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EFFECTS OF THE DRY EXTRACT OF *CITRUS AURANTIUM* ON THE METABOLIC PROFILE OF WISTAR RATS

Claudia R. P. Detregiachi[1], Patrícia Cincotto dos Santos Bueno[1], Sandra Maria Barbalho[1,2*], Karina R. Quesada[1], Juliana Santana Silva[1], Paula Caroline Simião dos Santos[1], Sarah Monique Sakuray dos Reis[1], Claudemir Gregório Mendes[1]

[1]University of Marilia (UNIMAR), Marılia – Av. Higino Muzzi Filho, 1001 Marília - São Paulo, Brazil.
[2]School of Food Technology (FATEC), Av. Castro Alves, 62 - Marılia – São Paulo, Brazil.

ABSTRACT

Many plants have been used to reduce the risk factors associated with conditions as obesity, diabetes and cardiovascular diseases. *Citrus aurantium* (CA) is popularly known as bitter orange and the extract is primary a product from the immature (green) fruits of the Seville orange. It is widely used in folk medicine as in the treatment of anxiety, insomnia, cardiovascular stimulant, digestive, stomachache, sedative and to weight loss. The aim of this study was to evaluate the effect of the dry extract of CA on the metabolic profile of Wistar rats. Animals were divided in 4 groups: control group and groups treated with 5, 10 and 20 mg/kg weight of CA dry extract containing 6% *p*-synephrine. Results show no significant modifications in the body weight, visceral fat, glycaemia and lipid profile. Our results contradict many findings of the literature, thus we suggest that more research need to be done to evaluate the real effects of CA on the metabolic profile of animals as well as it would be essential to determine the optimal doses and the correct time for the treatment in order to obtain the benefic effects.

Key words: *Citrus aurantium*, Weight, Cholesterol, Glycaemia.

INTRODUCTION

Lifestyle of the global population is deeply changing and the consumption of healthy food is decreasing and the physical inactivity is increasing. These changes lead to the raise of the indices of diseases such as obesity, type 2 diabetes mellitus (DM2), metabolic syndrome (MS) and cardiovascular diseases (CVD). Several plants have been used to reduce the risk factors associated with these diseases and many of them are efficient in the prevention or even in the treatment possibly due to the presence of bioactive compounds as the phytochemicals (Zhang *et al.*, 2015; Barbalho *et al.*, 2015; Vinayagan, Jayachandran, Xu, 2015). Among the plants with therapeutic potential in obesity, we may mention the *Citrus aurantium*, popularly known as bitter orange and its extract is primary a product from the immature (green) fruits of the Seville orange. It belongs to the family Rutaceae and its fruits, flowers and leaves have been widely used in folk medicine as an aid in the treatment of anxiety, insomnia and as an anticonvulsant. In the Mediterranean region it is used since medieval times as a cardiovascular stimulant, digestive, stomachache, sedative, tranquilizer as well as antidote against poisons. Authors also have shown that it may exhibit anti-inflammatory and anti-oxidant effects (Impelliezzerial *et al.*, 2015; Stohs, Preuss, Shara, 2012; Carvalho-Freitas, Costa, 2002; Arias, Ramon-Laca, 2005).

Citrus aurantium extract are often used for weight loss and its primary alkaloid constituent is *p*-synephrine that are commonly used as thermogenic but it may also exhibit metabolic effects as augment in the basal metabolic rate and lipolysis and appetite suppression (Stohs, Preuss, Shara, 2012, Verpeut, Walters, Bello,

*Corresponding Author

Sandra M. Barbalho
Email: smbarbalho@gmail.com

2013; Stohs, Shara, 2007). However, literature shows a concern related to the safety of extract of this plant and *p*-synephrine. This compound is a phenylethylamine derivative with a hydroxy group in the "para*"* position on the benzene ring and differs from the synthetic *m*-synephrine (phenylephrine) that possess a hydroxyl group in the "meta*"* position on the benzene ring. This synthetic compound may cause cardiovascular effects but it is not present in the bitter orange. This misunderstanding about these components usually leads to confusion and inappropriate references to *m*-synpephrine (Stohs, Preuss, Shara, 2012; Pellati, Benvenuti, 2007; Sander, Putzbach, Nelson, 2008; Evan *et al.*, 2008).

Another compound known in the *C. aurantium* is a biogenic amine named octopamine that has been identified as an important active component in this and in other *Citrus* species. Octopamine and synephrine are alkaloids with adrenergic activity. Synephrine is similar in structure to epinephrine, and octopamine is similar to norepinephrine (Oliveira *et al.*, 2013; Haaz *et al.*, 2006).

Literature presents many studies with *p*-synephrine and *C. aurantium* extracts mixed with other ingredients and herbal components and the safety and efficacy is confused due to the structural similarity between *p*-synephrine and ephedrine and other biogenic amines. It is worthy to say that the pharmacokinetics of these components and the receptor binding specificities are not the same because of the structural and stereochemical differences in the molecules (Bahmani *et al.*, 2015; Lopes *et al.*, 2013; Stohs, Preuss, Keith, 2011; Stohs, Preuss, 2011).

Given these considerations and based in the fact that the use of food supplements to weight loss has been observed Brazil and worldwide the aim of this study was to evaluate the effect of the dry extract of *Citrus aurantium* on the metabolic profile of Wistar rats.

MATERIAL AND METHODS

Ethical principles

This study had the approval from the Animal Research Ethics Committee of the University of Marília (UNIMAR/ Marília, SP, Brazil) with protocol number 17/2015 and during the experimental period, the animals were cared for according to the recommendations of the Canadian Council's "Guide for the care and use of experimental animals".

Animal groups

Animals used in this study included 40 females of Wistar albino rats (*Rattus norvegicus*) weighing approximately 220g to 240g, which were kept in the vivarium at University of Marilia (UNIMAR). The rats were housed in collective cages under a dark/light cycle of 12 hours, 22 ± 2°C in the room temperature, and relative air humidity of 60 ± 5%. After seven days of acclimation to the vivarium conditions, the rats were randomly divided in four groups (n=10):

G1 – Control group that was fed water and rat food ad libitum and daily received 0.5mL of water (gavage route).

G2 – Group that was fed water and rat food ad libitum and daily treated with gavage of 5 mg/kg of *Citrus aurantium* dry extract containing 6% *p*-synephrine dissolved in water.

G3 – Group that was fed water and rat food ad libitum and daily treated with gavage of 10 mg/kg of *Citrus aurantium* dry extract containing 6% *p*-synephrine dissolved in water.

G4 – Group that was fed water and rat food ad libitum and daily treated with gavage of 20 mg/kg of *Citrus aurantium* dry extract containing 6% *p*-synephrine dissolved in water.

The weight gain was evaluated every two days as well as the consumption of water and rat food and after 18 days of treatment and a 12-hour fast, the animals of G1, G2, G3 and G4 were euthanized with a lethal intraperitoneal injection of thiopental (200 mg/Kg) until complete sedation. After that blood samples were drawn from the vena cava to determine biochemical profile (glycaemia, total cholesterol, LDL-c (Low density lipoprotein), HDL-c (High density lipoprotein) and triglycerides. The visceral fat were collected and weighted.

Statistical analysis

Statistical analysis of quantitative data was carried out using BioEstat 5.0. To the evaluation of variables, it was used the one way ANOVA. Correlation analyses were performed after Pearson Test. The probability of significance considered was 5% ($p < 0.05$).

RESULTS

In table 1 it is possible to find that, at the beginning of the experimental period there was no significant differences in mean weight of the animals ($p = 0.3332$), a fact that remained until the end of the study ($p = 05,098$). However, the average weight gain was lower in G2 and G3 in relation to G1 and G4, but with no significant difference ($p = 0.3284$). The average daily consumption for animal feed was similar between groups ($p = 0.6952$).

The correlation between weight gain (g) and the weight of visceral fat (g) in the animals from group G1 and G4 was weak ($r = -0.3033$) and negligible negative ($r = -0.0705$) respectively, while in groups G2 and G3 we observed a moderate negative correlation of $r = -0.7176$ and $r = -0.5118$ respectively, and showing significance in the G2 group ($p = 0.0194$) (Figures 2-5). At the end of the experimental period, we did not observe significative differences in glycaemia and lipid profile (Table 2).

Figure 1. Weight of the visceral fat (g) of the experimental groups.

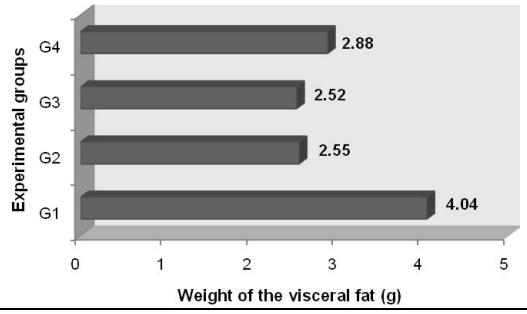

Figure 2. Correlation between weight gain and visceral fat in the animals of experimental group G1.

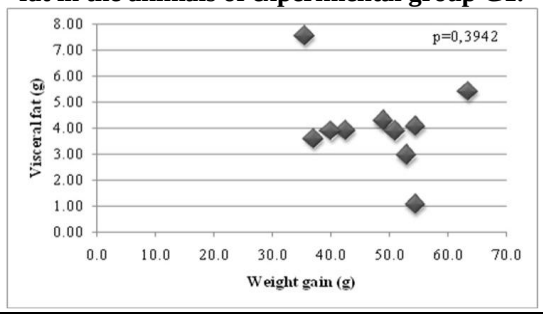

Figure 3. Correlation between weight gain and visceral fat in the animals of experimental group G2.

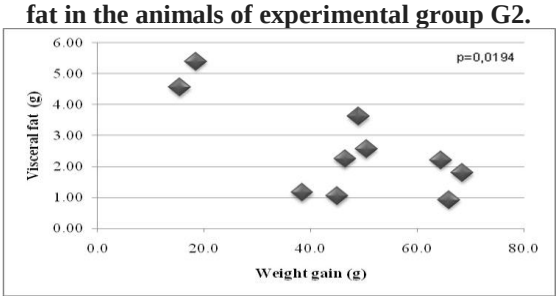

Figure 4. Correlation between weight gain and visceral fat in the animals of experimental group G3.

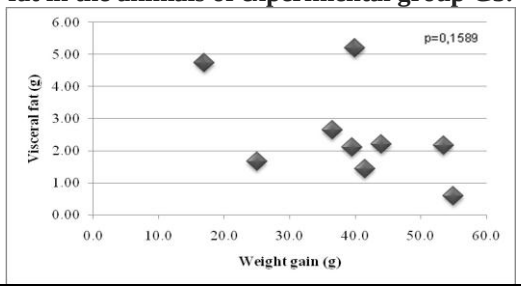

Figure 5. Correlation between weight gain and visceral fat in the animals of experimental group G4

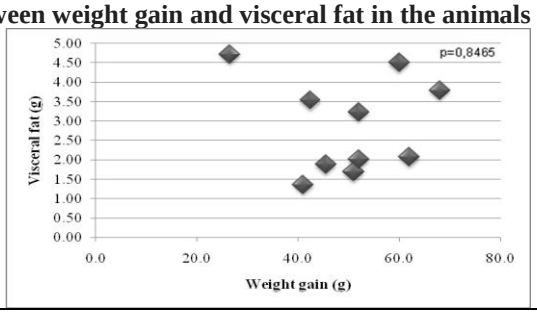

Table 1. Initial and final weight, percentage of body weight gain and feed consumption of the animals during the experimental period

Parameters	G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	*p*-value†
	mean ± standart deviation				
Initial weight (g)	128.4±24.65	120.4±33.03	141.66±25.96	122.1±23.91	0.3325
Final weight (g)	176.6±23.30	166.9±20.59	180.88±20.72	172.20±18.46	0.5098
Weight gain(g)	48.05±9.01	46.25±18.32	39.11±12.17	50.05±11.95	0.3284
Food comsumption (g)	16.1±1.74	15.36±1.54	16.27±2.18	15.6±1.37	0.6952

† Anova one way Test.

No significant differences (p=0.0953) were found in the visceral fato f the groups, however those treated with *Citrus aurantium* exhibit lower values.

Table 2. Biochemical parameters of G1, G2, G3 and G4.

Parameters	G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	*p*-value†
	mean ± standart deviation				
Glycaemia (mg/dL)	165.0±32.69	167.2±33.52	186.9±55.12	247.2±171.79	0.1942
Total cholesterol (mg/dL)	51.0±10.36	54.7±8.55	51.66±10.19	54.7±7.15	0.7192
HDL-c (mg/dL)	38.3±7,87	44.6±6.04	40.6±8.48	42.0±8,85	0.2661
Triglycerides (mg/dL)	65.9±25.11	65.9±20.34	68.7±21.76	64.8±22.92	0.9846

HDL-c: high-density lipoprotein cholesterol. † Anova one way Test.

DISCUSSION

Data from this study show that there was no interference of the dry extract of *Citrus aurantium* in food intake, or in the average weight gain of the animals, although the average weight gain was lower in G2 and G3 in relation to G1 and G4. We also did not observe significant modifications in the biochemical profile among the control group and treated groups.

Some studies have shown that the bitter orange extract alone or in combination with other constituents increase the metabolic rate (Stohs, Preuss, Keith, 2011; Sale *et al.,* 2006; Gougeon, Harrigan, Tremblay, 2006). Calapai et al (1999), demonstrated in a study conducted with rodents, that *Citrus aurantium* administration with 4% concentration and 6% synephrine, at doses of 2.5, 5, 10 and 20 mg/kg significantly reduced the food intake and weight gain.

Colker *et al.,* (1999) conducted a study with humans who receive daily 975 mg of *Citrus aurantium* containing 6% of synephrine and at the end of the experiment the data showed that there was significant weight loss, with an average reduction of 2.9% in percentage of fat. But it is noteworthy that along with the *Citrus aurantium* the tested product contained a generous amount of caffeine, which has thermogenic effect. According to Arbo *et al.,* (2008) and Haaz *et al.,* (2006), *C. aurantium* has effects on the metabolism, but also leads to the reduction of gastric motility, increase satiety what reduces food intake. Kim *et al.,* (2012) performed a study for 6 days with culture of cells that received two different concentrations of *C. aurantium* extract, and observed changes in adipogenesis, and the highest concentration of extract inhibited more strongly lipid accumulation during adipogenesis, contributed to adipocyte lipolysis and inhibited the differentiation of pre-adipocytes into adipocytes. In line with Peixoto *et al.,* (2012) *C. aurantium* extract and p-synephrine increase glycogenolysis, glycolysis, oxygen uptake and perfusion pressure, probably due to, at least in part, α- and β-adrenergic antagonism. p-synephrine increased glucose output that was only 15% smaller than those promoted by the extract containing p-synephrine. At lower concentrations, *C. aurantium* extract increased gluconeogenesis, but at high concentrations it was inhibitory. Authors also found that the action of the plant extract on liver metabolism is similar to adrenergic agents and may be partly attributed to p-synephrine and most of catabolic actions are compatible with the weight-loss effects promoted by *C. aurantium.*

Verpeut, Walters, Albello (2013) studied the effects of *C. aurantium* in normal-weight animals as well as in high-fat diet (60% fat) animals and showed that the acute administration of this plant (1-10 mg/kg) did not reduce deprivation-induced food intake and body weight, but the treatment of both *C. aurantium* (5.6 mg/kg) and *Rhodiola rosea* (20 mg/kg) produced a 10.5% feeding suppression and a 30% decrease in visceral fat weight. Authors have concluded that this association may promote actions on central monoamine pathways and exhibit benefic potential for the treatment of obesity.

Oliveira *et al.,* (2013) showed that octopamine (also present in *C. aurantium*) promoted increase in glycogenolysis, glycolysis, oxygen uptake, gluconeogenesis and accelerated the oxidation of exogenous fatty acids and concluded that octopamine increase catabolic and anabolic processes in the liver via adrenergic stimulation and the acceleration of oxygen uptake increase the oxidation of endogenous fatty acids, derived from lipolysis. They also postulate that these effects are compatible with reported weight-loss effects in experimental animals. Cui *et al.,* (2015) studied the effects of p-synephrine in rat liver and found suppressive effect on the glucose production but no effects were seen in the lipid accumulation in liver cells.

Compounds as hesperidin, naringenin, tangeretin, and nobiletinare are flavonoids found in *Citrus* species and are associated with properties as lipid-lowering, antidiabetic, anti-inflammatory, and antihypertensive. Many studies with animal models of metabolic syndrome and diabetes, show that these components may improve glucose tolerance, insulin sensitivity, and dyslipidemia, suggesting that they could be useful in the therapeutic approach of DM and its complications as MS and CVD (Evans *et al.,* 2015; Sharma *et al.,* 2011; Assini, Mulvihill, Sutherland, 2013; Alam, Kauter, Brown, 2013; Part *et al.,* 2013; Shin *et al.,* 2013).

Jia *et al.,* (2015) studied the effects of neohesperidin derived from *C. aurantium* in diabetic mice and observed significant reduction of fasting glucose and serum glucose as well as triglycerides and total cholesterol. Oliveira, Dourado, Cesar (2013) used hesperidin in rats and observed that this compound itself or in combination with swimming exercise, decrease total cholesterol, LDL-c, triglycerides and increase HDL-c levels. In the other hand, the use of hesperidin did not improve the lipid profile (total cholesterol and LDL-c) in men and women in moderately hypercholesterolemia (Demonty *et al.,* 2010). Assini *et al.,* (2015) studied the effects of naringenin (another component derive from *Citrus*) and observed its potential as lipid-lowering and as an insulin sensitizer in vivo. In another study, Costa *et al.,* (2013) observed hypolipidemic effects of *C. aurantium* essential oil.

Studies with polyphenolic fraction derived from other species of *Citrus* reduced total cholesterol, LDL-c, the LDL-c/HDL-c ratio in patients with hyperlipidemia (Gliozzi *et al.,* 2013). Jing *et al.,* (2013) used d-limonene in obese mice and observed reduction of LDL-c, triglycerides, fasting blood glucose and glucose tolerance, and increased HDL-c. Authors suggest that the use of d-

limonene derivate form *Citrus* species may help hyperlipidemic and hyperglycemic patients ameliorating metabolic disorders.

Our study shows that use *C. aurantium* did not exhibit significant effects on the body weight, visceral fat, glycaemia and lipids in the animals of the experimental groups, though when we performed the correlation between weight gain and the weight of visceral, only the G2 presented significance. This may suggest that smaller doses could have better effects than higher concentrations. Other possibility to obtain better results would conduct this study for a longer period of time.

CONCLUSION

Our results contradict many findings of the literature, thus we suggest that more research need to be performed to evaluate the real effects of *C. aurantium* on the metabolic profile of animals. It is also essential to determine the optimal doses and the correct time for the treatment in order to obtain the desired effects.

CONFLICT OF INTERESTS

All authors declare no conflict of interests.

AUTHORS' CONTRIBUTIONS

CRPD, SMB and PCCB: designed the study and wrote the final version of the manuscript; PCSB and PCSS: performed the statistical analysis; CGM and JSS wrote the protocol and performed the laboratorial analyses; CRPD, SMB and KRQ managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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