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# Associations of Flavonoids and Natural Dyes in the Control of Lipidic Metabolism

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**RESUMEN. "Asociaciones de flavonoides y colorantes naturales en el control del metabolismo lipídico". El presente trabajo estudia el efecto de rutina y naringenina, administradas en forma aislada o asociadas con antocianina y monascus, sobre el metabolismo lipídico de ratas. Para eso, dichos compuestos fueron disueltos en propilenglicol y suministrados dos veces por vía intraperitoneal a la dosis de 5 mg/kg de peso. La primera dosis fué administrada inmediatamente después de la administración de Tritón, sustancia responsable por la inducción de la hiperlipidemia, y la segunda veinte horas después. Después de 43 horas de la administración de la primera dosis y del Triton, se extrajo sangre y se determinó el contenido de colesterol total, colesterol-HDL y triacilglicéridos. Los resultados han evidenciado mayores porcentajes de reducción del colesterol para naringenina + monascus y naringenina + antocianina, rutina + monascus y rutina + antocianina. En el caso del colesterol-HDL los mejores resultados fueron obtenidos con naringenina sola y finalmente para los triacilglicéridos las mayores reducciones fueron halladas con naringenina, naringenina + monascus y rutina + antocianina.**

*SUMMARY.* The present work evaluates the effects of rutin and naringenin, isolated and in association with anthocyanin and monascus, on lipidic metabolism of rats. These compounds were dissolved in propylene glycol and administered by intraperitoneal route in two doses of 5mg/kg of body weight. The first dose was administered together with the Triton, compound responsible for induction of hyperlipidaemia, and the second, twenty hours later. After fourty three hours of the first dose and Triton administration, the blood was retreat and cholesterol, HDL-cholesterol, and triacylglycerols were dosed. Results evidence the largest percentual reduction of cholesterol for naringenin + monascus, naringenin + anthocyanin, rutin + monascus and rutin + anthocyanin . On the other hand, for HDL-cholesterol, the best results were obtained with naringenin alone. Finally, the best reduction of triacylglycerols levels was showed for naringenin, naringenin + monascus and rutin + anthocyanin associations.

## INTRODUCTION

Being myocardial infarction one of the main causes of death in industrialized countries, research in the last four decades has sought to identify situations which may lead to an increase in the probability of occurrence of coronary thrombosis.

Preventive measures involving mainly tests with several vegetal and synthetic substances have also been investigated [1]. Research with rabbits which received glycosylated anthocyanin to the rate of 6g/Kg (orally) or 500mg/Kg (intraperitoneally) did not suffer any change in blood pressure, while diuretic and vasodilation effects [2] were observed for the 25 mg/Kg dose.

Blood circulation disorders were treated with pharmaceutical preparations containing anthocyanin [3]. It was also observed their participation in the process of formation of prostaglandins and endoperoxides such as prostacyclins, which inhibit platelet aggregation in the prevention of thrombosis [4].

Another class of natural dyes, monacolins, presented strong inhibitory action in the synthesis of cholesterol *"in vivo"* [5-9].

Some flavonoids, such as biochanin A, for-

PALABRAS CLAVE: Antocianina, Colesterol, Colesterol-HDL, hiperlipidémicos, lipidos, Monascus, Naringenina, Ratas, Rutina, Triacilglicéridos.
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monometine, taxifoline, and quercetin have attested hypolipidemic properties in rats [10,11]. They were also effective in the treatment of cardiovascular diseases [12].

The aim of the present work is to test in hyperlipidemic rats the hypolipemic effect of flavonoids and natural dyes isolated or in association.

## MATERIAL AND METHODS

Male Wistar rats weighing 200+ 20g in average and provided by the Nutrition Department of the Universidade Federal of Viçosa were used for this work. They received commercial Labina and water freely. For the adequate development of the test, 8 experimental groups of 8 rats distributed at random were formed and treated as follows:

GROUP 1 (ration), GROUP 2 (ration + triton), GROUP 3 (ration + triton + naringenin), GROUP 4 (ration + triton + rutin), GROUP 5 (ration + triton + naringenin + monascus), GROUP 6 (ration + triton + naringenin + anthocyanin), GROUP 7 (ration + triton + rutin + monascus), GROUP 8- (ration + triton + rutin + anthocyanin).

The experimental design was totally randomic, with 8 treatments in 8 repetitions. The treatments were carried out in the control and treated groups as described above.

Before starting the experiment, the rats were submitted to a five-day adaptation period in proper environment and cages with controlled temperature (26 ± 2 °C) and twelve-hours periods of light and darkness.

To induce hyperlipidemy, Triton (Sigma), dissolved in physiologic solution of NaCl (0.9%), was administered at the dose of 300 mg/Kg of body weight by intraperitoneal route. All animals received two doses of the compounds being the first immediately after the administration of triton, and the second twenty hours later. The flavonoids naringenin and rutin (Sigma) were administered isolated and in association to natural dyes monascus and anthocyanin (Christian Hansen Industry), by intraperitoneal route in propileneglycol as vehicle, to the dose of 5 mg/Kg of body weight. Fourty-three hours after the beginning of the triton applications, the animals were anesthetized with ethyl ether by inhalatory route, and blood samples were taken by heart punction. These samples were centrifuged at 7,161.6 G per minute to obtain serum for cholesterol, HDL-cholesterol and triacylglycerols measurement by the LIMA method [13] and for quantification with a Hitachi spectrophotometer.

## RESULTS AND DISCUSSION

Results obtained on serum lipids are shown on Tables 1, 2 and 3.

The effects of different treatments were expressed as percentual variations from serum lipids values in hyperlipidemic rats induced by triton.

According to the results in Table 1, the animals treated with triton, Group 2, increased by ten times the average values of cholesterol levels. These hypercholesterolemic animals submitted to the several treatments established, showed statistically significant reductions of cholesterol levels. Among the treatments carried out, it was observed that the naringenin + monascus (Group 5), naringenin + anthocyanin (Group 6), rutin + monascus (Group 7), and rutin + anthocyanin (Group 8) treatments presented larger percentual reduction as shown by the Dunnet test in comparison with Group 2. By Tukey test differences can be observed between

| Groups | Cholesterol (mg/dL) | % variation |
|---|---|---|
| 1-ration | 26.36 ± 0.70 ' | - |
| 2-ration+ triton | 262.11 ± 13.96 | - |
| 3-ration+ triton + naringenin | 90.80 ± 2.37 a | -65.36 * |
| 4-ration+ triton + Rutin | 102.65 ± 1.45 a | -60.84 * |
| 5-ration+ triton + naringenin + monascus | 45.63 ± 1.47 b | -82.59 * |
| 6-ration+ triton + naringenin + anthocyanin | 39.12 ± 1.45 b | -85.07 * |
| 7-ration+ triton + rutin + monascus | 47.75 ± 0.84 b | -81.78 * |
| 8-ration+ triton + rutin + anthocyanin | 52.79 ± 1.50 b | -79.86 * |

**Table 1**. Average values of cholesterol ± standard error in male Wistar rats and percentual variations produced by treatments. Averages followed by the same small block letters do not differ by the Tukey test (P>0,05).
* Statistically different from the control ($_{ration}$ + triton) by the Dunnett test (P<0,05).

| Groups | Cholesterol-HDL (mg/dL) | % variation |
|---|---|---|
| 1-ration | 22.25 ± 0.79 | - |
| 2-ration + triton | 64.20 ± 0.18 | - |
| 3-ration + triton + naringenin | 74.38 ± 1.65 a | +15.86 * |
| 4-ration + triton + rutin | 64.45 ± 1.19 b | +0.39 |
| 5-ration + triton + naringenin + monascus | 47.62 ± 1.23 d | -25.83 * |
| 6-ration + triton + naringenin + anthocyanin | 56.39 ± 1.45 c | -12.17 * |
| 7-ration + triton + rutin + monascus | 68.45± 1.56 b | +6.62 |
| 8-ration + triton + rutin + anthocyanin | 36.21 ± 1.79 e | -43.60 * |

**Table 2**. Average values of serum cholesterol - HDL ± standard error in Wistar rats and percentual variations produced by treatments. Averages followed by the same small block letter do not differ by Tukey test (P>0.05). * Statistically different from the control (ration + triton) by Dunnett test (P<0.05).

| Groups | Triacylglycerols (mg/dL) | % variation |
|---|---|---|
| 1-ration | 160.68 ± 2.68 | - |
| 2-ration + triton | 308.90 ± 2.82 | - |
| 3-ration + triton + naringenin | 69.99 ± 1.99 cd | -77.34 * |
| 4-ration + triton + rutin | 71.48 ± 1.29 cd | -76.86 * |
| 5-ration + triton + naringenin + monascus | 66.28 ± 1.49 d | -78.54 * |
| 6-ration + triton + naringenin + anthocyanin | 127.92 ± 1.26 | -58.59 * |
| 7-ration + triton + rutin + monascus | 98.78 ± 1.41 b | -68.02 * |
| 8-ration + triton + rutin + anthocyanin | 74.66 ± 1.33 c | -75.83 * |

**Table 3**. Average values of serum triacylglycerols ± standard error in Wistar rats and percentual variations produced by treatments. Averages followed by the same small block letter do not differ by Tukey test (P>0.05). * Statistically different from the control (ration + triton) by Dunnett test (P<0.05).

the groups. It can be observed from these results that the association of flavonoids with dyes increased the hypolipidemic effect. These results are better than some useful medicines, and can be used in the prevention of cardiovascular diseases.

HDL-cholesterol values shown in Table 2 evidence that the best treatment was obtained with naringenin alone (Group 3), according to the Tukey test. On the other hand Dunnet test evidenced that Group 3 in comparison with Group 2 showed the best results, since it raised the levels of HDL-cholesterol by 15.86%. This is an advantage since HDL-cholesterol is responsible for transport of cholesterol from peripheric circulation to the liver.

Table 3 presents the results of rat serum triacylglycerols. It is noticeable that the treatments with naringenin (Group 3), rutin (Group 4), naringenin + monascus (Group 5), and rutin + anthocyanin (Group 8) presented the largest reduction percentages of triacylglycerols levels (by the Tukey test), although the other treatments have been statistically significant. On the other hand, Dunnet test evidenced that Group 5 in comparison with Group 2 showed the best results, although the other treatments have been statistically significant.

It is known that the parenteral administration of rutin, naringenin and hesperidin lead to biliar excretion of conjugated glycuronic acid with these glycosil flavonoid derivatives, since the glycosidase enzymes which hydrolyze them are not present in mammalian tissues [14]. These statements are based on results obtained by Griffiths [15], who isolated these glycosilated flavonoids from germ-free rats feces. This and other flavonoids have the ability to penetrate the cell membrane, either penetrating the cell interior or not. Verkman [16] verified the ability of this substance to penetrate the lipidic bilayer of cell membranes with a fluorescence spectroscopy technique.

The literature records [17] that catechin stimu-

lates the absorption of sugars and aminoacids, besides inhibiting the accumulation of p-aminohipurate and N-metil-nicotinamide in renal cortex of dogs, through a specific effect on the permeability of the basolateral plasmatic membrane of tubular cells, but which does not have an effect on the absorption of L-phenyl alanin in the intestines of guinea pigs.

Naringenin, on its turn, inhibits the transport of sugars and aminoacids in the lower intestine of dogs, guinea pigs and rats [18,19]. This inhibition has been partially explained by an effect of naringenin on the metabolism and in part by a direct action of naringenin on cell membranes. The reason for these actions seems to be associated to the fact that naringenin inhibits $Na^+$-dependent alanin absorption through the borders of the bowel cells or inhibits the flux of alanin through the basolateral plasmatic membrane of the enterocyte. Both effects inhibit aminoacid fluxes through the membrane. The impairment of the transports observed is attributed to a metabolic change and to the inhibition of the $Na^+$ - $K^+$-ATPase activity, which reduces the $Na^+$ gradient through the cell membrane, and in this route inhibits the $Na^+$ dependent absorption of aminoacids in the enterocytes.

Compounds that inhibit platelet aggregation, among which are the flavonoids [20], have potential use in the prevention or treatment of atherosclerosis. This mechanism could involve the cAMP increases because flavonoids inhibit cyclic nucleotide phosphodiesterases.

Results show that the action of flavonoids and their association to natural dyes favors the reduction of cholesterol. For HDL-cholesterol, naringenin alone show best results. On the other hand, for triacylglycerols naringenin, rutin, naringenin + monascus and rutin + anthocyanin were more effective in reducing these levels.

Considering that naringenin inhibits the transport of sugars and aminoacids, the reduction of triacylglycerols could be explained by the smaller availability of sugars for the synthesis of triacylglycerols. The aminoacids used in proteic synthesis and the apoproteins that make part of lipoprotein structure are also reduced by these same compounds [18,19].

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