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Comparative study of hypocholesterolemic potential of pineapple and passion fruit peels in rats and mice

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Abstract: Researchers showed the importance of diets rich in fibers on the prevention of cardiovascular disease. As the passion fruit mesocarp and the peel of pineapple are rich in fibers, the aim of this work was to evaluate their influence on lipid metabolism in hypercholesterolemic rats and mice. The rats and mice received a high calorie diet, which consisted of a mixture of standard normocaloric feed (Nuvilab®; 3.78 kcal / g), roasted peanuts (5.95 kcal / g), chocolate (5.4 kcal/ g) and corn starch cookies (4.25 kcal / g). The animals received this diet during a period of 30 days. Subsequently, animals were treated with passion fruit mesocarp (DHM), pineapple peels (DHA) or simvastatin (DS) for 15 days. The administration of high calorie diet increased the levels of total cholesterol in rats and mice. In rats, there was a significant reduction in total cholesterol levels in DHM, DHA and DHS groups, and in mice, only the group treated with simvastatin (DHS) had reduced plasma cholesterol levels. There were no significant changes on glucose levels of any groups. The high calorie diet caused plasma triglycerides augmentation only in rats. However, any of the treatments were able to reduce the hypertriglyceridemia in rats. Furthermore, treatments didn´t cause any changes on plasmatic AST and ALT activities. Therefore, it is possible to say that treatment with peels of pineapple and passion fruit mesocarp is efficient to reduce hypercholesterolemia in rats. Also, the treatments with fruits peels did not show signs of liver toxicity.

Keywords: *hypercholesterolemia; Passiflora edulis; Ananas comosus; cholesterol; fibers.*

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INTRODUCTION

Hypercholesterolemia is a major risk factor for cardiovascular disease. This chronicle evolution can result in organism complication such as atherosclerosis; also can cause angina pectoris, myocardial infarction, stroke, and others kidney or liver diseases (HERNANDEZ et al., 2010). As a result, the cholesterol has been a subject very discussed currently and there is a large preoccupation as regards the control of the same. For this reason it is great the interest on cholesterol-lowering drugs.

Many therapies can be used in the treatment of hypercholesterolemia with expressive reduction of blood cholesterol levels. However, are reported many undesirable effects due used some drugs like gastrointestinal disorders, insomnia, rashes and others (XAVIER, 2005).

In the last few years, some researchers showed the importance of diets rich in fruits and vegetables on the prevention of atherosclerosis, through the adjuvant effect in reducing total cholesterol levels. These foods are rich in antioxidant compounds (including phenolic compounds, carotenoid, anthocyanins and tocopherols), mineral, vitamins and fibers

(BARTOSZ, 1997, NACKZ; SHAHIDI, 2006), and that the concentration of this components is high not only in pulp of fruits but also in peels.

The dietary fibers are a complex association of different polysaccharides of plants and are classified into soluble fibers (pectins, gums, and mucilages) and insoluble (cellulose, hemicellulose and lignin) according to solubility of its components in water (MATOS and MARTINS, 2000).

As the passion fruit mesocarp and the peel of pineapple are rich in fibers, this work aimed at evaluating their influence on lipid metabolism in hypercholesterolemic rats and mice.

MATERIALS AND METHODS

Population

Male mice of the Swiss strain and rats of the Wistar strain were utilized, with 30 ±5 g and 250 ±15 g body weight, respectively; both proceeded from the biotery of the "Faculdade Integrado de Campo Mourão". The animals had free access to water and food and were maintained to temperature constant around 24°C with light/dark cycle of 12/12 h.

The rats were divided into five groups of six animals, being high calorie diet group (DH), high calorie diet group treated with passion fruit mesocarp (DHM), high calorie diet group treated with pineapple peels (DHA), and high calorie diet group treated with simvastatin (DHS). The control group (DC) received a standard normocaloric diet.

The experimental protocol of this study followed the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee on Animal Experiments of the "Faculdade Integrado de Campo Mourão".

Preparation of diets

The fruits of *Passiflora edulis* (passion fruit) were kindly donated by "Associação dos Produtores de Corumbataí do Sul" (APROCOR) and the fruits of *Ananas comosus* (pineapple) were purchased in local markets. The fruits were washed with mild detergent and sanitized with chlorinated solution (50 ppm). After the separation of the pulp, the peels were submitted to a bleaching process, staying immersed in boiling water for 10 minutes, to inactivate enzymes. For drying, the peels were placed in a ventilated oven at 60ºC for 48 hours, approximately. The peels were subsequently ground in a cutting mill and sieved (tamiz 150) to get a fine powder (flour).

Hypercholesterolemia induction and treatments

For induction of hypercholesterolemia the animals of groups DH, DHM, DHA, and DHS received the high calorie diet, previously standardized by Estadella et al. (2004), containing by weight: 19% of protein, 47% of carbohydrates, 16% of lipids, 3% of cellulose, 5% of vitamins and minerals with 4,79 kcal/g. The ingredients were ground and mixed in pellets, in the following proportion: 15 g of normocaloric standard feed Nuvilab® (3.78 kcal/g), 10 g of roasted peanuts (5.95 kcal/g), 10 g of chocolate (5.4 kcal/g) and 5 g of corn starch cookies (4.25 kcal/g). The animals received this diet for a period of 30 days. The control group (DC) received a commercial standard feed (Nuvilab®), containing by weight 19% protein, 56% carbohydrate, 3.5% lipids, 4.5% cellulose, 5% of vitamins and minerals with 3.78 kcal/g, during the same period. After that time, the animals of DHM and DHA groups continued to receive the high calorie diet and were also treated daily with a suspension containing the passion fruit mesocarp or pineapple peels in 0.9% saline at a dose of 1 g/kg body weight, by oral gavage, for a period of 15 days. The DHS group received 4 mg/kg of simvastatin and the DH group received 0.9% of saline, by oral gavage, for the same period of time.

Biochemical Analysis

After the treatment the animals were fasted for 15 hours and then were sacrificed for blood sampling. Plasma concentrations of total cholesterol, glucose, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined by enzymatic colorimetric methods with commercial kits on equipment Cobas XL. The blood glucose and plasma lipid fractions were expressed as mg/dL and transaminases as U/L.

Statistical Analysis

The data were presented as means ± standard error of mean (SEM). In order to verify the influence of diet on the significance of the model, ONE-WAY ANOVA and multiple comparison test Neumans-Keuls was carried out with a significance of 5%, using the statistical package for microcomputers "Statistica" (StatSoft, Inc.)

RESULTS AND DISCUSSION

Initially, it was confirmed the induction of hypercholesterolemia in animals. After 30 days with the high calorie diet, the hypercholesterolemia in rats and mice was confirmed, being observed a significant increase of total cholesterol levels of 27.5% in rats and 55% in mice (Figure 1). This demonstrates that the hypercholesterolemia induction using the Estadella model – demonstrated in rats - (ESTADELLA et al., 2004) was also reproduced in mice. It can be noted, however, a significantly higher plasma levels of cholesterol in mice submitted to diets, compared to the rats. This difference can be related by metabolic differences between this two species, in relation to the endogenous lipids synthesis. The cholesterol, as other lipids, is mainly biosynthesized in the liver through the Acetil-CoA resulting of carbohydrates and protein metabolism. Other authors have reported differences in the lipid biosynthesis between rats and mice in response to different diets. Some authors report, for example, lipogenesis in rats liver can be decreased through the administration of rich diets of saturated and unsaturated fatty acids (HILL et al., 1960). Studies with mice, however, show that only high polyunsaturated fats diets are able to reduce the hepatic lipogenesis (ALLAMANN e GIBSON, 1965; SABINE et al, 1969). Although the diet employed in this work is a complex mixture of different fatty acids (saturated and unsaturated), carbohydrates and proteins, it is plausible to speculate that, in this condition, there is a higher hepatic production of cholesterol in mice, compared to the rats.

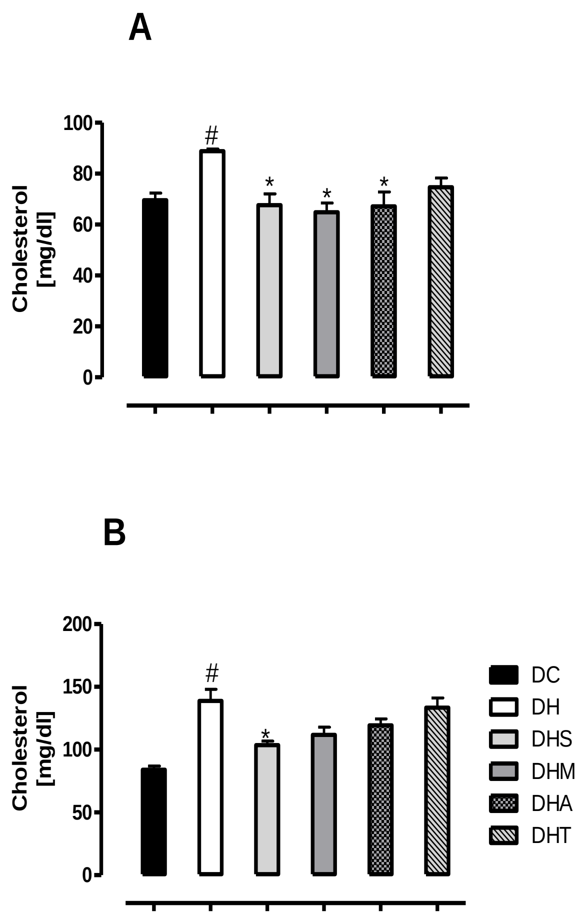

Figure 1 - Plasma concentration of total cholesterol of rats (A) and mice (B) treated with standard diet (DC, n=6), high calorie diet (DH, n=6 high calorie diet + simvastatin (DHS, n=5), high calorie diet +passion fruit mesocarp (DHM, n=5), high calorie diet +pineapple peels (DHA, n=5). The animals received standard or high calorie diet for 30 days, subsequently, the groups DHS, DHM and DHA, received a suspension of simvastatin, passion fruit mesocarp and pineapple peels for 15 days. The value represent mean ± standard error of mean *p<0.05 related with control high calorie diet (DH) # p<0.05 related with control group (DC).

Subsequently, it was evaluated the influence of passion fruit mesocarp and pineapple peels on the hypercholesterolemia installed in rats and mice, being observed different effects between these two species of animals. In rats, there was a significant reduction in total cholesterol levels in DHM, DHA and DHS groups of 27.2%, 23.5% and 23.8%, respectively, with a return to the baseline levels (Figure 1). In mice, only the group treated with simvastatin (DHS) showed reduced plasma cholesterol levels (25%) compared to the group treated with the high calorie diet (DH) (Figure 1).

The results achieved when the rats were the studied population are in agreement with various studies carried out in humans and in others animal species that confirm the hypocholesterolemic effect of passion fruit mesocarp (HSU et al., 2006; RAMOS et al., 2007). The high content of pectin found in passion fruit mesocarp, a soluble fiber of viscous consistency, change the gastric emptying time, increases satiety, delay the absorption time of simple carbohydrates and increases the excretion of cholesterol through complex formation (GALISTEO et al., 2008). Moreover, the passion fruit mesocarp also has insoluble fibers with proven hypocholesterolemic action (CHAU e HUANG, 2005).

The pineapple peels are also rich in insoluble fibers. Recent studies have evidenced its benefits on risk of cardiovascular diseases, colon cancer and obesity (XIE et al., 2005). Xie et al. (2005) shown that the extract of leaves of *Ananas comosus* has a hypocholesterolemic activity by the inhibition of HMG-CoA reductase enzyme, which regulates the endogenous biosynthesis of cholesterol. Furthermore, these authors claim that this activity is comparable to that of statins, but different of fibrates, and this way, the extract of leaves of pineapple can be auxiliary as an adjuvant in therapy with fibrates in patients intolerant to statins.

The mechanisms by which fiber reduces serum lipids include an increased conversion of cholesterol to bile acids, reduction of endogenous cholesterol synthesis and absorption of lipids, cholesterol and bile acids (MAZUR et al., 1990; GARCIA-DIEZ et al., 1996; MARLETT, 2001). The soluble fibers have the property of binding water, forming a gel that reduces lipids and sugar absorption, while the insoluble fibers are important to provide the mass required to peristaltic action in the gut (RIQUE et al., 2002).

Although it is a high calorie diet, its influence on the weight of the animals showed no difference compared to animals fed with the normocaloric diet (Figure 2). There was also no significant influence of both treatments (passion fruit mesocarp and pineapple peels) on animal´s weight (data not shown).

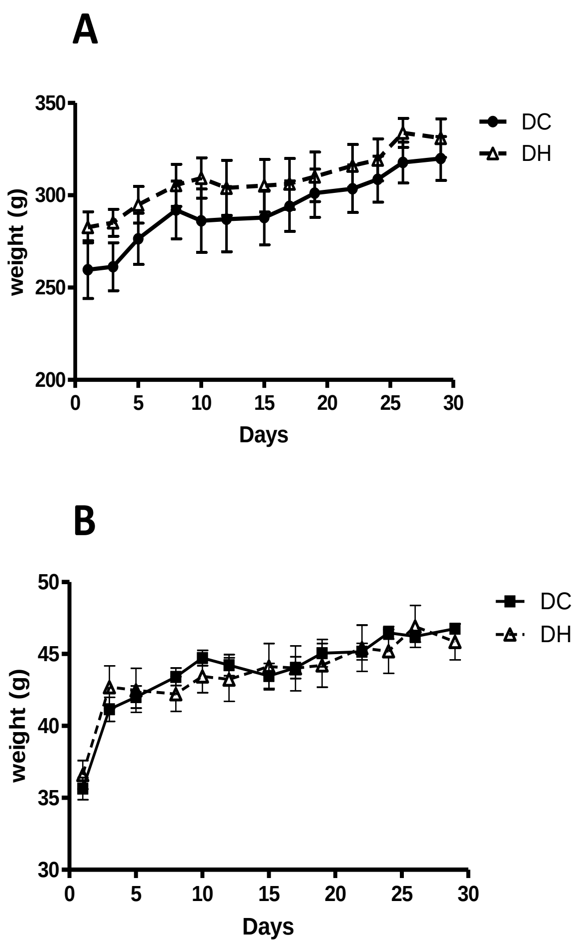

Figure 2 - The weight gain of rats (A) and mice (B) treated with standard diet (DC) and with high calorie diet (DH) for 30 days. The values represent mean ± standard error of mean.

For triglycerides, the high calorie diet was not able to significantly increase the plasma concentration of these lipid in mice (data not shown). Regarding to rats, however, there was an increase of 70% in plasma concentration of triglycerides in animals that received the high caloric diet compared to the control group (Figure 3). However, the treatments with the fruit peels, as well as simvastatin, were not able to decrease the triglyceridemy promoted by high calorie diet in rats (Figure 3). These results disagree with the studies of Silva et al. (2011) who shown a reduction of triglyceridemy in diabetics rats that received pectin extracted of passion fruit peels.

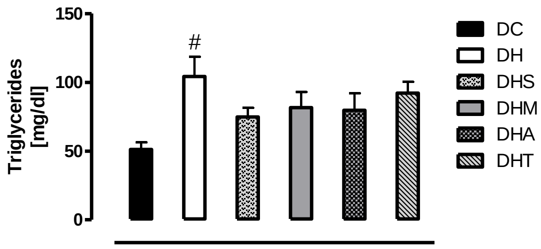

Figure 3 - Plasma concentration of triglycerides of rats treated with standard diet (DC, n=6), high calorie diet (DH, n=6), high calorie diet +simvastatin (DHS, n=5), high calorie diet +passion fruit mesocarp (DHM, n=5), high calorie diet +pineapple peels (DHA, n=5). The animals received standard or high calorie diet for 30 days, subsequently, the groups DHS, DHM and DHA, received a suspension of simvastatin, passion fruit mesocarp and pineapple peels for 15 days. The value represent mean ± standard error of mean # $p<0.05$ related with control group (DC).

The blood glucose of animals was also evaluated and the high calorie diet did not cause significant changes. Similarly, the treatment with fruit peels did not cause significant alterations in blood glucose levels of rats and mice (data not shown).

To evaluate the influence of high calorie diet employed in this work and of treatments with pineapple peels and the passion fruit mesocarp on liver functions, the plasma concentrations of an aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determinated. It can be observed in Table 1 that not only the administration of high calorie diet, but also the treatments with fruits peels did not show signs of liver toxicity. These findings corroborate those of Ramos et al. (2007) who carried out the assessment of liver function in humans who have received 30g of passion fruit peels for 60 days.

Table 1 – Plasma concentration of AST and ALT in U/L, in rats and mice of different experimental groups.

Groups	AST (U/L) (Rats)	AST (U/L) (Mice)	ALT (U/L) (Rats)	ALT (U/L) (Mice)
DC	104±13	95 ± 4	86±19	83 ± 6
DH	80 ± 8	83 ± 5	80 ± 8	48 ± 4
DHS	83 ± 8	79 ± 6	52 ± 6	45 ± 5
DHM	73 ± 7	75 ± 4	73 ± 5	44 ± 5
DHA	87 ± 15	96 ±18	53±13	48 ± 7

CONCLUSIONS

Front of results found, it is possible to say that the influence of the peels of pineapple and passion fruit mesocarp in rats and mice is different. The hypocholesterolemic effect was found only in rats, being the results comparable to those obtained with simvastatin. Also, it was proved that the treatments with fruits peels do not show signs of liver toxicity. The observed differences in relation to responses of rats and mice to hypercaloric diet used and the treatment with the peel of pineapple and passion fruit mesocarp only emphasize the premise that interspecies extrapolations should be performed with caution, due to metabolic differences between these species.

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