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Melatonin decreases oxidative stress in *Drosophila melanogaster* exposed to manganese

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Key words: *Drosophila melanogaster;* melatonin; oxidative stress; manganese.

Abstract. In a previous study we found that manganese induces a pro-oxidant effect on *Drosophila melanogaster*. In the present study the effect of melatonin (MEL; 0.43 mM) in oxidative stress markers of *D. melanogaster* after manganese (Mn; 30 mM) intoxication was determined after 6 days of treatment. Three groups were used: 1. Mn (exposed to Mn); 2. Control (maintained in standard medium); 3. MEL 6 d (treated with MEL). In four additional groups, the effect of MEL treatment for 40 days after the intoxication was determined: a. Mn-Control (exposed to Mn and no additional treatment for 40 days); b. Mn-MEL (exposed to Mn and treated with MEL for 40 days); c. Control 40 d (maintained in standard medium for 40 days); d. MEL 40 d (treated with MEL for 40 days). After 6 days, an increase in the concentrations of H_2O_2, NO•, MDA, protein carbonyl and 8-OHdG, and the activities of SOD, CAT, NOS and GSH-Px was observed in Mn intoxicated flies. A decrease of GSH was also detected. At 40 days, in the Mn-Control group, the increase in the concentrations of H_2O_2, protein carbonyl and 8-OHdG and in the activities of GSH-Px, CAT and SOD was maintained. In contrast, the concentration of GSH and MDA was decreased. Similarly the concentrations of H_2O_2, NO•, GSH and the activities of SOD, CAT, GSH-Px and NOS were decreased in the Mn-MEL group compared to the Mn-Control. In conclusion, the prolonged treatment with MEL mitigated the oxidative stress in *D. melanogaster* after exposure to Mn.

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La melatonina disminuye el estrés oxidativo en *Drosophila melanogaster* expuesta a manganeso.

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Palabras clave: *Drosophila melanogaster;* melatonina; estrés oxidativo; manganeso.

Resumen. En estudios previos se encontró que el manganeso induceun efecto prooxidante sobre *Drosophila melanogaster.* Se determinó el efecto de la melatonina (MEL, 0,43 mM) en marcadores de estrés oxidativo de *D. melanogaster* intoxicadas con manganeso (Mn; 30 mM). Tres grupos fueron utilizados: 1. Mn (expuesto a Mn); 2. Control (mantenido en medio estándar); 3. MEL 6 d (tratado con MEL). Se utilizaron cuatro grupos adicionales para determinar el efecto del tratamiento con MEL durante 40 días después de la intoxicación: a. Mn-Control (expuesto a Mn y luego sin tratamiento adicional durante 40 días); b. Mn-MEL (expuesto a Mn y tratado con MEL durante 40 días); c. Control 40 d (mantenido en medio estándar durante 40 días); d. MEL 40 d (tratado con MEL durante 40 días). A los 6 días, se observó un aumento significativo en las concentraciones de H_2O_2, NO•, MDA, proteínas carboniladas y 8-OhdG y en las actividades de SOD, CAT, NOS y GSH-Px en las moscas intoxicadas con Mn. También se detectó una disminución de GSH. A los 40 días, en el grupo Mn-Control se mantuvo el incremento en las concentraciones de H_2O_2, proteína carbonilada y 8-OHdG y las actividades de GSH-Px, CAT y SOD. En contraste, disminuyeron las concentraciones de GSH y MDA. Del mismo modo, decrecieron las concentraciones de H_2O_2, NO•, GSH y las actividades de la SOD, CAT, GSH-Px y NOS en el grupo Mn-MEL al compararlo con el grupo Mn-Control. En conclusión, el tratamiento con MEL disminuyó el estrés oxidativo en *D. melanogaster* expuestas a Mn.

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INTRODUCTION

Melatonin (MEL) is a hormone that has been shown to protect against oxidative stress due to its ability to scavenge free radicals and induce protective mechanisms through the stimulation of antioxidant enzymes (1-4).

Manganese (Mn) is a "transition metal" which is a co-factor of a number of enzymes such as hydrolases, kinases, decarboxylases and transferases (5). Although Mn has low toxicity, it may cause intoxication in humans, when they are exposed to high concentrations of this metal or to low doses for long periods of time (6). The major mechanism for induction of toxicity by Mn is unknown. However, oxidative stress has been suggested to play a key role in the neurotoxicity caused by the metal (7-9). The greatest risk of intoxication occurs in mining workers in the steel industry and those working in the manufacture of electrodes and dry cells or batteries (10).

Manganese intoxication in humans is characterized by two phases: acute (manganese madness) and chronic. Barbeau (6), suggested that chronic manganism can be considered as a model of bradykinesia accompanied by an extrapyramidal dystonic syndrome of striatal nature. Mn-induced motor disorders, neuronal degeneration, astrocytic

activation, and apoptosis, are some of the pathological, ultrastructural and biochemical changes observed in murine intoxicated with Mn. In these animals MEL antagonizes the Mn-induced oxidative injury through the activation of Keap1-Nrf2-ARE signaling pathway in the striatum. However, the effect of MEL in *D. melanogaster* exposed to Mn is little known (11).

Faust *et al.* (12), have suggested that *Drosophila* is a valuable model for preclinical testing of drugs with therapeutic potential for neurodegenerative diseases like Parkinson's disease. In fact, *D. melanogaster* is a good experimental model because: 1) has a short life cycle; 2) adults show many of the manifestations of cellular senescence observed in mammals, and 3) 75% of the genes that cause disease in humans, are present in *D. melanogaster* (13,14). The purpose of this study was to determine the effect of MEL on the manganese-induced oxidative injury in *D. melanogaster.*

MATERIALS AND METHODS

Animals stock

Male wild type flies of *D. melanogaster* (Oregon R strain) were used. Flies were maintained in a light/dark cycle of 12h/12h at a temperature of 25°C and fed standard corn meal prepared as described previously (15).

Treatment of flies with manganese and melatonin

Two days after emerging from the pupa, seven groups of flies (300 per group) were used for the assessment of oxidative parameters after acute intoxication with manganese chloride (30 mM): 1. Mn (exposed to Mn for 6 days); 2. Control 6d (maintained in standard medium for 6 days); 3. MEL 6d (treated with MEL for 6 days). Four additional groups were used to determine the effect of MEL treatment for 40 days after the intoxication: a. Mn-Control (exposed for 6 days to Mn and then no additional treatment for 40 days); b. Mn-MEL (exposed for 6 days to Mn and treated with MEL for 40 days); c. Control 40d (maintained in standard medium for 40 days); d. MEL 40d (treated with MEL for 40 days). The MEL concentration (0.43 mM) was standardized previously by Bonilla *et al.* (15). The flies were transferred daily to fresh medium during 40 days. Each experiment was carried out in triplicate and in turn in each experiment 10 determinations were made for a total of 30.

Hydrogen peroxide (H_2O_2) determination

A homogenate of whole body of *D. melanogaster* was prepared with 50 flies in 500 μL PBS. The homogenate was centrifuged at 10,000 *g* for 5 min. at 4°C. Ninety μL of the supernatant were removed to determine H_2O_2 using the OXISELEC™ hydrogen peroxide Assay Kit (STA-343, CELL BIOLABS, INC). The results were expressed in nmol H_20_2 /mg protein (16,17).

Determination of soluble protein

Quantification of soluble proteins was performed following the protocol described in the Bicinchoninic Acid Protein Assay Kit (BCA1, SIGMA). The absorbance was measured at 562 nm (SYNERGY HT, BIOTECK). The results were expressed in mg protein/mL (17,18).

Indirect determination of nitric oxide (NO•)

The QuantiChrom™ (D2NO-100) kit was used to determine the concentration of nitrites (NO_2^-). The assay is designed to measure the production of NO•, followed by the reduction of nitrate to nitrite using an improved Griess method. Whole body homogenates of *D. melanogaster* were prepared as described above. The supernatants were used for determination of NO_2^-, following the protocol described in the kit. Results were expressed in μM of nitrite (19).

Determination of glutathione (GSH)

The EnzyChrom™ assay kit GSH/GSSG was used (EGTT-100). To measure the concentration of glutathione 40 flies were ho-

mogenized in 200 μL of cold buffer [50 mM phosphate (pH 7.0), 1 mM EDTA and 20 μL of capturer. The homogenates were centrifuged at 10,000 *g* × 5 min at 4°C and the supernatants were transferred to clean tubes for deproteinization. All samples were deproteinized by preparing a solution of 5% metaphosphoric acid in distilled water (RX MPA). Absorbances were read at 412 nm at 0 min and 10 min. The results were expressed in μM of GSH (20).

Determination of the nitric oxide synthase (NOS) activity

Seventy flies per treatment group were homogenized in PBS (pH 7.4) and centrifuged at 10,000 *g* at 4°C. The supernatant was used to determine NOS activity which was assayed using the EnzyChrom™ kit (ENOS-100) (BioAssay SYSTEMS). The results were reported in U/L (21).

Determination of superoxide dismutase (SOD) activity

Whole body homogenates were prepared from *D. melanogaster* with 10 flies in 400 μL of ice-cold lyses buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA, 0.5% Triton X-100), using a glass-glass homogenizer. The homogenates were centrifuged at 12,000 *g* for 10 min at 4°C. The supernatant was removed to determine SOD activity using the OXISELEC™ Superoxide Dismutase Activity Assay Kit (STA-340, CELL BIOLABS, INC). Seventy μL of the supernatant were removed and the activity was determined following the kit instructions. The results were expressed in U/mg protein (22).

Determination of the catalase (CAT) activity

A homogenate of whole body of *D. melanogaster* was prepared with 10 flies in 500 μL ice-cold PBS with 1mM EDTA per gram of tissue, using a glass-glass grinder. The homogenate was centrifuged at 10,000 *g* for 15 min at 4°C and the supernatant removed to determine CAT activity using the OXISELEC™ Catalase Activity Assay kit (STA-341, CELL BIOLABS, INC). The results were expressed in U/mg protein (23).

Determination of glutathione peroxidase (GSH-Px) activity

The homogenates of whole body of *D. melanogaster* were prepared with 70 flies in 500 μL of PBS. The homogenates were centrifuged at 10,000 *g* for 10 min at 4°C and the supernatants were used to determine the glutathione peroxidase activity according to the protocol described in the EnzyChrom™ (EGPX-100) kit. The results of GSH-Px activity were expressed in U/L (24).

Determination of malondialdehyde (MDA)

Mitochondria from whole body of *D. melanogaster* were prepared by differential centrifugation, following the protocol described by Fernandez-Vizarra *et al*. (25) and modified by Mora *et al*. (17). All reagents were from SIGMA-ALDRICH, USA.

The mitochondrial fraction was re-suspended in phosphate-buffered saline (PBS), following the protocol described in the OXISELEC™ TBARS Assays kit (STA-330, CELL BIOLABS, INC). The results were expressed in nmol MDA/mg protein (17, 26).

Determination of protein carbonyl

The ELISA kit for the determination of protein carbonyl [OXISELEC™ (STA-310) (CELL BIOLABS, INC)] was used. Seventy flies per group were homogenized in PBS (pH 7.4) and centrifuged at 10,000 *g* at 4°C; the supernatants were used for the assay. Absorbance was read at 450 nm. The results were expressed in nmol/mg (27).

DNA extraction and Purification

For DNA purification seventy flies from each treatment were homogenized in 1 mL digestion buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0; 25 mM EDTA, pH 8.0; 0.5% SDS, 0.1 mg/mL proteinase K) and incubated in a water bath at 50°C for 16 hours, then centrifuged at 13,200 *g* at 4°C for 5 minutes.

Since RNA interferes with the determination of 8-OHdG, a digestion with RNase was performed. The DNA was re-suspended in 50 μL of TE buffer and stored at -20°C until used. Samples of DNA were separated and observed by agarose (0.7%) gel electrophoresis with ethidium bromide [0.5 mg/mL] (28). The DNA quantification was performed using the Bio-nano-spec spectrophotometer (SHIMADZU).

Determination of 8-hydroxy-d-guanosine

Oxidative damage to DNA was determined using the competitive ELISA kit OXISELEC™ from CELL BIOLABS, INC. (STA-320) for the quantification of 8-OHdG. Standards of 8-OHdG and unknown samples were initially added to a plate containing the conjugate pre-adsorbed 8-OHdG/ABS. After an incubation time, a monoclonal antibody (anti-8-OHdG) was placed. Subsequently a conjugated secondary antibody (HRP) was added. The content of 8-OHdG in the unknown samples was determined by comparison with a predetermined standard curve. The results were expressed in ng/mL (29).

Statistical analysis

Data were expressed as means ± S.E.M and the significance between specific means was determined by one-way ANOVA, and differences among groups were evaluated using the test of Dunnett´s for comparisons. Differences were considered significant when $p<0.05$ (30).

RESULTS

Reactive oxygen species (ROS), reactive nitrogen species (RNS), endogenous antioxidants, enzyme activities and oxidative damage in adult males *D. melanogaster* after 6 days of treatment with Mn (30 mM)

As shown in Table I after 6 days of Mn treatment induced a significant increase in the concentrations of H_2O_2 and NO•, and in the activities of SOD, CAT, GSH-Px and NOS when compared to the Control group ($p<0.001$). A significant reduction ($p<0.001$) in the concentration of GSH in the Mn group with respect to the Control was evident. Markers of oxidative damage to lipids (MDA), proteins (protein carbonyl) and DNA (8-OHdG) showed a significant increase following exposure to Mn, when compared to the Control ($p<0.001$).

MEL treatment increased the enzyme activities of SOD and GSH-Px ($p<0.001$) and reduced •NO and MDA levels as compared to the Control ($p<0.001$). No significant difference was observed between MEL and Control with respect to the concentrations of H_2O_2, GSH, protein carbonyl and 8-OHdG and the activities of CAT and NOS (Table I).

ROS, RNS, endogenous antioxidants, enzyme activities and oxidative damage in adult males of *D. melanogaster* 40 days after treatment with Mn (30 mM) for 6 days

Forty days after being intoxicated with Mn, the increase in the levels of H_2O_2, protein carbonyl and 8-OHdG and in the activities of SOD and CAT as well as the decrease in the concentrations of GSH when compared to the Control group persisted ($p<0.001$). On the other hand, a decrease ($p<0.001$) of MDA levels relative to the Control was also found (Table II). The Mn-MEL group showed a decrease in the concentrations of H_2O_2, •NO, GSH, and in the enzyme activities of SOD, CAT, GSH-Px and NOS when compared to the Mn-Control group ($p<0.001$) (Table II). The treatment with MEL for 40 days resulted in an increase in the enzymatic activities of SOD, CAT and GSH-Px, and a decrease in the concentrations of H_2O_2, NO•, GSH, MDA as in the activity of NOS with respect to the Control group ($p<0.001$). The levels of protein carbonyl and 8-OHdG in the MEL group were similar to the Control group (Table II).

TABLE I
OXIDATIVE STRESS MARKERS IN ADULT MALES OF *D. melanogaster* AFTER 6 DAYS OF TREATMENT WITH MANGANESE (30 mM) OR MELATONIN (0.43 mM).

	CONTROL 6D	MEL 6D	Mn
ROS, RNS			
H2O2 (nmol H2O2/mg protein)	0.8 ± 0.008	0.9 ± 0.008	1.1 ± 0.006*
NO● (μM nitrite)	39.2 ± 0.7	36.3 ± 0.5*	43.6 ± 0.4*
ENZYMES			
SOD (U/mg protein)	187.0 ± 1.0	229.9 ± 1.2*	494.3 ± 5.7*
CAT (U/mg protein)	0.14 ± 0.002	0.17 ± 0.003	0.24 ± 0.006*
GSH-Px (U/L)	6.3 ± 0.5	22.9 ± 0.3*	24.2 ± 0.8*
NOS (U/L)	0.007 ± 0.0002	0.005 ± 0.0002	0.009 ± 0.0005*
ENDOGENOUS ANTIOXIDANTS			
GSH (μM)	37.7 ± 0.6	39.2 ± 0.6	10.3 ± 0.5*
OXIDATIVE DAMAGE LIPID PEROXIDATION			
Mitochondrial MDA (nmol MDA/mg protein)	6.6 ± 0.06	4.8 ± 0.05*	11.1 ± 0.15*
PROTEIN DAMAGE			
Protein Carbonyl (nmol/mg)	6.3 ± 0.05	6.3 ± 0.14	9.5 ± 0.16*
DNA DAMAGE			
8-OHdG (ng/mL)	2.09 ± 0.02	2.09 ± 0.02	4.33 ± 0.04*

Data were expressed as mean ± standard error and analyzed by one-way ANOVA. Significant differences between experimental and control groups were measured using Dunnett's test; *p<0.001, when compared to Control. (H_2O_2 n=450; NO• n=450; SOD n=110; CAT n=110; GSH-Px=630; NOS n=630; MDA n=1050; protein carbonyl n=630; 8-OhdG n=1500).

TABLE II
OXIDATIVE STRESS MARKERS IN ADULT MALES OF *D. melanogaster* PRE-TREATED WITH MANGANESE (30 mM) FOR 6 DAYS AND POST-TREATED WITH MELATONIN (0.43 mM) FOR 40 DAYS.

	CONTROL 40D	MEL 40D	MN-CONTROL	MN-MEL
ROS, NOS				
H_2O_2 (nmol H_2O_2/mg protein)	0.8 ± 0.007	0.3 ± 0.005*+	4.1 ± 0.075*	2.3 ± 0.010*+
NO• (μM nitrite)	40 ± 0.26	30 ± 0.43*+	39 ± 0.62	18 ± 0.37*+
ENZYME				
SOD (U/mg protein)	160.1 ± 0.7	205.0 ± 1.3*+	179.3 ± 0.6*	159.9 ± 0.9+
CAT (U/mg protein)	0.16 ± 0.01	0.17 ± 0.001+	0.24 ± 0.006*	0.16 ± 0.002+
GSH-Px (U/L)	5.3 ± 0.3	12.2 ± 0.7*+	25.9 ± 0.1*	17.8 ± 0.3*+
NOS (U/L)	0.00645 ± 0.0002	0.0053 ± 0.0013*	0.0061 ± 0.0002	0.0045 ± 0.0013*+
ENDOGENOUS ANTIOXIDANTS				
GSH (μM)	49.3 ± 0.5	33.6 ± 0.5*+	26.5 ± 0.6*	19.7 ± 0.5*+
LIPID PEROXIDATION				
Mitochondrial MDA (nmol MDA/mg protein)	6.9 ± 0.05	4.5 ± 0.07*	4.9 ± 0.08*	4.4 ± 0.07*
PROTEIN DAMAGE				
Protein Carbonyl (nmol/mg)	6.8 ± 0.2	7.0 ± 0.3+	8.4 ± 0.2*	7.8 ± 0.1*
DNA DAMAGE				
8-OHdG (ng/mL)	2.5 ± 0.02	2.3 ± 0.01+	3.7 ± 0.16*	3.2 ± 0.03*

Data were expressed as mean ± standard error and analyzed by one-way ANOVA. Significant differences between experimental and control groups were measured using Dunnett's test; *$p<0.001$ when compared to Control. + $p<0.001$ as compared to Mn-Control. (H_2O_2 n=450; NO• n=450; SOD n=110; CAT n=110; GSH-Px=630; NOS n=630; MDA n=1050; protein carbonyl n=630; 8-OhdG n=1500).

DISCUSSION

Treatment with Mn for 6 days induced an oxidative effect on adult male *D. melanogaster* which was expressed by an increase in the levels of ROS (H_2O_2) and RNS (NO•), in the activities of the endogenous antioxidant defense enzymes (SOD, CAT, GSH-Px) and by a decrease in the concentrations of GSH.

Previous studies have demonstrated that Mn stimulates the production of hydrogen peroxide in the microglia of rat (31) and induced intracellular ROS production and a neurotoxicity that significantly dissipated the mitochondrial membrane potential (32). Mn-catalyzed autoxidation of dopamine (DA) involves redox cycling of Mn^{2+} and Mn^{3+} in a reaction that generates ROS

and DA-O-Quinone, thereby leading to oxidative damage. The high rate of of Mn-induced oxidation of DA can contribute to the death of dopaminergic neurons and the formation of cytotoxins (quinones and ROS) (33,34). The oxidative stress generated by high Mn concentrations lead to the induction of mitochondrial permeability transition (mPT), a Ca^{2+}-dependent process, characterized by the opening of the permeability transition pore in the inner mitochondrial membrane, resulting in increased solubility to protons, ions and solutes, loss of the mitochondrial inner membrane potential, mitochondrial swelling and deterioration of oxidative phosphorylation and ATP synthesis (35-38). Nitric oxide, produces cell damage by several mechanisms, such as induction of apoptosis by activation of the ERK pathway, release of p38-dependent cytochrome c and caspase activation. It can also reversibly inhibit cytochrome c oxidase, which may increase the reduction state of electrons in the electron transport chain and, consequently, superoxide anion production (39).

We found that the content of reduced glutathione decreases in the group of flies treated with Mn for 6 days with respect to control. Similar results were obtained by Marreilha dos Santos (40), who demonstrated that manganese chloride diminished the intracellular levels of reduced glutathione in RBE4 cells. Manganese decreases the mitochondrial membrane potential. This process is dependent on Ca^{2+}. Apoptosis is a secondary mechanism to changes in mitochondrial function, which has been implicated in the neurotoxicity induced by Mn. Ca^{2+} induces pore opening of mitochondrial permeability, allowing the activation of the Bcl-2 family of proteins, especially Bax/Bak, culminating in the release of cytochrome c (*cytc*) (41), which activate the Erk Signaling Pathway, the cysteine protease (caspase-3), that mediates the apoptosis, chromatin condensation and DNA fragmentation (42).

In the present work we demonstrate that manganese induces an increase in protein carbonyl and 8-hydroxyguanine (8-OHdG) levels at 6 and 40 days of exposure to this metal in *D. melanogaster*. Brenneman *et al.* (43), found that the inhalation of high concentrations of manganese did not significantly alter 8-OHdG levels in either mitochondrial or nuclear DNA. However, our results support the hypothesis that oxidative damage caused by ROS to the DNA (8-OHdG) and protein (protein carbonyl), is an important mechanism of action of the Mn-induced toxicity in *D. melanogaster*.

After 6 and 40 days of treatment, MEL increased the activity of SOD compared to the control group ($p<0.001$). Our results are similar to those reported by Liu and Ng (44), who observed an increase in SOD activity in kidney, liver and brain rat after a single injection of melatonin (5 mg/kg). In cultures of dopaminergic cells, melatonin induced the expression of genes of the antioxidant enzymes Cu/Zn-SOD, Mn-SOD and GSH-Px (45). Similarly, Kotler *et al., i*n 1998 (46), demonstrated that after chronic administration of melatonin (50 and 500 mg / kg) this neurohormone had a stimulatory effect on gene expression of the antioxidant enzymes GSH-Px, Cu- Zn-SOD and Mn-SOD. In mice, Deng et al. (47) demonstrated that pretreatment with MEL attenuated Mn-induced neurotoxicity by means of its antioxidant properties and promotion of the antioxidant defense mechanisms.

In our study, MEL induced an increase in the activity of GSH-Px in *D. melanogaster* after exposure to Mn. Barlow-Walden *et al.* (48), showed that MEL caused an increase in the activity of GSH-Px in the rat brain after 30 minutes of administration. Moreover, the activity of GSH-Px in the brain is greater at night than during the day and is correlated with high levels of cerebral MEL at night. It is believed that GSH-Px is the major enzyme in eliminating peroxides in the brain. This antioxidant enzyme reduces the formation of hydroxyl radicals from the Fenton type reaction. Since the hydroxyl radical is known as the most harmful ROS, induction of GSH-

Px in the brain could be an important mechanism by which MEL exerts its potent neuroprotective effects.

In *D. melanogaster,* it was found that the pro-oxidant effect of $MnCl_2$ at a concentration of 30 mM was mitigated by 0.43 mM of MEL as shown by the fact that MEL treatment for 6 days decreased the concentrations of NO• and MDA and the activity of NOS as compared to the control group. After 40 days, MEL decreased the concentrations of H_2O_2 and GSH with respect to the control. In the MEL group, no significant difference was observed in the concentrations of protein carbonyl and 8-OHdG at 6 or 40 days with respect to the control flies.

It has been shown that exposure of SH-SY5Y cells to Mn promotes the accumulation of oxidative DNA damage that is mitigated by chemical antioxidants, confirming the potential of antioxidants as therapeutic agents in the oxidative DNA damage and as a strategy for protection against damage induced by Mn^{2+} (49).

When MEL interacts with ROS and RNS the resulting metabolites are cyclic 3-hydroxymelatonin (C-3OHM), N^1-acetyl-N^2-formyl-5-methoxy-kynuramine (AFMK), and N-acetyl-5-methoxy-kynuramine (AMK) which also act as radical scavengers. Therefore, one molecule of MEL can detoxify up to 10 free radicals (50-54).

The decrease in lipid peroxidation by MEL and its metabolites can be explained by the reduction of the initiation of lipid peroxidation by scavenging the initiating agents, •OH, ONOO-, among others. Additionally, MEL and its metabolites AMK and C-3OHM interrupt the propagation of lipid peroxidation by scavenging the peroxyl radical. In vivo, MEL effectively protects lipids from peroxidation thereby preventing rancidity and preserving optimal membrane fluidity (55). Park and Chun (56), demonstrated that MEL also inhibited the lipid peroxidation induced by Mn and/or LPS in microglia.

In conclusion, the intoxication with Mn for 6 days induces a pro-oxidant effect in adult males *D. melanogaster* as shown by the increases in ROS and nitric oxide, and in the enzymatic activity of SOD, CAT, GSH-Px and NOS and by the decrease in the content of GSH. These changes induced damage to cell membranes, proteins and DNA. Forty days after treatment with Mn for 6 days, MEL mitigated the oxidative damage probably due to decreases in reactive oxygen and nitrogen species and in the activities of SOD, CAT, GSH-Px, and NOS.

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