Hypolipidemic Effect of Flavonoids and Cholestyramine in Rats

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SUMMARY. The present work evaluates the action of quercetin, rutin, morin and naringenin isolated or/and in association with cholestyramine on blood lipids of rats. These compounds were mixed to the diet (mark purina) containing 1% of cholesterol and 0.1% of cholic acid and they were administered in the dose of 30 mg for the flavonoids and 10 mg for cholestyramine. After thirty days, cholesterol, cholesterol-HDL and triacylglycerols were measured after retreat of blood. Results evidence that cholestyramine in association with quercetin present the largest percentual reduction of cholesterol. On the other hand, for cholesterol-HDL the level increase in the association of cholestyramine and naringenin showed a synergic effect, while the results for triacilglicerols indicated a reduction in all treatments being the best results obtained with association of cholestyramine and flavonoids.

RESUMEN. "Efectos Hipolipidémicos de Flavonoides y Colestiramina en Ratas" . Este trabajo evalúa la acción de quercetina, rutina, morina y naringenina aisladamente y en asociación con colestiramina en el suero sanguíneo de ratas. Estos compuestos fueron mezclados a la dieta (Marca Purina) conteniendo 1% de colesterol y 0,1% de ácido cólico y las sustancias fueron administradas en la dosis de 30 mg para los flavonoides y 10 mg para la colestiramina. Después de treinta días se determinó el contenido de colesterol, colesterol-HDL y triacilgliceroles en sangre. Los resultados evidencian que colestiramina en asociación con quercetina presentaran la mayor reducción de colesterol. Por otro lado, para colesterol-HDL los niveles fueron aumentados en la asociación de colestiramina y naringenina, presentando un efecto sinérgico, mientras los resultados para triacilgliceroles indicaron una reducción en todos los tratamientos, siendo la asociación de colestiramina con flavonoides la que ha mostrado los mejores efectos.

INTRODUCTION

Flavonoids are compounds widely distributed in nature [1], they are present in fruits, cereals, teas, coffee, wine and bear. They are not considered as nutrients, but due to their health promoting properties, such as anticarcinogenic [2], antioxidant [3,4], antiinflamatory [5], hypolipidemic [6-10], antiatherogenic and antitrhrombotic actions [11], they are classified into the group of functional foods [12].

Cell culture studies show that flavonoids inhibit LDL macrophage oxidation and therefore are antiatherosclerotic compounds present in the diet [13]. The literature [14-16] reported several activities for flavonoids mainly their antioxidant effects, responsible for atherogenic and throm-bogenic phenomena, and a series of metabolic and tissue disorders related to the lipid oxidation, such as changes in membrane related functions (enzymes, receptors, permeability), protein polymerization, DNA mutation, atherogenesis, changes on atherogenic and thrombogenic macrophage and platelet functions and on araquidonic acid cascade reactions. They also inhibit oxidative enzymes (phospholipase A2, ciclooxygenase and lipoxigenase).

MATERIALS AND METHODS

Male rats of the Wistar race, provided by the Department of Nutrition of Universidade Federal de Viçosa, weighing 200 ± 20 g, received water and food *"ad libitum"*. After five days adapta-

tion period, under the control of light and darkness of 12 h, the animals were separated in eleven experimental groups with eight animals in each. They were randomly distributed to receive the treatment shown in Table 1.

One group of animals received a Control Diet during the experimental period (PURINA Mark). The other groups received the Control Diet including 1% cholesterol (SIGMA) and 0.1% cholic acid in order to induce hypercholesterolemia. A daily oral dosis of 30 mg of the

flavonoids (quercetin, rutin, morin or naringenin) and / or 10 mg cholestyramine was given mixed to the diets of each group, except for groups 1 and 2 during 30 days. On the 31st, blood samples were taken by heart puncture. These materials were centrifuged at 7161.6 g.min⁻¹ to obtain the serum, which was analyzed for cholesterol and triacylglycerols, using enzymatic method described by Henry [17]. The method describes by Lima [18] was used for cholesterol- HDL analysis, both from Biomerrieux. Quantitative analysis was made by a Hitachi spectrophotometer.

Group	Treatment
1	Control Diet
2	Control Diet + cholesterol + cholic acid
3	Control Diet + cholesterol + cholic acid + quercetin
4	Control Diet + cholesterol + cholic acid + rutin
5	Control Diet + cholesterol + cholic acid + morin
6	Control Diet + cholesterol + cholic acid + naringenin
7	Control Diet + cholesterol + cholic acid + cholestyramine
8	Control Diet + cholesterol + cholic acid + cholestyramine + quercetin
9	Control Diet + cholesterol + cholic acid + cholestyramine + rutin
10	Control Diet + cholesterol + cholic acid + cholestyramine + morin
11	Control Diet + cholesterol + cholic acid + cholestyramine + naringenin

Table 1. Experimental groups.

RESULTS AND DISCUSSION

Results of serum lipids in rats are given in the Table 2. They are expressed in mg.dl-1, with their respective percentual variations.

According to the results shown in Table 2, the cholesterol levels of the animals that received cholesterol and cholic acid with their diet raised by 360%. The cholesterol level of these hypercholesterolemic animals has reduced to the highest degree, so this is the most efficient treatment accomplished with group 8 (cholestyramine + quercetin) (48.18%), followed by naringenin (42.75%) group 11. Cholestyramine showed the lowest percentage of reduction (14,72%), group 7.

Results of HDL- cholesterol, evidence that the best treatment was accomplished with cholestyramine + naringenin (group 11), even no being statistically different. That is an advantage since HDL-cholesterol is responsible for the transportation of cholesterol from peripheric tissues to the liver in human metabolization.

Group (mg/dl)	Total cholesterol	% of Variation	HDL-cholesterol (mg/dl)	% of Variation	triacylglycerols (mg/dl)	% of Variation
1	36.54 ± 3.90	-	25.18 ± 2.27	-	80.91 ± 6.51	-
2	167.82 ± 4.49	-	55.70 ± 3.29	-	173.78 ± 3.98	-
3	116.90 ± 4.87 bcd	-30.34 *	50.94 ± 2.45 ab	-8.55	98.00 ± 5.32 ab	-43.61 *
4	130.89 ± 5.84 abc	-22.01 *	51.74 ± 2.08 a	-7.11	88.82 ± 4.74 b	-48.89 *
5	135.88 ± 5.27 ab	-19.03	43.67 ± 3.42 ab	-21.60 *	86.94 ± 4.96 b	-49.97 *
6	129.28 ± 5.54 abc	-22.97 *	53.74 ± 4.14 a	-3.52	98.47 ± 4.58 ab	-43.34 *
7	143.11 ± 6.69 a	-14.72	35.71 ± 3.64 b	-35.89 *	118.95 ± 8.41 a	-31.55 *
8	86.96 ± 4.69 e	-48.18 *	53.55 ± 4.94 a	-3.86	77.22 ± 2.65 b	-55.56 *
9	104.95 ± 5.09 de	-37.46 *	52.20 ± 3.83 a	-6.28	83.40 ± 3.88 b	-52.01 *
10	107.53 ± 5.95 cde	-35.93 *	48.76 ± 3.14 ab	-12.46	81.98 ± 3.67 b	-52.83 *
11	96.08 ± 4.78 de	-42.75 *	57.76 ± 3.78 a	+3.70	88.16 ± 5.56 b	-49.27 *

Table 2. Mean levels of cholesterol (±SE), HDL-cholesterol (±SE), and triacylglycerols (±SE), in the serum of Wistar rats and their respective percentual variation. Means followed by same letters do not differ from each other in Tuckey test (P>0.05). * Statistic different data differentiated from the control (ration + cholesterol + cholic acid) by using Dunnett's t: est (P<0.05).

Finally, the results for triacylglicerols analysis of rat serums indicated a reduction in all treatments. The association of cholestyramine with flavonoids has produced a better effect than cholestyramine alone.

Dietary cholesterol absorption, in rats, occurs mainly on the distal ileum [19] through solubilization in the mixed micelles. Without these micelles, cholesterol precipitates so its absorption reduces. They contain free fatty acid, monoacylglycerols, diacylglycerols, triacylglycerols and bile acids. Flavonoids acts as a cofactor of the enzyme cholesterol esterase, enhancing its activity [20].

It has been shown that quercetin and rutin reduce platelet thrombus formation in endothelial tissue of rabbit aorta, reducing collagen synthesis in this process. This mechanism is associated with their ability of reacting with free radicals, to increase prostaciclyne synthesis and to inhibit lipid peroxidation, which is responsible for platelet aggregation [11].

The hypolipidemic effect of cholestyramine is associated with the anion exchange (Cl⁻ ions) for negative charged bile acids. Since these resins are not absorbed, bile acids are excreted. This results in a lower bile acid uptake by the liver, and therefore in a higher conversion of cholesterol to bile acids in the liver. It is possible that a lower absorption of cholesterol and neutral steroids also occurs, due to a higher excretion of bile acids. This may increase the levels of liver LDL receptors and HMG CoA reductase activity. As a consequence, cholesterol content of the hepatocytes is restored by the increased uptake of plasma LDL, mediated by the increased expression of LDL receptors and by the increased biosynthesis of endogenous cholesterol. These reduces plasma LDL levels and recover bile acids production [21].

Cholestyramine also acts by enhancing the activity of the enzyme responsible for 7-α hydroxycholesterol synthesis, an intermediate product of bile acids in humans [22].

It is also known that rat livers may synthesize an easily degraded lipoprotein-like LDL. This explains the low levels of LDL in rats. Some HDL fractions are responsible for cholesterol transport to the tissues [19]. Because rats have HDL_1, HDL_2, and HDL_3, this facilitates its transport, and explains the faster cholesterol metabolism [23].

Flavonoids activate multi enzyme systems, such as citocrome P450 and b5 [24] and this action affects the whole metabolism, as these systems are involved in the metabolism of xenobiotics, including drugs, insecticides, and pollutants, that have great importance on pharmacology and toxicology. Due to this effect, flavonoids act on body lipid constituents like steroids and bile acids, and influence lipid metabolism. They increase bile acid excretion because cytocrome P-450 enzymes bind some compounds to the bile acids and therefore reduce cholesterol level in the body [25].

Gomes [6] reported the triacylglycerol-lowering effect of flavonoids, while Kato [26] showed that quercetin added to the diet for 15 days reduced triacyglycerol levels in rats.

The mechanism of action seems to be done trough activation of AMPc synthesis. AMP activates protein kinase and this enzyme increases triacylglycerol hydrolysis, and hence reduces its levels in blood and liver [27]. Flavonoids also activate LDL receptors, containing 7 to 10% triacylglycerol in their structure [28].

On the other hand cholestyramine acts in gut lumen, by binding to bile acids and increasing their excretion, while flavonoids reduce lipid levels either by increasing citocrome P 450 and b5 activities or by increasing cholesterol and bile acid excretion [24].

CONCLUSION

As a consequence flavonoids and cholestyramine show a hypolipidemic effect either alone or in association. These results suggest the potential use of flavonoids in drug treatment or prevention of cardiovascular disease.

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