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Environment

White and brown crystal sugar diets: a high consumption effect in *D. Melanogaster*

Dieta à base de açúcar cristal branco e mascavo: efeito de alto consumo em *D. melanogaster*r

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ABSTRACT

The high consumption of sugars in their different forms has been of concern to the International Health Organization (WHO). In this study, D. melanogater (born in the dietary medium – Generation F1) males and females were subjected to a white (WS) and brown (BS) Crystal Sugar rich diet. Results obtained indicate an increase in oxidative stress with an increase in the consumption of sugar in the diet, as observed in the increase in the enzymatic activity of SOD, CAT, and GPx. These results are corroborated by analyses of lipid peroxidation (TBARS), carbonyl content, and ROS (DCFH), which clearly demonstrate an increase in the formation of reactive oxygen species with the increase in the consumption of both white and brown crystal sugars. Another effect observed by the increase in sugar consumption was the augmentation in glucose levels (white and brown sugars) and in iron levels (brown sugar). In this sense, the high consumption of iron in brown sugar has contributed more strongly to the formation of ROS in *D. melanogaster*, mainly in females.

Keywords: Sugar intake; Oxidative stress; Male and female flies; Enzyme activity; Sucrose; Iron

RESUMO

O alto consumo de açúcares em suas diferentes formas tem preocupado a Organização Internacional da Saúde (OMS). Neste estudo, machos e fêmeas de D. melanogater (nascidos no meio dietético – Geração F1) foram submetidos a uma dieta rica em Açúcares Cristal Branco (WS) e Mascavo (BS). Os resultados obtidos indicam um aumento do estresse oxidativo com o aumento do consumo de açúcar na dieta, conforme observado no aumento da atividade enzimática de SOD, CAT e GPx. Esses resultados são corroborados pelas análises de peroxidação lipídica (TBARS), teor de carbonila e ROS (DCFH), que demonstram claramente um aumento na formação de espécies reativas de oxigênio com o aumento do consumo de açúcares tanto branco quanto mascavo. Outro efeito observado pelo aumento do

consumo de açúcar foi o aumento dos níveis de glicose (açúcares branco e mascavo) e de ferro (açúcar mascavo). Nesse sentido, o alto consumo de ferro no açúcar mascavo tem contribuído mais fortemente para a formação de ROS em *D. melanogaster*, principalmente em fêmeas.

Palavras-chave: Ingestão de açúcar; Estresse oxidativo; Moscas machos e fêmeas; Atividade enzimática; Sacarose; Ferro

1 INTRODUCTION

Sucrose or sugar free is one of the most consumed foods in the world. Brazil is the 4th largest consumer of sucrose in the world, (Veja Saúde, 2021). The recommendation of the World Health Organization (WHO) is that only 10% of the food consumed per day comes from sugar, however, the Brazilian population consumes an average of 16.3%. Epidemiological studies have shown that dietary components are the most important environmental risk factors for the development of chronic metabolic diseases (Espositoa et al., 2010; Parillo and Riccardi, 2010). Thus, special attention should be given to carbohydrates, as reports in the literature show that diets rich in sucrose trigger an increase in diseases such as (Na et al., 2013); maternal obesity (Brookheart et al., 2017); insulin resistance (Morris et al., 2012); cellular and humoral immune responses (Yu et al., 2018); obesity (Rovenko et al., 2015) and diabetic phenotypes (Ecker et al., 2017).

The consumption of free sugar refers mainly to the white crystal sugar type, but the consumption of brown crystal sugar has increased due to its natural characteristics, maintaining sugarcane vitamins and minerals. The inclusion of sugar in drinks as soda, artificial juice and others has caused several health problems (Malik et al., 2010). Prasad et al., in your manuscript reviewed, indicated that (Prasad and Dhar, 2014) consumption of sugars induce atherosclerosis, hypertension, peripheral vascular disease, coronary artery disease, cardiomyopathy, heart failure, and cardiac arrhythmias and that these effects of added sugars are mediated through reactive oxygen species (ROS). Brown et al. demonstrated that daily consumption of every extra sugar-sweetened beverage

increased the systolic arterial pressure by 1.6 mmHg and the diastolic pressure by 0.8 mmHg (Brown et al., 2011). Increased levels of oxidative stress from a diet rich in sucrose and other sugars like fructose and glucose are already known. (Rovenko et al., 2015); (Stanhope et al., 2009); (Folmer, 2002).

The use of alternative in vivo testing models, such as Drosophila melanogaster (*D. melanogaster*), in the study of high sugar diets, has shown good results (Musselman et al., 2019) and (Schipanski et al., 2008). In addition, this model has been used in studies such as obesity, metabolic syndrome, diabetes, and other diet-induced diseases (Rovenko et al., 2015; Ecker et al., 2017; Musselman et al., 2011 and Matzkin et al., 2013). Despite the physiological differences between mammals and insects, studies with the *D. melanogaster* model have led to considerable achievements in understanding genetic mechanisms of nutritional sensing and metabolic regulation in mammals (Rovenko et al., 2015; Danielsen et al., 2013). Thus, the aim of the study was to verify the effect of white and brown sugars crystal on biochemical parameters, oxidative and motor stress in male and e female flies.

2 MATERIALS AND METHODS

2.1 Chemical

Phosphate Buffer 0.05 M pH 7.4, acid 3-(2-pyridyl) – 5,6 diphenyl-1, 2, 4-triazine-p,p'-disulfonic acid monosodium salt hydrate, Cumene Hydroperoxide, 2,4-Dinitrophenylhydrazine (DNPH), Quercetin, were obtained from Sigma (St. Louis, MO, USA). All other commercial reagents were of analytical grade.

2.2 Sample

Commercial white (WS) (Picinin Alimentos Ltda, Brazil) and brown (BS) (Guimarães Indústria e Comércio Ltda, Brazil) crystal sugar were purchased in Uruguaiana city (Brazil). The sugar in the solid state was used in all experiments

and mixed with medium corn bran, coarse, corn flour, powdered milk, salt and wheat germ.

2.3 Drosophilas melanogaster stock

D. melanogaster wild-type (Harwich strain) was obtained from the National Stock Stock Center (Bowling Green, OH, USA). The flies were maintained and grown in corn meal medium (1% w/v brewer's yeast, 2% w/v sucrose, 1% w/v powdered milk, 1% w/v agar and 0,08% v/w of nipagin) at a constant temperature and humidity (22 ± 1 ° C, 60% relative humidity, respectively), subjected to a light/dark 12 h cycle until treatment.

2.4 Drosophila melanogaster feeding

Male and female flies were treated according to Soares et al. with some modifications (Soares et al., 2017) Briefly, flies were divided into the groups control, 5% sugar, 10% sugar, 20% sugar, 30% sugar and 40% for WS and BS, respectively. After, the *D. melanogaster* was removed from the treatment medium and only *D. melanogaster* eggs remained in the medium sugar for further studies. The *D. melanogaster* flies, which were born in sugar rich medium, were kept throughout their breeding stage and after hatching, remained in the middle 5 day age (adult stage). After treatment, flies were anesthetized on ice for approximately 1 minute and separated into male and female for further studies.

2.5 Homogenate preparation

About 20 flies of each group (male and female) were homogenized in phosphate buffer, triturated and centrifuged at 3000 x g for 10 minutes in refrigerated centrifuge equipment at 4 °C. After centrifugation of the samples the supernatant was used for the tests. All experiments were performed in triplicate.

2.6 Antioxidant enzyme activity assays

Superoxide dismutase (SOD) activity was determined according to the method proposed by Misra & Fridovich (1972). The kinetic analysis of SOD was measured at 480 nm and the results were expressed as U/mg protein. Catalase (CAT) activity was measured spectrophotometrically according to the method proposed by Aebi (1984). The kinetic analysis of catalase was started after H2O2 was added and the reaction was determined at 240 nm. Results were calculated using the molar extinction coefficient of H2O2, corrected by protein content expressed as nmol H2O2/mg protein/min. Glutathione Peroxidase (GPx) was measured according to Pinto and Bartley, 1969 using a kit Ransel (Randox®). Briefly, 50 µL of a diluted haemolymph mixture was added to the reaction mixture containing 40 µL cumene hydroperoxide and 50 mM buffer (pH 7.2). The optical density of NADPH was measured at 340 nm and 37°C, and the rate of the reaction was estimated from the absorbance readings in the first 3 min after adding cumene hydroperoxide. The experiments were carried out in triplicate.

2.7 Lipid Peroxidation (TBARS) and Protein Carbonyl

Lipid peroxidation was measured according to Ohkawa et al., 1979. In brief, adding S1 samples (100 µL) to a medium containing 8.1% sodium dodecyl sulfate, acetic acid buffer (pH 3.5), and 0.8% aqueous solution of thiobarbituric acid. The medium was heated at 95 °C for 60 min and the red pigment produced was measured spectrophotometrically at 532 nm. Results were calculated using a standard curve constructed with malondialdehyde (MDA) at known concentrations and corrected by protein content. The results were expressed as nmol MDA/mL.

Protein Carbonyl levels were determined according to the method proposed by Levine et al.,1990. The carbonyl groups in the protein side chains were derivatized, to 2,4-dinitrophenylhydrazone (DNPH) by reaction with 2,4-dinitrophenylhydrazine. The results were expressed as nmol carbonyl/mg protein. The experiments were carried out in triplicate.

2.8 DCFH-DA oxidation assays

The assessment of 2,7 –dichlorofluorescein diacetate (DCFH-DA) oxidation in the head and body regions of the control and treated flies were performed to determine the level of intracellular reactive oxygen and nitrogen species (RONS) generation, a general index of oxidative stress. The assay reaction mixture consisted of 150 µL of 0.1 µM potassium phosphate buffer (pH 7.4), 40 µL of distilled water, 5 µL of DCFH-DA (200 µM, final concentration 5 µM), and 5 µL of the sample (1:10 dilution). The fluorescence emission of DCF resulting from DCFH oxidation was monitored for 10 min (30 s intervals) at 488 nm and 525 nm, excitation and emission wavelengths, respectively, using a SpectraMax plate reader (Molecular Devices, CA, USA). The rate of DCF formation was expressed as a percentage (% of the control group) (PÉREZ et al., 2004). The experiments were carried out in triplicate.

2.9 Glucose levels

Glucose levels were measured with a commercial Glicose Liquiform Kit (Labtest Diagnóstica Ltda®, Lagoa Santa, Minas Gerais, Brazil), using an analyzer equipment ChemWell T – Labtest® The result was measured spectrophotometrically at 505 nm. The experiments were carried out in triplicate.

3.0 IRON LEVEL IN BROWN CRYSTAL SUGAR

The determination of the iron in the BS was made according to Iron colorimetric assay kit (Ferrozine methods) (kit Vida Biotecnologia – Belo Horizonte, MG, Brazil) where the iron is released from the transferrin in buffered acid medium and reduced to ferrous ions by thioglycolic action. 3 – (2-Pyridyl) – 5,6-bis (4-phenylsulfonic acid) – 1,2,4-triazine reacts with these ions to form a complex of pink coloration, with intensity proportional to the concentration of Fe ions present in the sample. Its absorption peak on the UV/VIS spectrophotometer is 565 nm. Iron in WS was not detected (*data not demonstrated*). The experiments were carried out in triplicate.

4 STATISTICAL ANALYSIS

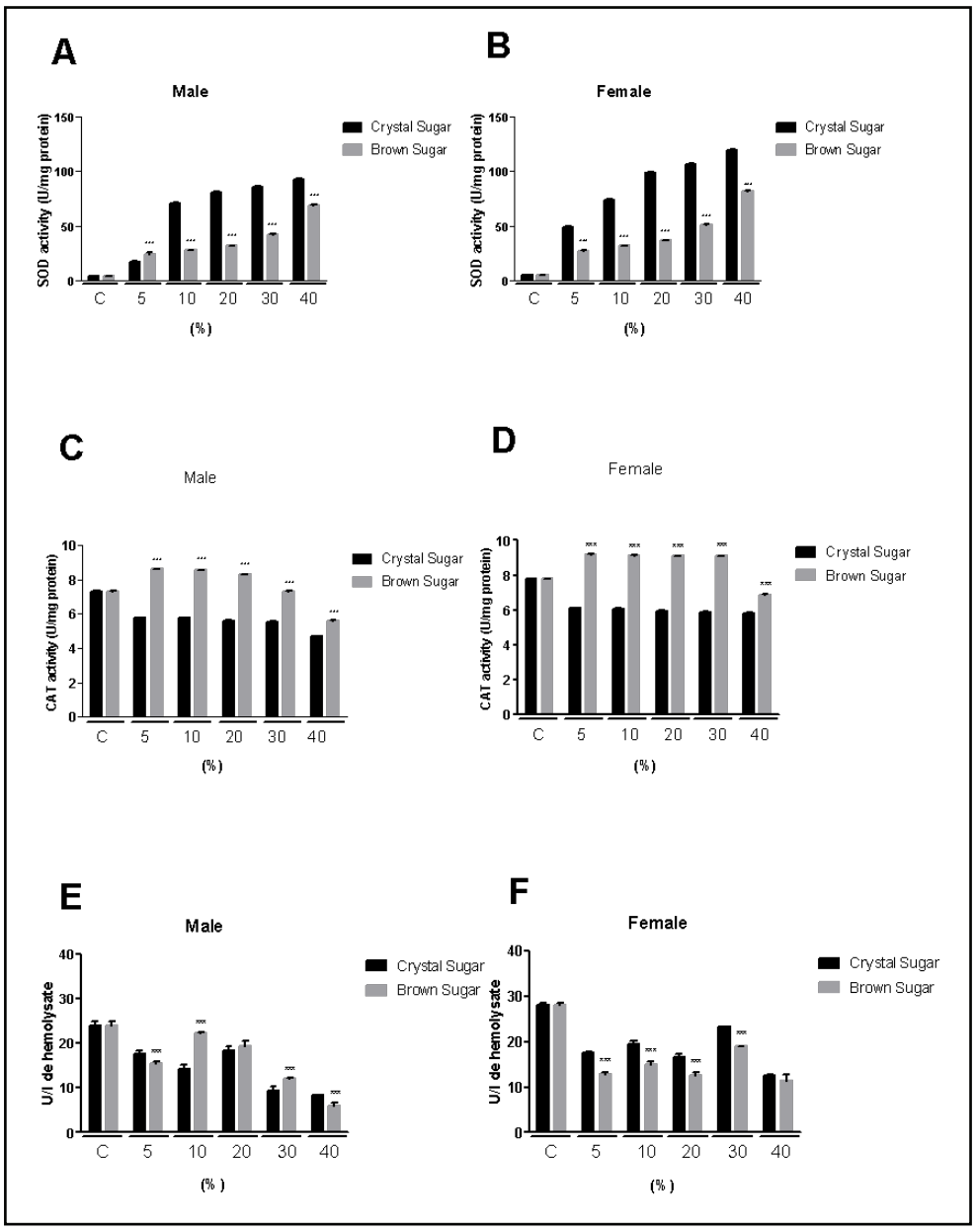
Statistical analyzes were performed using two-way ANOVA followed by Bonferroni's post-test to compare the effects between types of sugars at the same concentration for male and female flies separately. For the comparison between the concentrations in the iron dosage, we used one-way ANOVA followed by Tukey's post-test. For the comparison between male and female flies in the same type of sugar in the same concentrations, two-way ANOVA followed by the Bonferroni post-test of multiple pairs was used; these results were expressed in control variation (%). Differences between groups were considered significant when $P < 0.05$, $P < 0.01$ or $P < 0.001$, according to the assay. All results were expressed as the mean of the triplicate ± standard error. Statistical analyses were performed using GraphPad Prism 5.0 (San Diego, CA, USA).

5 RESULTS

5.1 Antioxidant enzyme activities

The antioxidant enzyme activities are summarized in figure 1. Results showed that in male *D. Melanogaster*, an increase in SOD values with increased WS and BS sugar concentration compared to control, but this effect was more pronounced in WS than BS (Figure 1A). Similar behavior was observed in female D*. Melanogaster* (Figure 1B). Male flies (Figure 1C) present decreased CAT activity with increased BS sugar concentration whereas WS sugar did not show significant differences. Female flies (Figure 1D) demonstrated similar behavior observed in male flies to BS and WS sugars. For GPx activity results showed a decrease in activity with the increase of the BS and WS sugars in male (Figure 1E) and female (Figure 1F) flies.

Figure 1 – Evaluation of the antioxidant activity of male and female flies submitted to a high brown or white crystal sugar diet by superoxide dismutase assay (SOD) (A-Male, B-Female), Catalase (CAT) assay (C-Male, D-Female), – Glutathione Peroxidase (GPx) assay (E-Male, F-Female). (*p<0.05, **p<0.01, ***p<0.001 values, statistically significance) comparing the different types of sugar in the same concentration



5.2 Oxidative stress parameters

The comparative results of oxidative stress markers in male and female flies

submitted to a high BS and WS sugars diet are shown in figure 2. The levels of oxidative peroxidation (Figures 2A and 2B), Carbonyl protein (Figures 2C and 2D) and ROS (Figures 2E and 2F) were significantly augmented with increases in both sugars diet (BS and WS sugars) for male and female flies compared to the control.

Table 1 presents the results regarding the comparison of antioxidant enzyme activities between male and female flies (*D. melanogaster*) for the same concentrations and types of sugar (BS and WS sugars) diet.

The results obtained from WS sugar (Table 1) indicate that SOD values, compared to control, were higher in female flies up to 5% sugar diet ($p < 0.001$), after predominating values obtained for male flies higher values up to 30% sugar diet. For the 40% sugar diet, the values were similar in both genders. Already in relation to the use of the BS sugar diet for male flies, the values were higher in all concentrations ($p < 0.001$). In relation to the CAT assay, a new effect was observed. For BS and WS sugars diet, comparative lower values than control were observed in male and female flies. In WS sugar, the behavior is similar in both genders until the 30% sugar diet; for 40% concentration, this effect is stronger in male flies. The same behavior was not observed in the results obtained for BS. Values obtained for males were slightly higher than controls at all concentrations tested while in females results indicated values much higher than the control until the 30% sugar diet ($p < 0.001$). The value obtained in the 40% concentration sugar diet was below the control. GPx levels show similar behavior for BS and WS sugars diet in relation to control values. All results were below to control values at all concentrations and both sexes. In the WS sugar diet, this effect was higher in male flies and in BS sugar until 20% concentration this effect was more in female flies. After this concentration, the values were higher in males than in female flies ($p < 0.001$).

Figure 2 – Evaluation of the antioxidant activity of male and female flies submitted to a high brown or white crystal sugar diet by lipoperoxidation assay (TBARS) (A-Male, B-Female), Carbonyl protein levels (C-Male, D-Female), – Chemical deacetylation of DFCH-DA compound (E-Male, F-Female). (*p<0.05, **p<0.01, ***p<0.001 values, statistically significance) comparing the different types of sugar in the same concentration

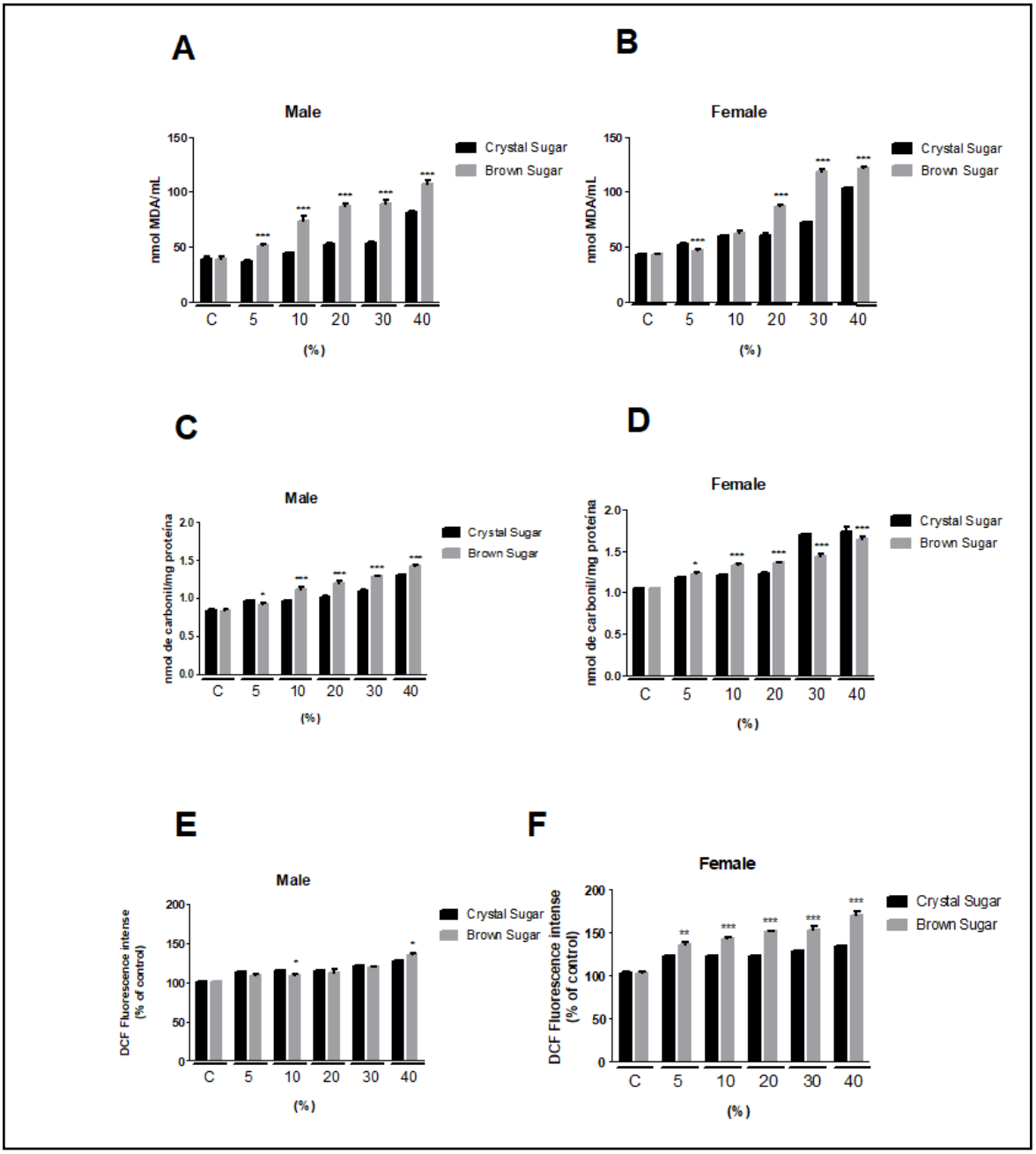


Table 1 – Comparison of antioxidant protection enzymes between males and females of the same concentrations and types of sugar

WHITE SUGAR						
C	CAT		GPX		SOD	
(%)	Male	Female	Male	Female	Male	Female
5	– 20.8 ±0.1	a-21.8 ± 0.1	– 26.0 ± 1.7	aaa-37.7 ± 0.7	341.6 ± 15.3	aaa882.4 ± 10.1
10	– 21.1 ± 0.2	a-22.2 ± 0.2	aa-40.3 ± 2.0	– 31.0 ± 1.7	aaa1742.3 ± 13.7	1375.7 ± 6.95
20	– 23.2 ± 0.3	-23.7 ± 0.2	– 23.1 ± 2.1	aaa-41.1 ± 1.6	aaa20181 ± 9.8	1879.2 ± 11.7
30	– 23.8 ± 0.3	-24.5 ± 0.1	aaa-60.4 ± 2.2	– 17.4 ± 0.4	aaa2140.1 ± 12.8	2027.4 ± 15.8
40	aaa-35.5±0.1	-25.4 ± 0.2	aa-64.9 ± 0.1	– 55.0 ± 0.1	2322.0 ± 11.1	2301.0 ± 0.2

BROWN SUGAR					
CAT		GPX		SOD	
Male	Female	Male	Female	Male	Female
1.2 ± 0.0	aaa17.9 ± 0.3	-35.7 ± 1.0	aaa-54.0 ± 1.0	aaa531.9 ± 24.1	452.2 ± 12.3
1.2 ±0.0	aaa17.0 ± 0.2	-7.7 ± 1.1	aaa-47.1 ± 0.9	aaa635.34 ± 4.4	533.8 ± 8.4
1.1 ± 0.0	aaa16.5 ± 0.1	-19.7 ± 3.3	aaa55.7 ± 1.6	aa737.7 ± 3.7	631.1 ± 11.2
1.0 ± 0.0	aaa16.4 ± 0.1	aaa-49.4 ± 0.4	-32.3 ± 0.2	aaa1009.2 ± 10.6	931.0 ± 8.2
aaa0.8. ± 0.0	-12.2 ± 0.3	aaa – 75.4 ± 2.0	-59.2 ± 2.6	aaa1703.8 ± 7.7	1537.2 ± 8.2

aFor significant increase over the equal concentration pair, $p < 0.05$; aa for $p < 0.01$ and aaa for $p < 0.001$. C=Concentration. Comparison of antioxidant protection enzymes between males and females of the same concentrations and types of sugar (BS and WS sugars) diet.

Table 2 presents the results obtained concerning the comparison of oxidative stress markers between male and female flies (*D. melanogaster*) for the same concentrations and types of sugar (BS and WS sugars) diet.

Analysis of the WS sugar results demonstrated that carbonyl levels show for males and females (Table 2) similar behavior until the 20% sugar diet, but above 30% was observed a predominance of the female flies ($p < 0.001$). Already, in BS sugar results showed higher values for males except in the lowest concentration (5%). TBARS

levels show similar values for male and female flies until 30% in WS sugar diet, only at 40% sugar diet prevail higher values in male flies. For BS sugar was observed at higher values in male flies until the 20% sugar diet above this concentration existed a predominance of female flies. Reactive oxygen species (ROS) levels using DCFH-DA assay show higher values for females compared to male flies, in both treatment sugar diets for all concentrations ($p < 0.001$).

Table 2 – Comparison of oxidative stress markers between males and females of the same concentrations and types of sugar

WHITE SUGAR						
C	Carbonil		DCF		TBARS	
(%)	Male	Female	Male	Female	male	female
5	14.3 ± 0.7	11.3 ± 0.0	12.9 ± 0.0	^{aaa}18.3 ± 0.0	-5.6 ± 2,9	0.0 ± 0.0
10	14,3 ± 0.7	14.1 ± 0.5	14.8 ± 0.0	^{aaa}18.3 ± 0.0	13.4 ± 1.3	^{a}22.4 ± 1.3
20	21.4 ± 0.7	16.0 ± 1.1	13.9 ± 0.0	^{aaa}19.2 ± 0.0	35.6 ± 0.8	40.0 ± 0.5
30	29.8 ± 1.4	^{aaa}60.7 ± 0.3	19.8 ± 0.0	^{aaa}24.0 ± 0.0	38,0 ± 0,8	41.2 ± 1.8
40	55.9 ± 0.0	^{aa}64.1 ± 3.03	26.1 ± 0.3	^{aaa}29.5 ± 0.3	^{aaa}109.7 ± 2.0	67.6 ± 1.0

BROWN SUGAR					
Carbonil		DCF		TBARS	
Male	Female	Male	Female	Male	Female
9.5 ± 1.2	a16.0 ± 1.1	7.3 ± 2.4	^{aaa}31.7 ± 2.4	^{aaa}31.1 ± 0.0	7.8 ± 2.6
^{aa}33.3 ± 1.8	25.5 ± 1.4	7.3 ± 2.3	^{aaa}38.1 ± 1.9	^{aaa}88.9 ± 6.4	44.6 ± 3.6
^{aaa}42.9 ± 1.4	28.0 ± 0.3	11.9 ± 3.2	^{aaa}46.5 ± 1.5	^{aaa}124.4 ± 3.1	102.6 ± 1.4
^{aaa}53.6 ± 0.7	35.8 ± 1.4	18.5 ± 0.9	^{aaa}48.4 ± 3.2	^{aaa}130.1 ± 3.8	176.4 ± 1.6
^{aaa}70.2 ± 0.7	55.7 ± 0.9	34.0 ± 1.4	^{aaa}64.1 ± 4.8	176.4 ± 3.5	182.9 ± 1.9

^{a} Por significant increase over the equal concentration pair, $p < 0.05$; ^{aa} for $p < 0.01$ and ^{aaa} for $p < 0.001$. C=Concentration. Comparison of oxidative stress markers between males and females flies for same concentrations and types of sugar (BS and WS sugars) diet.

5.3 Glucose levels

Glucose levels are presented in Fig. 3 (comparison between BS and WS sugars diet in male and female flies). In relation BS and WS sugars diet, an augmentation of glucose levels with the increase of the concentration of sugar for male and female flies was observed (Fig. 3A and 3B, respectively). Until 20% sugar diet concentration results are similar in male flies, above this concentration, higher values were observed for WS sugar. In female flies, BS sugar showed more values in relation to WS sugar.

Figure 3 – Glucose levels in male (Fig. 3A) and female (Fig. 3B) flies submitted to a high brown or white crystal sugar diet. (C=control). (*p < 0.05, **p < 0.01, ***p < 0.001 values, statist

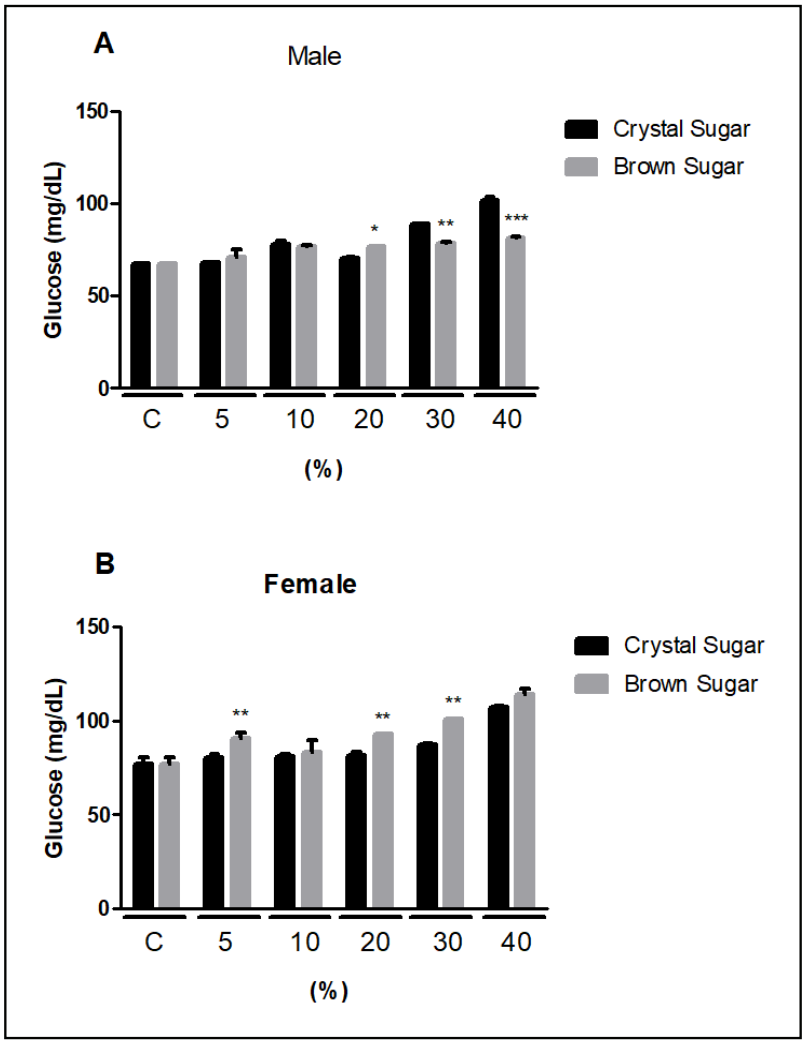
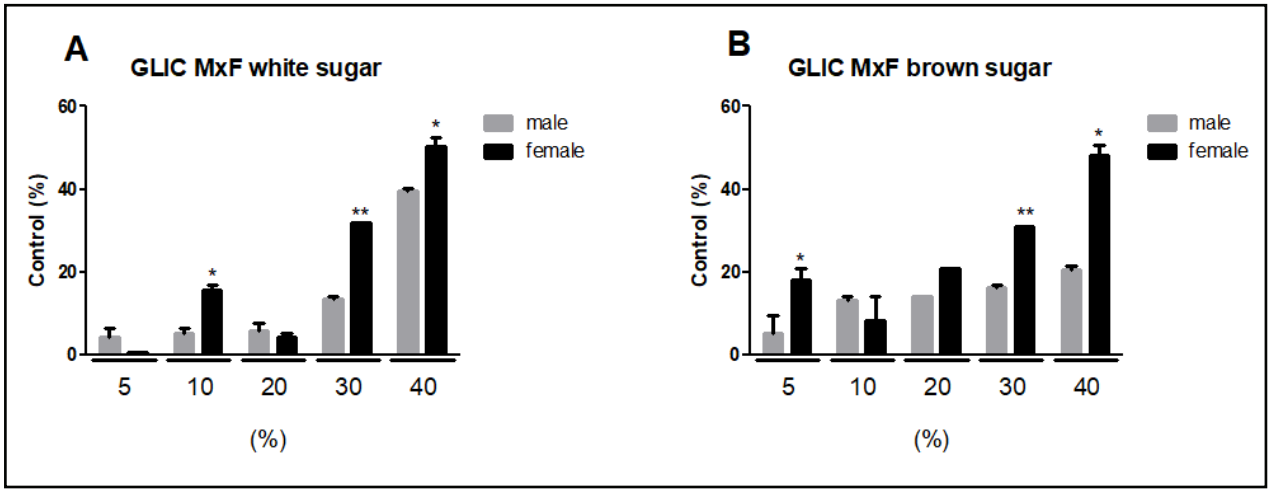


Fig. 4 compares the glucose levels for male and female *D. melanogaster* flies in the same sugar diet. Results showed elevated glucose levels with increasing sugar concentration in male and female flies with predominance for female flies. This behavior was observed in both WS and BS sugar treatment (Fig.4A and 4B).

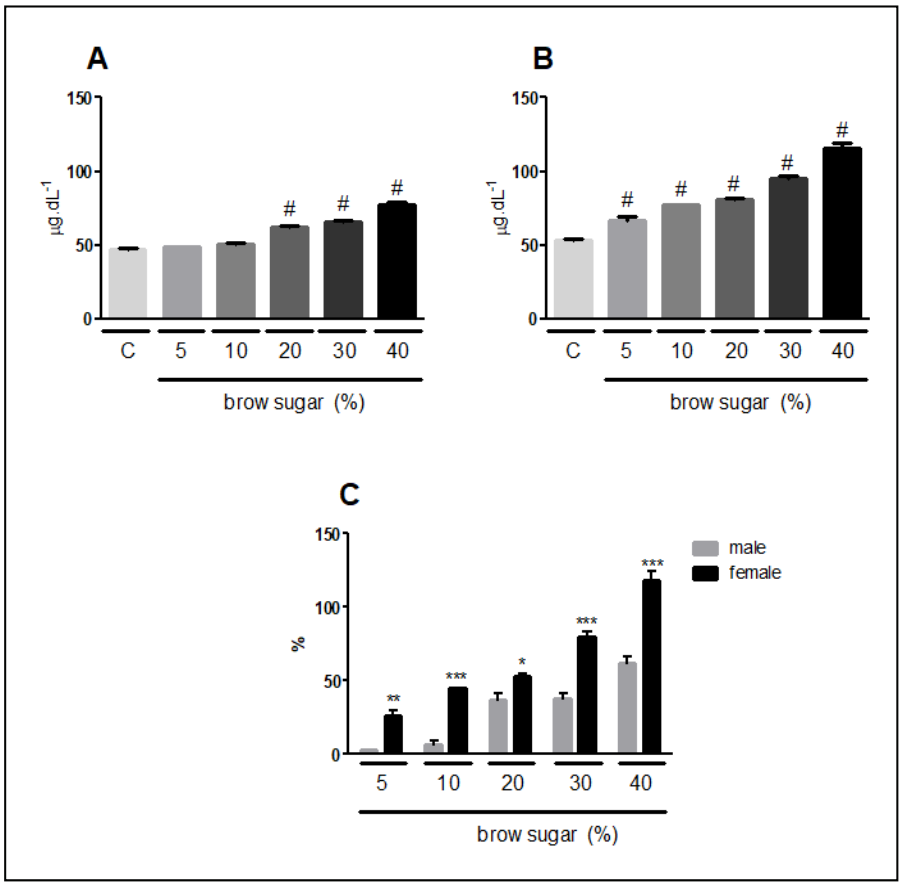
Figure 4 – Comparative Glucose levels between males and females of the same concentrations and types of sugar white (Fig. 4A) and brown (Fig. 4B) crystal sugar diet. (*p < 0.05, **p < 0.01 and ***p < 0.001 values, statistically significance).



5.4 Iron levels

The presence of the Iron level was determined in male and female flies for BS sugar treatment are shown in Fig. 5. For male (Fig. 5A) and female (Fig. 5B) flies results demonstrated an increase of iron level with an increase of the concentration sugar diet. When comparing male and female flies *D. melanogaster* (Fig. 5C), results show higher iron levels in females than in male flies. The iron level was not determined for WS sugar because this sugar type doesn't have iron in your constitution.

Figure 5 – Iron levels in BS sugar for males (Fig. 5A), females (Fig. 5B) and comparative values of iron levels (Fig.5C) between males and females of the same concentration sugar diet



6 DISCUSSION

The continuous increase in the consumption of sugars, mainly derived from sugar cane (sucrose), whether in free form, in soft drinks, sweets or other foods, has raised the concern of health agents from different countries and in general, from the Organization World Health Organization (WHO, 2015). The excessive consumption of sugars by the population, since childhood, along with a sedentary lifestyle and fatty foods has caused serious diseases such as diabetes, heart problems, hypertension, among others. Trying to understand the effect caused by high sugar consumption (sucrose), a study was developed for the first time that made it possible to monitor

this excess, using the *D. Melanogaster* fruit fly (Male and Female) as an alternative experimental model (MCGURK et al., 2015). *D. melanogaster* has been used as an experimental model in the study to assess oxidative stress (Sohal et al., 1995 and Galarisa et al., 2019), neurodegenerative diseases such as Parkinson and Alzheimer diseases, (Jeibmann and Paulus, 2009; Ramirez et al., 2011 and Finelli et al., 2004) among others. In this study, using *D. Melanogaster*, it was possible to evaluate the effect of a diet rich in sugar in the formation of the first generation (F1) of the flies, and its effect in adulthood through maintaining the diet. For the study two types of sugars were used, one processed namely white crystal sugar (WS) and the other natural named brown sugar (BS). Studies have shown that oxidative stress can contribute to the progression of signs and symptoms observed during the onset of diseases such as diabetes mellitus (Musselman et al., 2019), metabolic syndrome (Lang et al., 2019), Parkinson's (Aaseth et al ., 2018) among others.

Studies suggest that the consumption of high doses of sucrose associated in diets with the development of hyperglycemia in the fly is responsible for triggering oxidative events (Ecker et al., 2017). In this study, where the fruit fly was born and remained in a medium rich in sugar (excess of sugar), an increase in the enzymatic activity of SOD is observed for both WS and BS sugars in male and female *D. melanogaster* (Fig.: 1A-Male and 1B-Female). Higher values of SOD caused by the WS type, suggest a faster digestion process and consequently a higher production of ROS (Khatun et al, 2018). Although there is an increase in SOD values during a BS diet, their values are lower, possibly due to slower digestion. Comparatively, it appears that as flies promise higher values in relation to males, corroborating as described by Pickering et al., (2012) that as must flies they can adapt to the hydrogen peroxide stress (Table 1), both for the consumption of WS and BS.

Table 1 also indicates lower values for BS compared to WS when comparing equal concentrations between males and females. Studies suggest that the presence of iron in brown sugar induces an increase in Reactive Oxygen Species (ROS) (Khatun

et al, 2018). The increase in the activity of the enzyme Superoxide Dismutase (SOD) may be associated with a high formation of potentially harmful intermediates, such as the superoxide anion (O2-·) (Barbosa et al., 2010).

When evaluating the activity of the enzyme Catalase (CAT), responsible for the decomposition of hydrogen peroxide, whose comparative results between the WS and BS diets indicate higher values of CAT for BS compared to WS, with a slight tendency to decrease the values with the increase in the concentration of the diet used, when compared to the control group. The greatest decrease observed in WS may be related to an increase in H2O2 decomposition, produced through the Fenton reaction and a faster digestion process (Fig .: 1C-Male and 1D-Female), already in a BS type diet. , it is suggested that the effect may be related to the presence of iron and slower digestion, making the process slower. Comparatively between males and females (Table 1), results show higher values for male flies compared to females, suggesting that hormonal differences may be involved, altering the digestion metabolism of BS and WS sugars.

Results obtained in the analysis of Glutathione Peroxidase (GPx) indicate a tendency of decreasing values with the increase of the concentration of sugars in the diet, both for WS and BS (Fig.:1E – Male and 1F-Female) (Table 1). In males, there is a tendency for higher values in the BS diet compared to the WS diet, whereas for female flies higher values are observed in the WS diet. The behavior presented by the results of the antioxidant enzymes, suggests that the first line of enzymatic defense caused by SOD promotes the greatest defense against reactive species (RS) formed by excess sugar consumption. The antioxidant activity observed through the SOD, CAT and GPx assays from a diet rich in WS and BS sugars suggests that the excess sugar in *D. melanogaster* causes a greater generation of RS, especially the superoxide anion radical (O2-·), triggering, in a first stage of defense, the SOD (in the cytoplasm) producing the Hydrogen Peroxide (O^{2-}*+ SOD → H_2O_2). In a second stage, in the cytosol, through CAT, forming H_2O e O_2, (H_2O_2 + CAT → H_2O + O_2) (Barbosa et al., 2010).

Another process occurs when H_2O_2 reacts with Fe^{2+} through the Fenton reaction forming the Hydroxyl radical ($H_2O_2 + Fe^{2+} \rightarrow$ *OH), although the hydroxyl radical can also be formed, directly via superoxide ion. The small variation observed in the CAT results and the decrease in GPx with the increase in the concentration of sugars, corroborate this statement. Another important factor observed in the literature is the presence of iron. According to RAMIREZ et al. (2011), an increase in iron consumption in *D. melanogaster* reduces the lifespan and locomotor activity of the flies, due to neurodegeneration in the dopaminergic neurons of the flies.

The formation of superoxide anion through a high sugar diet can also trigger the formation of hydroxyl radicals (*OH) (Ighodaro, 2018). The presence of the hydroxyl radical can cause oxidative damage in biomolecules, as observed in the lipoperoxidation (TBARS), protein carbonylation and DCFH-DA assays (Figure 2 and Table 2) The results obtained due to the increase in sugar consumption in *D. melanogaster*, male and female indicate an increase in the rates of Lipid Peroxidation (TBARS) (Figures 2A and 2B), Carbonylated Protein (Figures 2C and 2D) and ROS (DCFH-DA) (Figures 2E and 2F) for flies of both sexes (male and female), corroborating the results previously observed in SOD, CAT and GPx. Lipid Peroxidation occurs from the reaction of a free radical with an unsaturated fatty acid and is propagated by means of peroxyl radical (ROO*) finally forming lipid and aldehyde hydroperoxides such as malonaldehyde. The presence of malonaldehyde can be determined by reacting with thiobarbituric acid (TBARS). In this sense, the results obtained indicate for WS sugar, similar behavior in males and females up to the 30% sugar diet, in 40% the effect was greater in males. The observed behavior is that the excessive consumption of WS sugar causes damage to the lipid wall in *D. Melanogaster*, mainly in male flies. Already in a diet rich in BS, the results presented that the increase in sugar consumption produces cellular damage (lipoperoxidation), affecting mainly surviving flies, according to the study by Chandegra et al., 2017, as female *D. melanogaster* will present an advantage in relation to male flies for most of the stress tested, with for example oxidative stress. The lipid damage caused by the

increased consumption of BS sugar, possibly is due to the higher iron content present in BS, producing more ROS (Fenton reaction), causing greater lipid damage in female *D. melanogaster* (Tower et al., 2020)..

The increase in protein damage was observed with the increase in sugar concentration when compared to the control, for both WS and BS for male and female flies (Figure 2 C and 2D). In male flies (Figure 2C), the greatest protein damage was caused by BS sugar, whereas for females (Figure 2D), a greater effect was observed caused by WS sugar. However, the results did not show significant variations between male and female flies (Table 2) for the WS diet, suggesting that the reactive species act similarly regardless of the sex of the flies. Although, in the diet using BS sugar, the effect was more pronounced in male flies (Table 2).

The increase in the formation of ROS with an increase in the concentration of sugars in the diet can be confirmed by the results obtained in the analysis by DCFH-DA. Figure 2E (males) and 2F (females) presented data showing an increase in the ROS rate, both for WS sugar ((Males: 114% (5% sugar) – 127% (40% sugar), Females (123% (5% sugar) – 135% (40% sugar)) as BS ((Males: 108% (5% sugar) – 135% (40% sugar)), Females (137% (5% sugar) – 171% (40 % sugar)), when compared to the control (100%).

In male flies, the difference between the two types of diets (WS and BS) showed little difference between them, however, in female flies the consumption of BS sugar showed higher values in relation to the WS diet, suggesting once again that one of the effects of increased ROS are linked to the presence of iron in BS sugar. Comparative evaluation between males and females (Table 2) shows a more pronounced effect in females, possibly due to reproductive investment, where the need for large production of biomass leads women to a more anabolic state (Chandegra et al. 2017).

In addition to the ROS formation, the increased consumption of a diet rich in sugars (sucrose) causes changes in glucose production, the results of which are shown in Figure 3 (Comparative WS and BS: A-males, B-females) and Figure 4 (Comparison male and female, for the same type of sugar). Results obtained indicate an increase in

the glucose rate with an increase in the sugar concentration for both diets (WS and BS) (Figure 3) when compared to the control. In male flies (Figure 3A) the effect was greater for the WS diet and in female flies (Figure 3B), the greater effect was caused by BS. When the results are compared between male and female flies for the same concentration of sugar consumed, there is a predominance of higher values for both sugars in female flies. It is observed in the literature that the increase in glucose levels, in addition to causing ROS (ROVENKO et al., 2015) causes hyperglycemia, leading in the future to diseases such as Diabetes Mellitus (Chatterjee et al., 2017), atherosclerosis (Bornfeldt, 2016) cardiovascular disease (Nishizawa and Bornfeldt, 2012), among others.

Our results clearly demonstrate a more pronounced effect in female flies, especially when evaluating the BS-rich diet. The results have suggested that these effects may be caused by the consumption of iron present in the BS. According to the results obtained (Figure 5), there is an increase in the levels of iron present in the flies, with an increase in the concentration of BS (5A-males, 5B-females). Comparing male and female flies (Figure 5C), the predominance of higher levels in female flies is clearly seen. The increase in iron levels in the body can contribute to the formation of ROS (Calap-Quintana et al., 2017) mainly through the Fenton reaction, corroborating to explain the results obtained through the enzymatic antioxidant defenses and oxidizing effects (TBARS, Carbonyl and DCFH-DA).

7 CONCLUSIONS

The results obtained in this study allow us to conclude that, under experimental conditions, a diet rich in white or brown crystal sugars causes oxidative stress in *D. melanogaster*. Results obtained indicated an increase in the enzymatic activity of SOD, CAT and GPx, as well as lipid peroxidation (TBARS), carbonyl content and ROS (DCFH). The increase in Glucose and Iron levels was again observed. The effects were more pronounced in female flies than in male flies. All results suggest that the iron in brown crystal sugar is responsible for the increase in oxidative stress and ROS damages.

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