

Propolis and swimming in the prevention of atherogenesis and left ventricular hypertrophy in hypercholesterolemic mice

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Abstract

Aims: The present study verified the effect of propolis alone and its association with swimming in dyslipidemia, left ventricular hypertrophy and atherogenesis of hypercholesterolemic mice. **Methods and Results:** The experiments were performed in LDLr^{-/-} mice, fed with high fat diet for 75 days, and were divided into four experimental groups (n=10): HL, sedentary, subjected to aquatic stress (5 min per day, 5 times per week); NAT submitted to a swimming protocol (1 hour per day, 5 times per week) from the 16th day of the experiment; PRO, sedentary, submitted to aquatic stress and which received oral propolis extract (70 uL/animal/day) from the 16th day of the experiment; HL+NAT+PRO, submitted to swimming and which received propolis as described above. After 75 days, blood was collected for analysis of serum lipids. The ratio between the ventricular weight (mg) and the animal weight (g) was calculated. Histological sections of the heart and aorta were processed immunohistochemically with anti-CD40L antibodies to evaluate the inflammatory process; stained with hematoxylin/eosin and picosirius red to assess morphological and morphometric alterations. The HL animals showed severe dyslipidemia, atherogenesis and left ventricular hypertrophy, associated with a decrease in serum HDLc levels and subsequent development of cardiovascular inflammatory process, characterized by increased expression of CD40L in the left ventricle and aorta. Swimming and propolis alone and/or associated prevented the LVH, atherogenesis and arterial and ventricular inflammation, decreasing the CD40L expression and increasing the HDLc plasmatic levels. **Conclusion:** Propolis alone or associated with a regular physical activity is beneficial in cardiovascular protection through anti-inflammatory action.

Keywords: dyslipidemia, left ventricular hypertrophy, propolis, LDLr^{-/-} mice.

Própolis e natação na prevenção da aterogênese e hipertrofia ventricular esquerda de camundongos hipercolesterolêmicos

Resumo

Objetivos: O presente estudo verificou o efeito do própolis associação ou não com a natação na dislipidemia, na hipertrofia ventricular esquerda e aterogênese de camundongos hipercolesterolêmicos. **Métodos e Resultados:** Os experimentos foram realizados em camundongos LDLr^{-/-}, alimentados com dieta hiperlipídica por 75 dias, e divididos em quatro grupos experimentais (n = 10): HL, sedentários, foram submetidos ao estresse aquático (5 min por dia, cinco vezes por semana); NAT foram submetidos a um protocolo de natação (1 hora por dia, cinco vezes por semana) a partir do 16^o dia do experimento; PRO, sedentários, submetidos a estresse aquático e que receberam extrato de própolis oral (70 uL / animal / dia) a partir do 16^o dia do experimento; HL + NAC + PRO, submetidos a natação e que recebeu a própolis, como descrito acima. Após 75 dias, foi coletado sangue para análise do perfil lipídico. Calculou-se a relação entre o peso ventricular (mg) e o peso do animal (g). Os cortes histológicos do coração e aorta foram processados imunohistoquimicamente com anticorpos anti-CD40L para avaliar o processo inflamatório, corados com hematoxilina / eosina e picrossírius red, para avaliar as alterações morfológicas e morfométricas. Os camundongos HL apresentaram dislipidemia grave, aterogênese e hipertrofia do ventrículo esquerdo, associada a uma diminuição dos níveis plasmáticos

de HDLc e o desenvolvimento subsequente do processo inflamatório cardiovasculares, caracterizada pelo aumento da expressão do CD40L no ventrículo esquerdo e na aorta. Natação e a própolis isolado e \ ou associados preveniram a HVE, a aterogênese e a inflamação tanto na artéria quanto no ventrículo, diminuindo a expressão de CD40L, aumentando os níveis plasmáticos de HDLc. **Conclusão:** A Própolis isolada ou associada a uma atividade física regular é benéfica na proteção cardiovascular através da ação anti-inflamatória.

Palavras-chave: dislipidemia, hipertrofia ventricular esquerda, própolis, camundongos LDLr-/-.

1. Introduction

Inflammatory process (Kai et al., 2005), dysfunction of the endothelium (Aubin et al., 2006) and oxidative stress (Lang et al., 2000), in the cardiovascular environment resulting from dyslipidemia are the conditions that promote and sustain atherosclerosis and cardiac hypertrophy

Atherosclerosis is an inflammatory disease (Lusis, 2000) and the main hypotheses of atherogenesis described in the last decades are the reverse cholesterol transport (Zhang et al., 2003) and LDL oxidation (Navab et al., 2004). Both hypotheses pinpoint the pivotal role of LDL oxidation as a starter, and HDL as an atherogenic procrastinator (Navab et al., 2004). Evidences support a central role in the interaction between ligand CD40 (CD40L) and CD40 (its membrane receptor) in the pathogenesis of atherosclerosis (Lutgens and Daemen, 2002; André et al., 2002). The expression of CD40L correlates with the severity of inflammation. Several studies have shown that CD40L affects the endothelial function process. The CD40-CD40L interaction may trigger the production of several proinflammatory cytokines and chemokines (Thienel et al., 1999; Chakrabarti et al., 2007) and of matrix metalloproteinase expression and vascular endothelial growth factor (Mach et al., 1999; Flaxenburg et al., 2004). The inhibition of CD40-CD40L signaling can effectively reduce atherosclerosis in rats (Mach et al., 1998).

Studies by Garcia and Incerpi (2008) showed that the hypercholesterolemia induced left ventricular hypertrophy through CD40L-CD40. The interaction between CD40 and CD40L activates Nf κ B pathway (Gelbmann et al., 2003) and promotes the phosphorylation of IKK (K κ B kinase inhibitor), resulting in translocation of nuclear factor kappa β (NF κ B) to the nucleus where it activates genes involved in inflammation and cell growth (Vellaichamy et al., 2005). The NF κ B activation participates in the development of cardiac hypertrophy in mice, characterized by increased collagen deposition (Vellaichamy et al., 2005). Studies have also shown that the prevention of left ventricular hypertrophy and resistance to neointimal lesion development in LDLr-/- mice fed with standard diets can be related to lower activity of the CD40/CD40L pathway due to increased plasma levels of HDL (Garcia et al., 2011).

The regulation of lipid metabolism with drugs, natural foods and physical activity is an important target to reduce the risk of cardiovascular disease. These treatments have been used empirically, lacking in study methodology that allows more reliable conclusions. In the last decades, the association of physical activity with herbal medicines in

the prevention of cardiovascular disease has been growing. The physical activity performed by individuals of varying ages and levels of fitness was beneficial in the modification of the levels and chemical composition of the fractions and subfractions of HDLc and LDLc cholesterol, from small and dense LDL-cholesterol processing, considered more atherogenic, into large and less dense. Only a few did not find significant changes in HDL-cholesterol and LDL-cholesterol levels with aerobic exercise (Hurley, 1989).

Propolis is a bee product (*Apis mellifera* L.) which presents in its composition mainly flavonoids and other compounds (Bankova et al., 2000; Marcucci et al., 2001), and has been used in folk medicine in many countries since ancient times, because it has antioxidant, antimicrobial and anti-inflammatory properties (Wang, 1993; Marcucci, 1995; Banskota et al., 2002). In this manner, the present study aimed to verify the effect of propolis alone and its association with swimming in dyslipidemia, left ventricular hypertrophy and atherogenesis of hypercholesterolemic mice that were receiving high-fat diet.

2. Material and Methods

2.1. Experimental protocol

The experiments were performed in male homozygous mice for the absence of the LDL receptor gene (LDLr-/-), generated in C57BL6 background, with three months of age and weight of 22 ± 3 g. The animals were bred in the vivarium of José do Rosário Vellano University (Alfenas, MG, Brazil) with controlled temperature and light / dark cycle (12 hours), were given throughout the experimental period a hyperlipidemic diet containing 20% total fat, 1.25% cholesterol and 0.5% cholic acid and were divided into four experimental groups: HL group (n = 10) – received a hyperlipidemic diet and were subjected to aquatic stress (5 min per day, 5 times per week, to mimic the aquatic stress associated with the experimental protocol) (Evangelista et al., 2003), NAT group (n = 10) – received a hyperlipidemic diet and were submitted to the swimming protocol five days a week, one hour a day for 60 days (Evangelista et al., 2003); PRO group (n = 10) – received a hyperlipidemic diet, were subjected to aquatic stress (Evangelista et al., 2003) and were treated oral with 70 μ L of 85.7% propolis extract / animal / day, for 60 days; HL + NAT + PRO group (n = 10) - received a hyperlipidemic diet, were submitted to the swimming protocol 5 days per week, one hour a day for 60 days (Evangelista et al., 2003) and were treated with 70 μ L of 85.7% propolis extract / animal / day, orally, for 60 days. The swimming protocol and treatments were administered to mice after 15 days

of the start of the high-fat diet. All animals received high fat diet and water ad libitum.

After 75 days of experiment, the mice remained fasting for 12 hours and right after were weighed and anesthetized intraperitoneally using Xylazine / Ketamine (Bayer AS[®] and Parke-Davis[®]) at dose of 6 and 40 mg / kg, respectively. Blood was collected by puncturing the retro-orbital venous plexus, immediately centrifuged (3000 rpm per 10 min), and serum was obtained for biochemical analysis of triglycerides, total cholesterol and its HDLc, LDLc and VLDLc fractions. Then, after thoracotomy, heart and aorta were removed. The use of animals and experimental protocol were approved by the Ethics Committee on Animal Use (CEUA) of José do Rosário Vellano University (UNIFENAS), under protocol number 04A/2011.

2.2. Qualification of the constituent classes from the propolis extract

We analyzed the propolis sample provided by the *Sul de Minas* Federal Institute's apiary – Muzambinho campus. It was determined the content of total phenols, total flavonoids and beeswax (Woisky and Salatino, 1998). High performance liquid chromatography techniques were employed coupled with mass spectrometry (HPLC/MS) and gas chromatography – mass spectrometry (GC/MS). Tests were performed in triplicate, which mean values, expressed in percentage, relative to the constituent classes from the sample were: total phenols (9,281±0,029%), total flavonoids (2.685±0.001%) and beeswax (14.514±0.144%).

2.3. Lipid serum analysis

Serum lipids (triglycerides, total cholesterol and HDLc) were measured by colorimetric enzymatic methods using protocols described in the commercial kits (In Vitro[®]) by automation (Hedrick et al., 2001). VLDLc was calculated by dividing the plasma triglyceride levels by 5 (Tian et al., 2006). LDLc was determined by the formula: LDLc = total cholesterol - (HDLc + VLDLc) (Friedewald et al., 1972).

2.4. Histological and immunohistochemical procedures

Immediately after removal, the heart was excised and the left ventricle was isolated, weighed and it was calculated the ratio of the left ventricular weight (mg) by the animal weight (g). Then, the ventricle was fixed for 24 hours in 10% formalin with the aorta. They were then embedded in paraffin for histological sections of four micrometers thick, according to Junqueira et al. (1979). In the immunohistochemical evaluation, histological sections of the aorta and the left ventricles were treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Nonspecific sites were blocked with 2% skimmed milk diluted in 10 mM PBS (phosphate-buffered saline) pH 7.4. The slides were incubated for 12 hours with rabbit polyclonal anti-CD40L antibody (Santa Cruz[®] 1:50) in a moist chamber. After incubation with primary antibody, incubation was performed with biotinylated secondary antibody (Dako[®] LSAB + kit) for one hour at 37 °C. To highlight immunoreactive areas, the sections were incubated with the peroxidase conjugated

complex (Dako[®] LSAB +) for 45 minutes at 37 °C and placed in a solution of chromogen (50 mg of DAB in 50 ml of PBS with 3 ml of 10% hydrogen peroxide) for three minutes. After counterstaining with Harris hematoxylin (Sigma[®]) for 25 seconds, the slides were mounted and analyzed by light microscopy. Photomicrographs were analyzed by LGMC-image software version 1.0 and the fractional percentages of the immunoreactive area to aorta and myocardium CD40L were acquired (Armstrong et al., 1998).

For analyzes of morphological and morphometric alterations, histological sections of the aorta and the left ventricles were stained with hematoxylin / eosin and picosirius red for qualitative analysis of collagen in the left ventricular wall. Four photomicrographs were obtained from the same pre-established point of the cross sections of the aortas of each animal using the digital camera coupled to the Leica IM50 program (version 1.20). Sections stained with picosirius red were analyzed with non-polarized light. Each photomicrograph was analyzed and areas marked in red with picosirius red were analyzed qualitatively. The LGMC-image vs 1.0 software, programmed to recognize colors and distinct shades, was used to highlight pixels of a particular color or specify shades within the field. The software highlighted a particular color within the field (based on the operator's threshold settings) and calculated the occupied area. Nontissue spaces of the field were recognized (with operator-threshold settings) by the software and subtracted to provide the correct area of total tissue in the field. The ratio of collagen deposit area to total tissue area ($\times 100\%$) was then calculated to provide a measure of percent area. Percent average was represented by the average of 11 fields from each histological section. In another analysis, the myocyte diameter within the field was measured using standard criteria. A point-to-point perpendicular line was placed across the longitudinal axis of the myocyte at the level of the nucleus, and this diameter was then measured by computer-imaging software. All longitudinally directed cardiomyocytes with a distinct cell border (at the level of the nucleus) within the sampling field were measured and averaged to provide a mean cardiomyocyte diameter. Transverse or oblique cut cardiomyocytes were excluded. We analyzed 5 different and complete cross-transverse cuts per ventricle, which produced 15 to 20 measurements of cardiomyocyte per histological section. The total measurements were 75 to 100 cardiomyocytes per animal.

Aorta were processed as previously described . Since early lesion were localized in the aortic root and ascending aorta, this area was chosen for analysis using serial transversal sections. Image Pro Plus Software (version 3) for image analysis (Media Cybernetics) was used. Each aortic lesion area was expressed as the sum of lesion areas from 10 equally spaced sections/mouse. Results from six mice/group are expressed in μm^2 . A single examiner performed the histological analyses under a double-blinded scheme.

2.5. Statistical methods

The data were expressed as mean \pm SEM. The variance analysis (ANOVA) followed by the Tukey test were used to compare the means of different groups. The value of $p < 0.05$ indicated significant differences.

3. Results

In the analysis of lipid profile, it was not observed significant differences in total cholesterol serum levels (TC) and the LDLc fraction of animals in the four experimental groups (Table 1). However, it was observed an increase of HDLc serum levels in mice of NAT, PRO and HL + NAT + PRO when compared with the ones from the HL group (Table 1). Nevertheless, mice of the PRO group had higher serum levels of this fraction compared with the mice of HL + NAT + PRO group. The mice of the latter showed greater HDLc serum levels than the mice of the NAT group (Table 1). With respect to serum levels of VLDLc fraction and TG, it was observed a decrease in mice from NAT and PRO groups compared to the HL and HL + NAT

+ PRO groups (Table 1). Yet, the TC / HDL, LDL / HDL and TG / HDL relations were lower in the PRO and HL + NAT + PRO when compared to the HL group (Table 1).

In morphological and morphometric analysis of the aorta, it was found that swimming and propolis alone in mice of NAT and PRO respectively prevented the development of atherosclerotic lesions by preventing the increase of the lesion area when compared to HL group (Table 2 and Figure 1). This was also followed by the prevention of the inflammatory process in the aorta, in which the aorta of the NAT and PRO mice exhibited a lesser immunoreactive area for CD40L when compared to HL group (Table 2 and Figure 1). The mice that underwent swimming and were treated with propolis, HL + NAT + PRO group, showed an area of injury similar to the one of HL and HL + NAT + PRO groups, but on the other hand, they showed a smaller immunoreactive area for CD 40L when compared with the NAT and PRO groups (Table 2 and Figure 1).

In morphological and morphometric analysis of the left ventricle, it was discovered that swimming and propolis alone or in mice of NAT and PRO respectively prevented

Table 1. Comparison of total cholesterol (TC) serum levels and its HDLc, LDLc and VLDLc fractions, Triglycerides (TG) and the TC / HDLc, LDLc / HDLc, TG / HDL relations among HL (hyperlipidemic), HL + NAT (hyperlipidemic submitted to swimming), HL + PRO (hyperlipidemic treated with propolis) and HL + NAT + PRO (hyperlipidemic submitted to swimming and treated with propolis) mice.

Parameters	HL Group	HL+NAT Group	HL+PRO Group	HL+NAT+PRO Group
N	10	10	10	10
Total Cholesterol (mg/dL)	869 \pm 15 ^a	868 \pm 23 ^a	882 \pm 25 ^a	918 \pm 11 ^a
HDLc (mg/dL)	20 \pm 1 ^a	36 \pm 6 ^b	75 \pm 5 ^c	57 \pm 7 ^d
LDLc (mg/dL)	814 \pm 29 ^a	793 \pm 21 ^a	763 \pm 64 ^a	840 \pm 20 ^a
VLDLc (mg/dL)	54 \pm 4 ^a	34 \pm 2 ^b	31 \pm 2 ^b	46 \pm 5 ^a
TG (mg/dL)	271 \pm 22 ^a	169 \pm 11 ^b	156 \pm 11 ^b	244 \pm 27 ^a
TC/HDLc	44 \pm 2 ^a	31 \pm 4 ^b	13 \pm 1 ^c	17 \pm 2 ^c
LDLc/HDLc	38 \pm 2 ^a	31 \pm 4 ^a	11 \pm 1 ^b	16 \pm 2 ^b
TG/HDLc	14 \pm 2 ^a	5 \pm 1 ^b	2 \pm 0.2 ^b	4.5 \pm 0.5 ^b

(ANOVA + Tukey test). Data were expressed as mean \pm Standard Error of the Mean (SEM). Same superscript letters in rows do not differ by Tukey test ($p < 0.05$).

Table 2. Comparison of aortic atherosclerotic lesion area, percentage of immunoreactive area for CD40L in the aorta and myocardium / endocardium, the left ventricular weight (LVW) / Animals Weight (AW) ratio and the diameter of cardiomyocytes from HL (hyperlipidemic), HL + NAT (hyperlipidemic submitted to swimming), HL + PRO (hyperlipidemic treated with propolis) and HL + NAT + PRO (hyperlipidemic submitted to swimming and treated with propolis) mice.

Parameters	HL Group	HL+NAT Group	HL+PRO Group	HL+NAT+PRO Group
N	10	10	10	10
Atherosclerotic lesion area (μm^2)	5552 \pm 1643 ^a	484 \pm 398 ^b	195 \pm 167 ^b	109 \pm 109 ^b
Immunoreactive area % for CD40L in the aorta	7.3 \pm 0.4 ^a	4.3 \pm 0.8 ^b	3.3 \pm 0.2 ^b	1.8 \pm 0.4 ^c
Immunoreactive area % for CD40L in the myocardial/endocardial	5.2 \pm 0.4 ^a	1.8 \pm 0.2 ^b	2.4 \pm 0.5 ^b	0.5 \pm 0.1 ^c
LVW/AW (mg/g)	4.5 \pm 0.1 ^a	3 \pm 0.1 ^b	2.9 \pm 0.1 ^b	3.2 \pm 0.1 ^b
Diameter of cardiomyocytes (μm)	25 \pm 0.6 ^a	23 \pm 0.7 ^{a,b}	20 \pm 0.8 ^b	21 \pm 1 ^b

(ANOVA + Tukey test). Data were expressed as mean \pm Standard Error of the Mean (SEM). Same superscript letters in rows do not differ by Tukey test ($p < 0.05$).

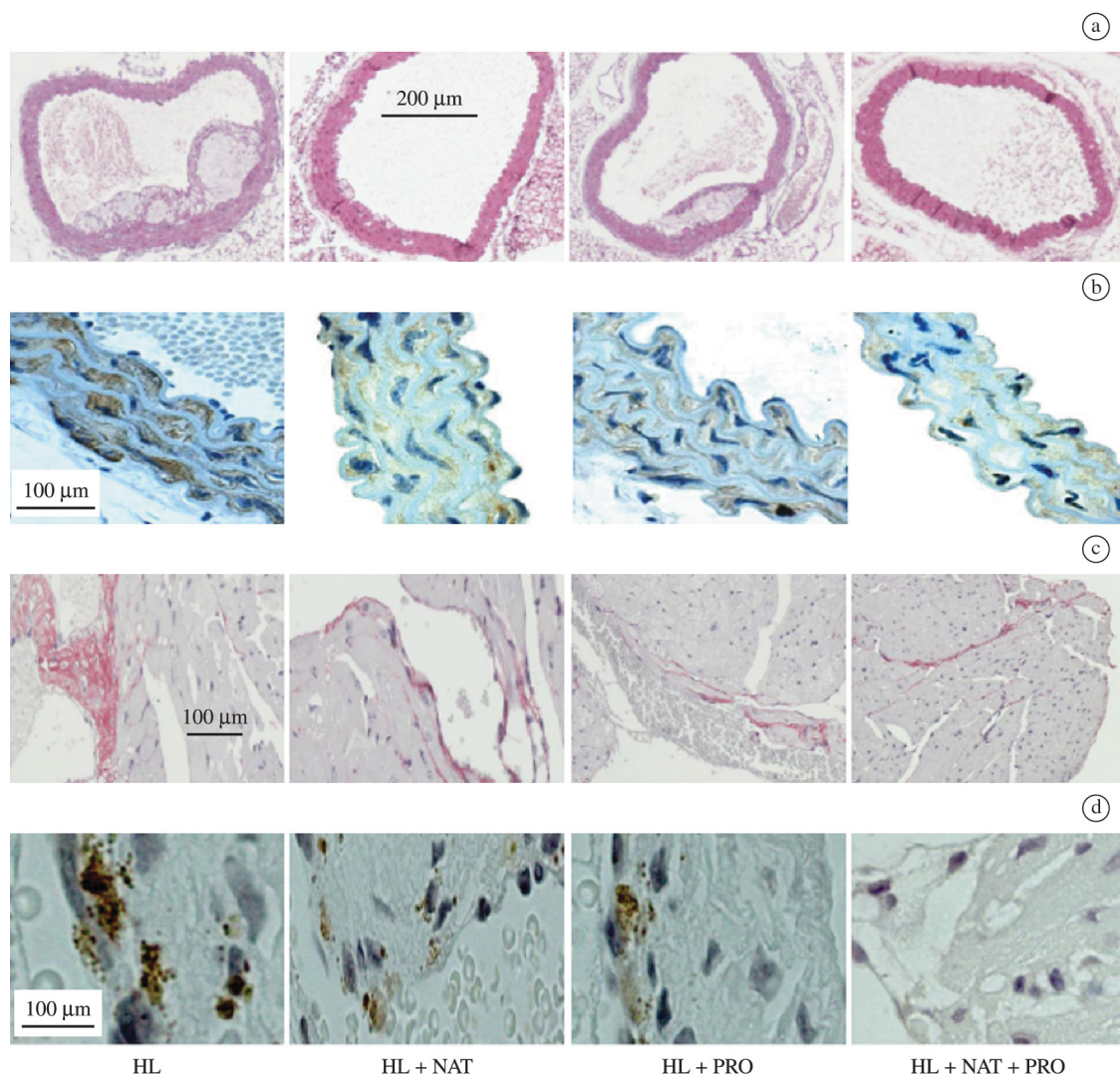


Figure 1. Photomicrographs of histological sections of the aorta (panel a: stained with HE showing atherosclerotic lesion area), Photomicrographs of transverse histological sections of the aorta (panel b: immunoreactive area for CD40L) Photomicrographs of myocardial / endocardial histological sections (panel c:stained with red picosirius showing interstitial collagen deposition) Photomicrographs of myocardial / endocardial histological section (panel d: immunoreactive area for CD40L in HL mice (hyperlipidemic), HL + NAT (hyperlipidemic submitted to swimming), HL + PRO (hyperlipidemic treated with propolis) and HL + NAT + PRO (hyperlipidemic submitted to swimming and treated with propolis).

the development of left ventricular hypertrophy, when compared with the HL group (Table 2 and Figure 1), preventing collagen deposition (Figure 1) without changing the cardiomyocytes diameter in the NAT group (Table 2). However, the diameters of ventricular cardiomyocytes of mice of PRO and HL+ NAT + PRO (Table 2) did not increase. The mice in NAT and PRO groups exhibited a lesser immunoreactive area for CD40L when compared to the HL group, showing a preventive effect in inflammatory processes of the myocardium/ endocardium in isolated conditions. However, when in associated conditions with the HL + NAT + PRO group, this effect was more exacerbated, presenting a smaller immunoreactivity area

in these mice when compared to the NAT and PRO groups (Table 2 and Figure 1).

4. Discussion

Studies in our laboratory showed that LDLr^{-/-} mice with standard diet for rodents showed increased HDL serum levels, when compared with its C57Bl6 background, which prevented the inflammatory process and the insulin resistance and consequently showed resistance to the development of atherosclerotic lesions (Garcia et al., 2011). In the present study, the LDLr^{-/-} mice fed with high-fat diet showed severe dyslipidemia associated with pronounced reduction

in serum levels of HDL and the subsequent development of cardiovascular inflammation process, characterized by the marked expression of CD40L both in the left ventricle and the aortic wall, formation of atheromatous plaque and left ventricular hypertrophy (LVH). The decrease in HDL serum levels associated with hypercholesterolemia, hypertriglyceridemia and the inflammatory process may be the common denominator for the atherogenesis and LVH in mice from the HL group in this study. Moreover, LDLr^{-/-} mice fed with hyperlipidemic diets showed insulin resistance (Garcia et al., 2011) and increased oxidative stress (Krieger et al., 2006), which sustain the atherogenesis and LVH in this study.

With the appearance of oxidative hypothesis for atherosclerosis (Steinberg et al., 1989; Meilhac et al., 2001), experiments *in vitro*, in animal and human models, have shown that oxidized lipids present pro-atherogenic effects (Meilhac et al., 2001). In early events of the atherogenic, oxidized low density lipoprotein (oxLDL) is rapidly internalized and accumulated in macrophages, thus forming the foam cells, which are found and deposited in the subendothelial space (Lusis, 2000; Libby, 2002). The oxLDL is cytotoxic for endothelial cells, promoting the expression of cytokines and cell proliferation, inhibiting vascular relaxation induced by nitric oxide (NO) and thereby triggering a cascade of inflammatory responses (Smith, 2001). According to this hypothesis, the increase in atheroma area of HL mice in the present study, associated with severe dyslipidemia was due to the inflammatory process, determined by the increased expression of CD40L with consequent generation of reactive oxygen species (ROS) and lower bioavailability of the NO, which induced an increase of LDL oxidation and atherogenesis.

Imbalance between production and removal of reactive oxygen species (ROS) is called oxidative stress. Several physiopathological and genetic situations can induce cardiac oxidative stress, such as hypercholesterolemia (Sato et al., 2004), mechanical stress in the myocardium (Aikawa et al., 2001) and inflammatory processes (Yao et al., 2006). Therefore, the marked cardiac inflammatory process in HL mice associated with oxidative stress due to the increased expression of CD40L in cardiac tissue and subsequently reduced bioavailability of nitric oxide (NO) hold the pathway of left ventricular hypertrophy observed in the HL mice in this study, characterized by the increase in collagen deposition and cardiomyocyte diameters.

In the present study, swimming and propolis alone and / or associated prevented LVH, atherogenesis, ventricular and arterial inflammation, decreasing the CD40L expression and increasing the plasma levels of HDLc.

Studies have shown that regular physical activity promotes the reduction of the inactivation of nitric oxide (Fogarty et al., 2004; Traverse et al., 2000), increases the production of nitric oxide (Takamura et al., 2002), increases the expression of endothelial nitric oxide synthase (eNOS) (Griffin et al., 2001) and promotes angiogenesis induced by vascular endothelial growth factor, and, with the net effect of increasing the bioavailability of nitric oxide.

The increased bioavailability of nitric oxide in response to the decreased expression of CD40L in this study may be the factor that prevented ventricular hypertrophy and atherogenesis in mice of NAT group. Therefore, LVH was attenuated by (1) an anti-inflammatory effect due to HDLc serum increase, detected as a decrease in CD40L overexpression, as well as fibrosis because decreasing CD40-CD40L activity could lead to less severe vascular inflammation, which in turn would lead to less fibrosis and cardiac derangements, (2) antioxidant activity and consequent decrease in oxidative stress, which resulted in anti-inflammatory effects and a return to homeostasis. Furthermore, the NO / cGMP pathway activates the sarcolemmal KATP channels (Baker et al., 2001), with probable inhibition of 70-kDa S6 kinase, attenuating the hypertrophic response in hyperlipidemic models (Lee et al., 2004).

The prevention of atherogenesis by swimming in the present study was due to the lower oxidative stress and increased bioavailability of the NO in response to the increase in HDL serum concentration and increase of shear stress on the vascular wall caused by the increase in cardiac output while swimming in animals of NAT group. The HDL cholesterol is a lipoprotein that carries out the reverse cholesterol transport, removing the excess of free cholesterol not only from cell membranes but also from the subendothelium and transporting it to the liver to be degraded (Shils et al., 2005), in addition to its anti-inflammatory and antioxidant effect (Silva et al., 2006). The anti-inflammatory and antioxidant effect of HDLc in NAT Group was visualized by the decrease in the immunoreactive area to CD40L in the aorta and myocardium / endocardium of these animals.

In our studies, propolis [total phenols (9,281±0,029%), total flavonoids (2.685±0.001%) and beeswax (14.514±0.144%)] alone or associated with swimming increased plasma levels of HDL and decreased expression of CD40L in both myocardium and aortic wall, preventing LVH and atherogenesis. Studies have shown that propolis is a natural antioxidant basically composed of flavonoids and phenolic compounds, which decrease the concentration and activity of superoxide dismutase (SOD), reducing lipid peroxidation (Marcucci, 1995; Isla et al., 2001; Krol et al., 1990) in the initiation stage, because they act as antioxidants, eliminating superoxide and hydroxyl radicals. It is proposed that flavonoids interrupt the chain reaction of free radicals by donating hydrogen atoms to peroxy radical, forming a flavonoid radical. The flavonoid radical then reacts with the free radical ending in this manner the propagation of the chain reaction (Bouziotas et al., 2004). The increase of HDL, an antioxidant and anti-inflammatory (Holvoet, 2008), in PRO and HL + PRO + NAT mice in this study explains the protective effect of propolis on the cardiovascular system, reducing oxidative stress and lipid peroxidation, preventing atherogenesis and LVH in these animals.

In Conclusion, propolis and Swimming alone and/or associated prevented the LVH, atherogenesis and arterial

and ventricular inflammation, decreasing the CD40L expression and increasing the HDLc plasmatic levels. Propolis alone or associated with a regular physical activity is beneficial in cardiovascular protection through anti-inflammatory action.

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