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CENTESIMAL COMPOSITION AND EFFECTS OF THE INTAKE OF SOLANUM AMERICANUM ON SATIETY AND METABOLISM OF RATS

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ABSTRACT

Within the diversity of Amazon fruits with potential functional activity, Solanum americanum which contains bioactive compounds that have potential to reduce weight gain, modulates metabolism, inflammatory and oxidative processes. Thus, this study aimed to evaluate the centesimal composition of this fruit and effects of Solanum americanum ingestion in rats. The centesimal composition was determined and two groups were constituted receiving by intubation (gavage) either water - control group (CO n=5) or 0.3214 ml of the fruit by 100 g body weight - experimental group (MP n=10). Feed intake and weight gain were measured (g) daily and at the end of the trial blood was collected for biochemical determinations (mg dl^{-1}). Solanum americanum presented 81.36 ± 0.77 g of humidity, 3.58 ± 0.43 g of ashes, 0.24 ± 0.003 g of protein, 0.13 g ± 0.01 of lipids, 14.70 g of total carbohydrates, with pH 4.39 ± 0.02 and titratable acidity of 0.69 ± 0.02 g citric acid 100 g^{-1}. MP group consumed less feed (25.05 ± 3.72) than CO group (28.34 ± 2.93) (p=0.018) suggesting that the fruit consumption may have provided a greater satiety that might have reflected on the trend to weight gain reduction in MP group (p=0.21). There was a tendency towards the blood glucose (90.90 ± 22.09) (p=0.47) and cholesterol (107.60±16.58) (p=0.54) reduction in MP group The aforementioned effects can be possibly attributed to the presence of bioactive compounds, which makes the consumption of this ripened fruit a sustainable option for management of chronic degenerative diseases.

Key words: Anthocyanins. Phenolic compounds. Weight. Glycaemia. Lipids.

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RESUMO

Composição centesimal e efeito da ingestão de solanum americanum na saciedade e metabolismo de ratos

Dentro da diversidade de frutos da Amazônia com potencial atividade funcional, Solanum americanum, que contém compostos bioativos com potencial para reduzir o ganho de peso, modula o metabolismo, processos inflamatórios e oxidativos. Assim, este estudo teve como objetivo avaliar a composição centesimal desse fruto e os efeitos da ingestão de Solanum americanum em ratos. A composição centesimal foi determinada e dois grupos foram constituídos recebendo intubação (gavagem), grupo controle de água (CO n = 5) ou 0,3214 ml da fruta por 100 g de peso corporal - grupo experimental (MP n=10). A ingestão de ração e o ganho de peso foram medidos (g) diariamente e no final do estudo, o sangue foi coletado para determinações bioquímicas (mg dl-1). Solanum americanum apresentou 81,36 ± 0,77 g de umidade, 3,58 ± 0,43 g de cinzas, 0,24 ± 0,003 g de proteína, 0,13 g ± 0,01 de lipídios, 14,70 g de carboidratos totais, com pH 4,39 ± 0,02 e acidez titulável de 0,69 ± 0,02 g ácido cítrico 100 g-1. O grupo MP consumiu menos ração (25,05 ± 3,72) do que o grupo CO (28,34 ± 2,93) (p=0,018), sugerindo que o consumo de frutas pode ter proporcionado uma maior saciedade que pode ter refletido na tendência de redução do ganho de peso no grupo MP (p=0,21). Houve uma tendência à redução da glicemia (90,90 ± 22,09) (p 0,47) e colesterol (107,60 ± 16,58) (p=0,54) no grupo MP Os efeitos mencionados acima podem ser atribuídos à presença de compostos bioativos, o que torna o consumo desse fruto amadurecido é uma opção sustentável para o manejo de doenças degenerativas crônicas.

Palavras-chave: Antocianinas. Compostos fenólicos. Peso. Glicemia. Lipídios.

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INTRODUCTION

The Amazon stands worldwide for its flora biodiversity that understands several species of plants and their fruits still little exploited scientifically (Bassoli et al., 2015).

It is noteworthy that the qualitative characteristics analyses of any food are very important to their physical-chemical characterization, especially in new and unfamiliar foods, such as fruits typical from the Brazilian Amazon region.

Within the wide variety of plant species, Solanum americanum, popularly known in Brazil as Maria Pretinha, is having a significant breakthrough in scientific studies, due to its composition characterization and identification of various bioactive compounds (Vagula et al., 2016).

Fruits from Solanum americanum species presents a particula centesimal composition and phytochemicals where stands out the presence of polyphenols such as flavonoids, among which anthocyanins (Vagula et al., 2016).

Reports claim that Solanum americanum fruits represent an unusual though feasible source of anthocyanins (Nachtigall et al., 2010).

Results were found ranging from 150-200 mg of anthocyanins by 100 g of Solanum americanum (Silva, 1996), while açaí (Euterpe oleracea) has shown levels around 111 ± 30.4 mg of anthocyanins by 100 g of fruit (Rufinus et al., 2010).

Furthermore, the ripe berries of Solanum americanum are considered sources of anthocyanins of high bioavailability (Silva, 1996).

Anthocyanins, flavonoids produced naturally by plants (Wallace and Giusti, 2015), are important antioxidants (Degaspari and Waszczynskyj, 2004) and its role in the prevention of cardiovascular diseases (Sozański et al., 2016, Wallace et al., 2016) is related to the protection against oxidative stress and lipid peroxidation (Plaza et al., 2016) and with other functions listed in review studies as contributing to the reduction of weight gain and inflammatory processes (Smeriglio et al., 2016).

Besides, once Solanum americanum presents significant amounts of fibers (Ijarotimi et al., 2010) and phenolic compounds like anthocyanins, it is possible that it can also control the effectiveness of both glycemic and lipid profiles since the referred compounds have the ability to reduce intestinal absorption of certain substances (Castro-Acosta et al., 2016).

Based on the above considerations, it is possible that the intake of this Amazonian fruit contributes to reducing the risk and control of chronic-degenerative cases such as obesity, diabetes and dyslipidemias, risk factors for the development of coronary artery disease.

Thus, the present study aims to characterize the composition of Solanum americanum from the Legal Amazon, also assessing both physiological (food intake and weight gain) and metabolic (glycemic and lipid profile) effects of the bioactive compounds present.

MATERIAL AND METHODS

Ethical Aspects

The animal experimentation protocol was executed in accordance with the norms established by the Brazilian Committee of Ethics, having been approved by the Committee of Ethics in Animal experimentation of UNIR-Campus Rolim de Moura, under protocol number PP020/1014.

Plant Material

Solanum americanum (Solanaceae family - taxonomy nomem number 100795) is an herbaceous plant, erect, branched, with single leaves, white flowers, measuring until 90 cm in height and 4-8 cm in length and produces berries that when ripened are purplish and have a sweet taste (Lorenzi et al., 2002).

Fruits under a suitable maturity stage for consumption were harvested in the city of Cacoal (coordinates 11˚ 26’ S and 61 ˚ 27’ W) - Rondônia State situated in the Legal Amazon region basin on the right bank of the BR 364.

The edible part of the Solanum americanum fruit (bark + pulp + seeds) was cleaned and stored in glass containers with lids in a freezer at -18 °C until time of use. It is important to note that prior to the procedures, Solanum americanum was macerated daily, once natural antioxidants or even synthetic sources, when present in low concentration relatively to the medium, do delay significantly or prevent the claimed medium oxidation (Halliwell and Gutteridge, 2000).

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Centesimal Composition Analysis

Protein, lipids, pH, total titratable acidity, moisture and ashes of the Solanum americanum fruit were determined by using official methodologies described in Association of Official Analytical Chemists (AOAC, 2005) and Instituto Adolfo Lutz (IAL, 2008).

The analyses were performed in triplicate, in the food analysis laboratory of the Faculdades Integradas de Cacoal Rondônia.

For the protein's determination, total nitrogen was accessed by Kjedahl method and a 6.25 conversion factor was adopted. For the digestion process, approximately 10.0 g of Solanum americanum were used, with 5.0 ml of sulphuric acid, 2.0 g of catalytic blend (Na_2SO_4 and $CuSO_4.5H_2O$) and 15.0 ml of distilled water. Such mixture was digested for 4 hours at 350 °C. The distillation step used a nitrogen Adamo distiller and 40% NaOH as neutralizer. Titration had methyl red 0.2% and 0.2% methylene blue (as indicators) and 10.0 ml of boric acid and 0.1 N HCl.

The lipid content was determined using Soxhlet hot extraction method. Approximately 10.0 g of Solanum americanum were weighted, placed on filter paper along with 3.0 ml of ammonium hydroxide, 5.0 ml of ethyl alcohol, 5.0 ml of petroleum ether and 250.0 ml of hexane. After 4 hours of extraction, the filter was placed in an oven for the solvent volatilization and soon after weighted again to obtain the lipids content.

For moisture, a 5.0 g sample of Solanum americanum was weighted, homogenized with a spatula and transferred into dry and previously tared crucibles, then placed with a clamp into a Quimis muffle at 550°C for 7 hours. Using tweezers, the crucibles were then placed in an Odontobras oven at 105 °C for 4 hours. After that, the crucibles were removed from the oven and placed in a desiccator for cooling and, finally, weighted.

Total carbohydrates (including food fibers) were calculated by difference (100 g - grams of moisture, ashes, protein and lipids).

After proper calibration, the pH was determined using a pH meter to check the homogenized mixture of 5.0 g of Solanum americanum and 50.0 ml of distilled water.

Total titratable acidity is the amount of acid in a sample that reacts with a base of known concentration. The most common is the quantitative analysis, which determines the total acidity by titration. The titration with 0,1 N sodium hydroxide was performed on the same sample of pH, using phenolphthalein as the final turning point.

Animal Experimentation

For this experiment, Federal University of Mato Grosso (UFMT) kindly provided 15 male Wistar rats (Rattus norvegicus albinus var), weighing between 120 and 140 g, which were then kept in the Vivarium of UNESC (Faculties of Cacoal Rondônia), with free access to pelletized rodent feed and water, under environmental controlled conditions of moisture, temperature and luminosity (light/dark cycle of 12:00).

The animals were divided in five for the control group and ten for experimental group, and were submitted daily for 14 days to administration of 0.3214 ml by 100 g body weight by gastric intubation (gavage) of either water (control group - CO) or the fruit of Solanum americanum (whole fruit macerated and filtered with a gauze) (experimental group - MP) in a dose that is equivalent to an intake of 225 ml of extract of Solanum americanum by a 70 kg human adult.

Every day the animals were weighed as well as the amount of feed consumed measured.

Biochemical Analysis

After the end of the period, a drop of blood from the tip of the tail of the animals was collected for blood glucose measurement (mg dl^{-1}) by a G Tech Free apparatus. Also, after anesthesia with an intraperitoneal injection with sodium pentobarbital (40 mg kg^{-1}) followed by a laparotomy, there was collection of 2 ml of blood from the abdominal aorta destined to the determination of the other biochemical parameters.

The biochemical analysis of triglycerides and total cholesterol (mg dl^{-1}) were performed in an automated Labmax240® device (Labtest) of high precision at a clinical analyses laboratory duly certified. Assay of cholesterol and triglycerides in serum was realized using colorimetric methodology (Trinder enzymatic).

Statistical Analysis

The experiment was randomly performed and data from both control (n = 5) and experimental (n = 10) groups were

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tabulated and analyzed to obtain the mean and standard deviation.

For data comparison of the biological parameters between control and experimental group, normality and homogeneity of variances conditions were checked and followed by Student's t-test. For the analysis, a 5% ($p < 0.05$) significance level was adopted, applying both Excel and Statistica 12.0 (Statsoft) softwares.

RESULTS

In assessing the centesimal composition of Solanum americanum coming from Legal Brazilian Amazon, it was observed a high moisture content (81.36 ± 0.77 g), low lipid level (0.13 ± 0.01 g), 3.58 ± 0.43 g ashes, 0.24 ± 0.003 g of protein, 14.69 g of total carbohydrates, pH 4.39 ± 0,02 and titratable acidity of 0.69 ± 0.02 g citric acid 100 g^{-1}, as shown on Table 1.

With regards to the physiological effects of Solanum americanum ingestion, it can be observed in Figure 1-A a significant reduction in feed consumption in the MP group (25.05 ± 3.72 g) comparing with control group (28.34 ± 2.93 g) ($p = 0.018$). Further to that, Solanum americanum intake showed a trend to a reduction in body weight (76.50 ± 35.05 g) paired with CO group (98.03 ± 12.17 g) ($p=0.21$) (Figure 1-B).

Table 1 - Solanum americanum centesimal composition.

Parameter	
Moisture (%)	81.36±0.77
Ashes (%)	3.58±43
Proteins (g)	0.24±0.003
Lipids (g)	0.13±0.13
Total carbohydrates (g)*	14.69
pH	4.39±0.02
Total titratable acidity (g citric acid 100 g^{-1})	0.69±0.02

Legend: Expressed as mean ± standard deviation of 03 samples. *Total carbohydrates by difference.

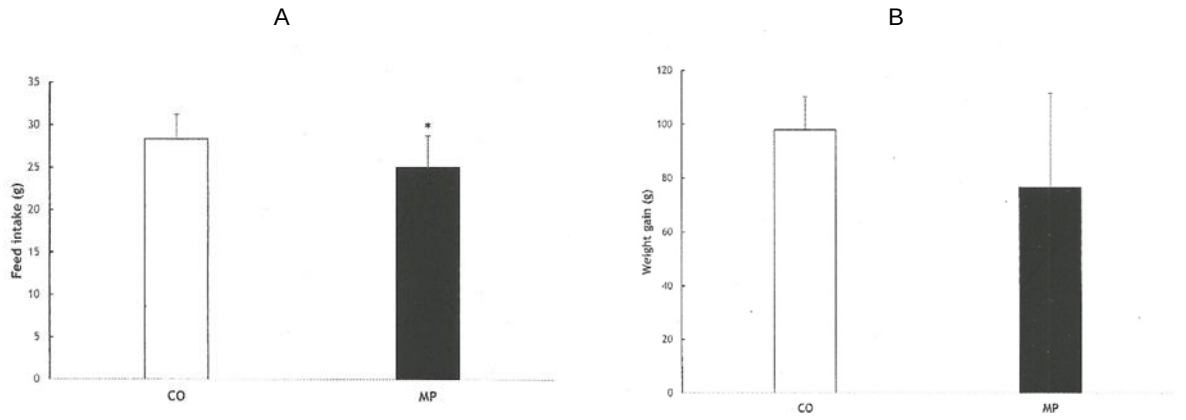

Figure 1 - Influence of Solanum americanum intake on (A) feed consumption (g) and (B) the weight gain (g), during the experimental period. Control Group (CO) and Experimental Group (MP). Values expressed as mean ± standard deviation (5 to 10 animals) and analysed by Student t test adopting 5% as significance level. *Statistically significant difference ($p<0.05$).

The chart below (Figure 2-A) shows the glycemic profile of the experimental group (90.90 ± 22.09 mg dl^{-1}), and the control group (100.60 ± 29.36 mg dl^{-1}). Thus, it was observed a tendency to glycemia reduction after sub-chronic ingestion of Solanum americanum fruit ($p = 0.47$).

Figure 2-B shows that the intake of Solanum americanum induced a trend in cholesterol level reduction (107.60 ± 16.58 mg dl^{-1}) in comparison with CO group (113.00 ± 8.45 mg dl^{-1}) ($p = 0.54$), having not promoted pronounced changes in the levels of triglycerides ($p = 0.88$). It should be noted that the found trend to lipid metabolism modulation probably reflects the effects of an sub-chronical administration (14 days).

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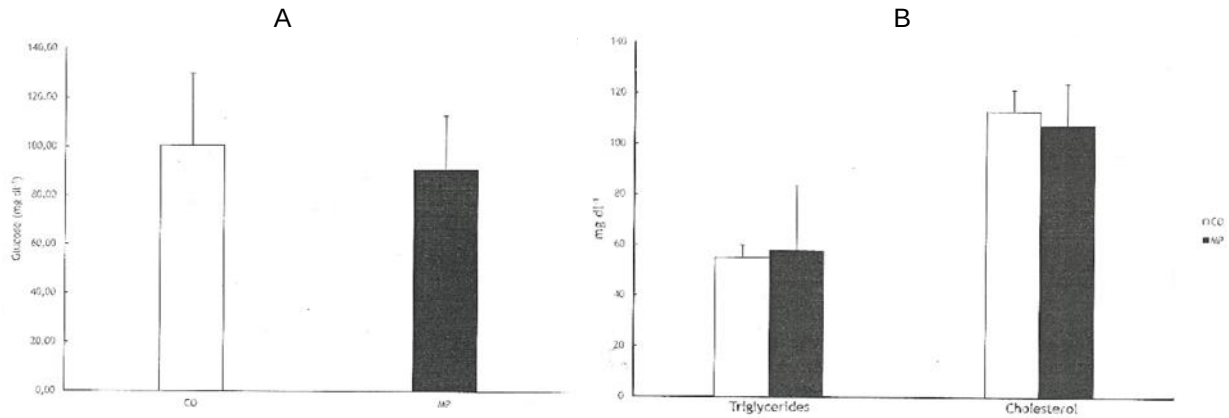

Figure 2 - Influence of Solanum americanum intake on (A) glycemia (mg dl^{-1}) and (B) lipidic profile (cholesterol and triacylglycerols (mg dl^{-1}). Control Group (CO) and Experimental Group (MP). Values expressed as mean ± standard deviation (5 to 10 animals) and analysed by Student t test adopting 5% as significance level.

DISCUSSION

In this work the determined moisture, pH and lipids levels in Solanum americanum samples was (Table 1), quite close to the findings by Vagula and collaborators (2013), which supports the characterization of Solanum nigrum (plant of the same family and gender) as a fruit with a high amount of water and low lipid content, characteristics that if associated with high concentrations of anthocyanins (Vagula et al., 2016), may be related to the effects of body weight reduction trend (Figure 1-B) in animals that ingested the fruit in an acute form.

Besides, according to Wu et al., (2014), the consumption of anthocyanins prevented the development of obesity induced by high-fat diet. The administration of 40 and 200 mg Kg^{-1} anthocyanins reduced body weight in 5.2 and 11.2%, respectively, in addition to reducing the size of adipocytes (fat cells), leptin secretion (body weight and appetite regulator hormone), levels of glucose, triglycerides, total cholesterol and LDL-cholesterol.

Moreover, in the present study, the acute ingestion of Solanum americanum promoted a significant reduction of feed intake (p=0.018) (Figure 1-A), which can possibly be attributed to greater satiety promoted by ingestion of this fruit as a whole that offers various bioactive compounds, among them fibers (Ijarotimi et al., 2010). In fact, there are indications in the literature that the viscous solutions formed by fibers interfere with the processes of intestinal peristalsis, digestion and absorption of nutrients and therefore also with the signals of satiety (Rebello et al., 2016).

It was also observed a tendency in the reduction of blood glucose (Figure 2-A) and total cholesterol (Figure 2-B) of the experimental group relatively to the control group, which probably can be attributed to the high concentration of anthocyanins in this fruit, bioactive compounds that belong to the class of phenolic compounds, which indeed inhibit the intestinal absorption of glucose (Bassoli, 2008; Castro-Acosta et al., 2016) and to the fibers that inhibit the intestinal absorption of lipids (Nwachukwu et al., 2015).

This glycemic reduction trend corroborates findings that report that anthocyanins are beneficial not only to reduce postprandial glycaemia (Castro-Acosta et al., 2016), but also act beneficially in other pathophysiological processes (Smeriglio et al., 2016; Wallace et al., 2016).

According to Sohrabipour et al., (2013), the administration of 1 g l^{-1} of the extract of Solanum nigrum (Solanaceae family), to diabetic rats during 8 weeks decreased plasma glucose levels. The author describes that the treatment improved glucose tolerance in diabetic rats, suggesting an improved insulin secretion or increased translocation of Glut4.

Sohrabipour et al., (2013) also showed that the administration of Solanum nigrum (Solanaceae family), promoted a reduction of both VLDL and LDL cholesterol, an increase in HDL cholesterol and triglycerides reduction. There is also evidence

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of the growing role of magnesium in modulation of serum lipids levels and absorption of lipids in macrophages, moreover, some research results indicate a significant antioxidant activity of Solanum nigrum, which can potentially be beneficial in preventing oxidation of LDL, the main component of the atheroma plates (Sohrabipour et al., 2013).

It was also observed a LDL cholesterol reduction in studies where anthocyanins was administered to dislipidemic patients (Quin et al., 2009) or rabbits with atherosclerosis (Sozański et al., 2016).

Future studies could be conduct in order to explore more complex food analysis and even further biological and molecular markers on the evaluation of the complementary biological effects of the ingestion of Solanum americanum helping to build a stronger overall evidence base.

As epidemiological studies reveal an association between diets rich in fruits and vegetables with the decrease of cardiovascular disease rate (Wallace et al., 2016), after additional scientific studies involving Solanum americanum it may represent a possible new functional food from Legal Brazilian Amazon.

CONCLUSIONS

This study contributed to characterize the composition and physiological effects of Solanum americanum from Legal Brazilian Amazon in a way that it may be possible to conclude that the consumption of this fruit ripe (rich in anthocyanins and with reduced levels of toxic compounds) and in moderation, may assist in promoting satiety and weight control as well as modulating the metabolism of carbohydrates and lipids, thus contributing to the promotion of health and reducing the risk of chronic degenerative diseases as obesity and cardiovascular disease in the Amazon region in a sustainable way.

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