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Bisphenol A exposure is involved in the development of Parkinson like disease in *Drosophila melanogaster*

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ABSTRACT

The pathogenesis of Parkinson's disease has not been fully clarified yet but its cause is known to be multifactorial. One of these factors is oxidative stress induced by exposure to environmental toxifiers. We studied the effect of Bisphenol A (BPA) at concentrations of 0.5 mM and 1 mM, the concentration of 1 mM corresponding to Lowest Observed Adverse Effect Level (LOAEL) for humans in adult *Drosophila melanogaster*. The BPA induced oxidative stress was established by increased levels of malondialdehyde, reactive species, and decreased activity of the antioxidant enzymes superoxide dismutase and catalase, and detoxificant enzyme glutathione-S-transferase. Associated with oxidative stress, there was a reduction of acetylcholinesterase activity and a reduction of dopamine levels, which are related to the decreased locomotion activity as observed in negative geotaxis, open field and equilibrium behaviors in group exposed to 1 mM of BPA. Oxidative stress also impaired mitochondrial and cellular metabolic activity in the head causing an increase in the mortality of flies exposed to both BPA concentrations. Therefore, BPA induced Parkinsonian-like changes in flies and it is possible that the oxidative stress is closely related to this effect, providing new insights for future studies.

1. Introduction

Bisphenol A (BPA) (Fig. 1) is a synthetic phenol widely used in polycarbonate manufacturing and epoxy resins (Jalal et al., 2018). Thus, BPA is present in kitchen utensils, packaging, heat-resistant plastic bottles and inner coating of canned food (Geens et al., 2012; Vandenberg et al., 2007). When these items are subjected to washing processes, heating or contact with acidic or basic pH, the hydrolysis of ester bonds of the molecule occurs, thus BPA monomers are released to the environment and contaminate food and beverages (Bae et al., 2002; Vandenberg et al., 2007).

Continuous exposure to BPA is directly related to changes in the central nervous system (Inadera, 2015). Among the target neurological systems, the dopaminergic system is strongly affected by exposure to BPA (Jones and Miller, 2008). In rodent studies, decreased nigra substance was observed due to the degeneration of dopaminergic neurons, reduction of tyrosine hydroxylase (TH) activity and dopamine (DA) transporters (Ishido et al., 2007). In this sense, it is worth noting that changes in the dopaminergic system result in debilitating behavioral disorders such as Parkinson's disease (PD) (Jones and Miller, 2008), characterized by the progressive loss of dopaminergic neurons (Ebrahimi et al., 2017).

The dopaminergic neurodegeneration has the oxidative stress as an important mediator (Jones and Miller, 2008). Qiu et al. (2016) found that there was induction to oxidative stress by prolonged exposure to low doses of BPA. The oxidative stress caused by BPA also compromised mitochondrial activity and reduced the activity of complex I (NADH ubiquinone oxidoreductase) (Ooe et al., 2005), and decreased mitochondrial enzyme activity (Khan et al., 2016). Even though many results have been evaluated in isolation, and some factors may contribute to the development of PD by exposure to BPA in rodents (Jones and Miller, 2008; Ooe et al., 2005), the results are still inconclusive, but still suggest that BPA is an important risk factor.

It is important to emphasize the characterization of new

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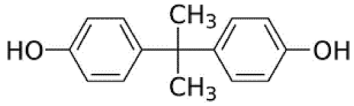

Fig. 1. Chemical structure of Bisphenol A (BPA).

experimental models of degenerative diseases, and it is fundamental to understand more about the appearance and progression of these diseases. *Drosophila melanogaster* is an alternative model valid for studies related to PD (Poddighe et al., 2013), because it has a structure and neurological function similar to mammals, but ordered in a simpler way, which makes it easier to observe the mechanism of action of xenobiotics in the development and progression of the disease (Nagoshi, 2018). In addition *Drosophila melanogaster* exhibits behaviors dependent of DA, controlled by dopaminergic neurons, widely distributed in the brain (Chen et al., 2014; Nagoshi, 2018). The fly is also feasible for studies related to oxidative stress, due to its biochemical and metabolic similarity to humans (Doran et al., 2017). Therefore, the objective of this study was to show the toxicity of BPA in the adult nervous system, elucidating the mechanisms involved in the development of Parkinson disease in *Drosophila melanogaster*.

2. Materials and methods

2.1. Chemicals

The BPA (Fig. 1) (> 99% purity; Sigma Aldrich, St. Louis, MO, USA) was diluted in 0.1% of Dimethylsulfoxide (DMSO), according to the methodology of Kaur et al. (2015). The DMSO and other analytical grade reagents used in this work came from the laboratory of the Federal University of Pampa, Campus Itaqui.

2.2. Drosophila melanogaster stock and culture

It was used *Drosophila melanogaster* of Harwich strain, wild type, obtained from the National Species Center (Bowling Green, Ohio, USA). The flies were kept in glass flasks, under controlled temperature of 25 ± 1 °C, humidity of 60–70% and circadian cycle light/dark of 12 h, fed with standard food composed of corn flour (76,59%), wheat germ (8,51%), sugar (7,23%), milk powder (7,23%), salt (0,43%) and antifungal methylparaben.

2.3. Experimental protocol

Flies of both sexes, aged between one and two days were divided into three groups, containing 50 flies each. The control group received only 0,1% DMSO. For the exposure to occur orally, BPA was incorporated in food in concentrations of 0.5 mM and 1 mM (Fig. 2). The highest concentration (1 mM) corresponds to the Lowest Observed Adverse Effect Level (LOAEL) of 50 mg/kg body/day for humans (Kaur et al., 2015).

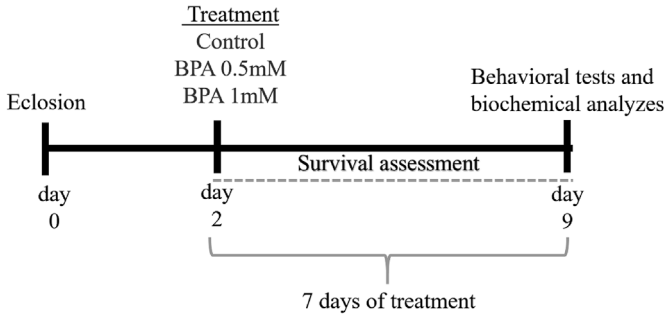

Fig. 2. Schematic diagram of the experimental treatment protocol.

2.4. Survival assessment

The influence of BPA on the survival of flies was valued. For this, the daily count (1x at the end of each day) of the number of alive flies was performed during the seven days of treatment of the control group, BPA 0.5 and 1 mM. Each group consisted of 50 adult flies of both sexes added randomly to the treatment. There was no exchange of food during these seven days to exclude the risk of death by the manipulation process. Three independent experiments were performed.

2.5. Behavioral tests

After 7 days of treatment, the flies of both sexes were submitted to behavioral tests. The tests were conducted in an acclimatized environment (25 ± 1 °C), between 10AM and 4PM, to avoid changes in the behavior, because according to Kaur et al. (2015), the circadian rhythm is related to changes in performance and variations in fly behavior.

2.5.1. Negative geotaxis

The negative geotaxis test corresponds to the innate escape response in flies. It is widely used in studies related to the development of neurodegenerative diseases, because it is sensitive to deficits in motor coordination and muscle tone (Chen et al., 2014). The method used was the one described by Jimenez-Del-Rio et al. (2010), with some adaptations.

The test was performed on five flies from each group, from four independent experiments. The flies were anesthetized and transferred individually to tubes. After 10 min of recovery, the tubes (one at a time) in an upright position were lightly tapped on a flat platform so that the flies remained at the base of the tube.

Then the timer was quickly triggered beginning the count of the time spent by each fly to reach the height of 8 cm, measured from the base of the tube. Each fly had a time maximum of 120 s to scroll the apparatus until reaching 8 cm of height. The time interval between one repetition and another for each fly was 3 min. During the test, the flies remained in their respective tubes. The test was repeated five times per fly, and the average of the times was calculated individually for the statistical analysis.

2.5.2. Open field test

Through the open field test, it is possible to evaluate the spontaneous locomotor capacity of the flies. The test was performed according to the methodology established by Connolly (1966), with some adaptations. Fifteen flies from each group from four independent experiments were used, totaling 60 flies per group used for the test application. The flies were lightly cryoanesthetized in a falcon tube and separately added to a transparent polycarbonate petri dish (9 mm in diameter) with the aid of a soft bristle brush.

The plate cover was graduated with squares measuring 1 cm^2 each, without spacing, slightly marked with a thin line, so it would not interfere with the behavior of the flies during the test. The test started 2 min after the flies were added to the plate. This amount of time is appropriate to reestablish the flies anesthesia and acclimate them to the plate, so it would not interfere with the test. After 2 min, the open field test was performed.

Thus, in order to evaluate the spontaneous locomotor activity of the flies, the number of quadrants walked by the flies was observed and visually counted with the help of a manual counter, triggered at each crossed square by the fly, during 60 s timed with the aid of a digital chronometer. The test was performed in duplicate and the mean values were calculated.

2.5.3. Test of motor coordination

Some modifications were performed in the methodology described by Iliadi et al. (2016), however the test is effective for measure the

motor coordination and locomotion of *Drosophila melanogaster*. Fifteen flies of both sexes were anesthetized with ice to enable the cutting of its wings, a procedure performed 3 days before the test. The test apparatus consists of an acrylic box, measuring (28 cm long, 11.8 cm wide and 2.4 cm high), with water (23–27 °C) up to a distance of 5 mm from the transparent nylon line (0.6 mm), which was secured between two platforms submerged in water.

The test was performed simultaneously in the 3 groups (Control, BPA 0.5 mM and BPA 1 mM). Thus, the flies were individually one added on the platform, with a brush, and it was timed when the fly passed the red zone (located 1 cm after the platforms). The maximum time for locomotion of 13 cm was 60 s. According to the literature, the test was performed in triplicate, each fly performed the test three consecutive times, without time interval, and the average of the three performances of each fly was evaluated.

Thus, the average rate of time observed (seconds) was used to assess the average distance traveled (mm) for each of the 15 flies.

2.6. Ex vivo assays

2.6.1. Homogenized preparation

The flies were first anesthetized and euthanized on ice after 7 days of treatment. Subsequently, flies were beheaded for sample production. Each sample is consisted for homogenization of a number of fly heads (the number of heads to be homogenized together depends on the protocol of each analysis). AChE activity was measured separately in flies head and body samples, similar to the others.

2.6.2. Determination of acetylcholinesterase (AChE) activity

Twenty heads and 20 bodies of each group were homogenized in 200 μL and 800 μL of HEPES buffer (20 mM, pH 7.0) respectively and centrifuged at 78G for 10 min, according to Ellman et al. (1961), with some adaptations. A mixture containing 0.25M KPi buffer (pH 8.0) and 5,5′-dithiobis (2-nitrobenzoic acid) (5 mM DTNB) was prepared. At the time of analysis, the supernatant sample heads (15 μL) were added sequentially to 935 μL of the mixture and 50 μL of acetylthiocholine (AcSCh) (7, 25 μM) and 50 μL of sample were used in the body samples for 900 μL of mixture and 50 μL of acetylthiocholine (AcSCh) (7.25 μM). The AChE activity was determined spectrophotometrically at 412 nm for 2 min, expressed as μmol AcSCh/h/mg protein.

2.6.3. Determination of reactive species levels

The oxidation of DCFDA as free radical generation index and oxidative stress were measured in the supernatant of the samples, according to Pérez-Severiano et al. (2004). Twenty heads of flies were homogenized in 1 mL of Tris buffer (10 mM, pH 7.0) and centrifuged at 1004G for 5 min. An aliquot of 34 μL sample supernatant was added to a mixture containing 964 μL HEPES buffer (pH 7.0) and 10 μL 2,7-dichloroflurescein diacetate (3,33M; DCFDA). Therefore, after 1 h of incubation, the emission of fluorescence from the DCFH resulting from the oxidation of DCFDA was monitored in a spectrophotometer in an excitation wavelength of 488 nm and an emission wavelength of 520 nm. The results obtained were expressed as a percentage of the control. The average of three independent experiments was used totaling 60 flies for each group.

2.6.4. Determination of thiobarbituric acid reactive substances

Considered a marker of oxidative stress, malonildialdehyde (MDA) is a final product resulting of lipid peroxidation. Its content in supernatants was measured according to Ohkawa et al. (1979) with some modifications. Fifteen fly heads were homogenized in 90 μL of 0.1M HEPES buffer (pH 7.0) and centrifuged at 78G for 10 min. The supernatant was transferred (50 μL) to assay tubes with solution containing 125 μL of thiobarbituric acid (TBA, 8%), 12 μL of acetic acid, 50 μL of Sodium dodecyl sulfate (1.2% SDS) and 25 μL of water, and taken to incubation in a water bath at 95 °C for 2 h. After cooling to room temperature, 200 μL were transferred to acrylic microplates for spectrophotometer reading at 532 nm.

2.6.5. Antioxidant and detoxifying enzymes activities

To evaluate the action of BPA on the antioxidant and detoxifying defense system, the activity of catalase (Cat), SOD (superoxide dismutase) and Glutathione-S-transferase (GST) enzymes was evaluated. The preparation of the samples to analyze the activity of both enzymes was similar, following the same protocol. Therefore, 20 heads of flies were homogenized in 200 μL of 0.1M HEPES buffer (pH 7.0), and centrifuged at 15366G for 30 min. The supernatant was reserved to carry out the analysis.

2.6.5.1. Determination of superoxide dismutase activity. The activity of SOD was evaluated by the method described by Kostyuk and Potapovich (1989), with modifications by Franco et al. (2009), by monitoring the inhibitory effect of SOD on quercetin oxidation. To produce the analysis samples, an aliquot of 10 μL of the supernatant was diluted in 90 μL of HEPES buffer. The reaction solution comprised sodium phosphate buffer (0.025M/0.1 mM EDTA, pH 10) and N, N, N-tetramethylethylenediamine (TEMED) was added (1 mL) along with 10 μL of diluted sample and 50 μL of quercetin in the cuvette for spectrophotometer reading at 406 nm for 2 min. The results were corrected for the absorbance of the amount of protein present in the supernatant sample, calculated as percentage inhibition of the oxidation of quercetin.

2.6.5.2. Determination of catalase activity. The activity of Cat was determined according to the methodology of Aebi (1987) with some modifications made by Paula et al. (2012), which consists in the enzyme's ability to degrade H_2O_2. It was added 30 μL of supernatant to a quartz cuvette along with 2 mL of reaction mixture composed of 0.25M KPi buffer/2.5 mM EDTA pH 7.0, and Triton H_2O_2. The reading was performed in a spectrophotometer at wave length of 412 nm for 2 min. The analysis was performed in 5 independent experiments, with samples in duplicate, and the results were corrected according to the protein concentration. The final result was expressed in Cat unit per mg protein (U/mg).

2.6.5.3. Glutatione-S-transferase activity. The GST activity was determined according to the method described by Habig et al. (1974), with some adaptations. The technique consists in the catalytic action of GST in the conjugation reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH) to obtain a thioether (4-dinitrophenyl glutathione).

To perform the reading in a spectrophotometer at 340 nm for 2 min, it was added to an acrylic cuvette 30 μL of supernatant, 1000 μL of mix (0.25M KPi buffer/2.5 mM EDTA pH 7.0, 100 mM GSH and distilled water) and finally, 20 μL of the CDNB primer substrate (50 mM). The results were corrected by value of protein and expressed in milli units of enzyme activity/mg protein (mU/mg protein).

2.6.6. Evaluation of mitochondrial metabolic activity by the reduction of MTT (3–4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide)

The methodology was performed according to Hosamani et al. (2010), with some modifications. Therefore, approximately 100 heads of flies were homogenized on ice Tris-sucrose (0.25M, pH 7.4) buffer containing Mannitol (220 mM), Sucrose (68 mM); KCl (10 mM), HEPES (10 mM) and BSA (0.1%). The homogenate was centrifuged at 999 G for 5 min. The supernatant was removed and the homogenate was centrifuged again to obtain the pellet. Then, 200 μL of suspension buffer were added (Tris 0.25M sucrose, pH 7.4 without BSA) to sediment. After 3 h on ice, the samples were incubated with MTT for 30 min at 37 °C and centrifuged at 7840G, for 5 min. Upon removing the supernatant, 200 μL of DMSO were added and the sample was re-incubated for 30 min at 37 °C and centrifuged at 7840 G for 5 min. Finally, 150 μL

of the supernatant were transferred to microplates for spectrophotometer reading at 540 nm. The results were expressed as a percentage of the control (n = 4–6).

2.6.7. Evaluation of cellular metabolic activity by the Resazurin reduction method

Cell viability was assessed according to Franco et al. (2009). The method consists in the ability of cells to reduce resazurin, forming resorufin that is a fluorescent molecule. Samples for analysis were obtained by homogenizing 10 fly heads in 100 mL of 20 mM Tris buffer (pH 7.0), and centrifuging at 999G for 10 min. The supernatant was removed, and 20 μL of the sample was added to an acrylic microplate along with 180 μL of 20 mM Tris (pH 7.0) and 10 μL of resazurin. Every 1 h, for the total of 4 h the readings were carried out at 573 nm. The value of the last reading was considered as the final result and it was expressed as a percentage of control.

2.6.8. Determination of dopamine levels

Dopamine levels were determined through High Performance Liquid Chromatography with a Diode Matrix Detector (HPLC-DAD) equipped with a quaternary pump and automatic sampler, as described by Soares et al. (2017). For the preparation of the samples, 30 heads of flies were homogenized in 288 μL of 0.9% NaCl and 12 μL of 0.5M HCl for 1 min. Subsequently, this homogenized solution was centrifuged for 10 min at 7840G at −4 °C. After centrifugation, the supernatants were filtered with 0.22 μm syringe filters. Next, 200 μL of sample were injected into the HPLC system by the automatic sampling device. Ultrapure water and methanol (12.5%), pH 2.5, were used in the mobile phase. The flow was maintained at 0.8 mL/min and the detection was performed at 198 nm.

2.6.9. Determination of protein concentration

The determination of protein concentration in replicate fly head samples were made according to the method of Bradford (1976), using bovine serum albumin as standard.

2.6.10. Statistical analysis

GraphPad Prism software, version 6 (San Diego, CA, USA) was used to perform the statistical analysis. The normality was assessed with the Shapiro–Wilk test. Fly survival rates during the 7 days of treatment were determined by the log-rank test (Mantel-Cox). Behavioral data analysis was done by Kruskal-Wallis one-way analysis of variance followed by Dunn's post hoc test. The other data were analyzed by one-way analysis (ANOVA), using Bonferroni test for multiple comparisons. The results of the nonparametric data analysis were represented as box graphs, and the parametric analysis data as mean and standard deviation (SD). Correlation analyzes were also performed using the Pearson correlation coefficient. Values of probability less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Results

3.1. Survival assessment

Adult flies were exposed for 7 days at two concentrations of BPA (0.5 mM and 1 mM) had a decrease in survival rate, when compared to the control group ($p < 0.0001$, Fig. 3).

3.2. Behavioral tests

All behavioral tests are shown in Fig. 4. In the negative geotaxis test, BPA increased the climbing time of adult flies exposed to higher BPA concentration (BPA 1 mM) compared to the control group (Fig. 4A $p < 0.0024$). In the open field test, locomotion of adult flies exposed to 1 mM BPA concentration also decreased compared to the control group (Fig. 4B $p < 0.0005$). In the negative geotaxis and open field tests

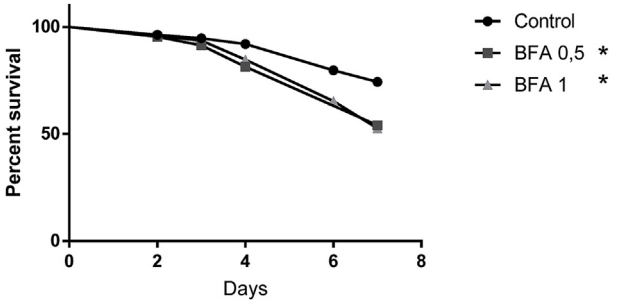

Fig. 3. Evaluation of the survival rate of adult flies exposed to Bisphenol A (BPA) for 7 days. The total number of flies represents the sum of 3 independent experiments. Mortality was determined by comparing the survival curves of the Mantel-Cox log-rank tests.

there was no statistically significant difference in relation to the groups exposed to concentration of BPA 0.5 mM. In the test that assessed the motor coordination of the flies, BPA at both concentrations affected the locomotion and balance of the flies compared to the control group (Fig. 4C $p < 0.0001$).

3.3. AChE activity

In the head samples, there was a decrease in the activity of the AChE enzyme in both groups exposed to BPA (0.5 mM and 1 mM), when compared to the control group (Fig. 5A $p < 0.0007$; F = 18.08), however the body samples. AChE activity did not change (Fig. 5B $p < 0.2738$; F = 1.620).

3.4. Oxidative stress and inhibition of the detoxifying and antioxidant protection system by exposure to BPA

It is possible to observe the significant increase in the production of reactive species in the heads of the flies in the group that was exposed to 1 mM BPA (Fig. 6 $p < 0.0005$; F = 20.16), whereas in the group exposed to the lowest concentration of BPA 0.5 mM had no statistically significant increase of reactive species when compared to the control group. In relation to the TBARS analysis (Fig. 7 $p < 0.0001$; F = 119.9), BPA increased lipid peroxidation, at both concentrations tested.

BPA decreased the activity of the antioxidant enzymes SOD (Fig. 8 A $p < 0.0005$; F = 20.16) and Cat (Fig. 8 B $p < 0.0001$; F = 50.47) at both concentrations. There was also a reduction in the activity of the detoxifying enzyme GST (Fig. 8 C $p < 0.0065$; F = 9.282) in both groups with BPA when compared to the control group.

3.5. Mitochondrial and cellular metabolic activity

There was a decrease in mitochondrial metabolic activity in both groups treated with BPA, when compared to the control group (Fig. 9 $p < 0.0001$; F = 39.41). There was also a decrease in the cell viability of the groups exposed to BPA at both concentrations compared to the control group (Fig. 10 $p < 0.0001$; F = 64.22).

3.6. Depletion of the levels of dopamine (DA) induced BPA

Exposition to BPA at the highest concentration (1 mM) decreased DA levels in the head of the flies (Fig. 11 $p < 0.0275$; F = 4.922), whereas the lowest concentration of BPA (0.5 mM) did not alter the DA levels. Decreases in dopamine levels correlate with decreased locomotion and fly balance (Table 1 $p < 0.05$), and it also increased oxidative stress at brain level (Table 2 $p < 0.05$).

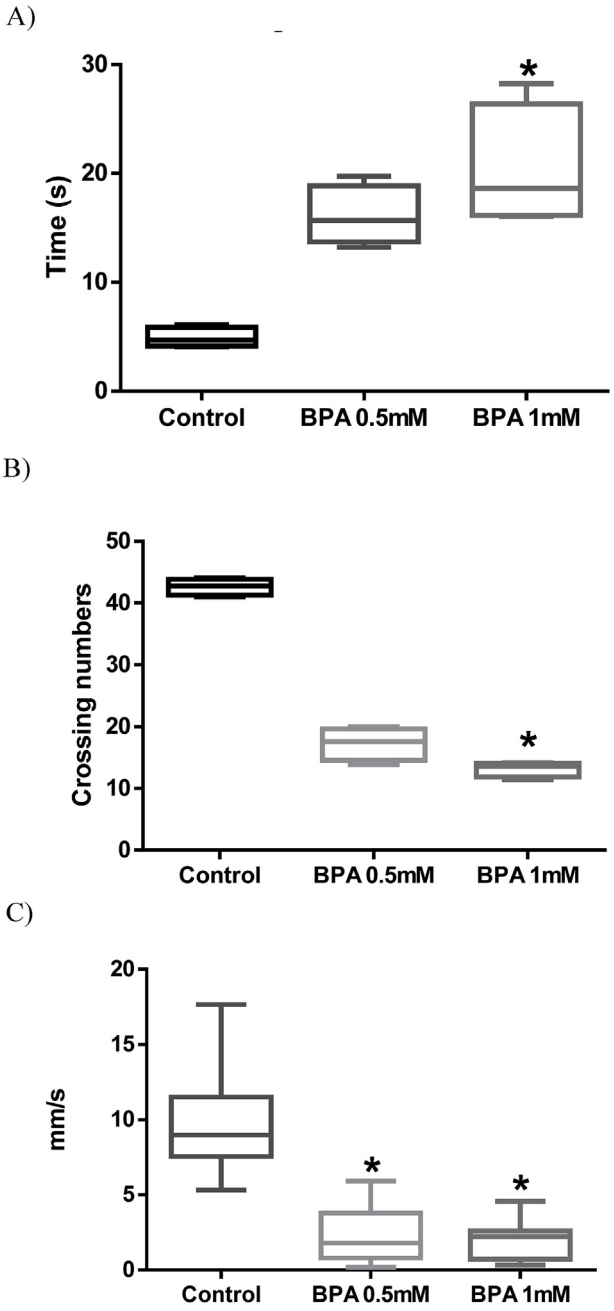

Fig. 4. Evaluation of locomotor activity of adult flies exposed to different concentrations of Bisphenol A (BPA) for 7 days, evaluated by A) Negative geotaxis, B) Open field and C) Balance test. Significance was determined by nonparametric analysis Kruskal-Wallis. Values are expressed as median and range (interquartile interval). *Significant difference compared to control group ($p < 0,05$).

4. Discussion

Environmental pollutants can cause various behavioral and biological changes in flies. The induction of oxidative stress mediated by BPA in *Drosophila melanogaster* can be one of the possible mechanisms for the development of various diseases. In this study we observed that BPA promoted oxidative stress in flies by increasing lipid peroxidation and free radicals levels, in addition to inhibiting antioxidant enzymes, causing a mitochondrial and cellular metabolic imbalance. There was a decrease in dopamine levels and a decrease in acetylcholinesterase activity, which are related to changes in the locomotor behavior, similar to PD. These biochemical changes induced by BPA are factors that influenced the survival of the flies during the 7 days of treatment. Thus, the groups that were exposed to BPA had an increase in mortality, when compared to the control.

It was observed that there was an increased production of reactive species (RS) in the head of the flies in the group exposed to the highest concentration of BPA (1 mM), which corresponds to LOAEL for humans. The group that was exposed to the lowest concentration of BPA (0.5 mM) had no statistical difference in relation to the control, however, a tendency to increase RS production was observed. This result is concomitant to the increase in lipid peroxidation, since BPA at both concentrations triggered the increase in MDA levels. As TBARS is a broad indicator of lipid peroxidation (Kabuto et al., 2003), the results indicate that exposure to BPA caused damage to the membranes of the brain tissue due to the increase in production of reactive species.

BPA also damaged the detoxifying and antioxidant system of *Drosophila melanogaster*. This compost at both concentrations decreased the activity of GST, a phase II detoxifying enzyme that solubilizes conjugated substances in phase I (Chahine and O'Donnell, 2011). The phase I system conjugates BPA converting into reactive species. Thus, our result indicates that reduction in GST activity promotes non-neutralization of these reactive species generated in the phase I conjugation, contributing to oxidative stress at the brain level. The antioxidant enzymes SOD and Cat also had decreased activity in both groups that received BPA. SOD catalyzes the conversion of the superoxide anion radical into H_2O_2. The H_2O_2 generated in the cells or from the action of SOD are converted by Cat into reactive species more soluble in water (Fridovich, 1997). Thus, the decrease in the activity of SOD and Cat reflect in the increase of the ROS and LPO levels, similar to a study by Anet et al. (2019), and emphasize that BPA is an inducer of oxidative stress.

Oxidative stress and disruption of the electron transport chain causes mitochondrial damage (Khan et al., 2016). It can be indicated by the MTT reduction assay that is mediated by dehydrogenase enzymes present in the mitochondria (Caughlan et al., 2004); therefore, the dehydrogenases inactivation decreases the MTT reduction, thus characterizing the mitochondrial damage. BPA was able to reduce the metabolism of MTT, at both concentrations, by asserting mitochondrial damage. The chemical structure of BPA confers greater affinity to mitochondrial membranes that are constituted in his internal by hydrophobic proteins rather than hydrophilic (Law et al., 1986; Nunez et al., 2001). Thus, BPA accumulates in the mitochondrial membrane inhibiting the complex I of the respiratory chain (Ooe et al., 2005; Khan et al., 2016), interfering in electron transport and increasing the production of reactive oxygen species, which may be involved in the immobilization of mitochondrial enzymes. Mitochondrial damage can lead to cell death (Shirani et al., 2019). Thus, through the resazurin reduction assay, as an indicator of cell damage, also mediated by dehydrogenases, it is possible to observe that BPA even at low doses decreased neuronal cell function.

Oxidative stress is also a primary mediator of dopaminergic neurodegeneration (Jones and Miller, 2008). Dopaminergic neurons are extremely vulnerable to degeneration, and the death of these neurons is related to the development of PD (Ishido and Masuo, 2014). In a study by Ishido et al. (2007), it was found that BPA decreased dopamine levels, tyrosine hydroxylase activity and dopamine transporters, and increased the death of dopaminergic neurons. In this study, we observed that the group exposed to 1 mM of BPA had their dopamine levels reduced in comparison to the control group. The decrease in dopamine correlated with all the oxidative stress markers mentioned above. Thus, we observed that the decrease in dopamine is related to the decrease in the activity of antioxidant (SOD and Cat) and detoxifying (GST) enzymes, and increased levels of RS and MDA.

The decrease dopamine is also related to the characteristic behavior of PD, such as tremor and loss of balance (Dauer and Przedborski, 2003). In the negative geotaxis test, flies exposed to a concentration of 1 mM of BPA increased climbing time, and in the open field test flies

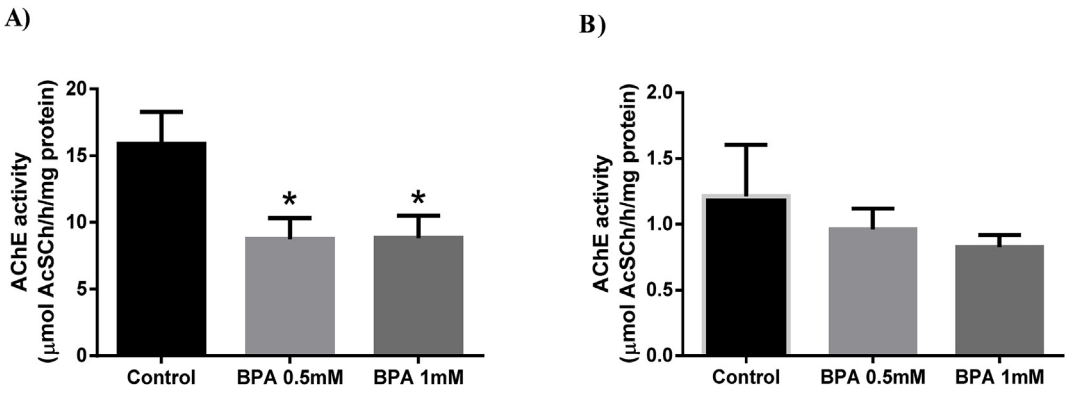

Fig. 5. Acetylcholinesterase enzyme activity in A) head and B) body of adult *Drosophila melanogaster* exposed for 7 days to different levels of Bisphenol (BPA). Significance was determined by one-way analysis of variance (ANOVA) followed by Bonferroni test. Values are expressed as means ± SD. *Significant difference compared to the control group ($p < 0.05$).

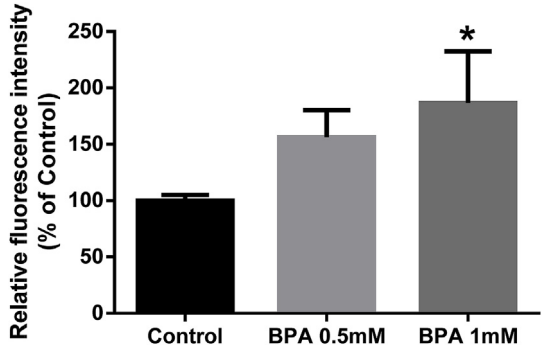

Fig. 6. Levels of reactive species (RS) in *Drosophila melanogaster* head samples exposed to different concentrations of Bisphenol A (BPA). Significance was determined by one-way analysis of variance (ANOVA) followed by Bonferroni test. Results were expressed as the percentage (%) of the control group. Values are expressed as means ± SD. * Significant difference compared to the control group ($p < 0.05$).

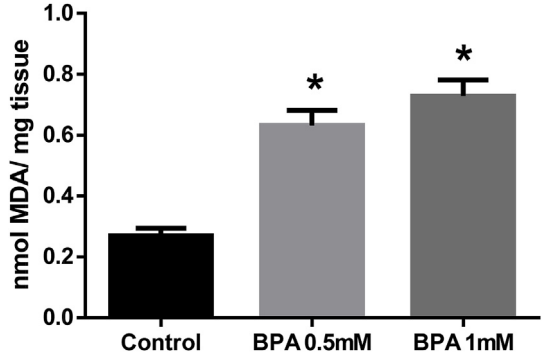

Fig. 7. Dosage of lipid peroxidation (LPO) levels in *Drosophila melanogaster* head samples exposed to different concentrations of Bisphenol A (BPA). Significance was determined by one-way analysis of variance (ANOVA) followed by Bonferroni. Values are expressed as means ± SD. *Significant difference compared to the control group ($p < 0.05$).

traveled fewer quadrants compared to the control group. In the equilibrium test, flies also had a reduction in this capacity when exposed to 0.5 mM and 1 mM BPA.

Through open field tests it is possible to assess general locomotion defects, but it is not sufficient to observe damage to fine motor and psychomotor movements because flies have the ability to compensate for small locomotion defects due to flexible mating between their six legs (Wosnitza et al., 2013). However, the balance test is quite effective for quantifying motor disorders at early stages of the development of neurodegenerative diseases (Iliadi et al., 2016). Our study shows that in a sub-chronic exposure to BPA, it was possible to observe that there was psychomotor and locomotion damage in adult *Drosophila melanogaster*.

Based on these data, it is important to remember that BPA is present in water, air and soil, but the greatest exposure is through the consumption of contaminated food and drink (Kang et al., 2006), so human exposure to this toxic is constantly, chronically. The behavioral results complement the results of dopamine and ROS levels, where it was significant in the group that received 1 mM of BPA, showing that there was induction of Parkinson's like disease in *Drosophila melanogaster*. Even though the results were not statistically significant for the groups exposed to 0.5 mM BPA, it is important to highlight the same as sub-chronic exposure, toxicity and brain level can be observed in these flies.

Any substance that causes interference in the neurotransmission components of dopamine may alter behaviors that are essential for locomotion and survival of flies (Figueira et al., 2017). Thus, in our study, BPA induced, in addition to the decrease in dopamine levels and impairment of locomotion, the increase in fly mortality. In already consolidated models of Parkinson's like disease in *Drosophila melanogaster*, such as rotenone, it is also observed the decrease of dopamine levels related to increased mortality and reduction of locomotion and balance (de Freitas Couto et al., 2019).

Another neurotransmitter directly related to locomotor regulation is acetylcholine, and neurodegeneration of dopaminergic neurons is directly related to the development of neurodegenerative diseases (Craig et al., 2011). There was a decrease in AChE activity at the brain level in the groups exposed to BPA at both concentrations. However, in the body samples, there was no significant difference between the 0.5 mM and 1 mM BPA when compared to the control. Some studies evidence the inhibition of AChE activity by the action of free radicals (Tsakiris et al., 2015; Wyse et al., 2004). In our results, the oxidative stress induced by the BPA at the cerebral level decreased the activity of the enzyme AChE.

The environmental risk factor for PD development consists of xenobiotics that cause oxidative damage, mitochondrial dysfunction and death of dopaminergic neurons, such as MPTP, paraquat and rotenone (Aryal and Lee, 2019). Through this study, the possible involvement of BPA in the development of Parkinson's like disease in *Drosophila melanogaster* was evident in the group exposed to 1 mM of BPA. We demonstrate the possible mechanisms involved in the development of a Parkinson's like disease in *Drosophila melanogaster* and thus design a new model for future studies related to the pathogenesis of this disease. We believe that this work can serve as a warning for the constant exposure to BPA, clearly demonstrating the toxicity of this monomer to the adult brain through biochemical and behavioral changes.

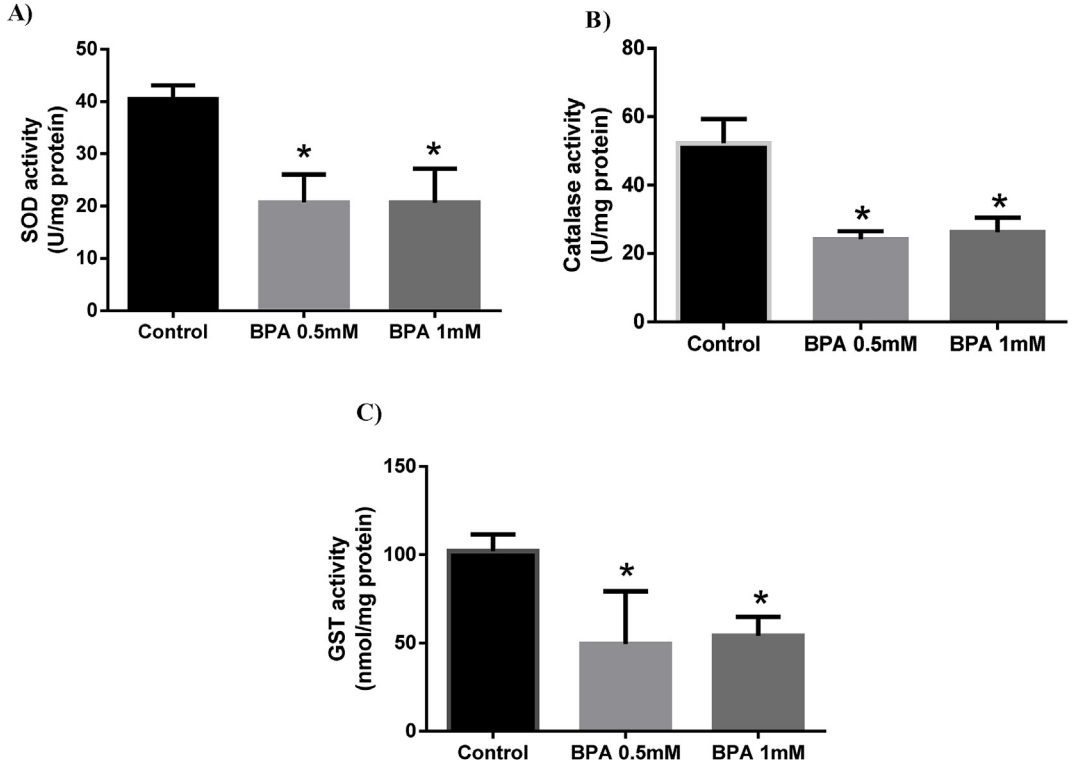

Fig. 8. Enzyme activity analyzes of *Drosophila melanogaster* head samples exposed to different concentrations of Bisphenol A (BPA). Enzyme activity A) Superoxide dismutase (SOD), B) Catalase (Cat) and C) glutathione S-transferase (GST). Significance was determined by one-way analysis of variance (ANOVA) followed by Bonferroni test. Values are expressed as means ± SD. *Significant difference compared to the control group ($p < 0.05$).

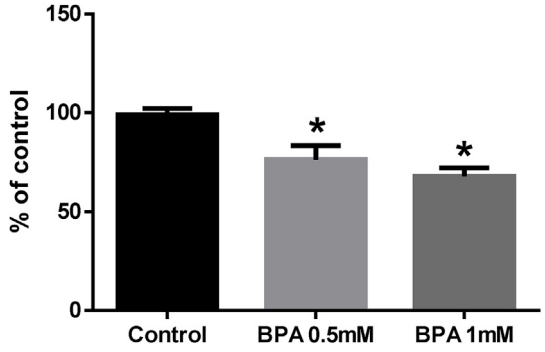

Fig. 9. Mitochondrial metabolic viability assessed by the (3–4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) bromide reduction method in the head homogenate of flies exposed to different concentrations of Bisphenol A (BPA), for 7 days. Significance was determined by a one-way analysis of variance (ANOVA) using the Bonferroni test. The results were expressed as percentage (%) of the control group. Values are expressed as means ± SD. *Significant difference in relation to the control group, ($p < 0.05$).

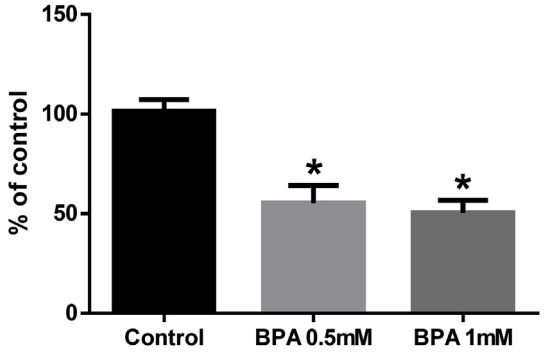

Fig. 10. Cell viability, assessed by resazurin reduction assay in homogenate of fly head samples exposed to different concentrations of Bisphenol A (BPA). Significance was determined by one-way ANOVA with the Bonferroni test. Results were expressed as the percentage (%) of the control group. Values are expressed as means ± SD. *Significant difference compared to the control group ($p < 0.05$).

Author contributions section

• M.P access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

• E.A.S.M and M.P conceived and designed the project.

• E.A.S.M, S.M.A, V.C.B, S.F.C, M.R.P, M.M.M.D, L.B.M, E.F.J, B.P.R, R.R and G.P.G performed the behavioral experiments.

• E.A.S.M, S.M.A, S.F.C, M.R.P, B.P.R, R.R and M.M.M.D performed the biochemical measurements and acquisition of data.

• E.A.S.M, S.M.A, S.F.C and M.P interpreted of data and statistical analysis.

• E.A.S.M drafting of the manuscript.

• Critical revision of the manuscript for important intellectual content: G.P.G, S.M.A and M.P.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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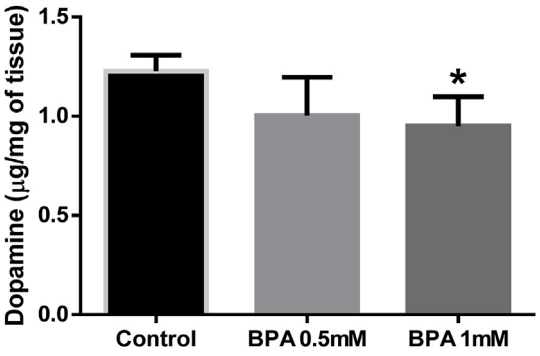

Fig. 11. Dopamine levels present in the head homogenate of flies exposed for 7 days at different concentrations of Bisphenol A (BPA). Significance was determined by one-way analysis of variance (ANOVA) using the Bonferroni test. Values are expressed as means ± SD. * Significant difference compared to the control group ($p < 0.05$).

Table 1
Effects of Bisphenol A (BPA) on *Pearson's* correlation analysis (*r*) between Dopamine and locomotor and balance behavior. *$p < 0.05$ is considered significant.

Dopamine (DA) and behavioral analysis	*r*	*P*	*N*
DA x Open Field	0.7852	0.0025*	12
DA x Negative geotaxis	−0.8329	0.0008*	12
DA x Equilibrist Test	0,7804	0,0006*	15

Table 2
Effects of Bisphenol A (BPA) on *Pearson's* correlation analysis (*r*) between Dopamine and oxidative stress markers. *$p < 0.05$ is considered significant.

Dopamine (DA) and oxidative stress markers	*r*	*P*	*N*
DA x Reactive species	−0.8664	0.0025*	9
DA x malonildialdehyde	0.8227	0.0010*	12
DA x Superoxide dismutase	0.9103	0.0001*	12
DA x Catalase	0.8497	0.0005*	12
DA x Glutathione-S-transferase	0.9040	0.0001*	12

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2020.111128.

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