

Full Length Research Paper

Influence of sweeteners in the biodistribution of radiopharmaceutical and laboratory tests in rats

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This study aimed to evaluate the effect of supplementation of sucralose and fructose on the metabolism of adolescent rats. Eighteen male Wistar rats were divided into 3 groups: control group (GC), fructose group (GF) treated with 50 mg/kg of fructose, and sucralose group (GS) receiving 50 mg/kg of sucralose for 24 days. The weight and feed intake were measured weekly. At the end of the experiment, some biochemical parameters, histopathology of the liver and biodistribution of the radiotracer ^{99m}Tc-sodium phytate in liver and blood were analyzed. The GF showed higher body weight only in the first week compared with GS and GC (p<0.05). Histopathology and % ATI/g radiotracer ^{99m}Tc-sodium phytate in liver and blood were not different between the groups. The GF showed higher values of aspartate aminotransferase activity, bilirubin, alkaline phosphatase activity and gamma glutamyl transferase activity, compared with the other groups (GC and GS) (p<0.05). Activity of alanine aminotransferase and albumin level of GF were higher than GS (p<0.05). For other parameters, no statistical difference was observed. It was concluded that the use of fructose during the experiment was able to alter hepatic enzymes, but on the other hands, the use of sucralose caused no change.

Key words: Sucralose, fructose, adolescent rats, radiopharmaceutical.

INTRODUCTION

Excessive consumption of carbohydrates is responsible for metabolic abnormalities in individuals, leading to morbidities such as diabetes mellitus (DM), obesity, and consequent increase in population mortality. An

alternative to control plasma glucose and weight gain are sweeteners and diets/lights products, created for food industry (Castro and Franco, 2002; Hossain et al., 2007; Oliveira and Franco, 2010).

Sucralose is a non-nutritive and non-caloric sweetener, derived from sucrose, with a sweeten potential 600 times greater than sucrose (Grice and Goldsmith, 2000). Sucralose has qualities that are interesting not only for the consumers but also to the food and beverage industry. Some of these qualities are: it is very stable at high temperatures and low pH; it does not leave an aftertaste like many others sweeteners do; it cannot be hydrolyzed during the digestion and metabolic processes since the carbon-chloro linkage is very stable; it is fairly soluble in water and it presents high stability both in crystalline and solution forms (Grotz and Munro, 2009; Rodero et al., 2009). Fructose is found naturally in fruits and honey, but may also be obtained through industrial process for the inversion of sucrose. Fructose is widely consumed by diabetics, because it does not require insulin to enter the cell (Barreiros et al., 2005).

However, studies show that fructose can cause changes in lipid profile and liver abnormalities in humans and animals (Bocarsly et al., 2010; Lunardelli et al., 2004).

Nuclear medicine is a medical specialty, which goal is the use of radioactive materials, named radionuclides, radioactive isotopes or radioisotopes for therapeutic and diagnostic purposes (Saha, 2010). In nuclear medicine, about 95% of radiopharmaceuticals are used for diagnostic purposes, while the remaining 5% is used for therapeutic treatment, for example, in the treatment of radiosensitive tumors. Most diagnostic techniques are based on labeling with radioactive molecules to generate images (mapping or scintigraphy) of the body where the radioactive material is concentrated, using special equipment for this purpose. Unlike other imaging methods, scintigraphic techniques cannot only evaluate the morphological structure of the organ, but also assess their physiological function (Owunwanne et al., 1995; Saha, 2010).

The main radionuclide currently used in nuclear medicine is technetium-99m, which has physical characteristics favorable to the formation of scintillation camera images. The radiocolloid sodium phytate labeled with technetium-99m (^{99m}Tc -sodium phytate) is a radiopharmaceutical that has been widely used to study the liver and spleen, since its introduction in 1973. Biodistribution to the liver and bone marrow has shown good correlation with the severity of liver diseases such as cirrhosis and fibrosis of the organs and its prognosis. Thus, quantification of the uptake of ^{99m}Tc -sodium phytate serves as an excellent index of liver function (Groshar et al., 2002; Pereira et al., 2008).

Although, the use of radiopharmaceuticals in nuclear medicine occurs on a large scale for many years, several problems related to the interaction of these

radiopharmaceuticals in the body have been observed as a change in the normal biodistribution of these radiopharmaceuticals. There are many factors, both external and internal to the body, that may affect the normal biodistribution of radiopharmaceuticals, such as surgical procedures, pathophysiological mechanisms, radiation, infections, smoking (tobacco), use of natural or synthetic drugs and some foods, and nutritional conditions (Barbosa et al., 2009; Bernardo-Filho et al., 2005; Cekic et al., 2011; Holanda et al., 2014; Passos et al., 2002; Valença et al., 2005; Vallabhajosula et al., 2010). Rocha et al. (2011), demonstrated that sucralose in different concentrations can change biodistribution of radiopharmaceuticals as sodium pertechnetate in kidney and ^{99m}Tc -DTPA in various organs such as pancreas, stomach, spleen, muscle, and thyroid.

It is important that health professionals recognize these factors and understand the mechanisms by which they alter the biodistribution of radiopharmaceuticals, not to compromise the interpretation of imaging studies. Due to the large number of patients and even normal individuals who make continuous use of sucralose and fructose, the aim of this study was to investigate possible toxic and metabolic effects of these sweeteners evaluating its interference on the biodistribution of the radiopharmaceutical ^{99m}Tc -sodium phytate in the liver and blood as well as in laboratory parameters and histopathology of rats.

MATERIALS AND METHODS

Animals and diet

Eighteen male Wistar rats of five weeks old were kept in metabolic cages under controlled temperature of $23 \pm 1^\circ\text{C}$ and light/dark cycle 12/12 h. The rats were divided into 3 groups: control group (GC), which was treated with distilled water; the fructose group (GF) treated with 50 mg/kg of fructose (Doce Menor® - WOW Nutrition); and sucralose group (GS) 50 mg/kg of sucralose (Finn® - Boehringer de Angeli Quim. Farm). All groups received supplementation addition, standard chow (Essence - Purina) and water *ad libitum*. Supplementation with sweetening was performed once a day by gavage and was administered 2 ml/100 g/day for 24 days. The feed intake and body weight gain were measured weekly. The research followed the experimental protocol according to the ethical guidelines of the National Institutes of Health (Bethesda, USA), with respect to animal care and protocol was approved by the Ethics Committee on Animal Use (CEUA n° 128-2013) of the Federal University of Campina Grande.

Biodistribution of ^{99m}Tc -sodium phytate

On the last day of treatment, all animals were anesthetized with xylazine (20 mg/kg) and ketamine (50 mg/kg) by intramuscular via and all groups received 0.1 ml of ^{99m}Tc -sodium phytate (0.66 MBq

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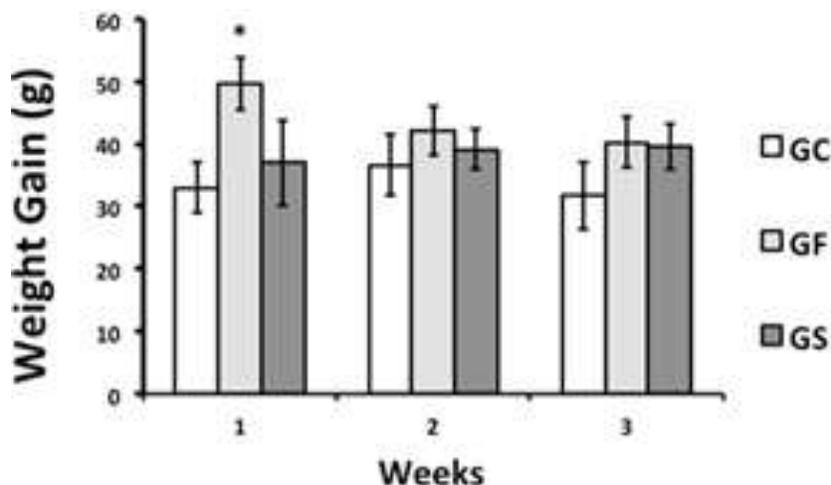


Figure 1. Weekly weight gain of the rats treated with fructose (GF) $n = 6$ and sucralose (GS) $n = 6$, compared with the control group (GC) $n = 6$, receiving distilled water. Data expressed as mean \pm SD (One-way ANOVA, Holm-Sidak). *= $p < 0.05$.

of radioactivity) by tail vein. The technetium-99m was eluted from a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator produced by the Institute of Energy and Nuclear Research, São Paulo/Brazil and the kits sodium phytate were kindly donated by the Liga Norteriograndense Contra o Câncer, Natal/RN. Forty minutes after the injection of the radiopharmaceutical, whole blood samples were taken by cardiac puncture of all animals, with heparinized syringes, and were placed in tubes for subsequent biochemical analysis and in racks for counting of radioactivity. Liver small samples from the same liver lobe from each animal were washed in 0.9% saline, weighed on a precision scale and the percentage of radioactivity per gram of tissue (%ATI/g) was determined in an automatic gamma counter (Wizard™ Perkin-Elmer, Finland), with automatic correction radiation decline. The percentage of total radioactivity injected organ gram (% ATI/g) of each organ was calculated by dividing the activity/g tissue to the total activity administered to each animal.

Biochemical and histopathological analysis

A liver tissue fragment was fixed for 24 h in 10% buffered formalin for histopathological analysis. After fixing, the material was dehydrated in an increasing alcoholic series, diaphanized in xylene, soaked and immersed in paraffin. The fragment had 5 μm of thickness and was stained with hematoxylin and eosin (HE). Blood samples obtained from the animals were used for biochemical analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamyl transferase (γGT), total bilirubin, direct and indirect bilirubin, total cholesterol and fractions (LDL and HDL), triglycerides and total protein, and fractions (albumin and globulin), using the biochemical autoanalyzer Konelab 60i (Weiner test kit, São Paulo, Brazil).

Statistical analysis

All data are presented as mean \pm standard deviation (SD). The body weight gain and food consumption, % ATI/g and biochemical analysis were compared by one-way ANOVA test, Holm-Sidak considering $p < 0.05$ as statistically significant. Sigma Start Program

was used for the data analysis.

RESULTS

Weight gain and food consumption

There was no significant difference in the initial body weight of the animals distributed in three groups: GC (125.86 ± 5.18); GF (128.00 ± 7.04); GS (122.86 ± 11.36). Data are expressed as mean and SD. The weekly gain of body weight of the experimental groups is as shown in Figure 1. The fructose group showed a statistically significant difference in the first week, compared to the control group and sucralose group ($p < 0.05$).

No statistical differences ($p > 0.05$) were observed in feed intake of young rats with fructose sweeteners (GF) and sucralose (GS) compared with the control group (GC) (Figure 2).

Biodistribution of $^{99\text{m}}\text{Tc}$ -sodium phytate

Table 1 shows the radioactivity per gram of tissue (% ATI/g) of $^{99\text{m}}\text{Tc}$ - sodium phytate in the blood and liver of animals treated with fructose, sucralose and distilled water. There was no significant difference in the biodistribution of radioactivity in the studied organs.

Biochemical and histopathological analysis

As shown in Table 2, the animals treated with fructose

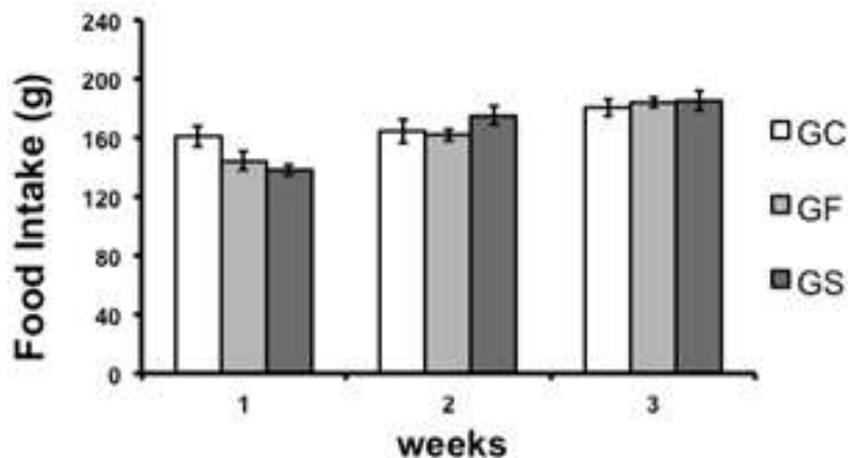


Figure 2. Weekly feed consumption of young rats treated with fructose (GF), sucralose (GS) and distilled water (GC). Data expressed as mean \pm SD (One-way ANOVA, Holm-Sidak).

Table 1. Biodistribution of the radiotracer in the liver and blood of rats treated with different kinds of sweeteners.

Organ	Control (%ATI g)	Fructose (%ATI g)	Sucralose (%ATI g)
Liver	2.72 \pm 1.71	2.36 \pm 1.59	2.18 \pm 1.38
Blood	0.06 \pm 0.03	0.06 \pm 0.04	0.08 \pm 0.02

Results expressed as Mean \pm SD; n = 6 per group (One-way ANOVA, Holm-Sidak); p > 0.05.

Table 2. Biochemical analysis of blood of rats treated with different sweeteners during 24 days.

Biochemical parameter	Groups		
	Control	Fructose	Sucralose
Total Bilirubin (mg/dl)	0.20 \pm 0.12	0.38 \pm 0.13	0.20 \pm 0.00
Direct Bilirubin (mg/dl)	0.05 \pm 0.06	0.03 \pm 0.00	0.08 \pm 0.04
AST (U/L)	43.25 \pm 3.20	268.00 \pm 54.04* [#]	60.00 \pm 55.74
ALT (U/L)	52.20 \pm 8.41	71.00 \pm 9.87 [#]	47.00 \pm 14.31
Bilirubin Indirect (mg/dl)	0.15 \pm 0.06	0.35 \pm 0.13* [#]	0.12 \pm 0.04
Total Protein (g/dl)	6.63 \pm 0.44	6.56 \pm 0.33	6.34 \pm 0.74
Albumin (g/dl)	4.27 \pm 0.15	4.76 \pm 0.22 [#]	4.06 \pm 0.62
Globulin (g/dl)	2.17 \pm 0.61	1.80 \pm 0.20	2.27 \pm 0.47
Total cholesterol (mg/dl)	78.00 \pm 13.30	64.80 \pm 8.53	73.29 \pm 12.80
HDL cholesterol (mg/dl)	53.17 \pm 8.57	39.00 \pm 6.04	54.43 \pm 11.40
LDL cholesterol (mg/dl)	11.67 \pm 10.94	15.00 \pm 2.83	12.73 \pm 12.59
Triglycerides (mg/dl)	62.33 \pm 19.55	45.00 \pm 13.47	54.71 \pm 18.02
Alkaline phosphatase (U/L)	212.50 \pm 73.20	775.20 \pm 177.01* [#]	323.14 \pm 28.02
γ GT (U/L)	1.68 \pm 0.36	8.25 \pm 2.06* [#]	2.04 \pm 0.23

Results expressed as Mean \pm SD; n=7 (One-way ANOVA, Holm-Sidak). AST: Aspartate aminotransferase; ALT: alanine aminotransferase; γ GT: gamma glutamyl transferase; *Versus control group-GC; [#]Versus sucralose group-GS.

(GF) had higher AST, bilirubin indirect, alkaline phosphatase, and γ GT, compared with the other groups

(GC and GS) (P<0.05). Albumin and ALT enzyme of fructose group, when compared with the GS (p=0.039),

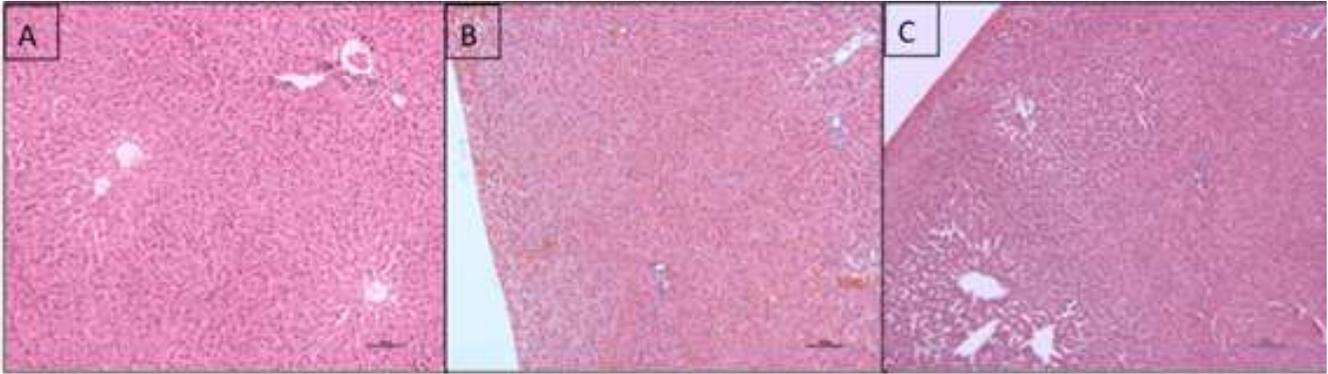


Figure 3. Histological section of the liver of the animals of the control group (A), fructose group (B) and sucralose group (C).

also showed significant statistical difference. There was no significant statistical difference in the biochemical parameters among the groups.

Figure 3 illustrates the histology of the liver of the control group (A) and the groups treated with fructose (B) and sucralose (C). In all three groups there was a healthy liver tissue characterized by cordonal arrangement of hepatocytes distributed radially from the periphery to the center of the lobe. No histologic changes were observed in groups.

DISCUSSION

This study dealt with adolescent rats with fructose and sucralose for three weeks and observed their effects on food intake and weight gain, the biodistribution of the radiopharmaceutical ^{99m}Tc -sodium phytate in the liver and blood, biochemical, and histopathological parameters.

The results showed that this treatment does not alter the body weight, the only exception was the fructose group (GF), which showed greater weight gain in the first week, the biodistribution of the radiopharmaceutical in the liver and blood, as well as histopathology. However, significant increase of AST, ALT, alkaline phosphatase, γGT , bilirubin and indirect bilirubin ($p < 0.05$) was observed in the GF. Although, the hepatic enzymes are altered in the fructose group; these damages were not able to alter the biodistribution of the radiopharmaceutical, as well as sucralose group also demonstrated no change.

It was observed that GF had an increase in weight gain in the first week of the experiment compared to the other groups. This increase in weight gain could be observed even with food intake and it was unchanged in GS and GC. Based on these data, it was concluded that the sweetener was responsible for this increase in weight gain and food intake did not. These data corroborate the studies by Sylvetsky et al. (2011), who demonstrated a positive association between the consumption of artificial sweeteners and weight gain in children.

In the present study, there was no changes in the

biodistribution of the radiopharmaceutical ^{99m}Tc -sodium phytate, which confirms the study made by Rocha et al. (2008) that treated rats with different concentrations of sucralose and also did not find any change in the morphology of red blood cells and on the biodistribution when administered sodium pertechnetate in organs and tissues (Rocha et al., 2008). Rocha et al. (2008) used rats at 8 weeks and feed them with sweeteners for 8 days, whereas in the present study, adolescent rats were treated for 24 days and this long period of exposure of sucralose did not alter the biodistribution of the radiopharmaceutical. Grice and Goldsmith (2000) showed that after intravenous administration of sucralose in rats, it was essentially eliminated of all tissues at approximately 6 h, possibly indicating lack of toxicity.

Roberts et al. (2000) examined the excretion of sucralose in humans and found that it is the major component in urine. This feature of this sweetener can justify the lack of interference with the biodistribution of the radiopharmaceutical. In this study, fructose was also not able to change this biodistribution. This result is consistent with the results obtained by Holanda et al. (2014), who investigated the biodistribution of ^{99m}Tc -sodium phytate in the liver, spleen and blood of rats fed food rich in fructose (soursop) and observed no change in their uptake.

Radiopharmaceuticals have a specific distribution and/or elimination patterns when administered. The presence of some biochemical or physiopathological changes can cause alteration on the biodistribution of the radiopharmaceutical and a normal disposal. This change in the biological behavior of the radiopharmaceutical is used in the diagnosis of diseases and knowledge of toxicity of substances due to interactions. In addition, it is possible to observe changes in the biodistribution due to the use of natural and synthetic products (Owunwanne et al., 1995). Thus, we saw the importance of using this method to assess whether sweeteners being studied are capable of causing the described changes.

As regards the changes in hepatic enzymes caused by consumption of fructose, they resemble the study in

which animals were fed with a diet rich in fructose (30%) and with a copper deficiency. The authors attribute this result, possibly inhibition of carnitine palmitoyl-transferase I enzyme (CPTI) involved in the beta oxidation, which leads to an accumulation of fatty acids in the liver and induces liver damage (Song et al., 2012). In a study by Holanda et al. (2014), it was observed that the use of *Annona muricata* extract (soursop) (25 mg/kg/day), which is a rich source of fructose, did not significantly alter the levels of AST and ALT. The changes in hepatic enzymes (AST and ALT) caused by consumption of fructose in studies by Song et al. (2012) were due to different concentrations of the *A. muricata* extract and high dose used by the authors (50 mg/kg/day).

The effects of diet rich in fructose in the liver are well known, and their use in experimental models, with the purpose of causing obesity and fatty liver is increasingly common. Bigoniya et al. (2012) observed that rats fed fructose had their weight and plasma lipids increased compared to the control group. Similar results were found by Bocarsly et al. (2010), who reported greater weight gain, abdominal fat and increased triglycerides. In another study, rats treated with diet rich in fructose, showed high liver steatosis, which is decreased when fructose was accompanied with resveratrol (Kopec et al., 2013). In the present study, it was not observed in GF group changes in triglycerides, LDL-cholesterol, HDL-cholesterol, and total cholesterol.

According to the histopathological analysis, which can identify possible toxicity of products used in research, no change was observed in the different groups. In mice fed with diet rich in fructose, this result was different, since fatty liver was found in the group fed with this sweetener, a result not found in the control group (fructose free) (Kopec et al., 2013).

Children and adolescents are major consumers of beverages containing added sugars. This consumption is positively associated with increased energy intake and is thought to be a significant contribution to the rapid increase in global obesity (Drewnowski and Bellisle, 2007). The choice of rats with five weeks for the experiment was because at that age the rats are weaned but not yet an adult, corresponding to adolescence (Sengupta, 2013).

Alternatively, to control excess carbohydrate sweeteners are used. Its use allows a marked reduction in the consumption of sugar and a significant decrease in calorie intake while maintaining the desirable palatability of food and non-alcoholic beverages (Vences-Mejia et al., 2006). So, the consumption of artificial sweeteners by children and adolescents is still little studied, and its adverse effects are not well known.

Conclusion

Artificial and natural sweeteners require a lot of

responsibility and study, since they are more and more present in the diet of people and determine which product brings more benefit. Fructose is known for its fatty and obesogenic effect, but these health risks should be determined through rigorous research and long-term and consider quantities typically ingested by people and not overestimated doses.

Based on the data obtained in this study, the amount of fructose used, probably, was not able to alter the biodistribution of radiopharmaceuticals, histopathological parameters and body changes were not lasting. But as liver enzymes were altered, investigations to assess liver damage for a longer period should be conducted. Sucralose showed no toxicity and no other harmful effects to the body in the investigated parameters in this study.

Conflict of Interests

The authors have not declared any conflict of interests.

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