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Short Communication

Use of spice extracts and its impact on lipid profile *in vivo*

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Abstract

This study aimed to evaluate the antioxidant and hypolipemiant effect of oregano and thyme oleoresins under the lipid profile and the oxidation of the LDL fraction in vivo. Wistar rats were used and fed with a hypercholesterolemic diet and oleoresin supplementation by gavage, for 40 days. Analyses of the lipid profile and LDL oxidation were performed. The isolated administration of oleoresins significantly reduced the values of total cholesterol when compared to the group that received only the hypercholesterolemic diet. The supplementation with oregano oleoresin was the most effective one, regarding the reduction of total cholesterol.

Introduction

The control of the accelerated global growth of cardiovascular diseases is a big challenge. In order to change this reality, it is necessary to focus on the reduction of risk factors, including hypercholesterolemia, which is considered to be one of their main causes (Salgado *et al*., 2008). According to guidelines from the National Cholesterol Education Program, it is estimated that, for each 1% reduction on blood cholesterol concentration, the risk of heart diseases is reduced in 2%. The dietary therapy is the first step to reduce blood cholesterol, which can favor a slow reduction of 10-13% of low-density lipoprotein (LDL). Consequently, a safe and well tolerated diet supplement may be useful in the non-pharmacological handling of hypercholesterolemia, aiming for a faster reduction of LDL (Grundy *et al*., 1993; Maki *et al*., 2001).

Although the positive relation between dyslipidemias and the development of cardiovascular diseases, particularly of atherosclerosis, is highly documented, studies indicate that, besides altering the lipid profile, oxidative modifications of lipoproteins are essential to the development and progression of atherosclerosis (Toshima *et al*., 2000; Huthe and Fagerberg, 2002).

Among the lipid metabolic disorders, one of the hypotheses of why the injury of atherosclerosis occurs proposes that the first step of the atherogenesis is the induced endothelial dysfunction, especially through the vascular edothelium exposure to the oxidized low-density lipoprotein (LDL-ox). The high-density lipoproteins (HDL) play a protective role against the development of atherosclerosis, inhibiting the oxidation of LDL (Assmann and Nofer, 2003).

A diet which is rich in antioxidants prevents oxidative stress and reduces the formation and absorption of peroxides and their decomposition products (Ursini and Sevanian, 2003). Scientific evidences indicate that foods contain physiologically active substances that are necessary for the promotion of health and prevention of diseases. Therefore, the diet compounds are fundamental to the oxidative stress modulation, determining the role of nutrition in the modification of atherosclerosis risk (Rani *et al*., 2013).

Among the several natural products being studied, herbs and spices stand out, due to their facility for incorporating in foods and their high antioxidant activity already mentioned (Yanishlieva *et al*., 2006). The oregano extracts present high content of polyphenols and intense antioxidant property, acting in an effective way in the prevention of all stages of the oxidative process, from neutralization of free radicals, to blockage of peroxidation catalyzed by iron and, finally, the interruption of the lipid radical in the reaction chain (Aranha and Jorge, 2012; Rotolo *et al.*, 2013). According to Teixeira *et al.* (2013) the oregano extracts and essential oil

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have strong potential to be used as alternatives to synthetic chemicals in industries whereas oxidation and microbial contamination are problems.

As well as in oregano, the phenolic compounds thymol and carvacrol are the main compounds of thyme extracts. Researches, over thyme essential oil, highlight the moderated inhibition of LDL oxidation (20-27%) (Rani *et al.*, 2013). Thus, this study aimed to evaluate the antioxidant and hypolipemiant effect of oregano and thyme oleoresins, under the lipid profile and oxidation of the LDL fraction in vivo.

Materials and Methods

The project was submitted to the Animal Experimentation Ethics Committee from São Paulo State University, UNESP - Botucatu Campus, São Paulo, Brazil, according to what is presented in the report from memorandum nº 21/07 - CEEA.

Animals and diets

In this study, 40 male Wistar rats (30 days old) coming from the animal facility laboratory of Centro Universitário de Rio Preto – UNIRP, São José do Rio Preto, São Paulo, Brazil, were used. The animals remained in collective cages made of polypropylene, in a temperature-controlled environment of 23 ± 2ºC, in clear/dark cycle of 12 hours with water and diet ad libitum. They were fed during a period of 40 days, including adaptation time of 5 days. Experimental diets were prepared by the company Pragsoluções Biociências, according to the standard diet for rodents, American Institute of Nutrition - AIN-93 G -Table 1 (Reeves *et al.*, 1993).

The materials used as supplements were spice extracts from oregano (*Oreganum vulgare* L.) and thyme (*Thymus vulgari*s L.) in form of oleoresins, supplied by Kalsec®, and their components are described in Table 2. Five groups were formed, each one with 8 animals distributed at random. The animals received the following treatments: Group 1 - standard diet; Group 2 - hypercholesterolemic diet; Group 3 - hypercholesterolemic diet and oregano oleoresin supplementation (weighing 16 mg/kg); Group 4 - hypercholesterolemic diet and thyme oleoresin supplementation (weighing 16 mg/kg) and Group 5 - hyperchlesterolemic diet and supplementation of the mixture of both oleoresins (16 mg/kg of each oleoresin).

The oleoresins were diluted in soybean oil without the addition of synthetic antioxidants. After the adaptation period, gavage was performed every day in all groups, due to the fact that it is a stress-generator process. Groups 1 and 2 were supplemented with soybean oil, so that the same energetic ingestion was maintained among the groups. The ingestion and weight of the animals were daily monitored during the whole assay in order to obtain the calculation parameter of the oleoresins that were administered and to analyze the weight gain curve and the feed efficiency of the diet (weight gain/diet consumption). The average was used as measurement of central tendency of each group.

Table 1. Composition of diets (%) used in the assay

Ingredients	Standard Diet (AIN-93G)	Hypercholesterolemic Diet
Corn starch	39.75	39.75
Casein (Labsinth)	20.00	20.00
Starch dextrinated	13.20	13.20
Saccharose	10.00	10.00
Soybean oil	7.00	1.00
Lard	-	5.00
Cholesterol (Sigma)	-	1.00
Microcrystalline cellulose	5.00	5.00
Mineral mix	3.50	3.50
Vitamins mix	1.00	1.00
L-cystine	0.30	0.30
Choline bitartrate	0.25	0.25
Butylated hydroxytoluene	0.0014	0.0014

After the experiment and a 12 hour fast, the animals were anesthetized by inhalation with ethyl ether, and blood samples were collected by cardiac puncture in order to have the lipid profile analyzed concerning the levels of total cholesterol, fractions (HDL, LDL and VLDL), triglycerides and LDL-ox.

In vivo *analyses*

The serum dosages to determine the lipid profile, content of total cholesterol, HDL fraction and triglycerides were performed with enzymatic kits provided by Katal Biotecnológica. The LDL fraction was determined according to the Friedewald et al. (1972). The LDL oxidation was determined by the method of indirect solid-phase immunoenzymatic assay (ELISA), through the determination of auto-antibodies against oxidized LDL (LDL-ox) (Havel *et al.*, 1955; Puhl *et al.*, 1994; Fernvik *et al.*, 2004).

Statistical analysis

Results of the biological assay (Total cholesterol, HDL, LDL, triglycerides and LDL-ox) were subjected to analyses of variance using delineation in casualized blocks with 8 repetitions. Analyses of variance and Tukey tests for the averages at 5% were obtained through the ESTAT program (Statistical Analyses System), version 2.0 (UNESP, Jaboticabal, São Paulo, Brazil).

Results and discussion

According to De Luca *et al.* (1996), the average rate of feed ingestion for rats varies from 12 to 15 g per day. An average ingestion of 11 g per day was observed in the five groups studied, therefore being a little lower than the values reported by literature. During 40 days of treatment, the total consumption of feed among the animals varied from 410 to 471 g. However, this variation was not enough to influence animal weight gain. Average values of body weight gain in function of different diets and supplementations used are presented in Table 3, as well as the Feed Efficiency Coefficient (FEC) obtained from the ratio between animal weight and feed consumption.

It is possible to observe that group 3 (Table 3), supplemented with oregano oleoresin, presented FEC value lower than the control group. However, such value was similar to those presented by the other groups. These data indicate that the compounds present in oleoresins did not interfere in the employment of nutrients present in the feed. Data also demonstrated that the supplementation enabled animals to develop and grow normally, excluding the possibility of an anti-nutritional effect of oleoresins.

Table 4 presents the averages of serum biochemical parameters of animals in function of the different diets and supplementations used. Wistar rats can be resistant to developing hypercholesterolemia and atherosclerosis, possibly due to the increase in the conversion of cholesterol into biliary acids in the liver (Machado *et al.*, 2003). Such resistance was not confirmed in this study. It was possible to observe a sharp increase of total cholesterol (60%) and LDL fraction (97%) with the ingestion of hypercholesterolemic diets (group 2) comparing to the control group.

Reactivity Index (RI), of the animals regarding the different diets and supplements used.

According to Table 4, the isolated administration of oleoresins in groups 3 and 4 significantly reduced the values of total cholesterol when compared to group 2, which only received a hypercholesterolemic diet. The supplementation with oregano oleoresin was the most effective in reducing cholesterol, not differing from group 1, which received a normocholesterolemic diet. However, it is possible to observe that the mixture of oleoresins was not effective in promoting the reduction of total cholesterol. This fact can be related to the high dose of supplemented bioactive compounds, which is due to the combination of the oleoresin concentrations.

Table 2. Constituents of oleoresins (%) studied

Components	Oleoresins	
	Oregano	Thyme
Natural extract	60 - 70	55 - 65
Canola oil	30 - 40	35 - 45
Mono and diglycerides	-	0 - 10

Ribeiro *et al.* (2010) highlight that groups of animals that receive high concentrations of bioactive compounds as a supplementation to the diet may present pro-oxidant effect, increasing lipid peroxidation in the serum and potentialization of the oxidative stress. However, the use of doses which are equivalent to the human consumption level may have the effect of antioxidant protection.

Regarding the HDL-c fractions, it is possible to observe that groups with oleoresin supplementation presented higher values, differing significantly from groups 1 and 2. Group 3, supplemented with oregano oleoresin, presented an average HDL value which is 17% higher than LDL value. This relation is opposite to what was found in the other groups that were supplemented by oleoresins, in which HDL is lower than LDL. Besides that, the atherogenic index obtained by the relation LDL/total cholesterol (De La Cruz *et al*., 2000) was lower in group 3 (0.37), which reinforces the fact that the supplementation with oregano oleoresin reduces the risk of cardiovascular diseases (CVD), considering that LDL increase has an essential role in the pathogenesis of atherosclerosis. The average values for the other groups were 0.54; 0.66, 0.56 e 0.54 for groups 1, 2, 4 and 5 respectively.

Rodrigues *et al*. (2003) found similar values for the LDL/total cholesterol relation in animals treated with antioxidant nutritional supplementation (0.30) in study concerning the effect of flavonoid rutin in Wistar rats. Results are in agreement with those obtained by Assmann and Nofer (2003), whose studies obtained significant reduction of total cholesterol and triglycerides, besides LDL, as well as increase of HDL levels in the plasma of hyperlipidemic rats, induced by diet, when treated with different flavonoids.

In this study, the supplementation of oleoresins did not present positive effects in relation to the triglycerides indexes, since groups 3, 4 and 5, which received such supplementation, did not differ from group 2, remaining with higher triglycerides than in group 1, control.In the auto-antibodies dosage by ELISA, the LDL oxidized by the copper ion is fixed in the micro porous of the plate as an antigen, where the antibodies, if present in the sample, will be specifically linked. The levels of auto-antibodies provide indirect evidence of the importance of LDL-

Table 3. Mean values of body weight gain and Feed Efficiency Coefficient (FEC) of the animals regarding the different diets and supplements used

Parameters	Groups				
	1	2	3	4	5
Weight gain (g)	194[a]	192[a]	193[a]	185[a]	187[a]
FEC	0.47[a]	0.43[ab]	0.41[b]	0.42[ab]	0.42[ab]

Group 1: standard diet; Group 2: hypercholesterolemic diet; Group 3: hypercholesterolemic diet and oregano oleoresin supplementation (weighing 16 mg/kg); Group 4: hypercholesterolemic diet and thyme oleoresin supplementation (weighing 16 mg/kg) and Group 5: hyperchlesterolemic diet and supplementation of the mixture of both oleoresins (16 mg/kg of each oleoresin). (n = 8/group). Means followed by same letter do not differ by Tukey test (p > 0.05).

Table 4. Mean values of serum biochemical parameters and the determination of autoantibodies against LDL-ox, expressed as Reactivity Index (RI), of the animals regarding the different diets and supplements used

Biochemical parameters	Groups				
	1	2	3	4	5
Total cholesterol (mg/dl)	65[c]	104[a]	67[c]	80[bc]	91[ab]
HDL (mg/dl)	23[b]	24[b]	30[a]	25[a]	31[a]
LDL (mg/dl)	35[bc]	69[a]	25[c]	45[b]	49[b]
Triglycerides (mg/dl)	35[b]	59[a]	62[a]	52[a]	53[a]
LDL-ox	1.2665[a]	1.2175[a]	1.3605[a]	1.2459[a]	1.3419[a]

Group 1: standard diet; Group 2: hypercholesterolemic diet; Group 3: hypercholesterolemic diet and oregano oleoresin supplementation (weighing 16 mg/kg); Group 4: hypercholesterolemic diet and thyme oleoresin supplementation (weighing 16 mg/kg) and Group 5: hyperchlesterolemic diet and supplementation of the mixture of both oleoresins (16 mg/kg of each oleoresin). (n = 8/group). Means followed by same letter do not differ by Tukey test (p > 0.05).

ox in cardiovascular disease (Duarte *et al.*, 2008).

Table 4 presents the Reactivity Index (RI) averages for all groups based on the different diets and supplementations used. The average values of auto-antibodies against LDL-ox are not significantly different among themselves. However, it is possible to observe a tendency to increase auto-antibodies against LDL-ox in the groups supplemented with oregano oleoresin.

The relation between the levels of these antibodies and the coronary artery disease is inconsistent. Some studies indicate a positive correlation between the levels of these antibodies and the coronary artery disease, while others do not show any associations (Leinonem *et al.*, 1998; Sherer *et al.*, 2001). In spite of the plausible role of oxidative stress in the atherosclerosis pathogenesis, many controlled prospective studies did not indicate a reduction of the coronary artery disease with the use of antioxidant supplements (Yusuf *et al.*, 2000).

Oliveira *et al.* (2000) studied the effect of vitamin E over levels of auto-antibodies against LDL-ox in hyperlipidemic hamsters, and verified that the groups that were fed with a hypercholesterolemic diet presented three times increase in serum cholesterol, and two times increase in triglycerides concerning the control group. The supplementation of vitamin E did not change the levels of serum lipoproteins, but led to a smaller formation of auto-antibodies against LDL-ox.

Conclusion

In the evaluation in vivo, the results are promising concerning the future use of oregano oleoresin supplementation to control the lipid metabolism, especially in the reduction of total cholesterol and the increase of HDL; consequently, it may play a secondary role in the prevention of cardiovascular disorders. However, further studies should be conducted to identify and quantify the bioactive molecules present in each oleoresin.

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