Full Paper

Biological Evaluation of Chalcones and Analogues as Hypolipidemic Agents

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In order to evaluate the anti-hyperlipidemic effect of synthetic chalcones and some analogues, nineteen compounds with different substituents in both rings were synthesized and hypolipidemic activities were measured *in vivo* using Triton WR1339 acute and hypercaloric chronic assays. 4′,4-dichlorochalcone, 3′,4′,4-trichlorochalcone, and 4′-chlorochalcone gave an excellent decrease in serum total cholesterol and triglycerides in the acute assay. These compounds also showed significant anti-lipidemic activity in the chronic assay.

Keywords: Chalcones analogues / Cholesterol / Hypolipidemic activity / Triglycerides

Received: February 21, 2006; accepted: April 24, 2006

DOI 10.1002/ardp.200600034

Introduction

Today, coronary artery disease and the atherosclerotic process with which it is closely associated constitute the major cause of death in Western society, and hypercholesterolemia is a major and modifiable risk factor for coronary heart disease [1]. Low serum high-density lipoprotein cholesterol (HDL-C) levels together with an increase in the level of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides are strong predictors of coronary heart disease [2]. The cause of hyperlipidemia is thought to be related to increased lipid synthesis, decreased lipid clearance from the blood, or a combination of these two processes.

Chalcones are natural compounds belonging to the flavonoid family. Chemically, they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α-, β-unsaturated carbonyl system. They carry out biological activities, the most important being estrogenic, antifungal, antinociceptive, antibacterial, antiviral, and anti-inflammatory [3, 4]. Their antifungal and antileishmanial activities suggest that the mode of action of some chalcones is by inhibiting the activity of an enzyme that participates in the biosynthesis of ergosterol [5, 6]. In this study, we tested the hypolipidemic effects of chalcones in rats.

Results and discussion

As shown in Scheme 1 and Table 1, nineteen chalcones were synthesized by a base-catalyzed condensation of appropriate acetophenones and aldehydes. 2,3-dibromo-1,3-diphenylpropanones were obtained by reacting the appropriate chalcones with bromide. All synthesized compounds were screened for hypolipidemic activity in an acute experimental model and then some of these were screened in a chronic experimental model.

The concentrations of total cholesterol, VLDL-cholesterol and triacylglycerides in the acute assay showed that all compounds tested were able to decrease total serum cholesterol as shown in Table 2.

Some chalcones were selected for the chronic assay according to the results obtained in this screening test

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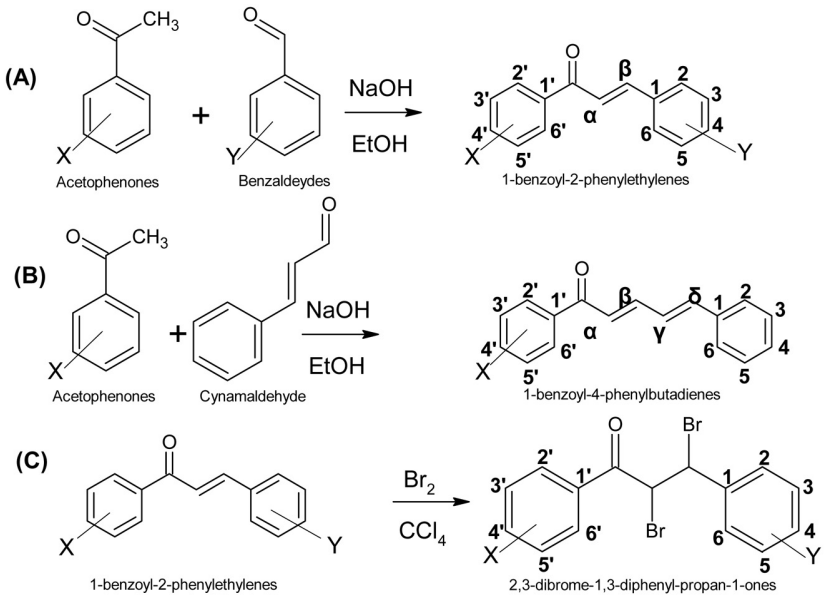

Scheme 1. Synthesis of chalcones (A) and related compounds (B), with substitutions (X), (Y), and (n), according to the method previously described by Guider *et al.* [12]. Novel syntheses were indicated as ns.

Table 1. Synthesized chalcones and related compounds.

Compounds (A)	X	Y	Reference
1	4′-H	4-H	18
2	4′-Cl	4-Cl	3
3	4′-Br	4-Cl	3
4	4′-Br	4-H	3
5	4′-Cl	4-H	3
6	3‘,4′-Cl	4-H	3
7	3‘,4′-Cl	4-Cl	3
10	4′-H	4-CH_3	19
11	4-H	4-NO_2	20
12	4′-H	4-OCH_3	20
13	4′-H	2-OCH_3	ns
14	4′-H	3-OCH_3	19
Compounds (B)			
8	4′-OCH_3	4-H	ns
9	2′-OH	4-H	ns
15	4′-H	4-H	ns
Compounds (C)			
16	4′-Cl	4-Cl	ns
17	4′-H	4-CH_3	ns
18	4′-H	4-NO_2	ns
19	4′-H	4-OCH_3	ns

(Triton WR1339). Hence, the compounds (**1**, **2**, **3**, **4**, **5**, **7**, **11**), which simultaneously decreased cholesterol (72%) and triglyceride levels (58%), along with compounds **13** and **17**, which gave a large reduction in cholesterol levels (80 and 51%) in spite of a low reduction in triglyceride (34 and 31%) were selected for the second experimental model. Thus, the other compounds were not tested in the chronic assay.

The chalcones tested lowered the serum levels of total cholesterol, triglycerides, LDL-C, and VLDL-C, as well as hepatic cholesterol and triglycerides in the model of chronic hyperlipidemia (Tables 3 and 4). The AAI% (antiatherogenic index), a measure of the antiatherogenic potential of a given drug, was significantly higher in the groups treated with **2** and **5** chalcones. These results support the hypothesis that the chalcones expresses hypolipidemic activity *in vivo* in acute and chronic assays.

The most active compounds in the acute assay were 4′,4-dichlorochalcone **2**, 3′,4′,4-trichlorochalcone **5**, and 4′-chlorochalcone **7**; they decreased total serum cholesterol by 78, 72, and 79%, and triglycerides by 94, 65, and 89%, repectively. Furthermore, these compounds exhibited antilipidemic activity in the chronic assay, decreasing the total serum cholesterol by 70, 77, and 69%, and triglycerides by 83, 75, and 84%, respectively.

The 4′,4-dichlorochalcone also gave a greater reduction in LDL-C and VLDL-C and a greater increase in the HDL-C concentration with respect to the unsubstituted compound. It is important to note that the AAI% of this compound was higher than that of lovastatin, a specific inhibitor of the rate-limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase. However, it must be noted that 4′,4-dichlorochalcone was used in a molar ratio of 5 : 1 with respect to lovastatin.

According to Garattini *et al.* [7], a decrease in hyperlipemia 8 h after intraperitoneal (*i.p.*) administration of a drug, given at the same time as Triton WR1339 there is evidence of activity resulting from the inhibition of cholesterol and fatty acids synthesis. Furthermore, previous studies have shown that *in vivo* inhibition of lipoprotein lipase with Triton WR1339 blocks VLDL catabolism, inhi-

Table 2. Effect of the tested chalcones (12.5 mmol/kg, b.w.), VLDL-cholesterol, and lovastatin (2.5 mmol/kg, b.w.) on serum total cholesterol (TC) and triglyceride (TG) levels and control group results in acute Triton WR1339 assays.

Compound	TC (mg/dL)	TC Reduction (%)[a)]	TG (mg/dL)	TG Reduction (%)[a)]	VLDL-C (mg/dL)	VLDL-C Reduction (%)[a)]
1	57.6 ± 12.8***	72	228.9 ± 9.6***	58	45.8 ± 9.6	58
2	45.4 ± 4.6***	78	30.2 ± 4.4***	94	6.0 ± 4.4***	94
3	53.9 ± 7.7***	74	39.1 ± 5.4***	93	7.8 ± 5.4***	93
4	45.6 ± 5.8***	78	37.0 ± 11.8***	93	7.4 ± 11.8***	93
5	66.3 ± 7.4***	72	190.7 ± 9.2***	65	38.1 ± 10.2	65
6	100.2 ± 13.6***	51	447.5 ± 5.8***	17	89.5 ± 5.8	17
7	43.4 ± 6.9***	79	57.1 ± 4.4***	89	11.4 ± 4.4***	89
8	76.4 ± 8.4***	63	438.6 ± 19.4***	19	87.7 ± 19.4	19
9	53.2 ± 7.2***	74	331.1 ± 13.1***	39	66.2 ± 13.1	39
10	47.0 ± 7.2***	77	442.5 ± 16.1***	18	88.5 ± 16.1	18
11	44.0 ± 6.7***	79	37.8 ± 10.1***	93	7.6 ± 10.1***	93
12	68.8 ± 6.4***	66	396.4 ± 19.1***	27	73.9 ± 19.1	32
13	40.8 ± 7.1***	80	359.1 ± 7.7***	34	71.8 ± 7.7	34
14	53.7 ± 20.0***	74	307.7 ± 18.5***	43	61.5 ± 18.5	43
15	74.7 ± 16.6***	64	277.6 ± 11.9***	49	55.5 ± 11.9	49
16	64.9 ± 13.0***	58	105.9 ± 10.5***	65	16.2 ± 10.5**	85
17	100.7 ± 10.4***	51	371.5 ± 8.2***	31	74.3 ± 8.2	31
18	79.8 ± 9.8***	61	104.6 ± 10.2***	65	16.3 ± 10.4**	85
19	55.6 ± 6.9***	73	760.8 ± 14.3	–	152.2 ± 14.3	–
Lovastatin	72.4 ± 3.4***	65	632.5 ± 25.3**	–	126.5 ± 25.3	–
Normal control	98.6 ± 7.4	–	96.46 ± 3.4	–	19.3 ± 3.4	–
Triton WR-1339 control	205.4 ± 10.3	–	541.6 ± 23.9	–	108.3 ± 23.9	–

Table 3. Lowering effect of the tested chalcones (12.5 mmol/kg, b.w.) and lovastatin (2.5 mmol/kg, b.w.) on serum total cholesterol (TC), serum triglycerides (TG), hepatic cholesterol (HC), and hepatic triglycerides (HTG) levels and control group results in chronic hyperlipemia diet assay.

Compound	TC (mg/dL)	TC Reduction (%)[a)]	TG (mg/dL)	TG Reduction (%)[a)]	HC (mg/mg protein)	HC Reduction (%)[a)]	HTG (mg/mg protein)	HTG Reduction (%)[a)]
1	49.4 ± 9.3***	71	30.9 ± 7.3***	93	38.2 ± 0.5***	60	407.4 ± 3.3***	51
2	49.9 ± 9.8***	70	57.0 ± 9.6***	83	18.0 ± 0.2***	85	205.7 ± 4.6***	69
3	65.6 ± 7.1***	31	112.5 ± 9.7***	61	–	–	–	–
4	70.2 ± 2.9***	58	104.7 ± 6.8***	67	–	–	–	–
5	39.1 ± 2.3***	77	89.1 ± 9.6***	75	25.9 ± 1.8***	79	205.6 ± 2.6***	69
7	52.4 ± 3.9***	69	45.3 ± 3.9***	84	30.4 ± 0.5***	75	395.9 ± 5.1***	46
11	47.7 ± 8.0***	71	71.8 ± 5.8***	80	–	–	–	–
13	52.3 ± 7.8***	69	85.9 ± 7.4***	77	–	–	–	–
17	95.0 ± 6.7***	43	81.4 ± 6.9***	64	–	–	–	–
Lovastatin	69.6 ± 4.9***	58	54.2 ± 9.7***	71	54.1 ± 0.6***	55	429.5 ± 5.7***	54
Normal	97.0 ± 6.8	–	67.1 ± 5.9	–	52.7 ± 0.9	–	244.9 ± 5.1	–
Hyperlipemic control	167.8 ± 3.6	–	188.7 ± 4.3	–	121.6 ± 1.8	–	672.6 ± 4.8	–

[a)] Percent reduction compared to hyperlipemic control group. Each group was composed of six rats and the determinations were performed in duplicate. All values were expressed in terms of mean ± SEM. *** indicates statistical significance in relation to hyperlipemic control group, $P < 0.001$.

biting the degradation of VLDL particles and causing accumulation of blood VLDL particles rich in triglycerides formed in the liver [8]. These and previous results, which have shown that 2′,6′-dihydroxy-4′-methoxychalcone is an inhibitor of some enzyme involved in ergosterol biosynthesis [6], suggest that the mode of action of these chalcones in their hypocholesterolemic effect could be associated with the inhibition of an enzyme

Table 4. Effect of the tested chalcones (12.5 mmol/kg, b.w.) and lovastatin (2.5 mmol/kg, b.w.) on total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and antiatherogenic index (AAI) in chronic assay (hyperlipemic diet).

Compound	HDL-Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)	VLDL-C (mg/dL)	AAI (%)
1	20.4 ± 8.9**	32.8 ± 4.2***	17.8 ± 9.6	70.6 ± 0.5
2	42.8 ± 3.5	18.5 ± 5.6***	11.4 ± 10.6	602.8 ± 7.7
3	35.5 ± 6.5	52.6 ± 1.5***	22.5 ± 10.0	117.9 ± 0.6
4	27.9 ± 4.2*	63.2 ± 0.1***	20.9 ± 6.8	65.9 ± 1.4
5	26.9 ± 4.4*	30.0 ± 3.9***	17.8 ± 9.6	222.3 ± 2.1
7	33.9 ± 3.5	27.6 ± 0.3***	9.06 ± 3.9	183.2 ± 0.5
11	16.5 ± 1.9***	45.7 ± 4.9***	14.4 ± 5.8	52.7 ± 6.1
13	15.3 ± 2.2***	49.0 ± 4.2***	17.2 ± 7.4	41.3 ± 5.6
17	23.2 ± 7.4**	88.1 ± 2.3***	16.3 ± 6.9	32.3 ± 0.6
Lovastatin	57.9 ± 3.9	22.5 ± 1.5***	10.8 ± 12.7	495.6 ± 1.0
Control	31.0 ± 3.6	89.4 ± 1.3	13.4 ± 5.9	–
Hyperlipemic control	81.6 ± 2.1	124.1 ± 1.3	37.7 ± 4.3	–

Each group was composed of six rats and the determinations were performed in duplicate. All values are expressed in terms of mean ± SEM.

***, **, * indicate statistical significance in relation to hyperlipemic control group, $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively.

that participates in cholesterol biosynthesis. Further studies are necessary to define clearly the precise mechanism of action.

Reduced triglyceride and VLDL-C levels could result also from decreased secretion and/or increased rates of catabolism. It is important to note that the hypotriglyceridemic activity of some tested chalcones was very strong and a study group of the European Atherosclerosis Society has recommended that more attention must be paid to hypertriglyceridemia as a risk factor for coronary heart disease [9].

Conclusion

The development of an anti-hyperlipidemic agent to lower blood total cholesterol and triglycerides simultaneously is important in the management of hyperlipidemic patients [10, 11]. This study suggests that chalcones, especially 4′,4-dichlorochalcone, may be used as a reference in the search for new active molecules for the treatment of hyperlipidemia, since they cause a decrease in total cholesterol, triglyceride, and LDL-C levels.

The authors wish to thank the Nucleo de Investigações Químico Farmacêuticos (NIQFAR) of Universidade do Vale de Itajaí for chemical syntheses of the chalcones examined and the Central Analysis Laboratory of the Chemistry Department of Universidade Federal de Santa Catarina for the physico-chemical analyses. The authors are grateful to CNPq and FAPESC for financial support.

Experimental

Materials

All chemicals were of the highest commercially available purity. Diagnostic kits for total cholesterol, HDL-cholesterol, and triglyceride determinations were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were purchased from Aldrich-Chemie (Steinheim, Germany).

General procedure for the preparation of chalcones 1–20 and their bromide derivatives

Compounds **1–20** were obtained by reaction of acetophenone and benzaldehyde (1:1) in the presence of sodium hydroxide and ethanol and further addition (to cool) of diluted acetic acid, according the method previously described by Guider *et al.* [12]. The respective products were recrystallized from ethanol. All compounds were synthesized in good yields (55–98%) and characterized by ^{1}H-NMR, IR, and microanalyses. TLC using several solvent systems of different polarity was used to determine the purity of these compounds.

Chemical characterization

Melting points (m.p.) were obtained on a MELTEMPII apparatus (Laboratory Devices, USA) and were uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 597 infrared spectrophotometer (Perkin Elmer, Wellesley, MA, USA). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a Bruker Nuclear AC-200F 80 MHz and a Bucker 400 MHz spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS) and signals are given as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Elemental analyses were performed with a Perkin-Elmer 2400 CHN analyzer. Percentages of C and H were in agreement with the product formula (within ± 0.4% of theoretical values). The novel compounds synthesized to our best knowledge are the following:

(2E,4E)-1-(4′-Methoxybenzoyl)-4-phenylbutadiene ***8***

MW = 264 g; m.p.: 84 – 86°C; yield: 80%; FT-IR (KBr disk, cm^{-1}): 1653 (ν C=O), 1602 (ν C=C); H^1-NMR (DMSO-d_6, ppm): 3.86 (s, 3H, OCH_3), 7.09 (d, 2H, H3′, H5′, J = 8), 7.22 – 7.62 (m, 5H, H2 – H6), 7.37 (d, 2H, H9, H10, J = 16), 7,45 (d, 2H, H7, H8, J = 16), 8.04 (d, 2H, H2′, H6′, J = 8). $C_{16}H_{14}O_2$, Anal. calc. C: 80.57, H: 5.88, found C: 80.75, H: 5.90.

(2E,4E)-1-(2′-Hydroxybenzoyl)-4-phenylbutadiene ***9***

MW = 250 g; m.p.: 118.5 – 129.2°C; yield: 96%; FT-IR (KBr disk, cm^{-1}): 1650 (ν C=O), 1605 (ν C=C); H^1-NMR (DMSO-d_6, ppm): 6.81 (d, 2H, H3, H5, J = 8), 6.89-7.63 (m, 5H, H3′ – H6′, H2, H4, H6), 7.37 (d, 2H, H9, H10, J = 16), 7.45 (d, 2H, H7, H8, J = 16), 7.88 (d, 1H, H6'). $C_{15}H_{12}0$, Anal. calc. C: 80.35 H: 5.35, found C: 80.47, H: 5.39.

(2E)-2-(2-Methoxyphenyll)-1-phenylethylene ***13***

MW = 238 g; m.p.: 59 – 60°C; yield: 94%; FT-IR (KBr disk, cm^{-1}): 1661 (ν C=O), 1601 (ν C=C); H^1-NMR (DMSO-d_6, ppm): 3.91 (s, 3H, OCH_3), 7.06 – 8.03 (m, 7H, H3 – H6, H3′ – H5′), 7.56 (d, 1H, H_a, J = 14), 7.63 (d, 1H, H_β, J = 14), 8.13 (d, 2H, H2′, H6′, J = 8). $C_{16}H_{14}O_2$, Anal. calc. C: 80.57, H: 5.88, found C: 80.48, H: 5.92.

(2E,4E)-1-Benzoyl-4-phenylbutadiene ***15***

MW = 234 g; m.p.: 97 – 99.5°C; yield: 50%; FT-IR (KBr disk, cm^{-1}): 1656 (ν C=O), 1600 (ν C=C); H^1-NMR (DMSO-d_6, ppm): 7.37 (d, 2H, H3′, H5′, J = 8), 7.24 – 7.67 (m, 6H, H2 – H6, H4′), 7.58 (d, 2H, H9, H10, J = 14), 7.65 (d, 2H, H7, H8, J = 14), 8.02 (d, 2H, H2′, H6′, J = 8). $C_{17}H_{14}O$, Anal. calc. C: 87.17, H: 5.90, found C: 87.07, H: 5.87.

2,3-Dibromo-1,3-bis(4-chlorophenyl)-propan-1-one ***16***

MW = 437 g; m.p.: 159 – 162°C; yield: 85%; FT-IR (KBr disk, cm^{-1}): 1687 (ν C=O); H^1-NMR (DMSO-d_6, ppm): 5.83 (d, 1H, H-8, J = 12), 6.73 (d, 1H, H-7, J = 12), 7.53 8 d, 2H, H3, H5, J = 8), 7.74 (d, 2H, H3′, H5′, J = 8), 7.88 (d, 2H, H2, H6, J = 8), 8.31 (d, 2H, H2′, H6′, J = 8). $C_{15}H_{12}OBr_2$, Anal. calc. C: 48.91, H: 3.26, found C: 48.99, H: 3.31.

2,3-Dibromo-3-(4-methoxyphenyl)-1-phenylpropan-1-one ***17***

MW = 382 g; m.p.: 140 – 144°C; yield: 90%; FT-IR (KBr disk, cm^{-1}): 1685 (ν C=O); H^1-NMR (DMSO-d_6, ppm): 4.74 (s, 3H, OCH_3), 5.79 (d, 1H, H-8, J = 12), 6.70 (d, 1H, H-7, J = 12), 7.42 – 7.98 (m, 7H, H2, H3, H5, H6, H3′-H5′), 8.29 (d, 2H, H2′, H6′, J = 8).$C_{16}H_{14}O_2Br_2$, Anal. calc. C: 48.24, H: 3.51, found C: 48.32, H: 3.57.

2,3-Dibromo-3-(4-nitrophenyl)-1-phenylpropan-1-one ***18***

MW = 413 g; m.p.: 128 – 131°C; yield: 96%; FT-IR (KBr disk, cm^{-1}): 1682 (ν C=O); H^1-NMR (DMSO-d_6, ppm): 5.99 (d, 1H, H8, J = 12), 6.79 (d, 1H, H7, J = 12), 7.62 – 8.32 (m, 9H, H2′, H3′, H5′, H6′, H2 – H6). $C_{15}H_{11}O_3N$, Anal. calc. C: 43.47, H: 2.65, found C: 43.58, H: 2.60.

2,3-Dibromo-3-(3-methoxyphenyl)-1-phenylpropan-1-one ***19***

MW = 398 g; m.p.: 94 – 99°C; yield: 97.6%; FT-IR (KBr disk, cm^{-1}): 1687 (ν C=O); H^1-NMR (DMSO-d_6, ppm): 3.84 (s, 3H, OCH_3), 5.75 (d, 1H, H8, J = 12), 6.69 (d, 1H, H7, J = 12), 6.93-7.78 (m, 7H, H2, H4 – H6, H3′ – H5′), 8.30 (d, 2H, H2′, H6′, J = 8). $C_{16}H_{14}O_2Br_2$, Anal. calc. C: 48.24, H: 3.51, found C: 48.17, H: 3.59.

Animals

Male Wistar-EPM-M1 rats, weighing 130 ± 30 g and 230 ± 25 g at the age of seven weeks and four months, respectively, were kept under controlled conditions (12 h light-dark cycle, 22 ± 2°C, 60% air humidity) and had free access to standard laboratory chow and water. Animals were received from the Central Bioterio of the University of Vale do Itajaí (Itajaí, Brazil). All animals were allowed to acclimatize for at least five days prior to the first treatment. They were fasted for 12 h before experiments and allowed water *ad libitum*. All animal procedures were conducted in accordance with legal requirements relating to the species.

Acute hypolipidemic activity

An aqueous solution of Triton WR1339 was administered *i.p.* (400 mg/kg, body weight, b.w.) to male Wistar rats (230 ± 25 g, n = 6) and after 30 min the compounds investigated (12.5 mmol/kg, b.w.) or lovastatin (2.5 mmol/kg, b.w.) dissolved in saline solution, or saline solution (control group) only, was given orally [7, 8]. After 8 h, blood was taken from the occular vein and used for the determination of serum total cholesterol (TC) and triglyceride (TG) concentrations by enzymatic methods [13, 14]. The concentration of VLDL-cholesterol (VLDL-C) in the rats was determined indirectly by the use of a mathematical formula [10]: VLDL-C = TG/5.

Chronic hypolipidemic activity

One group of rats (control group, 130 ± 30 g, n = 6) was fed on a hypercaloric-diet (cholesterol 2%, sodium cholate 2%, vitamin mixture 2%, oligoelements 0.2%, salt mixture 5.8%, corn oil 20%, cellulose 4%, sucrose 44%, casein 5%, protein 15%) for 30 days and two groups were fed on a hypercaloric diet plus 0.5 mL of the compounds investigated (12.5 mmol/kg, b.w., n = 6) or 0.5 mL of lovastatin (2.5 mmol/kg. b.w., n = 6), administered by oral gavage for the same period [15]. Blood samples for the assays were collected after the last day of the treatment for the lipid determinations: TC, high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C). Rats were killed and a portion of the liver was removed, homogenized, and tissue cholesterol was extracted by the Folch method [16] and analyzed using the cholesterol oxidase method [13]. LDL-C was estimated using the Friedewald formula [17].

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