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NEUROCIÊNCIAS

O ASPARTAME PODE PREJUDICAR O DESENVOLVIMENTO COGNITIVO E CAUSAR ALTERAÇÕES METABÓLICAS SIGNIFICATIVAS EM RATOS JOVENS

ASPARTAME CAN IMPAIR COGNITIVE DEVELOPMENT AND CAUSE SIGNIFICANT METABOLIC CHANGES IN YOUNG RATS

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RESUMO

Nas últimas décadas, um aumento significativo no consumo de adoçantes artificiais, como o aspartame, foi detectado na população mundial. Neste estudo, levantamos a hipótese de que o uso crônico de aspartame poderia levar a distúrbios do metabolismo no cérebro. Oitenta ratos Wistar foram utilizados desde o desmame até os 60 dias de idade. Esses animais foram divididos em quatro grupos: controle (água), ASP35 (35mg/kg de aspartame), ASP80 (80mg/kg de aspartame) e ASP160 (160mg/kg de aspartame), e receberam essas administrações uma vez ao dia por gavagem oral por 40 dias. No último dia, os animais foram submetidos à evitação inibitória após serem sacrificados e seu sangue foi coletado. Além disso, o nível de açúcar no sangue de todos os animais foi medido no primeiro e no último dia do experimento. Os animais foram pesados durante toda a investigação e sua ingestão alimentar foi monitorada.Os resultados mostraram que o aspartame, especialmente a 160mg/kg, mas também em doses mais baixas, induziu comprometimento cognitivo e alterações metabólicas, como ganho de peso, aumento dos níveis de glicose e triglicérides no sangue no final do tratamento. Os dados sugerem que a administração crônica de aspartame pode levar a danos metabólicos e cerebrais e

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enfatiza a necessidade de revisões em relação à sua ingestão diária aceitável e uso pretendido.

Palavras-chave: aspartame; cognição; estresse oxidativo; peso corporal; glicemia; perfil lipídico.

ABSTRACT

Over the past decades, a significant increase in the consumption of artificial sweeteners such as aspartame has been detected in the world population. In this study, we hypothesized that the chronic use of aspartame could lead to metabolism disorders in the brain. Eighty Wistar rats were used from weaning up to 60 days of age. These animals were divided into four groups: control (water), ASP35 (35mg/kg aspartame), ASP80 (80mg/kg aspartame), and ASP160 (160mg/kg aspartame), and received these administrations once a day by oral gavage for 40 days. On the last day, the animals underwent inhibitory avoidance after being sacrificed, and their blood was collected. Also, the blood sugar level of all animals was measured on the first and last day of the experiment. The animals were weighed throughout the investigation, and their food intake was monitored. Results showed that aspartame, especially at 160mg/kg, but also at lower doses, induced cognitive impairment and metabolic changes such as weight gain, an increase in blood glucose and triglyceride levels at the end of the treatment. The data suggest that chronic administration of aspartame can lead to metabolic and brain damage and stresses the need for revisions regarding its acceptable daily intake and intended use.

Keywords: aspartame; cognition; oxidative stress; body weight; blood glucose; lipid profile.

1. INTRODUCTION

Aspartame is an artificial sweetener derived from two amino acids, L-aspartic acid, and L-phenylalanine, linked by a methyl ester. It was accidentally discovered in 1965 in the United States by James M. Schlatter when he was trying to develop a drug for treating ulcers[1,2].

Aspartame has the same caloric value as sucrose (4 kcal/g), but its sweetening power is 180-200 times higher than sucrose when used in low concentrations, making it useful and classifying it as a non-nutritive sweetener[2,3]. It is estimated that over 200 million people worldwide consume aspartame as a sweetener, which is a significant number[4-6]. Aspartame is present in over 6,000 products, including foodstuffs, table sweeteners under different brands, and over 600 medicines and other pharmaceutical items[8].

After ingestion, aspartame is metabolized by enzymes in the intestinal lumen, and it is hydrolyzed into phenylalanine (50%), aspartic acid (40%), and one molecule of methanol (10%)[9,10]. Elevations in plasma phenylalanine and aspartic acid concentrations may increase the transport of these amino acids to the brain. They may cross the blood-brain barrier (BBB), leading to changes in brain neurochemical composition[10,11]. In addition, recent studies have pointed to the association of methanol with redox imbalance since its metabolism is accompanied by the formation of free radicals in the body[12-17].

Surprisingly, numerous epidemiological studies have shown that the increase in the use of artificial sweeteners has coincided with the rise in the percentage of the obese population[18-20]. While some studies suggest that artificial sweeteners may help control body weight[21-23], others question their effectiveness in weight loss and maintenance[18,24-28] and their effects on lipid and glycemic profiles[29-32]. Various mechanisms have been proposed to explain the association between sweetener consumption and metabolic changes. However, there is no consensus on which mechanisms are at play, and synergistic action between them is possible. Among the main hypothesized mechanisms are the activation of sweet taste receptors[33,34], compensatory eating behavior[29,35], and alteration of brain neurotransmitters[14,24,36]. More recently, changes in intestinal microbiota[37-39] and induction of oxidative stress[40,41] have also been associated. These mechanisms can directly reflect on

weight gain and changes in blood glucose, cholesterol, and triglyceride levels because obesity may contribute to the later onset of other chronic diseases.

Food regulatory authorities in Canada and Europe have established an acceptable daily intake (ADI) for aspartame at 40 mg/kg of body weight per day[42]. In the United States, the FDA has defined the aspartame intake limit at 50 mg/kg of body weight per day[43]. Despite these authorities confirming its safety within these limits, aspartame remains the subject of numerous studies, particularly its long-term effects on the human body. While some people did not experience adverse or side effects with aspartame use[44,45], others found concerning changes, especially in neurobehavioral levels[14,17,46-48] and body metabolism[24-26,49,50]. This evidence suggests that aspartame consumption may affect various systemic functions such as energy balance and metabolism and brain processes such as learning and memory. Thus, this study aims to test the hypothesis that chronic use of aspartame can lead to metabolic disorders in the brain.

2. METHODS AND MATERIALS

2.1. ANIMALS

This study used eighty Wistar rats, 40 males and 40 females, from weaning (21 days after birth) up to 60 days of age. The animals were housed in polyethylene boxes (5 per box and separated by gender). They were maintained at 22 ± 1°C in a 12-hour light/dark cycle and had free access to water and food. This project was a post-graduation thesis[51], and it was approved by the Ethics Committee for the Use of Animals (CEUA) of the University of the Extreme South of Santa Catarina (UNESC), followed by protocol 055 / 2018-2. All experimental procedures were performed according to Conselho Nacional de Controle de Experimentação Animal (CONCEA) recommendations for treating and using laboratory animals.

2.2. ASPARTAME

The aspartame used was diluted daily in heated water according to the dosage of 35mg/kg, 80mg/kg, and 160 mg/kg. The literature varies widely regarding the doses of aspartame used. Therefore, we chose to use a typical moderate, high, and very high dose from a consumer of this sweetener.

2.3. EXPERIMENTAL GROUPS

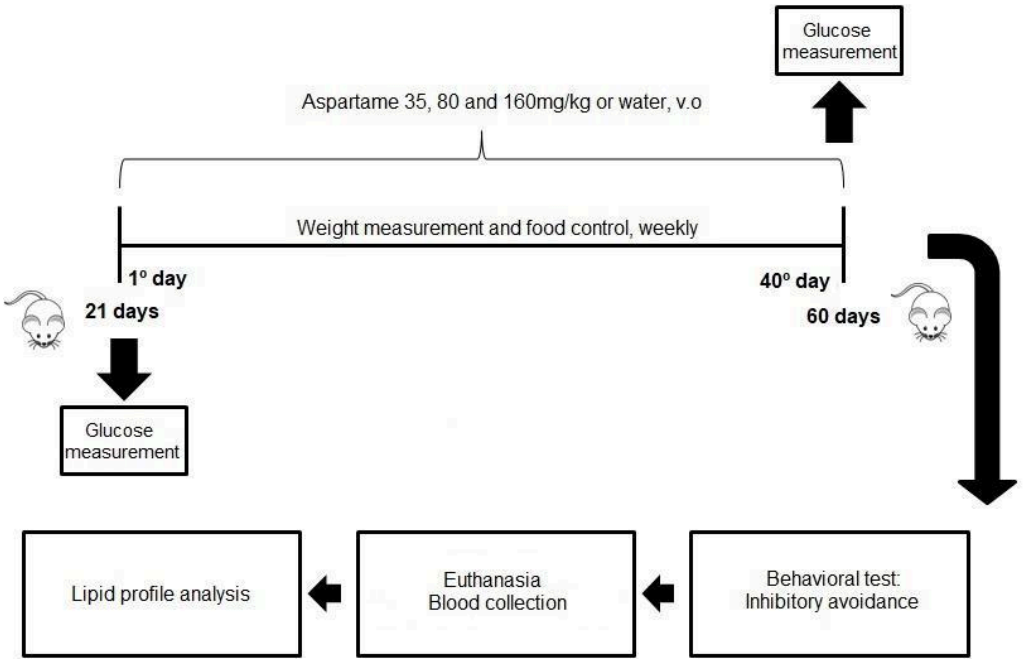
The animals were randomly divided into 4 groups (n = 10 animals) for each gender, as described below:

1. Control group (CG) - administrated with water.
2. ASP35 group - administrated with aspartame at a 35mg/kg dose by gavage.
3. ASP80 group - administrated with aspartame at a dose of 80mg/kg by gavage.
4. ASP160 group - administrated with aspartame at a dose of 160mg/kg by gavage.

2.4. EXPERIMENTAL DESIGN

Aspartame was administered by gavage once a day at doses of 35mg/kg, 80mg/kg, and 160 mg/kg, according to each group, from the first day of weaning (21 days after birth) until 60 days of life, totaling 40 days of treatment. Animals that did not receive aspartame received water (control group). On the last day of the experiment, the animals were subjected to the inhibitory avoidance behavioral test. They were euthanized by guillotine decapitation at the end of the trial, followed by blood collection. The collected blood was centrifugated and then stored at -20ºC until lipid profile analysis could be performed. The blood glucose of all animals was also measured on the first and last day of the experiment. The animals were weighed and monitored for food intake; both evaluations occurred weekly (Figure 1).

Figure 1. Experimental design.



2.5. BEHAVIORAL ANALYSIS

2.5.1. Inhibitory Avoidance

The behavioral test started with a training session, which occurred 24 hours after the last administration of aspartame. The apparatus used in the inhibitory avoidance test consists of a Perspex box in which the floor is composed of parallel metal bars, and a platform is placed on the left wall of the device[8,52]. In the training session, the animals were placed on the platform, and the time required to step down from the platform with all four legs (latency) onto the floor was measured. Immediately after leaving the platform, the animal received a shock of 0.4 mA for 2 seconds. In the test session, the animals were again placed on the platform, and the time required to step down (latency) was measured; however, no shock was given. The interval between the training and test sessions (5 seconds) was measured immediately after training to assess the working memory of the rats. One and a half hours after the initial training and test sessions, the test was repeated to measure the animals' short-term memory and, after 24 hours, to evaluate their long-term memory[53,54].

2.6. METABOLIC PARAMETERS

2.6.1. Bodyweight and food intake

The animals were weighed weekly throughout the experiment. Food intake was calculated weekly, twice a week (beginning and end), by considering the total amount of food (g) provided to the animals by subtracting the remaining food (g) in the cage.

2.6.2. Glycemic profile

Blood glucose was measured on the first and last day of treatment for comparison purposes. To perform this procedure, it was necessary to pierce the end of the mouse syrup with a needle (13 x 0.45 mm) and place the blood on the

glucose-measuring tape to determine serum glucose utilizing a glucose meter (TRUEread®). The procedure was performed before the administration of aspartame or water treatments.

2.6.3. Lipid profile

For the lipid profile analysis, the blood collected during decapitation was centrifuged at 3,000 rpm at room temperature for 10 minutes to obtain the blood serum, which was frozen and stored at -20ºC until the analysis of the lipid fractions. Serum concentrations of total cholesterol, high-density lipoprotein (HDL), and triglycerides were followed by colorimetric enzyme assays using commercial kits (Vida Biotechnology and Labtest).

2.7. STATISTICAL ANALYSIS

Data were expressed as the mean and standard error of the mean (SEM). The inhibitory avoidance test, lipid profile, body weight, and food intake were statistically analyzed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. For blood glucose analysis, the Dunn-Sidak comparison test was used after ANOVA. The significance level established for the statistical test was $p < 0.05$. GraphPad Prism 8.0.1 was used as a statistical package.

1. RESULTS

1.1. INHIBITORY AVOIDANCE

Figure 2 shows the results of the inhibitory avoidance test in male and female rats. The results show that males and females in the control group (water) showed increased latency in all memory types ($p<0.05$) (working, short-term, and long-term memory) when compared to the same training group. In the aspartame groups (ASP35, ASP80, and ASP160), it was impossible to point out the animals' learning compared to their respective groups' training.

In addition, the animals from groups ASP35, ASP80, and ASP160 showed short and long-term working memory decreases compared to the same memory types of the control group ($p<0.05$).

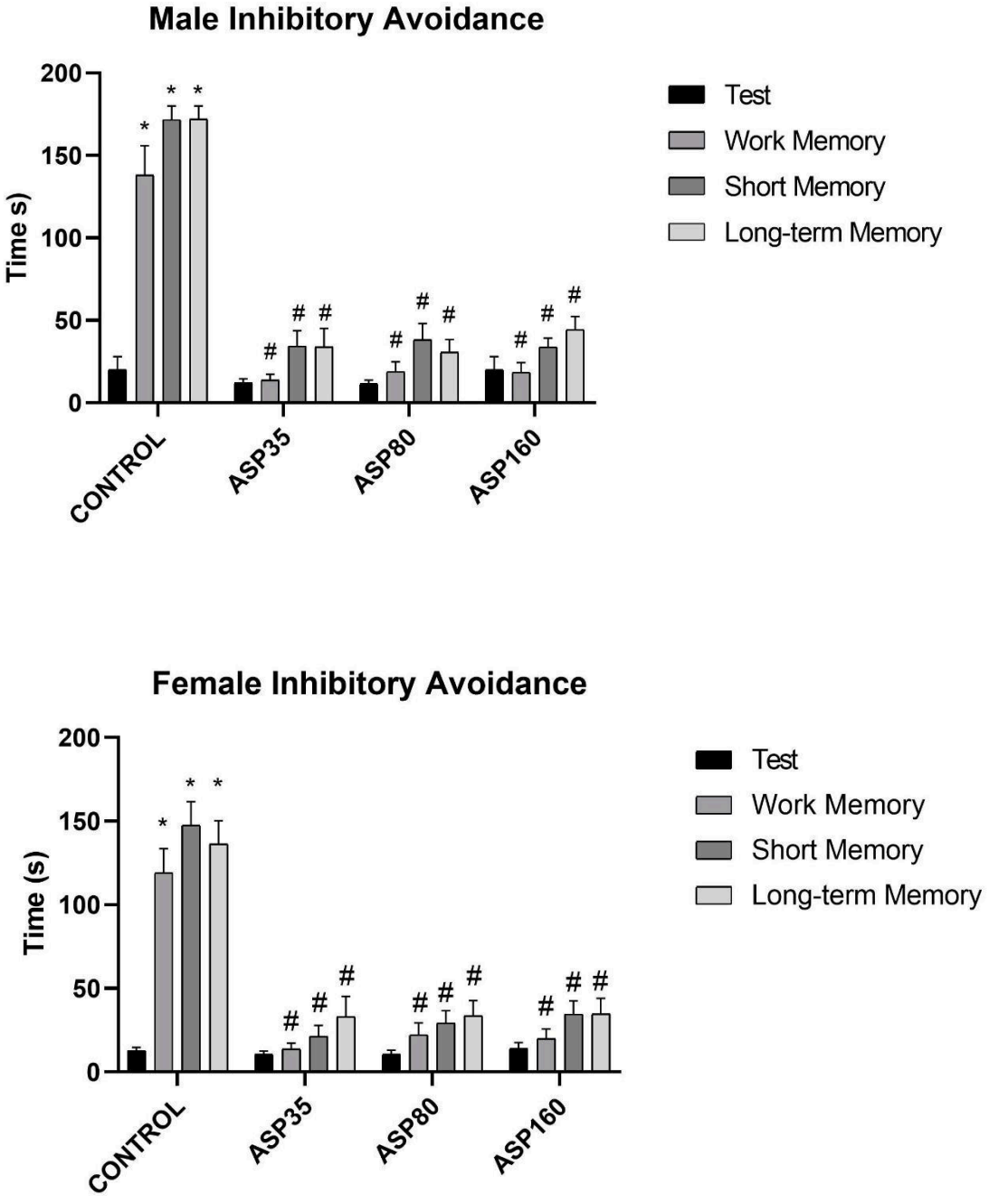


Figure 2. Effect of chronic administration of aspartame at doses of 35 mg/kg, 80 mg/kg, and 160 mg/kg or water on the inhibitory avoidance test in male (A) and female (B) Wistar rats. Values were expressed as mean ± standard error of the mean. (n = 10 animals per group). * different from the training of each group. # different from the same type of memory as the control group. $p < 0.05$ (one-way ANOVA followed by Tukey's post hoc).

1.1. BODY WEIGHT

Table 1 shows the males' average weekly weight gain until the last day/week of treatment. Figure 3 shows the total body weight curve regarding the first and previous weight assessments.

	Control (g)	ASP35 (g)	ASP80 (g)	ASP160 (g)
1st week	64,9 ± 1,24	65,7 ± 3,46	65,4 ± 3,38	64,9 ± 3,38
2st week	97,5 ± 3,7	98 ± 4,98	98,3 ± 5,23	99,3 ± 5,33
3st week	140,8 ± 4,67	139,9 ± 4,15	142,5 ± 5,07	144,7 ± 6,07
4st week	182,2 ± 5,55	183,3 ± 5,95	187,7 ± 5,42	189,9 ± 6,2
5st week	228,3 ± 6,93	230,1 ± 7,7	229,7 ± 8,16	233,2 ± 8,58
6st week	257,5 ± 6,91	260,3 ± 7,53	261,4 ± 9,08	267,2 ± 9,5
7st week	287,6 ± 7,3	293,4 ± 7,57	293,5 ± 6,45	309,9 ± 9,4*

Table 1. Weekly body weight in g of male Wistar rats that received water or aspartame (35 mg/kg; 80 mg/kg; 160 mg/kg) for 40 days (equivalent to 7 weeks). Data are expressed as mean ± standard error of mean body weight each week until the end of the treatment (n = 10 animals per group). * different from the weight gain of the control group. p <0.05 (one-way ANOVA followed by Tukey's post hoc).

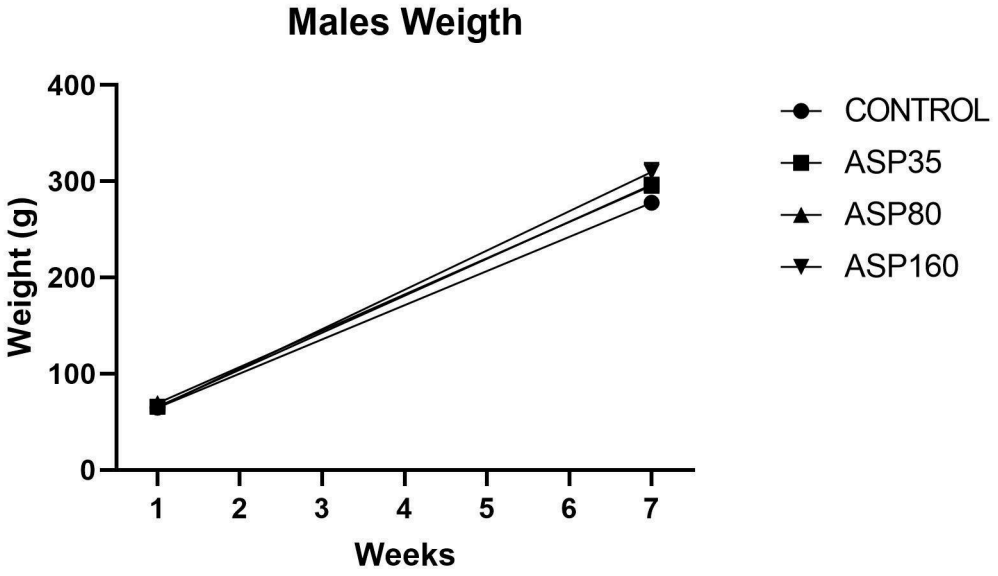


Figure 3. Total body weight (g) curve of male Wistar rats, which received water or aspartame in doses of 35 mg/kg, 80 mg/kg, and 160 mg/kg for a period of 40 days (equivalent to 7 weeks). Values were expressed as mean ± standard error of the mean. (n = 10 animals per group). * different from the control group. p <0.05 (one-way ANOVA followed by Tukey's post hoc).

Table 1 reveals that the basis weight of the water, ASP35, ASP80, and ASP160 groups did not present significant differences. Regarding the weight gained by the rats over the weeks, 160 mg/kg aspartame-treated male rats had a significant increase in body weight in the last week compared to the gain in the control group ($p<0.05$). By observing the total body weight curve and comparing the rats' initial and final weight gain, it was possible to maintain a statistically significant increase in the group receiving aspartame of 160mg/kg ($p<0.05$) compared to the control.

Table 2 shows the female average weekly weight gain until the last day/ week of treatment. Figure 4 shows the total body weight curve regarding the first and previous weight assessments.

	Control (g)	**ASP35 (g)**	**ASP80 (g)**	**ASP160 (g)**
1st week	50,6 ± 1,29	52,5 ± 2,57	54,4 ± 2,47	52,8 ± 2,85
2st week	84,1 ± 3,74	85,8 ± 3,2	87,2 ± 2,52	89,7 ± 4,96
3st week	107,2 ± 2,97	112,1 ± 2,23	116,4 ± 3,36*****	121,2 ± 4,51*****
4st week	130,7 ± 3,07	139,1 ± 2,85	144,8 ± 3,04*****	153,6 ± 4,51*****
5st week	153,3 ± 3,76	165,7 ± 3,65	172,3 ± 2,72*	183,1 ± 4,79*
6st week	165,7 ± 2,99	181,6 ± 3,85	189,7 ± 3,85*	201,7 ± 4,41*
7st week	174,3 ± 2,56	201,4 ± 3,59*****	200,8 ± 2,37*****	215,5 ± 3,73*****

Table 2. Weekly body weight in g of female Wistar rats that received water or aspartame (35 mg/kg; 80 mg/kg; 160 mg/kg) for 40 days (equivalent to 7 weeks). The data are expressed as mean ± standard error of the mean body weight each week until the end of the treatments. (n = 10 animals per group). * different from the weight gain of the control group. $p < 0.05$ (one-way ANOVA followed by Tukey's post hoc).

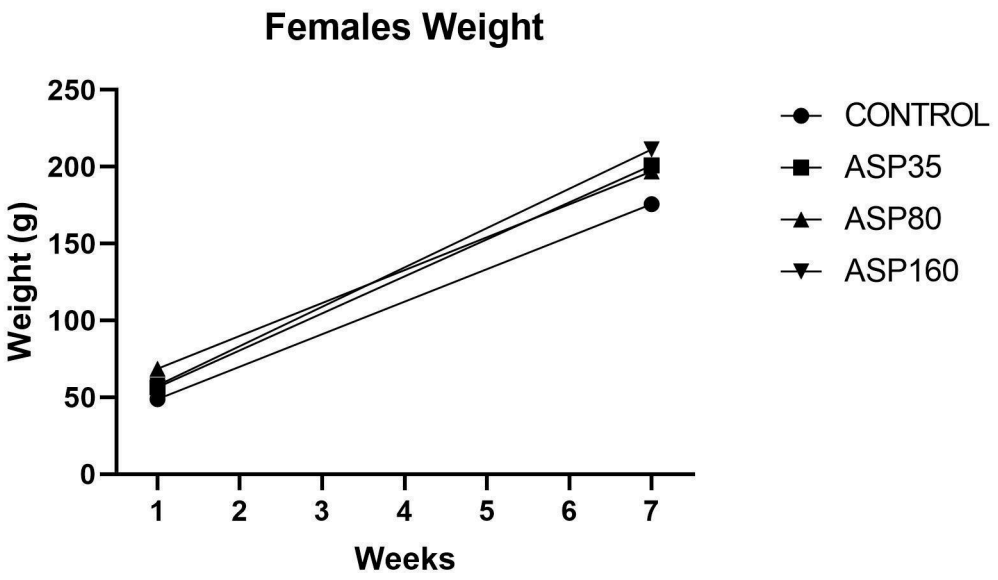


Figure 4. Total body weight (g) curve of female Wistar rats received water or aspartame in doses of 35 mg/kg, 80 mg/kg, and 160 mg/kg for 40 days (equivalent to 7 weeks). Values were expressed as mean ± standard error of the mean. (n = 10 animals per group). * different from the control group. p <0.05 (one-way ANOVA followed by Tukey's post hoc).

According to Table 2, it is possible to observe that the baseline weight of the water groups, ASP35, ASP80, and ASP160, did not present any significant differences. From the third week of the experiment onwards, it was possible to observe significantly higher weight gain in the ASP80 and ASP160 groups compared to the control group ($p<0.05$). In the last week, the ASP35 group showed a statistically higher weight gain than the control group ($p<0.05$). By observing the total body weight curve and comparing the rats' initial and final weight gain, it was possible to maintain a statistically significant increase in all groups that received aspartame (35mg/kg, 80mg/kg, and 160mg/kg) when compared to the control group ($p <0.05$).

1.3. FOOD INTAKE

Table 3 shows the average final food intake of the control group and the ASP35, ASP80, and ASP160 groups over the entire experimental period.

	Control (g)	**ASP35 (g)**	**ASP80 (g)**	**ASP160 (g)**
Males	4141 ± 72,49	4360 ± 95,52	4412 ± 19,52	4512 ± 125,5

Females	2897 ± 23	3080 ± 43,5	3190 ± 64	3282 ± 72,5

Table 3. Total intake of animal feed in g, by male and female Wistar rats, which received water or aspartame (35 mg/kg; 80 mg/kg; 160 mg/kg) for 40 days. The data are expressed as mean ± standard error of the mean of total feed intake. (n = 10 animals per group). Non-significant data.

When evaluating the average food intake of males and females, an increase in the groups that received aspartame at the three doses (35mg/kg, 80mg/kg, and 160mg/kg) was observed in both sexes compared to the control group. However, there was no statistically significant difference ($p<0.05$).

1.4. GLYCEMIC PROFILE

Figures 5 and 6 show the measured blood glucose values in males and females.

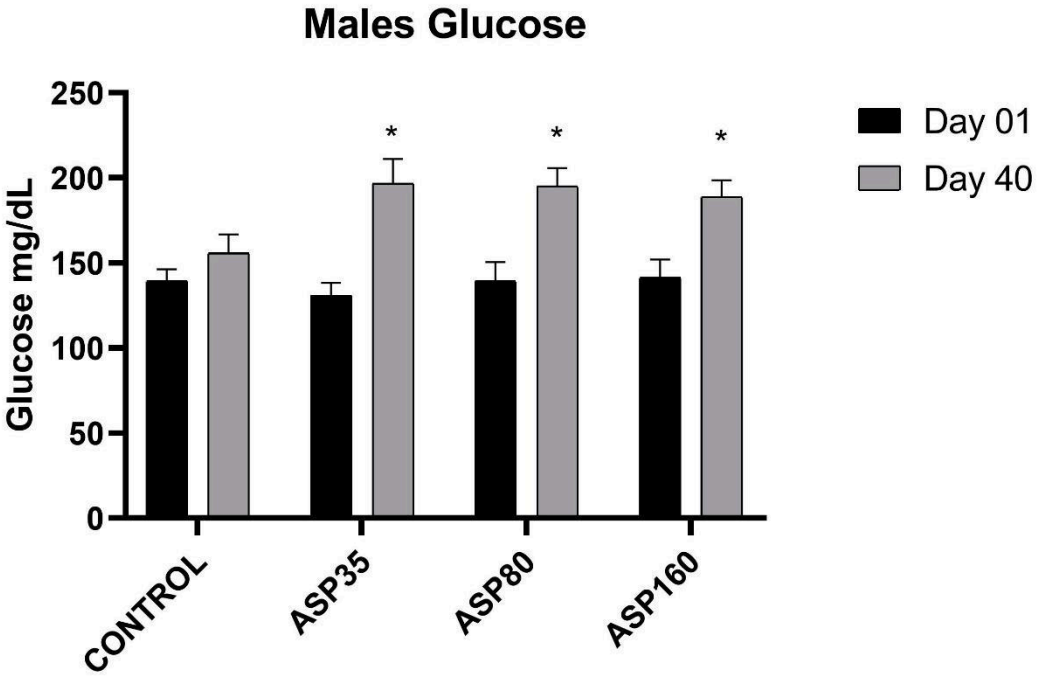


Figure 5. Effect of chronic administration of aspartame at doses of 35 mg/kg, 80 mg/kg, and 160 mg/kg or water, on the glycemic profile of male wistar rats. Values were expressed as mean ± standard error of the mean. (n = 10 animals per group) * different from the first day of the group itself. $p < 0.05$ (one-way ANOVA followed by the Dunn-Sidak test).

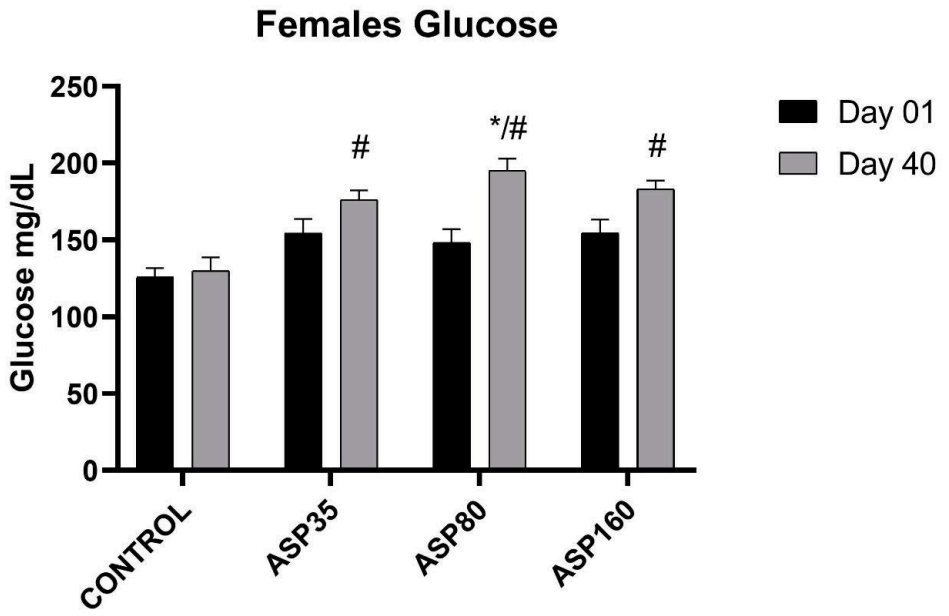


Figure 6. Effect of chronic administration of aspartame at doses of 35 mg/kg, 80 mg/kg, and 160 mg/kg or water on the glycemic profile of female Wistar rats. Values were expressed as mean ± standard error of the mean. (n = 10 animals per group). * different from the first day of the group itself. # different from the last day of the control group. p <0.05 (one-way ANOVA followed by the Dunn-Sidak test).

The male results show that the initial glycemia, measured on the first day of the treatment, did not differ significantly from the aspartame and control groups. The values obtained for blood glucose levels on the last day of treatment indicate an increase in blood glucose in the groups ASP35, ASP80, and ASP160 ($p<0.05$), compared to the mean values observed on the first day in the same groups.

In females, the results show that the initial glycemia, measured on the first day of the treatments, did not differ significantly from the aspartame, control groups, and males. However, at the end of the treatment, the blood glucose values obtained on the last day indicate a significant increase in the ASP35, ASP80, and ASP160 groups ($p<0.05$) compared to the control group. The results also demonstrate an increase in blood glucose on the last day of treatment in the ASP80 group ($p<0.05$) compared to the mean values observed on the first day in the same group.

1.2. LIPID PROFILE

Table 4 shows the mean values of lipid assessments measured in serum from male and female Wistar rats, which received only 35mg/kg, 80mg/kg, and

160mg/kg of aspartame or water over the entire experimental period. The table results show that, in males and females, 160 mg/kg of aspartame increased plasma triglyceride values compared to the control group ($p<0.05$). An increase was also observed in females who received aspartame at 35mg/kg ($p<0.05$). There was no significant difference ($p<0.05$) between the total cholesterol and HDL fraction values groups.

	CT (mg/dL)	HDL (mg/dL)	TG (mg/dL)
	MALES		
Control	62 +- 2,66	44 +- 1,43	100,2 +- 5,91
ASP35	62,71 +- 2,36	41,29 +- 2,38	119,2 +- 10,58
ASP80	61,71 +- 2,57	38,86 +- 1,53	110, 8 +- 13,3
ASP160	77 +- 4,41	48,71 +- 2,89	182,9 +- 20,67*
	FEMALES		
Control	66,86 +- 3,59	49,29 +- 2,6	79,61 +- 7,06
ASP35	71 +- 3,84	50,14 +- 2,89	117,8 +- 8,39*
ASP80	59,86 +- 3,31	43,86 +- 3,69	80,81 +- 4,16
ASP160	64,71 +- 3,22	50,14 +- 2,46	119,1 +- 11,47*

Table 4. Total cholesterol, HDL and triglycerides (mg / dL) of male and female Wistar rats, which received water or aspartame (35mg / kg; 80mg / kg; 160mg / kg) for a period of 40 days. The data are expressed as mean ± standard error of the mean of total cholesterol, HDL fraction, and triglycerides (n = 7 animals per group). * different from the control group. $P<0.05$ (one-way ANOVA followed by Tukey's post hoc). CT: Total cholesterol; HDL: High-density lipoprotein; TG: Triglycerides.

4. DISCUSSION

In the last decades, consumption of diet and low-sugar foods has increased among consumers with dietary restrictions and those seeking to lose weight. In addition, the use of sweeteners in foods, especially artificial ones such as aspartame, is widespread in diet products and general products used by the

population[5]. These findings have raised many concerns and questions about the long-term safety of these substances in human health. The debate on the use of aspartame has existed since its approval by the FDA and currently persists with many controversies. Our results support our initial hypothesis and indicate that chronic consumption of aspartame, especially at 160mg/kg, and lower doses, induced cognitive impairment and metabolic changes, such as weight gain and increased blood glucose and triglyceride levels.

The animals underwent the inhibitory avoidance test to assess their cognitive profile. The inhibitory avoidance task is one of the most widely used memory tests; it inhibits the exploration of the environment by shocking the animal. Thus, the idea is that the animal learns not to get off the platform. As a result, male and female animals subjected to chronic aspartame consumption in the three doses administered demonstrated cognitive impairment compared to their respective groups' training. In addition, they also presented impairment in all memory types (working, short-term and long-term memory) compared to the control groups. These data show cognitive impairment associated with chronic aspartame consumption and corroborate other studies described in the literature that evaluated learning and memory by different protocols.

Erbas[48] demonstrated cognitive impairment by passive avoidance testing in adult male rats receiving aspartame at 3mg/kg/day for six weeks. Similarly, Abdel-Salam studied memory functions in male mice, giving them different dosages of aspartame for 2 weeks[14]. They reported that a 5.625 mg/kg aspartame dose significantly affected animal performance in the Morris Water Maze test. In another study, Abu-Taweel[15] found that chronic individual and combination ingestion of aspartame (32mg/kg) and monosodium glutamate (8mg/kg) for 30 days caused significant disruptive effects on cognitive responses and memory retention in mice.

These findings support the evidence of neurobehavioral effects associated with aspartame, such as impaired learning and memory. They may be supported by the fact that aspartame metabolites can cause significant neurochemical changes. Choudhary and Lee[11] reported that the substantial increase in plasma phenylalanine due to chronic aspartame consumption might lead to brain disorders. These disorders may be related to the significant increases in phenylalanine in the brain and the decrease in the entry of other amino acids, such as tyrosine and tryptophan, precursors of serotonin (5-HT) and dopamine (DA) neurotransmitters, respectively.

These neurotransmitters, in turn, perform critical cognitive functions, and such a decrease has already been demonstrated in the brain of rodents treated with aspartame[14,55]. Furthermore, studies have also found that in addition to modifying brain neurotransmitter concentrations, aspartame disrupts neuronal function as its degradation product, aspartate, can indirectly lead to an imbalance in brain homeostasis, including neurodegeneration[10,55]. These studies have shown that even slight increases in aspartate levels in the brain can lead to excitotoxicity in neurons due to their ability to open cationic channels, leading to uncontrollable calcium influx and thus triggering a cascade of reactions that culminate in cell death[47,56].

Researchers have postulated that aspartame is a chemical stressor associated with exacerbated free radicals production in the body. At high concentrations, these products may reduce synaptic plasticity by weakening long-term potentiation (LTP), affecting synaptic neurotransmission, and thus increasing the brain's vulnerability to oxidative stress, causing cellular damage and adverse effects on neurobehavioral health[57,58]. These actions are mainly related to methanol. For example, it has been shown that in albino Wistar rats, methanol accelerated the action of reactive oxygen species since, when metabolized, it promoted the formation of the superoxide radical anion and hydrogen peroxide[59,60].

In addition to the brain effects of aspartame use, this sweetener appears to be related to metabolic disorders. Studies have questioned the effectiveness of aspartame in losing and maintaining body mass and its results on lipid and glycemic profiles. A priori, this concern seems unfounded, as it would be very logical to think that replacing sugar with low or no-calorie sweeteners would contribute to lower caloric intake and reduction or maintenance of blood glucose levels, which would contribute to lost weight and blood glucose control. However, this hypothesis has generated controversy, prompting research on such parameters[61].

Our results on body weight showed that male rats receiving aspartame at a dose of 160mg/kg gained weight at the end of treatment compared to the control group. In females, it was possible to observe this weight gain in all groups (ASP35, ASP80, and ASP160). Moreover, at the 80mg/kg and 160mg/kg doses, this increase in their weight was evident from the third week of the experiment.

The results found in this research corroborate other studies that used aspartame-treated animals in different protocols. Silva[62] demonstrated an increase in body weight of 2mL/100g in aspartame-treated adolescent Wistar rats. Souza[30]

observed higher body weight in the offspring of aspartame-treated rats at a dose of 25mg/kg. Also, A study by Reis[63] in adult Wistar rats observed more significant weight gain in the aspartame-treated group (0.4%) over 12 weeks.

These studies showed a high food intake by the animals, concluding that aspartame stimulates appetite. This increase in appetite induces weight gain since calories not eaten during sugar substitution by sweeteners can be ingested later as a form of compensation. Other authors have also reported a similar increase in appetite. From the preclinical studies by Swithers and Davidson[25-27], using non-caloric sweeteners weakens the ability of sweet taste perception and activation of the cephalic phase of digestion - the preparation of the digestive tract for food consumption. In addition, the reward sensation triggered by food consists of two pathways: sensory and post-ingestion[64]. The signaled post-ingestion pathway depends on the metabolic products of the food[65]. Since the loss of caloric contribution characterizes non-nutritive sweeteners, this usually eliminates the post-ingestion component. Thus, it has been suggested that dissociation between sweet taste perception and caloric intake may increase appetite, triggering food-seeking behaviors and thus contributing to obesity[49]. Also, this increase in appetite may be related to the reduced DA and 5-HT concentrations in the presence of aspartame[55]. Although our research results showed a difference between the aspartame and control groups in terms of food intake, the difference was not statistically significant, suggesting that aspartame may induce weight gain through other mechanisms.

Changes in microbiota have been strongly associated with consuming artificial sweeteners and triggering metabolic changes[25,32,37]. One of the reasons for these results would be the possibility of artificial sweeteners altering the intestinal microbiota composition. A decrease in bifidobacteria combined with an increase in enterobacteria can lead to endotoxemia, which results in a low-grade inflammatory state, and is associated with some metabolic conditions such as obesity, insulin resistance, and increased intestinal permeability. Thus, it is postulated that metabolic endotoxemia can be a driving force behind obesity and insulin resistance induced by artificial sweeteners[66,67]. In a study published by Suez[31], it was found that all rats that consumed sweeteners developed weight gain and glucose intolerance; however, none of the control rats had that outcome. Several bacterial taxa that changed after consuming artificial sweeteners were previously associated with type 2 diabetes in humans.

Our studies involving the rats' glycemic profile showed that aspartame increased blood glucose in all males between the first and last day of treatment. The same correlation could not be seen in the control group. In females, the results indicated that the glycemia of the animals that received 80 mg/kg of aspartame at the end of the experiment increased. The glycemic values of the three aspartame groups were higher than the control group. A study by Souza[30] also observed more elevated mean glucose in the offspring of aspartame-treated rats at a dose of 50mg/kg.

Regarding the lipid profile, our results showed that the triglyceride plasma levels at the end of the experiment were significantly higher in male and female animals that received 160 mg/kg aspartame compared to the control group. In addition, aspartame at a dose of 35mg/kg could also cause such an effect in females. Von Poser Toigo[29], in assessing metabolic changes associated with prenatal aspartame exposure, also found increased triglyceride levels in the offspring of rats treated with this sweetener.

These results related to glycemic and lipid profiles can be partially explained by the studies and mechanisms described above (food consumption, oxidative stress, intestinal microbiota), either by direct actions or as a consequence of obesity. However, studies involving such analyzes are still scarce and contradictory.

Finally, in our results, observing metabolic and brain alterations in the three doses of aspartame used was generally possible. Thus, the present study provides evidence that aspartame, a sweetener widely used in the human diet, is capable of impairing cognitive development as well as causing significant metabolic changes. Therefore, we emphasize the need to review aspartame ADI and raise awareness of its consumption.

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