Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.56822

Pitaya [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose] effect on glycemia and oxidative stress in aloxan-induced diabetic mice

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Abstract

Pitaya is a rich source of bioactive compounds, such as polyphenols and betalains. Moreover, these compounds has been associated with reduced risk of type 2 diabetes mellitus. The aim of this study is to evaluate the effect of this fruit on glycemia and oxidative stress in aloxan-induced diabetic mice. Thus, considering that In the in vivo assays, the mice were divided into 5 groups (n = 6): (a) healthy group treated with water; (b) diabetic mice treated with metformin at 200 mg/kg body weight diluted in water; (c) untreated diabetic mice; (d) diabetic mice treated with pitaya at 200 mg/kg and (e) diabetic mice treated with pitaya at 400 mg/kg of body weight diluted in water. The results shows that pitaya was able significantly to reduce blood glucose ($p < 0.05$) (200 mg/kg); significantly reduce cholesterol (200 and 400 mg/kg) and significantly increase HDL-c (400 mg/kg) levels. In the oxidative stress experiment, Malondialdehyde levels in the liver were significantly reduced ($p < 0.05$) in the groups treated with pitaya, when compared to the other groups, suggesting lower lipid peroxidation. The consumption of pitaya reduced the blood glucose and cholesterol and increase the HDL, in addition, the lipid peroxidation - which is common in diabetic patients, was reduced.

Keywords: diabetes *mellitus*; pitaya; *Hylocereus polyrhizus*; oxidative stress.

Practical Application: Effect of pitaya concentrations on glycemia and oxidative stress

1 Introduction

Diabetes is a metabolic disorder characterized by chronic hyperglycemia (Ayua et al., 2021) and is one of the most common causes of death in the world (Takım, 2021).

Between 1980 and 2014, there was a global increase in diabetes cases, from 108 to 422 million, with a higher incidence in low- and middle-income nations (World Health Organization, 2020). It still stands out, a huge expenditure by the government, affected individuals and their families on diabetes treatment (International Diabetes Federation, 2019). Therefore, there is a growing interest in decreasing and controlling diabetes and its effects.

Currently, for the treatment of diabetes, medical and nutritional approaches are made. For nutritional treatment, diets that help in the low production of postprandial glucose in the blood are recommended, while medical treatment has used drugs such as metformin, thiazolidinediones, meglitinides, miglitol, among other medications. However, most of these drugs used have side effects such as diarrhea, hypoglycemia, weight gain, flatulence, stomach distension and are not recommended for people with liver disease (Maideen, 2019; Wang et al., 2019).

There has been growing interest in recent years in the beneficial effects of plant-based diets for the prevention of chronic non-communicable diseases (Gao et al., 2021). There are a large number of studies suggesting dietary intake of polyphenols, especially flavonoids, associated with reduced risk of type 2 diabetes mellitus (DM2) (Guasch-Ferré et al., 2017; Guo et al., 2019; Zhou et al., 2018). These studies shows an antioxidant and anti-inflammatory effects of these compounds (cellular and tissue level), which influence glucose metabolism through several mechanisms (Gao et al., 2021). Thus, it is known that the consumption of fruits and vegetables can reduce the risk of chronic degenerative diseases such as heart disease and type II diabetes, due to the presence of secondary metabolites such as phenolic compounds and betalains (Montiel-Sánchez et al., 2021).

Pitaya has also been considered a rich source of bioactive compounds. Pitaya or dragon fruit comprises a group of exotic cactus species of the genus Hylocereus (Paśko et al., 2021) and is originally from the tropical and subtropical Americas. The most commercialized species are *Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose (Angonese et al., 2021), characterized

Received 19 June, 2022
Accepted 12 Dec., 2022
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by pulp and peel of intense red color. Recent research suggests that pitaya exibihit biological properties, including antioxidant, hypocholesterolemic (Holanda et al., 2021), cytotoxic (Paśko et al., 2021; Luo et al., 2014), antimicrobial, prebiotic (Choo et al., 2016) and anxiolytic activities (Lira et al., 2020). It is known that dragon fruit has several bioactive compounds, such as betalains, polyphenols and flavonoids (Arivalagan et al., 2021). Several of these compounds have shown protective effects in experimental diabetes by enhancing the activity of antioxidant enzymes (Sharma et al., 2021). Thus, the aim of this study was to investigate the effect of pitaya on glycemia and oxidative stress in aloxan-induced diabetic mice

2 Materials and methods

2.1 Plant material

Pitaya [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose] was obtained from Frutacor, which is located in Vale do Jaguaribe - CE (05º 53' 26" S; 38º 37' 19" W). The fruits were washed, sanitized, and processed in Embrapa Agroindústria Tropical (Fortaleza-CE). Thus, the pitaya was processed using a pulp-finisher (ITAMETAL/BONINA 0.25 DF) equipped with sieves of different meshes (2.5 and 0.8 mm) to separate the pulp (containing seeds) and the peel, which was discarded. In the end, the pulp and seed (edible portion) was obtained, frozen at −20 °C, lyophilized (LIOTOP LP 510) and tritured using and a mortar/pestle. This lyophilized material was used in all the experiments.

2.2 Pitaya characterization

The moisture, protein, and ash contents were determined, respectively, using the Association of Official Analytical Chemists (2005) methods no. 934.01, 984.13, and 923.03. The lipids were determined by analyzing the ethereal extract using a ANKOM model XT15 high-temperature extractor, using the Am 5-04 method proposed by of the American Oil Chemists' Society (Association of Official Analytical Chemists, 2005). The total amount of carbohydrates was calculated using the following Equation 1

$$\textbf{Difference} = 100 - \left(\%\ \textbf{water} + \%\ \textbf{protein} + \%\ \textbf{lipids} + \%\ \textbf{ash}\right) \quad (1)$$

The insoluble (FDI) and soluble (SDF) fractions of the food dietary fiber were determined according to AOAC method 991.43 (Association of Official Analytical Chemists, 2005) using an ANKOM Automatic Nutrient TDF analyzer (Ankon Technology Corporation). The total dietary fiber (TDF) was calculated as the sum of the insoluble and soluble fractions.

2.3 Pancreatic α-amylase inhibition assay

The pancreatic α-amylase inhibition assay was adapted from Sudha et al. (2011). The assay was performed in a microtiter plate based on the starch- iodine test where absorbance was read at 620 nm in a microplate reader, and 5 mg/mL of pitaya was used. The control reaction represented 100% of the enzyme activity did not contain any lyophilized pulp from the pitaya. The inhibition assay was performed using the chromogenic DNSA (3,5- dinitrosalicylic acid) method.

2.4 Animals and treatments

Thirty mice (*Mus musculus*) Swiss, adult, female, with an average weight of 25-30 g and age of 8-12 weeks were used. They were kept in collective cages, at a controlled temperature of 22 ± 2 °C, in light-dark cycles of 12/12 h for twenty eight days. The animals received water and feed ad libitum.

The mice were distributed into 5 groups (n = 6), which were called: SAUD (healthy group): treated with water (0.2 mL of water); MET 200: diabetic mice treated with metformin at 200 mg/kg body weight diluted in water; DNT: untreated diabetic mice; PIT 200: diabetic mice treated with pitaya at 200 mg/kg of body weight diluted in water; PIT 400: diabetic mice treated with lyophilized pitaya at 400 mg/kg of body weight diluted in water. Dosages were adjusted according to the weight of each animal, with daily preparation. The groups received the pitaya, via gavage, according to the adapted protocol of Barbosa et al. (2013).

All protocols described in this research were submitted to the Animal Research Ethics Committee of the State University of Ceara, under protocol nº 7255099/2018, according to the precepts of law nº 11,794, of October 8, 2008, of Decree No. 6,899, of July 15, 2009, and with the rules issued by the National Council for the Control of Animal Experimentation.

2.5 Diabetes induction

Diabetes induction was performed through a single injection of alloxane monohydrate (Sigam® - St. Louis, MO) at a dose of 150 mg/kg of animal weight. Alloxane was dissolved in a sterile saline solution (0.9% NaCl) and administered intraperitoneally. The animals were fasted for 12 hours before alloxane injection [adapted from Vareda (2013)]. After a period of 7 days, the mice were again fasted for eight hours for blood collection through the retroorbital plexus and subsequent determination of blood glucose. Animals with blood glucose equal to or greater than 200 mg/dL were considered diabetic (Aragão et al., 2010). Healthy animals, in order to suffer the same stress as alloxane-induced mice, received intraperitoneally the same volume of saline solution.

2.6 Biochemistry determinations

Blood samples were collected from the orbital plexus of mice, fasted for 4 h, in two moments: after the induction period (0 days) and at the end of the treatment period (28 days). The samples were centrifuged immediately after collection (11000 rpm for 3 min and 28 °C), the supernatant was removed and the samples were again centrifuged at 11000 rpm for only 1 min (28 °C). The serum was separated for analysis, which was subjected to standardized techniques of commercial kits based on kinetic, enzymatic and colorimetric methods (Bioclin/Quibasa, Minas Gerais, Brazil) in a spectrophotometer with a multimode ELISA microplate reader (BioTek, Winooski, USA), according to adaptation by Arruda et al. (2017). The parameters analyzed were glycemia, creatinine, aspartate aminotransferase (AST),

alanine aminotransferase (ALT), albumin, triglycerides, urea, total cholesterol and HDL cholesterol.

The serum atherogenic index (AI) is a measure of the extent of atherosclerotic lesions based on serum lipids. It was determined in all groups. The atherogenic index is calculated by the formula IA = CT/HDL (Balzan et al., 2013).

Determination of lipid peroxidation

Liver samples (up to 150 mg) were cut and then homogenized in ice-cold 50 mM phosphate buffer, pH 7.4 to give 10% homogenate. Lipid peroxidation was determined by measuring thiobarbituric acid reactive species (TBARS) through Malondialdehyde (MDA), the results were expressed in nmol/g of wet tissue. The method used was described by Ohkawa et al. (1979).

Determination of non-protein sulfide groups (GSH)

Reduced glutathione (GSH) was determined according to the method described by Sedlak & Lindsay (1968). Aliquots (2 mL) of liver tissue homogenate were mixed with 1.6 mL of distilled water and 0.4 mL of 50% (w/v) trichloroacetic acid (TCA) in glass tubes and centrifuged at 3000 rpm for 15 min. The supernatants (2 mL) were then mixed with 4 mL of Tris buffer (40 mM, pH 8.9) and 5,5'-dithiobis (2-nitrobenzoic acid) (10 mM DTNB) was added. After the lesions were evaluated by mixing reactions, their absorbance at 412 nm was measured within 5 min of the addition of DTNB against the blank without homogenate. The GSH concentration (μg/g of wet tissue) was calculated from a standard curve constructed using different GSH standard concentrations.

3 Statistical analysis

For biochemical analysis and lipid peroxidation and the determination of non-protein sulfide groups (GSH) measurement, the data were expressed as mean ± standard error of the mean (EPM). One-Way Analysis of Variance (ANOVA) was used to analyze the significance of the differences between the animals in the groups, followed by the Tukey test, with a significance level of $p < 0.05$. The statistical comparisons, creation and editing of the graphs were performed using the GraphPad Prism version 5.0 program.

4 Results and discussion

4.1 Diabetes

The chemical and physico-chemical composition of pitaya (pulp and seeds) is shown in Table 1. Results from the total carbohydrates and fibers (total, soluble and insoluble) were 11.03% ± 0.12 for carbohydrates, and 13.36% ± 0.45 for total fiber, soluble and insoluble, respectively. This is in accordance with Santos et al. (2020), that shows that the pitaya pulp was mainly composed of carbohydrates, mostly consisting of dietary fiber. As expected, the protein levels was low, and lipid (mainly concentrated in the seed) shows 0.69% levels. Santos et al. (2020) shows that the predominant saturated fatty acids (FAs) in pitaya seed oil were palmitic, stearic, arachidic, and meristic acids, representing around 22% of the total FAs. Up to 86% of the total FAs identified in seed oil consisted of unsaturated FAs, and linoleic and oleic acids were the majority compounds found in the seeds.

After pitaya characterization, the in vivo assays were performed. The results of the biochemical parameters shows that pitaya was able to reduce blood glucose (200 mg/kg); reduce cholesterol (200 and 400 mg/kg) and increase HDL-c (400 mg/kg) in aloxan-induced diabetic mice (see Figure 1 and Table 2).

For glucose results, it is observed that the group treated with pitaya at a dose of 200 mg/kg significantly decreased glycemia, compared to the untreated group and metformin. Several authors suggest that betalains, phenolic compounds (quercetin, for instance) and oligossacharides may exert a beneficial effect on glycemia. Thus, these compounds may be related to the decrease of glucose blood levels after pitaya treatment, since it is a rich source of these

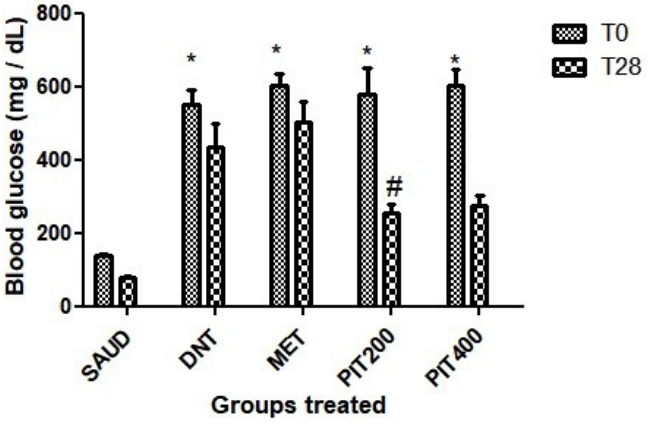

Figure 1. Effect of pitaya on bood glucose levels. SAUD: healthy group; DNT: untreated group; MET: Metformin; PIT200 and PIT 400: pitaya at a dose of 200 and 400 mg/kg/day, respectively. Data are expressed as mean ± standard error of the mean of 6 mice and each group. To analyze the significance of the differences between the groups, we used the analysis of variance (ANOVA) followed by Tukey's comparison test, with *$p < 0.05$ *versus* SAUD group; #$p < 0.05$ *versus* DNT group.

Table 1. Effect of pitaya and metformin on the wet weight of diabetes-induced *swiss* mice organs.

	GROUPS				
	SAUD	DNT	MET	PIT 200	PIT 400
Liver	3.91 ± 0.27	4.84 ± 0.23*	5.08 ± 0.16*	4.91 ± 0.12*	4.73 ± 0.09
Kidney	1.15 ± 0.02	1.26 ± 0.07	1.47 ± 0.09*	1.10 ± 0.04	1.13 ± 0.05
Pancreas	0.16 ± 0.04	0.12 ± 0.02	0.21 ± 0.01	0.30 ± 0.05#	0.31 ± 0.03#
Spleen	0.27 ± 0.01	0.37 ± 0.04	0.28 ± 0.01	0.34 ± 0.02	0.32 ± 0.02

SAUD: healthy group; DNT: untreated group; MET: Metformin; PIT200 and PIT 400: pitaya at a dose of 200 and 400 mg/kg/day, respectively. Relative weight of liver; Relative weight of kidneys; Relative weight of the pancreas; Relative weight of the spleen (28 days of treatment). Values given as mean ± standard error of 6 mice per group. To analyze the significant difference between groups, analysis of variance (ANOVA) was used, followed by a comparison Tukey test. *$p < 0.05$ *versus* SAUD group. #$p < 0.05$ *versus* DNT group.

Table 2. Effect of pitaya on biochemical parameters of diabetes-induced *swiss* mice.

	GROUPS				
Day	SAUD	DNT	MET	PIT200	PIT400
Cholesterol (mg/dL)					
0	68.27 ± 4.63	73.84 ± 4.76	49.13 ± 3.88*#	66.49 ± 5.52	57.48 ± 3.90
28	62.07 ± 1.64	67.31 ± 1.77	58.55 ± 6.00	49.27 ± 0.99#	53.01 ± 3.66#
HDL-C (mg/dL)					
0	99.01 ± 2.74	80.22 ± 4.96	76.94 ± 1.99	98.19 ± 19.11	87.53 ± 13.78
28	99.47 ± 2.95*	47.40 ± 9.01	88.98 ± 8.46#	66.38 ± 8.18*	86.46 ± 1.42#
Triglycerides (mg/dL)					
0	198.10 ± 16.75	373.10 ± 36.16*	313.90 ± 45.26	355.40 ± 26.32*	346.90 ± 51.73
28	145.50 ± 9.10	240.60 ± 33.02*	184.80 ± 16.06	230.40 ± 27.04	263.70 ± 15.19*
ALT (U/L)					
0	13.34 ± 0.44	23.66 ± 1.99	26.27 ± 1.96	15.72 ± 1.37	16.13 ± 1.70
28	35.96 ± 1.19	46.20 ± 2.61	35.76 ± 0.86	21.97 ± 4.08*#	20.69 ± 2.43*#
AST (U/L)					
0	20.09 ± 1.06	33.93 ± 3.97*	23.87 ± 1.86	21.08 ± 3.01#	20.23 ± 0.85#
28	20.33 ± 1.54	31.74 ± 4.80*	18.43 ± 1.01#	13.43 ± 1.13#	13.09 ± 1.00#
Creatinine (mg/dL)					
0	1.02 ± 0.18	1.71 ± 0.10*	1.71 ± 0.20*	1.22 ± 0.12	1.35 ± 0.12
28	0.93 ± 0.11	1.65 ± 0.15*	0.89 ± 0.090#	0.54 ± 0.046#	0.61 ± 0.06#
Urea (mg/dL)					
0	51.72 ± 2.54	90.44 ± 8.28*	81.71 ± 4.53	100.6 ± 5.53*	99.17 ± 12.32*
28	36.25 ±1.02	73.98 ± 2.25*	76.61 ± 7.41*	71.13 ± 2.35*	66.55 ± 6.07*
Albumin (mg/dL)					
0	2.84 ± 0.04	3.13 ± 0.12	3.21 ± 0.12	2.63 ± 0.09#	2.49 ± 0.04#
28	2.69 ± 0.04	2.58 ± 0.06	2.67 ± 0.03	2.42 ± 0.03*	2.19 ± 0.04*#

SAUD: healthy group; DNT: untreated group; MET: Metformin; PIT200 and PIT 400: pitaya at a dose of 200 and 400 mg/kg/day, respectively; ALT: alanine aminotransferase; AST: aspartate aminotransferase. Values given as mean ± standard error of 6 mice per group. To analyze the significant difference between groups, analysis of variance (ANOVA) was used, followed by a comparison Tukey test, *$p < 0.05$ *versus* SAUD group. #$p < 0.05$ *versus* DNT group.

bioactive compounds, as demonstrated by our group recently (Lira et al., 2020; Holanda et al., 2021). Lugo-Radillo et al. (2012) shows that the betanidin significantly reduces blood glucose levels in BALB/c mice fed with an atherogenic diet, in addition Sadowska-Bartosz & Bartosz (2021) which also showed that betalains reduce blood glucose and Wootton-Beard et al. (2014) shows the effect of a beetroot juice with high neobetanin content on the early-phase insulin response in healthy volunteers. For phenolic compounds, Mahesh & Menon (2004) and Vessal et al. (2003) demonstrate that quercetin allievates oxidative stress and has antidiabetic effects in streptozocin-induced diabetic rats; and Kobori et al. (2011) shows that a chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57/BL6J mice. Furthermore, Kim et al. (2011) shows that quercetin attenuates fasting and postprandial hyperglycemia in animal models of diabetes mellitus. Finally, oligosaccharides of pitaya were identified by Wichienchot et al. (2010) and their prebiotic properties were demonstrated by in vitro assays.

The positive effect shows in the animals glicemia levels can be reinforced by the alpha amylase inhibition assay, where a 100% inhibition of this enzyme was observed. These results shows that one of the mechanisms of blood glucose reduction that pitaya promotes is through the reduction of digestion and absorption of carbohydrates, which delays the release of glucose to the body, consequently reducing blood glucose (Kazeem et al., 2013; Gautam et al., 2013; Ademiluyi & Oboh, 2013; Zheng et al., 2020). Therefore, as discussed previously, these effects could be related to the quercetin (Najafian, 2015) and the oligossacharides (Wichienchot et al., 2010), both constituints of pitaya. Due to the negative side effects of synthetic products for the treatment of diabetes (Najafian, 2015) this result of the pitaya is relevant, showing this fruit as an hypoglycemic potential.

There are some common abnormalities in diabetic individuals, such as hypertriglyceridemia and hypercholesterolemia (Hosseini et al., 2015). However, in this study, hypercholesterolemia was not observed in the group treated with pitaya. Total cholesterol decrease in the groups treated with pitaya at doses of 200 and 400 mg/kg ($p < 0.05$) when compared to the untreated group, which was not observed when comparing with metformin. On the other hand, there was an increase in the HDL level in the 400 mg/kg group ($p < 0.05$). Considering that cholesterol and HDL are a risk criteria for coronary heart disease (Bock, 2012; Chang & Robidoux, 2017), the pitaya can help to reverse the cholesterol pathways. These results are in agreement with the literature, since Sani et al. (2009) shows the effectiveness of *Hylocereus polyrhizus* extract in decreasing serum lipids and liver MDA-TBAR level in hypercholesterolemic rats. Moreover, Wroblewska et al. (2011) shows the effect of beetroot crisps (a importante souce of betalains) in dyslipidaemic diets of rats. And, Jeong et al. (2012) shows that quercetin was able to ameliorates

hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice.

In addition, it is observed in this study that there was a decrease in the atherogenicity index, mainly in the group treated with pitaya at doses of 200 and 400 mg/kg and in the group treated with metformin, as shows in Figure 2 ($p < 0.05$). Atherosclerosis is determined by chronic inflammation in the arterial wall, resulting from a series of specific cellular responses (Koenen et al., 2009). Atheromas are the formation of plaques or streaks fats that are originated by the large amount of inflammatory cells, fibrous elements and lipids that are deposited in the artery walls and that normally cause the obstruction of these plaques. When these plaques rupture, they usually trigger thrombus formation. Some factors may contribute to this thrombus formation, such as arterial hypertension, diabetes mellitus and hypercholesterolemia (Gottlieb et al., 2005; Stein et al., 2002; Hulthe & Fagerberg, 2002). It is known that pitaya (*Hylocereus polyrhizus*) has some constituents such as quercetin and betalain, which may influence these results. Quercetin plays an important role in protecting blood vessels, reducing the risk of heart disease and atherosclerosis (Bischoff, 2008). Studies showed that betanidine and betanin were able to inhibit LOX (Kanner et al., 2001; Silva et al., 2022). Betanin and betaxanthines were also able to inhibit COX-1 and COX-2 (Reddy et al., 2005). The anti-inflammatory activity of betalains can be justified due to their action against cellular mediators of inflammation, such as the intercellular adhesion molecule-1 (ICAM-1) (Gentile et al., 2004; Silva et al., 2022). All these facts justify the reduction in the atherogenicity index presented by pitaya. In relation to hypertriglyceridemia, common in diabetes, the lyophilized pitaya pulp at a dose of 200 mg/kg ($p < 0.05$) reduced the level of triglycerides when compared to the untreated animal, although this difference is not statistically significant. On the other hand, the group treated at the dose of 400 mg/kg failed to reduce the level of plasma triglycerides. This effect is possibly due to the impairment of the triglyceride hydrolysis process caused by alloxane, which may favor the mobilization of lipids into the plasma, as shown by Oliveira et al. (2002). To assess renal function, urea and creatinine were measured. Creatinine did not differ between the groups treated with pitaya and the healthy group, only differed from the untreated animal. This may suggest that this untreated group had diabetic nephropathy, since creatinine is a widely used marker for the non-invasive measurement of glomerular filtration rate (GFR) (Parreira, 2008). The urea dosage in the present study increased, both in the untreated animal groups and in the groups treated with pitaya, this may have been due to several factors, such as renal failure or a nephropathy, which is common in diabetics (Cardenas, 2005), or due to the inducer causing this change. Urea is an indirect and approximate measure of kidney function and glomerular filtration rate (if there is normal liver function). It is partially excreted by the kidneys (Oh, 2012). The high level of urea in plasma is related to the level of protein catabolism, which in diabetics increases when there is hypoinsulinemia, impaired renal function and the hydration status, which is altered because of hyperglycemia (Wyckoff & Abrahamson, 2005; Motta, 2003), as it was observed at the end of the experiment that there was an increase in water consumption. Pitaya failed to reduce the damage caused by the inducing drug, but this does not mean that it is toxic, as studies show that it did not show toxicity (Hor et al., 2012; Luo et al., 2014) and that it was also seen by previous studies carried out by our team (Lira et al., 2020).

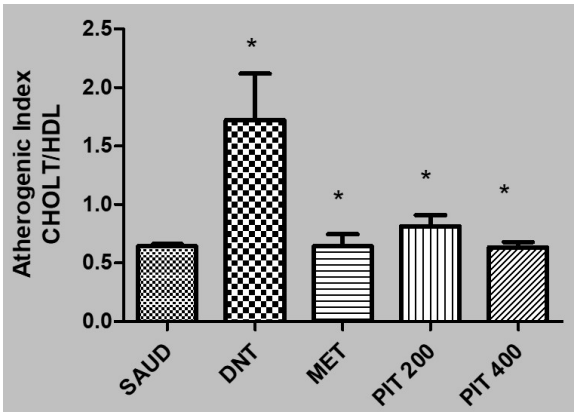

Figure 2. Effect of pitaya on the atherogenicity index. SAUD: healthy group; DNT: untreated groups; MET: Metformin; PIT200 and PIT 400: pitaya at a dose of 200 and 400 mg/kg/day, respectively. Data are expressed as mean ± standard error of the mean of 6 mice in each group. Analyze the significance of the differences between the groups, the analysis of variance (ANOVA) was used, followed by the Tukey test, with *$p < 0.05$ *versus* DNT group.

The pitaya 200 and 400 mg/kg failed to reverse the loss of albumin in diabetic mice when compared to control groups, a common situation in diabetics (Wang et al., 2019). This reduction may be associated with non-enzymatic glycations caused by the hyperglycemic state of diabetes mellitus, or it may also be justified by microalbuminuria caused by diabetic nephropathy presented by diabetic mice (Vilar et al., 2001). Diabetes also has consequences on liver tissue. The enzyme ALT (alanine amino transferase) did not differ from the healthy group. In relation to AST (aspartate amino transferase), the dose used in this study of 200 and 400 mg/kg ($p < 0.05$) of the pitaya pulp reduced the level of this enzyme when compared to the control groups. It is suggested with this result that there was no liver damage, since there is no increase in these two enzymes and that AST is an enzyme present in higher concentrations in skeletal muscle cells and in hepatocytes (Meyer et al., 1995; Dzoyem et al., 2014). As they are intracellular enzymes, serum levels of AST and ALT are generally very low. Therefore, any significant tissue alteration gives rise to high levels of serum transaminase (Rosa, 2009).

Regarding the weight of the organs of mice that was performed, it can be seen in the results presented in Table 2 that there was an increase in liver weight, in the untreated, metformin and PLP (pitaya pulp)-treated groups at a concentration of 200 mg/kg ($p < 0.05$) this effect could probably have been caused due to the diabetes inducer alloxane that often causes this damage (Mahmoud & Al-Salahy, 2004; Can et al., 2005; Dornas et al., 2006). The liver is an organ that performs numerous vital functions, highlighting the regulation of the metabolism of various nutrients, protein synthesis and other molecules, hormonal degradation and inactivation and excretion of drugs and toxins, essential purposes for body homeostasis (Nunes & Moreira, 2007), so it is not interesting to change this body. The kidneys perform numerous functions,

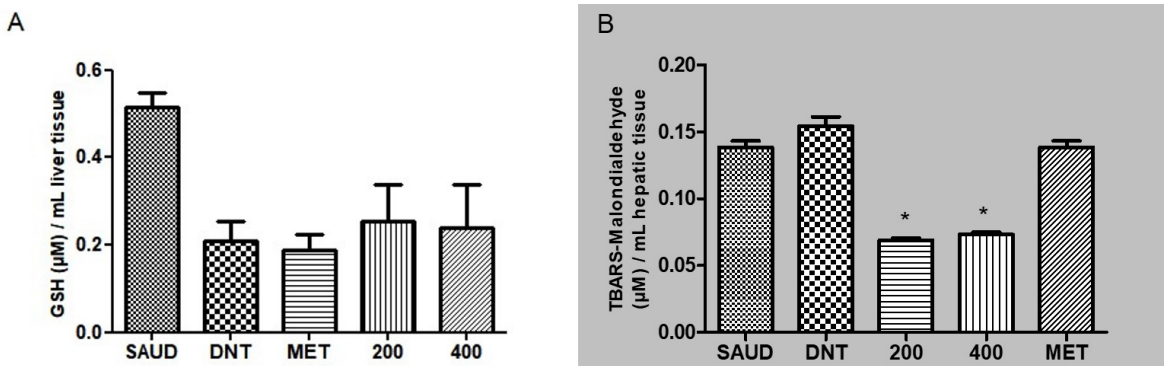

Figure 3. Effect of pitaya and metformin on GSH and lipid peroxidation. SAUD: healthy group; DNT: untreated groups; MET: Metformin; PIT200 and PIT 400: pitaya at a dose of 200 and 400 mg/kg/day, respectively. A- GSH; B- TBARS. Data are expressed as mean ± standard error of the mean of 6 mice in each group. To analyze the significance of the differences between the groups, we used the analysis of variance (ANOVA) followed by the Tukey test, with *$p < 0.05$ *versus* DNT group.

such as filtration, reabsorption, homeostasis, metabolic and endocrine functions (Sodré et al., 2007). The kidneys did not show a significant difference in weight in the untreated animal groups and in the groups treated with pitaya, there was only a difference in the group treated with meformin, as there was an increase in them. This renal change may indicate a chronic progressive nephropathy (Greaves, 2012). In relation to the spleen, there was no difference between any of the analyzed groups. The results in this study show that there was no weight change in the pancreas of animals fed with the lyophilized pulp of pitaya, when compared with the pancreas of healthy animals and standard drug. On the other hand, there is a significant reduction in the pancreas of untreated sick animals. It is suggested that these changes may have been caused by alloxane, as reported in other studies (Dunn et al., 1943; Junod et al., 1967).

4.2 Level of Reactive Oxygen Species (ROS) in the liver

And finally, in the oxidative stress levels experiment, MDA (malondialdehyde) levels in the liver were significantly reduced in the groups treated with the lyophilized pulp, when compared to the other groups, suggesting lower lipid peroxidation (Figure 3B) ($p < 0.05$) (Yigit et al., 2018). This result is relevant since the reduction of MDA suggests a decrease in liver oxidative stress (ROS) relieving inflammation (Rio et al., 2005). It was also observed that animals treated with PLP showed a tendency to increase GSH levels (Figure 3A). In this sense, it can be suggested that PLP helps to reduce oxidative stress and cardiovascular diseases (Sies, 1999). The increase in oxidative stress is very common in diabetics, causing several complications (Ighodaro, 2018), soon the importance of consuming antioxidant foods is realized. Flavonoids, which are present in pitaya, play a role in gene regulation of several molecules and enzymes involved in atherogenesis, promoting a decrease in lipid peroxidation (Kaliora et al., 2006). Betanin and betanidine present in pitaya are also efficient inhibitors of lipid peroxidation (Kanner et al., 2001).

5 Conclusion

The consumption of pitaya promoted a reduction in blood glucose and cholesterol and an increase in HDL, in addition reduce the lipid peroxidation, which is common in diabetic patients. These results are possibly associated with constituents present in pitaya, such as betalain, phenolic compounds (such as quercetin), and oligossacharides, with prebiotic properties. However, more studies are needed to elucidate the mechanism of action of the pitaya to help in an alternative treatment for diabetes, in order to encourage its regular consumption.

Conflict of interest

The authors declare that they do not have any conflict of interest regarding the publication of this work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

MIF, Guedes; Lira, SM: Dionísio, AP; Holanda, MO; Marques, CG; Correa, LC; Santos, GBM; Dantas, JB; Costa, JTG; Lima, CLS; Zocolo, GJ; Silva, JYG, Silva, G Contributed to the methodological aspects of the present study, as well as helped in the interpretation of the data and the assembly of the graphs. Guedes, MIF; Lira, SM, Dionísio, AP; Holanda, MO; Marques, CG; Correa, LC; Santos, GBM, Dantas, JB; Costa, JTG; Lima, CLS; Zocolo, GJ, contributed to the Biochemistry determinations, animal experimental. Guedes, MIF contributed in the experimental conduction and advices, aid in the cost to obtain materials and reagents necessary for the analyzes of the present study.

Acknowledgements

We thank the Biotechnology Post-graduation Program (RENORBIO) focal point of State University of Ceará and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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