ZOOLOGY

Effects of sucrose-rich diets and pterostilbene on oxidative status and intrinsic innervation of the jejunum in rats

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ABSTRACT. Obesity is a chronic disease related to gastrointestinal disorders. Pterostilbene has already demonstrated beneficial activity. This study aimed to evaluate the effects of a sucrose-rich diet and pterostilbene on oxidative status, intestinal wall, and myenteric neurons of the jejunum. Wistar rats comprised the groups Control (C), Sucrose (S), Control+Pterostilbene (CP), and Sucrose+Pterostilbene (SP). For 180 days, C and CP received water and chow *ad libitum*, while S and SP groups received chow and water plus 40% sucrose *ad libitum*. Pterostilbene (40 mg Kg⁻¹) was administered daily in the final 40 days. Jejunal samples were collected to assess oxidative status, wall morphometry, goblet cells, and myenteric neurons. Pterostilbene increased lipid peroxidation in the CP group. Catalase activity increased in groups S and CP. The myenteric neuron number did not change by sucrose; however, it reduced their number in CP. SP animals had a higher density of neurons than CP. Groups S, CP, and SP showed hypertrophy of the neuronal cell body. There was maintenance of the intestinal wall and goblet cells. In conclusion, pterostilbene has a possible pro-oxidant and cytotoxic activity on jejunal myenteric neurons of rats on a standard diet; however, when combined with 40% sucrose-added water, it minimized the oxidative stress, evidencing a neuroprotection activity.

Keywords: Antioxidant; free radicals; goblet cells; intestinal wall; myenteric neurons; sucrose solution.

Introduction

Foods and drinks rich in glycids are being increasingly consumed by the world population. These products, with high caloric content, when ingested in excess and associated with a sedentary lifestyle, are responsible, in large part, for the obesity epidemic observed in the 21st century (Stenvinkel, 2015). Considered a growing chronic and epidemic disease, obesity is a risk factor for other chronic diseases, such as type 2 diabetes (Reddy & Rao, 2006; Caixàs, Albert, Capel, & Rigla, 2014), cardiovascular diseases (Sloboda et al., 2014), non-alcoholic fatty liver disease (Reddy & Rao, 2006; Manne & Saab, 2014; Lima et al., 2016; Souza Cruz et al., 2020), and gastrointestinal tract (GIT) disorders (Xing & Chen, 2004; Mushref & Srinivasan, 2013).

Excessive food intake leads cells to a state of oxidative stress, which occurs when the production of free radicals exceeds their antioxidant capacity (Vincent, Russell, Low, & Feldman, 2004) and can damage important components of the GIT, with changes in morphology of intestine layers (Scoaris et al., 2010; Soares, Beraldi, Ferreira, Bazotte, & Buttow, 2015), in addition to impair the intrinsic intestinal innervation (Soares et al., 2006; Beraldi et al., 2015; Stenkamp-Strahm et al., 2015; Anitha et al., 2016; Bhattarai et al., 2016; Reichardt et al., 2017), responsible for the control of motility, secretion, and blood flow of the digestive tract (Wade & Cowen, 2004).

Sucrose (industrial sugar) is among the most consumed carbohydrates and is composed of glucose and fructose molecules (Sloboda et al., 2014). Several studies have shown the harmful effects of fructose consumption, which is often pointed out as the harmful component of sucrose (Manne & Saab, 2014; Mamikutty, Thent, & Suhaimi, 2015; Lima et al., 2016). Several natural compounds are being studied to minimize the damage and complications caused by a poor diet. Vazquez Prieto et al. (2015) revealed that treatment with quercetin and catechin was able to mitigate inflammation, oxidative stress, and insulin resistance in rats fed sucrose.

In this scenario, pterostilbene, a synthetic resveratrol analogue, has shown interesting results. The treatment of animals with pterostilbene was able to reduce blood glucose (McCormack & McFadden 2013), improve the lipid profile (Rimando, Nagmani, Feller, & Vokoyama, 2005), inhibit the production of reactive oxygen species (Riche et al., 2013; Wang et al., 2016; He et al., 2018; Zhang et al., 2020), increase neuronal survival in brain (Hou et al., 2014; Zhou et al., 2015; Li et al., 2016), and in spinal cord (He et al., 2018), reduce neuronal apoptosis (Zhou et al., 2015; Fu, Yang, Wei, & Li, 2016), stimulate neurogenesis (Yang et al., 2019), inhibit colon carcinogenesis (Chiou et al., 2010, Sun et al., 2016), alleviate intestinal damage (Zhang et al., 2020), in addition to modifying the intestinal microbiota (Etxeberria et al., 2017). However, specifically on the behavior of intrinsic intestinal morphology and innervation, no studies have been found in our search that evaluate the efficacy and safety of pterostilbene in rats on a diet rich in sucrose.

Thus, this research can reveal the harmful effects of sucrose intake on the intestine, besides the potential benefits of an alternative therapy to minimize the gastrointestinal damage associated with obesity. Therefore, this study aimed to evaluate the effects of a solution rich in sucrose and the treatment with pterostilbene on oxidative status, intestinal wall, and myenteric neurons of the jejunum in rats.

Material and Methods

Animals and treatment

Twenty male Wistar rats (*Rattus novergicus*), approximately 60 days of age, were obtained from the Animal Facility of the *Universidade Estadual de Maringá* (UEM). Animals were housed and treated in the Animal Facility of the *Universidade Estadual do Norte do Paraná* (UENP) in individual boxes under controlled temperature and light (25°C; 12h/12h light/dark). The animals remained for seven days without being handled for previous acclimatization.

Animals were distributed into groups: Control (C), Sucrose (S), Control + Pterostilbene (CP), and Sucrose + Pterostilbene (SP). For 180 days, groups C and CP received filtered water and commercial chow *ad libitum*; groups S and SP received commercial chow and water plus 40% sucrose *ad libitum*. Pterostilbene (Tokyo Chemical Industry Co. LTD., Tokyo, Japan) at a dose of 40 mg Kg^{-1} (Satheesh & Pari, 2008) was administered daily via gavage for 40 consecutive days, starting on day 141 of the experiment (de Morais et al., 2021). The pterostilbene solution was prepared daily, diluted in 0.5% methylcellulose containing 0.2% Tween 80 (de Morais et al., 2021). Groups C and S received saline solution as a placebo during this period.

The establishment of the model and nutritional status of the animals was preliminarily performed at the Laboratory of Metabolic Biochemistry/UENP (Souza Cruz et al., 2020; de Morais et al., 2021).

Tissue collection

At the end of the experimental period, animals were euthanized by an overdose of barbiturates followed by cardiac puncture for midline laparotomy (Souza Cruz et al., 2020). Samples of the jejunum were collected, measured, and sent for the assessment of oxidative status, morphometric analysis of the intestinal wall, determination of the number of goblet cells, and morpho-quantitative study of myenteric neurons.

All the procedures described in this work were approved by the Animal Research Ethics Committee of the *Universidade Estadual do Norte do Paraná*, protocol 05/2017, and agree with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA).

Assessment of oxidative status

Approximately 200 mg jejunum was homogenized in L-Beader 3®, with ultrapure zirconium spheres, in 0.1M sodium phosphate buffer, pH 7.0 (1: 1 w/v). Homogenates were centrifuged at 10,000 rpm for 10 minutes in a refrigerated centrifuge at −4°C (MSE - HAWK 15/05®), with the supernatants analyzed for total proteins by colorimetric method in a spectrophotometer (CELM SB-190®) with the Gold Analisa® kit, as well as for oxidative status.

As a marker of oxidative stress, the concentration of malondialdehyde (MDA) was quantified in a protocol adapted from Buege and Aust (Buege & Aust, 1978) by testing thiobarbituric acid reactive substances (TBARS) by mixing 1 mL sample with 2 mL reagent color (0.25N HCl, 15% trichloroacetic acid and 0.67% beta-mercaptopyrimidine). The mixture was heated to 95°C for 15 minutes, cooled, and centrifuged (4,500 rpm, 10 minutes) to obtain the supernatant and later read on a spectrophotometer (FEMTO 800XI®) at 535 nm.

The activity of the catalase enzyme (EC 1.11.1.6) in the homogenate was determined in a spectrophotometer (FEMTO 800XI®) at 240 nm by monitoring the variation in hydrogen peroxide absorption, according to Peixoto, Cambraia, Sant´ana, Mosquim, and Moreira (1999). For the test, 10 µL protein extract was added to 990 µL of a 50 mM potassium phosphate buffer, pH 7.0, supplemented with hydrogen peroxide at a final concentration of 12.5 mM. The variation in absorbance (ΔE) was calculated in an 80 seconds interval and the enzyme activity was calculated using the molar extinction coefficient $\varepsilon = 39.4 \text{mM}^{-1}.\text{cm}^{-1}$. The specific activity ($\mu\text{Kat}.\mu\text{g}.\text{Prot}^{-1}$) of the catalase took into account the concentration of soluble protein in the test.

Histological processing

Samples of the jejunum were opened at the mesenteric border, washed with saline, fixed in Bouin for 6 hours, stored in 70% alcohol, dehydrated in a series of increasing concentrations of ethanol (80%, 90%, and 100%), cleared in xylol and embedded in paraffin to obtain 5 µm thick semi-serial sections in a Leica RM 2145® microtome.

Morphometric analysis of the intestinal wall

Histological sections were stained by the Hematoxylin-Eosin (H.E.) method for the morphometric evaluation of the intestinal wall. We measured 100 points of the total wall, tunica mucosa, muscularis externa, height of villi, and longitudinally oriented crypts in 10 histological sections/animal from images captured at 100X total magnification under a Motic BA410® light microscope coupled to a Moticam 2300® camera and analyzed with the Image-Pro Plus® 4.5 software. The results were expressed in µm.

Histochemistry and quantitative analysis of goblet cells

Histological sections were stained by Periodic Acid-Schiff (P.A.S.) to show the goblet cells producing neutral mucins. Slides were analyzed under a Motic BA410® light microscope at 400X total magnification; 2,500 cells were counted per animal to obtain the percentage of marked/unmarked cells in the jejunal mucosa. The goblet cell index was calculated by the number of marked cells x 100/total number of cells counted.

Disclosure of myenteric neurons

The total population of myenteric neurons in the jejunum was evidenced by Giemsa staining based on methylene blue (Barbosa, 1978). For staining, the collected samples were washed in 0.9% saline solution and later stored in Giemsa fixative. After fixation, whole-mount preparations of the muscle layer were obtained by removing the tunica mucosa and submucosa from jejunum samples by dissection under a Motic SMZ 168® stereo microscope with trans-illumination. Membrane preparations obtained were stained for 24 hours with the Giemsa method in 0.1N Sorensen phosphate buffer (pH 6.9) at room temperature and protected from light. Subsequently, they were dehydrated in a series of increasing concentrations of ethanol (80%, 90%, and 100%), cleared in xylol, and mounted between a slide and a coverslip using Permount® synthetic resin.

Morpho-quantitative neuronal analysis

For quantification of the neuronal population, neuronal cell bodies were counted in 60 microscopic fields per animal, per muscularis externa preparations, in images captured randomly in the intermediate and antimesenteric regions of the intestinal circumference (30 images/region), using a 40X objective. The total area analyzed was 5.69 mm²/animal. The neuronal density was expressed as the number of neurons per cm².

For neuronal morphometric analysis, from the captured images, the area (μm^2) of cell bodies and nuclei of 100 randomly selected neurons/animal (50 neurons/region) of the jejunum of each animal was measured, making a total of 500 cells/nuclei per group. The area of the cytoplasm was estimated by subtracting the area of the nucleus from the area of the cell body. Only neurons with an intact cell body, and nuclear profile, and well-defined boundaries were analyzed.

Images were captured using a Zeiss Axio Scope A1® microscope coupled to an AxioCam Erc 5s® camera, and the morpho-quantitative analysis was performed with the analysis software Image-Pro Plus® 4.5 - Media Cybernetics.

Statistical analysis

Numerical data were tested for normality by the Kolmogorov-Smirnov test. All data were parametric and tested by Analysis of Variance (one-way ANOVA) followed by Tukey's test, with the results expressed as mean

± standard error, with a significance level of 5% (p <0.05). The GraphPad Prism® 7.0 software (GraphPad Software, Inc.) was used for statistical analysis.

Results and discussion

Experimental model

Experimental models of obesity induced by a hypercaloric and/or hyperlipidemic diet (Scoaris et al., 2010; Reichardt et al., 2017) are conditions for the study of the enteric nervous system since such pathology can compromise the gastrointestinal tract (Mushref & Srinivasan, 2013, Beraldi et al., 2015). The use of natural compounds as a form of treatment (Vazquez Prieto et al., 2015) has been increasingly highlighted; however, morpho-quantitative analyses of enteric neurons of animals receiving sucrose solution, treated or not with pterostilbene currently lack literature.

The diet rich in sucrose (40%) caused a significant increase in body weight, total energy ingested, visceral, retroperitoneal, and epididymal adipose tissue, total fat, as well as the adiposity index, and lower feed intake. Treatment with pterostilbene caused an increase in feed intake in the CP group compared to the C and SP groups. However, body weight, total fat, and total energy intake continued to be higher in groups that received sucrose, with no difference between groups S and SP. BMI, Lee index, specific weight gain, and blood glucose did not differ significantly between groups (Souza Cruz et al., 2020; de Morais et al., 2021).

Oxidative status

Pterostilbene increased lipid peroxidation in animals fed a standard diet (CP) without changing the levels of MDA in rats receiving the solution added with sucrose (SP). Group S did not differ statistically from group C, although MDA levels were higher (p> 0.05) (Figure 1a). Catalase activity significantly increased in groups S (96.97%) and CP (204.38%) compared to C. The use of pterostilbene combined with a sucrose-rich diet (SP group) promoted no significant difference from group S but rather (p <0.05) from group CP (Figure 1b). Our findings are contrary to the antioxidant action of pterostilbene reported in the intestine (Sun et al., 2016), brain (Zhou et al., 2015; Yang et al., 2016), and nerve cell culture (Fu et al., 2016; Wang et al.; 2016; Yang et al., 2016; Yang et al., 2017; He et al., 2018).
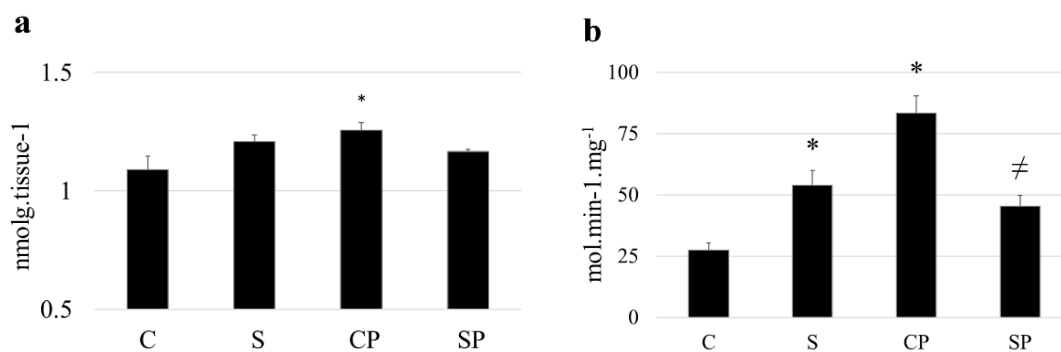
Figure 1. Malondialdehyde (MDA) levels (a) and catalase activity (b) in the jejunum of rats in the control (C), sucrose (S), control + pterostilbene, (CP) and sucrose + pterostilbene (SP) groups. Mean ± standard error (n=5). (*) p <0.05 compared to C; (≠) p<0.05 compared to CP. One-way ANOVA followed by Tukey's post-test.

According to Sun et al. (2016), pterostilbene (20 mg Kg^{-1} dose) can inhibit oxidative stress and increase the antioxidant status in the jejunum of Wistar rats with injury caused by intestinal ischemia/reperfusion. Zhou et al. (2015) reported that oral pterostilbene attenuates acute cerebral ischemia-reperfusion injuries in mice in a dose- and time-dependent manner. The study tested pterostilbene at doses of 2.5, 10, 40, and 80 mg Kg^{-1}, and found that the dose of 10 mg Kg^{-1} has a neuroprotective effect with suppression of apoptosis and oxidative stress in neurons. In vitro tests on primary spinal cord neurons have shown that doses of 40, 60, and 80 μM pterostilbene increase cytotoxicity and decrease cell viability, while doses 1, 5, 10, and 20 μM do not influence this parameter. However, the 20 μM dose reduced the production of reactive oxygen species (He et al., 2018).

The assessment of oxidative status allows us to infer there was (1) an increase in the level of free radicals with the sucrose-rich solution (group S) since a higher activity of the enzyme catalase was found; (2) an increase in the level of free radicals in animals fed a standard diet and pterostilbene (CP group), with an

increase in MDA and catalase probably due to the antioxidant activity of this compound, which in excess, can act as a tissue pro-oxidant (Bjelakovic, Nikolova, Gluud, Simonetti, & Gluud, 2012); and (3) an antioxidant action of pterostilbene when combined with a diet containing sucrose (SP group), as this ensured less catalase activity compared to the treated control animals (CP group) but without changing the enzymatic activity when comparing SP to S and C groups.
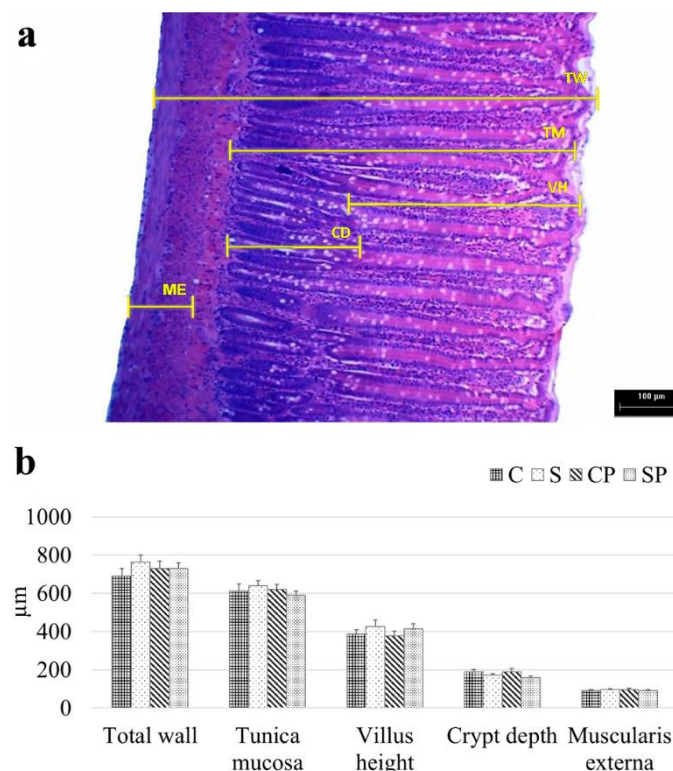
Intestinal morphometry

Biometric parameters of the small intestine (length, circumference, and area) showed no significant changes (p> 0.05) by the use of sucrose and pterostilbene (Table 1). Scoaris et al. (2010) observed an increase in the length of the small intestine in animals fed a cafeteria diet, while Soares et al. (2015) reported a reduction in this parameter in animals receiving a high-fat diet. Such results differ from those found herein and demonstrate the diets tested promote different effects on the small intestines of experimental animals.

Table 1. Biometric parameters of the small intestine of rats in the control (C), sucrose (S), control + pterostilbene (CP) and sucrose + pterostilbene (SP) groups. Mean ± standard error (n=5).

Parameters	C	S	CP	SP
Length (cm)	99.7 ± 6.06	96 ± 3.75	104.5 ± 1.24	103.9 ± 4.52
Circumference (cm)	0.94 ± 0.06	0.90 ± 0.04	0.94 ± 0.05	0.96 ± 0.06
Area (cm^2)	96.74 ± 8.79	86.78 ± 6.62	100.1 ± 5.49	100.3 ± 10.01

Non-significant difference (p>0.05). One-way ANOVA followed by Tukey's post-test.

In the morphometric analysis of the intestinal wall, no significant changes were detected in the layers, demonstrating maintenance in the total wall, tunica mucosa, height of villi, depth of crypts, and the thickness of the muscularis externa in all groups studied (Figure 2). Similar results were reported in mice jejunum (Hamaoka & Kusunoki, 1986) and rat ileum (Soares, Schoffen, De Gouveia, & Natali, 2006) in conditions of obesity induced by monosodium glutamate. These data indicate that the absorptive capacity and motility of the intestine were possibly maintained in these two study models.
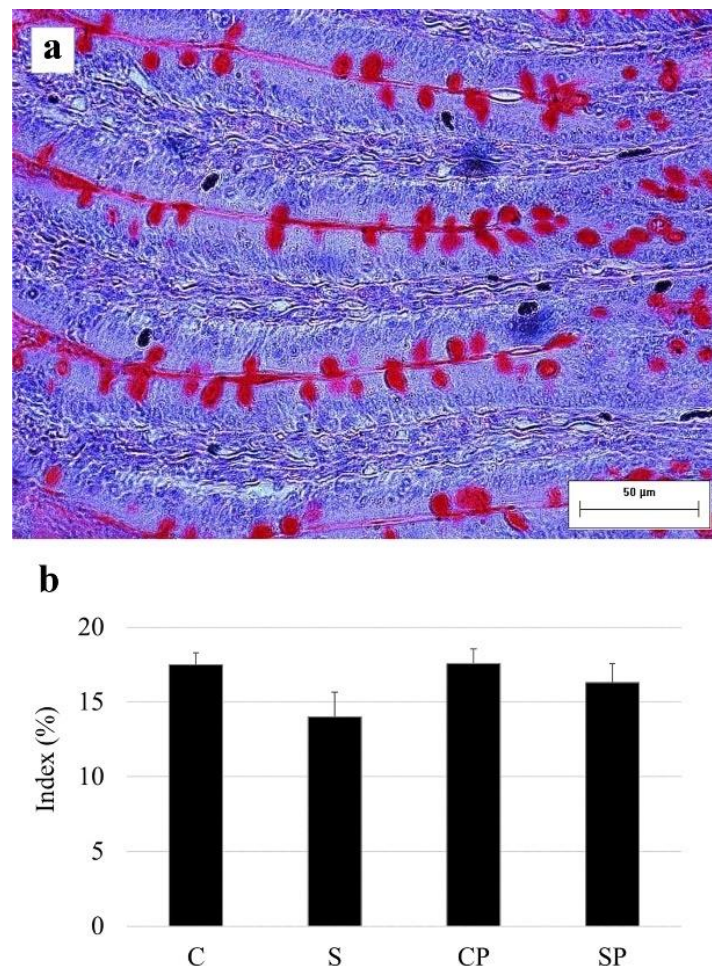

Figure 2. (a) Photomicrograph of the jejunum showing total wall (TW), tunica mucosa (TM), villus height (VH), crypt depth (CD), and muscularis externa (ME). H&E staining. (Total magnification: 100X). (b) Morphometric parameters of the intestinal wall of the control (C), sucrose (S), control + pterostilbene (CP) and sucrose + pterostilbene (SP) groups. Mean ± standard error (n=5). Non-significant difference (p>0.05). One-way ANOVA followed by Tukey's post-test.

Divergences were found comparing our findings to the results obtained with the cafeteria diet and the high-fat diet. The cafeteria diet caused an increase in villi height, crypt depth, and the thickness of the total wall of the jejunum in rats, suggesting that the type of diet was responsible for the hypertrophic effect observed in the intestinal villi and crypts of obese animals (Scoaris et al., 2010). In the hyperlipidemic diet, there was an increase in the same parameters but with a reduction in the crypt depth of the jejunum in mice (Soares et al., 2015). Such changes indicate adaptations related to digestibility (Santoro et al., 2006), and the consistency of the hyperlipidemic diet (Pluske, Hampson, & Williams, 1997), or increased food retention in the jejunum caused by decreased motility in these segments (Soares et al., 2015).

Goblet cell population

Regarding goblet cells, which produce neutral mucins (P.A.S.), there was no change in the percentage of this cell type despite the downward trend (p> 0.05) in the number of these cells in group S compared to the other groups (Figure 3). The reduction in the number of goblet cells in the jejunum of rats and mice fed a cafeteria diet and a high-fat diet have already been recorded in the literature (Scoaris et al., 2010; Soares et al., 2015). According to Soares et al. (2015), the decrease in the population of goblet cells is associated with changes in the permeability of the intestinal barrier and inflammation. Changes in the intestinal wall are accompanied by changes in specific cell populations (Soares et al., 2015); thus, the maintenance observed in the jejunum mucosa of the animals in this study justifies the conservation of the population of goblet cells, ensuring protection and lubrication of the intestinal epithelium surface.


Figure 3. (a) Photomicrograph of the jejunum with goblet cells stained in red by the P.A.S. technique (Total magnification: 400X). (b) Index of goblet cells in the jejunal mucosa of the control (C), sucrose (S), control + pterostilbene (CP) and sucrose + pterostilbene (SP) groups. Mean ± standard error (n=5). Non-significant difference (p>0.05). One-way ANOVA followed by Tukey's post-test.

Intrinsic intestinal innervation

When counting by regions (intermediate and antimesenteric), there were no significant differences between them. For this reason, the results were presented together.

The sucrose solution did not modify the number of neurons in the jejunum myenteric plexus (group S) in relation to the control animals. However, a significant reduction by 31.83% in the number of these cells was found in the control group treated with pterostilbene (CP) (Figure 4a). This result indicates cytotoxicity of pterostilbene at a dose of 40 mg kg^{-1} to myenteric neurons of the jejunum in rats fed a standard diet *ad libitum*.
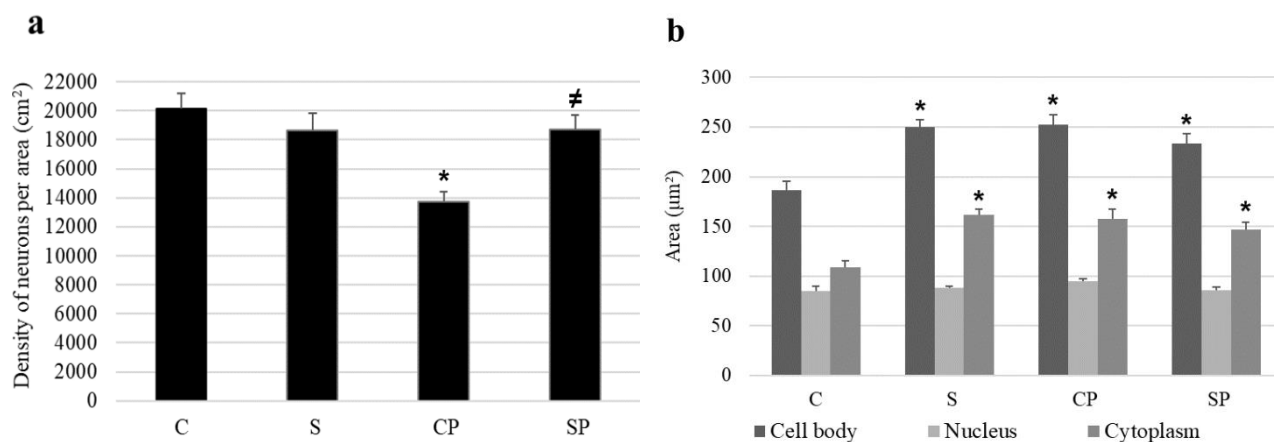
Figure 4. Mean number (a) and cell body area, nucleus area, and cytoplasm area of myenteric neurons (b) in the jejunum of rats in the control (C), sucrose (S), control + pterostilbene (CP) and sucrose + pterostilbene (SP) groups. Mean values were obtained by counting neurons in 60 microscopic fields per animal, with a total area of 5.69 mm²/animal. Mean ± standard error (n=5). (*) $p < 0.05$ compared to C; (≠) $p < 0.05$ compared to CP. One-way ANOVA followed by Tukey's post-test.

Pterostilbene showed cytotoxic and antiproliferative effects in gastric cancer cells (Pan, Chang, Badmaev, Nagabhushanam, Ho, 2007) and human colon cells treated in vitro (Wawszczyk, Kapral, Hollek, & Węglarz, 2014). Kong et al. (2016) observed that pterostilbene was able to prevent carcinogenesis in human lymphoma B cells through the ability to inhibit cell viability, generate reactive oxygen species, and induce apoptosis.

High-dose antioxidants can become pro-oxidants through interaction with other compounds (Bjelakovic et al., 2012). Herbert (1996) reports that all antioxidants, including vitamin antioxidants, are redox agents (reduction-oxidation), protecting against free radicals in some circumstances and promoting the generation of free radicals in others. Studies have revealed pro-oxidant effects of antioxidant vitamins, vitamin E (Burkitt & Milne, 1996) and C (Podmore et al., 1998), under certain circumstances. Antioxidant and apoptotic activities of resveratrol have already been elucidated (Ahmad, Syed, Singh, & Hadi, 2005; Murias et al., 2005); however, their analogues showed better results in eliminating free radicals and inhibiting tumor cell growth (Murias et al., 2004).

In this sense, pterostilbene may have acted as a pro-oxidant and triggered apoptosis (Kong et al., 2016), presenting a neuronal cytotoxic effect. Nonetheless, studies on the cytotoxicity of pterostilbene in myenteric neurons must be conducted.

Contrary to the CP group, animals that received sucrose and pterostilbene (SP group) showed a higher density of myenteric neurons than rats that were given a standard diet plus this compound but without statistically differing from the control group (Figure 4a), demonstrating a neuroprotective capacity of pterostilbene, as it prevented neuronal loss in the jejunum. The neuroprotective activity of pterostilbene has already been observed in cerebral ischemia (Zhou et al., 2015; Li et al., 2016; Wang et al., 2016; Yang et al., 2016) and in Alzheimer's disease (Fu et al., 2016). This finding implies that the diet can modulate the action (cytotoxic or protective) of pterostilbene on myenteric neurons.

The increase in MDA and catalase activity observed in this study suggests that oxidative stress may be responsible for the neuronal loss recorded in animals fed a standard diet and pterostilbene. The lower activity of catalase and MDA ($p > 0.05$) seen in SP rats shows a possible antioxidant activity of pterostilbene, which would be able to minimize the oxidative damage caused by the elevation of free radicals with the intake of sucrose, which justifies the neuroprotection verified in SP. These data corroborate that the dose of 40 mg kg^{-1} pterostilbene was cytotoxic to myenteric neurons of the jejunum of the control animals. On the other hand, the combination of pterostilbene with a 40% sucrose diet presented a neuroprotective action.

The effect of pterostilbene is dependent on dose and time (Zhou et al., 2015; He et al., 2018), as well as on the components ingested in the diet, as reported in this research. Da Silva et al. (2021) showed that consumption of sucrose solution attenuated liver injury in a murine model of chronic cirrhosis. Similarly, our

study reaffirms these findings and suggests that the diet component is a relevant factor that can modulate cellular responses to natural compounds. Future studies are required to confirm this hypothesis.

Regarding morphometric analysis, groups S, CP, and SP showed an increase in the area of the cell body and neuronal cytoplasm compared to group C (Figure 4b). Control animals supplemented with pterostilbene showed compensatory cell hypertrophy due to neuronal loss, as the remaining neurons had to respond to functional tissue demand and increase their activity (Phillips, Kieffer, & Powley, 2003). In turn, in the animals receiving 40% sucrose solution, the observed hypertrophy is possibly due to a metabolic/oxidative impairment in neurons caused by excessive sucrose consumption.

Although not statistically significant, pterostilbene showed a tendency to minimize neuronal hypertrophy in the SP group compared to the S group (Figure 4b). This indicates the capacity of pterostilbene to preserve or diminish probable damage to myenteric neurons of animals that ingested 40% sucrose daily. There was no significant change in the area of the neuronal nucleus in rats on diets enriched with sucrose and supplemented or not with pterostilbene, demonstrating the functional maintenance of nuclear activity.

Figure 5 illustrates the myenteric neurons of the jejunum of rats from the four study groups of this research.
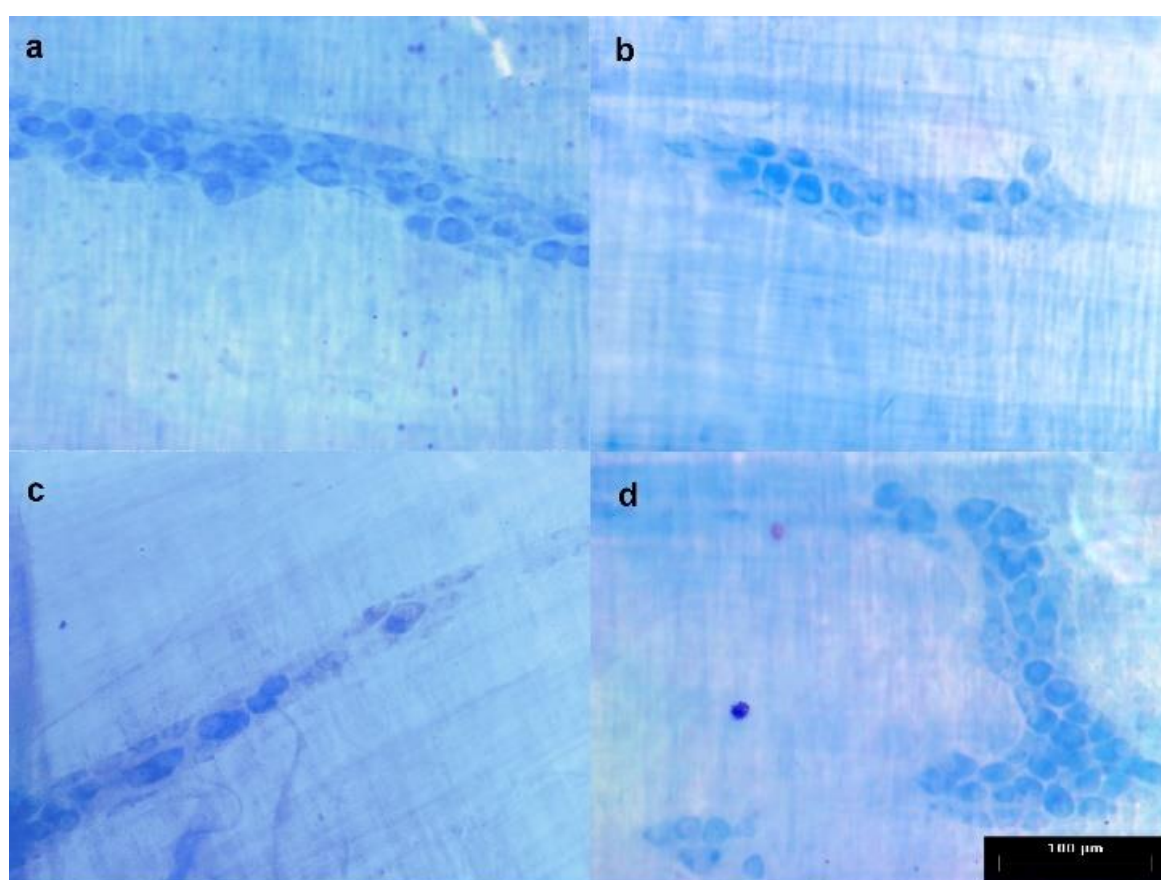
Figure 5. Photomicrograph of the myenteric neurons evidenced by Giemsa staining in the jejunum of rats in the control (a), sucrose (b), control + pterostilbene (c), and sucrose + pterostilbene (d) groups (Total magnification: 400X).

Conclusion

In conclusion, pterostilbene (40 mg Kg^{-1}) has a possible pro-oxidant and cytotoxic activity on the myenteric neurons of the jejunum in rats fed a standard diet *ad libitum*. However, combined with a 40% sucrose solution diet, it minimized oxidative stress and played a neuroprotective role, without interfering with the morpho-quantitative aspects of the intestinal wall. Further studies testing doses higher and lower than 40 mg Kg^{-1} of pterostilbene must be developed.

Acknowledgments

Laboratory.

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