid, vitamin B, magnesium, phosphorus, calcium, and potassium as minor compounds. The phenolic fraction (0.40%) of *L. caerulea* berries contains 20.1% of polyphenolics, 18.5% of which are anthocyanins (glycosides of cyanidin, delphinidin, and pelargonidin), 0.9% flavonoids (quercetin and its glycosides, epicatechin and apigenin), and 0.7% phenolic acids (chlorogenic, caffeic, ferulic, protocatechuic, gentisic, rosmarinic, and vanillic). Phenolic compounds, including anthocyanins are known strong antioxidants (*6*−*8*).

All cells in the body are exposed to oxidants from both endogenous and exogenous sources. Oxidation of lipids, nucleic acids, and proteins, if uncontrolled, can contribute to the etiology of the development of several chronic diseases. For example, oxidatively modified low density lipoprotein has been hypothesized to be a causative agent in the development of cardiovascular diseases (*9*). Nutrients comprise an important aspect of the antioxidant defense system which humans have evolved. This is the reason why a large body of research has investigated the potential role of antioxidant nutrients in the prevention of chronic diseases (*9*).

Blue honeysuckle has been used as food for centuries because of its high nutritive value. Reports on their biological activity are scarce. Apart from composition and antioxidant activity (*10*), the suppression of ocular inflammation in endotoxin-induced uveitis in rats (*11*) has been the only aspect of *L. caerulea* biological activity published up until very recently. The protective effect of

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L. caerulea phenolic fraction against UVA-induced damage to human keratinocytes due to its scavenging activity was studied recently in our laboratory (*12*). We have therefore investigated the effect of the phenolic fraction against oxidative damage to rat liver microsomes, low density lipoproteins, rat hepatocytes, and human umbilical vein endothelial cells in vitro.

MATERIALS AND METHODS

Reagents. *t*BH (70% in water), TBA (98%), trypan blue for cell cultures, Williams' medium E, MTT, NADPH, Tris (99%), BHA, bovine serum, and additives were purchased from Sigma-Aldrich Ltd., Czech Republic, DMEM/F12 and additives from Gibco Invitrogen, Czech Republic, collagenase from Sevapharma, Czech Republic, saccharose from BioBasic, Canada, and EDTA (97%) from Fluka, Czech Republic. Other chemicals and solvents were of analytical grade from Pliva-Lachema, Czech Republic.

Plant Material. The phenolic fraction was prepared as follows: *L. caerulea* berries (4.84 kg) were grown in Central Moravia (Czech Republic, 2007). The fruits were harvested manually over a total period of 6 h and frozen 1 h after harvest at −20 °C. Frozen berry fruits were extracted with an aqueous solution of phosphoric acid (0.1%; v/v; 50 °C) on a battery of percolators for 14 h. The obtained primary extract was purified on a column packed with nonionic polystyrene−divinylbenzene resin Sepabeads SP 207 (Mitsubishi Chemical, Japan). The column was washed with deionized water, and the absorbed organic compounds were desorbed by ethanol. The ethanol extract was then concentrated by evaporation (20.3 g). The process is described in more detail in the CZ patent 29 28 34 (*13*).

Animals. The study and obtaining rat livers, hepatocytes, and microsomes were approved by the Ethics Committee, Ministry of Education, Czech Republic and conducted in compliance with the Experimental Animals Protection Act No. 167/1993 L.C. Male Wistar rats (n=5; 340 ± 10 g) were purchased from BioTest Ltd., Konarovice, Czech Republic. The rats were acclimatized 1 week before the experiment. They were kept in plastic cages containing dust-free sawdust, two animals per cage. The conditions in the animal room were as follows: temperature (23 ± 2 °C; checked daily); relative humidity (30−70%); light/dark cycle 12 h/12 h. The animals had free access to a standard laboratory diet (KrmiMo s.r.o., Tetcice, CZ) and water, and the health of the animals was checked daily.

Rat Liver Microsomes. To obtain liver tissue, the rats were sacrificed under total anesthesia (*14*). The liver was excised immediately and stored at −80 °C for later use. The tissue was thawed at 4 °C and mixed with ice-cold isolation buffer (3 mM Tris/HCl; 0.25 M sacharose; 0.1 mM EDTA) to prepare 20% homogenate. After homogenization, the mixture was centrifuged (15 min; 800*g*; 4 °C) to separate nuclei, tissue residues, and corpuscles. The supernatant was ultracentrifuged (ultracentrifuge Optima; Beckman Instruments, USA; 20 min; 10000*g*; 4 °C) to separate mitochondria. The supernatant was then ultracentrifuged (60 min; 105000*g*; 4 °C) to obtain microsomes. The pellet was resuspended in 50 mM Tris/HCl containing 1 mM EDTA and 100 mM KCl, and ultracentrifugation was repeated. The acquired microsomal pellet was resuspended in 0.5 mL of 1.15% KCl and diluted in 50 mM Tris/HCl containing 0.1 mM EDTA and stored at −80 °C. The protein content was determined according to Bradford (*15*). Microsomes (200 μg/mL in phosphate buffered saline; PBS) were incubated (1 h; 37 °C) in the presence of the phenolic fraction (0−1000 μg/mL; PBS) with or without *tert*-butyl hydroperoxide (*t*BH; 10 mM). Lipid peroxidation was evaluated as the amount of the thiobarbituric acid reactive substances (TBARS) at 532 nm using a spectrophotometer (Sunrise Remote; Tecan, Austria) (*16*). The effect of the phenolic fraction was expressed as IC_{50} ± SD.

Low Density Lipoproteins (LDL). LDL were isolated from fresh human serum by stepwise ultracentrifugation (*17*). Plasma was first adjusted to a density of 1.006 g/mL (0.09 M NaCl; 1 mM EDTA) and centrifuged (11 h; 45000*g*; 10 °C). The infranatant was then adjusted to a density of 1.063 g/mL (0.38 M KBr; 1 mM EDTA) and centrifuged again (12 h; 45000*g*; 10 °C) to obtain LDL. The protein content was determined by BCA assay (*18*).

For testing the antioxidative properties of the phenolic fraction, LDL (100 μg/mL; PBS) were incubated in the presence of 0.0, 0.5, 1.0, and 2.0 μg/mL phenolic fraction or 5 μM ascorbic acid in PBS. LDL oxidation, induced by 10 μM $CuSO_4$ (*19*), was monitored spectrophotometrically (UV−vis spectrophotometer UV-2401PC; Shimadzu, Japan) as the formation of conjugated diene lipid hydroperoxides at 37 °C. The resistance of LDL to oxidation was estimated in terms of the length of the lag phase.

For application to the cell cultures, EDTA and diffusible low molecular mass compounds were removed from the LDL by gel filtration on a PD-10 Sephadex G-25 M gel. The protein content was determined by BCA assay. Separated LDL were diluted to a concentration of 0, 1, 5, 10, 25, 50, 100, and 200 μg/mL and treated with 10 μM $CuSO_4$ for 2, 4, 8, or 24 h at 37 °C. The oxidation was terminated by addition of 100 μM EDTA and 200 μM BHA, and these solutions were applied to the cells.

Rat Hepatocyte Primary Cultures. Rat hepatocytes were isolated by two-step collagenase perfusion of rat liver (*20*). Liver was removed from the enterocoele after intraperitoneal anesthesia (Xylazin 2%−32 mg/kg and Narkamon 5%−200 mg/kg) and portal vein cannulation. The liver was washed for 4 min with Hanks buffer I with EGTA and 6 min with Hanks buffer II with collagenase and Ca^{2+}. After shaking, the cell suspension was filtered and washed three times with centrifugation buffer (160 mmol/L NaCl, 3.09 mmol/L KCl, 0.69 mmol/L Na_2HPO_4, 32.7 mmol/L HEPES, 6.75 mmol/L $CaCl_2$) and centrifuged (2 min/50 g). After the last centrifugation, the cell suspension was washed with Krebs−Henseleit buffer. Cell viability was determined by measuring trypan blue exclusion. Yields of 2−4 × 10^8 cells/liver, with a viability greater than 80%, were routinely obtained. The hepatocytes were then dispersed in sterile conditions in Williams' medium E supplemented with penicillin (10 IU/mL), streptomycin (0.1 mg/mL), glutamine (2 mmol/L), and bovine serum (10%), and then cultivated in collagen-coated 12-well dishes in a humidified atmosphere of 5% CO_2 and 37 °C. After 4 h of culture stabilization, the cultivation medium was replaced by a serum-free one, and the tested sample (final concentration 0−1000 μg/mL) was added to the incubation medium.

For the toxicity studies, the hepatocyte monolayers were incubated with the phenolic fraction for 4, 6, 24, and 48 h, and the quality of the culture was controlled using the following parameters: cell viability−MTT (*21*) and activity of released lactate dehydrogenase (LDH) test (*22*).

To study the cytoprotective effects against *t*BH-induced damage, the primary cultures were preincubated with the phenolic fraction (0−1000 μg/mL) for 30 min. After that, cells were intoxicated with *t*BH for 1.5 h (final concentration 0.5 mmol/L) without media removal. The quality of the culture was monitored by cell viability (MTT test, LDH assay, and TBARS formation). All of the methods were adapted for measurement in microtiter plates.

Human Umbilical Vein Endothelial Cells (HUVEC). HUVEC were obtained from human umbilical cords of healthy nonsmoking women (18−35 years) at the Department of Obstetrics and Gynecology, University Hospital in Olomouc. All volunteers had to fulfill requirements for study submission and sign the personal awareness approbation. The choice of volunteers was in accord with the principles of the International Ethics Committee for Biomedical Research (CIOMS, Geneva 1993), and the study was approved by the Ethic Committee of the University Hospital and the Faculty of Medicine and Dentistry, Palacký University in Olomouc, Czech Republic. After delivery, a part of the umbilical cord (minimum 100 mm) was cut off and placed in sterile Earle's Balanced Salt supplemented with antibiotics at 4 °C. Endothelial cells were isolated by collagenase digestion up to 20 h after removal. The umbilical cord was washed with EBS and infused with a solution of collagenase in EBS. After incubation (30 min, 37 °C), the cord was washed with Hanks buffer without Ca^{2+} or Mg^{2+}, and the cells were cultivated in Endothelial Cell Basal Medium with growth factors (Promocell) and used at passages 2−6. After dissociation, the cells were collected and cultured on gelatin-coated culture dishes in DMEM/F12 until confluence.

To determine the effect of the phenolic fraction and oxLDL, HUVEC were incubated for 2, 4, 8, and 24 h in the presence of 0−1000 μg/mL of phenolic fraction or 0−200 μg/mL of oxLDL. At the end of the stimulation, mitochondrial dehydrogenase activity was used as an index of cell viability and was assessed using the MTT test. LDH leakage was determined to test the loss of plasma membrane integrity (LDH assay).

To evaluate the cytoprotective effect against *t*BH induced damage, HUVEC were intoxicated by *t*BH for 1.5 h (0.5 mmol/L) after preincubation with the phenolic fraction (0−1000 μg/mL) for 30 min. The quality of the culture was monitored by an MTT test and an LDH assay.

To determine the cytoprotectivity of the phenolic fraction against oxLDL-induced cytotoxicity, HUVEC were first incubated with the

phenolic fraction (0−1000 μg/mL) for 2 h and then stimulated with oxLDL (200 μg/mL; 24 h oxidation) for 2 h. The potential protective effect of the phenolic fraction against oxidation was determined by MTT and LDH assays and by the level of lipoperoxidation products as TBARS.

Statistics. Data are expressed as means ± SD. Three independent experiments were performed with three replicates for each sample. Statistical analysis was performed using Student's *t*-test for dependent samples. Differences were considered statistically significant when $*p$ was < 0.05.

RESULTS

Lipid Peroxidation of Rat Liver Microsomes. Rat liver microsomes were prepared by fractional centrifugation and were incubated for 1 h at 37 °C in PBS in the presence of a phenolic fraction (0−1000 μg/mL) and *t*BH (10 mM). Lipid peroxidation was evaluated by the formation of TBARS at 532 nm as malondialdehyde. The phenolic fraction showed significant dose-dependent protective activity in rat hepatocyte microsomes oxidatively damaged by *t*BH with IC_{50} values of 160 ± 20 μg/mL (**Figure 1**). The values above a concentration of 500 μg/mL were superior to 100% effectivity (data not shown).

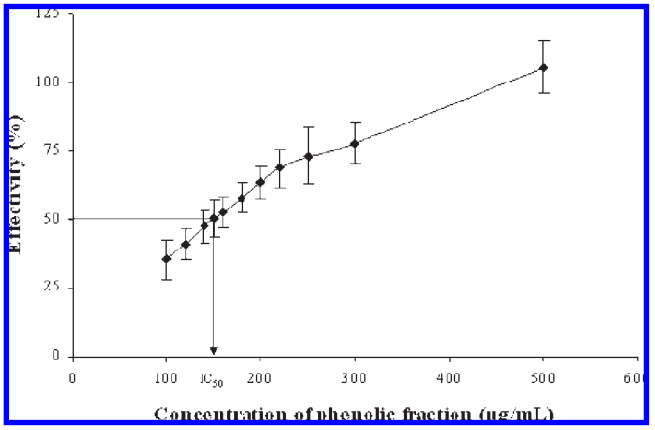

Figure 1. Lipid peroxidation of rat liver microsomes. Rat liver microsomes were incubated for 1 h at 37 °C in PBS in the presence of phenolic fraction (0−1000 μg/mL) and *t*BH (10 mM). Lipid peroxidation was monitored spectrophotometrically as TBARS formation. The phenolic fraction showed significant dose-dependent protective activity with IC_{50} values of 160 ± 20 μg/mL. The values above the concentration of 500 μg/mL are superior to 100% efficiency.

Oxidation of Human LDL. LDLs (100 μg/mL; PBS) isolated from human serum by stepwise ultracentrifugation were treated with different concentrations of the phenolic fraction. The resistance of LDL to oxidation was estimated as the length of the lag phase. The phenolic fraction (0.5, 1.0, and 2.0 μg/mL) delayed the Cu^{2+}-induced LDL oxidation in a dose-dependent manner by 130 ± 20%, 200 ± 30%, and 400 ± 10%, respectively. The elongation of the lag phase by ascorbic acid (5 μM = 880 μg/mL), used as a model compound, was 150 ± 20% (**Figure 2**).

Cytotoxicity. The cytotoxicity of the phenolic fraction was determined in the rat hepatocyte and human umbilical vein endothelial cell primary culture models. The phenolic fraction of *L. caerulea* at all tested concentrations (0−1000 μg/mL) had no influence on either the ability of the cells to reduce MTT to violet colored formazan or cell wall permeability, expressed as LDH leakage into medium after 4, 6, 24, and 48 h of incubation (data not shown).

Cytoprotectivity. In a typical nontreated rat hepatocyte primary cell culture, the MTT test gave $A_{540\ nm} = 0.86 \pm 0.07$, and the activity of LDH was 1.22 ± 0.09 μkat/L. After intoxication with *t*BH, these values changed to 0.100 ± 0.006 (MTT) and 5.45 ± 0.11 μkat/L (LDH). In the cell culture preincubated with 1000 μg/mL of phenolic fraction before *t*BH application, $A_{540\ nm} = 0.300 \pm 0.02$, and LDH activity = 4.05 ± 0.37 μkat/L.

In the case of HUVEC, the MTT test gave $A_{540\ nm} = 0.136 \pm 0.002$, and the activity of LDH was 0.31 ± 0.01 μkat/L. After intoxication with *t*BH, these values changed to 0.039 ± 0.004 (MTT) and 2.41 ± 0.19 μkat/L (LDH). The phenolic fraction at 1000 μg/mL restored the control values to 0.4900 ± 0.0005 (MTT) and 1.38 ± 0.06 μkat/L.

For comparison of the phenolic fraction activity at various concentrations in the cell cultures, we expressed the cytoprotective effect as % of nontreated control (**Figure 3**).

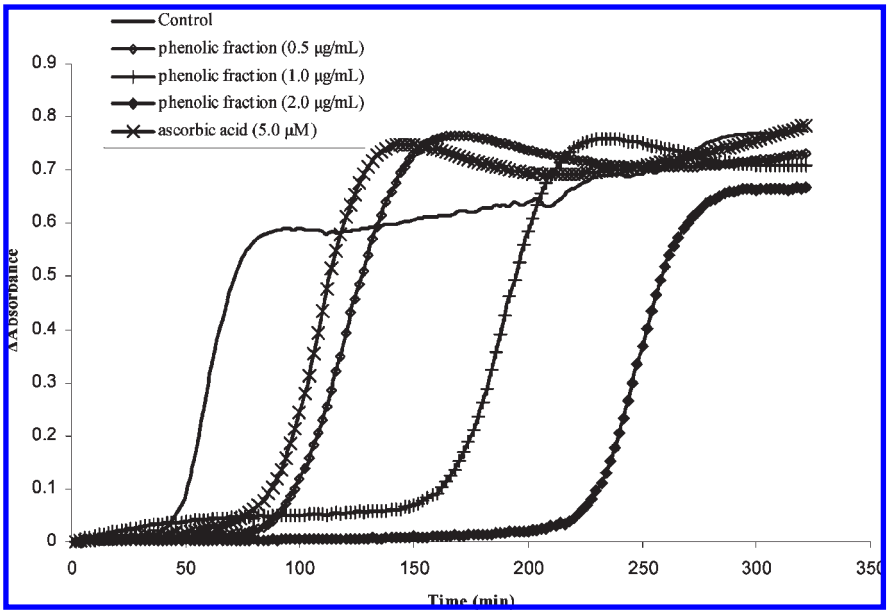

Figure 2. Cu^{2+}-induced LDL oxidation. The protective effect of phenolic fraction 0.5, 1.0, and 2.0 μg/mL and 5 μM ascorbic acid against LDL oxidation was monitored spectrophotometrically as the formation of conjugated diene lipid hydroperoxides at 37 °C. The resistance of LDL to oxidation was estimated in terms of the length of the lag phase. Representative results from 1 of 18 independent experiments are shown.

Cytoprotectivity of the Phenolic Fraction and oxLDL on HUVEC. LDL (200 μg/mL) were oxidized by Cu^{2+} for 2, 4, 8, and 24 h. The HUVEC were then treated with the oxLDL for 2, 4, 8, and 24 h. Treatment of HUVEC with oxLDL induced an increase in LDH leakage and a decrease in formazan formation from MTT and NR uptake (data not shown). The results of the MTT correlated well with results of NR. The toxicity effect was most pronounced for the concentration 200 μg/mL (24 h oxidation) and 2 h of oxidation. The time of incubation had only a small effect. **Figure 4** shows the example of 2 h of incubation. Native LDL had no detrimental effect on HUVEC (data not shown).

In the second series of experiments, the HUVEC were pretreated with different concentrations of the phenolic fraction (0−1000 μg/mL) for 2 h and then treated with oxLDL (200 μg/mL, 24 h oxidation) for 2 h. The protective effect against cell damage caused by oxLDL was registered by the LDH and MTT assay at the phenolic fraction concentration of 0.1 μg/mL. The TBARS formation is also positively influenced but not significantly (**Figure 5**).

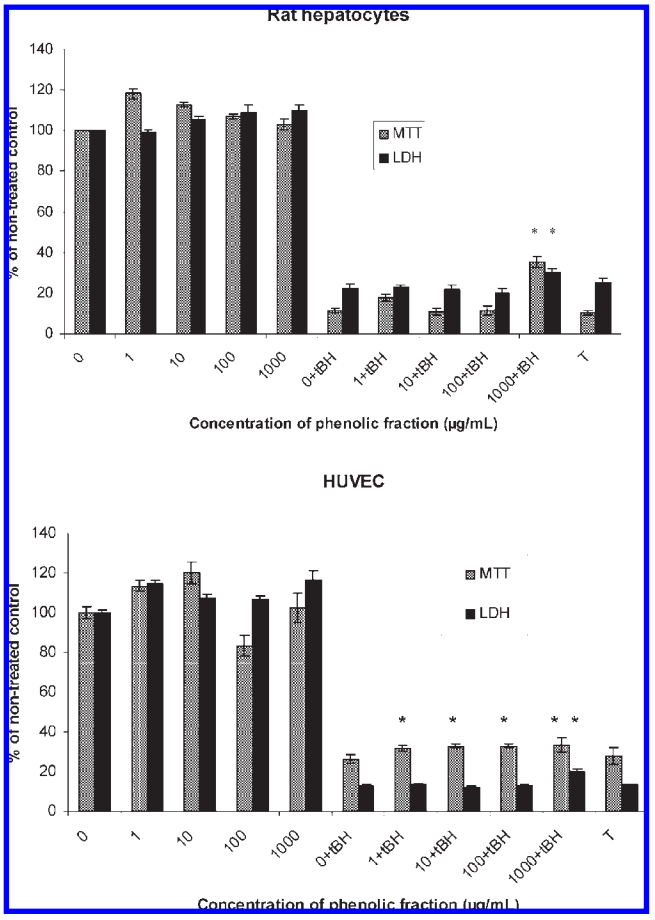

Figure 3. Protective effect of the phenolic fraction on *t*BH-induced damage of rat hepatocytes and HUVEC. After 30 min of preincubation with tested fractions, the cell monolayers were treated with *t*BH (0.5 mmol/L) during 1.5 h. Results are expressed as means ± SD, $n = 4$; cytoprotective effects are expressed as % of nontreated cells. Significant differences from *t*BH intoxicated controls (*$p < 0.05$) are indicated; T, triton.

DISCUSSION

These days, the use of in vitro subcellular and cellular model systems is irreplaceable for the determination of the biological activity, toxic properties, and metabolic mechanisms of xenobiotics (*23*). In this study, we chose two different primary cell cultures that model main human organs in contact with xenobiotics. The first cellular model was hepatocytes since the liver is the main organ responsible for metabolizing xenobiotics and the first target for the potentially toxic actions of these compounds.

The second cell culture was HUVEC given that all compounds and their metabolites are transported through cell walls into target tissues. Endothelial cell function is tied with plasma lipoproteins, especially low-density lipoproteins. Over the past decade, evidence that oxidized LDL play a critical role in atherosclerosis has been steadily accumulating (*24*). We chose oxidative modification by copper since oxidation of LDL using metal ions is the most widely used model for in vitro oxidative modification of lipoproteins. Exposure of HUVEC to LDL oxidized to varying degrees shows the effect of these particles on the cell wall and the development of vein injury.

Various xenobiotics are used to determine the protective effects of natural substances. For liver alteration using *t*BH and for HUVEC impairment, Cu^{2+}-induced LDL damage is an example of the most widely used models. *t*BH and oxLDL particles induce oxidative attack by oxygen free radicals (*25*, *26*).

Plant extracts containing a number of components may act against this kind of damage in different ways both in plants and humans. In this sense, polyphenols play a crucial role. Our phenolic fraction of *L. caerulea* berries contains 20.1% phenolic compounds and previously displayed Folin−Ciocalteau reagent reducing (335 ± 15 μg of gallic acid equivalent/mg), DPPH and superoxide scavenging activity (IC_{50} 12.1 ± 0.1 and 115.5 ± 6.4 μg/mL) (*10*).

In the present work, we extended the testing first to liver microsomes and low density lipoproteins. In the case of microsomes oxidatively damaged by *t*BH, the phenolic fraction showed significant dose-dependent protective activity with IC_{50} values of

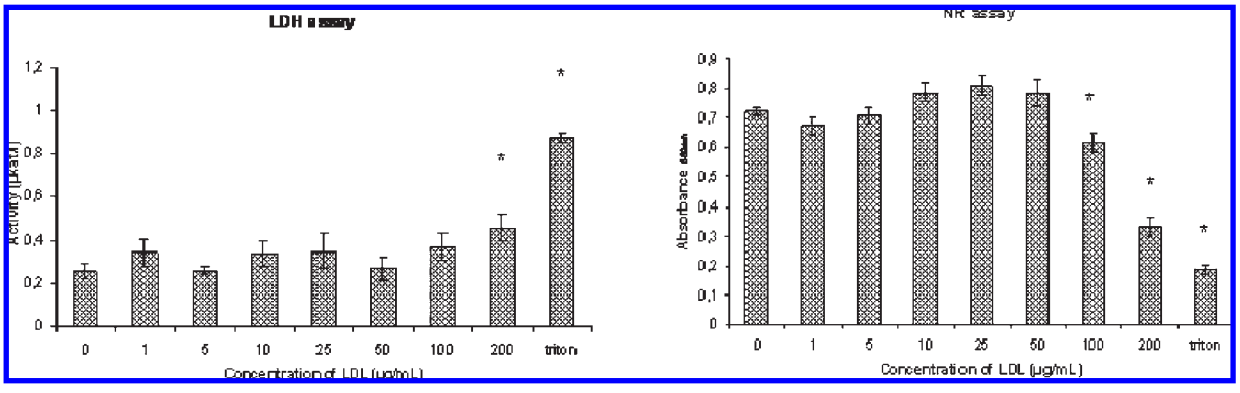

Figure 4. The effect of oxLDL to oxidative damage of HUVEC. LDL (200 μg/mL) were oxidized by Cu^{2+} for 24 pph and HUVEC were then treated with the oxLDL for 24 h. Treatment of HUVEC with oxLDL induced an increase in LDH leakage and decrease in NR uptake.

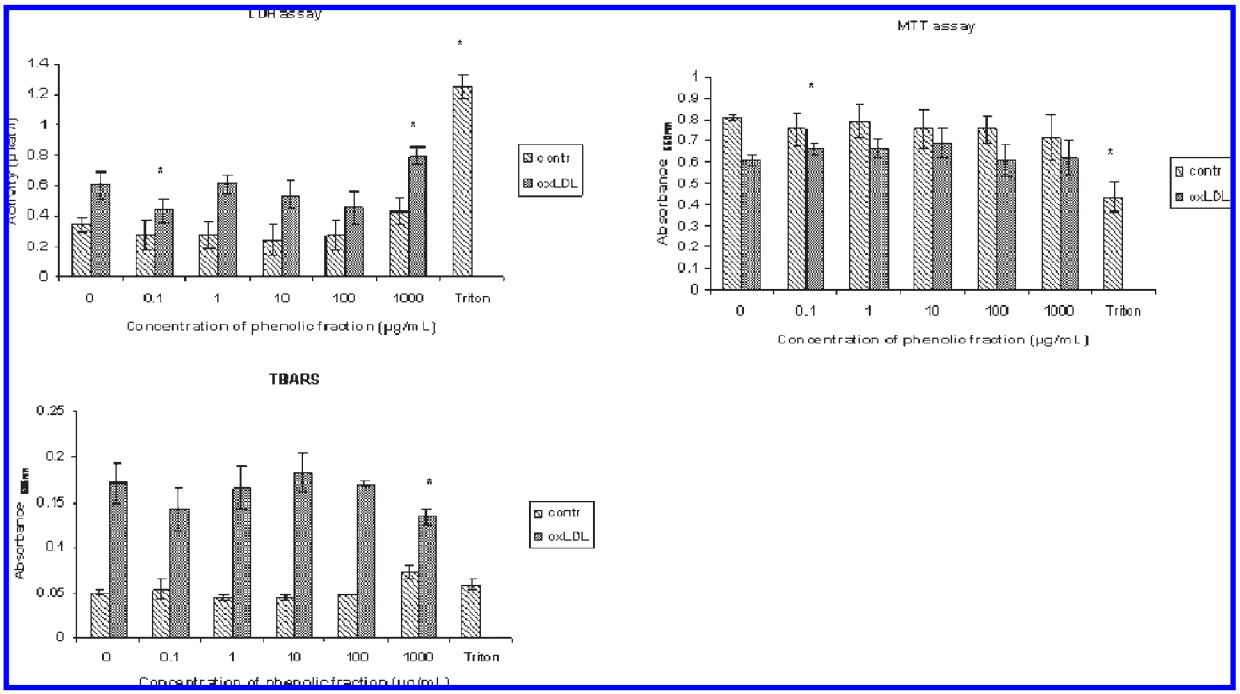

Figure 5. Phenolic fraction effect on oxLDL-damaged HUVEC. HUVEC were pretreated with the phenolic fraction (0−1000 μg/mL) for 2 h and then treated with oxLDL (200 μg/mL; 24h of oxidation) for 2 h. The concentration 0.1 μg/mL significantly influenced the LDH decrease and MTT increase and insignificantly influenced the TBARS formation decrease. The phenolic fraction at the highest concentration provides confusing data. Significant differences from LDL intoxicated controls (*$p < 0.05$) are indicated.

160 ± 20 μg/mL (**Figure 1**). In the case of Cu^{2+}-induced oxidation of LDL, the phenolic fraction showed the same trend, and in terms of length of the lag phase, it inhibited the damage in a dose-dependent manner (**Figure 2**). In these two experiments, the phenolic fraction concentrations were very low, and hence, the results may give support to the beneficial effect of the *L. caerulea* phenolic fraction to human health.

The effects of *L. caerulea* phenolic fraction on cell cultures were determined in rat hepatocytes and human umbilical vein endothelial cells by MTT test and LDH assay after 4, 6, 24, and 48 h of incubation. The phenolic fraction was nontoxic to both cell cultures at all tested concentrations and time intervals (**Figure 3**).

Then the protective effect of *L. caerulea* polyphenols against oxidative damage of the cells was studied. Compared to control cells, *t*BH-treated rat hepatocytes pretreated with phenolic fraction displayed larger amounts of formazan after the MTT viability test and lower levels of LDH in the culture medium. In HUVEC, the effect of the phenolic fraction was much greater. However, these results are significant only at the highest phenolic fraction concentration, which is unfortunately not reachable in human plasma (*27*). Hence, this effect seems to be important only in vitro. To the best of our knowledge, the protective activities of *L. caerulea* phenolic fraction in rat hepatocytes and HUVEC have not been determined before. Earlier studies have shown that *L. caerulea* extract significantly suppresses ocular inflammation in endotoxin-induced uveitis in a dose-dependent manner in rats (*11*). The possible mechanisms for this effect include the ability of *L. caerulea* polyphenols to inhibit the activation of NF-κB and subsequent production of proinflammatory mediators such as TNF-α, PGE_2, and NO (*11*). *L. caerulea* phenolic fraction has also shown protective effects against UVA-induced oxidative damage to human keratinocytes (*12*), and recently, an aqueous extract of the berries reduced the tumor volume when administered continuously during the tumor growth and development stages in Wistar rats (*28*).

To study protective activity of *L. caerulea* phenolic fraction against oxidative damage, the reaction of oxLDL particles with human endothelial cells was initiated. HUVEC were treated with oxLDL (200 μg/mL; 24 h) for 2 h, which induced an increase in LDH leakage and decreased formazan formation from MTT uptake. The protective effect of phenolic fraction pretreatment (2 h) was investigated by the LDH, MTT assay, and TBARS formation. This test provided contradictory results. The phenolic fraction concentration of 0.1 μg/mL displayed cells protection, which results from the LDL leakage decrease, MTT increase (significant), and TBARS formation decrease (insignificant). The effect of the highest concentration (1000 μg/mL) was confusing because it increased LDH leakage but decreased TBARS formation. Nevertheless, this high concentration is usually not achievable in the human body. The mechanisms of polyphenolic compound action against cardiovascular diseases include the protection of endothelial cells against apoptosis (*29*). Jeong et al. (*30*) suggest that flavonoids may differentially prevent Cu^{2+}-oxidized LDL-induced apoptosis and promote cell survival as potent antioxidants.

Overall, the results confirm the possible protective beneficial effect of *L. caerulea* against oxidative damage to microsomes, low density lipoproteins, and human umbilical vein endothelial cells at the concentrations affordable in human body. Futher investigation is under way.

ABBREVIATIONS USED

BCA, bicinchoninic acid; BHA, butyl hydroxyanisole; EDTA, ethylendiaminetetraacetic acid; HUVEC, human umbilical vein endothelial cells; LDH, lactate dehydrogenase; LDL, low density lipoproteins; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; NADPH, nicotine amide adenine dinucleotide phosphate; oxLDL, oxidized low density lipoproteins; PBS, phosphate buffered saline; SPE, solid phase extraction; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid

reactive substances; *t*BH, *tert*-butyl hydroperoxide; Tris, tris-(hydroxymethyl)aminomethane; μLC/MS, microliquid chromatography/mass spectrometry.

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