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Hypolipidemic Effects of *Citrus bergamia* Risso et Poiteau Juice in Rats Fed a Hypercholesterolemic Diet

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Citrus bergamia Risso et Poiteau fruits have been traditionally utilized, in Calabria (Italy), as a popular remedy for their hypolipidemic properties. *C. bergamia* juice total phenol content (2474.35 ± 38 μg/mL) was evaluated by the Folin–Ciocalteu method; moreover, HPLC analysis led to the identification of naringin (520 ppm), neoeriocitrin (370 ppm), and neohesperidin (310 ppm). The present study was designed to investigate the hypolipidemic effects of *C. bergamia* juice and its protective effect on liver of hyperlipidemic rats. Chronic administration of *C. bergamia* (1 mL/rat/day) provoked a significant reduction in serum cholesterol, triglycerides, and low-density lipoprotein (LDL) levels and an increase in high-density lipoprotein (HDL) levels; moreover, histopathological observations showed, in rats submitted to *C. bergamia* treatment, a protection of hepatic parenchyma. In addition, fecal neutral sterols and fecal bile acid excretion was found to be increased after *C. bergamia* treatment. These results suggest that the hypocholesterolemic effect of *C. bergamia* may be mediated by the increase in fecal neutral sterols and total bile acids excretion. In addition to the hypolipidemic effect, the juice shows radical scavenging activity in the diphenylpicrylhydrazyl (DPPH) test; probably the two effects are related. These observations suggest that the positive intake of *C. bergamia* may reduce the risk of some cardiovascular diseases through its radical scavenging function and hypocholesterolemic action.

KEYWORDS: ***Citrus bergamia*** **juice; flavonoids; hypolipidemic activity; fecal bile acids; DPPH test**

INTRODUCTION

Citrus bergamia Risso et Poiteau (bergamot) is a small tree belonging to the family Rutaceae. The fruit is pale yellow, pyriform, and 7.5–10 cm in diameter (*1*).

C. bergamia appeared in southern Italy before 1700 and is defined as a hybrid of *Citrus aurantium* L. and *Citrus limon* L. Burm. Fil., by some authors, or of *C. aurantium* L. and *Citrus aurantifolia* (Christm.) Swing. by others (*2*).

To date, 95% of worldwide bergamot production occurs in the Ionic area of Calabria (Italy), where soil characteristics and pH (<6.5–7.5) are particularly suitable for its cultivation (*3*). Particularly, the province of Reggio Calabria yields 55% of the production, and the remaining part comes from the Calabrian Ionic coast (*4*). The annual Italian production of bergamot amounts to 25000 tons (*5*).

C. bergamia fruit is used mostly for the extraction of its essential oil from the peel, which is widely used in the cosmetic, pharmaceutical, and food industries (*5*). *C. bergamia* juice, obtained from the endocarp after essential oils extraction, is considered to be just a secondary and discarded product (*6*).

Recently, some authors have reported information on the flavonoid composition of *C. bergamia* juice (*7, 8*). It is well-known that neoeriocitrin, naringin, and neohesperidin are the flavanone-*O*-glycosides to be found in the highest amounts in bergamot juice (257.0–295.8, 248.1–274.6, and 206.6–235.7 mg/L, respectively) (*8*). The same authors have identified another eight flavonoids, lucenin-2, stellarin-2, isovitexin, scoparin, orientin 4′-methyl ether, rhoifolin 4′-*O*-glucoside, chrysoeriol 7-*O*-neohesperidoside-4′-*O*-glucoside, and chrysoeriol 7-*O*-neohesperidoside, in bergamot juice (*8*).

Bergamot juice, moreover, is characterized by the high content of fatty acids constituting 79.50% of total acids [oleic

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acid (33.98%), linoleic acid (24.00%), and palmitic acid (15.34%)], by an acidity value of 0.710 g/L, and by a total sugar content of 72.760 g/L (*2*).

Previous studies have shown that a chronic administration of some *Citrus* species fruit juice positively influences plasma lipid levels and can be associated with reduced risk of coronary heart disease (*9, 10*). Literature data show that the hypolipidemic effects can be correlated to several components of *Citrus* juices, such as flavonoids (naringin and hesperidin), pectins, and ascorbic acid, which have a high antioxidant potential and interfere with cholesterol metabolism (*10–12*).

Few studies have focused on the biological activities in *C. bergamia* juice; in Calabria the fruit juice has been traditionally utilized as a popular remedy for its hypolipidemic properties (*6*). However, the scientific basis for this use in folk therapy has not been examined. The present study was designed to investigate the hypolipidemic effects of *C. bergamia* juice and its protective effect on liver of hyperlipidemic rats.

MATERIALS AND METHODS

Plant Material. *C. bergamia* Risso et Poiteau fruits were collected from plants growing in a cultivation located in Mortara di Pellaro (Reggio Calabria, Italy).

Preparation of Juice. Bergamot juice was obtained, as a blend of three cultivars, 'Fantastico' (95%), 'Femminello' (5%), and 'Castagnaro' (5%), by hand-pressing; after it was filtered, small aliquots (25 mL) were stored at −20 °C and defrosted immediately before the experiments.

Determination of Total Phenolic Content. The phenol content was measured by using the Folin–Ciocalteu (reagent) method (*13*). One hundred microliters of *C. bergamia* juice, previously centrifuged (4 °C, 10 min, 3000 rpm), was mixed with 0.2 mL of Folin–Ciocalteu reagent, 2 mL of H_2O, and 1 mL of 15% Na_2CO_3, and the absorbance was measured at 765 nm, after 2 h of incubation at room temperature, with a model UV-1601 spectrophotometer (Shimadzu, Milan, Italy). All reagents were purchased from Sigma-Aldrich. Gallic acid was used as a standard, and the total phenols were expressed as micrograms of gallic acid equivalents per milliliter of juice. The value of total phenolic content was obtained as the average of three determinations, and the result is expressed as mean ± standard deviation (SD).

Determination of Flavonoids. The HPLC analysis of *C. bergamia* juice was performed using a Shimadzu HPLC system (Kyoto, Japan) equipped with an SCL-10Avp controller, two LC-10ADvp pumps, a DGU-14A degasser, and an SPD-M10vp diode array detector. The column used was a Luna C18 (250 × 4.6 mm i.d., 5 μm) purchased from Phenomenex (Torrance, CA). A mixture of water, acetonitrile, isopropanol, and formic acid (78:12:10:0.1, v/v) was used as a mobile phase in isocratic mode. The mobile phase flow rate was 0.8 mL min^{-1}. Sample volume injected was 20 μL of undiluted bergamot juice, centrifuged (3000 rpm) and filtered through 0.45 μm membrane filters before analysis. Data were acquired using a photodiode array detector in the range of 190–370 nm, and the chromatograms have been extracted at 283 nm. Time constant was 0.64 s and frequency 1.5625 Hz. Data acquisition was performed by Shimadzu LC solution software.

Pure standards (neoeriocitrin, naringin, neohesperidin) were purchased from Extrasynthese (Genay, France), and the flavonoids in the sample were identified by comparison of retention times and spectra for each peak with the corresponding flavonoid standard.

In addition, HPLC-ESI-MS analysis was carried out for further confirmation of peak identification. The HPLC conditions were the same as for HPLC-PDA analysis.

The following ESI-MS parameters were applied: mass spectral range, *m*/*z* 200–800; interval, 0.2 s; scan speed, 4000; nebulizer gas flow, 1.5 L/min; curved desolvation line (CDL) temperature, 300 °C; CDL voltage, –34 V; probe voltage, +5 kV; Q array (quadrupole array): scan; detection gain, +1.5 kV. MS data acquisition was performed by Shimadzu LabSolutions V2.04 software.

Free Radical Scavenging Activity. Radical scavenging activity was assayed according to the method of Ohinishi et al. (*14*). A methanol 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution (0.1 mM) was mixed with different concentrations of *C. bergamia* juice (100, 50, 10, 5 μL) previously centrifuged (4 °C, 10 min, 3000 rpm); the optical density change at 517 nm was measured for 4 min after the initial mixing with a model UV-1601 spectrophotometer (Shimadzu). The scavenging activity was measured as the decrease in absorbance of samples versus DPPH standard solution. Ascorbic acid (Sigma-Aldrich) was used as reference. The mean values were obtained from triplicate experiments. Radical scavenging activity was expressed as the inhibition percentage (*15*).

Hypolipidemic Activity. *Animals.* Male Wistar rats (Harlan Italy), weighing 180–200 g, were used for the experiment. The animals were kept in standardized conditions (temperature, 22 ± 2 °C; humidity, 60 ± 4%; natural light), with water ad libitum. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192), as well as with the EEC regulations (*Off. J. Eur. Communities* **1986**, *L 358*).

Treatment. The effects of *C. bergamia* juice on lipid metabolism were evaluated by diet-induced hypercholesterolemia experimental model in rat (*16*). The hypercholesterolemic diet (cholesterol, 2%; sodium cholate, 2%; vitamin mixture, 2%; oligoelements, 0.2%; salt mixture, 5.8%; coconut oil, 20%; cellulose, 4%; sucrose, 44%; casein, 5%; drakettprotein, 15%) was supplied by Altromin-Rieper (Bolzano-Italy).

The rats were divided into three groups of 10 animals each:

- Group I (normolipidemic controls) was kept on a standard diet (S. Morini, Mil rat GLP) for 30 days.
- Group II (hyperlipidemic controls) received the hypercholesterolemic diet for 30 days.
- Group III received the hypercholesterolemic diet for 30 days; from the 1st to the 30th day, each rat was administered by gavage with *C. bergamia* juice (1 mL/rat).

During the experiment, animals were weighed weekly, and 24 h food consumption was recorded daily. On day 29, rats were individually housed in metabolic cages and feces were collected for 24 h.

At the end of the study, the animals were fasted overnight; blood samples were collected from the carotid artery of the rats under light diethyl ether anesthesia, and serum was separated and stored at −20 °C until analyzed. Livers and aorta artery were removed and kept at −80 °C until microscopic observations. Fecal samples were collected and kept at −20 °C until analyzed.

Serum Lipids. Total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and triglycerides in serum samples were determined colorimetrically and enzymatically using commercial assay kits (BioSystems S.A., Barcelona, Spain). The atherogenic index (AI) was calculated by using the following equation: (total cholesterol – HDL-cholesterol)/HDL-cholesterol.

Microscopic Observation: Histology. Liver and aorta artery pieces for histological investigation (5 mm diameter) were fixed in neutralized 4% paraformaldehyde in 0.2% phosphate buffer for 6 h at 4 °C. The samples were dehydrated in graded ethanols and, finally, embedded in Bioplast (Biooptica, Milano, Italy). The liver sections (5 μm thick), obtained by a rotative microtome, were stained with hematoxylin-eosin. All samples were observed and photographed with Leica DMLD microscopy.

Fecal Neutral Sterols and Bile Acids Determination. Neutral sterols and bile acids in the fecal samples were extracted according to the method of Crowell and Macdonald (*17*). Feces were freeze–dried for 48 h, weighed, and ground into fine powder. To sample aliquots (200 mg) was added 1 mL of KOH solution, and the mixtures were saponified by autoclaving at 120 °C for 1 h; after the addition of 1 mL of NaCl solution, neutral sterols were extracted twice with ethyl ether (20 mL). The organic phases (upper phases) were pooled, evaporated with a rotary evaporator, and dried under nitrogen. The lower phase was acidified with 0.2 mL of concentrated HCl and extracted six times with ethyl ether (20 mL). The upper phases were pooled, evaporated with a rotary evaporator, and dried under nitrogen. The concentration of fecal neutral sterols was determined by gas chromatographic (GC) analysis. Before the GC-analysis, all samples were diluted 1:10 v/v in *n*-hexane, and α-cholestanol was added as internal standard to a final concentration of 500 ppm.

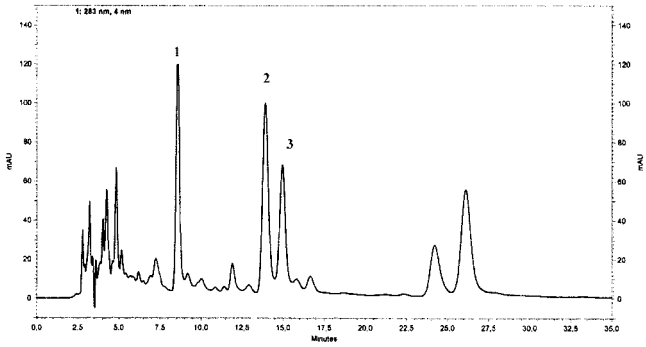

Figure 1. HPLC chromatogram of *C. bergamia* Risso et Poiteau juice at 283 nm. Peaks: 1, neoeriocitrin (370 ppm); 2, naringin (520 ppm); 3, neohesperidin (310 ppm).

The GC analysis of the sterols fraction was carried out using a Shimadzu GC-2010 gas chromatography system. The split/splitless injector was held at a temperature of 300 °C, and a split ratio of 1:10 was applied. Carrier gas was helium, at a constant linear velocity of 30.1 cm/s and a pressure of 130.5 kPa. All of the analyses were carried out on a 30 m × 0.25 mm i.d. × 0.25 μm d_f SLB-5 ms column (Supelco, Milan, Italy). The temperature was programmed as follows: 200 °C held for 5 min, then increased at 5 °C/min to 250 °C and at 2 °C/min to 300 °C. The FID temperature was set at 305 °C (sampling rate = 40 ms), and gas flows were 50 mL/min for hydrogen, 40 mL/min for makeup (N_2/air), and 400 mL/min for air, respectively. Data were collected by using GC solution software (Shimadzu).

The amount of fecal bile acids was determined using a commercially available enzymatic kit (Biomedis, Italy).

Statistical Analysis. Values of the indices investigated in vitro are given as mean ± SD of three determinations.

The data referred to the in vivo tests are reported as mean ± SD of 10 determinations; the results were statistically analyzed by Student's *t* test. Differences of $P < 0.05$, versus hyperlipidemic controls, were considered to be significant.

RESULTS

Determination of Total Phenolic Content. The total phenolic content of *C. bergamia* juice, evaluated by the Folin–Ciocalteu method, was 2474.35 ± 38 μg/mL.

Determination of Flavonoids. The flavonoid profile of *C. bergamia* juice is shown in **Figure 1**. The main flavonoids identified in the present study were (1) neoeriocitrin (370 ppm), (2) naringin (520 ppm), and (3) neohesperidin (310 ppm). Quantitative values were obtained as the average of triplicate analyses and were in accordance with those reported in the literature (*8*).

Free Radical Scavenging Activity. *C. bergamia* juice showed, at the concentrations assayed, radical scavenging effects on DPPH radical. The DPPH scavenging abilities of 0.1 mL of bergamot juice and of ascorbic acid at a concentration of 0.2 mg/mL were superimposable (**Figure 2**).

Hypolipidemic Activity. There was no significant difference in weekly mean body weight, in *C. bergamia* treatment, versus hyperlipidemic controls; moreover, no reduction in 24 h food consumption was observed (data not shown).

Serum Lipids. The administration of *C. bergamia* juice for 30 days provoked a significant reduction in serum levels of cholesterol (29.27%), triglycerides (46.12%), and LDL (51.72%) and an increase in HDL (27.61%) levels versus hyperlipidemic controls (**Figure 3**).

There was a significant decrease in AI in *C. bergamia*-treated rats. The AI was 1.09 ± 0.10 in the *C. bergamia*-treated group as compared with 3.09 ± 0.20 in the hyperlipidemic group ($P < 0.01$) (**Figure 4**).

Microscopic Observation: Histology. The hepatic parenchyma of normolipidemic controls (group I) shows a normal configuration both of hepatic architectural pattern of epithelial cells and of centrilobular vein; moreover, portal triad is well represented (**Figure 5A**).

In the liver sections of hyperlipidemic controls (group II) infiltrating macrophages and several vacuolated cells are evident in the whole lobule; around the centrilobular vein, large confluent lipidic droplets can be observed. In portal space, wide-caliber vessels are evident, which give place to an extensive perilobular vessels network. In the peripheral region of the lobule, hepatocytes with large confluent lipidic droplets are also present (**Figure 5B**).

After treatment with *C. bergamia* juice (group III), the lobule revealed a normal configuration and a reduction of degenerative lipidic droplets around the centrilobular vein. In both the middle and periferic zones of the lobule, the cytoplasm of hepatic cells is normal in appearance. Vascular elements of portal Triad show a normal architecture. Moreover, a reduction in the caliber of the vessels of perilobular network, in comparison with the hyperlipidemic controls, is evident. A decrease of infiltrating macrophages can also be seen (**Figure 5C**).

Morphological evaluation shows microscopic histological changes both in endothelial cells and within intimal tunica in hyperlipidemic rats compared to normolipidemic animals (**Figure 6A**,**B**). Edema in the wall of the vessel and degenerative changes of endothelium were reduced in aortic specimen of the *C. bergamia*-treated group compared to hyperlipidemic controls (**Figure 6C**).

Fecal Neutral Sterols and Bile Acids. Fecal output of total bile acids and neutral sterols was found to be enhanced, significantly, by 42.22 and 30.96%, respectively, in the *C. bergamia*-treated group compared to the hyperlipidemic group (**Figures 7** and **8**).

DISCUSSION

Atherosclerosis is the primary cause of heart disease and stroke. Epidemiological studies have revealed several important environmental and genetic risk factors associated with atherosclerosis, such as hypertension and diabetes mellitus. Hence, blood lipid content is probably the major determinant of the development of cardiovascular disease. Several authors have reported the hypolipidemic effects of *Citrus* juice (*10–12*); moreover, some studies suggest that a high dietary intake of orange or grape juice might reduce hypercholesterolemia, and this was postulated to be largely due to the principal *Citrus* flavanones, hesperidin from orange and naringenin from grapefruit, as their glycosides, hesperitin and naringin (*18*).

The current study demonstrates the *C. bergamia* juice modified serum lipid levels by reducing total cholesterol content in rats fed a hypercholesterolemic diet for 4 weeks. This diet, enriched in cholesterol (2%), sodium cholate (2%), and oil (20%), was used because the combination of these components in a diet is commonly employed to induce hyperlipidemia and aortic atherosclerosis lesion formation in rats and mice (*19*).

Chronic administration of *C. bergamia* juice for 30 days, at the dose of 1 mL/rat/day, provoked a significant reduction in serum levels of cholesterol, triglycerides, and LDL, and an increase in HDL levels versus hyperlipidemic controls.

The results of the diet intake, and body weight, after 4 weeks of feeding, showed no significant difference among all groups, suggesting that neither the hypercholesterolemic diet nor *C. bergamia* treatment had an adverse effect on the growth of rats. Moreover, the rats fed a hypercholesterolemic diet had a higher

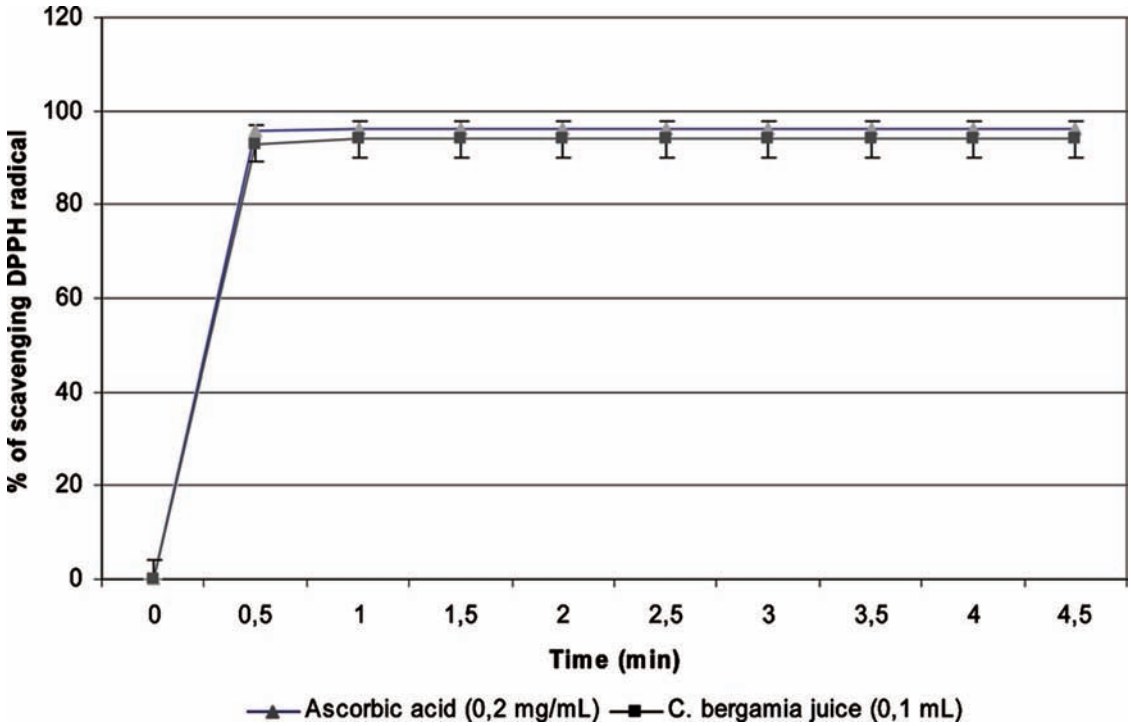

Figure 2. Kinetics of DPPH scavenging effects of *C. bergamia* juice.

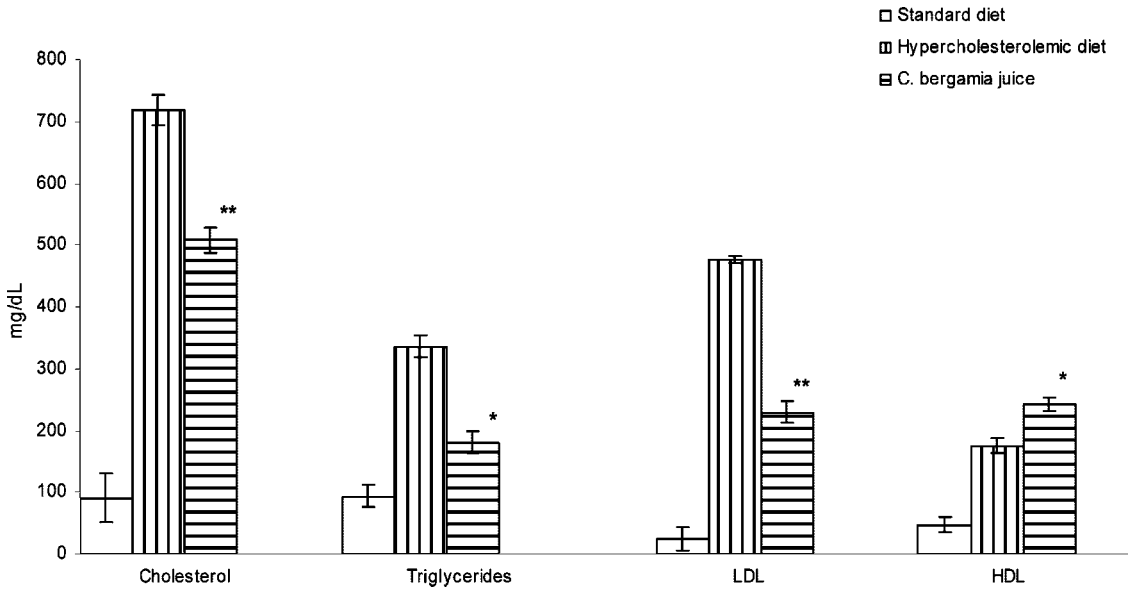

Figure 3. Effects of *C. bergamia* juice treatment on serum total cholesterol, LDL, triglycerides, and HDL levels of rats fed a hypercholesterolemic diet. Values are expressed as mean ± SD (vertical bars) for 10 animals per group. **, $P < 0.01$, and *, $P < 0.05$, represent significant differences when compared with the hyperlipidemic controls.

concentration of total cholesterol, triglycerides, and LDL in serum than those fed a standard diet, indicating that the hypercholesterolemic model was successfully established.

It is widely accepted that elevations in cholesterol and LDL plasma levels are major factors for coronary heart disease (*20*). The relationship between LDL and atherosclerosis and the suggestion that the pathological process could be reversed by reducing the serum LDL have been reported by several researchers (*21*). When there is excessive LDL in the blood, it is deposited in the blood vessel walls and becomes the major component of atherosclerosis plaque lesions.

In this study *C. bergamia* treatment reduces significantly cholesterol and LDL serum levels. These results indicated that *C. bergamia* might be a candidate to prevent atherosclerosis by lowering serum cholesterol and LDL levels.

Moreover, *C. bergamia* treatment increases, significantly, serum HDL levels. HDL is considered to be "good" cholesterol in the circulation; it, in fact, carries the cholesterol or cholesterol ester from peripheral tissues or cells to the liver for catabolism (*22*). This pathway plays a very important role in reducing the cholesterol levels in blood and peripheral tissue and inhibiting the atherosclerosis plaque formation (*23*). Therefore, the increase in HDL may slow the atherosclerosis process (*24*). Our results showed that *C. bergamia* juice treatment increased the concentration of serum HDL when compared with hyperlipidemic controls. The AI is believed to be an important risk factor of

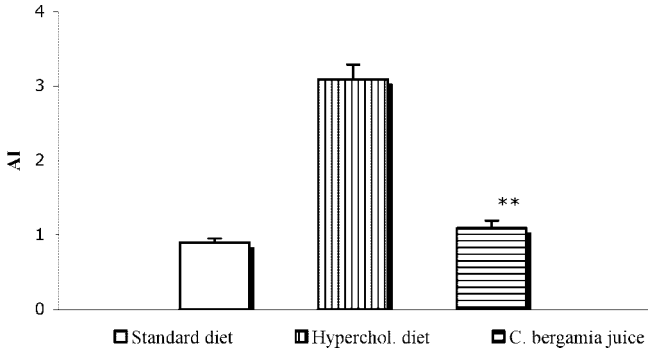

Figure 4. Atherogenic index (AI) = (CHOL-HDL)/HDL. Values are expressed as mean ± SD (vertical bars) for 10 animals per group. **, $P < 0.01$, represents significant differences when compared with the hyperlipidemic controls.

atherosclerosis. Because cholesterol was significantly suppressed and administering *C. bergamia* resulted in increased HDL, the AI value was significantly decreased. This decrease in AI was another positive change after *C. bergamia* treatment.

C. bergamia treatment produced a significant decrease in serum triglyceride levels. As plasma triglyceride level is determined by hepatic triglyceride synthesis, release from the liver, and activity of lipoprotein lipase, we can assume that *C. bergamia* juice has an effect on these processes.

In addition to the hypolipidemic effect, the juice shows radical scavenging activity in the DPPH test; probably the effect on lipid metabolism can be related to the free radical scavenging properties of flavonoid compounds, as neoeriocitrin, naringin, and neohesperidin, contained in bergamot juice. Flavonoids have been demonstrated to inhibit, in vitro, the oxidation of LDL (*25*), which has been recognized to play an important role in atherosclerosis (*26*). Oxidative modification of LDL alters its structure, allowing LDL to be taken up by scavenger receptors on macrophage, endothelial, and smooth muscle cells, leading to the formation of lipid-laden foam cells, the hallmark of early atherosclerotic lesions (*27*). Flavonoids can reduce LDL lipid peroxidation by scavenging reactive oxygen/nitrogen species; they can also reduce macrophage oxidative stress by inhibiting cellular oxygenases or by activating cellular antioxidants. Flavonoids might inhibit the free radical formation and the propagation of free radical reactions chelating of transition-metal ions, particularly those of iron and copper (*25*). Therefore, the hypolipidemic activity shown by *C. bergamia* juice could be related, at least partly, to the free radical scavenging properties of flavonoids contained in the juice.

Fecal output of total bile acids and neutral sterols was found to be enhanced in the *C. bergamia* juice treated group compared to the hyperlipidemic group. The conversion of cholesterol to bile acids is the major pathway of cholesterol elimination, and it accounts for about 50% of daily cholesterol excretion (*28*). Cholesterol 7α-hydroxylase, the rate-determining enzyme in the conversion of cholesterol to bile acids, is mainly regulated by feedback inhibition of bile acids reabsorbed from the intestine (*29*). Lipids fecal excretion was found to be increased in hyperlipidemic rats, and *C. bergamia* treatment enhanced, significantly, their excretion. The increase in the excretion of bile acids seems to activate cholesterol 7α-hydroxylase, enhancing the conversion of liver cholesterol to bile acids for excretion. This leads to a decrease in hepatic cholesterol content, which in turn stimulates LDL receptor expression and lowers blood cholesterol levels.

Thus, the hypocholesterolemic activity of *C. bergamia* could be due to the promotion of cholesterol and bile excretion.

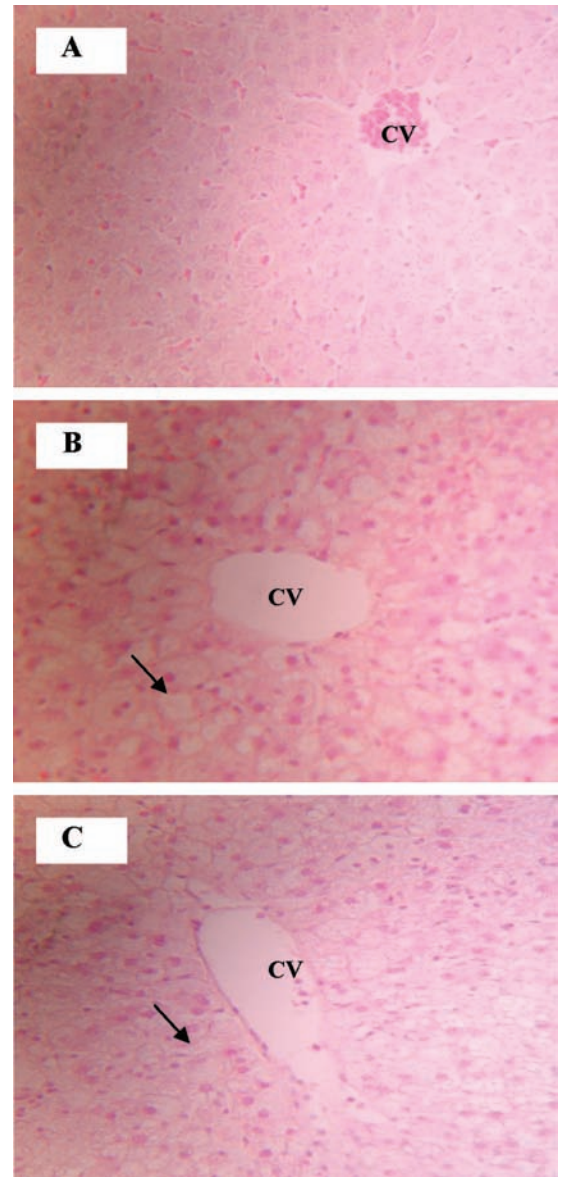

Figure 5. Light microscopy observations of rat liver sections (hematoxylin-eosin staining): (**A**) normolipidemic controls [the hepatic parenchyma shows a normal configuration both of hepatic architectural pattern of epithelial cells and of centrilobular vein (CV) (magnification ×20)]; (**B**) hyperlipidemic controls [several vacuolated cells are evident in the whole lobule; around the centrilobular vein, large confluent lipidic droplets (→) can be observed (magnification ×40)]; (**C**) *C. bergamia* juice treatment [the lobule reveals a normal configuration and a reduction of degenerative lipidic droplets (→) around the centrilobular vein (magnification ×40)].

Citrus juices, it is well-known, contain polyphenol compounds, pectins, and ascorbic acid (*9*). The hypocholesterolemic activity of some phenolic compounds from fruits and vegetables has been attributed to an increase in biliary cholesterol and bile acids concentrations and a subsequent increase in the fecal excretion of these compounds (*30*). A similar mechanism was observed for some flavonoid compounds (*31*). *Citrus* juices, moreover, contain pectins; dietary pectins have previously been shown to increase the synthesis and fecal excretion of bile acids in rats (*32*) and to increase the excretion of fecal neutral steroids in rabbits (*33*). Therefore, the effect of *C. bergamia* treatment on lipid metabolism could be related, also, to the pectin content.

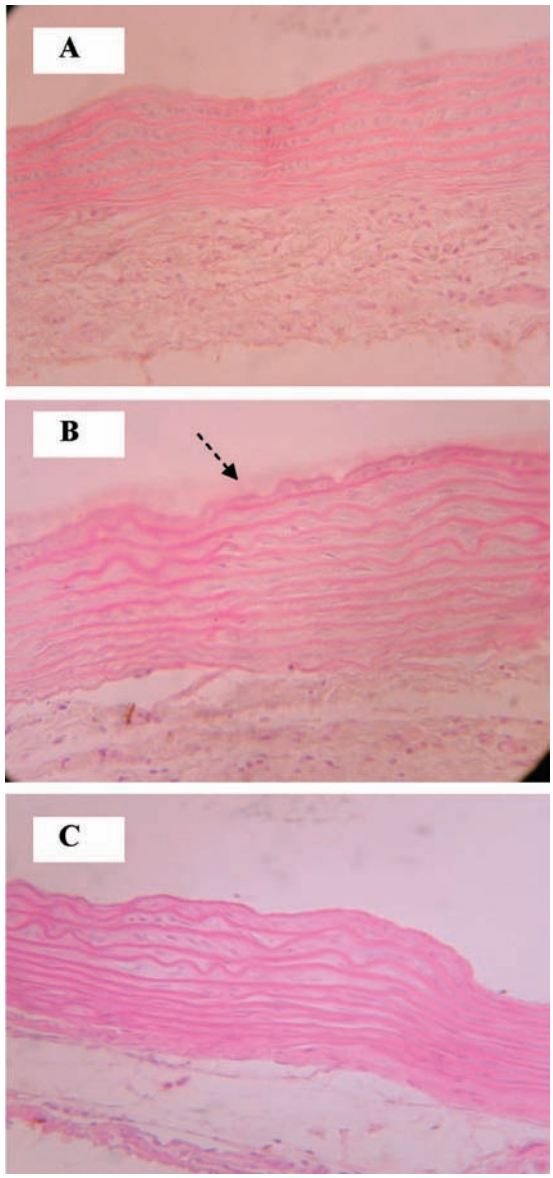

Figure 6. Light microscopy observations of rat aorta artery sections (hematoxylin-eosin staining) (magnification ×40): (**A**) normolipidemic controls; (**B**) hyperlipidemic controls (microscopic histological changes both in endothelial cells and within intimal tunica; (**C**) *C. bergamia* juice treatment (edema in the wall of the vessel is reduced).

Furthermore, the histopathological analysis of the liver supported the biochemical data and indicated a protective effect of *C. bergamia* on the development of liver damage due to hyperlipidemic diet. Histopathological observation pointed out, in rats submitted to *C. bergamia* treatment, a protection of hepatic parenchyma; in fact, in the hepatocytes medium- and small-caliber and not confluent vesicles were observed. Moreover, the cytoplasm vesicles are confined around centrilobular vein, so it can be assumed that the hepatic injury does not involve the whole lobule. The more interesting data concerned the vascular district; in fact, a reduction in the vessel caliber was noted, both in the portal Triad and in the centrilobular vein, in comparison with the hyperlipidemic controls.

The results of microscopic observation suggest that *C. bergamia* juice could influence cholesterol metabolism by acting directly on the liver.

C. bergamia juice contains high concentrations of polyphenols; HPLC analysis led to the identification and quantification of some of the most abundant flavonoid derivatives contained

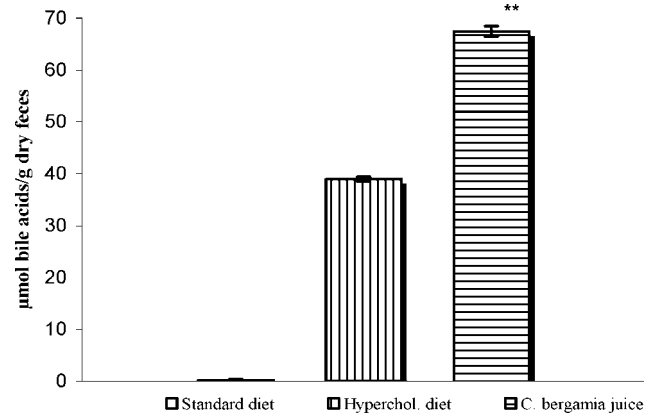

Figure 7. Effect of *C. bergamia* juice treatment on fecal bile acids excretion. Values are mean ± SD (vertical bars) for 10 animals per group. **, $P < 0.01$, represents significant differences when compared with the hyperlipidemic controls.

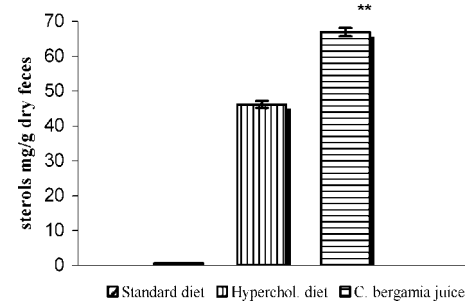

Figure 8. Effect of *C. bergamia* juice treatment on fecal neutral sterols excretion. Values are mean ± SD (vertical bars) for 10 animals per group. **, $P < 0.01$, represents significant differences when compared with the hyperlipidemic controls.

in juice, neoeriocitrin, narigin, and neohesperidin; however, further studies are under way to identify other polyphenols.

Therefore, the effects on the lipid metabolism could be related to the high content of polyphenols in the juice; it can be supposed that these compounds are able to influence lipid metabolism by acting directly on hepatic parenchyma. Wilcox et al. (*34*) reported that the *Citrus* flavonoids, naringenin and hesperetin, decrease apolipoprotein B secretion and cholesterol esterification in human hepatoma cell line HepG2; therefore, the reduction of plasma cholesterol could have its origin in the hepatocyte.

In conclusion, the present results indicate that *C. bergamia* juice reduces diet-induced hyperlipidemia in rats. It significantly lowers the concentration of serum cholesterol, triglycerides, and LDL and elevates the serum HDL level. This hypocholesterolemic effect might be related both to radical scavenging activity and to an increase of fecal neutral sterols and total bile acids excretion.

Therefore, *C. bergamia* juice could be considered a valuable supplement to prevent coronary diseases.

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