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Effect of *Psidium guajava* (cv. Pedro Sato) fruit and extract on the lipidemia in hypercholesterolemic rats

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The objective was to analyze the bioactive compounds of the fruit and the ethanolic/acetonic extract of the Pedro Sato guava and to study their effect on the liver mass versus body mass ratio, hepatic lipids, C-reactive protein and levels of total and fractionated cholesterol in hypercholesterolemic Wistar rats. 6 groups were used: non-hypercholesterolemic control, hypercholesterolemic control, non-hypercholesterolemic treated with fruit, hypercholesterolemic treated with fruit, non-hypercholesterolemic treated with extract, hypercholesterolemic treated with extract. The treatments received the samples at a dosage of 50 mg kg^{-1} daily for 42 days. This study showed that the daily consumption of a guava (100 g) can contribute 10.08% of the fiber and 68.47% of the vitamin C recommended, besides supplying considerable amounts of phenolic compounds. All the hypercholesterolemic animals treated with the samples presented a reduction in the total and fractionated cholesterol levels; however there was no difference for the liver mass versus body mass ratio, hepatic lipids and C-reactive protein. These results show the importance of the guava consumption in the control of the hypercholesterolemia in rats and it may prevent and reduce the risk of heart diseases.

Key words: Antioxidants, cholesterol, dietary fiber, *Psidium guajava,* rats.

INTRODUCTION

In recent decades, the cardiovascular diseases started to be the main cause of morbidity and mortality in developed countries and in increasing proportions in the developing countries. Among the cardiovascular events, the disease atherosclerosis is responsible for approximately 50% of the deaths in western countries and in Brazil, about 300,000 people per year are victims of this disease (Rehrah et al., 2007).

The increase in the incidence of cardiovascular diseases in our country is due to the transition of nutritional patterns and to physical inactivity, which enabled the increase in the prevalence of dyslipidemia, considered one of the main risk factors for cardiovascular diseases and characterized by the elevation of the serum levels total and fractionated cholesterol, associated with the decrease in the high density lipoprotein (HDL)-c

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values (Bruckner, 2008).

Various studies show the benefit of the effect of fruit and vegetable consumption on the cardiovascular disease risk development reduction and the reduction of the disease itself. That effect is due to the presence of dietary fiber and antioxidant substances (Salgado et al., 2008). The soluble dietary fibers act by sequestering the biliary salts and cholesterol present in the small intestine, thus impeding its absorption for the organism, since the insoluble fiber increases the evacuation frequency. Among the antioxidant substances, the phenolic compounds have been receiving great attention, because they act by increasing the low density lipoproteins (LDL)-c resistance to oxidation.

Experiments using rats with induced hypercholesterolemia, phenolic compounds in green tea (Yokozawa et al., 2002) and in the mango (Anila and Vijayalakshmi, 2003) have demonstrated a reduction of the cholesterol and LDL levels. The same results were related in garcinia and white mulberry (Koshy, 2001; El-beshbishy, 2006). The guava possesses high nutritional value, with various biological activities, among them the antioxidant activity. Its antioxidant activity has been attributed to the variety of phenolic compounds which are: isoquercetine, quercetin, myricitine, anthocyanins, caffeic acid and ferulic acid, among others (Thaipong et al., 2005).

Considering the importance of guava to the economy and the diet of the Brazilians, and the importance of research for substances that act towards the prevention of the cardiovascular diseases, the objective if this work was to evaluate the level of phenolic compounds, dietary fiber and the antioxidant activity of the guava fruit, as well as its effect on the total serum and fractionated cholesterol levels, hepatic lipids and C-reactive protein.

MATERIALS AND METHODS

Characterization of guava fruits

Pedro Sato *Psidium guajava* fruits were selected in the city of Lavras, Minas Gerais, Brazil, where they were taken to the Biochemistry Laboratory of Federal University of Lavras (UFLA). Those were cut and washed, frozen in liquid nitrogen and lyophilized to obtain the flour. The fruits were identified by the College of Agriculture Lavras Herbarium, where the voucher specimen was deposited which received voucher number 26277. The ethanolic/acetonic extracts of the fruits were maintained under maceration in ethanol/acetone (70/30) at a 1:50 (p/v) proportion, for 24 h, and soon afterwards, filtered. The supernatant was collected and the residue re-extracted under the same conditions. The two supernatants were joined, submitted to evaporation and later lyophilized to obtain the dry extracts (Rufino et al., 2007). The soluble and insoluble dietary fiber content was determined through the Sigma Total Dietary Fiber Kit. The results were expressed in g 100 g^{-1} of sample (Association of official analytical chemistry-AOAC, 2005). The phenolic compounds were measured using Folin-Denis reagent (AOAC, 2005), the results were expressed in mg of tannic acid g^{-1} dry material (DM). The antioxidant activity was determined by the DPPH and beta-carotene/linoleic acid methods. The results were expressed through the sample concentration necessary to inhibit 50% of the radical DPPH• (Thaipong et al., 2006). The results were expressed as percentage of protection against the lipidic oxidation of linoleic acid (Rufino et al., 2007).

Biological analysis

The commercial diet was triturated and cholesterol, and cholic acid was added. The mixture was moistened with water, shaped and taken in a ventilated oven at 35°C for two days (Rocha, 2009). The animals of the non-hypercholesterolemic group received the same commercial diet, however, without the cholesterol and cholic acid addition. The animals in hypercholerterolêmic groups treated began receiving hypercholesterolemic diet at the beginning of the experiment along with the samples. The fruit and the extracts were administered to the animals by gavage, once a day, for 42 days, at a concentration of 50 mg Kg^{-1} of body weight, while the control groups (non-hypercholesterolemic and hypercholesterolemic control) received water by the same administration method. At the beginning of the experiment and 3 days before sacrifice, the animals of control groups were analyzed for total cholesterol by puncture of the tail vein in order to confirm the induction of hypercholesterolemia.

The *in vivo* study was conducted according to the ethical principles for animal experimentation adopted by the Brazilian School of Animal Experimentation (COBEA), approved in 11/11/2010 by the animal research ethics committee of the Federal University of Alfenas (UNIFAL), protocol number 326/2010. 30 male Wistar rats were used (*Ratus norvegicus*) with 400 ± 50 g, obtained from the vivarium of UNIFAL. The animals were randomly divided into 6 groups with 5 animals each: NC: non-hypercholesterolemic control; HC: hypercholesterolemic control; NE: non-hypercholesterolemic animals treated with the fruit extracts; HE: hypercholesterolemic animals treated with the fruit extracts; NF: non-hypercholesterolemic animals treated with the fruit; HF: hypercholesterolemic animals treated with the fruit.

At the end of the experiment, the animals remained under fast for 12 h, and later were anesthetized with Sodium Thiopental (35 mg Kg^{-1}) and the blood removed by the heart puncture technique, and soon afterwards the liver was removed for analyses. The total and fractionated cholesterol levels were determined in the blood samples using the Labtest® enzymatic-colorimeter kit. The non-HDL cholesterol levels were determined by the difference between the total and HDL cholesterol levels. The liver mass ratio versus body mass was determined dividing the mass of the whole liver by the body mass of the animal. The hepatic lipids were determined by the methodology proposed by AOAC (2005). The C-reactive protein levels were determined in the blood serum of the animals by the turbidimetric method using the Human® kit.

Statistical analysis

A completely randomized design in a 2 × 2 + 2 factorial outline was used; being two forms of extract preparation (fruit and ethanol/acetone extract), two diet types (hypercholesterolemic and non-hypercholesterolemic) and two additional treatments (hypercholesterolemic control and non-hypercholesterolemic control), totaling 6 treatments with 5 repetitions. The statistical analysis was conducted using the Sisvar program (Ferreira, 2000), the averages being compared by the Tukey test to 5% of probability.

Table 1. Chemical characterization and antioxidant activity of guava fruit and extracts.

Parameter	Fruit	Ethanolic/acetonic extract
Soluble dietary fiber (g 100 g^{-1} DM)	1.57±0.42	ND
Insoluble dietary fiber (g 100 g^{-1} DM)	11.27±0.56	ND
Phenolic compounds (mg g^{-1} DM)	7.45±0.16	14.66±0.58
Antioxidant activity ($IC_{50\%}$ mg ml^{-1})	0.438±0.01	0.106±0.01
Antioxidant activity (inhibition %)	39.65±0.64	4.52±0.152

ND: not detected.

RESULTS AND DISCUSSION

Chemical characterization and antioxidant activity

By the data presented in Table 1, it can be seen that the insoluble dietary fiber content is superior to that of the soluble fiber. Literature data indicates that the insoluble and soluble dietary fiber content is between 9.96 and 12.18 g 100 g^{-1} DM and 1.48 to 1.58 g 100 g^{-1} DM (Guerra et al., 2004). The dietary fiber act impeding to cholesterol absorption in the intestine of the organism and increasing its excretion in the form of biliary salts. The Food and Drug Administration (FDA) recommends the consumption of 25 g of dietary fiber per day in a diet of 2,000 calories, thus the consumption of 100 g of guava "*in natura*" supplies approximately 10.08% of the recommended ingestion. In the extract, the dietary fiber content was not detected by the methodology used. The extract presented higher phenolic compounds content than the fruit. The content found in the fruit is in agreement with that found in the literature (Hassimoto et al., 2005; Melo et al., 2006). This fruit can be considered a good source of antioxidant substances because it possesses a high concentration of phenolic compounds when compared the other fruits consumed daily (Brat et al., 2006; Melo et al., 2006).

Various works have demonstrated that fruits are a natural source of dietary fiber and antioxidant substances and that a diet rich in fruits has a positive influence on the serum levels of lipids and its metabolism and on the antioxidant activity in experiments with laboratory animals (Leontowicz et al., 2001; Leontowicz et al., 2002; Salgado et al., 2008). The antioxidant activity of the fruits is related to their antioxidant substance content. The DPPH method evaluates the capacity of the sample to sequester the DPPH radical formed through the donation of hydrogen and as such, the lower the $IC_{50\%}$ value expressed in mg ml^{-1}, the higher the antioxidant activity.

In Table 1, it can be seen that the extract presented higher antioxidant activity and of phenolic compound contents than the fruit. The extract and fruit had 0.106 and 4.52 mg ml^{-1} (antioxidant activity) and 14.66 and 7.45 mg g^{-1} DM (contents phenolic compound), respectively.

The beta-carotene/linoleic acid method evaluates the inhibition percentage of the linoleic acid by the lipophilic antioxidant substances present in the fruit and in the extract of the fruits. By this antioxidant method, the fruit (39.65%) presented higher antioxidant activity, in other words, higher inhibition percentage than the extract of the fruit (4.52%).

Biological analysis

The liver mass/body mass ratio is presented in Table 2, where one can see that the animals of the HC (0.030) presented a higher ratio than that of the NC (0.023) animals. Similar results were observed in the literature (Machado et al., 2005; Lima, 2008). When analyzing the animals of the HF and HE with the hypercholesterolemic control, 0.029 and 0.028, respectively, a significant difference was not observed. The animals of the NF and NE, 0.024 and 0.024, respectively, presented a ratio to that of the NC. The percentage of hepatic lipids was significantly higher for the animals of the HC with 13.3% of hepatic lipids. The hypercholesterolemic animals treated with the guava fruits presented a reduction of the 22.56 and 12.78% for HF and HE, respectively in the lipid percentage compared to the animals of the HC. It is known that, besides increasing the dietary cholesterol absorption, the colic acid can also inhibit its conversion into biliary salts and favor the accumulation of hepatic lipids (Machado et al., 2005). This situation can lead to hepatic cell overload, due to a higher physiologic demand. These results observed in the present study corroborate with the literature data (Machado et al., 2005; Rocha, 2009). The C-reactive protein is a protein synthesized by the liver in response to the cytokines that reflect active systemic inflammation, in other words, it is an inflammation biomarker. Significant alterations were not observed in the levels of that marker.

From the data of Table 3, one can observed that the animals of the NC presented total serum cholesterol levels of 46.23 mg dL^{-1}, while the animals of the HC presented an increase of 109% in the levels, a significant difference, indicating hypercholesterolemia. These values

Table 2. Levels of hepatic lipids, liver mass/body mass ratio and C-reactive protein levels in Wistar rats treated with flour and ethanol extract of guava.

Treatments	Hepatic lipids (%)	Liver mass x body mass	PCR[1] (mg L^{-1})
NF[2]	2.95[c]	0.024[b]	1
NE[3]	3.31[c]	0.024[b]	0.6
HF[4]	10.32[b]	0.029[a]	0.8
HE[5]	11.63[b]	0.028[a]	0.6
NC[6]	3.56[c]	0.023[b]	0.2
HC[7]	13.30[a]	0.030[a]	1

Means followed by same letter in columns do not differ by the Tukey test at 5% probability;[1]C-reactive protein; [2]non-hypercholesterolemic animals treated with the fruit; [3]non-hypercholesterolemic animals treated with the fruit extracts; [4]hypercholesterolemic animals treated with the fruit; [5]hypercholesterolemic animals treated with the fruit extracts; [6]non-hypercholesterolemic control; [7]non-hypercholesterolemic animals treated with the fruit.

Table 3. Total serum cholesterol, triglycerides, HDL and non-HDL cholesterol in Wistar rats treated with flour and ethanol extract of guava.

Treatments	Cholesterol (mg dl^{-1})	HDL (mg dl^{-1})	Triglycerides (mg dl^{-1})	Non-HDL cholesterol (mg dl^{-1})
NF[1]	46.12[d]	19.20[a]	37.55[b]	26.92[d]
NE[2]	47.24[d]	18.40[ab]	37.50[b]	29.50[d]
HF[3]	60.66[c]	15.40[b]	41.89[ab]	46.00[c]
HE[4]	77.20[b]	20.20[a]	30.10[b]	58.00[b]
NC[5]	46.23[d]	15[b]	40.06[ab]	31.08[d]
HC[6]	96.70[a]	8.80[c]	50.78[a]	87.90[a]

Means followed by same letter in columns do not differ by the Tukey test at 5% probability; [1]non-hypercholesterolemic animals treated with the fruit; [2]non-hypercholesterolemic animals treated with the fruit extracts; [3]hypercholesterolemic animals treated with the fruit; [4]hypercholesterolemic animals treated with the fruit extracts; [5]non-hypercholesterolemic control; [6]non-hypercholesterolemic animals treated with the fruit.

are close to those found in others works (Lima, 2008; Guerra et al., 2004). The fruit and the extract caused a reduction of 37.27% and 20.17%, respectively, in the levels of total cholesterol when compared to the HC. The highest total cholesterol reduction by the fruit can be due to the presence of dietary fiber (Table 1), which was not detected for the extract.

The non-hypercholesterolemic animals treated with the fruit and extract of the fruits did not present a difference in the cholesterol levels in relation to the animals of the non-hypercholesterolemic. That can be justified by the need of the organism to maintain the cholesterol at basal levels for serum membrane, biliary salt and hormone synthesis. Even not being acquired in the diet, the hepatocytes possesses enzymes capable of their synthesis. Various studies (Camire and Dougherty, 2003; Liu et al., 2000; Rocha, 2009). show that the soluble fiber promotes reduction in the cholesterol levels in the blood. Because this fiber bond to the cholesterol leading to its elimination, and thus this reduces its absorption by the liver. Simultaneously, those fibers favor the synthesis of biliary salts in the liver from the cholesterol present in the blood.

The fruit and extract of the fruits promoted a significant reduction in the serum triglyceride levels. Table 3 displays a reduction of 17.51 and 40.72% for the fruit and extract samples, respectively, when compared to HC. The highest reduction in the triglyceride levels by the extracts can be justified by the higher phenolic compound content and higher antioxidant activity by the DPPH method (Table 1).

The non-HDL cholesterol levels were calculated through the difference of the total cholesterol and the HDL cholesterol levels. These values represent an estimate of the cholesterol concentration in the atherogenic lipoproteins, in other words, of very low density lipoproteins (VLDL) and LDL. In Table 3, a reduction of 47.66 and 34.02% for the fruit and guava

extract, respectively, compared to HC can be seen. This reduction is important, because various studies have shown that the reduction of total cholesterol and non-HDL cholesterol represents an effective measure to reduce cardiovascular mortality associated to patients with cardiovascular diseases or to prevent the appearance of that disease (Martinella, 2006).

The HDL is responsible for the inhibition of the cholesterol deposition moderated by LDL in the walls of the arteries and by the reverse transport of cholesterol, providing the removal of the cholesterol from the body cells to the liver, to reuse it or to convert it into biliary acids. Therefore, the higher the level in the organism, the lower the risk of cardiovascular disease occurrence. The fruit and the extract of the guava promoted an increase in the HDL level of 75 and 129.55%, respectively.

Studies involving the progression of arteriosclerosis have been intensifying in recent years, in function of the increase in the incidence of cardiovascular diseases. The progression of the arteriosclerosis appears to be linked to the oxidative stress and pathologies such as myocardial infarction and cerebral vascular accidents, among others (Duarte et al., 2009). This justifies the investigation of dietary fiber and natural substances with antioxidant activity that can contribute to the prevention and progression of this evil.

Conclusion

From the obtained results, it can be concluded that the guava tree fruits are a source of antioxidant substances and dietary fiber. The fruit and the extracts of the Pedro Sato guava tree fruits were effective in the control of the hypercholesterolemia. This supports the hypothesis that those phytochemicals found in the guava promote health benefits, reducing cardiovascular diseases risk development. Based on these results, the consumption of the fruit is recommended with the purpose of increasing the ingestion of these phytochemicals.

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