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LIPID PROFILE AND ACTIVITY OF γ-GLUTAMYL TRANSPEPTIDASE IN MICE TREATED WITH HESPERIDIN AND COFFEE

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Abstract

Hesperidin, a flavonoid with many biological activities that contribute to the protection of human health, is present at high concentrations in citrus fruits. Clinical and epidemiological studies suggest that the consumption of coffee increases total cholesterol and low-density lipoprotein levels. The aim of this study was to evaluate the effects of oral administration of brewed coffee (900 mg/kg/day) and the combination of hesperidin in drinking water (100 mg/ml in solution) and brewed coffee (900 mg/kg/day) on the lipid profile and activity of γ-glutamyl transpeptidase (γ-GT) in Swiss strain mice. Administration of brewed coffee and the combination of hesperidin in drinking water and brewed coffee extract did not cause significant changes in total cholesterol, HDL-cholesterol, and LDL-cholesterol levels in mice. Serum triglyceride levels significantly decreased in the group treated with hesperidin in drinking water and brewed coffee extract compared to those in the control group ($P < 0.0001$) and the group treated with coffee ($P = 0.0204$). The γ-GT activity increased in the group treated with brewed coffee compared to that in the control group ($P = 0.0002$). The enzyme activity of the group treated with the combination of hesperidin in drinking water and brewed coffee extract was not significantly different from that of the control ($P = 0.2027$), but was reduced compared to that of the group treated with coffee ($P = 0.0014$). These results reinforced the beneficial effects of the flavonoid hesperidin, and highlighted the risk of coffee and the need to control coffee intake for a healthy life.

Keywords: *hesperidin, brewed coffee, caffeine, lipid profile, γ-glutamyl transpeptidase.*

Introduction

Hesperidin (hesperetin 7-rhamnoglucoside) is an important component of plants with anti-inflammatory activity, which belongs to the class of flavonoids known as flavonones [1][2]. This compound, which is present in high concentrations in citrus fruits, exhibits many biological activities that contribute to the protection of human health [3]. Flavonoids have shown beneficial effects in the treatment of cardiovascular diseases, certain cancers, AIDS, Alzheimer's disease and other diseases associated with aging, and atherosclerosis through the inhibition of low density lipoprotein oxidation (LDL) [4][5][6][7].

This group of substances has attracted attention because of their important biological effects, such as anticancer and anti-inflammatory activity, hypotensive reduction of LDL cholesterol (LDL-C), platelet aggregation, and reduced risk of coronary heart disease (CHD) [3][8][9][10][11][12]. Biochemically, flavonoids may inactivate the free radicals in aqueous and lipophilic media [13] and modify the metabolism of blood lipids and atherogenic factors, prevent the release of mast cells and inflammation in heart tissue, which protects against coronary heart disease (CHD) [14]. Weight gain with hesperidin treatment and a saturated fat diet was reported by Vinueza et al. [15], but other studies demonstrated no significant difference in weight gain or organ weight gain between control and experimental groups treated with hesperidin [9][16].

In contrast, Phillips et al. [17] observed a positive correlation between coffee and/or tea ingestion and the reduction of LDL-cholesterol in humans. Clinical and epidemiological studies suggested that the consumption of coffee increases total cholesterol and low-density lipoprotein [18]. Other studies, however, suggest that it is not the caffeine in coffee that is responsible for its hypercholesterolemic effect [15][16]. Two diterpenoid alcohols, cafestol and kahweol, which are found at significant levels in boiled coffee, have been identified as the hypercholesterolemic components. Although these components are largely trapped by the use of a paper filter during coffee preparation, there is some evidence that the consumption of filtered coffee is associated with small increases in serum cholesterol levels [19]. Although the roasting process changes the number of constituent molecules, such as polyphenols, other antioxidant molecules that contribute to the antioxidant activity of roasted coffee remain unchanged [20]. Coffee consumption is associated with reduced insulin sensitivity, as caffeine increases the synthesis of epinephrine or antagonizes the adenosine receptor. Furthermore, epinephrine may influence the metabolic rate through the stimulation of insulin secretion, glycogenolysis, and †he mobilization of fatty acids [21].

The objective of this study was to evaluate the effects of hesperidin in drinking water and the oral administration of coffee on the lipid profile and γ-glutamyl-transpeptidase activity in Swiss strain mice.

Methods

The experiments were conducted at the Research Laboratory in Analytical Biochemistry and Toxicology, Department of Biological Sciences – *Universidade Estadual do Centro Oeste* (UNICENTRO - Paraná State - Brazil). The effect of hesperidin in drinking water and brewed coffee extract was evaluated in thirty Swiss mice (*Mus musculus*). The handling, care, and welfare of the animals followed international standards of conduct, and were approved by the Ethics Committee on Animal Use (CEUA) UNICENTRO (Protocol number 018/2016).

Eight-week-old male Swiss albino mice were obtained from TECPAR Laboratory Animal Center (Curitiba, Paraná, Brazil). After a 5 day adaptation period, the mice were divided and collectively housed in cages (34 × 41 × 17 cm) with ten animals per cage. The animals were kept in a temperature- and humidity-controlled room (23°C ± 1°C and 60% ± 5% relative humidity) with a 12 h light/dark cycle, and were given free access to food and distilled water for 36 days.

On day 0 (zero), the animals (average weight: 25 g) were divided into three groups: animals administered a control treatment (control group), animals administered a coffee extract (coffee group), and the animals administered both hesperidin and coffee extract (the combination group). Hesperidin was prepared at 100 mg/ml in distilled water,

with the addition of 50 mg sucrose to increase acceptance by the animals.

The coffee extract was prepared from *Coffea arabica* (catuaí red variety), obtained commercially. The hesperidin (99%) was obtained in high purity by Merck® and was prepared in aqueous solution. The coffee extract was obtained by the infusion of 22,4 g of roasted and ground coffee with 100 ml of distilled water (224 mg/ml) at 90 °C for 10 min. After extraction, the material was filtered through paper filter.

The animals in the coffee group received a daily administration of 100 µL of the brewed coffee extract by gavage (~900 mg/kg/day coffee) for 36 days. The concentration of caffeine in each dose/day was ~3 mg/kg/day [22]. The amount of coffee administered to the mice was based on the half the quantity of caffeine from coffee drinks consumed by an European adult man (6 mg/kg/day) [23]. The per capita consumption level of caffeine (of all ages) in U.S. consumers is approximately 120–180 mg per day, equivalent to a mean intake of 1.73–2.57 mg/kg body weight/day [24][25][26]. During the experiment, the animals were weighed every 3 days.

Biochemical analysis

At the end of the treatment, the animals were anesthetized with xylazine (10 mg/kg intraperitoneal - IP) and ketamine (80–100 mg/kg IP) was used for terminal anesthesia and a sample of blood was quickly collected by using cardiac puncture [27]. Blood samples were immediately processed to separate the serum and plasma to determine serum cholesterol, serum triglycerides, serum HDL- and LDL-cholesterol and the enzyme γ-glutamyl-transferase (γ-GT) by using specific kits from Labstore® as appropriate, with measurements conduced on a Gehaka® UV-340G spectrophotometer.

Data analysis

The data are expressed as the mean ± SD. The effects of †he treatments were determined by one-way ANOVA. Differences among treatment groups were assessed by the Tukey test (SPSS version 11.0; SPSS, Chicago, IL). Differences were considered significant when $P < 0.05$.

Results

The body weight of the animals increased until the end of the experiment (day 36) (Figure 1A). However, animals treated with the combination of hesperidin in drinking water and brewed coffee exhibited a lower weight gain ($P = 0.0176$) than the control and coffee groups ($P < 0.0001$) between the beginning and the end of the treatment (Figure 1B). The combination group did not exhibit a significant weight gain between days 11 and 36 ($P > 0.05$) (Figure 1A and 1B) compared to that of the control group.

In the coffee group and the combination group, the levels of serum total cholesterol, serum HDL-, and LDL-cholesterol (Table 1) were not altered compared to those of the control ($P > 0.05$). However, serum triglyceride levels significantly decreased in the combination group compared to those of the control group ($P < 0.0001$) and the coffee group ($P = 0.0204$).

The γ-GT enzyme activity (Figure 2) was significantly increased in the coffee group compared to that in the control group ($P = 0.0002$). The combination group exhibited no significant change in enzyme activity compared to that in controls ($P = 0.2027$), but was significantly lower than the activity in the coffee group ($P = 0.0014$).

Discussion

The combination of hesperidin and coffee was administered to determine whether hesperidin altered the action of coffee, or even the effects of the components present in the coffee, on the analyzed parameters. Coffee consumption has been associated with higher concentrations of serum total cholesterol and LDL-cholesterol in some observational studies, but not in others [18][28]. The results of this study showed that coffee *per se* did not alter the levels of cholesterol; further, when administered concomitantly with hesperidin, no alterations were observed in serum total cholesterol, HDL-cholesterol, and LDL-cholesterol levels. In another study, no significant differences were observed in serum total cholesterol or LDL-cholesterol levels between the group that consumed filtered coffee and the group that consumed no coffee [28]. Coffee contains cholesterol-increasing compounds, such as diterpenes including cafestol and kahweol [29]. Boiled coffee has higher concentrations of diterpenes because

they are extracted from coffee beans by prolonged contact with hot water. Conversely, brewed (filtered) coffee has lower concentrations of diterpenes because of much shorter contact time with hot water and the retention of diterpenes by filter paper [29][30].

It should be taken into account that the caffeine present in coffee extract is rapidly and almost completely absorbed in the stomach and small intestine and distributed to all tissues, including the brain [31]. In the liver, cytochrome P450 isoform CYP1A2 is responsible for almost 95% of the primary metabolism of caffeine [24][25]. Therefore, we hypothesized that the amount of caffeine present in brewed coffee extract (3 mg/kg/day) was not sufficient to alter the lipid profile of mice, although the concentration of caffeine in the coffee drink [22] administered to animals was the half amount consumed by an average adult human in Europe [23].

Coffee significantly affects body weight loss when body weights are above the normal range, such as in overweight or obese individuals, but it is possible that this effect is not seen in individuals of a normal weight [32]. Thus, this study also tested whether the combination of coffee extract and hesperidin in drinking water could reduce weight gain in normal weight animals. The control group and the coffee group exhibited significant weight gain until the last day of the experiment. However, the combination group treated exhibited significant weight gain until day 11 of the experiment, but not between day 11 and day 36.

Gardner et al. [33] and Nawrot et al. [23] highlighted the biological properties of coffee, citing a positive correlation between the antioxidant activity and its diminishing effect on the risk of developing chronic diseases. These effects can be attributed to the presence of compounds such as trigonelline, chlorogenic acids, and caffeine in coffee. Some components, for example terpenes, are considered to offer benefits, such as liver protection and anticarcinogenic effects; some components also exert undesirable effects, such as elevated cholesterol level caused by cafestol [22][31]. We observed that the combination treatment of hesperidin and coffee did not alter the total cholesterol in mice.

It is known that in the liver, the metabolite of glycosylated hesperidin, hesperidin-glucuronic acid, inhibits HMG-CoA reductase and cholesterol synthesis in the liver, which in turn leads to a reduction in plasma cholesterol [9][34]. The flavonoid conjugate can be excreted in the bile fluid or transported to peripheral tissues by the bloodstream where they perform functions such as antioxidation, prevention of oxidative stress, and amelioration of chronic diseases [11][35]. We hypothesized that the concentration of hesperidin in the drinking water and/or the time of treatment have were not sufficient to meet the metabolic levels in the liver for hesperidin-glucuronic acid to inhibit the HMG-CoA reductase to a sufficient level to reduce cholesterol synthesis in the liver.

We also considered that the increase in γ-GT activity in the coffee group might arise from the bioactive compounds, such as caffeine, that are present in coffee extract. This enzyme is localized in the canaliculi of liver cells and in the epithelial cells of the bile ducts. Owing to its key location, the enzyme appears altered in nearly all hepatobiliary disorders. However, as caffeine metabolism predominantly occurs in the liver, caffeine may have caused hepatic injury in the group administered only coffee. The activity of γ-GT in the combination group and the coffee group was unchanged compared with the control group, which suggested that the flavonoid hesperidin act as an antioxidant, protecting liver cells and maintaining normal γ-GT levels in the blood.

The combination group exhibited lower weight gain than the control group and coffee group; this result was in accordance with those of Ohara et al. in humans [36], who demonstrated that the administration of a combination of 500 mg glycosylic hesperidin and 50 or 75 mg caffeine for 12 weeks significantly reduced abdominal fat, body weight, and body mass index in subjects with a moderately high body mass index. Therefore, a combination of glycosylic hesperidin and caffeine may be useful for the prevention or treatment of obesity [36].

Studies should predominantly consider pregnant women and fetuses that may be

particularly vulnerable to the effects of caffeine. Caffeine is a biologically active molecule with multiple targets that can affects numerous functions in a positive or negative manner [37]. Caffeine ingested by pregnant women will be present in the umbilical cord and breast milk; furthermore, the half-life of caffeine is much long in neonates (65–130 h) than in adults (3–7 h) because of their immature kidneys and liver [25].

In conclusion, this study demonstrated that the administration of a combination of hesperidin in drinking water and brewed coffee extract was able to decrease weight gain in mice, reduce triglyceride levels, and maintain γ-GT enzyme activity within normal values. The coffee extract increased γ-GT enzyme activity, but did not affect animal weight gain or the lipid profile. Therefore, there are many remaining uncertainties with regard to the effects of coffee, caffeine, and potential associated flavonoids on mammalian enzyme systems that warrant further research.

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Table 1: Total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol of control mice and mice administered brewed coffee extract and a combination of hesperidin + brewed coffee extract for 36 days. The data are presented as the mean ± standard deviation. *****$P < 0.05$.

	Control	Brewed coffee extract	Hesperidin + Brewed coffee extract
Total cholesterol	93.34 ± 2,45	97.02 ± 3.556	91.96 ± 6.166
Triglycerides	216.2 ± 3.48	222.6 ± 5.69	172.5 ± 5.634 *
HDL-cholesterol	38.25 ± 2.394	44.20 ± 4.283	45.02 ± 3.162
LDL-cholesterol	65.29 ± 2.567	54.88 ± 4.691	61.88 ± 2.765

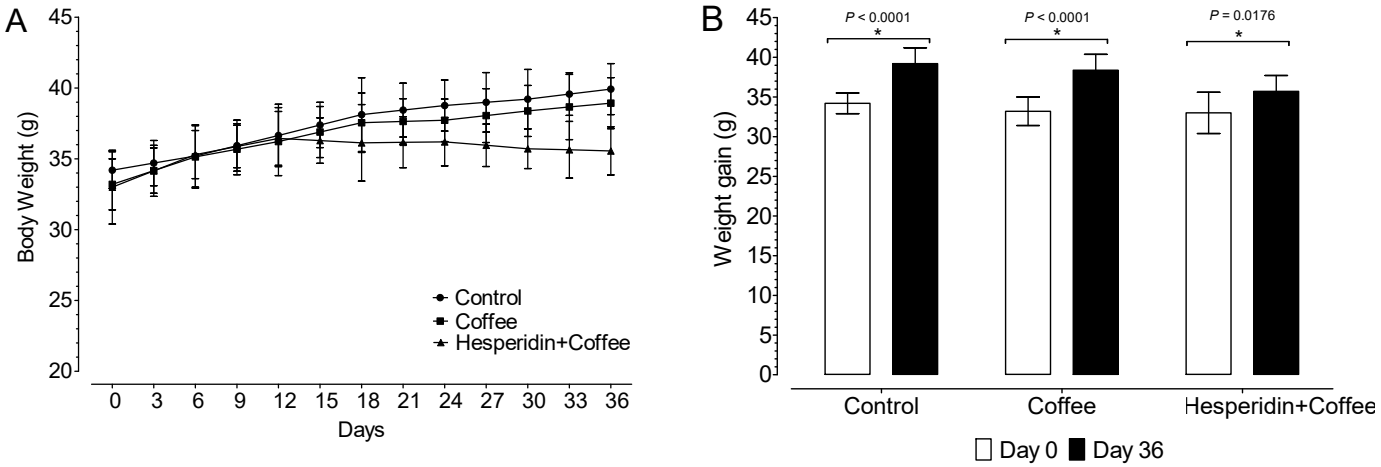

Figure 1: Body weight (**A**) and weight gain (**B**) of mice in the control group (Control) or mice administered brewed coffee extract (Coffee) or mice administered a combination of hesperidin + brewed coffee extract (Hesperidin+Coffee). The data are expressed as the mean ± standard deviation of the evolution of the weight of the mice at the beginning and end of treatment.

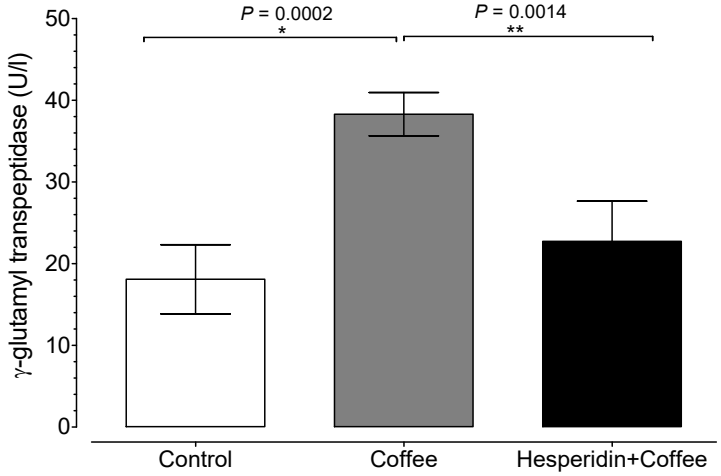

Figure 2: Activity of γ-glutamyl-transpeptidase of mice in the control group, mice administered coffee brewed extract, and mice administered a combination of hesperidin in drinking water + brewed coffee group after 36 days. The data are expressed as the mean ± standard deviation.