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CELLULAR AND MOLECULAR BIOLOGY

Ora-pro-nobis (*Pereskia aculeata*) supplementation promotes increased longevity associated with improved antioxidant status in *Drosophila melanogaster*

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Abstract: This study evaluated the effects of ora-pro-nobis (*Pereskia aculeate*) flour supplementation on the *in vivo* basal antioxidant system of *Drosophila melanogaster*, and its action on the neural modulation observed by the enzyme acetylcholinesterase (AChE). The flies will receive a standard diet with flour incorporated at 5, 10 and 20% for 7 days. There was no change in food consumption, body weight, protein thiol levels and negative geotaxis behavior. The flies showed a reduction in the basal production of reactive species at concentrations of 10 and 20%, while there was a reduction in lipid peroxidation and catalase activity at all concentrations, accompanied by an increase in the levels of non-protein thiols. Superoxide dismutase activity was reduced in the 5 and 20% groups, while the reduction of superoxide anion in the 10% group may have contributed to the increase in longevity also in the 10% group. Longevity increased in groups 5 and 10%. The open field test may be related to the reduction in AChE activity in the 5, 10 and 20% groups. In general, the data show that supplementation with ora-pro-nobis flour at the concentrations tested did not cause toxicity and modulated the cholinergic system, demonstrating a therapeutic potential.

Key words: *Pereskia aculeata, Drosophila melanogaster*, antioxidants, oxidative stress, acetylcholinesterase, longevity.

INTRODUCTION

Pereskia aculeata, popularly called ora-pro-nobis, is a plant from the *Cactaceae* family native to South and Central America and tropical America, with a natural distribution on the Northeast to the South of Brazil (Sharif et al. 2013), where it is also known as poor man's meat, due to its high protein content (Sharif et al. 2013, Takeiti et al. 2009). According to the Ministry of Agriculture, Livestock and Supply, ora-pro-nobis is considered a non-conventional food plant, that is, it has food potential, but is not usually used in the daily diet of the population in general, although its consumption may be common in certain regions (Cruz et al. 2021).

Ora-pro-nobis leaves, part of the plant predominantly used as food, are consumed fresh, in salads or drinks (de Almeida & Corrêa 2012). In addition to the fresh form, ora-pro-nobis leaves can be in the form of flour or isolated extract (e.g. mucilage), thus improving and maintaining the nutritional content and technofunctional properties (Nogueira Silva et al. 2023). When dehydrated and transformed into flour, it can be incorporated into various food products (Manetta et al. 2023, Sommer et

al. 2022). Flour made from ora-pro-nobis leaves stands out for its high concentration of fiber and proteins, with reported values between 12 and 55% fiber and between 15 and 28% protein, calcium, magnesium, zinc and iron, in addition to significant levels of vitamins A, C and folic acid (de Almeida & Corrêa 2012, Sommer et al. 2022, Takeiti et al. 2009). The beneficial health effects attributed to ora-pro-nobis are related to its great antioxidant potential resulting from the high levels of phenolic compounds (Hoff et al. 2022, Mattila & Hellström 2007). Given the observed medicinal effects and nutritional composition, ora-pro-nobis is a technologically promising plant, and research that encourages innovation and the development of new products is in vogue (Sá et al. 2024).

Experimental data show that plant-based diets may be related to integrated antioxidant and anti-inflammatory mechanisms, associated with a phytochemical matrix present in these foods. Synthetic antioxidants have been increasingly used, but excessive or prolonged use can cause harm to health such as liver damage and carcinomas, as shown in *in vivo* studies (Ramalho & Jorge 2006). In this sense, the inclusion of ingredients with natural antioxidants, containing phenolic compounds, with the capacity to promote the stability of free radicals is an interesting alternative (Ghasemzadeh & Ghasemzadeh 2011). The high levels of iron and bioactive compounds present in ora-pro-nobis reinforce the plant's ability to be used as an ingredient in food enrichment and predict its potential as a nutraceutical source, which can be applied as a complementary alternative to combat iron deficiency and eliminate radicals (Teixeira et al. 2023).

Bioactive compounds complement endogenous antioxidant defenses, preventing the body from entering a state of oxidative stress, which is often associated with premature aging and the emergence of various chronic diseases (Oliveira et al. 2017). The body naturally produces a variety of reactive species (RS), and oxidative stress occurs when there is an imbalance between the production of free radicals and antioxidant defenses. Therefore, antioxidant substances can be applied as therapeutic strategies, preventing or reducing oxidative damage to DNA, proteins or lipids, through the neutralization of reactive oxygen species, thus preventing the onset or progression of pathologies (de Souza et al. 2019). Furthermore, among the effects already observed, there is a gap when evaluating ora-pro-nobis at a neurological and behavioral level. Therefore, carrying out research that highlights the antioxidant potential of ora-pro-nobis can serve as support for the bioprospecting of new functional food and pharmaceutical products.

In this sense, demonstrating *in vivo* the action of a given substance more reliably conveys the real effect it will have on the body. For this, we used an alternative model of *Drosophila melanogaster*, popularly known as fruit fly, which has been widely used in toxicological experimental protocols (Musachio et al. 2023), and in the investigation of many common cellular development processes in higher eukaryotes, including humans (Adams et al. 2000). There have been no previous experimental investigations using the *Drosophila melanogaster* model to evaluate the effect of ora-pro-nobis. As it is a well-established model in studies on oxidative stress and evaluation of natural compounds (Poetini et al. 2018), the model proved to be excellent for carrying out this research. Therefore, given this context, objective of this study was to evaluate the effects of ora-pro-nobis flour supplementation on the basal antioxidant system *in vivo*, and its action on the neural modulation observed by the enzyme acetylcholinesterase (AChE).

MATERIALS AND METHODS

Preparation of raw material

Obtaining plant leaves

From an ora-pro-nobis plant cultivated in the urban area of the municipality of Itaqui/RS, Brazil (latitude 29° 9' 9'' South, longitude 56° 33' 3'' West), with the aid of pruning shears, branches containing leaves were cut, placed in polyethylene plastic bags and immediately sent to the Chemistry laboratory of the Universidade Federal do Pampa – Itaqui.

Drying and elaboration of flour

In the laboratory, the leaves were manually separated from the branches, washed under running water and heated in a microwave (ME28S, Electrolux) at high power for two minutes, placed in aluminum dishes and dried in an oven with air circulation and renewal (SL 102/480, Solab) at 60 °C until they appear dry and brittle (about 16 hours). Subsequently, the dehydrated leaves were ground in a micromill (A11, IKA) to result in ora-pro-nobis flour, which was placed in a polyethylene terephthalate (PET) plastic pot and stored at -18 °C until preparation of experimental diets.

The flour obtained through this method, in a previous study by our research group, showed greater antioxidant properties when compared to other flours from the same plant, but obtained through different production methods (Arena et al. 2023). 100g of ora-pro-nobis flour used in this study has 4.72% moisture, 14.59% ash content, 5.53% lipids, 16.14% protein, 57.16% fiber food and 1.84 of digestible carbohydrates (Sommer et al. 2022).

Biological test

Drosophila melanogaster stock

Wild flies of the *Drosophila melanogaster* species (Harwich lineage), obtained from the Laboratory of Pharmacological and Toxicological Assessments Applied to Bioactive Molecules (LAFTAMBIO), from the Universidade Federal do Pampa - Itaqui, were used in this study. The flies were maintained in a controlled environment with a 12h/12h light/dark cycle, a temperature of 25 °C ± 1, and 60% humidity. The flies were fed a standard diet consisting of corn flour (76.59%), sugar (7.23%), wheat germ (8.51%), salt (0.43%), powdered milk (7.23%) and antifungal Nipagin® (0.08%).

Experimental design

Flies aged 1 to 4 days old, of both sexes, were used, divided into four groups, with 50 flies each. For seven days, the flies were exposed to the following experimental diets:

- Control (5 g of standard diet, without adding ora-pro-nobis flour);
- 5% (4.75 g of standard diet added with 0.25 g of ora-pro-nobis flour);
- 10% (4.5 g of standard diet added with 0.5 g of ora-pro-nobis flour);
- 20% (4 g of standard diet added with 1 g of ora-pro-nobis flour).

After the period of exposure to the diets, the flies were used to perform *in vivo* (behavioral tests and survival and longevity*)* and *ex vivo* analyses (Figure 1). In the *ex vivo*, flies were cryoeuthanized (placed at -2°C for 10 min), homogenized and centrifuged according to the specific protocol for sample production, and the supernatant was collected to perform the measurements biochemical analyses.

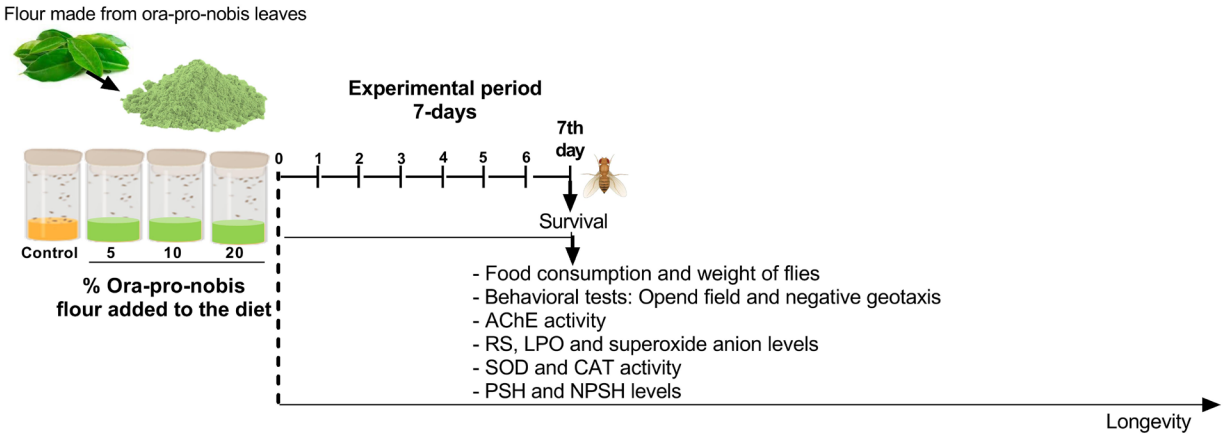

Figure 1. Schematic representation of the methodology.

In vivo analyzes

Consumption test

Food consumption was analyzed according to Lushchak et al. (2011), with modifications. For this analysis, 15 flies (representatives of each group) were used. After 30 minutes of fasting, the flies were exposed to experimental diets (containing different concentrations of ora-pro-nobis) with the addition of 0.5% non-brilliant blue FD&C. The flies remained exposed (feeding) for 2 h, and after this time they were removed from the medium and cryoeuthanized. The whole bodies of 15 flies (corresponding to each group) were homogenized in 200 µL of 20 mM HEPES, pH 7.5, and then placed in a centrifuge at 14.000 rpm for 15 minutes. Flies that fed on diets without the dye were used as blanks for optical density. The samples were read in the supernatant obtained, at a wavelength of 629 nm. For this analysis, five independent experiments (n=5) were used, and the results were expressed as a percentage in relation to the control group.

Body weight

Body weight was measured as described by Meichtry et al. (2020). The initial weight was obtained by weighing 50 flies before starting treatment. After the 7-day treatment, the surviving flies were weighed again to assess whether there was any change in weight. As the number of flies weighed at the end of the treatment was not the same as the initial one, a rule of three was used to correct the values. Five independent experiments were carried out (n=5), and the results were expressed in grams (g).

Survival and longevity

Survival (mortality rate) was assessed by counting the number of flies killed every 24 hours during seven days of exposure. Fly longevity was assessed as described by Farombi et al. (2018), 50 flies per group were added to their respective treatment bottles. Every two days, the flies were transferred to new vials containing the respective diets. In general, the flies are changed bottles every 7 days, however, it was necessary to change them every 2 days because, even adding Nipagin®, the proliferation of fungi occurred, from the third day onwards, in the diets containing ora-pro-nobis. The flies were exposed throughout their lives, and the number of dead flies was recorded every 24 hours. The observation carried out until the seventh day was used to draw the survival curve, then

longevity was assessed, with an initial protocol the same as that for survival, but the flies were counted daily until there were none left alive. In both tests, three independent experiments (n = 3) were carried out for each.

Open field test

For locomotor and exploratory activity, the open field test was performed as described by Connolly (1966), with modifications made by Musachio et al. (2020). Five flies from each group were used, totaling 20 flies, which were previously immobilized on ice, then transferred individually to a Petri dish divided into squares measuring 1 cm^2 each. After 2 minutes to recover from anesthesia and acclimatize to the arena, the number of crossings between the squares by each fly was evaluated during 60 seconds. The test was performed twice individually and the average of these data was calculated. For this analysis, five independent experiments (n=5) were used.

Negative geotaxis test

The negative geotaxis test was performed to analyze the fly's climbing ability, as described by Paula et al. (2012). In each test, five flies from each group were used, which were previously immobilized on ice and placed individually and vertically in a test tube with a diameter of 1.5 cm. After 10 minutes, the flies were gently shaken to descend to the bottom of the test tube and the time required for them to rise to the 8 cm mark on the wall of the test tube was timed. This test was repeated 5 times, with 60 second intervals. For this analysis, five independent experiments (n=5) were used. The results were analyzed according to the average time of each climb.

Ex vivo analyzes

AChE enzyme activity

According to the method described by Ellman et al. (1961), 10 whole fly bodies were homogenized in 400 µL of 20 mM hydroxyethyl piperazineethanesulfonic acid (HEPES) buffer at pH 7.0, and centrifuged at 10.000 rpm, for 10 min. For reading, 950 µL of MIX (8 mL of Kpi buffer, 1M pH 8.0 without EDTA, 6 mL of distilled water and 2 mL of DTNB), 50 µL of sample (supernatant) and 25 µL of acetylthiocholine 25 mM were placed in a cuvette. The reading was performed at a wavelength of 412 nm for 120 seconds. For this analysis, five independent experiments were used (n=5). Results were expressed as µmol AcSCh/h/mg protein.

Determination of superoxide dismutase (SOD) activity

According to the method proposed by Kostyuk & Potapovich (1989), 10 whole bodies of flies from each group were separated, which were homogenized with the addition of 500 µL of 20 mM HEPES buffer (pH 7.0) for 60 seconds. Soon after, the samples were centrifuged at 1000 rpm/10 minutes at 4 °C. For reading, 10 µL of diluted sample, 1 mL of MIX (50 mL of 0.025 M Kpi buffer/0.1 mM EDTA pH 10, 65 µL of TEMED) and 50 µL of quercetin were added to a cuvette. The results were expressed in terms of the amount of protein required for 50% inhibition of quercetin oxidation. Five independent experiments were performed (n=5). Enzymatic activity was expressed as U/mg of protein.

Determination of catalase activity (CAT)

Catalase activity was measured as described by Aebi (1987), with modifications. So, 10 whole bodies of flies from each group were separated and homogenized with the addition of 500 µL of 20 mM HEPES buffer (pH 7.0) for 60 seconds.

For reading, 30 µL of the supernatant and 2 mL of mix (10 mL Kpi buffer 0.25 M/EDTA 2.5 mM pH 7.0, 35 mL of water, 43 µL of H_2O_2 30% and 10 µL Triton X100) were placed in a cuvette. For this analysis, five independent experiments were carried out (n=5). Enzymatic activity was monitored for 2 minutes, at a wavelength of 240 nm and expressed as U/mg of protein.

Determination of lipid peroxidation by thiobarbituric acid reactive species (TBARS)

Lipid peroxidation (LPO) was analyzed by estimating thiobarbituric acid reactive species (TBARS) according to the method described by Ohkawa et al. (1979), with modifications. The 20 flies from each group were used, and their entire bodies were homogenized in 1.000 µL of 20 mM HEPES (pH 7.0), which were then centrifuged at 1.000 rpm/10 minutes at 4 °C. The supernatant was removed and added with thiobarbituric acid (0.8% TBA, pH 3.2), acetic acid buffer (20%, pH 3.4) and sodium sulfate (8.1% SDS). Afterwards, the samples were incubated for two hours at 95 °C and the absorbance was measured on a microplate reader at a wavelength of 532 nm. For this analysis, five independent experiments were carried out (n = 5). The values were normalized by protein concentration and expressed as nmol of malondialdehyde (MDA)/mg protein.

Content of protein thiols (PSH) and non-protein thiols (NPSH)

Non-protein thiols (NPSH) and protein thiols (PSH) determination was estimated as described by Ellman (1959). Briefly, twenty flies were homogenized in 350 µL of Tris buffer (pH 8.0) and centrifuged at 10.000 rpm for 5 min. For protein thiol (PSH) measurements, the supernatant was used (the pellet was reserved for later use) which was pipetted into the microplate, and then 5,5'-dithiobis 2-nitrobenzoic acid (DTNB), after waiting 15 minutes in room temperature protected from light and the reading was carried out on a plate reader at 412 nm. For non-protein thiol measurements of the above samples, the pellet was suspended in 0.5 M Tris/HCl buffer pH 8.0, the supernatant was removed and added to 5 mM DTNB and left for 15 minutes at room temperature protected from light and at reading was on the plate reader at 412 nm. PSH and NPSH were expressed as µm GSH/mg of tissue. Only the NPSH values were corrected for the value of the protein contained in the sample. For this analysis, five independent experiments were carried out (n=5).

Quantification of superoxide anion

As described by Morabito et al. (2010), 10 flies per group were homogenized in 500 µL of 20 mM HEPES buffer (pH 7.0) for 60 seconds. The homogenate was centrifuged at 2.000 rpm for 5 minutes. Aliquots of 90 µL of the supernatant were removed and transferred to a dark Eppendorf® (wrapped in aluminum foil), along with 10 µL of blue tetrazolium chloride (NBT), where they remained incubated at 37 °C for 3 hours. Afterwards, the samples were centrifuged again at 14.000 rpm. From the Eppendorf® 80 µL of the supernatant was discarded and 80 µL of DMSO was added. The samples were incubated at 37 °C for 20 minutes to dissolve the formazan crystals. The content was transferred to microplates, where reading was performed at a wavelength of 550 nm. For this analysis, five independent experiments (n=5) were carried out and the results were expressed as a percentage in relation to the control group.

Protein quantification

The analyzes of SOD, CAT, AChE activity, PSH and MDA quantification had the final results corrected by the protein value of the sample. For this, bovine serum (BSA) was used as standard protein, according to the method of Bradford

(1976). The technique consists of the interaction between the dye Coomassie brilliant blue BG-250 and portions of proteins that contain amino acids with basic or aromatic side chains. The interaction between the proteins and the BG-250 dye causes the dye's equilibrium to shift toward the anionic form, which is strongly absorbed at 595 nm.

Statistical analysis

Data were analyzed in the statistical software GraphPad Prism version 8 (San Diego, CA, USA). Data normality was verified by Shapiro-Wilk test and homoscedasticity was verified using the Bartlett test. One-way analysis of variance (ANOVA) followed by Tukey post hoc was used to analyze homoscedastic data with Gausean distribution. Kruskal-Wallis followed by Dunns post hoc were used for non-parametric or heteroscedastic data. All values were expressed as mean (s) ± standard error of the mean (SEM). The results were presented in bar graphs, showing the comparison of different concentrations of ora-pro-nobis to the control group. For survival and longevity curves, the Mantel-Cox log-rank test was used. In all analyzes the statistical difference was considered significant when $p < 0.05$.

RESULTS

Food consumption test and body weight

Initially, it was observed whether the flies were consuming the food containing different concentrations of ora-pro-nobis (Figure 2a). The flies demonstrated good acceptance of the addition of ora-pro-nobis flour to the diet at all concentrations (5, 10 and 20%), with no statistical difference between the treated groups and the control ($F_{(3.16)} = 1.754$; DF 19; $p = 0.5955$; $p = 0.9993$; $p = 0.7393$, respectively). Body weight was also evaluated (Figure 2b), and consequently no change in the weight of the flies was evident, thus there was no statistical difference between the groups added with ora-pro-nobis flour, 5, 10 and 20%, compared to the control group ($F_{(3.16)} = 1.754$; DF 19; $p = 0.9503$; $p = 0.7168$; $p = 0.6192$, respectively) .

Assessment of survival (seven days of treatment) and longevity

The survival of the flies was observed for seven days, that is, the total time of the treatment (Figure 3a). Flies exposed to diets containing 5 and 10% had a higher survival rate, while the group that received 20% ora-pro-nobis flour showed no statistical difference when compared to the control group (Long-rank (Mantel-Cox)

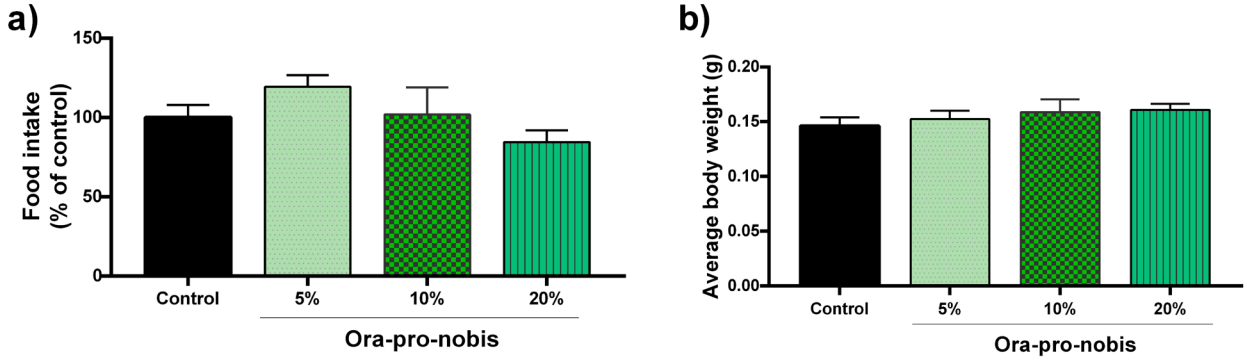

Figure 2. Assessment of food consumption (a) and verification of body weight (b) of *Drosophila melanogaster* exposed for seven days to diets containing the addition of 5, 10 and 20% ora-pro-nobis flour. The bar graphs express the comparison between different concentrations of ora-pro-nobis (5, 10 and 20%, separately), to the control group. The results are expressed as mean ± standard error of the mean (SEM).

DF = 3; p = 0.0369; p = 0.0015, respectively; p = 0.1397). In the test that evaluated the longevity of flies (Figure 3b), flies that consumed the diet containing 10% ora-pro-nobis flour lived had greater longevity, when compared to the control group (Long-rank (Mantel-Cox) DF= 3; p = 0.0019). While no statistical difference was observed between groups 5 and 20%, when compared to the control group (Long-rank (Mantel-Cox) DF = 3; p = 0.2115; p = 0.3865, respectively)

Behavioral tests and Acetylcholinesterase (AChE) activity

In evaluating the ability to move around and explore the environment, carried out using the open field test (Figure 4a), flies fed diets containing different concentrations of ora-pro-nobis (5, 10 and 20%) had greater movement within the arena, compared to the control group ($F_{(3.16)}$ = 5.027; DF = 19; p = 0.0232; p = 0.0234; p = 0.0398, respectively). However, in the negative geotaxis (Figure 4b) test there was no statistically significant difference between the groups with added ora-pro-nobis flour 5, 10 and 20%, in relation to the control ($F_{(3.16)}$ = 0.1117; DF = 19; p = 0.9575; p = 0.9900; p = 0.9999, respectively).

However, when evaluating the activity of the AChE enzyme (Figure 4c), a significant increase was found in all groups that consumed a diet added with ora-pro-nobis flour 5, 10 and 20%, when compared to the group control (Kruskal-Wallis statistic = 11.83; DF = 19; p = 0.0160; p = 0.0382; p = 0.0465, respectively).

Biomarkers of oxidative parameters

Quantification of RS, lipid peroxidation, superoxide anion levels, and enzymatic and non-enzymatic antioxidant defenses

Regarding the production of reactive species, flies exposed to diets containing concentrations of 10 and 20% ora-pro-nobis produced a smaller amount of reactive species when compared to the control group (Figure 5a) ($F_{(3.16)}$ = 1.754; DF = 19; p = 0.0012; p = 0.0005, respectively). No statistical difference was observed between the group that received 5% ora pro-nobis flour and the control group (p = 0.9626). Lipid peroxidation was also evaluated using the method of quantifying to thiobarbituric acid species reactive (TBARS), in this case malondialdehyde (MDA) (Figure 5b), and it was observed that, at all concentrations

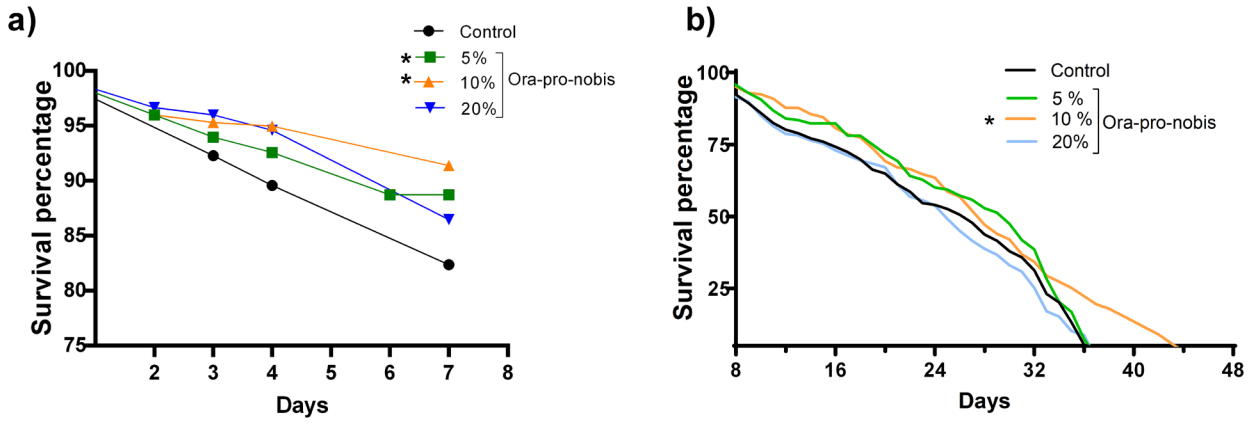

Figure 3. Assessment of survival of *Drosophila melanogaster* exposed for seven days to diets added with ora-pro-nobis flour (a) and longevity (flies observed until the end of life) (b). Survival and longevity curves were compared using the Mantel-Cox log-rank test. *Indicates significant statistical difference (p < 0.05) in relation to the control group.

of ora-pro-nobis (5, 10 and 20%), there was a decrease in MDA levels when compared to the control group ($F_{(3.16)}$ = 10.90; DF = 19; p = 0.026; p = 0.0040; p = 0.0004, respectively). When quantifying the levels of superoxide anion (Figure 5c), it was possible to identify that the organism of flies that received the diet containing 10% ora-pro-nobis flour produced less quantity compared to the control group ($F_{(3.16)}$ = 10.01; DF = 19; p = 0.0454). There was no statistical difference when comparing groups 5 and 20% to the control (p = 0.1387; p = 0.6704, respectively). As an enzymatic antioxidant defense, the activity of the SOD (Figure 5d) was evaluated, in which it was observed that flies exposed to concentrations of 5 and 20% ora-pro-nobis had reduced activity enzyme, compared to the control group ($F_{(3.16)}$ = 8.131; DF = 19; p = 0.0236 and p = 0.0016, respectively). There was no difference between the group that received 10% ora-pro-nobis flour and the control group (p = 0.5490). As for CAT (Figure 5e), flies exposed to all concentrations of ora-pro-nobis (5, 10 and 20%) showed a reduction in the activity of this enzyme when compared to the control group ($F_{(3.16)}$ = 8.381; DF = 19; p = 0.0025; p = 0.0099; p = 0.0040, respectively). The non-enzymatic antioxidant defenses observed in the study were the levels of protein, PSH (Figure 5f) and NPSH (Figure 5g). For these, there was no statistical

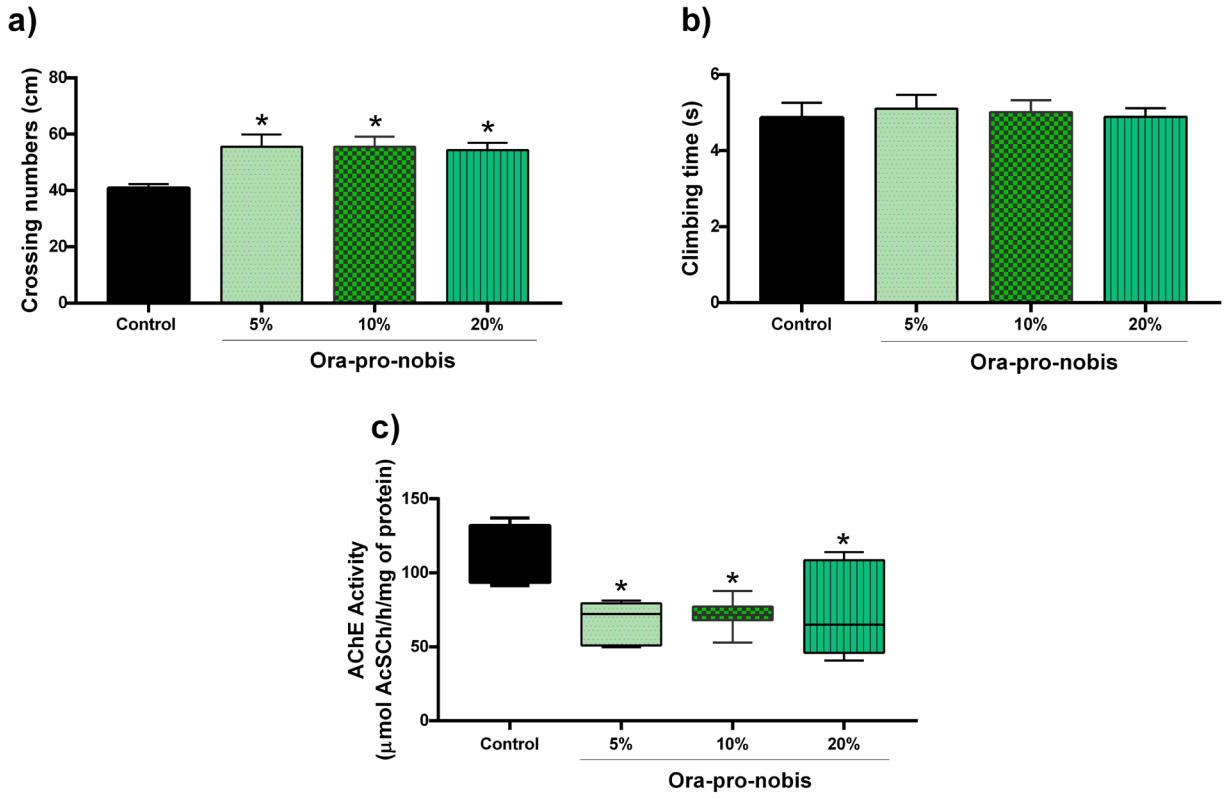

Figure 4. Assessment of exploratory capacity by open field test (a), negative geotaxis (b) and acetylcholinesterase (AChE) enzyme activity (c) in *Drosophila melanogaster* exposed for seven days to diets with addition of 5, 10 and 20% of ora-pro-nobis flour. The bar graphs express the comparison between different concentrations of ora-pro-nobis (5, 10 and 20%, separately), to the control group. The results are expressed as mean ± standard error of the mean (SEM). The box and whiskers graph expresses the result of AChE activity, as it is non-parametric data, showing the median (measure of centrality) and interquartile range (measure of variability). *Indicates significant statistical difference (p < 0.05) in relation to the control group.

difference in the PSH quantification analysis ($F_{(3.16)}$ = 0.9272; DF = 19; p = 0.4985; p = 0.7526; p = 0.4855). However, all groups that received a diet enriched with ora-pro-nobis flour (5, 10 and 20%) had an increase in NPSH levels in relation to the control group ($F_{(3.16)}$ = 16.87; DF = 19; p = 0.0001; p = 0.0014; p = 0.0023, respectively).

DISCUSSION

In this study, ora-pro-nobis flour appears as a supplement to the diet of *Drosophila melanogaster* and we evaluate the effect of different concentrations of it on biomarkers of oxidative parameters, showing a modulation of the basal redox effect and behavioral profile related to the neural activity exerted, at least in part, by the AChE enzyme modulation. To do this, first, before anything else, we carried out the test to evaluate the acceptability of flies to different concentrations of ora-pro-nobis. No difference was observed in the amount ingested by them, demonstrating good acceptance and non-aversion to diets containing 5, 10 and 20% ora-pro-nobis flour. This result guarantees that all the effects observed here can be attributed to supplementation with ora-pro-nobis flour. In addition, the weight of the flies was also unchanged, and this can be attributed to equal consumption between the groups. Another important factor to be observed was the survival of the flies during seven days of treatments, which showed that exposure throughout the entire treatment time is safe, highlighting the concentrations of 5% and 10%, where the flies demonstrated a greater survival when compared to the control group. These first data certify that it is possible to carry out the study using these quantities of ora-pro-nobis flour to evaluate the basal conditions of the redox status of the fly organism.

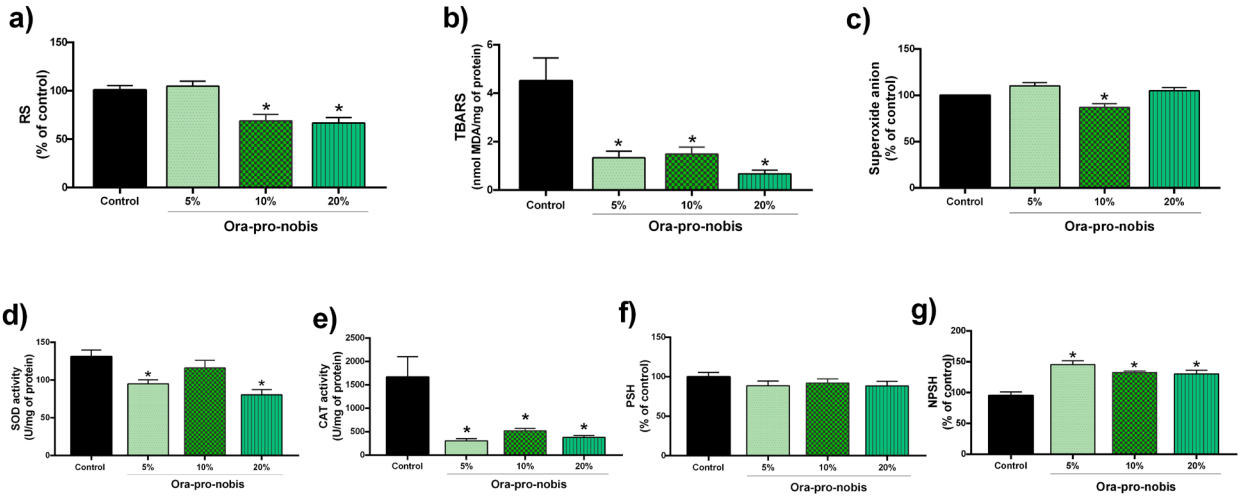

Figure 5. Quantification of reactive species (RS) (a), assessment of lipid peroxidation by the levels of thiobarbituric acid reactive species (TBARS) verified by the amount of malondialdehyde (MDA) (b) and superoxide anion (c). Activity of the enzyme superoxide dismutase (SOD) (d) and catalase (CAT) (e). Quantification of levels of protein thiols (PSH) (f) and non-protein thiols (NPSH) (g) in *Drosophila melanogaster* flies, exposed for seven days to diets containing concentrations of 5, 10 and 20% ora-pro-nobis. The bar graphs express the comparison between different concentrations of ora-pro-nobis (5, 10 and 20%, separately), to the control group. The results are expressed as mean ± standard error of the mean (SEM). *Indicates significant statistical difference (p < 0.05) in relation to the control group.

The data obtained from behavioral tests suggest that there was no negative effect on the flies' locomotor activity. The negative geotaxis test, which aims to verify the flies' climbing ability and which is a marker of damage, also did not show a significant difference between the diets tested. However, in the open field test, flies on diets containing ora-pro-nobis in all concentrations obtained greater locomotor activity within the arena, obtaining a greater number of crossings compared to the control group. It was observed that in all diets containing the addition of ora-pro-nobis flour (5%, 10% and 20%), the activity of the AChE enzyme was reduced. AChE regulates cholinergic synaptic transmission by hydrolyzing acetylcholine to acetate and choline (Kim et al. 2011). This result highlights that activity increased of this enzyme, the lower the breakdown of acetylcholine molecules, and thus, the greater availability of this neurotransmitter in the synaptic cleft, resulting in increased fly activity within the arena interpreted as agitation behavior.

To date, a single experiment has evaluated the effect of ora-pro-nobis on AChE activity. This study was *in vitro*, developed by Torres et al. (2021), where the ora-pro-nobis extract had an anticholinesterasic effect, that is, it reduced the activity of the AChE enzyme, and the authors attribute this effect to the alkaloids, terpenes, flavonoids and phenolic compounds present in the plant. Natural products for therapeutic purposes targeting neurological diseases (Custódio et al. 2023) are in vogue, with the aim of reducing the use of synthetic compounds, which can act on various neuropsychiatric, neurodegenerative and neurodevelopmental diseases (Piletsky et al. 2022). Furthermore, the AChE enzyme has been the focus of studies to discover new treatments for Alzheimer's disease, considering that the medications currently used work to a certain extent, and can increase the formation of β-amyloid plaques (Anand & Singh 2013). The mechanistic effect exerted by ora-pro-nobis flour in reducing AChE activity is a limiting factor in this study. Therefore, this inhibitory property of the AChE enzyme needs to be further studied. However, our results encourage further research demonstrating the potential effect on the modulation of the cholinergic system exerted by ora-pro-nobis flour consumption, can be applied to various treatments for neurological diseases.

Next, flour from ora-pro-nobis leaves demonstrated a positive modulation effect on the *in vivo* basal antioxidant status of *Drosophila melanogaster*. The quantification of total RS levels showed that flies that consumed diets containing concentrations of 10% and 20% ora-pro-nobis were lower compared to the control group, which represents an effect per se, quite common in antioxidant substances (Day 2014). At the 5% concentration, there was no reduction in RS basal levels, but it remained similar to the control, which does not minimize the other effects exerted by this concentration. The antioxidant effect of ora-pro-nobis has already been demonstrated, but in extract form (Pinto et al. 2015). The decline in LPO was also observed in flies that consumed diets containing ora-pro-nobis flour, indicating improvements in the oxidation-reduction relationship of fly cells.

LPO occurs in membrane lipids induced by the presence of radicals and ROS. LPO is perhaps the most important type of oxidative damage in biological systems; because the metabolites generated in peroxidation also continue the chain reaction that ends up compromising the membrane as a whole, thus increasing oxidative damage (Valgimigli 2023). Although many LPO by-products, such as MDA, exert toxicity at certain concentrations, it can induce an adaptive response, thus increasing tolerance against oxidative damage (Niki 2009).

According to studies, the main antioxidant agents present in ora-pro-nobis leaves are phenolic compounds, and among them isorhamnetin and rutin stand out (Garcia et al. 2019), which stimulate the intracellular production of GSH, protecting against lipid peroxidation (Cruz et al. 2021). Complementary to this, these phenolic compounds may be acting as chelators of ferric ions that act as LPO catalysts, thus reducing MDA levels. Another assumption is that these major phenolic compounds may be effective in the LPO initiation phase, which is when an antioxidant inhibits the formation of the first lipid radical, inhibiting the chain reaction.

In relation to the reduction in CAT activity that was observed in flies exposed to all concentrations of ora-pro-nobis flour and can be attributed to the increase in NPSH, as it is 90% composed of glutathione (GSH), a thiol present in higher concentrations in cells and responsible for most of the reduction in H_2O_2 (Quintana-Cabrera & Bolaños 2013). It is worth mentioning that the increase in GSH production is induced by phenolic compounds, which are mainly found in ora-pro-nobis flour, isorhamnetin and rutin (Garcia et al. 2019, Cruz et al. 2021). Thus, CAT can be reduced due to the reduction of its substrate, since according to Day (2014), the activity of antioxidant enzymes may be a consequence of the sparing effect of dietary antioxidants, reducing the need for enzymatic antioxidant function when high concentrations of exogenous antioxidants are present in the body. Even though the fly does not have glutathione peroxidase, the enzyme responsible for converting hydrogen peroxide to water, oxidizing GSH to GSSG, it is not yet fully understood how the absence of this enzyme is compensated in insect cells, but everything indicates that thioredoxin reductase be involved in this process (Radyuk et al. 2001).

In the case of SOD enzyme activity, although the intermediate concentration (10%) did not differ from the control diet, the diets containing 5 and 20% ora-pro-nobis had a significant reduction in the activity of this enzyme compared to the control. However, the fact that superoxide anion levels were lower in the group that consumed the diet with 10% ora-pro-nobis added, at the same time that the group showed greater activity of the SOD enzyme, indicates that the enzyme is catalyzing the dismutation of the superoxide anion (Ferreira & Matsubara 1997). It is worth mentioning that the flies that consumed a diet supplemented with 10% ora-pro-nobis, where the SOD enzyme had greater activity and a lower amount of superoxide anion, also showed greater longevity. This result is very positive as this effect can reflect on the population, attributed to a positive modulation of the antioxidant system in this group, considering that superoxide anion can be a very aggressive free radical to the body, being the most common cause of aging.

Therefore, in general and in line with other works found in the literature, such as the anti-inflammatory and healing potential of ora-pro-nobis observed in some studies, which helps reduce physical wear and tissue recovery (Pinto et al. 2015, Torres et al. 2021), we recorded the antioxidant effect in redox state *in vivo* evidenced in *Drosophila melanogaster*. Regarding other plants, evaluated the addition of blueberries and Yerba mate, respectively, to *Drosophila melanogaster* diets, but did not report significant differences in the activity of SOD and CAT enzymes (Peng et al. 2012, Portela et al. 2019), unlike the present study. The set of results demonstrated above suggests lower oxidative stress in flies that had ora-pro-nobis added to their diet, which may be related to the presence of phenolic compounds with antioxidant capacity in the plant's flour.

The antioxidant activity of ora-pro-nobis is mainly due to the high concentration of phenolic compounds present in the plant. According to Sousa et al. (2014), 100 g of ora-pro-nobis leaves contain chlorogenic acid (6.16512 mg), caffeic acid (0.92060 mg), *p*-coumaric acid (0.39825 mg) and ferulic acid (0.57243 mg). In a previous study, it was found that the incidence of total phenolic compounds and antioxidant capacity, measured using the ABTS method (2,2'-azinobis-3-ethyl-benzothiazoline6-sulfonated) via TEAC assay (Trolox Equivalent Antioxidant Capacity), in ora-pro-nobis flours, showed that the method used to produce the one used, obtained significantly higher values of total phenolic compounds and demonstrated greater antioxidant capacity compared to other flours (Sommer et al. 2022). In addition, the plant contains minerals in 100g of leaf such as Zinc (2.148 mg), Manganese (1.301 mg), Calcium (41.186 mg), Magnesium (9.674 mg), Copper (686.28 mg) and (Potassium 21.387 mg) (Teixeira et al. 2023). Given this, it is also worth highlighting that many of these minerals act as enzyme cofactors or interact with residues of certain amino acids, improving the link between enzyme and substrate. Thus, in addition to neutralizing RS, ora-pro-nobis can act to modulate the enzymatic antioxidant defense system.

In a study (Sommer et al. 2022), found 1201.86 mg of gallic acid equivalent/100 g and 3803.19 µM Trolox/100 g of phenolic compounds and antioxidant capacity, respectively, in an ora-pro-nobis flour prepared by the same procedure and obtained in the same location of the present study. According with (Arena et al. 2023), who studied the obtaining of protein concentrates from ora-pro-nobis leaves, reported an even higher antioxidant capacity, of 4193.79 µM Trolox/100 g of flour. Even when dealing with natural antioxidants, information related to toxicity is very important, but there is little information related to the biological activity of ora-pro-nobis (Sousa et al. 2014). Some *in vitro* studies provide information on cytotoxicity, showing lethal concentrations (LC50) in non-tumor cells are in the range of 200 µL/mL (Silva et al. 2017, Souza et al. 2016). In human hepatocytes, LC50 was greater than 400 µL/ mL (Garcia et al. 2019) and the only *in vivo* study to date observed that the consumption of dry extract did not induce toxicity in rats (Silva et al. 2017). Therefore, given this, ora-pro-nobis is classified as a class 5 plant in toxicity, the lowest class, which indicates low toxicological risk (Silva et al. 2017).

CONCLUSIONS

The results obtained contribute, in a favorable and promising way, to the use of ora-pro-nobis in food. The addition of plant flour to the diet has a protective effect on the body, slowing down harmful metabolic processes, through the reduction of RS and LPO. Furthermore, a modulatory effect on the cholinergic system was observed, providing greater locomotor activity in flies, thus instigating future studies regarding the therapeutic action of ora-pro-nobis in neurodegenerative diseases, for example. We highlighted a positive modulation in the enzyme, which reduced superoxide anion that may have contributed to the increased longevity of the flies. This result is encouraging in order to extrapolate to the population something evidenced in the laboratory, showing that the reduction of superoxide anion exerted by the concentration of 10% can increase longevity. Thus, it can be concluded that it is possible to enjoy the benefits of ora-pro-nobis without the need to consume high doses of the plant, as up to a concentration of 20% there is no enhancement of the positive effects, although it also does not present toxicity.

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REFERENCES

ADAMS D ET AL. 2000. The genome sequence of *Drosophila melanogaster*. Sci 287(5461): 2185-2195. https://doi.org/10.1126/science.287.5461.2185.

AEBI H. 1987. Catalase *in vitro*. Method. Enzymol 105: 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3.

ANAND P & SINGH B. 2013. A review on cholinesterase inhibitors for Alzheimer's disease. Arch Pharm Res 36: 375-399. https://doi.org/10.1007/s12272-013-0036-3.

ARENA RVP, RIBEIRO PF DE A & KAMINSKI TA. 2023. Obtenção e caracterização físico-química de concentrados proteicos das folhas de ora-pro-nóbis. Res Soc Dev 12(6): e14112642058. https://doi.org/10.33448/rsd-v12i6.42058.

BRADFORD MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3.

CONNOLLY K. 1966. Locomotor activity in Drosophila. II. Selection for active and inactive strains. Anim Behav 14(4): 444-449. https://doi.org/10.1016/S0003-3472(66)80043-X.

CRUZ TM, SANTOS JS, DO CARMO MAV, HELLSTRÖM J, PIHLAVA J-M, AZEVEDO L, GRANATO D & MARQUES MB. 2021. Extraction optimization of bioactive compounds from ora-pro-nobis (*Pereskia aculeata* Miller) leaves and their in vitro antioxidant and antihemolytic activities. Food Chem 351: 130078. https://doi.org/10.1016/j.foodchem.2021.130078.

CUSTÓDIO L, VIZETTO-DUARTE C, CEBECI F, ÖZÇELIK B, SHAROPOV F, GÜRER ES, KUMAR M, IRITI M, SHARIFI-RAD J & CALINA D. 2023. Natural products of relevance in the management of attention deficit hyperactivity disorder. EFood 4(1): 1-11. https://doi.org/10.1002/efd2.57.

DAY BJ. 2014. Antioxidant therapeutics: Pandora's box. Free Radic. Biol Med 66: 58-64. https://doi.org/10.1016/j.freeradbiomed.2013.05.047.

DE ALMEIDA MEF & CORRÊA AD. 2012. Utilização de cactáceas do gênero Pereskia na alimentação humana em um município de Minas Gerais. Cienc Rural 42(4): 751-756. https://doi.org/10.1590/S0103-84782012000400029.

DE SOUZA WFM, MARIANO XM, ISNARD JL, DE SOUZA GS, DE SOUZA GOMES AL, DE CARVALHO RJT, ROCHA CB, SIQUEIRA JUNIOR CL & MOREIRA RFA. 2019. Evaluation of the volatile composition, toxicological and antioxidant potentials of the essential oils and teas of commercial Chilean boldo samples. Food Res Int 124: 27-33. https://doi.org/10.1016/j.foodres.2018.12.059.

ELLMAN GL. 1959. Tissue sulfhydryl groups. Arch Biochem Biophys 82(1): 70-77. https://doi.org/10.1016/0003-9861(59)90090-6.

ELLMAN GL, COURTNEY KD, ANDRES V & FEATHER-STONE RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88-95. https://doi.org/10.1016/j.freeradbiomed.2013.05.047.

FAROMBI EO, ABOLAJI AO, FAROMBI TH, OROPO AS, OWOJE OA & AWUNAH MT. 2018. Garcinia kola seed biflavonoid fraction (Kolaviron), increases longevity and attenuates rotenone-induced toxicity in *Drosophila melanogaster*. Pestic Biochem Physiol 145: 39-45. https://doi.org/10.1016/j.pestbp.2018.01.002.

FERREIRA ALA & MATSUBARA LS. 1997. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. Rev Assoc Med Bras 43(1): 61-68. https://doi.org/10.1590/S0104-42301997000100014.

GARCIA JAA, CORRÊA RCG, BARROS L, PEREIRA C, ABREU RMV, ALVES MJ, CALHELHA RC, BRACHT A, PERALTA RM & FERREIRA ICFR. 2019. Phytochemical profile and biological activities of 'Ora-pro-nobis' leaves (*Pereskia aculeata* Miller), an underexploited superfood from the Brazilian Atlantic Forest. Food Chem 294: 302-308. https://doi.org/10.1016/j.foodchem.2019.05.074.

GHASEMZADEH A & GHASEMZADEH N. 2011. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J Med Plant Res 5(31): 6697-6703. https://doi.org/10.5897/JMPR11.1404.

HOFF R, DAGUER H, DEOLINDO CTP, DE MELO APZ & DURIGON J. 2022. Phenolic compounds profile and main nutrients parameters of two underestimated non-conventional edible plants: *Pereskia aculeata Mill.* (ora-pro-nobis) and Vitex megapotamica (Spreng.) Moldenke (tarumã) fruits. Food Res Int 162(PtA): 112042. https://doi.org/10.1016/j.foodres.2022.112042.

KIM W ET AL. 2011. Pharmacogenetic regulation of acetylcholinesterase activity in drosophila reveals the regulatory mechanisms of AChE inhibitors in synaptic plasticity. Neurochem Res 36(5): 879-893. https://doi.org/10.1007/s11064-011-0418-1.

KOSTYUK V & POTAPOVICH A. 1989. Superoxide- Driven oxidation of quercitin and a simple sensitive assay for determination of superoxide dismutase. Biochem Int 19(5): 1117-1124. https://pubmed.ncbi.nlm.nih.gov/2561443/.

LUSHCHAK OV, ROVENKO BM, GOSPODARYOV DV & LUSHCHAK VI. 2011. *Drosophila melanogaster* larvae fed by glucose and fructose demonstrate difference in oxidative stress markers and antioxidant enzymes of adult flies. Comp Biochem Physiol A Mol Integr Physiol 160(1): 27-34. https://doi.org/10.1016/j.cbpa.2011.04.019.

MANETTA GB, ROMANO BC, BRAGA COSTA TM & TRIFFONI-MELO AT. 2023. Utilização de farinha de Ora-Pro-Nobis (*Pereskia aculeata miller*) em preparação de biscoito de polvilho. Braz J Dev 9(1): 1494-1508. https://doi.org/10.34117/bjdv9n1-103.

MATTILA P & HELLSTRÖM J. 2007. Phenolic acids in potatoes, vegetables, and some of their products. J Food Compos. Anal 20(3-4): 152-160. https://doi.org/10.1016/j.jfca.2006.05.007.

MEICHTRY LB ET AL. 2020. Addition of Saturated and Trans-fatty Acids to the Diet Induces Depressive and Anxiety-like Behaviors in *Drosophila melanogaster*. Neuroscience 443: 164-175. https://doi.org/10.1016/j.neuroscience.2020.07.042.

MORABITO G ET AL. 2010. Antioxidant properties of 4-methylcoumarins in *in vitro* cell-free systems. Biochimie 92(9): 1101-1107. https://doi.org/10.1016/j.biochi.2010.04.017.

MUSACHIO EAS ET AL. 2020. Bisphenol A exposure is involved in the development of Parkinson like disease in *Drosophila melanogaster*. Food Chem Toxicol 137: 111128. https://doi.org/10.1016/j.fct.2020.111128.

MUSACHIO EAS, POETINI MR, JANNER DE, FERNANDES EJ, MEICHTRY LB, MUSTAFA DAHLEH MM, GUERRA GP & PRIGOL M. 2023. Safer alternatives? Bisphenol F and Bisphenol S induce oxidative stress in *Drosophila melanogaster* larvae and trigger developmental damage. Food Chem Toxicol 175: 113701. https://doi.org/10.1016/j.fct.2023.113701.

NIKI E. 2009. Lipid peroxidation: physiological levels and dual biological effects. Free Radic Biol Med 47(5): 469-484. https://doi.org/10.1016/j.freeradbiomed.2009.05.032.

NOGUEIRA SILVA NF, SILVA SH, BARON D, NEVES ICO & CASANOVA F. 2023. "*Pereskia aculeata Miller* as a Novel Food Source: A Review". Foods 12(11): 2092. https://doi.org/10.3390/foods12112092.

OHKAWA H, OHISHI N & YAGI K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2): 351-358. https://doi.org/10.1016/0003-2697(79)90738-3.

OLIVEIRA PM, MATOS BN, PEREIRA PAT, GRATIERI T, FACCIOLI LH, CUNHA-FILHO MSS & GELFUSO GM. 2017. Microparticles prepared with 50–190 kDa chitosan as promising non-toxic carriers for pulmonary delivery of isoniazid. Carbohydr Polym 174: 421-431. https://doi.org/10.1016/j.carbpol.2017.06.090.

PAULA MT, ZEMOLIN AP, VARGAS AP, GOLOMBIESKI RM, LORETO ELS, SAIDELLES AP & POSSER T. 2012. Effects of Hg(II) Exposure on MAPK Phosphorylation and Antioxidant System in *D. melanogaster*. Environ Toxicol 29(6): 621-630. https://doi.org/10.1002/tox.21788.

PENG C, ZUO Y, KWAN KM, LIANG Y, MA KY, CHAN HYE, HUANG Y, YU H & CHEN ZY. 2012. Blueberry extract prolongs lifespan of *Drosophila melanogaster*. Exp Gerontol 47(2): 170-178. https://doi.org/10.1016/j.exger.2011.12.001.

PILETSKY SA, BEDWELL TS, PAOLETTI R, KARIM K, CANFAROTTA F, NORMAN R, JONES DJL, TURNER NW & PILETSKA EV. 2022. Modulation of acetylcholinesterase activity using molecularly imprinted polymer nanoparticles. J Mater Chem B 10(35): 6732-6741. https://doi.org/10.1039/d2tb00278g.

PINTO NDCC, MACHADO DC, DA SILVA JM, CONEGUNDES JLM, GUALBERTO ACM, GAMEIRO J, CHEDIER LM, CASTAÑON MCMN & SCIO E. 2015. *Pereskia aculeata Miller* leaves present in vivo topical anti-inflammatory activity in models of acute and chronic dermatitis. J Ethnopharmacol 173: 330-337. https://doi.org/10.1016/j.jep.2015.07.032.

POETINI MR, ARAUJO SM, TRINDADE DE PAULA M, BORTOLOTTO VC, MEICHTRY LB, POLET DE ALMEIDA F, JESSE CR, KUNZ SN & PRIGOL M. 2018. Hesperidin attenuates iron-induced oxidative damage and dopamine depletion in *Drosophila melanogaster* model of Parkinson's disease. Chem Biol Interact 279: 177-186. https://doi.org/10.1016/j.cbi.2017.11.018.

PORTELA JL, BIANCHINI MC, BOLIGON AA, CARRIÇO MRS, ROEHRS R, SOARES FAA, DE GOMES MG, HASSAN W & PUNTEL RL. 2019. *Ilex paraguariensis* Attenuates Changes in Mortality, Behavioral and Biochemical Parameters Associated to Methyl Malonate or Malonate Exposure in *Drosophila melanogaster*. Neurochem Res 44(9): 2202-2214. https://doi.org/10.1007/s11064-019-02862-w.

QUINTANA-CABRERA R & BOLAÑOS JP. 2013. Glutathione and γ-glutamylcysteine in hydrogen peroxide detoxification. Methods Enzymol 527: 129-144. https://doi.org/10.1016/B978-0-12-405882-8.00007-6.

RADYUK SN, KLICHKO VI, SPINOLA B, SOHAL RS & ORR WC. 2001. The peroxiredoxin gene family in *Drosophila melanogaster*. Free Radic Biol Med 31(9): 1090-1100. https://doi.org/10.1016/S0891-5849(01)00692-x.

RAMALHO VC & JORGE N. 2006. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos.

Quim Nova 29(4): 755-760. https://doi.org/10.1590/s0100-40422006000400023.

SÁ KM ET AL. 2024. Technology Prospection of Ora-pro-N (*Pereskia aculeata Mill.):* A Non-conventional Food Plant. Recent Pat Biotechnol 18(2): 144-151. https://doi.org/ 10.2174/1872208317666230502101802.

SHARIF KM, RAHMAN MM, ZAIDUL ISM, JANNATUL A, AKANDA MJH, MOHAMED A & SHAMSUDIN SH. 2013. Pharmacological elevance of primitive leafy cactuses pereskia. Res J Biotec 8(12): 134-142.

SILVA DO, SEIFERT M, NORA FR, BOBROWSKI VL, FREITAG RA, KUCERA HR, NORA L & GAIKWAD NW. 2017. Acute Toxicity and Cytotoxicity of *Pereskia aculeata*, a Highly Nutritious Cactaceae Plant. J Med Food 20(4): 403-409. https://doi.org/10.1089/jmf.2016.0133.

SOMMER MC, RIBEIRO PF DE A & KAMINSKI TA. 2022. Obtention and physicochemical characterization of ora-pro-nobis flour. Braz J Health Rev 5(2): 6878-6892. https://doi.org/10.34119/bjhrv5n2-256.

SOUSA RMF, LIRA CS, RODRIGUES AO, MORAIS SAL, QUEIROZ CRAA, CHANG R, AQUINO FJT, MUÑOZ RAA & DE OLIVEIRA A. 2014. Atividade antioxidante de extratos de folhas de ora-pro-nóbis (*Pereskia aculeata Mill.)* usando métodos espectrofotométricos e voltamétricos *in vitro.* Biosci J 30(3): 448-457.

SOUZA LF, CAPUTO L, INCHAUSTI DE BARROS IB, FRATIANNI F, NAZZARO F & DE FEO V. 2016. *Pereskia aculeata Muller* (Cactaceae) Leaves: Chemical Composition and Biological Activities. Int J Mol Sciences 17(9): 1478. https://doi.org/10.3390/ijms17091478.

TAKEITI CY, ANTONIO GC, MOTTA EMP, COLLARES-QUEIROZ FP & PARK KJ. 2009. Nutritive evaluation of a non-conventional leafy vegetable (*Pereskia aculeata Miller*). Int J Food Sci Nutr 60(1): 148-160. https://doi.org/10.1080/09637480802534509.

TEIXEIRA VMC ET AL. 2023. A Critical Appraisal of the Most Recent Investigations on Ora-Pro-Nobis (*Pereskia* sp.): Economical, Botanical, Phytochemical, Nutritional, and Ethnopharmacological Aspects. Plants 12(22): 3874. https://doi.org/10.3390/plants12223874.

TORRES TMS, ÁLVAREZ-RIVERA G, MAZZUTTI S, SÁNCHEZ-MARTÍNEZ JD, CIFUENTES A, IBÁÑEZ E & FERREIRA SRS. 2021. Neuroprotective potential of extracts from leaves of ora-pro-nobis (*Pereskia aculeata*) recovered by clean compressed fluids. J Supercrit Fluids 179: 105390. https://doi.org/10.1016/j.supflu.2021.105390.

VALGIMIGLI L. 2023. Lipid Peroxidation and Antioxidant Protection. Biomolecules 13: 1291. https://doi.org/10.3390/biom13091291.

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