Environmental Toxicology and Pharmacology 56 (2017) 76–85

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Research paper

# Pre-treatment with melatonin decreases abamectin induced toxicity in a nocturnal insect *Spodoptera litura* (Lepidoptera: Noctuidae)

Subramanian P. Subala[a], Eduardo E. Zubero[b], Moises A. Alatorre-Jimenez[c], Muthugounder S. Shivakumar[a,*]

[a] Molecular Entomology Laboratory, Department of Biotechnology, School of Biosciences, Periyar University, Salem, 36011, Tamil Nadu, India
[b] Department of Pharmacology and Physiology, University of Zaragoza, Zaragoza, Spain
[c] Department of Cellular and Structural Biology, Health Science Center, University of Texas, San Antonio, USA

ARTICLE INFO

*Keywords:*
Antioxidant enzyme
Hemolymph
Lipid peroxidation
Melatonin
Midgut
Oxidative stress

ABSTRACT

*Aim:* Oxidative stress is an important component of the mechanism of pesticide toxicity. The aim of the present study was to investigate the time-dependent melatonin effects against abamectin-induced oxidative stress in a *S.litura* model. Larvae were divided into 5 different groups; (1) control group,(2) Melatonin group ($4.3 \times 10^{-5}$ M/100 ml diet), (3) Abamectin group 1.5 ml/L, (4) Pre-melatonin treated group (PM) ($4.3 \times 10^{-5}$ M/100 ml diet) before abamectin exposure 1.5 ml/L, (5) Post-melatonin treated group (TM) after abamectin exposure. Melatonin was supplemented via artificial diet in PM and TM animals during 24 h.

*Main methods:* Midgut, fatbody, and hemolymph, were collected for the analysis of oxidative stress markers (Total ROS, GSH, nitrite, TBARS, LPO), antioxidant enzyme levels (SOD, GST, CAT, POX, APOX) in fifth instar larvae. Midgut damage was examined by using morphological analysis.

*Key findings:* Our results observed that ABA group showed significant changes ($p < 0.001$) in the ROS and carbonyl content in midgut. The increase of antioxidant enzyme levels (SOD, CAT, POX, and APOX) in midgut was led by the continuous free radical scavenger cascade of melatonin. Significant ($p < 0.01$) increases in CAT and APOX levels were seen in the fatbody of PM and TM treated insects.

*Significance:* In conclusion, the results of the study revealed that abamectin toxicity generates oxidative stress in the insect, while pre-melatonin treatment reduces this damage due to its antioxidant properties, especially POX levels in midgut, fatbody, and hemolymph. Therefore, indoleamine can play a vital role curtailing the abamectin toxicity in time dependent manner in *S.litura*.

## 1. Introduction

Abamectin (ABA) belongs to the family of avermectins, which are the macrocyclic lactones produced by a soil actinomycete, *Streptomyces avemitilis* (Fisher and Mrozik, 1989; Burg and Stapley, 1989). It is characterized by having an unparalleled comprehensive antiparasitic activity, being effective against a diverse range of nematodes, arachnids, and insects (Molinari et al., 2009; Huang et al., 2011). It is used as a pest control agent in livestock and as an active substance of nematicides and insecticides for agricultural use (Kolar et al., 2008). The frequent and widespread usage of synthetic pesticides like avermectin can have deleterious effects in the environment (Wang et al., 2013). The avermectins increase the membrane conductance of chloride ions, followed by blocking the electrical activity in nerves and muscles of organisms (Clark et al., 1995).This interaction increases the permeability of cell membranes to chloride ions, which disrupts the neural signal transmission (Novelli et al., 2012). Particularly in insects and nematodes, avermectin and ivermectin targets the glutamate-gated chloride channel (GluCl), GABA-gated chloride channel (GABR), and the histamine-gated chloride channel (HisCl) (McCavera et al., 2007). However, the mode of action of avermectins is not specific to parasitic arthropods and nematodes (McKellar, 1997). Recent studies showed that abamectin was highly toxic to afrotropical dung beetles, non-target invertebrates, and aquatic organisms (Coles, 2005; Shen et al., 2011; Tisler and Erzen., 2006; Novelli et al., 2012; Kolar et al., 2006; Sommer et al., 1993; Na et al., 2009). In addition, it is well known that the

*Abbreviations:* CAT, catalase; POX, peroxidase; SOD, superoxide dismutase; AI, active ingredient; LPO, lipid peroxidation; GABA, gamma-aminobutyric acid; GluCl, glutamate-gated chloride channel; PM, pre-melatonin group; TM, post-melatonin group; GPx, glutathione Peroxidase; DNPH, dinitrophenyl hydrazine; GSH, reduced Glutathione; ROS, reactive oxygen species; RNS, reactive nitrogen species

* Corresponding author at: Molecular Entomology Laboratory, Department of Biotechnology, School of Biosciences, Periyar University, Salem, 36011, Tamil Nadu, India.
*E-mail address:* skentomol@gmail.com (M.S. Shivakumar).

http://dx.doi.org/10.1016/j.etap.2017.08.025
Received 1 September 2016; Received in revised form 10 August 2017; Accepted 28 August 2017
Available online 01 September 2017
1382-6689/ 

family of ivermectins generates damage in midgut regions, fatbody, and hemolymph in insects via its capacity to generate free radicals (Wang et al., 2013). Due to that, this pesticide has been included in the Integrated Pest Management programs (IPM) to evaluate its toxicity (Abd-Elhady and Heikal, 2011).

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine synthesized from the amino acid tryptophan in the pineal gland. It is present in all living organisms, including insects, and is observed to be a highly effective antioxidant agent as well as a free radical scavenger (Mauriz et al., 2013; Tan et al., 2015). Melatonin is effectively scavenging the free radicals than glutathione (GSH) and mannitol for the free radicals scavenging (Tan et al., 1993). Several studies observed that the indoleamine is an important regulator of redox-active enzymes, (GPx, CAT, Mn-SOD, and Cu/Zn-SOD) (Barlow-Walden et al., 1995; Liu and Ng, 2000; Reiter et al., 2000; Tan et al., 2002; Rodriguez et al., 2004; Galano et al., 2011). These effects have been shown against a wide spectrum of chemicals and drugs, including pesticides (Hardeland and PandiPerumal. Melatonin, 2005; Hardeland et al., 2006; Buyukokuroglu et al., 2008; Bharti and Srivastava, 2009; Rao and Chhunchha, 2010; Esteban-Zubero et al., 2016a). Our earlier study showed the protective effect of melatonin on the antioxidant enzymes in synthetic pyrethroid insecticide effect in *Spodoptera litura* (Karthi and Shivakumar, 2015). *S.litura*, is a nocturnal and polyphagus pest causing enormous loss of many economically important crops (Venkateswarlu et al., 2006; Qin et al., 2004). This nocturnal insect was chosen as a model for this study because of availability, fast-growing and polyphagous in nature.

Melatonin has become one of the most popular therapeutic agents for many oxidative stress related diseases in vertebrates; for example some pesticide disturbs (Cutando et al., 2007; Cutando et al., 2011; Antunes et al., 1999). However, the antioxidant role of melatonin is not completely understood in invertebrates, especially in insects. The aim of the present study is to evaluate melatonin's effect against abamectin toxicity in an insect model. In order to do that, we measured the pesticide toxicity in the midgut, fatbody and hemolymph of the lepidopteran insect *(S.litura)*. The indoleamine activity as an antioxidative agent was measured previously and after the exposure to abamectin with the aim to differentiate the protective and therapeutic melatonin effects against the pesticide.

## 2. Materials and methods

### 2.1. Animals

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

### 2.2. Experimental groups

Third instar larvae (n = 450) were divided randomly into five groups as follows:

(1) Control group: 0.1 M Phosphate buffered saline containing $Na_2HPo_4$ and $NaH_2Po_4$; pH 7.5).
(2) Melatonin group: $4.3 \times 10^{-5}$ M/100 ml diet (Karthi and Shivakumar, 2015).
(3) Abamectin group (ABA); 1.5 ml (28 mg AI)/L.

Preliminary study was done to select the dosage of abamectin in prior starting the experiment. Different concentrations of abamectin (0.2–10 ml/L) were used to calculate the $LC_{50}$ values. *S.litura* larvae (n = 90) were placed on different dosage of abamectin (0.2, 0.4, 0.6, 0.8, 1, 1.5, 2.5, 5, 10 ml/L) sprayed castor leaves (Leaf dip method). For each dosage 10 larvae were used and the doses were selected based upon a dose response (0.2–10 ml/L) in lethality. Mortality of larvae was checked for every 24 h. From this preliminary data, sub-lethal dosage ($LC_{50}$) of abamectin used was 1.5 ml (28 mg AI)/L for further experiments. The same dosage (1.5 ml; 28 mg AI)/L) of abamectin was used to against *Deraeocoris brevis* in the previous study (Kim et al., 2006).

(4) Pre-melatonin group (PM): ($4.3 \times 10^{-5}$ M/100 ml diet) before abamectin exposure 1.5 ml (28 mg AI)/L.
(5) Post-melatonin group (TM): ($4.3 \times 10^{-5}$ M/100 ml diet) after abamectin exposure 1.5 ml (28 mg AI)/L.

The melatonin solution was dissolved into ethanol (> 0.01%) (Giehs et al., 2010). Twenty four hours before/after melatonin exposure, an equal volume of cold PBS saline (0.1 M), was added to the abamectin solution and incorporated into larval diet until they reached the fifth instar stage in PM and TM-treated larvae. From each group (n = 90), we obtained 3 replicates (each replicate was formed by 30 larvae) which were kept in plastic containers fed with 100 ml of artificial diet according to previous studies (Kranthi, 2005). The food was replaced every three days. All the animals were dissected under cold conditions (–4 °C). Midgut, fatbody, and hemolymph were collected carefully using ophthalmic scissors and sterile needle and placed rapidly into a deep freezer at −20 °C until the time of analysis. This experiment was carried out in three independent biological replicates.

### 2.3. Chemicals

3-carboxy-4-nitrophenyl disulphide (DTNB), 2-thiobarbituric acid (TBA), and melatonin, were purchased 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 with 0.15 EC. Melatonin ($4.3 \times 10^{-5}$ M) was supplemented daily in the melatonin group; whereas in PM and TM groups, melatonin was administered 24 h before or after abamectin exposure. The chemicals and reagents used in this study were of analytical grade.

### 2.4. Insect bioassay and growth measurements

The larval survival rate was assessed by gentle probing with a fine camelin brush every 24 h for all experimental groups (Busvine, 1980). Animals were categorized as dead (no movement), moribund (unable to move), or alive (able to move > 1 body length). Dead and moribund were considered as dead. Mortality rate (%) was corrected according to Abbott's formula (Abbott, 1925). Larval length (cm) and weight (g) were measured using vernier scale and electronic weighing balance respectively. The larval development time were observed in all the experimental groups for every 24 h. It was done on 3 replicates of five groups. Values are expressed as mean ± standard deviation (SD).

### 2.5. Sample preparation

Fifth instar larvae alive were randomly selected from each group (n = 20). Midgut and fatbody tissues from each individual larva were dissected out using a sterile blade. Tissues were pooled and homogenized in 0.1 M ice cold phosphate buffer saline (pH 7.5) and then filtered. Subsequently, hemolymph was collected by gently removing an anterior proleg, using sterile blade and was collected directly into a chilled 1.5 mL microcentrifuge tube on ice and was diluted 1:24 with ice-cold PBS. All samples were prepared by centrifugation (1000 *x*g, 4 °C for 15 min) and used as an enzyme source in the subsequent biochemical analysis.