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EVALUATION OF GLUCOSE TOLERANCE IN OBESE RATS TREATED WITH *Citrus bergamia* FRUIT EXTRACT

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RESUMO

O objetivo foi avaliar o efeito do extrato aquoso do fruto de *C. bergamia* (BFE) na tolerância à glicose de ratos obesos. Ratos Wistar machos com obesidade induzida por dieta rica em gordura e açúcar (HSF) foram utilizados durante 30 semanas após desmame. Na 20ª semana, iniciou-se o tratamento com BFE por 10 semanas. Ao fim do tratamento, os animais foram submetidos ao Teste Oral de Tolerância à Glicose (TOTG) para cálculo da Área Sob a Curva (AUC) e foram eutanasiados para coleta de sangue. A glicemia dos tempos 0 (jejum) e 120 minutos do TOTG e a AUC nos grupos obesos (HSF e HSF+BFE) foram maiores do que as dos grupos C e C+BFE. No entanto, o HSF+BFE apresentou AUC menor do que o HSF, confirmando um menor nível de glicose circulante. Portanto, o tratamento com BFE foi eficaz em melhorar a tolerância à glicose em ratos obesos.

Palavras-chave: obesidade, intolerância à glicose, bergamota, roedor.

ABSTRACT

The objective was to evaluate the role of *C. bergamia* aqueous fruit extract (BFE) in the glucose tolerance of obese rats. Male Wistar rats with obesity induced by a high-fat and high-sugar diet (HSF) were used for 30 post-weaning weeks. In week 20, the BFE treatment was started, lasting ten weeks. At the end of the treatment, the animals were subjected to an Oral Glucose Tolerance Test (OGTT) to determine the Area Under the Curve (AUC), were anesthetized, and killed for blood collection. At time points 0 (fasting) and 120 minutes, and the AUC, the glycemia was higher in the obese groups (HSF and HSF+BFE) than C and C+BFE groups. However, the AUC was reduced in the HSF+BFE group than HSF group, confirming a lower circulating glucose level. Therefore, BFE treatment effectively improved the glucose tolerance of the obese rats.

Keywords: obesity, glucose intolerance, bergamot, rodent.

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1. INTRODUCTION

World Health Organization (WHO) defines obesity as an abundant accumulation of body fat associated with several health consequences, such as cardiovascular disease, diabetes, musculoskeletal diseases, and some types of cancer (Hruby; Hu, 2015). In 2022, the WHO reported that approximately 2 billion adults presenting the age of 18 or more were overweight, of which 650 million were obese, and at least 2.8 million adults annually die because of these conditions (WHO, 2024).

Obesity leads to increased oxidative stress, which is related to an imbalance between reactive oxygen species (ROS) and antioxidants, and inflammation, directly impairing the sensitivity of peripheral tissues to insulin. Therefore, obesity is a risk factor for developing Insulin Resistance (I.R.) (Barazzoni *et al*., 2012). Clinical research has ethical limitations and control of variables (nutritional and socioeconomic) that can influence the pathophysiological mechanisms related to obesity and its complications. To escape these limitations, several studies in the literature use animal models to study obesity and its repercussions (Róvero Costa *et al*., 2019; Nakandakare-Maia *et al*., 2023).

In recent decades, several forms of treatment have been used to reduce obesity; among them is the use of phenolic phytochemicals or natural extracts, such as the phytochemicals found in pomegranate (*Punica granatum* L.) that have been associated with several beneficial properties, including the anti-obesity effect (Machado *et al*., 2022; Laurindo *et al*., 2024). Among the plants used for this purpose, bergamot (*Citrus bergamia*), found in southern Italy, in the Calabria region, has been a scientific target due to its safety in use and efficacy (Cicero; Colletti, 2016; Carresi *et al*., 2020).

An experimental and clinical study used *C. bergamia* to evaluate the glycemic and lipid profile of the polyphenols of this plant, demonstrating that in both animals and patients, treatment with *C. bergamia* has hypolipidemic and hypoglycemic effects (Mollace *et al*., 2011). In addition, bergamot has antioxidant and anti-inflammatory action with proven benefits in some metabolic diseases (Ferlazzo *et al*., 2016).

Studies evaluating the effects of *C. bergamia* extract on glycemia and biochemical parameters after chronic ingestion of Western diets are scarce in the literature. We hypothesized that *C. bergamia* treatment induces glucose tolerance in obese rats. Therefore, our study aimed to evaluate the effect of bergamot fruit extract (*C. bergamia*) on the glucose tolerance of obese rats.

2. METHODOLOGY

2.1 ANIMALS

For the experiment, adult male Wistar rats (n=40), weighing approximately 160 grams, were used. These rats constituted four

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experimental groups (n=10 animals/group). Food and water were provided ad libitum. Diet consumption and caloric intake were monitored daily. The product of consumption and energy content of the diets determined caloric intake, and the animals' body weight was measured weekly. The rats were kept in individual cages enriched with crumpled paper towels in the shape of balls to reduce stress. Temperature (24±2ºC) and humidity (55±5%) were controlled, and the light-dark cycle (12-12hs) was maintained. The Ethics Committee on Animal Experimentation of the local institution (1337/2019) approved the study protocols and followed the recommendations of the Guide for the Care and Use of Experimental Animals.

2.2 TREATMENT WITH A STANDARD OR HIGH-SUGAR FAT (HSF) DIET

The standard and HSF diets were formulated at the Experimental Research Unit (Unipex) of the Botucatu Medical School (FMB/Unesp), following the ingredients and nutritional composition standard proposed by Francisqueti *et al.* (2017). The high-sugar fat diet had 34.2% of energy from lipids. In addition to the diet, the rats received filtered drinking water with 25% sucrose (HSF). Control animals received a standard diet and only filtered drinking water without added sucrose. The animals received the diet according to their group after weaning for 20 weeks.

2.3 PREPARATION OF BERGAMOT FRUIT EXTRACT AND ROUTE OF ADMINISTRATION

The fruit containing the albedo (white, fibrous part found internally to the skin of the fruit) of bergamot (*Citrus bergamia*) was sliced and mixed with water to extract the polyphenols following the protocol of Vedova *et al.* (2023). The animals received bergamot extract daily by intragastric route (gavage) at a dosage of 250 mg/kg of body weight, equivalent to 2.43 g for an adult human individual with a mean weight of 60 kg of body weight. This preparation followed other studies using *Citrus bergamia*, which confirmed this dose was non-toxic (Abdelghffar *et al.*, 2021). The non-treated animals received only drinking water (vehicle), similar to those treated with BFE.

2.4 EXPERIMENTAL GROUPS

Male Wistar rats (n=40) were distributed after weaning into four experimental groups (n=10 animals/group): 1) Rats given a standard diet and drinking water (Control-C); 2) C rats treated with bergamot fruit extract (C+BFE); 3) Rats given a high-fat, high-sugar diet (HSF) and drinking water; 4) HSF rats treated with BFE (HSF+BFE). After 20 weeks, the animals given the standard diet or HSF according to their experimental group received BFE or vehicle for ten successive weeks, performing 30 weeks under the experiment.

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2.5 ORAL GLUCOSE TOLERANCE TEST (OGTT) AND AREA UNDER THE CURVE (AUC)

After ten weeks of treatment with BFE, all rats were fasted for eight hours to perform the OGTT following the protocol by Quintanilha-Gallego *et al*. (2024). The total circulating glucose response was assessed by calculating the Area under the curve (AUC) (Tai, 1994).

2.6 EUTHANASIA OF THE ANIMALS AND COLLECTION OF BLOOD SAMPLES

At the end of the experimental period, the animals were exposed to an 8-hour fast and then anesthetized with Ketamine (225 mg/kg, i.p.) and Xylazine (30 mg/kg, intraperitoneal route). The animals were euthanized and decapitated for blood collection and assessments.

2.7 ASSESSMENT OF TRIGLYCERIDE LEVELS AND TYG INDEX

After collecting total blood, the plasma samples were obtained for triglyceride measurement and determined in a private laboratory. Subsequently, the TyG index was calculated using the following equation: TyG= Ln [fasting triglycerides (mg/dL) × fasting blood glucose (mg/dL)/2], used as a tool to assess insulin resistance (Guerrero-Romero *et al.*, 2010).

2.8 STATISTICAL ANALYSIS

Considering four experimental groups based on previous results from our laboratory on triglyceride concentration, power of 90%, and reliability of 95%, the sample size was ten rats per group. ANOVA followed by Tukey's Multiple Comparison Test was used to compare the mean values of blood glucose, triglycerides, and TyG. $P< 0.05$ was considered as limit of statistical significance.

3. RESULTS AND DISCUSSION

The present study aimed to evaluate the efficacy of treatment with bergamot fruit extract (*Citrus bergamia*) on glycemia, total cholesterol, triglyceride levels, and TyG index (fasting triglyceride and glucose product) in post-weaning male Wistar rats fed a high-fat, high-sugar diet (HSF). The animals that received BFE showed no difference in glycemic levels, while the HSF group had higher glycemia. However, the animals that ingested HSF and were treated with BFE showed lower glycemia during the OGTT, resulting in a lower concentration of circulating glucose in the 120 minutes of the test. This confirms the beneficial effect of BFE on the glycemia of adult rats fed a high-fat and high-sugar diet.

Figure 1 presents blood glucose levels in the Oral Glucose Tolerance Test (OGTT) (A) and the Area Under the Curve (AUC) values of circulating glucose at 120 minutes (B). During fasting, the HSF and HSF+BFE groups had higher blood glucose levels than those not

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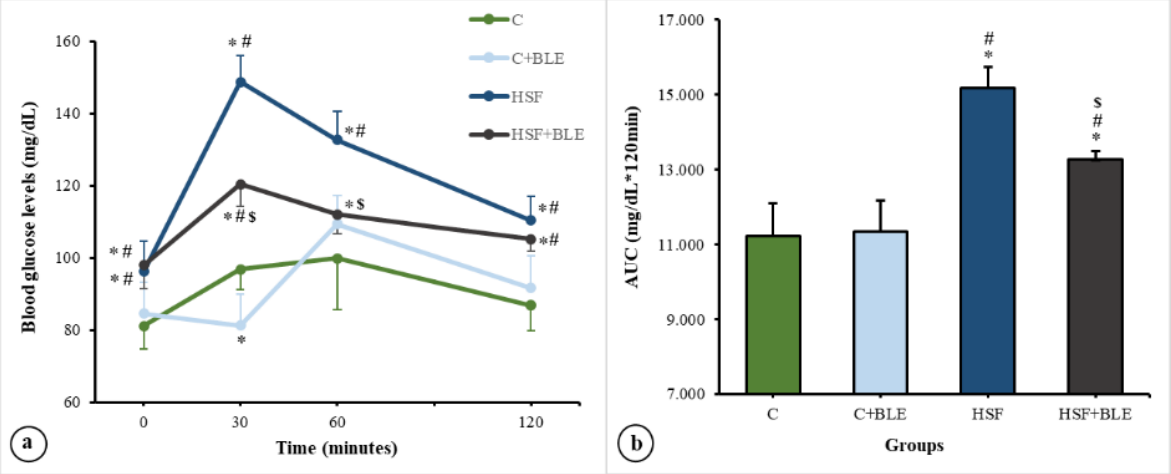
consuming the HSF diet (C and C+BLE). After the glucose overload at 30 minutes, the groups that received the diet, regardless of the treatment, showed higher levels of blood glucose than the control groups (C and C+BFE). The C+BLE group had lower blood glucose levels than the control (C). After 60 minutes of the glucose overload, the HSF group had higher blood glucose levels than the control groups (C and C+BLE), and the HSF+BLE group showed an increase in the glycemic levels compared to the control group and a decrease compared to the HSF group. At 120 minutes, regardless of treatment, both groups that received the diet had higher blood glucose levels than the control groups (C and C+BFE). The AUC calculations for the HSF and HSF+BFE groups were higher than the C and C+BFE groups. However, the HSF+BFE group had a lower AUC than the HSF group.

Figure I. a) Blood glucose levels of Oral Glucose Tolerance Test (OGTT); b) Area Under the Curve (AUC) calculated by OGTT in 120 minutes (min). Experimental groups (n=10 animals/group): control diet and placebo (C), control diet and Bergamot fruit extract (C+BFE), a diet high in fat and sugar and placebo (HSF), and a diet rich in fat and sugar and BFE (Bergamot fruit extract) (HSF+BFE).



Data are expressed as mean ± standard deviation,
*p<0.05 compared to the C group; #p<0.05 compared to the C+BFE group; $p<0.05 compared to the HSF group (ANOVA followed by Tukey's Multiple Comparison Test).

The high-fat and high-sugar diet was developed to mimic Western eating habits. Literature has shown that animals fed the HSF diet present obesity, metabolic syndrome, hyperleptinemia, hypertension, and hypertriglyceridemia when compared to control rats (Siqueira *et al*., 2022; Nakandakare-Maia *et al*., 2023). In addition, HSF diets are rapidly absorbed due to their lower molecular weight, and consequently, there is an increase in fat deposition. Animals that consumed HSF and were treated with BFE had a reduction in glycemia. There is evidence that naringenin is one of the main factors responsible for the

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increase in AMPK activity and glucose uptake in muscular cells and the liver (Hwang *et al*., 2009; Zygmunt *et al*., 2010), which reduces the glycemia in the OGTT and AUC. The BFE has flavonoids, including flavanones (such as naringenin, hesperetin, and eriodictyol glycosides), flavones (apigenin, luteolin, chrysoeriol, and diosmetin glycosides), and their 3-hydroxy-3-methyl-glutaryl (HMG) derivatives (Baron *et al*., 2021).

As shown in Table I, the HSF and HSF+BFE groups had higher levels of fasting blood glucose, triglycerides, and TyG than the C and C+BFE groups.

Hypoglycemic, antioxidant, and anti-inflammatory activities have been described (Nauman; Johnson, 2019; Perna *et al*., 2019; Carresi *et al*., 2020). Although studies demonstrate that bergamot has polyphenols and flavonoids responsible for reducing triglyceride concentration (Miceli *et al*., 2007; Nakandakare-Maia *et al*., 2023), our results showed no changes in triglyceride levels and TyG index. This finding might be related to the use of the fruit and not this plant's leaf extract or juice (Baron *et al*., 2021; Della Vedova *et al*., 2023).

Table I. Fasting glycemia, triglyceride levels, and TyG index of the blood samples from adult male Wistar rats fed a standard diet (C) or a high-fat, high-sugar diet (HSF) for 20 weeks and treated with placebo (P) or fruit extract of bergamot (Bergamot fruit extract) (BFE) for 10 weeks.

	Groups			
	C (n=10)	**C+BLE** (n=10)	**HSF** (n=10)	**HSF+BLE** (n=10)
Fasting blood glucose (mg/dL)	81.8 ± 6.2	84.5 ± 8.7	96.2 ± 8.4$^{*\#}$	98.0 ± 6.6$^{*\#}$
Triglycerides (mg/dL)	24.2 ± 7.5	26.9 ± 13.6	76.4±22.5$^{*\#}$	75.2±20.0$^{*\#}$
TyG	6.8 ± 0.3	6.9 ± 0.4	8.2 ± 0.3$^{*\#}$	8.2 ± 0.2$^{*\#}$

Legend: TyG: product of fasting triglycerides and glucose.
Values are expressed as mean ± standard deviation.
*p<0.05 compared to the group C; $^{\#}$p<0.05 compared to the C+BFE group (ANOVA followed by Tukey's Multiple Comparison Test).

4. CONCLUSION

Therefore, our study shows important findings because it demonstrates that treatment with bergamot fruit extract was able to improve the glycemic levels of obese rats.

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