Effect of commercial extract of *citrus aurantium* in obese rats induced by cafeteria diet

Efeito do extrato comercial de *citrus aurantium* em ratos obesos induzidos por dieta de cafeteria

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ABSTRACT

Obesity is the result of a higher caloric energy expenditure increasing the risk of developing diabetes and cardiovascular diseases. *Citrus aurantium*, has been used for weight reduction by having p-Synephrine, a compound associated with this action. The objective of this work was to evaluate the effects of commercial extract of *Citrus aurantium* in weight alteration in the biochemical parameters of rats induced to obesity by High fat / cafeteria diet. The animals were divided into four experimental groups: Group 1: Standard commercial feed; Group 2: standard commercial feed, and solution of commercial extract of *Citrus aurantium* via gavage; Group 3: high fat/cafeteria feed. Group 4: high fat/cafeteria feed commercial extract of *Citrus aurantium*, via gavage. In order to maintain the same experimental conditions in all groups, the animals in groups 1 and 3, received filtered water via gavage. Tests were performed to evaluate the antioxidant capacity of commercial extract of *Citrus aurantium*: total phenolic compounds, DPPH and FRAP.E in the plasma of animals: FRAP in addition to analyzes of blood biochemical parameters. Results obtained with the commercial extract showed positive results, however, the antioxidant activity in plasma, was not able to alleviate oxidative stress. Significant differences were found in the values of Aspartate aminotransferase, alanine aminotransferase, VLDL cholesterol, HDL and triglycerides, however there was no reduction of weight. The commercial extract of *Citrus aurantium*, is important in the prevention of hepatic damage and diseases linked to elevations in the levels of VLDL and triglyceride levels, especially when this increase is associated with obesity.
RESUMO

A obesidade resulta de uma ingestão calórica superior ao gasto energético aumentando o risco de desenvolver diabetes e doenças cardiovasculares. O *Citrus aurantium*, tem sido utilizado para redução de peso por possuir p-sinefrina, composto associado a esta ação. O objetivo deste trabalho foi avaliar os efeitos do extrato comercial de *Citrus aurantium* na redução de peso e alteração nos parâmetros bioquímicos de ratos induzidos à obesidade por dieta hiperlipídica/cafeteria. Os animais foram divididos em quatro grupos experimentais: Grupo 1: Ração comercial padrão; Grupo 2: ração comercial padrão, e solução de extrato comercial de *Citrus aurantium*, via gavagem; Grupo 3: ração hiperlipídica/cafeteria. Grupo 4: ração hiperlipídica/cafeteria e extrato comercial de *Citrus aurantium*, via gavagem. A fim de manter as mesmas condições experimentais em todos os grupos, os animais dos grupos 1 e 3, receberam água filtrada via gavagem. Foram realizados testes para avaliar a capacidade antioxidante do extrato comercial de *Citrus aurantium*: compostos fenólicos totais, DPPH e FRAP, e no plasma dos animais: FRAP além de análises dos parâmetros bioquímicos sanguíneos. Resultados obtidos com no extrato comercial, mostram resultados positivos, no entanto, a atividade antioxidante no plasma, não foi capaz de atenuar o estresse oxidativo. Diferenças significativas foram encontradas nos valores de aspartatoaminotransferase, alanina aminotransferase, colesterol VLDL, HDL e triglicerídeos, contudo não houve redução de peso. O extrato comercial de *Citrus aurantium*, apresenta-se como importante na prevenção de danos hepáticos e doenças ligadas a elevações nos níveis de VLDL e triglicerídeos, principalmente quando este aumento está associado a obesidade.


1 INTRODUCTION

Obesity is a result of dietary intake which exceeds the energy expenditure, resulting in accumulation of fat and excess weight. However, obese individuals differ among themselves in the amount and location of body fat, so it is important to distinguish more severe type, android obesity, where there is a greater accumulation of fat in the abdomen, from less severe, gynoid obesity when fat is distributed more uniformly throughout the body.

There is also a classification for obesity as a chronic non-communicable disease (NCD) which consists of a group of diseases of difficult definition, since it also covers the non-infectious diseases and chronic degenerative diseases, being the NCD triggered by multiple factors and unknown etiologic agents. The excess weight is also associated with a higher risk of developing non-insulin dependent diabetes, type 2 diabetes and cardiovascular diseases.

Identifying the cause of obesity is not a simple task since there is more than one etiological agent involved, among them the genetic and environmental factors, which are extremely comprehensive.
Obese individuals present imbalance among the fat, body weight and lipoprotein, which affects the body regarding the vulnerability to oxidative stress, pointed out as important alteration in the case of obesity, a fact that deserves highlighting as it clarifies possible approaches to this disease⁵.

Protection against oxidative stress is performed by the body by means of endogenous or exogenous systems and⁶, being the antioxidants, bioactive compounds present in fruits, often used in the prevention of diseases associated with oxidative stress and in promoting the health of the body⁷.

As a result of industrialization and urbanization, there was a decrease in the practice of physical exercises, increased caloric intake and the replacement of meals consisting of rice, beans, meat and salads for snacks, soft drinks, cookies and sandwiches, contributing to the increase in the number of people with excess weight. Thus, obesity has become an epidemic in developed countries and has affected all economic classes and ethnicities, resulting from the change in eating habits, physical activity and psychosocial conditions⁸,⁹.

According to data from the World Health Organization (WHO), in 2014 more than 1.9 million adults aged 18 years presented with excess weight, with 38% of men overweight and 11% with obesity and among women, this number is even higher with 40% overweight. The global prevalence of obesity grew by more than half between 1980 and 2014¹⁰.

According to projection of the Brazilian Association for the Study of Obesity and Metabolic Syndrome (ABESO) until 2025 approximately 2.3 billion adults will be overweight and more than 700 million will be obese. Regarding the worldwide number of obese or overweight, if no measure is taken, it might reach 75 million. In a survey conducted by the Brazilian Institute of Geography and Statistics (IBGE) in 2008/2009 showed that obesity or overweight is already presented in ascending way. In Brazil, approximately 50% of the population is above the weight and 15% of children are obese¹¹.

The human eating behavior is influenced by family and cultural habits, media, food fads, psychological needs, body image and food preferences¹².

Combating obesity means to reduce the risk of developing associated diseases, even presenting some failures and recurrences¹³,¹⁴. Analyzing habits of different populations, assists in the understanding of the diseases associated to feeding imbalances such as hypercholesterolemia, diabetes and obesity⁴.
It is important to start the prevention measures for obesity in childhood in order to minimize the serious consequences of excess weight in adulthood, by means of nutritional education and specific legislation for the labelling and publicity and advertising of foods\textsuperscript{12}.

For the choice of the type of appropriate treatment the severity of the problem and the complications that are associated with obesity must be considered. Change in the style of life, through the increase of knowledge, is fundamental to the successful treatment associated with nutrition and, in some cases, surgical procedures\textsuperscript{11}. Among the treatments available, bariatric surgery is an option for patients with severe obesity\textsuperscript{13}, and there is no evidence to recommend this surgical treatment for patients with BMI (body mass index) of less than 35 kg/m\textsuperscript{2}, nor to glucose control for diabetic patients, regardless of BMI values\textsuperscript{11}.

Phyto therapy is a form of treatment with easy access, based on natural compounds that plants possess and has shown effectiveness in the treatment of obesity\textsuperscript{15}. The bioactive compounds of citrus fruits have been highlighted as prosperous for the control of lipid metabolism of obese patients\textsuperscript{16}.

\textit{Citrus aurantium}, belonging to the family of Rutaceae, known as bitter orange or sour orange, is used as a substitute of ephedra, which is a substance indicated for weight reduction by promoting lipolysis, however, has effects that are harmful to health, discouraging its consumption\textsuperscript{15,16}.

Synephrine is the active compound of \textit{Citrus aurantium} with adrenergic action and beta-adrenergic making it the main source for dietary industry by the fact that this bioactive component is reported as responsible for weight reduction\textsuperscript{17,18,19}.

Considering the above, the objective of this study was to evaluate the effects of commercial extract of \textit{Citrus aurantium} extract in weight reduction and the variation of metabolic parameters associated with obesity in obese rats induced by cafeteria diet.

2 METHODS

To evaluate the antioxidant activity, the commercial extract of \textit{Citrus aurantium}, produced from the fruit of the plant, was subjected to quantitative analysis for complementation of information of constituents present in the extract, in addition to those offered by the supplier.

The determination of total phenolic compounds of commercial extract of \textit{Citrus aurantium} was determined in accordance with the Folin-Ciocalteu method (20). For an aliquot of 2.0 mL of the sample suitably diluted, it was added 0.3 mL of sodium carbonate -
Na$_2$CO$_3$. 1.9 M and 0.1 mL of phenol reagent of Folin Ciocalteu 1 mol/l. After 1 hour in the dark, the absorbances were determined at 725 nm on a spectrophotometer Micronal. Gallic acid (AG) (Sigma) was used as a reference. The results were expressed in mg/mL equivalent of AG.

The antioxidant activity - DPPH assay was performed in accordance with the methodologies of Thaipong et al. and Choi et al.$^{21,22}$ DPPH (1,1-diphenyl-2-picrylhydrazyl - 0.024g was diluted in 100 mL of methanol. A volume of 10 mL of this solution was added to 45 mL of methanol, absorbance was read at 515 nm, correcting it to close to 1.1. At 2.85 mL of solution 0.15 mL of the sample was added. After 24 hours in the dark at ambient temperature, the absorbance was measured at 515 nm. The sequestering activity was expressed as % of efficiency of the sequestration of free radicals. \( \% = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100 \). The synthetic antioxidant butyl-hydroxy-toluene (BHT) (0.2mg/mL) was used as positive control.

The assay FRAP (Ferric Reducing Antioxidant Power) was performed in accordance with the authors$^{23,24}$ and is based on the ability of the bioactive compounds in reducing Fe$^{+3}$ in Fe$^{+2}$ in a redox reaction coupled to a colorimetric method. The reagent solution was prepared on the day of use in the following way: 2.5 mL of a solution of TPTZ (2,4,6-tripyridyl-s-triazine) 10 mmol/L in HCl 40 mmol/L, plus 2.5 mL of FeCl$_3$.6H$_2$O (ferric chloride) 20 mmol/L in water and 25 mL of sodium acetate buffer 300 mmol/l pH 3.6. For the reaction it was used: 900 \( \mu \)L of FRAP reagent prepared on the day, 90 \( \mu \)L of distilled water, and the following samples were used (30 \( \mu \)L of extract and/or 50 \( \mu \)L of plasma), and the standard or blank (water), the reaction time was 30 min at 37°C, and centrifuged for 5 min (3500 rpm). It was determined the absorbance at 595 nm against the blank (blank: 120 \( \mu \)L of distilled water + 900 \( \mu \)L of FRAP reagent) and the standard used was Trolox$^\text{®}$ (Sigma).

The research was approved by the Committee on Ethics in Research Involving Animal Experimentation (CEPEEA) of the University of Paraná - UNIPAR, as protocol number 31932/2017.

For the experiment 20 (twenty) male Wistar rats (Rattus norvegicus) were used, with a mean age of 30 days, and average weight of 253 g, originated from Experimental Facilities of Universidade Estadual de Maringá - UEM.

For the purposes of adaptation, the rats stayed 15 days receiving water and feed Purina$^\text{®}$ (Purina Labina for rodents; Nestlé Purina Company) ad libitum, housed in collective boxes of polypropylene with three animals per box, wood shavings type bed, and it was also included as environmental enrichment, pipes of poly vinyl chloride (PVC). Controlled
conditions of temperature of 22±2 º C and luminosity light-dark cycle of 12 hours) were maintained.

12 days before the beginning of the experimental period the animals were housed individually, from the mean ± 10% of the weight, distributed in four groups of different treatments, totaling five animals per group, remaining in this condition until the end of 89 days of experiment.

The groups were composed of five animals each, and followed the treatment protocol described below:

- **Group 1 (G1)** - control group where the animals received standard commercial feed and water *ad libitum*, in addition to receiving 1.0 mL of purified water, via gavage;
- **Group 2 (G2)** - the animals received standard commercial feed and water *ad libitum*, in addition to solution of commercial extract of *Citrus aurantium* at a dose of 14.74mg/ mL/day via gavage;
- **Group 3 (G3)** - the animals received high fat feed and water *ad libitum*, daily doses were also administered of 1.0 mL of purified water via gavage;
- **Group 4 (G4)** - the animals received high fat feed and water *ad libitum*, in addition to solution of commercial extract of *Citrus aurantium* at a dose of 14.74mg/ mL/day via gavage;

The experimental period lasted 89 days and was divided into 2 phases: induction of obesity by high fat /cafeteria diet within the first 70 days of the experiment, followed by treatment with commercial extract of *Citrus aurantium*, with a duration of 19 days.

During the induction period of obesity, the animals were weighed every three days. With the beginning of the period of treatment, the weighing of the groups that received the commercial extract of *Citrus aurantium*, began to be performed with an interval of one day, for adjusting the dose of food supplement.

The feed consumption was calculated considering the difference of the weight of the feed supplied, measured on a digital scale, and the wastes and the leftovers of the feed every two days.

The commercial extract of *Citrus aurantium* was administered once a day, via gavage, always at the same time, between 01 p.m. and 03 p.m. The animals that did not receive the extract, made the ingestion of 1.0mL of filtered water, under the same experimental
conditions, to eliminate the variable of the stress suffered by the animals at the time of this technique.

The definition of dose for the rat, was based on already stipulated human dose of 600 mg/day\textsuperscript{26}, for the calculation of the allometric extrapolation the following formulas and parameters were used:

\begin{align*}
\text{Calculation based on basal metabolic rate (BMR)} \\
\text{BMR of animal-reference (BMR}_\text{ref}) \\
\text{BMR}_\text{ref} &= k \times m^{0.75} \\
\text{BMR of animal-target (BMR}_\text{target}) \\
\text{BMR}_\text{target} &= k \times m^{0.75} \\
\text{Dose} &= \frac{\text{total dose animal-reference (mg)}}{\text{BMR}_\text{ref}} \\
\text{Dose (BMR}_\text{ref}) \times \text{BMR}_\text{target}
\end{align*}

Where:

- \text{BMR} = \text{Basal Metabolic Rate}
- \text{K} = \text{constant of large taxonomic groups (placental mammals = 70)}
- \text{m} = \text{mass index}

The value obtained was 14.74 mg/day for rat with an average weight of 500g, from this pattern, the doses were calculated according to the weight of the animal obtained in weighing on the day of the administration of commercial extract of \textit{Citrus aurantium}.

The animals were observed daily regarding their behavior and general appearance, abnormal clinical signs, assessment of morbidity, especially during the treatment period.

The induction of obesity in animals was by the administration of high fat/cafeteria diet, where 1000 grams (g) of this feed was composed by: 22.3\% of ham pate and 11.1\% of type "shoestring” potatoes, bacon, sausage, biscuits of cornstarch, chocolate powder, whole milk powder and commercial feed following experimental model, adapted\textsuperscript{27,28,29}.

The proposed feed was produced manually, for the weekly intake, with the aid of a mill of knives and homemade multiprocessor for processing of solid ingredients. After milling, all ingredients were incorporated, in order to obtain a homogeneous mass, repelleted at hand and then the feed was dried in an oven at 55°C for 48 hours.

After removing the \textit{pellets} of feed of the greenhouse, the same was stored in jars with airtight and opaque closure to avoid the contact of the feed with room light, thus avoiding the oxidation of components. The animals were fed \textit{ad libitum} during the entire experimental period.
At the end of the experimental period, the rats were weighed and anesthetized with isoflurane in saturated chamber, with monitoring of vital signs, the animals were under anesthesia, a ventral incision was performed in alba line, for exposure of the caudal vein for blood collection, because volumes higher than 3mL were needed for achievement of biochemical tests of animals that have been performed in the Clinical Analysis Laboratory of Universidade Paranaense, after the harvest of blood the animals were then killed by overexposure to anesthetic.

Then  the adipose tissue related to retroperitoneal and periepididymal fat was collected which was weighed on an analytical balance of precision.

The serum was used to assess the hepatic integrity by means of enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT). In addition, other biochemical analyzes were performed, triglycerides, total cholesterol, high density lipoproteins (HDL) and low-density lipoproteins (LDL).

The results were analyzed using the program Bioestat 5.0. The differences between the treatments for the analyzed parameters, were compared by means of analysis of variance (ANOVA). Where relevant the averages were compared by the Tukey test. The results, expressed by means of mean ± standard error of the mean (mean ± SEM), were considered statistically significant when p< 0.05.

### 3 RESULTS

The Determination of Total Phenolic Compounds in commercial extract of *citrus aurantium* is represented in Table 1, showed 0.40 mg/eq. AG/mg of extract.

The antioxidant activity of commercial extract of *Citrus aurantium* expressed as EC\textsubscript{50} (% of efficiency of the sequestration of free radicals), the DPPH assay was 0.40 mg/mL (Table 1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic compounds</th>
<th>DPPH</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrus aurantium</em></td>
<td>0.40± 0.013</td>
<td>0.404</td>
<td>991.21±8.56</td>
</tr>
</tbody>
</table>
In relation to the oxidative capacity measured by the FRAP assay showed that the extract shows 99.21 mmol eq. Trolox/g extract (Table 1). On the other hand, in the plasma of animals there was no significant difference among the treatments (Table 2).

Table 2. Values of FRAP (mmol eq. Trolox/mL plasma) obtained from the blood plasma of rats of different groups.

<table>
<thead>
<tr>
<th>Groups - Rats (n= 3)</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1</td>
<td>12.66±0.12</td>
</tr>
<tr>
<td>G2</td>
<td>11.00± 0.66</td>
</tr>
<tr>
<td>G3</td>
<td>11.13± 0.42</td>
</tr>
<tr>
<td>G4</td>
<td>11.47± 0.34</td>
</tr>
</tbody>
</table>

ANOVA (p > 0.05)

G1: Standard commercial diet and gavage with filtered water; G2: Standard commercial diet and Gavage with Commercial extract of Citrus aurantium; G3: high fat /cafeteria diet and gavage with filtered water; G4: high fat/cafeteria diet and Gavage with commercial extract of Citrus aurantium.

As to the weight of the animals during the induction period of obesity, the animals in groups G3 and G4, had a weight gain statistically higher, when compared to the control group, a factor that defines the beginning of the period of treatment with commercial extract of Citrus aurantium, the second stage of the research. In this second phase, no significant difference was observed among the groups, as shown in table 3.
Table 3. Mean ± standard error of the weight (g) at the beginning of intake of the high fat/cafeteria diet (day 0); on the day of initiation of treatment with commercial extract of *Citrus aurantium* (initial weight of treatment) and on the day of the euthanasia of the animals (final weight); weight of periepididimal and retro peritoneal fat, after autopsy of animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight at day 0</td>
<td>318.21±15.40</td>
<td>297.01±6.55</td>
<td>303.13±5.54</td>
<td>306.38±8.90</td>
</tr>
<tr>
<td>Initial Weight of treatment</td>
<td>445.58±20.33</td>
<td>429.04±13.65</td>
<td>452.48±15.02</td>
<td>465.81±13.29</td>
</tr>
<tr>
<td>Final Weight</td>
<td>436.53±18.12</td>
<td>412.68±13.55</td>
<td>448.27±17.34</td>
<td>450.20±14.46</td>
</tr>
<tr>
<td>Periepididimal Fat</td>
<td>7.69±2.08</td>
<td>11.11±2.26</td>
<td>11.70±1.27</td>
<td>14.08±2.39</td>
</tr>
<tr>
<td>Retro peritoneal fat</td>
<td>14.36±1.63&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.49±1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.90±2.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.94±2.95&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by different letters on the line differ by Tukey test.

G1: Standard commercial diet and gavage with filtered water; G2: Standard commercial diet and Gavage with Commercial extract of *Citrus aurantium*; G3: high fat/cafeteria diet and gavage with filtered water; G4: high fat/cafeteria diet and Gavage with commercial extract of *Citrus aurantium*.

The animals of group G2, which received commercial extract of *Citrus aurantium* and standard commercial feed, presented retro peritoneal fat significantly lower than the animals of group G3<sub>p</sub> <0.01, which received high fat/cafeteria diet and were not supplemented with the commercial extract (Table 3).

The animals of group G4 that received high fat/cafeteria diet and were supplemented, even not showing significant statistical difference when compared to the group G3, showed a tendency to reduction of retro peritoneal fat which can be observed by the lower mean weight of this group and by the fact that this average is lower than the G1 group, indicating that the consumption of commercial extract of *Citrus aurantium*, when not associated to a high fat/cafeteria diet, has a tendency to reduce tissue (Table 3).

No significant differences were found (p <0.05), with relation to the weight of the periepididymal fat among the groups (Table 3).

Regarding the average consumption of feed, the animals of all groups educed the intake after the beginning of the second stage, as shown in table 4, event that is related to the animal’s maturity.
Table 4. Mean ± standard error of the mean daily consumption (g) during the time period 1 (time regarding the induction of obesity), and period 2 (treatment time with commercial extract of *Citrus aurantium*).

<table>
<thead>
<tr>
<th>Groups</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
</table>
| Consumption period 1 | 27.88±0.90
t | 26.35±0.64
t |
| Consumption period 2 | 21.57±0.32
t | 20.48±0.82
t |

Means followed by different letters on the line differ by Tukey test. (p<0.01)

*G1: Standard commercial diet and gavage with filtered water; G2: Standard commercial diet and Gavage with Commercial extract of *Citrus aurantium*; G3: high fat/cafeteria diet and gavage with filtered water; G4: high fat/cafeteria diet and Gavage with commercial extract of *Citrus aurantium*.

About the values of triglycerides, the groups that ingested the commercial extract *Citrus aurantium* showed reduced levels of this component, since significant differences were observed (p < 0.05) between the G1 group, which received commercial feed and was not supplemented, and the group G4, which received the supplement and high fat/cafeteria diet (Table 5).

Table 5. Mean ± standard error of the blood levels (mg/dL), total cholesterol, HDL, LDL, VLDL, triglycerides, hepatic enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT).

<table>
<thead>
<tr>
<th>Groups</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>77.6±6.21</td>
<td>84.8±5.72</td>
<td>100.83±5.1</td>
<td>98.33±6.40</td>
</tr>
<tr>
<td>HDL*</td>
<td>35.08±0.9a</td>
<td>36.6±0.74a</td>
<td>43.83±1.32b</td>
<td>40.33±2.01ab</td>
</tr>
<tr>
<td>LDL</td>
<td>20.75±5.26</td>
<td>24.75±9.23</td>
<td>37.66±5.27</td>
<td>39.83±4.72</td>
</tr>
<tr>
<td>VLDL*</td>
<td>25.4±1.16a</td>
<td>24.25±3.81ab</td>
<td>19.33±1.30b</td>
<td>18.16±0.70b</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>131±6.32a</td>
<td>121.5±19.22abh</td>
<td>96.33±6.00abh</td>
<td>91.66±3.55b</td>
</tr>
<tr>
<td>AST*</td>
<td>303.2±40.53ab</td>
<td>344.2±25.42a</td>
<td>212.2±34.23abc</td>
<td>91.83±5.67d</td>
</tr>
<tr>
<td>ALT**</td>
<td>714.7±2.9a</td>
<td>78.8±6.19a</td>
<td>38±2.65b</td>
<td>36±1.31b</td>
</tr>
</tbody>
</table>

Means followed by different letters on the line differ by Tukey test.

* significant at 5%

** significant at 1%

*G1: Standard commercial diet and gavage with filtered water; G2: Standard commercial diet and Gavage with Commercial extract of *Citrus aurantium*; G3: high fat/cafeteria diet and gavage with filtered water; G4: high fat/cafeteria diet and Gavage with commercial extract of *Citrus aurantium*.

The levels of HDL cholesterol showed a significant difference (p < 0.01) between the G1 and the G3 group, which received commercial ration and high fat/cafeteria, where the...
levels were lower in G1. No difference was found (p > 0.05) between groups G3 and G4, however, the supplemented animals had reduced levels of HDL, as shown in table 5.

The values of VLDL showed a significant difference (p < 0.05) between groups G1 and G4, indicating that the commercial extract can induce a reduction in the levels of VLDL. Despite not presenting significant difference (p > 0.05), a lower average is observed of the G2 group, which received commercial feed and commercial extract of *Citrus aurantium*, in relation to the G1 group, which received only the commercial feed and was not supplemented indicating a trend in reduction of the values of this type of cholesterol (Table 5).

Regarding the values of AST, the commercial extract of *Citrus aurantium* promoted the reduction of these enzymes in the blood, since no significant differences (p <0.01) were observed among the animals of group G1 and G4 (Table 5).

The reduction in the values of the hepatic enzyme ALT of group G4 compared to G1 group, was significant (p < 0.01), indicating that the supplementation with commercial extract of *Citrus aurantium* promotes hepatic protection, however, when the supplementation is performed in animals that received commercial diet there is no protective effect that was observed in the statistical differences in group G2 in relation to group G3 (p < 0.01) and in group G2 and the G4 group (p < 0.01), indicating that the use of commercial extract of *Citrus aurantium* when is no overweight can incur in hepatic damages.

4 DISCUSSION

The antioxidant activity of *Citrus aurantium* is in large part attributed to the phenolic compounds (bioactive compounds) that promote the benefit to the health of the organism. The amount of total phenolic compounds of commercial extract of *Citrus aurantium* and its EC$_{50}$ demonstrate that the antioxidant activity is directly related with contents of total phenolic compounds present in the extract. Studies with different species of the genus *Citrus* showed results that reinforce the relationship between the content of total phenolic compounds present in the extract with its antioxidant activity.$^6$

The commercial extract of *Citrus aurantium* showed total antioxidant activity (FRAP) in vitro, as shown in table 1, however, it was not found in the in vivo tests, significant difference in the different studied groups (Table 2).

These results showed the relationship of exposure time with the analysis of bioactive compounds with antioxidant activity in vitro. The extract should be able to maintain normal
levels of antioxidant activity in the plasma of rats supplemented with *Citrus*, however, this is not observed (Table 1).

FRAP levels in the plasma is an important indicator/factor in the balance between cellular oxidative/antioxidant state. This factor plays an important role in the maintenance of homeostasis under normal physiological conditions.

A recent study demonstrated the relationship between phenolic compounds of the hydroalcoholic extract of grape brand *Merlot* and its antioxidant FRAP activity in the plasma of arthritic rats. The results showed that the extract of grape pomaces kept the normal levels of FRAP in plasma of rats treated, attenuating the effects of oxidative stress caused by the disease.

In the present study, it was demonstrated that the commercial extract of *Citrus aurantium* did not attenuate the cellular oxidative/antioxidant state of obese rats in the FRAP levels in the plasma (Table 2). However, there was no significant difference in the FRAP in the plasma when compared with rats of the G1 group, which received no high fat/cafeteria diet.

Thus, it can be assumed that obesity, in physiologic levels, does not possibly alter drastically the cellular oxidative state, maintaining the levels of FRAP int he plasma similar, even using the extract with antioxidant activity.

It is notorious that the *Citrus aurantium* has bioactive compounds with antioxidant activity *in vitro*, but it is difficult to infer that the extract had physiological effects in obese rats, since there are no more detailed studies on the molecular mechanisms and their biochemical effects in the organism. The compound synephrine of *Citrus* is the most relevant and studied *in vivo* and is related to weight loss.

Oxidative stress is one of the causes of many diseases in humans, and the use of therapeutic agents (bioactive compounds) may contribute to the antioxidant defense system.

Obesity is resulting from the ingestion of food in excess, however, young rats adjust their food intake according to their fat reserves, in addition to that, the caloric content of the diet prevails over other factors that influence food ingestion by the animals. The mechanism of satiety in these animals becomes a hindrance to the etiology of obesity, since that the ingestion of food promotes physiological changes either temporary or permanent, controlled by the central nervous system, which respond to the mechanisms of intestinal distension, animal nutrition, calorie content and palatability of the diet.
In the case of animals, obesity can be identified when the group that receives a hypercaloric diet presents weight gain of more than 10% of the control group, which received a standard diet\textsuperscript{39}.

In a paper, which used high fat /cafeteria diet with the same ingredients used in feed developed in this study, effectiveness was noted in the induction of obesity in animals, since the weight of the animals that received the diet prepared in the laboratory showed a significant difference when compared to the weight of the animals that received the commercial feed\textsuperscript{29}.

A study evaluated the variation in body weight, feed consumption and lipid biochemical profile of female swiss rats treated with \textit{Citrus aurantium}, for 30 days, associated with swimming. These authors associated significant weight loss to exercise practice, control group, and not the isolated use of the extract, since the control group, which received aqueous vehicle, showed no significant loss when compared to the group which ingested \textit{Citrus aurantium}\textsuperscript{40}.

Similar results were found in a study where rats induced with obesity, with a high fat diet and were treated for 10 days, with \textit{Citrus aurantium} extract at a dose of 5.6 mg/kg, showed no changes in relation to body weight or the ingestion of the animal \textsuperscript{41}.

One of the justifications for the reduction in the efficacy of the use of commercial extract of \textit{Citrus aurantium} on weight reduction, when not used the isolated components of the extract, because the commercial extract, are associated with different compounds, making the results of weight reduction to be uniquely assigned to this phytotherapeutic\textsuperscript{26}.

The commercial extracts of \textit{Citrus aurantium} have from 3 to 6% of p-Synephrine, active compound present in \textit{Citrus aurantium}, which is responsible for the effects on Beta-3 receptors (\( \beta-3 \)) of the fat tissue, which when stimulated are responsible for lipolysis\textsuperscript{17,42,43}.

In a study conducted by Arbo et al. in 2009\textsuperscript{43} the authors tested different doses of \textit{Citrus aurantium} (400, 2000 and 4000 mg/kg), which corresponds to 30, 150 and 300 mg of p-Synephrine, respectively. Different from the one found in the present study, the authors found lower weight in relation to the control group, with no adverse effects regarding the dosage\textsuperscript{44}. The dose used in this study, 14.74 mg/mL/day was obtained based on the indicated dosage for humans that is 600 mg/day, from the allometric extrapolation, and this may be one of the factors that explain not occurring weight loss in the animals.

The longest research and with the highest dose of p-Synephrine, involving humans, used 46 individuals with a daily dosage of 98 mg of p-Synephrine, divided into 2 doses, for 60 days, while 23 people ingested placebo. The authors reported that no adverse effect was
observed. It was not mentioned if there was a change in body weight of the participants, however, these values of daily dosing, may indicate a safe limit of intake of *Citrus aurantium* for humans\(^{45}\).

It is not common to find values of triglycerides levels in an isolated manner, since increased levels of plasma component are accompanied by elevation in the values of total cholesterol and LDL and also reduced HDL levels\(^{46}\). This fact can be observed in medium-sized groups, related to these components, where the same animals that showed increased values of triglycerides also had reduced levels of HDL (Table 5).

A study conducted in rats induced experimentally to diabetes, the values of triglycerides and increased HDL decreased; after the beginning of the administration of alcoholic stratum of *Citrus aurantium*, the values of triglycerides and reduced HDL values increased, maintaining this condition in all the studied groups\(^{47}\).

Female rats during the period of pregnancy treated with cafeteria diet, obtained similar results regarding the triglyceride and HDL, where increased levels of triglycerides and reduced levels of HDL were observed, however the values of total cholesterol showed no significant difference when compared to the groups, results that corroborate with this study\(^{48}\).

An increase in the consumption of cholesterol and lipids in the food activates the intrinsic control, inactivating the enzyme 3-hydroxy-3-methyl-glutaril-CoA reductase (HMG-CoA-reductase), which is important for the production of endogenous cholesterol, thus enabling a system of intrinsic control of cholesterol in order to prevent an excessive increase in serum levels of this element\(^{49}\).

Associated to this is the age of rats used in this study, because they are young rats, the metabolism of lipids is more accelerated and this makes them more resistant to develop dyslipidemia, which is demonstrated in a study involving different types of lipids and various ages of male Wistar rats, where it was established that the total cholesterol levels did not change significantly until 12 months of age, however, relevant alterations of this component were observed only when the animals reached 18 months\(^{50}\).

Regarding the significant reduction in the values of VLDL, found in obese animals and treated with Commercial extract of *Citrus aurantium* (G4) when compared to the levels of the G1 group (Table 5), studies conducted in diabetic rats and treated with alcoholic extract of *Citrus aurantium*, treated for 21 days, the results corroborate the findings, the authors conclude that the ethanolic extract showed possible protection against hypercholesterolemia, with a beneficial effect on the situations that this condition can cause\(^{47}\).
In research with mice that were treated with essential oil of *Citrus limon*, at concentrations of 50, 100 and 150 mg/kg of body weight, a significant reduction was found in the levels of AST in the group that received the highest dose of *Citrus limon*, when compared to the control group, however, the values of ALT, showed no significant difference compared to the control group, but it is noted an increase in the mean values of this component in the highest dosage. The authors conclude that the increase in the levels of AST, in mice treated with doses of 50 and 100 mg, suggest possible hepatic overload\textsuperscript{51} in the same way that the AST values found in the G2 group, where there was an increase in the level of AST of rats supplemented with commercial extract of *citrus aurtantium*, but that received a balanced diet, suggesting that the use of that phytotherapeutic should be restricted to cases in which there is an increase in weight (Table 5).

A study conducted in male rats, with *Citrus aurantium* and p-Synephrine with the objective of evaluating the sub chronic toxicity and its action on biomarkers of oxidative stress, showed that the animals that received the dose of 4000mg/kg presented values of ALT reduced compared to the control\textsuperscript{42}.

5 CONCLUSION

The commercial extract of *Citrus aurantium* used in this study, showed antioxidant activity *in vitro*, however, the same result was not observed *in vivo*. Based on the results, the commercial extract was not effective in reducing body weight of the animals, however, showed a positive effect in reducing triglyceride and VLDL cholesterol, two important markers for the control of dyslipidemia. The reduction of the hepatic enzyme ALT, when associated to a high fat diet, shows that the commercial extract of *Citrus aurantium* exhibits hepato-protective function, suggesting that in cases of obesity this supplement can be used as adjuvant in the treatment.

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