Biomedicine & Preventive Nutrition 4 (2014) 527–533

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Naringin modulates the impairment of memory, anxiety, locomotor, and emotionality behaviors in rats exposed to deltamethrin; a possible mechanism association with oxidative stress, acetylcholinesterase and ATPase

Vinayagam Magendira Mani [a], Abdul Majeeth Mohamed Sadiq [b,*]

[a] Department of Biochemistry, Islamiah College (Autonomous), Vaniyambadi 635753, Tamil Nadu, India
[b] Department of Biochemistry, Adhiparasakthi College of Arts and Science (Autonomous), Kalavai 632 506 Tamil Nadu, India

ARTICLE INFO

Article history:
Received 24 July 2014
Accepted 10 August 2014

Keywords:
Deltamethrin
Oxidative stress
Lipid peroxidation
Acetylcholinesterase
ATPase
Neurotoxicity
Neurobehavioral analysis
Flavonoids
Naringin
Antioxidant

ABSTRACT

Exposure to pyrethroid pesticides has been associated with adverse neurodevelopmental outcomes, like neurodegenerative disorder, low IQ, pervasive developmental disorder, attention problems. Thus, we investigated the relationship between pyrethroid deltamethrin exposure to acetylcholine esterase, ATPase, oxidative stress biomarkers, and impaired behavior performance, and the possible ameliorating mechanism of dietary flavonoid naringin in male Wistar rats. Adult male wistar rats were divided into four different groups. Group I: control group; group II received DLM dissolved in corn oil 12.8 mg/kg BW orally (1/10 LD_{50}) for three weeks; group III received DLM as group II and naringin (100 mg/kg BW for 21 days) orally. Group IV: naringin alone. DLM exposure leads to reduction in the levels of acetylcholinesterase, Na^+/K^+, Ca^{2+}, Mg^{2+} ATPase, enzymic and non-enzymic antioxidants activities in cortex and hippocampus region and increase the activities of TBARS. DLM-induced neuronal alterations was evidenced by impairment behavioral performance, like memory, anxiety, locomotor, and emotionality behaviors. This is also supported by histopathological findings of cortex and hippocampus region of rats. However, naringin treatment modulates the abnormalities of DLM-induced alterations in oxidative stress biomarkers, acetylcholine esterase, ATPase, and behavioral performance of rats. These findings highlight the efficacy of naringin as neuroprotectant. In conclusion, our results demonstrated that DLM cause neurobehavioral and biochemical alterations. Oxidative stress, free radical mechanism play major role on DLM-induced neurotoxicity. Naringin could be a suitable agent for preventing the toxicity of DLM by its potent antioxidant, free radical scavenging and neuroprotective activity.

1. Introduction

Deltamethrin (DLM) is a broad spectrum synthetic type II α-cyano group insecticide widely used to protect agricultural crops, vegetables and fruits against pests, such as ants, mites, beetles and weevils. It is also used for public health nurseries, golf courses, urban basic, residential, garden for pest and mosquito control. Moreover, it is used topically in farm animals as an ectoparasiticide against ticks, mites, fleas and flies [1]. DLM insecticide has been used for the treatment of mosquito nets to control malaria and vector borne diseases all over the world [2]. To maintain coverage in Africa alone, 50 million nets a year are needed [3], pyrethroids have become a top choice for household pest control. Thus, we can conclude that numerous individuals are being exposed to DLM. So, human exposure to DLM are becoming increasingly common widespread. The most important sources of the animal and human exposure to DLM are polluted food and water, and it is readily absorbed by the oral route [4]. Chronic exposure leads to behavioral and molecular alterations leads to neurodegenerative disorders, developmental deficits, birth defects, and learning disabilities [5]. The association between pesticide exposure and neurobehavioral and neurodevelopmental effects is an area of increasing concern. Neurodegenerative disorders are characterized by loss of structure and function of neuronal path leading to behavioral impairment, and therefore, behavioral science helps to identify symptoms of neurodegenerative disorders as well as to verify the efficacy of

* Corresponding author. Department of Biochemistry, Adiparasakthi College of Arts and Science (Autonomous), Kalavai 632 50, Tamil Nadu, India.
Tel.: +91 9 443 449 881, +91 4 173 242 644; fax: +91 4 173 242 646.
E-mail address: mohamed68@rediffmail.com (A.M.M. Sadiq).

http://dx.doi.org/10.1016/j.bionut.2014.08.006
2210-5239/

neuropharmacological agents [6]. Pyrethroid insecticide DLM has been shown to cause apoptosis both in vitro [7] excessive apoptosis contribute to pathological cell death observed in several neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease [8].

Behavior is the product of various sensory, motor and associative functions of the nervous system, and the hypothesis is that neurotoxic pesticides can adversely affect one or more of these functions, disrupt learning and memory processes, cause harmful behavioral effects [9]. Behavioral paradigms like novel object recognition test for memory performance, open field test for anxiety-like behavior and motor deficits, hanging wire test for neuromuscular strength, gait analysis for nerve conduction velocity, and locomotion test for motor functions allow a comprehensive assessment of neurological functions during neurodegenerative disorders [10].

Induction of oxidative stress is one of the main mechanisms of many pyrethroid pesticides [11]. During DLM exposure, reactive oxygen species (ROS) are generated and result in oxidative stress, lipid peroxidation [12] leads to changes in protein and DNA structure [13], and influence on antioxidant defence system [14] in different organs. DLM caused a 52% decrease in acetylcholinesterase of the cerebellum [15] and hippocampus region was more than 5-fold in rats exposed to DLM. Interaction with neuronal voltage-sensitive sodium channels is the primary mode of action of DLM [16]. The mechanism by which pyrethroids is thought to exert neurotoxicity is by prolonging the opening of Na^+ channels, voltage gated calcium channels, chloride channels, $GABA_A$ receptors [17]. These altered sodium channels result in repetitive firing or depolarizing block of the neuron.

The generation of reactive oxygen species is well balanced by the counterbalancing act of antioxidant defences. The natural antioxidants may be helpful in preventing or reducing the harmful effects of ROS. Many flavonoids are their ability to scavenge free radicals. It occurs ubiquitously in the plant kingdom and common components of the human diet [18]. The various effects of flavonoids related to their structural similarity to ATP and hence to their capability to transferable in blood brain barriers and compete with ATP binding sites of various enzymatic sites [19]. Naringin is one of the flavonoids, which is a major bittering principal in citrus juices. It is extensively distributed in citrus and occurs in grapefruits, mandarins etc. [20]. Many biological activities of naringin have been recognized, such as antioxidant, antiradical agents, and antilipid peroxidation activities, anti-inflammatory [21] antimutagenic effects [22] inhibition of tumor development and suppress tumor cell proliferation [23], neuroprotective [24]. Hence, the present investigation was undertaken to assess the toxicological effects of deltamethrin on various biochemical, neurobehavioral changes and the possible alleviative effects of naringin by its antioxidant and free radicals scavenging activity.

2. Materials and methods

2.1. Chemicals

Deltamethrin commercial formulation Decis, deltamethrin 97.3% EC (denotes 97.3% of technical grade deltamethrin [W/W] in emulsifiable concentrate) was procured from Bayer crop science limited, Mumbai, India. Naringin were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade.

2.2. Animal model

Wistar male albino rats weighing between 200–240 g were housed in animal cages with food and water ad libitum. Six animals were housed per cage, and maintained on 12/12 h day and night cycle. The animals were fed with commercial pellet diet (Hindustan lever Ltd., Bangalore, India). The experiments were conducted according to ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Approval no. IAEC/APCAS/01/2013/01).

2.3. Experimental design

Experimental animals were divided into four groups of six rats each as follows:

- group I: control rats;
- group II: rats induced with deltamethrin dissolved in corn oil 12.8 mg/kg BW orally (1/10 LD_{50}) for three weeks (21 days);
- group III: rats administered with deltamethrin 12.8 mg/kg BW orally and simultaneously administered100 mg/kg BW of naringin dissolved in water orally for 21 days;
- group IV: naringin alone.

After the experimental period, the animals were fasted overnight, anaesthetized with sodium pentothal and blood collected from jugular vein for serum isolation and sacrificed by cervical decapitation. The brain tissue was excised immediately and a portion of the tissue was homogenized in 0.1 M Tris buffer, pH 7.4 and used for various biochemical assays.

2.4. Histological examination

A portion of the cortex and hippocampus region of brain tissue was fixed in 10% neutral buffered formalin and embedded in paraffin wax for histological evaluation. Sections with thickness 5 μm were stained with hamatoxylin and eosin (H–E), examined under high power light microscope.

2.5. Biochemical assays

The collected serum and tissue homogenate was used to estimate antioxidant enzymes, such as superoxide dismutase SOD [25], catalase CAT [26], glutathione peroxidase GPx [27], glutathione reductase GR [28], glutathione-*S*-transferase GST [29] and nonzymic antioxidants reduced glutathione GSH [30], vitamin C [31], vitamin E [32]. Lipid peroxidation level was determined by measuring thio barbituric acid reactive substances (TBARS) according to the method of Ohkawa et al. [33]. The activity of AChE in the homogenate was assayed by the method of Ellman et al. [34]. Na^+K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase were determined by the methods [35–37], respectively.

2.6. Behavioral studies

Behavioral analyses were conducted at I, II, IIIrd weeks of experimental period. Behavioral analyses, like novel object recognition [38], open field paradigm test [39] for gait abnormality and locomotion tests like grid runway, inclined plane runway, wide runway, narrow beam runway, staircase runway, swimming test for nerve and muscular function [40], were analyzed.

2.7. Statistical method

All the results were expressed as mean ± SD for six rats in each group. All the grouped data were statistically evaluated with SPSS/12.0 software. Hypothesis testing method included one-way analysis of variance (ANOVA), followed by least significant