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Original Article

Short, Mid, and Long-Term Melatonin Exposure Alleviates the Effects of Abamectin on the Antioxidant Defense Mechanism in the Midgut of *Spodoptera litura* (Insecta: Lepidoptera)

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ABSTRACT

Introduction: Melatonin (N-acetyl-5-methoxytryptamine), a serotonin derivate, is a powerful antioxidant due to its capacity to stimulate the innate immune response in plants and animals. However, in invertebrates, few studies have been conducted to verify the protective effect of melatonin in the antioxidant defense system. **Objectives:** This study aimed to evaluate melatonin antioxidant role in short-term (24 hr), intermediate (72 hr), and long-term (144 hr) effects against abamectin, the family of avermectins exposure in the midgut of *S. litura*. **Materials and Methods:** Melatonin affects ROS generation, nitrite content, protein carbonyl content, TBARS levels, antioxidant enzymes (SOD, GST, CAT, and GPx), ascorbic acid levels, and AChE levels were studied. **Results:** Results showed that abamectin exposure substantially increases nitrite, TBARS, and ascorbic acid levels ($p<0.001$). In contrast, long-term melatonin exposure (144 hr) declined ROS, carbonyl content, SOD, GPx, AChE, and ascorbic acid levels ($p<0.05$). Short-term melatonin exposure (24 hr) was also observed to be effective by significantly reducing ($p<0.05$) ROS, GST, and Gpx levels in abamectin-exposed animals. Similarly, long-term melatonin exposure (144 hr) showed small DNA fragments similar to control and expression of 100 and 75kDa proteins because of its protective mechanism in insects. Similarly, luzindole, a melatonin antagonist, has small DNA fragments because of its blocking actions on the melatonin receptors in insects. **Conclusion:** The present study shows that melatonin reduces free radical generation and has protective effects on pesticide-exposed insects, especially in long-term exposure to animals. This effect of indoleamine may be a possible strategy to prevent the damage caused due to abamectin exposure in animals.

Keywords: Abamectin, Antioxidant enzymes, Hormone, Lepidoptera, Midgut, Oxidative stress, Pesticide.

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INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine), a serotonin derivate, is produced in the pineal gland as well as several organs, including ovary, testes, bone marrow, gut, placenta, and liver invertebrate animals,[1-4] but it is also present in bacteria, protozoa, plants, fungi, and invertebrates.[5,6] Recent studies observed that the first melatonin metabolite in the antioxidant pathway is N-acety l-N-formyl-5-methoxykynuramine (AFMK), which prevents free radicals damage. The process by which melatonin and its metabolites successively scavenge Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) is referred to as the free radical scavenging cascade. This indoleamine effect is observed to be highly effective, even at low concentrations, protecting organisms from oxidative stress damage.[7] This activity has been observed in *Caenorhabditis elegans*[8] and humans.[9] Melatonin benefits have been studied in several invertebrate animals. However, few studies have suggested the immunotoxicity effects of indoleamine in insects.[10]

Abamectin (ABA) is a broad-spectrum insecticide, acaricide, and nematicide. It belongs to the family of avermectins and has been used in high potencies for a broad spectrum of invertebrate pests.[11] In addition, it is believed to be a GABAergic agent and is known to induce oxidative tissue damage due to its capacity to generate ROS.[12] ROS, the derivatives of cellular oxygen, cause damage to the organism reacting with critical biological macromolecules, such as lipids and proteins, leading to cell death. Insects have

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developed a complex network of enzymatic and non-enzymatic antioxidant systems.[13] In the enzymatic ROS-scavenging pathways, significant components of the antioxidant enzyme system of insects include Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidases (POX).[14] Several studies have verified the influence of melatonin in the antioxidant defense system regulating the activity of Glutathione Peroxidase (GPx), Mn-superoxide dismutase (Mn-SOD), and Cu/Zn-superoxide dismutase.[7] It is observed that the indoleamine stimulates the 'antioxidant cascade,' increasing the antioxidant enzymes' activities, improving the total antioxidative defense capacity of the organism.[15] In organisms, increased levels of Lipid Peroxidation (LPO) and protein carbonyls are considered markers of oxidative stress.[16] To prevent oxidative stress damage, it is well known that several antioxidant enzymes may decrease these disturbs due to their direct and indirect effects on the organism. By its immediate effects, melatonin is observed to reduce LPO levels and protein and DNA damage. In addition, indoleamine and its metabolites play a pivotal role in the antioxidant system scavenging both ROS and RNS.[17]

Among lepidopteran insects, *Spodoptera litura* Fabricus (Lepidoptera: Noctuidae), is a polyphagous insect that has about 172 species of host plants from 40 families in India. It is also known as the Cluster caterpillar, Cotton leafworm, Tobacco cutworm, and Tropical armyworm. It is one of the most economically important insect pests in many countries including India, Japan, China, and other countries of Southeast Asia. In India, *S. litura* is proving to be a chief constraint in the production of crops. The higher reproductive capacity and migration ability over long distances make *S. litura* a serious pest of many economically important crops such as cotton, groundnut, rice, tomato, tobacco, citrus, rubber, castor, millets, sorghum, maize and many other vegetables. This nocturnal insect was chosen as a model for this study because of its availability, fast-growing and polyphagous in nature.[18] In insects, brain and midgut tissue secretes high levels of melatonin.[19] Midgut has a rich antioxidant enzymatic source which diminishes the pro-oxidant conditions and decreases the free radicals generation.[18,20]

Numerous clinical trials have examined the therapeutic use of melatonin for the last 20 years in different fields of medicine. Our earlier study[21] proved that pre-treatment of melatonin decreases the abamectin pesticide-induced toxicity in *S. litura*. The efficacy of melatonin has been assessed to be a coadjutant treatment for several diseases with encouraging results due to the lack of serious toxicity and its natural source.[22-24] Several studies have verified melatonin antioxidant effect of invertebrate animals in a time-dependent manner.[25-27] However, more studies are needed to know about the safe use of melatonin in medicine. Nowadays, it is still unknown the time-dependent free radical scavenging potential of melatonin, especially in insects. Given the above, the purpose of this study is to evaluate melatonin's role in the antioxidant defense system in a short-term (24 hr), intermediate (72 hr), and long-term (144 hr) effect against abamectin exposure in the midgut of *S. litura* model.

MATERIALS AND METHODS

Chemicals

3-carboxy-4-nitrophenyl disulfide (DTNB), 2-thiobarbituric acid (TBA), melatonin, and Luzindole were purchased in technical grade from Sigma Aldrich (India). Abamectin (N-acetyl 2-benzyl tryptamine) was procured from Insecticides Pvt. Ltd., New Delhi (India) and has the commercial-grade name of Agrimek. Leaf-dip method[28] was used to find out the mortality (%) for 24 hr using castor leaves as substrates. From this preliminary data, the dosage of abamectin used was 1.56 ml (28 mg AI)/L for further experiments, according to previous studies.[29] The chemicals and reagents used in this study were of analytical grade.

Animals

Spodoptera litura (National Accession No NBAII-MP-NOC-02) was obtained from the National Bureau of Agricultural Insect Resources (NBAIR), formerly the National Bureau of Agricultural important insects (Bangalore, India). All the animals were maintained in our laboratory conditions (12-hr light/12-hr dark cycle, 70±10% humidity, 24±1°C) and provided*ad libitum*. The larvae were fast 8 hr before dissection. In this study, biochemical assays were performed with fifth instar larvae.

Experimental Design

Third instar larvae (*n*=630) were divided randomly into five groups as follows:

(1) Control group (*n*=90): 0.1M Phosphate buffered saline containing Na_2HPO_4 and NaH_2PO_4; pH 7.5).

(2) Luzindole group (*n*=90): (250 mM).[30]

(3) Melatonin group (*n*=90): 4.3×10^{-5} M/100 ml diet.[31]

(4) Abamectin group (*n*=90): 28 mg AI/L.[29]

(5) Melatonin+abamectin group (N=270 totally; *n*=90/each group): (MT; 4.3×10^{-5} M/100 ml diet and ABA; 28 mg AI)/L). Three different measurements were performed for the time of melatonin effects in this group:

(a) 24 hr (*n*=90): short-term treated larvae;

(b) 72 hr (*n*=90): intermediate treated larvae;

(c) 144 hr (*n*=90): long-term treated larvae;

Melatonin (4.3×10^{-5} M) along with abamectin was supplemented in three different time-dependent groups (24 hr: short-term, 72 hr: intermediate, and 144 hr: long-term). In the short-term group, melatonin was administered for 24 hr, in the intermediate group during 72 hr, and the long-term group was exposed during

144 hr to melatonin. After this period, larvae were provided with melatonin free diet until they reached the fifth instar stage.

In the melatonin solution preparation, ethanol was used to dissolve melatonin (>0.01%).[32] From each group (*n*=90 larvae), we obtained three replicates (30 larvae formed each replicate) which were kept in plastic containers fed with 100 mL of artificial diet according to previous studies.[33] All experimental animals were kept in this diet until they reached the 5th instar, replaced every three days. All the animals were dissected under cold conditions. Midgut was collected carefully using ophthalmic scissors and a sterile needle and placed rapidly into a deep freezer at -20ºC until the time of analysis.

Insect bioassay

Larval survival rate was assessed by gentle probing with a fine camelin brush every 24 hr in all experimental groups.[34] It was categorized as dead (no movement), moribund (unable to move), and alive (able to move>1 body length). Dead and dying insects were considered dead. The mortality rate (%) was corrected according to Abbott's formula.[35]

Preparation of midgut extract

Larvae were immobilized for 30 min maintaining them at -20°C previous to the dissection. The midgut was removed and weighed. Midgut homogenate (10%) was prepared using 0.1M ice-cold phosphate buffer (pH 6.4), and centrifugation was repeated 2-3 times (1000*x*g, 4ºC for 15 min), filtered. The supernatant was used as an enzyme source for subsequent biochemical analysis.

Assays of antioxidant enzyme activities

The measurement of SOD, GST, CAT, GPx, and non-enzymatic antioxidant molecules, including ascorbic acid activities, was determined spectrophotometrically. In every biochemical assay, protein content was measured using UV-visible Spectrophotometer (Systronics, India).

Superoxide Dismutase (SOD; EC 1.15.1.1) activity was determined based on the studies of Marklund and Marklund.[36] The reaction was started by adding Pyrogallol (15 mM) and followed by the rate of autoxidation at 440 nm. One unit of total SOD activity was defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation. Total SOD activity was expressed as units/min/mg protein.

Glutathione S–transferase (GST; EC 2.5.1.18) activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to the method of Habig *et al.*[37] The formation of GSH–CDNB conjugate was monitored by the change in absorbance at 340 nm and had an extinction coefficient of 9.6 mM^{-1} cm^{-1}. One unit of GST activity was defined as the amount that catalyzes the conjugation of 1 mM/L GSH with CDNB per minute per mg protein. GST enzyme activity was expressed as μM/min/mg of protein.

Catalase (CAT; EC 1.11.1.6) activity was determined by monitoring the clearance of hydrogen peroxide (H_2O_2) at 240 nm.[38] One unit of CAT activity was defined as the amount that decomposes H_2O_2 per second per g protein. Its activity was expressed as μM H_2O_2 decomposed/min/mg protein using the molar extinction coefficient (ε) of 0.0394 mM^{-1} cm^{-1} for H_2O_2 conjugate.

Glutathione Peroxidase (GPx; EC 1.11.1.19) activity was measured in absorbance at 412 nm. Reduced GSH reacts with DTNB and gets reduced to a yellow-colored complex with an absorption maximum at 412 nm.[39] GPx activity was expressed as Units/mg of protein. One unit of GPx is defined as the amount of the enzyme that converts 1 μM of GSH to Glutathione disulfide (GSSG) in the presence of H_2O_2 per min.

Furthermore, non-enzymatic antioxidant molecules such as ascorbic acid levels were determined by the methodology of Roe and Kuether.[40] It was assessed by coupling dehydro-ascorbic acid with DNPH, and the resulting derivative was treated with sulfuric acid (H_2SO_4) to produce a red color that absorbs maximally at 520 nm. The enzyme activity was expressed as μM/mg protein.

Acetylcholinesterase (AChE) activity

AChE activity was measured by Ellman *et al.*,[41] method using 5,5'-dithio-bis-2-nitrobenzoate (DTNB) as an oxidizing agent. The rate of hydrolysis of acetylthiocholine iodide was calculated by following an increase in absorbance at 412 nm. AChE activity was expressed as μM/min/mg protein using the molar extinction coefficient (ε) of the chromophore is 1.36×10^4 mM^{-1} cm^{-1}.

Total Protein concentration

According to Folin's Phenol method, total protein content was determined using Bovine Serum Albumin (BSA) as the standard.[42]

Measurement of ROS generation

ROS generation was performed according to Beauchamp and Fridovich[43] (with minor modifications), adding 1 mL of KOH (2M) and 1 mL of DMSO to the sample, and its absorbance was measured at 630 nm. ROS generation was calculated by comparing OD values of ROS with a standard curve elaborated with Nitro Blue Tetrazolium chloride (NBT) and was expressed as μM NBT equivalent/10 mg tissue.

Griess reaction for nitrite concentration

Nitrate levels were measured according to Ding *et al.*,[44] Nitrate converts nitrite to a deep purple azo compound using Griess reagent (1% sulfanilamide, 0.1% naphthylene diamine dihydrochloride, and 2.5% H_3PO_4) at 540 nm. All measurements were performed in triplicate. Nitrate concentration (μM/mg) was determined using a standard curve of sodium nitrate.

Oxidative damage markers

The oxidative damage to proteins was measured by estimating carbonyl proteins according to a modification of the technique of Levine *et al*.,[45] using 2, 4 dinitrophenylhydrazine (DNPH) as a reagent. Carbonyl content was determined from the difference in absorbance (λ = 370 nm) between DNPH-reacted and unreacted HCl samples. Protein carbonyl content was expressed as nmol protein carbonyl per mg protein.

As an index of lipid peroxidation, the formation of Thiobarbituric Reactive Substances (TBARS) was measured as described by Janero.[46] It was based on a Thiobarbituric Acid (TBA) method in which Malondialdehyde (MDA) reacts with TBA to generate a red species (with a maximum absorbance at 532 nm). Results were expressed as *nmol* malondialdehyde formation per g tissue.

DNA Fragmentation Assay

10% homogenate was collected by centrifugation at 12,000*x*g for 10 min at 4°C using 10mM cold Phosphate Buffered Saline (PBS). The pellet was washed twice with PBS and suspended in 750 µL of TES [10 mM Tris-HCl, 1 mM EDTA pH 8.0% SDS] with 50 µg/mL of proteinase K. The result was incubated at 37°C for 3 hr. The DNA was extracted twice with an equal volume of phenol (saturated with 100 mM Tris/HCl pH-8)/chloroform/ isoamyl alcohol (25:24:1) and then once with chloroform alone. The quantification of DNA samples was conducted using a UV-visible spectrophotometer. Additionally, the ratios of A260/280 and A260/230 were calculated to evaluate the sample integrity and contamination of proteins or other organic substances. High integrity and purity DNA preparations were accepted for electrophoretic analysis. The extracted DNA was precipitated in ethanol and dissolved in Tris-acetate EDTA (TAE) buffer with RNAse. The result was incubated at 37°C for 2 hr. Separation of DNA samples was performed using horizontal gel electrophoresis (1.5% agarose) under standard conditions. DNA fragments were detected using ethidium bromide staining and UV transilluminator.

Polyacrylamide gel electrophoresis

Gut enzyme extracts from the third instar of *S. litura* larvae were prepared according to Johnston *et al*.,[47] method with some modifications. The midguts were homogenized in ice-cold 0.2 M glycine-NaOH buffer, pH 8 containing 2 mM DTT, and 10% PVP (10 guts/mL buffer). The homogenates were kept for 2 hr at 10°C and centrifuged at 11,200*x*g for 15 min at 4°C. The resultant supernatant was used in the Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), using 10% gel. Bromophenol blue was used as the tracking dye. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 staining solution (0.025% Coomassie Blue R-250, 40% methanol, 7% acetic acid). The gel was destained with a solution I (40% methanol, 7% acetic acid, in distilled water) for 30 min. Then the gel was placed in destaining solution II (7% acetic acid, 5% methanol in distilled water) for 2 hr with intermittent shaking. Destaining was continued until blue bands and a transparent background was obtained, is then photographed. Molecular weights of unknown proteins were calculated from the standard molecular marker.

Statistical analysis

All the data were analyzed by one-way ANOVA using GraphPad Prism 5 (Version 5.0, San Diego, CA). The results in the graphs

Table 1: Larvae and Pupal length (cm) (Mean±SD) of experimental groups in *Spodoptera litura*.

			Larval stages (Instar)						
Sl. No.	Experimental Groups		I	II	III	IV	V	VI	Pupae
1.	Control		0.75±0.05	1.21±0.03	1.68±0.02	2.59±0.03	3.16±0.03	3.35±0.04	1.65±0.02
2.	Luzindole		0.64±0.07	0.99±0.03	1.41±0.03	1.99±0.04	2.47±0.02	3.18±0.04	1.36±0.05
3.	Melatonin		0.77±0.04	1.37±0.04	1.60±0.02	2.52±0.02	3.10±0.03	3.22±0.03	1.67±0.04
4.	Abamectin		0.21±0.07*	0.76±0.06*	1.17±0.04	1.67±0.03*	1.97±0.23*	2.10±0.05*	1.20±0.05
5.	Melatonin+abamectin	24 hr (Short-term)	0.53±0.03	0.89±0.04	1.20±0.06	1.66±0.07*	2.12±0.04	2.30±0.06	1.50±0.05
6.		72 hr (Midterm)	0.25±0.05*	0.78±0.03*	1.20±0.07	1.71±0.06	1.93±0.05*	2.13±0.04*	1.20±0.03
7.		144 hr (Long-term)	0.58±0.04	1.00±0.04	1.50±0.04	2.03±0.05	2.50±0.05	2.50±0.06	1.60±0.05

Data are expressed as mean ± SD of three independent biological replicates. Asterisk (*) showed the significant differences ($p< 0.05$) (marked as bold) with control group by one-way ANOVA.

I-First larval instar; II-Second larval instar; III-Third larval instar; IV-Fourth larval instar; V-Fifth larval instar; VI-Sixth larval instar; cm-centimeter; SD- Standard deviation; hr-hours.

Melatonin+Abamectin exposure during 24 hr, 72 hr and 144 hr.**; $p<0.01$, ***: $p<0.001$, compared with control group; ns; No significant.

represent the mean values ± SD. Statistical testing of significance was analyzed using Bonferroni multiple comparison test.

RESULTS

Different timing of Melatonin exposure on *S. litura* growth and Development

Increased larval mortality (50%) was observed in the abamectin group ($p<0.01$) compared to the control group. Abamectin toxicity was significantly reduced ($p<0.05$) in long-term melatonin+abamectin (144 hr) treated animals compared to the abamectin group (Figure 1A). Attending to the larvae survival rate, there were observed statistically significant differences ($p<0.001$) in intermediate melatonin+abamectin (72 hr) exposed animals. The number of moribund and dead larvae was observed to be increased significantly ($p<0.001$) in the abamectin group (Figure 1B). Our results also showed that the larval growth was affected in abamectin exposed animals. The length and weight of all life stages of larvae (from the first instar to the sixth instar) and pupae were displayed in Tables 1 and 2. While in melatonin+abamectin treated insects during 24 hr and 144 hr, there were no observed differences compared to the control group; the melatonin+abamectin 72 hr animals showed statistically significant differences ($p<0.05$) in length (I, II, V, VI instars) and weight (I, II, VI instars) compared to control group (Tables 1 and 2). The reduced number of hatching larvae and the increase of larvae development time in all life stages of larvae in the abamectin group was recovered in the melatonin exposed groups (24 hr and 144 hr). These results were not observed in melatonin+abamectin 72 hr group (Table 3). The results suggest that marked reduction of oxidative stress was retained in melatonin+abamectin 24 hr and 144 hr (Short-term and Long-term) treated insects attending to the physiological parameters such as length, weight, and developmental time.

Different timing of Melatonin exposure on ROS and nitrite levels

A significant increase ($p<0.001$) of ROS levels in *S. litura* larvae was observed when insects were exposed to abamectin. ROS levels were significantly reduced in all melatonin+abamectin treated animals (24 hr and 144 hr, $p<0.01$; 72 hr, $p<0.05$) compared to the abamectin group (Figure 2). Nitrite content was markedly elevated in the abamectin group ($p<0.001$) and decreased

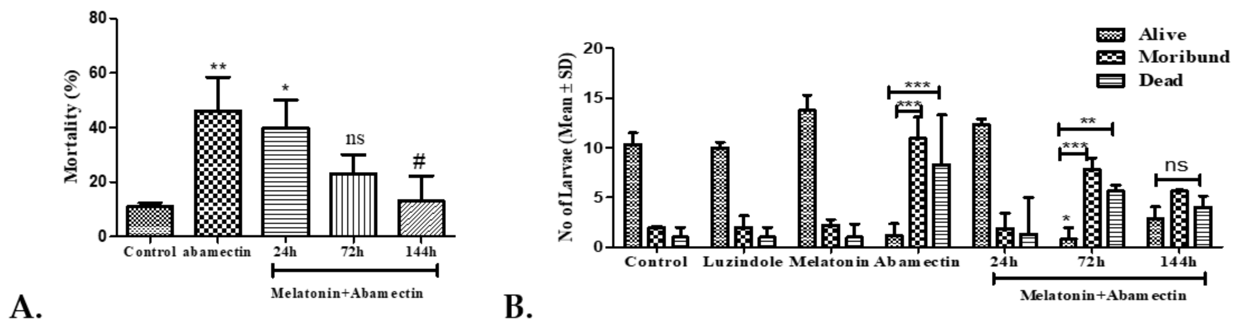


Figure 1: (A) Mortality (%) and (B) Larvae survival ratio (%) in *Spodoptera litura* midgut of different experimental groups. (There was no mortality ratio (%) in the Melatonin and Luzindole insects; therefore it was not mentioned in this mortality graph). *; $p<0.05$, **: $p<0.01$, ***: $p<0.001$, compared with control group by one-way ANOVA. #: $p<0.05$, compared with abamectin group by one-way ANOVA; ns; No significant.

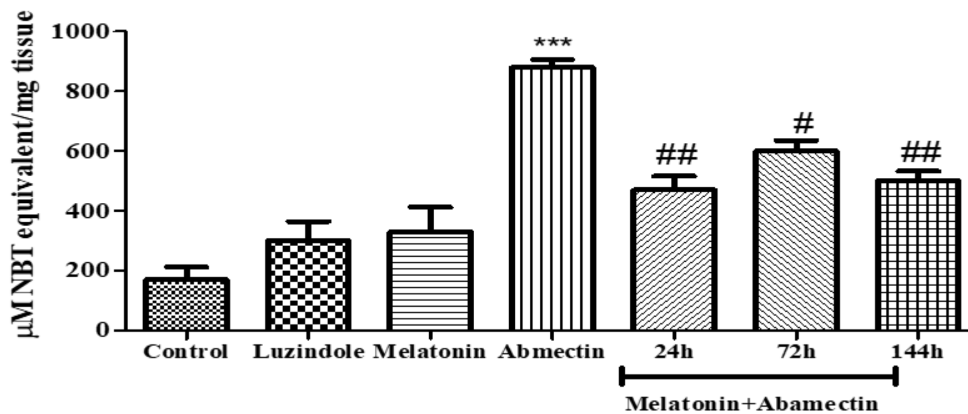


Figure 2: Total ROS levels (µM NBT equivalent/ mg tissue) in *Spodoptera litura* midgut of the different experimental groups. ROS- Reactive oxygen species. ***: $p<0.001$, compared with the control group by one-way ANOVA; #: $p<0.05$, ##; $p<0.01$ compared with the abamectin group by one-way ANOVA.

significantly ($p<0.05$) in melatonin+abamectin treated animals during 24 hr compared to the abamectin group (Figure 3).

Different timing of Melatonin exposure on Oxidative damage

Markers of oxidative damage were assessed by different timing of melatonin exposure (Figures 4 and 5). melatonin+abamectin animals treated during 24 hr, 72 hr, and 144 hr showed a statistically significant ($p<0.05$) decrease of protein carbonyl content compared to the abamectin group (Figure 4).

We observed a statistically significant increase ($p<0.001$) of the TBARS levels in the abamectin group compared to control animals. Supplementation of melatonin+abamectin during 24 hr ($p<0.05$) and 72 hr ($p<0.01$) showed a decrease of TBARS levels compared to the control group (Figure 5).

Different timing of Melatonin exposure on Antioxidant enzymes

A marked elevation ($p<0.05$) of SOD activity was observed when larvae were exposed to melatonin+abamectin during 144 has compared to the abamectin group. A significant decrease ($p<0.001$) of SOD activity was observed in abamectin-treated insects compared to control (Figure 6A). A statistically significant difference ($p<0.05$) of GST activity was observed in melatonin+abamectin 72 hr exposed insects compared to the control group. By contrast, decreased GST levels in the abamectin group were observed compared to control larvae (Figure 6B). CAT activity was also increased in the melatonin+abamectin group

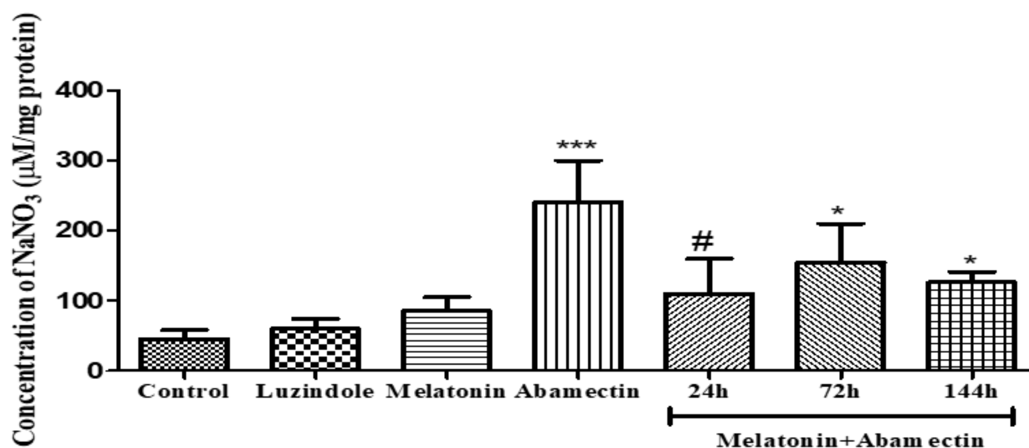


Figure 3: Nitrite content (µM/mg protein) in *Spodoptera litura* midgut of the different experimental groups. *; $p<0.05$, ***: $p<0.001$, compared with the control group by one-way ANOVA; #: $p<0.05$, compared with the abamectin group by one-way ANOVA.

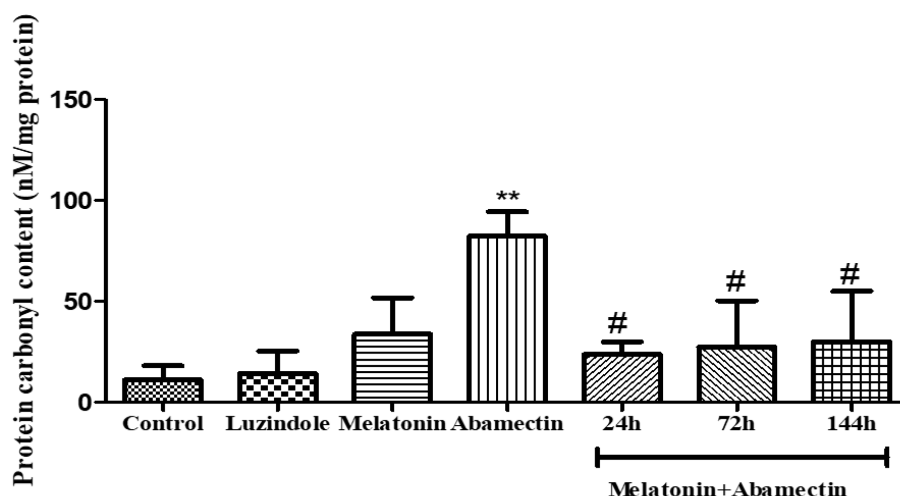


Figure 4: Protein carbonyl content (nM/ mg protein) in *Spodoptera litura* midgut of the different experimental groups. **: $p<0.01$, compared with the control group by one-way ANOVA; #: $p<0.05$, compared with the abamectin group by one-way ANOVA.

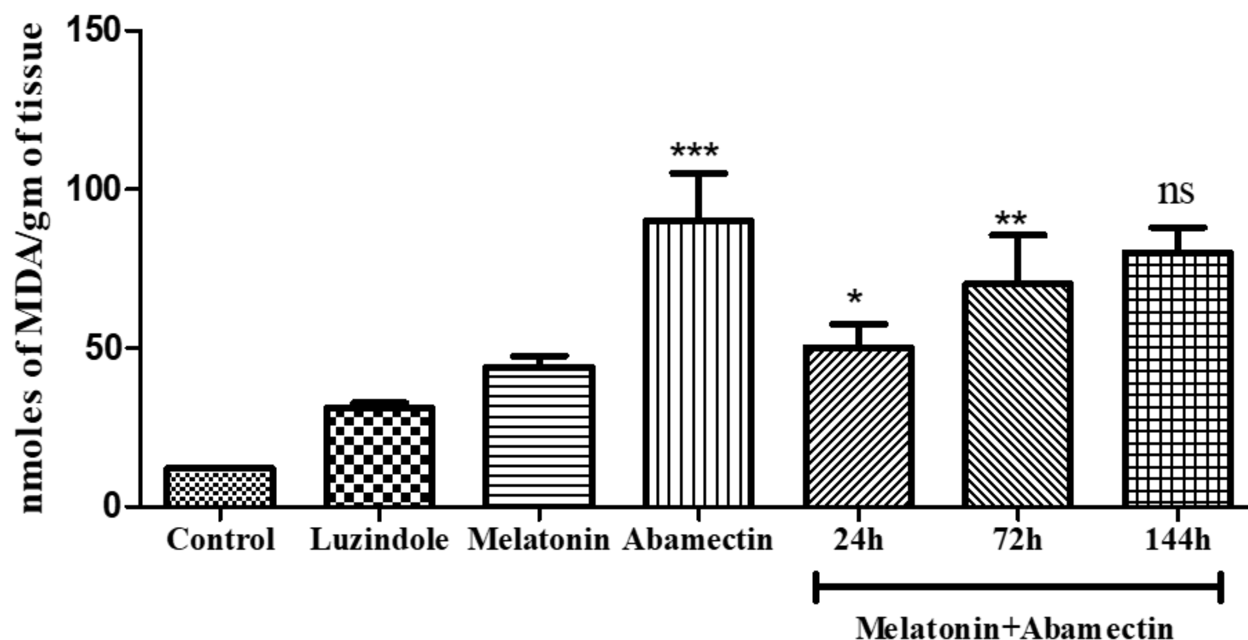


Figure 5: TBARS levels (n moles MDA/gm tissue) in Spodoptera litura midgut of the different experimental groups. TBARS- Thiobarbituric acid; ns; No significant. *; $p<0.05$, **: $p<0.01$; ***: $p<0.001$, compared with the control group by one-way ANOVA.

Table 2: Larvae and Pupal weight (g) (Mean±SD) of experimental groups in *Spodoptera litura*.

Larval stages (Instar)									
Sl. No.	Experimental Groups		I	II	III	IV	V	VI	Pupae
1.	Control		0.17±0.08	1.21±0.04	1.34±0.04	1.63±0.08	1.97±0.05	2.35±0.06	1.30±0.07
2.	Luzindole		0.09±0.01*	0.46±0.02*	1.27±0.05	1.39±0.07	1.93±0.07	2.40±0.04	1.18±0.06
3.	Melatonin		0.21±0.03	0.75±0.05	1.36±0.06	1.43±0.06	1.94±0.05	2.42 ±0.03	1.29±0.04
4.	Abamectin		0.04±0.01*	0.35±0.04*	1.12±0.07	1.28±0.05	1.41±0.04	1.62±0.05	1.05±0.07
5.	Melatonin+abamectin	24 hr (Short-term)	0.17±0.02	0.76±0.06	1.25±0.05	1.34±0.06	1.89±0.03	2.26±0.08	1.19±0.05
6.		72 hr (Midterm)	0.06±0.03*	0.63±0.07*	1.21±0.06	1.38±0.05	1.41±0.76	1.51 ±0.05*	1.22±0.06
7.		144 hr (Long-term)	0.20±0.01	0.87±0.08	1.32±0.07	1.54±0.05	1.90±0.45	2.39±0.03	1.34±0.08

Data are expressed as mean ± SD of three independent biological replicates. Asterisk (*) showed the significant differences ($p< 0.05$) (marked as bold) with control group by one-way ANOVA.

I-First larval instar; II-Second larval instar; III-Third larval instar; IV-Fourth larval instar; V-Fifth larval instar; VI-Sixth larval instar; g-gram; SD- Standard deviation; hr-hours.

24 hr ($p<0.05$), 72 hr ($p<0.01$; as compared to control group), and 144 hr ($p<0.001$) compared to abamectin animals, where a statistically significant decrease ($p<0.001$) was observed compared to abamectin insects (Figure 6C). GPx activity was significantly increased in melatonin+abamectin exposed animals during 72 hr ($p<0.05$) and 144 hr ($p<0.01$) compared to the abamectin group. A decrease in GPx activity was also observed due to the pesticide effect ($p<0.01$; Figure 6D). A significant increase of ascorbic acid (APOX) levels was observed in the melatonin+abamectin group 72 hr ($p<0.01$) compared to the abamectin group. A marked decrease ($p<0.05$) of APOX levels was observed in abamectin, and Luzindole exposed animals (Figure 6E). AChE activity increased significantly in melatonin+abamectin larvae exposed during 24 hr ($p<0.01$; as compared to control) and 144 hr ($p<0.05$; as compared to abamectin group). There were no observed differences in melatonin+abamectin 72 hr exposed animals (Figure 7A). It showed a statistically significant increase ($p<0.001$) of protein levels in the abamectin group compared to the control. This disturb was prevented due to the indoleamine effect in melatonin+abamectin exposed insects during 144 hr ($p<0.05$) as compared to abamectin (Figure 7B).

Different timing of Melatonin exposure on DNA fragmentation

Analysis of DNA extracted from midgut after exposure to melatonin+abamectin 24 hr, 72 hr, abamectin, and luzindole on*S. litura* showed a clear ladder pattern, whereas slight fragments were observed in melatonin+abamectin 144 hr exposed animals. No DNA fragmentation was observed in melatonin and control samples (Figure 8).

Different timing of Melatonin exposure on proteins of *S. litura* midgut

The midgut extract of proteins was resolved in 10% SDS-PAGE (Figure 9). Short-term Melatonin exposed animals (24 hr) were resolved into ten protein bands ranging (120, 115, 90, 50, 45, 35, 30, 25, 10, and 7 kDa), while long-term melatonin+abamectin exposed animals (144 hr) showed the same bands in addition to 100 and 75 kDa, resulting by this way a total of twelve bands. These two bands may be responsible for the long-term melatonin supplementation in the midgut. Because of the low concentration of the intermediate group samples (72 hr), we analyzed only the short-term and long-term melatonin+abamectin exposed midgut samples.

DISCUSSION

Recent studies have shown evidence that melatonin provides an important antioxidant activity in invertebrate animals such as insects.[21,31] Melatonin, maintains the oxidative stress of many pesticides, reflecting the protective role of the indoleamine on cell physiology.[20] The protective effects of melatonin against oxidative stress are aided by its ability to cross all biological membranes. It

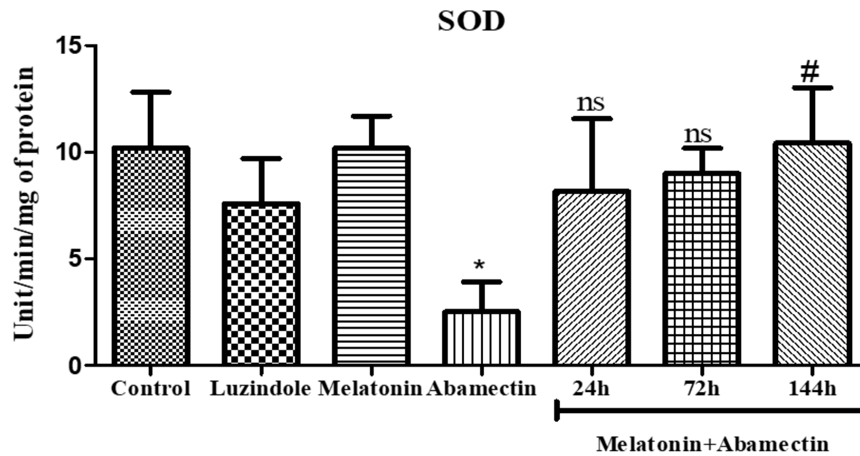
Table 3: Development time (days) (Mean±SD) of experimental groups in *Spodoptera litura*.

S.No.	Larval stage (Instar)	Control		Luzindole		Abamectin		Melatonin		Melatonin+abamectin					
										24 hr		72 hr		144 hr	
		No.of Larvae (Mean±SD)	Days (Mean±SD)	No.of Larvae (Mean±SD)	Days (Mean±SD)	No.of Larvae (Mean±SD)	Days (Mean±SD)	No.of Larvae (Mean±SD)	Days (Mean±SD)	No.of Larvae (Mean±SD)	Days (Mean±SD))	No.of Larvae (Mean±SD)	Days (Mean±SD)	No.of Larvae (Mean±SD)	Days (Mean±SD)
1.	I	10 ± 8.4	1.3	5 ± 5.2*	1.7	4 ± 2.0*	2.2*	10 ± 1.4	1.5	10 ± 1.7	1.9	7 ± 9.8	1.3	9 ± 1.4	1.5
2.	II	10 ± 2.0	3.1	5 ± 3.6*	3.4	3 ± 3.7*	3.8	10 ± 2.0	3.0	9 ± 1.9	4.3	10 ± 7.8	3.6	8 ± 5.0	4.0
3.	III	10 ± 1.0	3.6	7 ± 6.2	3.0	2 ± 2.1*	4.5*	10 ± 4.0	3.4	10 ± 3.1	4.1	9 ± 4.3	4.0	8 ± 7.0	3.7
4.	IV	10 ± 5.5	2.8	5 ± 1.7*	3.7*	3 ± 2.0*	5.1*	10 ± 7.2	2.7	10 ± 4.0	4.7	6 ± 4.6*	4.8*	8 ± 8.0	2.5
5.	V	10 ± 5.5	2.8	5 ± 2.0*	4.1*	3 ± 1.5*	5.8*	10 ± 2.7	2.7	7 ± 5.4	5.3	5 ± 3.4*	4.4*	8 ± 3.4	2.5
6.	VI	10 ± 8.3	3.1	4 ± 3.0*	4.2*	2 ± 1.4*	4.0*	10 ± 5.5	3.2	7 ± 5.6	5.4	4 ± 1.8*	4.7*	7 ± 2.8	4.3
7.	Pupae	10 ± 1.6	3.1	3 ± 7.7*	6.0*	2 ± 2.0*	7.2*	10 ± 6.3	3.0	10 ± 2.2	1.0	3 ± 2.1	6.0*	6 ± 7.0	4.2

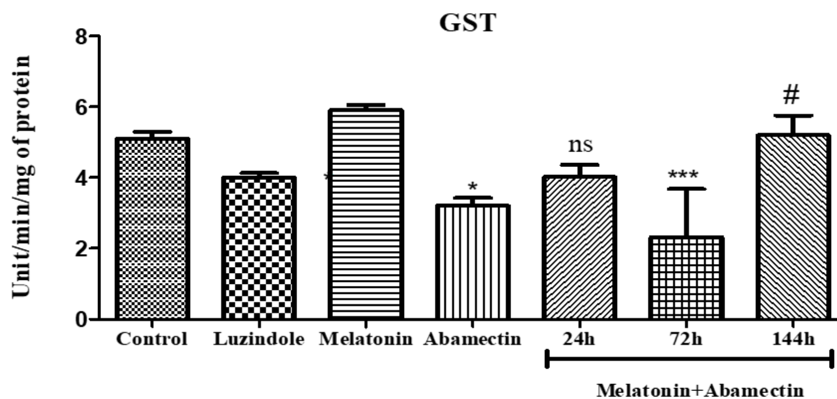
Data are expressed as mean ± SD of three independent biological replicates. Asterisk (*) showed the significant differences ($p<0.05$) (marked as bold) with control group by one-way ANOVA. I-First larval instar; II-Second larval instar; III-Third larval instar; IV-Fourth larval instar; V-Fifth larval instar; VI-Sixth larval instar; SD- Standard deviation; hr-hours.

is also known to be a powerful direct free radical scavenger as well as an indirect antioxidant in animals.[4] Moreover, it is observed to be a regulator of circadian rhythms due to its rhythmic secretion pattern serves as a biochemical signal for 'darkness',[48] measurement of day length[49] reduce the redox status[18] influence in the seasonal timing of reproduction, metabolism and immune response in animals.[50]

In recent years, an increasing research interest is focused on melatonin, which is a protective agent for various clinical and pharmaceutical applications. To prove this protective nature, we try to elucidate the antioxidative nature in invertebrates, particularly insects. Because of that, the present study has assessed melatonin's effect in a time dependent manner (short, mid and long-term exposure to the melatonin) in response to abamectin toxicity in the midgut of the lepidopteran insect. In insects, high levels of antioxidant enzymes are produced in metabolically active tissues such as midgut and fat body.[19,51] Our preliminary study has shown that the higher melatonin levels observed in the midgut act as an antioxidant source, and preventing ROS production.[48]



A.



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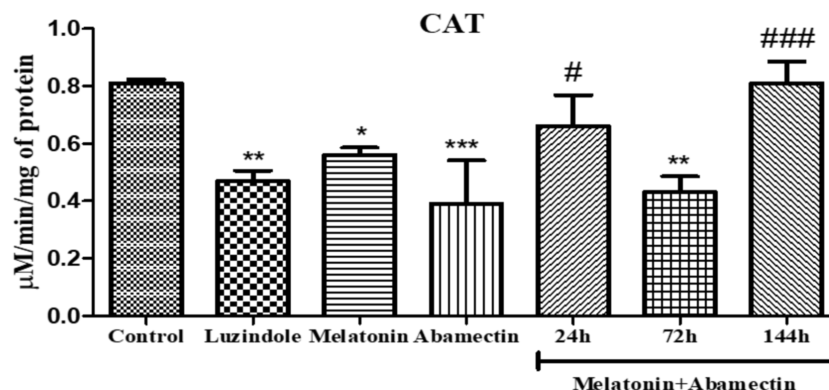

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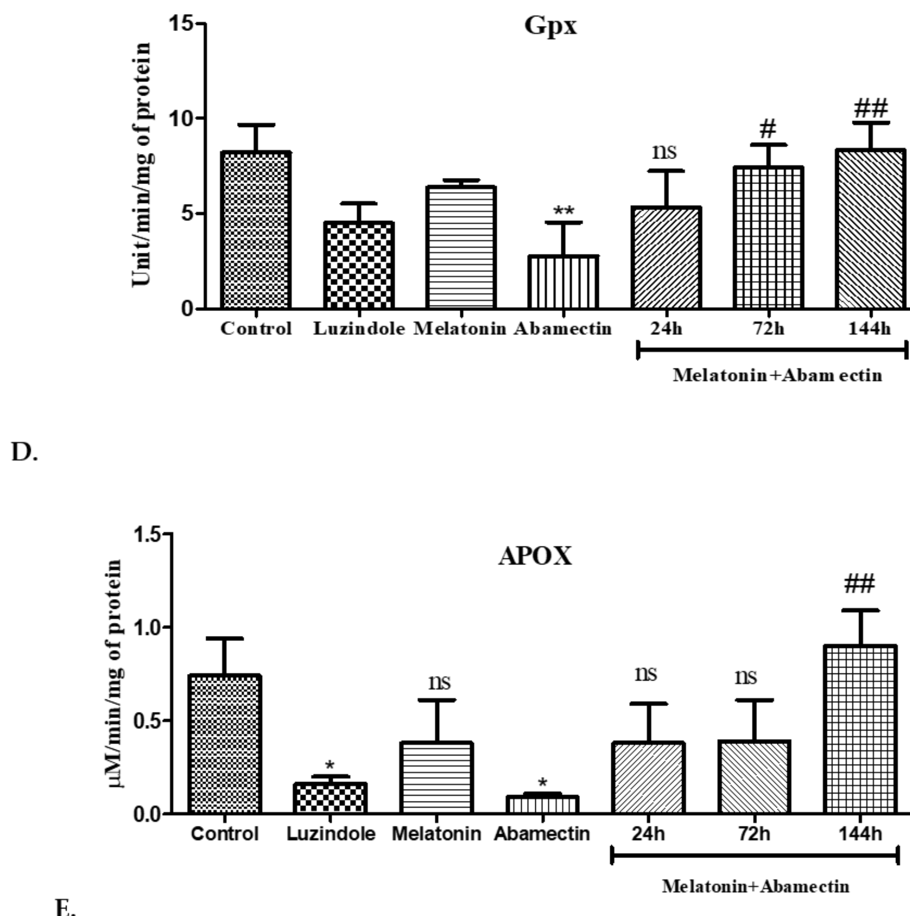

Figure 6: (A-D) Antioxidant enzyme activities in *Spodoptera litura* midgut of different experimental groups. (SOD, μM/mg protein; GST, Unit/min/mg Protein; CAT, μM/min/mg Protein; GPX, Unit/min/mg Protein; APOX, μM/min/mg Protein). SOD – Superoxide Dismutase, GST - Glutathione-S-transferase, CAT - Catalase, Gpx- Glutathione Peroxidase, APOX -Ascorbic acid, ns; No significant. *; $p<0.05$, **: $p<0.01$, ***; $p<0.001$, compared to control group by one-way ANOVA. #: $p<0.05$, ##: $p<0.01$, ###; $p<0.001$ compared to abamectin group by one-way ANOVA.

Larval Mortality, length, weight and developmental time

To identify the protective role of melatonin, primarily we looked into survival rate (%). Melatonin administration significantly increased the survival rate in larvae exposed to abamectin, showing that the indoleamine reversed the toxic effects of the pesticide. Studies have shown that the antioxidant activity of melatonin increased the life span in mice a dose-dependent manner.[52] This study was agreed with these results because we observed that melatonin exposure positively correlates with larval survival rate in a time dependent manner. It suggests that melatonin increases the number of larvae in livable conditions due to its capacity to reduce oxidative stress.

Melatonin is a well-known scavenger of ROS and RNS. Studies observed that the indoleamine reduced ROS levels in rats generated due to atrazine toxicity.[53] ROS has multiple functions,[54] plays an important role in cell signaling and several physiological processes.[55,56] However, the excess of ROS levels as a response of oxidative stress situations is harmful to living systems.[57,58] Our results observed that abamectin increased ROS levels in the midgut of *S. litura*, but this effect was prevented due to melatonin effect in a time-dependent manner. Moreover, Nitric Oxide (NO) is a free radical molecule released by cells in picomolar to nanomolar ranges with a very short life.[59] It was assessed on the basis of its stable oxidation end-products, nitrate (NO_3^-) and nitrite (NO_2^-).[60] We observed that nitrite content was increased in larvae exposed to abamectin compared to their normal counterparts. The ability of melatonin to scavenge the free radicals such as NO is an important property in its protective role against the oxidative stress.[4] Interestingly, melatonin+abamectin treatment reduced nitrite content in a significant manner. Our results are agreed with previous studies where the indoleamine prevented these disturbs in nitrite content due to paraquat toxicity.[61] Our findings showed that the abamectin administration is an important source of oxidative stress and it was modulated by melatonin exposure (MT+ABA 24 hr, 72 hr, 144 hr) due to the continuous scavenging cascade of melatonin. These melatonin modulating effects were also showed in ROS scavenging activities in the nocturnal insect. These findings are consistent with previous studies where

melatonin protects against oxidative stress damage of other ROS generating agents.

Oxidative stress has been shown to increase protein oxidation and generation of protein carbonylation.[44] Protein carbonyls tend to get accumulated on the side chains of proteins as a result of oxidative stress and is widely used as a marker of oxidative protein damage.[20] Similarly, oxidative stress also clearly affects lipids.[62] Our results showed that melatonin prevented the increase of protein carbonyl content due to abamectin toxicity. This effect was also observed previously.[21,53] MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, has been used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress.[63,64] In our study, the induction of oxidative stress in *S. litura* larvae exposed to abamectin is reflected by the substantial increase in TBARS levels. Our results observed that this trend was mitigated due to melatonin effect, which is in agreement with previous studies in rats,[65] insects,[21,66] and humans.[67]

In higher animals, daily variations of enzymatic antioxidant activities or non-enzymatic antioxidant contents were also reported in various tissues of vertebrates.[5] To counteract this, animals have developed a suite of antioxidant enzymes to cope with oxidative stress. Antioxidant enzymes provide a major line of defense against free radical damage either by metabolizing them to less reactive species or to nontoxic byproducts. Various antioxidant enzymes may decrease the level of lipid peroxidation as well as protein and DNA damage.[13]

Midgut Antioxidant Defense System (ADS) in *S. litura* larvae

The study was aimed to investigate whether melatonin protects midgut from oxidative stress injury of generated by abamectin through the capacity of the indoleamine to stimulate the activity of antioxidant enzymes in a time dependent manner. Abamectin was used to induce the generation of free radicals, interfering by this way in the antioxidant defense system of *S. litura*. It is known that the pesticide induces oxidative stress tissue damage decreasing the activities of SOD, GPx, and GST in rats.[68] GST in

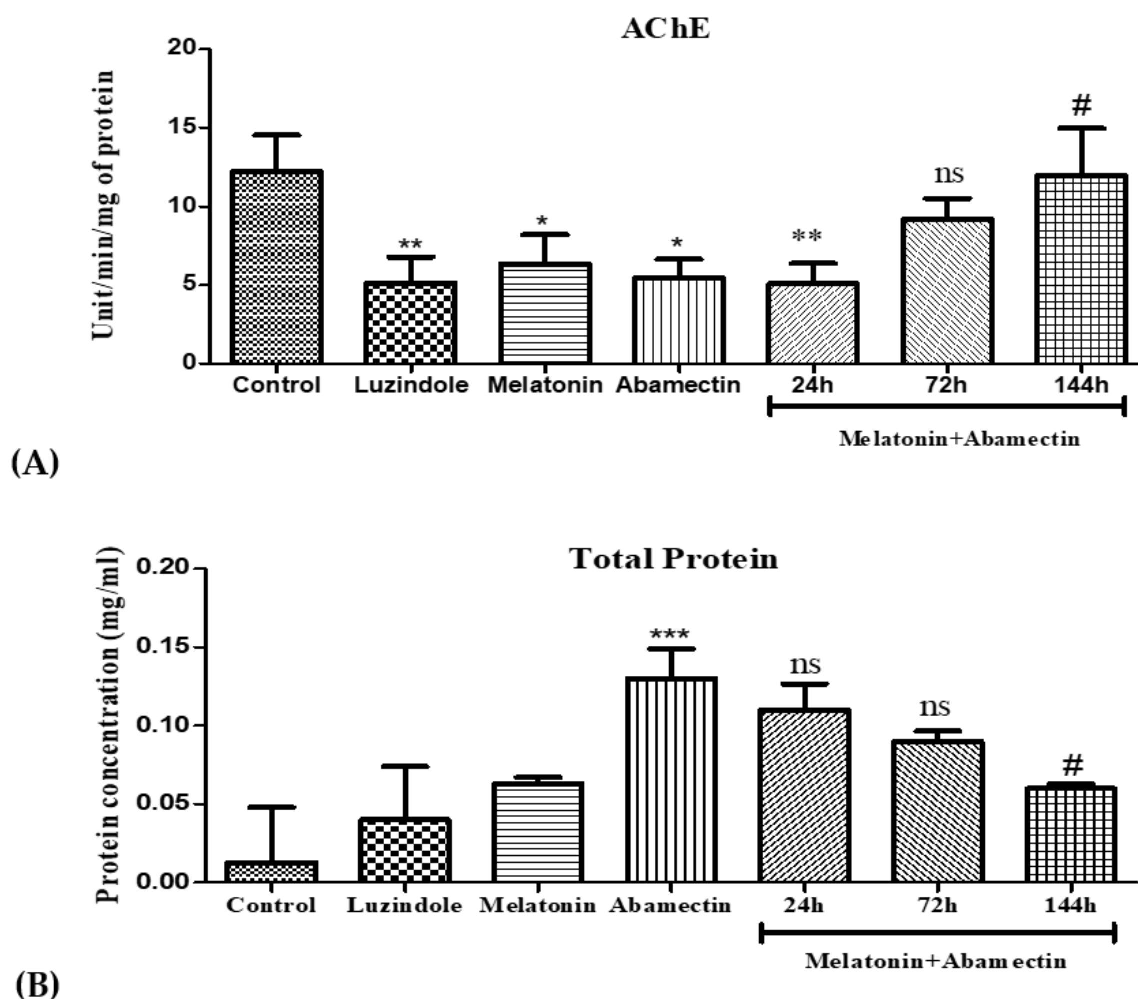

Figure 7: (A) AChE (Acetylcholine esterase) and (B) Total Protein (mg/ml) levels in *Spodoptera litura* midgut of different experimental groups. *; $p<0.05$, ***; $p<0.001$, compared to control group by one-way ANOVA. #: $p<0.05$, compared to abamectin group by one-way ANOVA; ns; No significant.

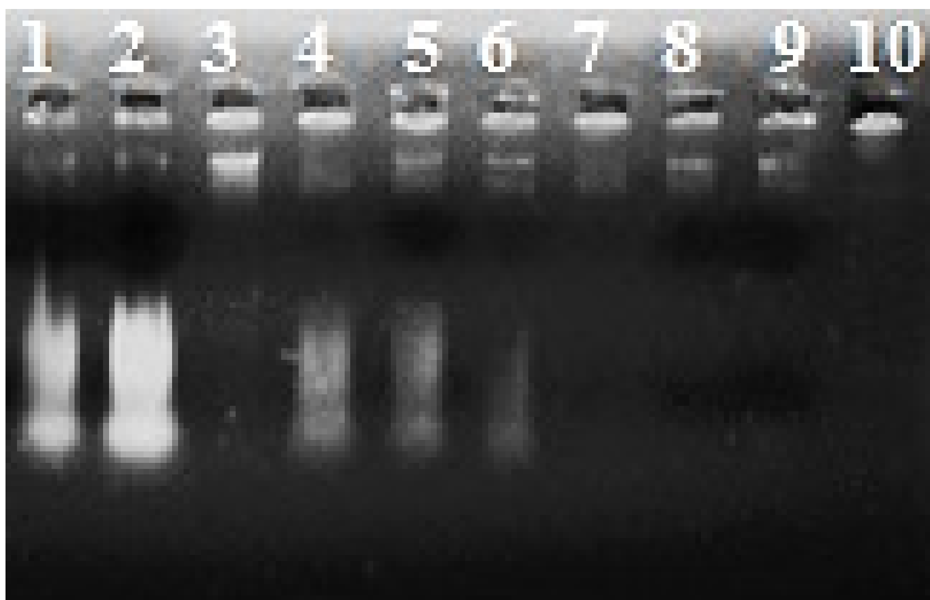

Figure 8: Electrophoretic detection of DNA fragmentation from *Spodoptera litura* midgut of the different experimental groups. [1] Melatonin+abamectin (24 hr) [2]. Melatonin+abamectin (72 hr) [3]. Melatonin+abamectin (144 hr) [4]. Abamectin [5]. Luzindole [6]. Melatonin [7]. Control.

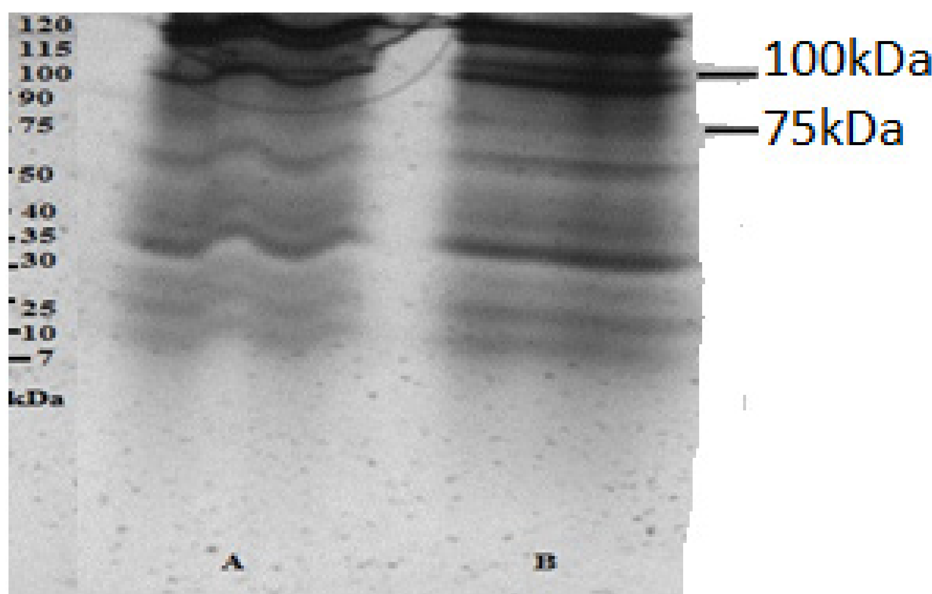

Figure 9: SDS-PAGE (10%) analysis of proteins isolated from *Spodoptera litura* midgut of the different experimental groups. (a) Melatonin+abamectin [24 hr] (b) Melatonin+abamectin [144 hr]. Arrows represent the molecular weight of proteins (100 and 75kDa).

insects is observed to remove the products of lipid peroxidation or hydroperoxides from cells.[69,70] In addition, as well as vertebrate and invertebrate animals, SOD, CAT, and POX are major components of the antioxidant enzyme system in insects.[13,14]

Our results showed that abamectin exposure decreased the activity of SOD, CAT, GST, APOX, and GPx. The indoleamine was observed to mitigate this effect, especially when melatonin was provided during 144 hr. Our results are in agreement with previous studies in rats,[71-74] insects,[75] and humans.[67] These studies observed melatonin benefits in an increased time exposure. Due to that, and in agreement with the theory that melatonin benefits are increased in a time-dependent manner, our results may be better in melatonin+abamectin insects exposed during 144 hr. However, while it is well known that the indoleamine effects are increased in a dose-dependent manner.[76,77] More studies are needed to improve the knowledge about melatonin effect in a time dependent manner.

Like melatonin, ascorbic acid also plays a role against oxidative stress damage to insect cellular constituents,[13,60] thus limiting the formation of cytotoxic lipid-hydroperoxides, protein carbonyls,

and depletion of glutathione levels. It is also known to be a direct scavenger of reactive oxygen species.[78] Attending to our results, it may suggest that APOX may trigger alone or combined with melatonin the reduction of free radicals as a result of its antioxidant activity.

AChE is a key enzyme in the insect nervous system, terminating neurotransmission by the hydrolysis of the neurotransmitter acetylcholine. As most of the insecticides are neurotoxic, acetylcholine esterase plays an important role reducing the toxic effect of insecticides through by its overproduction in insects.[79] As well as the other antioxidant enzymes analyzed in our study, AChE activity was substantially decreased ($p<0.05$) in abamectin exposed animals. This effect was ameliorated in melatonin+abamectin insects, especially in short-term ($p<0.001$ significant difference with control group) and long-term exposed larvae ($p<0.05$ compared to abamectin group). These results are in agreement with previous studies developed in human lymphocytes in a chlorpyrifos-induced toxicity model.[80] Our results of the antioxidant enzymes suggest that melatonin may play an important role against abamectin-induced toxicity through its capacity to regulate the oxidant–antioxidant status resulted from the generation of free radicals in larval midgut exposed to the pesticide.

Melatonin is a commonly used treatment in children and adolescents with insomnia, and therefore concerns have been raised regarding the short-term and long-term adverse consequences. The major gap of knowledge on short-term and long-term safety of melatonin treatment in children and adolescents calls for cautious use and for more research to inform clinicians.[81] Clinical study[82] revealed the long-term melatonin treatment in combination with adequate sleep hygiene interventions may afford clinical benefits to children with Neurodevelopmental disorders and potentially elevates their well-being. Overall this, Melatonin generally has a good safety record and has been shown in numerous trials to be well tolerated in both adults and children.[83] However, it is widely agreed that the long-term effects of taking exogenous melatonin have been insufficiently studied and warrant additional investigation.[84]

Melatonin Protective mechanism

Analysis of proteins in the midgut was used to verify the expressed proteins in the short- and long-term melatonin administration insects. SDS-PAGE in long-term melatonin+abamectin exposed animals (144 hr) showed the same ten protein bands expressed in short- term (24 hr) Melatonin+abamectin ranging (120, 115, 90, 50, 45, 35, 30, 25, 10, and 7 kDa) in addition to 100 and 75 kDa, resulting by this way a total of twelve bands. These two bands (100 and 75 kDa) observed in long-term melatonin supplemenation insects, may be generated due to the long-term exposure of the indoleamine (144 hr) in the midgut of *S. litura*. Further studies are needed to analyse these two bands may elucidate the role of proteins involving the long-term melatonin administration in *S. litura* larvae.

CONCLUSION

In conclusion, midgut disturbance generated by the pesticide showed amelioration in Melatonin+abamectin exposed insects. This effect was most significant in Melatonin+abamectin 144 hr larvae, suggesting by this way that the activity of the indoleamine is related to time exposure. However, while the studied antioxidant enzyme activities mainly were modulated in Melatonin+abamectin 144 hr exposed larvae, DNA modifications were less observed in short-term exposed insects. It suggests that the increase of the activity of the Antioxidant Defense System (ADS) reverses DNA disturbances, returning by this way the ADS system to its regular activity. However, future studies are needed to improve the knowledge of the different mechanisms of Melatonin involved in oxidative stress, especially in relation to the exposure time effect. It may be useful because it is observed that indoleamine has no important adverse effects in high-dose administration, which may be important to prevent the effects of oxidative stress, including several diseases in medicine.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

REFERENCES

1. Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem. 2003; 2: 181-97.
2. Reiter RJ, Tan DX, Rosales-Corral SD, Manchester LC. The universal nature, unequal distribution and antioxidant function of melatonin and its derivatives. Mini Rev Med Chem. 2013; 13(3): 373-84. doi: 10.2174/1389557511313030006, PMID 23190034.
3. Acuña-Castroviejo D, Escames G, Venegas C, Díaz-Casado ME, Lima-Cabello E, López LC, et al. Extrapineal melatonin: sources, regulation, and potential functions. Cell Mol Life Sci. 2014; 71(16): 2997-3025. doi: 10.1007/s00018-014-1579-2, PMID 24554058.
4. Tan DX, Manchester LC, Esteban-Zubero E, Zhou Z, Reiter RJ. Melatonin as a potent and inducible endogenous antioxidant: synthesis and metabolism. Molecules. 2015; 20(10): 18886-906. doi: 10.3390/molecules201018886, PMID 26501252.
5. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms for radical detoxification to radical avoidance. Endocrine. 2005; 27(2): 119-30. doi: 10.13 85/endo:27: 2:119, PMID 16217125.
6. Paredes SD, Korkmaz A, Manchester LC, Tan DX, Reiter RJ. Phytomelatonin: a review. J Exp Bot. 2009; 60(1): 57-69. doi: 10.1093/jxb/ern284, PMID 19033551.
7. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res. 2007; 42(1): 28-42. doi: 10.1111/j.1600-079X.2006.0 0407.x, PMID 17198536.
8. Migliori ML, Romanowski A, Simonetta SH, Valdez D, Guido M, Golombek DA. Daily variation in melatonin synthesis and arylalkylamine N-acetyltransferase activity in

the nematode Caenorhabditis elegans. J Pineal Res. 2012; 53(1): 38-46. doi: 10.1111/j.1600-079X.2011.00969.x, PMID 21995323.

9. Stehle JH, Saade A, Rawashdeh O, Ackermann K, Jilg A, Sebestény T, et al. A survey of molecular details in the human pineal gland in the light of phylogeny, structure, function and chronobiological diseases. J Pineal Res. 2011; 51(1): 17-43. doi: 10.1111/j.1600-079X.2011.00856.x, PMID 21517957.
10. Vengateswari G, Subala SP, Shivakumar MS. Protective effect of melatonin administration on abamectin-induced immunotoxicity in Spodoptera litura (Insecta: Lepidoptera). Int J Pest Manag. 2018; 64(4): 333-44.
11. Bloomquist JR. Toxicology, mode of action, and target-site mediated resistance to insecticides acting on chloride channels. Comp Biochem Physiol C Comp Pharmacol Toxicol. 1993; 106(2): 301-14. doi: 10.1016/0742-8413(93)90138-b, PMID 7904908.
12. Kaplan RM, Courtney CH, Kunkle WE, Zeng QY, Jernigan AD, Eagleson JS. Efficacy of injectable abamectin against gastrointestinal tract nematodes and lungworms of cattle. Am J Vet Res. 1994; 55(3): 353-7. doi: 10.2460/ajvr.1994.55.03.353, PMID 8192257.
13. Felton GW, Summers CB. Antioxidant systems in insects. Arch Insect Biochem Physiol. 1995; 29(2): 187-97. doi: 10.1002/arch.940290208, PMID 7606043.
14. Wang Y, Oberley LW, Murhammer DW. Antioxidant defense systems of two Lepidopteran insect cell lines. Free Radic Biol Med. 2001; 30(11): 1254-62. doi: 10.1016/S0891-5849(01)00520-2.
15. Subala SP, Shivakumar MS. Changes in light and dark periods affect the arylalkylamine N-acetyltransferase, melatonin activities, and redox status in the head and hemolymph of nocturnal insect Spodoptera litura. J Biol Rhythms.Res. 2017; 49(1): 13-28.
16. Lopez-Martinez G, Elnitsky MA, Benoit JB, Lee Jr RE, Denlinger DL. High resistance to oxidative damage in the Antarctic midge Belgica antarctica, and developmentally linked expression of genes encoding superoxide dismutase, catalase and heat shock proteins. Insect Biochem Mol Biol. 2008; 38(8): 796-804. doi: 10.1016/j.ibmb.2008.05.006, PMID 18625403.
17. Hardeland R, Pandi-Perumal SR. Melatonin, a potent agent in antioxidative defense: actions as a natural food constituent, gastrointestinal factor, drug and prodrug. Nutr Metab (Lond). 2005; 2: 22. doi: 10.1186/1743-7075-2-22, PMID 16153306.
18. Subala SP, Shivakumar MS. Circadian variation affecting biology and digestive profiles of a nocturnal insect Spodoptera litura (Insecta: Lepidoptera). J Biolrhythm Res. 2016; 48(2): 207-26.
19. Huether G. The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. Experientia. 1993; 49(8): 665-70. doi: 10.1007/BF01923948, PMID 8359272.
20. Krishnan N, Kodrík D. Antioxidant enzymes in Spodoptera littoralis (Boisduval): are they enhanced to protect gut tissues during oxidative stress? J Insect Physiol. 2006; 52(1): 11-20. doi: 10.1016/j.jinsphys.2005.08.009, PMID 16242709.
21. Subala SP, Zubero EE, Alatorre-Jimenez MA, Shivakumar MS. Pre-treatment with melatonin decreases abamectin induced toxicity in a nocturnal insect Spodoptera litura (Lepidoptera:Noctuidae). Environ Toxicol Pharmacol. 2017; 56: 76-85. doi: 10.1016/j.etap.2017.08.025, PMID 28886429.
22. Sánchez-Barceló EJ, Mediavilla MD, Tan DX, Reiter RJ. Clinical uses of melatonin: evaluation of human trials. Curr Med Chem. 2010; 17(19): 2070-95. doi: 10.2174/092986710791233689, PMID 20423309.
23. Reiter RJ, Tan DX, Korkmaz A, Ma S. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation, and melatonin suppression. Ann Med. 2012; 44(6): 564-77. doi: 10.3109/07853890.2011.586365, PMID 21668294.
24. Esteban-Zubero E, García-Gil FA, López-Pingarrón L, Alatorre-Jiménez MA, Ramírez JM, Tan DX, et al. Melatonin role preventing steatohepatitis and improving liver transplantation results. Cell Mol Life Sci. 2016 Mar 29 ; 73(15): 2911-27. doi: 10.1007/s00018-016-2185-2, PMID 27022943.
25. Pieri C, Marra M, Moroni F, Recchioni R, Marcheselli F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci. 1994; 55(15): PL271-6. doi: 10.1016/0024-3205(94)00666-0, PMID 7934611.
26. Pozo D, Garcia-Maurino S, Guerrero JM, Calvo JR. mRNA expression of the nuclear receptor RZR/ROR alpha, melatonin membrane receptor MT, and hydroxindole-O-methyl transferase in different populations of human immune cells. J Pineal Res. 2004; 1: 48-54.
27. Barlow-Walden LR, Reiter RJ, Abe M, Pablos M, Menendez-Pelaez A, Chen LD, et al. Melatonin stimulates brain glutathione peroxidase activity. Neurochem Int. 1995; 26(5): 497-502. doi: 10.1016/0197-0186(94)00154-m, PMID 7492947.
28. IRAC. Insecticide Resistance Action Committee. Method No: 007: leaf eating Lepidoptera and Coleoptera; 2010.
29. Kim DS, Brooks DJ, Riedl H. Lethal and sublethal effects of abamectin, Spinosad, methoxyfenozide and acetamiprid on the predaceous plant bug Deraeocoris brevis in the laboratory. BioControl. 2006; 51(4): 465-84. doi: 10.1007/s10526-005-1028-0.
30. Niva CC, Takeda M. Effects of photoperiod, temperature and melatonin on nymphal development, polyphenism and reproduction in Halyomorphahalys (Heteroptera: Pentatomidae). Zool Sci. 2003; 20(8): 963-70. doi: 10.2108/zsj.20.963.
31. Karthi S, Shivakumar MS. The protective effect of melatonin against cypermethrin-induced oxidative stress damage in Spodoptera litura (Lepidoptera: Noctuidae). Biol Rhythm Res. 2015; 23: 1-12.
32. Geihs MA, Vargas MA, Maciel FE, Caldas SS, Cruz BP, Primel EG, et al. Effect of melatonin in the antioxidant defense system in the locomotor muscles of the estuarine crab Neohelice granulate (Decapoda, Brachyura). Gen Comp Endocrinol. 2010; 166(1): 72-82. doi: 10.1016/j.ygcen.2009.09.018.
33. Kranthi KR. Insecticide resistance-monitoring, mechanisms and management manual. Nagpur, India: CICR; 2005. p. 12-25.
34. Busvine JR. Revised method for spider mites and their eggs (e.g., Tetranychus spp. and Panonychus ulmi Koch). FAO method No.10a, Recommended Methods for Measurement of Pest Resistance to Pesticides. FAO Plant Prod Prot Pap. 1980; 21: 49-53.
35. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925; 18(2): 265-7. doi: 10.1093/jee/18.2.265a.
36. Marklund SL, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47(3): 469-74. doi: 10.1111/j.1432-1033.1974.tb03714.x, PMID 4215654.
37. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase: the first enzymatic step in mercapturic acid formation. Int J Biol Chem. 1974; 249: 7130-9.
38. Beers RF, Sizer IW. Spectrophotometric methods for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952; 195(1): 133-40. doi: 10.1016/S0021-9258(19)50881-X, PMID 14938361.
39. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biological role as a component of glutathione peroxidase. Science. 1973; 179(4073): 588-90. doi: 10.1126/science.179.4073.588, PMID 4686466.
40. Roe JH, Kuether CA. The determination of ascorbic acid Article: in whole blood and urine through the 2,4-dinitrophenylhydrazine Derivative of dehydroascorbic 2,4-dinitrophenylhydrazine through the Acid in whole blood and urine. J Biol Chem. 1943; 147(2): 399-407. doi: 10.1016/S0021-9258(18)72395-8.
41. Ellman GL, Courthy KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7: 115-21.
42. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagent. J Biol Chem. 1951; 193(1): 265-75. doi: 10.1016/S0021-9258(19)52451-6, PMID 14907713.
43. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971; 44(1): 276-87. doi: 10.1016/0003-2697(71)90370-8, PMID 4943714.
44. Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from murine peritoneal macrophages: comparison of the activation cytokines and evidence of independent production. J Immunol. 1988; 141(7): 2407-12. doi: 10.4049/jimmunol.141.7.2407, PMID 3139757.
45. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990; 186: 464-78. doi: 10.1016/0076-6879(90)86141-h, PMID 1978225.
46. Janero DR. Malondialdehyde and thiobarbituric acid-reactive as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990; 9(6): 515-40. doi: 10.1016/0891-5849(90)90131-2.
47. Johnston KA, Gatehouse JA, Anstee JH. Effects of soybean protease inhibitors on the growth and development of larval Helicoverpa armigera. J Insect Physiol. 1993; 39(8): 657-64. doi: 10.1016/0022-1910(93)90071-X.
48. Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev. 1991; 12(2): 151-80. doi: 10.1210/edrv-12-2-151, PMID 1649044.
49. Arendt J, Skene DJ. Melatonin as a chronobiotic. Sleep Med Rev. 2005; 9(1): 25-39. doi: 10.1016/j.smrv.2004.05.002, PMID 15649736.
50. Maldonado MD. Perez-San-Gregorio MA. Reiter RJ. The role of melatonin in the immune neuropsychology of mental disorders. Rec Pat CNS Drug Discov. 2009; 4: 61-9.
51. Zhang HM, Zhang Y. Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. J Pineal Res. 2014; 57(2): 131-46. doi: 10.1111/jpi.12162, PMID 25060102.
52. Anisimov VN, Alimova IN, Baturin DA, Popovich IG, Zabezhinski MA, Rosenfeld SV, et al. Dose-dependent effect of melatonin on life span and spontaneous tumor incidence in female SHR mice. Exp Gerontol. 2003; 38(4): 449-61. doi: 10.1016/s0531-5565(02)00240-1, PMID 12670632.
53. Bhatti JS, Sidhu IP, Bhatti GK. Ameliorative action of melatonin on oxidative damage induced by atrazine toxicity in rat erythrocytes. Mol Cell Biochem. 2011; 353(1-2): 139-49. doi: 10.1007/s11010-011-0780-y, PMID 21404018.
54. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006; 160(1): 1-40. doi: 10.1016/j.cbi.2005.12.009, PMID 16430879.
55. Lecour S, Van der Merwe EV, Opie LH, Sack MN. Ceramide attenuates hypoxic cell death via reactive oxygen species signaling. J Cardiovasc Pharmacol. 2006; 47(1): 158-63. doi: 10.1097/01.fjc.0000198520.28674.41, PMID 16424801.
56. England K, Cotter TG. Direct oxidative modifications of signaling proteins in mammalian cells and their effects on apoptosis. Redox Rep. 2005; 10(5): 237-45. doi: 10.1179/135100005X70224, PMID 16354412.

57. Maia L, Vala A, Mira L. NADH oxidase activity of rat liver xanthine dehydrogenase and xanthine oxidase-contribution for damage mechanisms. Free Radic Res. 2005; 39(9): 979-86. doi: 10.1080/10715760500210962, PMID 16087479.
58. Cesaratto L, Vascotto C, Calligaris S, Tell G. The importance of redox state in liver damage. Ann Hepatol. 2004; 3(3): 86-92. doi: 10.1016/S1665-2681(19)32099-X, PMID 15505592.
59. Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. Circ Res. 1990; 66(6): 1561-75. doi: 10.1161/01.res.66.6.1561, PMID 2160870.
60. Felton GW. Antioxidant defenses of vertebrates and invertebrates. Oxidative Stress and Antioxidant gels. Anal Biochem. 1995; 44: 276-87.
61. Singhal NK, Srivastava G, Patel DK, Jain SK, Singh MP. Melatonin or silymarin reduces maneb- and paraquat-induced Parkinson's disease phenotype in the mouse. J Pineal Res. 2011; 50(2): 97-109. doi: 10.1111/j.1600-079X.2010.00819.x, PMID 20964710.
62. Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids. 2003; 25(3-4): 207-18. doi: 10.1007/s00726-003-0011-2, PMID 14661084.
63. Rael LT, Thomas GW, Craun ML, Curtis CG, Bar-Or R, Bar-Or D. Lipid peroxidation and the thiobarbituric acid assay: standardization of the assay when using saturated and unsaturated fatty acids. J Biochem Mol Biol. 2004; 37(6): 749-52. doi: 10.5483/bmbrep.2004.37.6.749, PMID 15607036.
64. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis. 2005; 15(4): 316-28. doi: 10.1016/j.numecd.2005.05.003, PMID 16054557.
65. Mehta KD, Mehta AK, Halder S, Khanna N, Tripathi AK, Sharma KK. Protective effect of melatonin on propoxur-induced impairment of memory and oxidative stress in rats. Environ Toxicol. 2014; 29(6): 705-13. doi: 10.1002/tox.21798, PMID 24733834.
66. Medina-Leendertz S, Paz M, Mora M, Bonilla E, Bravo Y, Arcaya JL, *et al*. Long-term melatonin administration alleviates paraquat mediated oxidative stress in Drosophila melanogaster. Invest Clin. 2014; 55(4): 352-64. PMID 25558754.
67. Sadowska-Woda I, Wójcik N, Karowicz-Bilińska A, Bieszczad-Bedrejczuk E. Effect of selected antioxidants in beta-cyfluthrin-induced oxidative stress in human erythrocytes in vitro. Toxicol in vitro. 2010; 24(3): 879-84. doi: 10.1016/j.tiv.2009.11.022, PMID 19961921.
68. Nahla S, Shenawy E. Effect of insecticide fenitrothion, endosulfan and abamectin on antioxidant parameters of isolated rat hepatocytes. Toxicol Vitro. 2010; 24(4): 1148-57.
69. Ahmad S, Duval DL, Weinhold LC, Pardini RS. Cabbage looper antioxidant enzymes: tissue specificity. Insect Biochem. 1991; 21(5): 563-72. doi: 10.1016/0020-1790(91)90111-Q.
70. Dubovskiy IM, Martemyanov VV, Vorontsova YL, Rantala MJ, Gryzanova EV, Glupov VV. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of Galleria mellonella larvae (Lepidoptera, Pyralidae). Comp Biochem Physiol C Toxicol Pharmacol. 2008; 148(1): 1-5. doi: 10.1016/j.cbpc.2008.02.003, PMID 18400562.
71. Bongiovanni B, De Lorenzi P, Ferri A, Konjuh C, Rassetto M, Evangelista de Duffard AM, et al. Melatonin decreases the oxidative stress produced by 2, 4-dichloro phenoxy acetic acid in rat cerebellar granule cells. Neurotox Res. 2007; 11(2): 93-9. doi: 10.1007/BF03033388, PMID 17449452.
72. Umosen AJ, Ambali SF, Ayo JO, Mohammed B, Uchendu C. Alleviating effects of melatonin on oxidative changes in the testes and pituitary glands evoked by subacute chlorpyrifos administration in Wistar rats. Asian Pac J Trop Biomed. 2012; 2(8): 645-50. doi: 10.1016/S2221-1691(12)60113-0, PMID 23569987.
73. Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, et al. Regulation of antioxidant enzymes: significant role for melatonin. J Pineal Res. 2004; 36(1): 1-9. doi: 10.1046/j.1600-079x.2003.00092.x, PMID 14675124.
74. Oaknin-Bendahan S, Anis Y, Nir I, Zisapel N. Effects of long-term administration of melatonin and a putative antagonist in the ageing rat. NeuroReport. 1995; 6(5): 785-8. doi: 10.1097/00001756-199503270-00020, PMID 7605949.
75. Medina-Leendertz S, Paz M, Mora M, Bonilla E, Bravo Y, Arcaya JL, et al. Long term melatonin administration alleviates paraquat mediated oxidative stress in Drosophila melanogaster. Invest Clin. 2014; 55(4): 352-64. PMID 25558754.
76. Succu S, Berlinguer F, Pasciu V, Satta V, Leoni GG, Naitana S. Melatonin protects ram spermatozoa from cryopreservation injuries in a dose-dependent manner. J Pineal Res. 2011; 50(3): 310-8. doi: 10.1111/j.1600-079X.2010.00843.x, PMID 21214627.
77. Wang FW, Wang Z, Zhang YM, Du ZX, Zhang XL, Liu Q, et al. Protective effect of melatonin on bone marrow mesenchymal stem cells against hydrogen peroxide-induced apoptosis in vitro. J Cell Biochem. 2013; 114(10): 2346-55. doi: 10.1002/jcb.24582, PMID 23824714.
78. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. 3rd ed. UK: Oxford University Press: Oxford; 1999.
79. Byrne FJ, Toscano NC. An insensitive acetylcholinesterase confers resistance to methomyl in the beet armyworm Spodoptera exigua (Lepidoptera: Noctuidae). J Econ Entomol. 2001; 94(2): 524-8. doi: 10.1603/0022-0493-94.2.524, PMID 11332849.
80. Ghayomi F, Navaei-Nigjeh M, Baeeri M, Rezvanfar MA, Abdollahi MA. A mechanistic approach for modulation of chlorpyrifos-induced toxicity in human lymphocytes by melatonin, coenzyme Q10, and vinpocetine. Hum Exp Toxicol. 2016 Aug; 35(8): 839-50. doi: 10.1177/0960327115607945, PMID 26519479.
81. Händel MN, Andersen HK, Ussing A, Virring A, Jennum P, Debes NM, et al. The short-term and long-term adverse effects of melatonin treatment in children and adolescents: a systematic review and GRADE assessment. EClinicalMedicine. 2023; 61: 102083. doi: 10.1016/j.eclinm.2023.102083, PMID 37483551.
82. Yuge K, Nagamitsu S, Ishikawa Y, Hamada I, Takahashi H, Sugioka H, et al. Long-term melatonin treatment for the sleep problems and aberrant behaviors of children with neurodevelopmental disorders. BMC Psychiatry. 2020; 20(1): 445. doi: 10.1186/s12888-020-02847-y, PMID 32912180.
83. Lucius K. Melatonin beyond sleep, Part I: An overview. Integr Med Int. 2022; 28(3): 138-45. doi: 10.1089/ict.2022.29025.klu.
84. Givler D, Givler A, Luther PM, Wenger DM, Ahmadzadeh S, Shekoohi S, et al. Chronic administration of melatonin: physiological and clinical considerations. Neurol Int. 2023; 15(1): 518-33. doi: 10.3390/neurolint15010031, PMID 36976674.

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