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EFFECTS OF THE LEAVES OF *PERESKIA ACULEATA* ON INGESTION AND BIOCHEMICAL PARAMETERS IN WISTAR RATS

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ABSTRACT

Pereskia Aculeata Miller, also known as *Ora-Pro-Nóbis* (OPN), is an underutilized species that belongs to the Cactaceae family. It is mainly distributed in the southern United States and Brazil, from Bahia to the Rio Grande do Sul state, growing in stony and sandy coastal plains. The aim of this study is to evaluate the effects of the use of OPN leaves on the metabolic parameters of Wistar rats. The animals were randomly divided into three groups: G1 (n = 8), control group, that received water and commercial rat feed; G2 (n = 8), group treated with commercial rat feed incorporated with OPN leaves (20%); and G3 (n = 8), group treated with commercial rat feed included with OPN leaves (30%). The experimental protocol lasted 40 days, and after that, biochemical and anthropometric data were evaluated. The groups that received OPN (G2 and G3) showed similar feed intake, weight gain, Lee index and amount of visceral fat, as well glycemia, when compared to the control group. Significant differences were observed only for Total Cholesterol, urea, and iron. The use of OPN leaves may result in positive effects on some metabolic parameters of Wistar rats. Nevertheless, there are controversies between the studies found in the literature. For these reasons we suggest more studies using this plant to verify the amounts that should be consumed to improve anthropometric and biochemical parameters.

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INTRODUCTION

Pereskia Aculeata Miller, also known as *Ora-Pro-Nobis* (OPN), is an underutilized species that belongs to the Cactaceaefamily. It is mostly distributed in the southern United States and Brazil, from Bahia to the Rio Grande do Sul state, growing in stony and sandy coastal plains(Vieira *et al*, 2019; Garcia *et al*, 2019). OPN qualifies as an *Unconventional Food Plant*, a term that includes leaves, flowers, seeds, rhizomes, fruits, and other parts of plants that are not often consumed and not by a large number of people. These plants are present in certain localities or regions, with a significant influence on the diet of the local population. However, they are usually not organized as a productive system and thus arouse no interest from seed, fertilizer, or agrochemical companies. In general, *Unconventional Food Plants* are recognized for their high nutritional value, because they are composed of nutrients in proper quantities, and have a characteristic of functional effect (CFN, 2017;Brasil, 2010). OPN has many nutrients, including protein, fiber, iron, calcium, magnesium, manganese, vitamin A, C, and folic acid, and is a source of essential oils and phenolic compounds. It is free of toxicity, and its rustic, vigorous and natural cultivation and, for these reasons, presents an affordable monetary value (Amaral *et al*, 2019; Silva *et al*., 2017). Studies have shown the high protein content present in OPN. Among the amino acids found are the essential leucine, lysine, and valine, as well as tryptophan, which is the most abundant, on average 20% of the total amino acids (Maciel, Yoshida, Goycoolea, 2019; Souza *et al*., 2015; Martinevski *et al*., 2013). OPN exhibits antioxidant, antibacterial, and antifungal activity and also presents analgesic potential (Souza *et al*., 2016; Pinto *et al* 2015a; Pinto *et al*, 2015b).

Table 1. Anthropometric data and feed and water intake of the animals of G1, G2, and G3 groups

Parameters	G1 (n=8)	G2 (n=8)	G3 (n=7)	p-value
	Mean ± standard deviation (median)			
Weight at the beginning (g)	174.6 ± 34.9 (161)	182.9 ± 37.2 (182)	184.2 ± 29.6 (170)	0.8385*
Final Weight (g)	256.9 ± 16.4 (253)	260.3 ± 14.3 (259)	259.6 ± 19.77 (257)	0.9186*
Weight gain (%)	50.6 ± 20.1 (58)	46.5 ± 25.0 (49)	42.8 ± 16.1 (51)	0.7780*
Visceral fat (g)	8.5 ± 2,9 (9)	8.1 ± 1.7 (9)	7.1 ± 2,4 (7)	0.5093*
Lee Index (g/cm)	29.7 ± 0.5 (30)	29.9 ± 0.5 (30)	29.7 ± 0.6 (30)	0.6914*
Rat food consumption (g/animal/day)	20.2 ± 1.2 (20)	20.9 ± 4.0 (21)	22.3 ± 5.2 (22)	0.2299**
Water intake (mL/animal/day)	31.3 ± 2.4 (31)^{A}	24.9 ± 8.1 (24)^{Ba}	27.8 ± 7.9 (26)^{Ba}	0.0397**

*Anova one way. **Kruskal-Wallis. Different superscript letters, upper or lower case, indicate a significant difference between pairs (p≤0.05), according to Dunn's complimentary test.

Pinto *et al* (2015a) showed that the hexane fraction of this plant produces strong chronic and acute topical anti-inflammatory properties against different irritant agents. The action mechanism is associated with the inhibition of Interleukin-6 and Tumor Necrosis Factor-α. Furthermore, these authors showed a glucocorticoid-like effect, possibly due to the presence of significant amounts of phytosterols. Barbalho *et al* (2016) showed that OPN flour might reduce total cholesterol, LDL-c, VLDL-c, and triglycerides and increase HDL-c levels. For the reasons we exposed above, the aim of this work is to evaluate the effects of the use of OPN leaves on the biochemical and anthropometric parameters of Wistar rats.

MATERIALS AND METHODS

This study was conducted following the approval of the Animal Use Ethics Committee of the University of de Marilia – UNIMAR (Protocol nº 01/2018/2). Twenty-three healthy adult female Wistar rats from the UNIMAR Animal Modeling Center were used. The animals were cared according to the Guide to Care and Use of Experimental Animals, which describes the principles of the Canadian Council for the Care of Laboratory Animals. The animals were randomly divided into three groups: G1 (n = 8), control group, that received water and commercial rat feed (CR1 Nuvilab®); G2 (n = 8),group treated with CR1 Nuvilab® commercial rat feed incorporated with OPN leaves (20%); and G3 (n = 8), group treated with CR1 Nuvilab® commercial rat feed included with OPNleaves (30%). The experimental protocol lasted 40 days. The animals were watered and fed*ad libitum.* Before experimentation, all animals were acclimated for seven days to the vivarium conditions. They were placed in plastic boxes (40x30x17cm), stocking four animals each, being kept in a room with a temperature between 20°C to 25°C and 12/12 hours controlled light / dark cycle and 60 ± 5% relative humidity. The supplemented feed was prepared by incorporating leaves from the fresh plant to the feed in the proportions of 20% and 30%. The OPN leaves were obtained from the University of Marilia - UNIMAR. The leaves were taken from different randomly drawn branches, then washed in running water, immersed for 15 minutes in a chlorinated solution prepared with 2.5% sodium hypochlorite at 200 ppm dilution and finally rinsed in running water. After the excess water had been removed, the OPN was cut into small pieces and incorporated into the CR1 Nuvilab® feed crushed in an industrial blender. This mixture was homogenized with the addition of a minimum amount of water, sufficient to obtain a moldable semi-pasty consistency mass and cut into cylinders of approximately three centimeters in length and one in diameter. Then this ration was taken to oven drying at 60ºC for five hours, with an air circulation capacity of 800 L (Model 214 - Fabbe Primar). The product obtained was then packed in uncapped polyethylene packaging for up to seven days and stored in the dark until use. Feed and water intake were measured three times a week, and the animals' weight was measured once a week. After 40 days of the experimental period, the animals were euthanized with anesthetic overdose (thiopental). Blood samples were then collected by lower vena cava puncture for blood glucose, total cholesterol (TC), high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c), triglycerides (TG), urea, creatinine, iron and Transaminases Aspartate Amino transferase (AST) and Alanine Aminotransferase (ALT). From the blood lipid examination, the atherogenic index (AI) and the protection index (PI) were calculated. The AI was calculated using the formula of Schulpis and Karikas (1998): AI: (TC - HDL-c)/HDL-c. The PI was calculated based on Dhandapani (2007): {[AI (G1) - AI (G2 or G3)] / AI (G1)} x100. Based on the bodyweight of the last day of the experiment and the naso-anal length measurement, the Lee index for obesity measurement was calculated. This index is determined by dividing the cubic root of the weight in grams by the naso-anal length in millimeters and multiplied by 100 (Kang, Yun, Park, 2010). Abdominal fat content was also evaluated. Thoracic and abdominal circumference were also performed by measuring respectively the circumference immediately after the front and hind legs of the animals.

RESULTS

The groups that received OPN (G2 and G3) showed similar feed intake, weight gain, Lee index, and amount of visceral fat (Table 1) when compared to the control group (G1). G2 and G3 showed significant lower TC values. No significant differences were found for all the other parameters (Table 2). Urea level was lower in OPN groups (G2 and G3). Creatinine levels were lower in G2 and higher in G3 compared to control. The animals of the experimental groups presented significantly lower serum iron in relation to the control group. The animals of the treated groups presented lower Atherogenic Index about the control group, but not significantly (Table 3).

DISCUSSION

The data from our study showed that the use of OPN leaves significantly reduced TC, urea, creatinine, and iron levels, but there were no significant differences in weight gain and visceral fat percentage and food intake. No significant changes in glycemia were observed either. Souza *et al* (2015) investigated the use of OPN juice on the biochemical and anthropometric profile of female and male Wistar rats and also observed reduction of AST and ALT levels.

Table 2. Biochemical Parameters of the animals of G1, G2, and G3 groups

Parameters	G1 (n=8)	G2 (n=8)	G3 (n=7)	p-value
	Mean ± standard deviation (median)			
TC (mg/dL)	63.8 ± 12.0 (65)^{A}	59.6 ± 13.5 (63)^{Aa}	47.7 ± 6.2 (45)^{Ba}	0.03281*
HDL-c (mg/dL)	56.8± 9.00 (56)	54.9 ± 11.1 (55)	45.1 ± 5.6 (43)	0.04823*
LDL-c (mg/dL)	7.0 ± 5.1 (7)	5.2 ± 3.2 (5)	9.9 ± 2.4 (11)	0.0714*
VLDL-c (mg/dL)	13.9 ± 4.7 (12)	9.9 ± 4.8 (8)	12.4 ± 2.6 (12)	0.1797*
TG (mg/dL)	70.1 ± 23.2 (61)	49.6 ± 23.5 (41)	62.7 ± 12.8 (60)	0.1605*
Glycemia (mg/dL)	277.8 ± 71.9 (276)	225.4 ± 42.6 (225)	236.4 ± 47.9 (236)	0.1700*
ALT (U/L)	79.8 ± 24.4 (73)	68.0 ± 21.6 (61)	70.9 ± 13.5 (76)	0.5119*
AST (U/L)	105.5 ± 27.2 (103)	109.2 ± 42.9 (99)	97.5 ± 4.8 (98)	0.7686**
Urea (mg/dL)	57.9 ± 5.1 (56)^{A}	53.5 ± 6.1 (54)^{Aa}	42.8 ± 2.8 (43)^{Bb}	0,0001*
Creatinine (mg/dL)	0.48 ± 0.04 (0.5)^{A}	0.45 ± 0.05 (0.4)^{Aa}	0.51 ± 0.02 (0.5)^{Ab}	0.0450*
Iron (mg/dL)	324.6 ± 50.7 (317)^{A}	233.6 ± 43.9 (225)^{Ba}	316.3 ± 74.5 (303)^{Ab}	0.0083*

TC= total cholesterol. HL-c= high density lipoprotein. LDL-c= low-density lipoprotein. VLDL-c= very-low-density lipoprotein. TG= triglycerides. ALT = alanine aminotransferase. AST: aspartate aminotransferase.*Anova one way. **Kruskal-Wallis. Different superscript letters, upper or lower case, indicate significant difference between pairs (p≤0.05), according to Tukey's complimentary test.

Table 3. Atherogenic indices of the animals of G1, G2, and G3 groups

Parameters	G1 (n=8)	G2 (n=8)	G3 (n=7)	p-value
	Mean ± standard deviation (median)			
Atherogenic Index	0.12 ± 0.07 (0.12)	0.08 ± 0.07 (0.09)	0.06 ± 0.03 (0.05)	0.1940*

*Anova one way. **Test t de imdependênia.

On the other hand, these authors did not note modifications in the levels of TC and found an increase in glycemia. The study of these authors did not evaluate the levels of serum iron. The study of Barbalho *et al* (2016) investigated the effects of OPN flour on the biochemical and anthropometric parameters of Wistar rats and showed that the consumption of this product reduced TC, LDL-c, VLDL-c, AST, body weight, visceral fat, and Lee Index, and augmented HDL-c. Similarly to Souza *et al* (2015), they also showed an increase in the levels of blood glucose. Vieira *et al* (2019) evaluated the effects of OPN flour on the adhesion of probiotics to intestinal epithelial cells and also assessed the impact of a product produced with this flour on gastrointestinal symptoms, body fat, weight, lipid profile, and glycemia in overweight men. In the end, the authors did not observe significant modifications to the biochemical and anthropometric parameters and concluded that the product maintained the nutritional status of the patients. Almeida, Correa (2012) studied the effects of other species of *Pereskia* (*P. grandifolia*) flour and found that animals fed a hypercaloric diet that received this product showed effectiveness in decreasing weight gain and showed reduced Lee index, body mass index, triglycerides, and glycemia.

Increased levels of AST and ALT may result from the destruction of liver cells. Our results showed a reduction of these parameters, suggesting that this plant is safe for consumption (Cai *et al*, 2011). Silva *et al* (2017) studied, for the first time, the acute oral toxicity of OPN in Wistar rats and showed no effects for signs of toxicity, body weight, food intake, and fecal excretion. They also did not observe histopathological alterations and concluded that this plant has potential to be used as a functional food. Authors have attributed the beneficial effects of OPN to the presence of several components such as vitamin C, carotene, fibers, and phenolic compounds such as caffeic acid derivatives and flavonoids (kaempferol, quercetin, and isorhamnetin glycoside derivatives). These substances, together or separated are associated with the prevention or reduction of risk factors for diabetes, metabolic syndrome, and cardiovascular diseases. These effects occur due to several pathways, including a decrease of free radicals, oxidative stress, and inflammation that are strictly related to the recovery of the homeostasis (Garcia *et al* 2019; Ben Salem *et al*, 2019; Kumar *et al*, 2019). Serum ferric levels for groups that consumed 20 or 30% OPN were lower than in the control group. Although considered a high-iron plant, according to Gallagher (2014), OPN has non-heme iron, and absorption is only 5% of the total ingested.
The Atherogenic and Protection Indices may indicate the increase of cardiovascular risks and may contribute tothe clinical practice as a tool of stratification of these diseases (Ikewuchi, 2012; Choi *et al*, 2017). However, the consumption of OPN leaves did not interfere in these indices. The use of OPN leaves may result in positive effects on some metabolic parameters of Wistar rats. Nevertheless, there are controversies between the studies found in the literature. For these reasons, we suggest more studies using this plant to verify the amounts that should be consumed to improve anthropometric and biochemical parameters.

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