Invest Clin 53(3): 250 - 261, 2012

The life span of *Drosophila melanogaster* is affected by melatonin and thioctic acid.

Raikelin Terán[1], Ernesto Bonilla[2,3], Shirley Medina-Leendertz[3], Marylú Mora[3], Virginia Villalobos[1], Milagros Paz[1] and José L Arcaya[2].

[1]Departamento de Biología, Facultad Experimental de Ciencias,
[2]Instituto de Investigaciones Clínicas "Dr. Américo Negrette", Facultad de Medicina, Universidad del Zulia,
[3]Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Instituto Venezolano de Investigaciones Científicas (CIB-IVIC). Maracaibo, Venezuela.

Keywords: aging, free radicals, melatonin, thioctic acid, *Drosophila melanogaster*, motor activity, phenotypic changes.

Abstract. Aging and reduced longevity are due in part to the action of free radicals (FR). Melatonin (Mel) and thioctic acid (TA) are effective in protecting against the damage caused by FR. In this study, the effect of Mel and TA on the life cycle of Drosophila melanogaster was determined. We used a control group of flies, another group that was provided with Mel (0.43 mM) throughout their life cycle (Mel-c), a third group received Mel upon reaching adulthood (Mel-a) and two groups were fed with TA (2.15 mM) in the same manner (TA-c and TA-a). The number of eclosed, survival, phenotype changes, motor activity and the content of malondialdehyde (MDA) was evaluated in each group. Mel-c increased the eclosion rate and the motor activity of the flies. Mel-c and Mel-a increased the life span and decreased the concentrations of MDA. By contrast, TA-c diminished the eclosion rate, produced phenotypic changes and increased MDA levels and motor activity of the flies. TA-a extended the life span of flies, and did not alter MDA levels and motor activity when compared with the control group. In conclusion, Mel mitigated the effects caused by FR generated during aging, while TA-c increased lipid peroxidation and altered the phenotype of flies.

Autor de correspondencia: Shirley Medina-Leendertz. Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Instituto Venezolano de Investigaciones Científicas (CIB-IVIC), Maracaibo, Venezuela. Email: sml24567@gmail.com

El ciclo de vida de la *Drosophila melanogaster* es afectado por la melatonina y el ácido tióctico.

Invest Clin 2012; 53(3): 250 - 261

Palabras clave: envejecimiento, radicales libres, melatonina, ácido tióctico, *Drosophila melanogaster,* actividad motora, cambios fenotípicos.

Resumen. El envejecimiento y la disminución de la longevidad se deben, en parte, a la acción de los radicales libres (RL). La melatonina (Mel) y el ácido tióctico (AT) son antioxidantes efectivos contra el daño ocasionado por los RL. En este estudio se determinó el efecto de la Mel y el AT en el ciclo de vida de la Drosophila melanogaster. Se utilizó un grupo de moscas control, otro grupo al que se le suministró Mel (0,43 mM) durante todo su ciclo de vida (Mel-c), un tercer grupo recibió Mel al alcanzar la adultez (Mel-a) y dos grupos a los que se le suministró AT (2,15 mM) de la misma manera (AT-c y AT-a). Se evaluó el número de eclosionados, la sobrevida, el fenotipo, la actividad motora y el contenido de malondialdehído (MDA) en cada uno de los grupos. Mel-c incrementó la tasa de eclosión y aumentó la actividad motora. Mel-a y Mel-c aumentaron la sobrevida y disminuyeron las concentraciones de MDA. Por el contrario, el AT-c disminuyó la tasa de eclosión, produjo cambios fenotípicos, no afectó la sobrevida de las moscas, aumentó los niveles de MDA y la actividad motora. El AT-a extendió la duración de la vida de los animales, no alteró los niveles de MDA, ni la actividad motora al comparar con el grupo control. En conclusión, la Mel mitigó los efectos causados por los RL generados durante el envejecimiento, mientras que el AT-c aumentó la peroxidación lipídica y alteró el fenotipo de las moscas.

Recibido: 10-05-2012. Aceptado: 19-07-2012

INTRODUCTION

The theory of free radicals (FR) proposed by Denham Harman, explains that the effect of FR on the cells is the cause of aging and death of living beings. Mitochondria are responsible for more than 90% of oxygen consumption, and produce the greatest amount of reactive oxygen species (ROS) (1, 2). Acuña-Castroviejo *et al.* (3) found that age induces a significant oxidative status in lung mitochondria, which exhibited a reduced activity of the respiratory chain and ATP production. After 9 months of melatonin administration in the drinking water, the hyperoxidative status and functional deficiency of aged mice lung mitochondria were totally counteracted and ATP production was increased indicating that melatonin administration maintained fully functioning lung mitochondria during aging.

The damage resulting from excessive ROS has been associated with at least 100 human diseases, including cancer, cardiovascular diseases such as atherosclerosis, myocardial infarction, hypertension and neurological diseases such as amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease (4). Neurophysiologically, aging leads to a general slowness of the metabolic processes and loss of speed

in motor activities (5); besides, tissue levels of antioxidants are reduced due to, among other factors, the effect of FR.

Lipids represent the group of compounds most susceptible to free radicals due to the presence of double bonds in their fatty acids; besides, they are a structural part of the most exposed cell organelle: the cell membrane. Lipid peroxidation is associated with the etiology of various pathological processes such as aging (6). Among the final products of lipid peroxidation is malondialdehyde (MDA) which can be used as a biochemical measure of oxidative damage (7).

Melatonin (Mel) is a highly effective antioxidant; it is a neurohormone that prevents mitochondrial injury and helps to maintain its bioenergetic capacity (2). With aging, a gradual decrease in the levels of Mel is produced. This hormone has been shown to prevent oxidative stress and death of neurons exposed to amyloid protein and to enter all subcellular compartments without the aid of molecular transporters since it is both lipid and water soluble (2). The protective effects of Mel are mediated by two mechanisms: first, its ability to directly scavenge hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite anion, superoxide anion y peroxyl radical (8). Second, Mel also regulates the expression and activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRD) and glucose 6-phosphate dehydrogenase (G6PD) (9) and increases the intracellular content of reduced glutathione (GSH) (2,10, 11).

To thioctic acid (TA) and its derivative α-dihydrolipoic acid, have been attributed four antioxidant properties: 1. Their ability to reduce reactive oxygen species (ROS), 2.The capacity to regenerate endogenous antioxidants and to increase the effects of SOD, GSH and coenzyme Q10; 3. The ability to repair oxidative tissue damage and, 4. Their activity as chelators. However, pro-oxidant effects of TA have also been reported (12, 13). TA effectively crosses the blood-brain barrier (14), is amphipathic, allowing it to participate directly in the antioxidant defense mechanisms (15). It also increases by 30-70%, the levels of intracellular GSH (15, 16,). Thioctic acid may also be effective in improving immune function in aging through decreasing oxidative damage and revitalizing antioxidants in blood (17).

Drosophila melanogaster has many of the manifestations of senescence observed in mammals (18). In fact, in cellular and molecular biology, it is used as an experimental model because the fly has orthologs to 177 of the 289 human disease genes, which provides the foundation for the rapid analysis of basic processes involved in human disease (19).

The aim of this study was to determine the effect of Mel and TA in the life cycle of *Drosophila melanogaster* in order to provide information regarding the role of FR in aging, and their potential usefulness in the therapeutic procedures used to treating diseases related to oxidative stress. We also made observations of the phenotype of *Drosophila melanogaster* to use it as an indicator of possible genotoxic effects in the animals treated with the antioxidants.

MATERIALS AND METHODS

Drosophila melanogaster stocks

Male and females wild-type flies of *Drosophila melanogaster* (Oregon wild strain) were used. Flies were reared in a light/dark (LD) cycle of 12 h:12 h at a temperature of 25°C. The standard corn meal contained: 0.3 g of agar-agar, 5 g of corn flour, 1.5 g of yeast, 1.25 mL of 100% ethanol, 5 mL of a brown sugar solution (100 g of sugar in 100 mL distilled water), 0.65 g

of methyl p-hydroxybenzoate (Sigma Chemistry Co. MO. USA), and 43.75 mL of water. Flies treated with Mel and TA were fed with the same ingredients used to prepare the control medium with the addition of antioxidants at a concentration of 0.43 mM for Mel and 2.15 mM for TA added separately.

Selection of virgin females

To obtain virgin females, newborn emerging from the source culture collection were transferred at a rate of one newborn fly per assay tube that contained corn-meal medium. In this way, adult females reach sexual maturity without contact with males. Newborn females of *Drosophila melanogaster* remain virgins approximately the first 6 hours when they reach sexual maturity (20).

Study of the life cycle

The life cycle of *Drosophila melanogaster* in the control groups, and Mel-c and TA-c, was monitored daily. The day of laying the first eggs, larvae, pupae and hatching of the first and last eclosed flies were recorded. The number of eggs were not counted. After 10 days the parents were removed from the cultures.

Registration of eclosed

This was performed every 24 hours using a stereomicroscope (Lieder). The newborn flies born in the control media and in the media treated with antioxidants were transferred to empty glass bottles to be anesthetized with ether, counted and sexually differentiated.

Observation of phenotypic changes

The presence of phenotypic changes in the progeny was verified by stereoscopic microscopic observation of the phenotypic characteristics of the wild type *Drosophila melanogaster* as described: the eyes are red, oval with many facets; they have smooth-edged wings with uniform venation and extend beyond the abdomen, the body is beige with a pattern of light and dark areas.

Longevity

One day old males were transferred to glass vials (Pyrex culture 9.6 × 100 mm) containing 1 mL of the test food. The flies were held in group of five per vial. Fresh solutions of melatonin (Sigma) and thioctic acid (Sigma) were prepared daily at a concentration of 0.43 mM and 2.15 mM, respectively, in standard corn meal. In each vial 1 mL of the control food or of the melatonin or thioctic acid containing food was added. The vials were closed with cotton stoppers. Every day at 10 a.m., the dead flies were counted and survivors were transferred to freshly prepared food. Three replicates of each treatment and control were done. Two hundred controls, 300 melatonin and 300 thioctic acid fed male flies were employed in each of the triplicate studies that were carried out between January and December of 2011.

Malondialdehyde (MDA) determination

The MDA concentration was evaluated by measuring the thiobarbituric acid reactive substances (TBARS) according to the thiobarbituric acid (TBA) test described by Ohkawa (21) with modifications. For the determination of MDA whole body homogenates of control and treated flies were used. Ten males from each of the replicate of each treatment group were homogenized in 500 μL of Phosphate Buffered Saline (PBS) containing Butylated hydroxytoluene (BHT) at 2%. All homogenates were centrifuged (SORVALL RT6000): 6.000 rpm for 10 min to 4°C. A dilution series of triplicate MDA standards in the concentration range of 0 μM-250 μM was prepared by diluting the MDA standards in deionized water. One hundred μL of each unknown sample, MDA standards and blank were added

to separate microcentrifuge tubes containing 50 μL of 8.1% SDS, 375 μL of 0.8% TBA, 375 μL of 20% Acetic Acid pH 3.5 and 150 μL of deionized water. Each tube was closed and incubated at 95°C for 1 hour. They were removed and cooled in an ice bath for 5 min. To each tube 250 μL of deionized water and 1250 μL of Butanol-Piridine (15:1) were added. They were mixed and centrifuged at 3000 rpm × 10 min. The absorbance at 532 nm was measured in the supernatants. The results are expressed in nmoles of MDA/mg of protein.

Soluble protein concentrations

The Bicinchoninic Acid Protein Assay Kit was used to determine soluble protein concentrations in whole body homogenates of treated and control flies. To 0.1 mL of supernatant, BSA (Bovine Serum Albumin) standard or blank, 2 mL of the BCA working Reagent (Bicinchoninic acid + Copper (II) Sulfate) were added. The tubes were thoroughly mixed for 30 seconds using an orbital shaker prior to 2 hour incubation at room temperature. Absorbance was measured at 562 nm (Spectronic, Genesys 5). The amount of soluble protein in each of the three replicates for each treatment and control was expressed in μg/mL.

Motor activity

The number of movements of individual *Drosophila melanogaster* from the treatments and control groups were determined using the DAM2 Drosophila Activity Monitor (Trikinetic). The monitor measures the simultaneous individual activity of 32 flies, each in a separate tube. As a fly walks its passage is detected and counted by an infrared beam, which bisects the tube, and the accumulated count totals are reported to the host computer at the conclusion of each reading period. Flies were placed within glass capillary tubes 153 (5 mm in diameter and 65 mm in length) used for monitoring activity levels. Activity levels for each of the three total replicates in the treatment and control flies were measure at 15 minute time intervals for a 9 hours time period between 15:00 p.m. and 0:00 a.m. (when we detected the peak of highest activity) to identify variations in movements in controls and in flies treated with melatonin or thioctic acid. During experimentation, environmental conditions were held at a constant temperature of 25°C with a 12 hour light/dark cycle. Within the glass tubes, the flies were supplied with a food source in one of its extremes.

Statistical analysis

Data are expressed as mean ± SEM and were analyzed by means of the Analysis of Variance and the Bonferroni´s multiple comparison tests where appropriate. Differences were considered statistically significant when $p<0.05$.

RESULTS

In the study of the life cycle of the fruit fly, the treatment with antioxidants did not produce changes in the time of onset and duration of the phases egg, larva, pupa and imago when compared to control. A highly significant increase ($p<0.001$) in the number of eclosed in the Mel-c flies and a significant decrease ($p<0.01$) in the TA-c flies as compared to the control group were detected (Fig. 1).

Of the population born under treatment with TA-c, 4.08% was affected by changes in the external morphology: 1.02% decreased in body size and 3.06% had alterations in the form of the wings (Figs. 2-5). The flies born under treatment with Mel-c were not affected.

The mean life span of the animals treated with Mel-a was 36 ± 0.88 days, and in the control population 46 ± 0.00 day; the difference was significant ($p<0.01$).

The mean life span of Mel-c (40 ± 1.73 days), AT-c (50 ± 2.08 days) and AT-a (38 ± 3.76 days) treated flies was not significantly different from that of control group (46 ± 0.00 days) (Fig. 6). Mel treatment increased the maximum life span of *D. melanogaster* compared with the control group (67 ± 0.33 days). Statistically significant results ($p<0.05$) were obtained for Mel-c (79 ± 2.18 days) and for Mel-a (84 ±

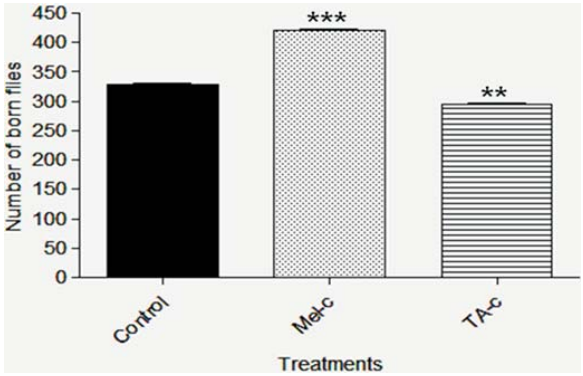

Fig. 1. Eclosion rate in *Drosophila melanogaster* control and treated with melatonin (Mel) and thioctic acid (TA). **($p<0.01$) TA-c vs control; ***($p<0.001$) control vs Mel-c, and Mel-c vs TA-c.

Fig. 2. Change in size in a female fly treated with TA-c, The size was decreased to approximately 1.5 mm, when comparing with a normal female *Drosophila melanogaster* wild type that measured approximately 2.5 mm. Olimpus SPS70VZ Digital Camera Macro mode.

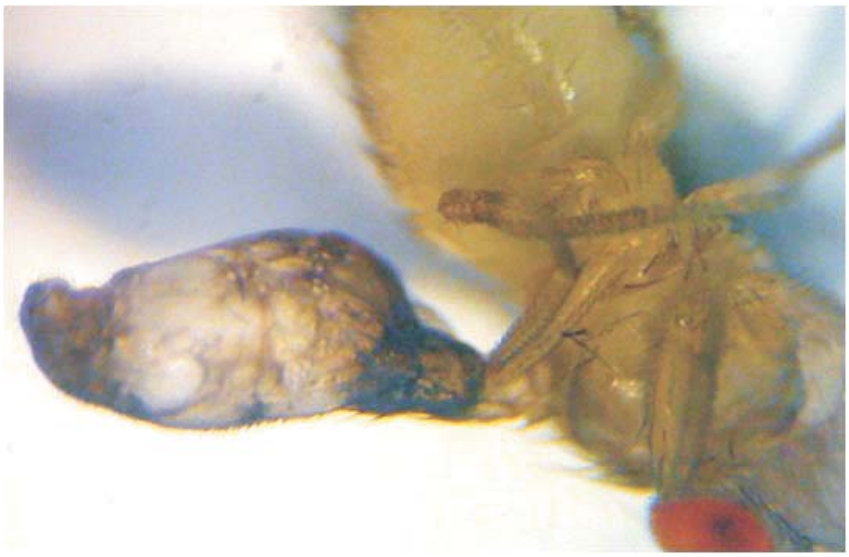

Fig. 3. Malformation in the wing of a *Drosophila melanogaster* treated with TA-c. A fluid-filled compartment was commonly observed. 10x.

Fig. 4. Comparison of a male with short wings to a male with wings of normal size. Olimpus SPS70VZ Digital Camera Macro mode.

Fig. 5. Comparison of a normal male fly with extended wing to a fly with rough not extended wing (right). Olimpus SPS70VZ Digital Camera Macro mode.

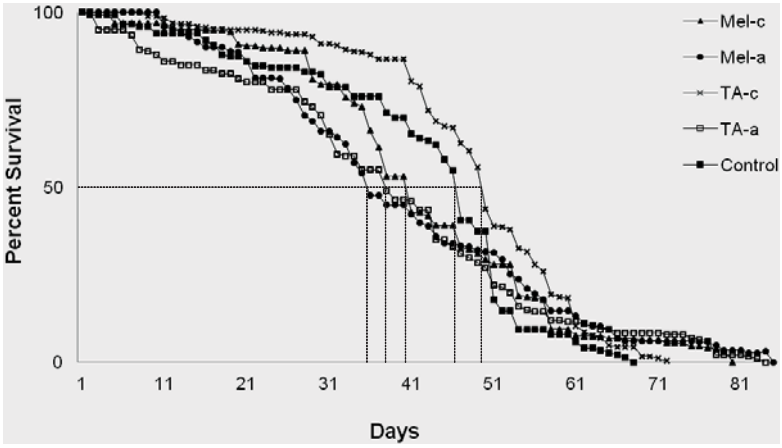

Fig. 6. Life span of *Drosophila melanogaster* control and treated with Mel and TA. Mean life span: Mel-a vs. control ($p<0.01$), 0% survival: Mel-c vs. control ($p<0.05$), Mel-a vs. control ($p<0.01$), AT-a vs. control ($p<0.01$).

0 days) ($p<0.01$), (Fig. 6). No significant difference in the life span was detected between TA-c (71 ± 1.20 days) and control (67 ± 0.33 days). In contrast, a significant increase ($p<0.01$) (83 ± 0.88 days) of maximum lifespan of the TA-a treatment group was observed when compared with the control group (Fig. 6).

The content of MDA in animals treated with Mel-c (10.7 ± 0.66 nmol of MDA/mg of protein) and Mel-a (10.3 ± 0.89 nmol of MDA/mg of protein) decreased significantly ($p<0.05$) when compared to the control group (15.7 ± 0.84 nmol of MDA/mg of protein) when the flies reached the age of 50% survival (Fig. 7). Interestingly, in flies treated with TA-c an increase in the concentration of MDA (16.7 ± 0.84 nmol of MDA/mg of protein) ($p<0.01$) was observed when compared with the control group. There was no significant difference in MDA levels when compared TA-a flies (17.7 ±

1.12 nmol of MDA /mg of protein) with control (15.7 ± 0.84 nmol of MDA / mg protein) (Fig. 7).

The control group had a score of spontaneous motor activity of 2269 ± 35.9 movements. A significant increase ($p<0.001$) in animals treated with Mel-c (4096 ± 9.3) and a significant decrease ($p<0.05$) in the group treated with Mel-a (1956 ± 19.9) was observed. The activity of flies treated with TA-c was significantly increased (2884 ± 26.4) ($p<0.01$) whereas no difference was observed in flies treated with TA-a (2156 ± 69.9) (Fig. 8).

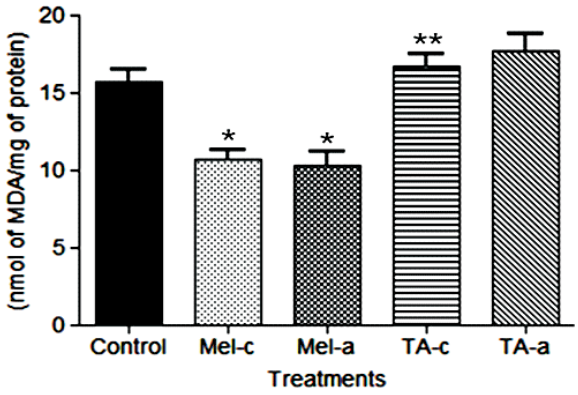

Fig. 7. MDA concentration (nmol of MDA/mg of protein) in *Drosophila melanogaster* when they reached the mean life span. *($p<0.05$) vs. control; **($p<0.01$) vs. control.

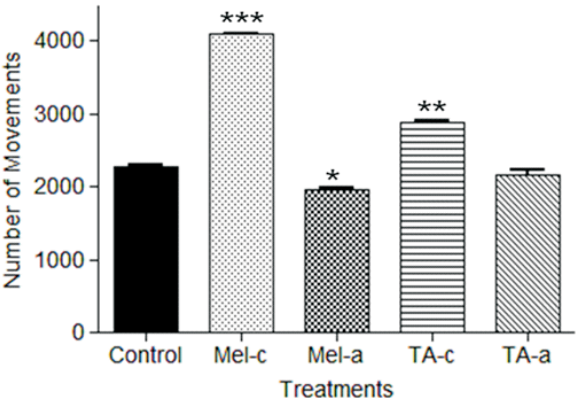

Fig. 8. Spontaneous motor activity of *Drosophila melanogaster* treated with Mel and TA when they reached the mean life span. *($p<0.05$) vs. control **($p<0.01$) vs. control; ***($p<0.001$) vs. control.

DISCUSSION

The egg-adult viability is the product of each of the viabilities given in the passage from one stage to the next throughout the ontogeny of *Drosophila melanogaster*. Thus, in this organism three basic types of viability are present: egg, larva, pupa and larva-pupa-imago. The fact that an egg does not hatch can be due to changes in the genotype or to environmental factors (22).

The decrease in viability in the TA-c flies could be due to several causes, namely: 1. The TA-c group could have a decreased fertility 2. The egg-larva hatching could have increased causing an increase in population density, 3. The TA-c may have had a genotoxic effect in the early stages of development, 4. Another possibility is that this antioxidant may have affected cell signaling cascades essential for patterning of developmental stages. Experimental evidence shows that ROS are not only toxic to the organism but also are important regulators of cell signaling pathways. For example, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which regulates the expression of a variety of immune genes and plays a central role on cell death and survival has been demonstrated to be regulated by ROS (23). The presence of NF-κB and several orthologs of the mitogen-activated protein kinase (MAPK) signaling cascades have been shown in *Drosophila* (24). In addition, the functional crosstalk of these pathways is also conserved in *Drosophila* (25).

Extensive experimental evidence shows that programmed cell death plays a critical role during embryogenesis (26), during eye differentiation (27, 28) and in the central

nervous system after eclosed (29, 30). One possible mechanism explaining the phenotypic alterations observed in the flies treated with TA during developmental stages may be the modulation of transcriptional pathways by the modification of ROS levels which in turn could have altered the normal pattern of cell death.

Baena-Lopez and Garcia-Bellido (31), conducted studies with *Drosophila melanogaster*, concluding that the size and shape of organs depend on cellular processes such as cell proliferation, cell survival and spatial arrangement of cells. They also determined that the pattern of gene expression leads to the formation of imaginal discs of the wings, and that the shape and size of this organ depends on the genome. Martín and Morata (32) analyzed the growth of the wing imaginal disc of *Drosophila*, in which the parameters of development and growth are well known. They observed that the imaginal discs have an autonomous mechanism through which growth in the anterior and posterior compartments is independent. The mechanisms that control organ growth during development are the least known. The final size is determined once the developmental process is finished.

Markow (33) studied the reproductive behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* and observed that food plays a key role in reproductive success. Santos *et al.* (34), reported that the accumulation of larvae affects survival due to changes in biological efficiency, suggesting that traits such as fertility of the female, the male mating ability, longevity and starvation resistance are dependent on the ontogenetic development. Another factor that may influence the decrease of the population of flies treated with TA-c is that the third larvae state did not emerge at a suitable height. On the walls of the medium the larvae did not reach the maximum height possible compared with that of Mel-c flies and control. This might be due to inadequate food intake because of a decreased larval foraging activity. Fong *et al.* (22), found that in *Drosophila melanogaster* pupae mortality decreased to 0% when the height of pupation reaches 31 to 41 mm above the medium. In this study, such parameter was not evaluated. However, it is possible that TA treatment affected larval motility or foraging activity. Modulation of ROS production by the antioxidant treatment could have affected programmed cell death on early adulthood and this could also explain wings morphology alterations observed in the present study. Several authors have shown that abdominal muscles required for eclosed and spreading of the wings and their innervating neurons go through a process of apoptosis within 12 hours of eclosed (35-37). Milton and colleages (38) recently showed that both excessive and defective ROS production is associated with defects of the neuromuscular junction formation and functioning in *Drosophila*.

Miller (39) found that when larval density increases, the time it takes to complete the development increases. This is due to a selection effect in which the larvae with smaller and greater capacity of foraging efficiently exploit the nutrition medium.

Izmaylov and Obukhova (40) studied the duration of the life cycle of *Drosophila melanogaster* under the effect of Mel. The compound was added to the culture medium during development. The geroprotector effect of the hormone was demonstrated by the increase in the life span of treated flies of a relatively low life span in the population from which the control and experimental groups were formed. However, for a relatively high life span the effect of the hormone was either not detected or appeared as a toxic reduction in life span (up to 10%) in the experimental group.

According to Bonilla *et al.* (41), Mel, when added to the nutrient medium, significantly increased the life span and stress resistance of adult *Drosophila melanogaster*. The maximum life span was 61.2 days in controls and 81.5 days in Mel treated flies (an increase in 33.2% in maximum life span). Furthermore, in a test of superoxide mediated toxicity Mel treatment increased the resistance of the flies to Paraquat and to an ambient temperature of 36°C.

For a long time researchers have used animal survival to identify genetic and pharmacological interventions that prolong life. Accordingly, Bauer *et al.* (42), conducted a trial with molecular biomarkers to identify drugs that prolong the life span of *Drosophila melanogaster*. In their study, treatment with TA was beneficial, because it prolonged the life span of flies in normal laboratory conditions. These results are similar to our results with TA-a but differ from those obtained with TA when administered throughout the life cycle (TA-c). Further research on the effect of this compound in the different experimental models is needed to clarify this observation.

The effect of TA on lipid peroxidation and the antioxidant status was studied in the blood of young and adult rats. The levels of enzymatic and non enzymatic antioxidants decreased with age, but this decrease was attenuated by TA. Lipid peroxide concentrations increased with age for controls, and it was reduced with TA administration. These results suggest that biochemical lesions that are considered part of normal aging process are neutralized by TA (43).

Several studies provide evidence that supplementation with TA decreases oxidative stress and restores to normal the reduced levels of other antioxidants in vivo. However, there is also evidence that TA and dihydrolipoic acid can exert prooxidant properties *in vitro*. In a study by Moini *et al.* (44), using rats as experimental model, these compounds stimulated superoxide anion production in mitochondria. In our study, the increased content of MDA detected in the flies treated with TA-c suggests that this compound could have increased the production of FR. These results warrant further investigation in vivo.

In conclusion, Mel increased fertility of flies and/or the survival of egg-adult and also increased the eclosion rates and life span of the flies. It also reduced the concentration of MDA and increased the motor activity of the flies. The results obtained with Mel in this study suggest that this hormone can be a therapeutic option to mitigate the damage caused by FR. Thioctic acid administered throughout the life cycle decreased the fertility of flies, led to changes in the phenotype, and increased the concentration of MDA and the motor activity in flies when compared to the control group. In flies treated in adulthood with TA, MDA levels were not altered but lifespan and spontaneous motor activity was increased when compared with the control group Moreover, the effects of TA show that this compound could cause side effects since it appears to be able to act as a prooxidant.

ACKNOWLEDGEMENTS

To the Consejo de Desarrollo Científico y Humanístico de la Universidad del Zulia (CONDES), for co-financing this study.

REFERENCES

1. **Harman D**. The free radical theory of aging. Antioxid Redox Signal 2003; 5: 557-561.
2. **Acuña-Castroviejo D, Escames GLC, Lopez LC, Ortiz F, Lopez A, García JA.** Evidencias de la utilidad de la melatonina frente al envejecimiento y los procesos

neurodegenerativos. Psicogeriatría 2009; 1: 3-21.
3. **Acuña-Castroviejo D, Carretero M, Doerrier C, López LC, García-Corzo L, Tresguerres JA, Escames G.** Melatonin protects lung mitochondria from aging. Age (Dordr) 2012, 34: 681-692.
4. **Mckee T, Mckee J.** Bioquímica. La base molecular de la vida. 3ra Ed. España: Editorial McGraw-Hill Interamericana; 2003. p: 320-322.
5. **Fleming J, Reveillaud I, Niedzwiecki A**. Role of oxidative stress in *Drosophila* aging. Mutat Res 1992; 275: 267-279.
6. **Velásquez PM, Prieto GB, Contreras PR**. El envejecimiento y los radicales libres. Ciencias 2004; 75: 36-43.
7. **Venereo GJR**. Daño oxidativo, radicales libres y antioxidantes. Rev Cubana Med Milit 2002; 31: 126-133.
8. **Reiter RJ**. Melatonin: Lowering the high price of free radicals. News Physiol. Sci. 2000; 15: 246-250.
9. **Tan DX, Manchester LC, Reiter RJ, Qi W, Karbownik M, Calvo JR**. Significance of melatonin in antioxidative defense system: reactions and products. Biol Signals Recept 2000; 9: 137-159.
10. **Reiter RJ, Tan DX, Manchester LC, Qi W**. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species. Cell Biochem Biophys 2001; 34: 237-256.
11. **Cantú BC, Baizábal CF, Aldret VJ, Chávez GE.** Aplicaciones clínicas del ácido tióctico (ácido α-lipoico) en pacientes con neuropatía diabética sintomática: revisión. Med Int Mex 2007; 23: 151-158.
12. **González-Pérez O, Moy-López NA, Guzmán-Muñiz J**. El alfa-tocoferol y el ácido alfa-lipoico. Una sinergia antioxidante con potencial en medicina preventiva. Rev Invest Clin 2008; 60: 58-67.
13. **Packer L, Kraemer K, Rimbach G**. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition 2001; 17: 888-895.
14. **Packer L, Trischeler HJ, Wessel K**. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. Free Radic Bio Med 1997; 22: 359-378.
15. **Aoyama S, Okimura Y, Frujita H, Sato EF, Umegaki T, Abe K, Inoue M, Utsumi K, Sasaki J.** Stimulation of membrane permeability transition by alpha-lipoic acid and its biochemical characteristics. Physiol Chem Phys Med NMR 2006; 38: 1-20
16. **Hagen TM, Moreau R, Sub JH, Visioli F.** Mitochondrial decay in the aging rat heart: evidence for improvement by dietary supplementation with acetyl-L-carnitine and/or lipoic acid. Ann NY Acad Sci 2002, 959:491-507.
17. **Palaniyappan A, Alphonse R.** Immunomodulatory effect of DL-α-lipoic acid in aged rats. Exp Gerontol 2011, 46: 709-715.
18. **Zorrilla GAE**. El Envejecimiento y el estrés oxidativo. Rev Cubana Invest Biomed 2002; 21: 178-185.
19. **Rubin GM, Yandell MD, Wortman JR, Gabor GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, Cherry JM, Henikoff S, Skupski MP, Misra S, Ashburner M, Birney E, Boguski MS, Brody T, Brokstein P, Celniker SE, Chervitz SA, Coates D, Cravchik A, Gabrielian A, Galle RF, Gelbart WM, George RA, Goldstein LSB, Gong F, Guan P, Harris NL, Hay BA, Hoskins RA, Li J, Li Z, Hynes RO, Jones SJM, Kuehl PM, Lemaitre B, Littleton JT, Morrison DK, Mungall C, O'Farrell PH, Pickeral OK, Shue C, Vosshall LB, Zhang J, ZhaoQ, Zheng XH, Zhong F, Zhong W, Gibbs R, Venter JC, Adams MD, Lewis S.** Comparative genomics of the eukaryotes. Science 2000; 287: 2204-2215.
20. **Demerec M; Kaufman BP**. *Drosophila* Guide. The life cycle of *Drosophila melanogaste*r. 10th Ed. USA: Carnegie Institution of Washington; 1996. p: 6-9.
21. **Ohkawa H, Ohishi N, Yagi K**. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
22. **Fong C, Díaz F, Osorio J, Castano L, González F, Jurado L, Castillo K, Cárdenas H**. Effect of eggs population density on viability and time of development of

Drosophila melanogaster (Drosophilidae). Acta Biol 2008; 13: 123-132.
23. **Thannickal VJ, Fanburg BL**. Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 2000; 279: L1005-L1028
24. **Silverman N, Maniatis T.** NF-κB signaling pathway in mammalian and insect immunity. Genes Dev 2001; 15: 2321-2342.
25. **Nakano H, Nakajima A, Sakon-Komazawa S., Piao J-H, Xue X, Okumura K**. Reactive oxygen species mediate crosstalk between NF-κB and JNK. Cell Death Differ 2006; 13: 730-737.
26. **Abrams JM, White K, Fessler LI, Steller H**. Programmed cell death during *Drosophila* embryogenesis. Development 1993; 117: 29-43.
27. **Cagan RL, Ready DF**. The emergence of order in the *Drosophila* pupal retina. Dev Biol 1989; 136: 346-362.
28. **Wolff T, Ready DF**. Cell death in normal and rough eye mutants of *Drosophila*. Development 1991; 113: 825-839.
29. **Truman JW, Thorn RS, Robinow S**. Programmed neuronal death in insect development. J Neurol 1992; 23: 1295-1311.
30. **Truman J W, Talbot WS, Fahrbach SE, Hogness DS**. Ecdysone receptor expression in the CNS correlates with stage-specific responses to ecdysteroids during *Drosophila* and *Manduca* development. Development 1994; 120: 219-234.
31. **Baena-Lopez LA, García-Bellido A**. Control of growth and positional information by the graded vestigial expression pattern in the wing of *Drosophila melanogaster*. Proc Natl Acad Sci 2006; 103: 13734-13739.
32. **Martín FA, Morata G**. Compartments and the control of growth in the *Drosophila* wing imaginal disc. Development 2006; 133: 4421-4426.
33. **Markow TA**. Reproductive behavior of *Drosophila melanogaster* and *D. nigrospiracula* in the field and in the laboratory. J Comp Psychol 1988; 102: 169-173.
34. **Santos M, Borash D, Joshi A, Bounlutay N, Mueller LD**. Density-Dependent natural selection in *Drosophila:* evolution of growth rate and body size. Evolution 1997; 51: 420-432.
35. **Demerec M.** Biology of Drosophila. Cold Spring Harbor Laboratory Press. Facsimile edition. New York. 1994.
36. **Truman JW**. Programmed cell death in the nervous system of an adult insect. J Comp Neurol 1983; 216: 445-452.
37. **Truman JW**. Cell death in invertebrate nervous systems. Annu Rev Neurosci 1984; 7: 171-188.
38. **Milton VJ, Jarrett HE, Gowersa K, Chalak S, Briggs S, Robinson IM, Sweeney ST**. Oxidative stress induces overgrowth of the Drosophila neuromuscular junction. PNAS 2011; 108: 17521-17526.
39. **Miller RS**. Larval competition in *Drosophila melanogaster* and *D. simulans*. Ecology 1964; 45: 132-147.
40. **Izmaylov DM, Obukhova LK**. Geroprotector effectiveness of melatonin: investigation of lifespan of *Drosophila melanogaster*. Mechec Ageing Dev 1999; 106: 233-240.
41. **Bonilla E, Medina S, Dias S**. Extention of life span and stress resistance of *Drosophila melanogaster* by long-term supplementation with melatonin. Exp Gerontol 2002; 37: 629-638.
42. **Bauer JH, Goupil S, Garber GB, Helfand SL**. An accerelated assay for the identification of life span- extending interventions in *Drosophila melanogaster*. Proc Natl Acad Sci USA 2004; 101: 12980-12985.
43. **Arivazhagan P, Juliet P, Panneerselvam C.** Effect of DL-α-Lipoic Acid on the status of lipid peroxidation and antioxidants in aged tats. Pharmacol Res 2000; 41: 299-303.
44. **Moini H, Packer L, Saris NEL.** Antioxidant and prooxidant activities of α-Lipoic acid and dihydrolipoic acid. Toxicol Appl Pharmacol 2002; 182: 84-90.