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Protective effect of α-lipoic acid against α-cypermethrin-induced changes in rat cerebellum

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ABSTRACT

Alfa cypermethrin is a pyrethroids extensively used as ectoparasiticide in domestic animals, insecticidal spray on cotton, vegetables and other crops and to kill cockroaches, fleas and termites in house and other buildings. Previous studies have shown the adverse effect of α -cypermethrin on brain. This study was planned to evaluate the possible role of α-lipoic acid in α -cypermethrin induced toxicity in brain of male albino rats. Rats were divided into four groups. The control, α-cypermethrin, α-lipoic acid and α -cypermethrin plus α-lipoic acid treated groups. The duration of the experiment was four weeks. Our results showed that the administration of α-cypermethrin caused a significant decreased in γ- aminobutyric acid level, acetylcholinesterase, catalase, superoxide dismutase activities and increase in lipid peroxidation in cerebellum. Furthermore, the co-administration of α-lipoic acid mitigates the toxicity of α-cypermethrin by partially normalizing the biochemical parameters. The biochemical observations were supported by histopathological examinations. The findings of this investigation suggest that α-lipoic acid may play a protective role against α-cypermethrin induced toxicity in cerebellum of treated rats.

1. Introduction

Pesticides (insecticides, herbicides and fungicides) constitute the major potential environmental hazard to humans and animals as these are present and concentrated in the food chain (Ahmed et al., 2012). Pyrethroids occupy 25% of the worldwide insecticide marker (Environment Health Perspective, 2005). Although they have less mammalian toxicity among pesticide, pyrethroids have been reported to affect physiological activities and produce pathological entities in animals (Khan et al., 2009). Alfa cypermethrin (α-CYP) is a pyrethroids extensively used as ectoparasiticide in domestic animals, insecticidal spray on cotton, vegetables and other crops and to kill cockroaches, fleas and termites in house and other buildings (Noaishi et al., 2013). Consistent with its lipophilic nature, α-CYP had been found to accumulate in brain (Tao et al., 2008) causing pathological changes (Yousef et al., 2003) and modifying biochemical parameters of brain (Dahamna et al., 2011). In brain, α-CYP crosses the blood-brain barrier and induced neurotoxicity (Azeez and A.L-Hussary, 2012). Acetylcholinesterase (AChE) is found at mainly neuromuscular junctions and cholinergic brain synapse, where its activity serves to terminate synaptic transmission (Singh and Sharma, 2013). δ-aminobutytic acid (GABA) neurotransmitter is one of the most predominating neurotransmitters, which regulates chloride channels in brain (Ullah et al., 2006). Its level is more in the cerebellum than the rest of the brain (Manna et al., 2005). GABA is the chief inhibitory neurotransmitter in the vertebrate central nervous system, which prevents over firing of the nerve cells (Bist and Bhatt, 2009). α-CYP effectively decreased GABA level leading to hyperexcitability and neurotoxicity symptoms (Ullah et al., 2006). Also, it inhibited AChE activity (Kumar et al., 2009). Furthermore, α-CYP-mediated neurotoxicity is contributed by its ability to induce free radical generation (Giray et al., 2001). Various studies have been conducted on certain substances with antioxidant properties that are taken into the body from the diet to overcome stress associating oxidant-mediated disorders (Ogutcu et al., 2006). Alpha-lipoic acid (ALA) is usually found in small amounts in our diet mainly in animal foods such as meat and liver and at low or undetectable levels in plant food such as potato (Lachman et al., 2000). ALA was first discovered in 1951 as part of an enzyme complex within the cell, which is responsible for energy production and assists in acyl group transfer and as coenzyme in the Krebs cycle (Lachman et al., 2000). It is a powerful lipophilic antioxidant *in vivo* and *in vitro* (Scott et al., 2009). ALA differs from antioxidants in that it neutralizes free radicals both in the fatty

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and in the watery regions of cells, in contrast to vitamins C (water soluble) and E (fat soluble) (Hurdag et al., 2008). It is easily absorbed from the gastrointestinal tract, is easily transported across cell, and can cross the blood brain barrier without exhibiting any serious side effects (Malinska and Winiaska, 2005). Several studies have revealed ALA protective effects in cases such as neurodegenerative diseases in which free radicals are involved (Wollin and Jones, 2003). The aim of this study was to evaluate the possible protective effects of ALA against toxicity induced by α-CYP in brain tissue, using biochemical parameters and histological assessment.

2. Materials and methods

2.1. Chemicals

Determination of acetylcholine esterase activity was done using Amplite™ Colorimetric Acetylcholinesterase Assay Kit (Co.11400) (AAT Bioquest, Inc., Sunnyvale, CA) and gamma-aminobutyric acid level using Rat Gamma-aminobutyric acid, GABA ELISA Kit (Co. E0900r) (SunLong Biotech Co., LTD, HangZhou, China). Malondialdehyde level, as an indicator for lipid peroxidation, Superoxide dismutase, Catalase and glutathione peroxidase activities were determined using commercial kits obtained from Diagnostic, Giza, Egypt.

2.2. Animals

Twenty adult male rats weighing about 180–200 mg were obtained from King Faisal University- Kingdom of Saudi Arabia. All animals were housed in plastic cages (5 rats/cage) and kept under the same laboratory conditions of temperature (25 °C), humidity (60%) and lighting (12 h light/12 h dark) for one week prior to start experiment for acclimatization. The rats were free access to food and water. They were fed standard commercial rat chow and water.

2.3. Experimental design

Twenty male rats were randomly divided into four groups of five animals each. Experimental groups was designed as follows: Control group: Rats received oral dose of saline followed by corn oil orally by gavaging. α-CYP group: rats received oral daily dose of α-CYP (14.5 mg/kg b.w.) by gavaging dissolved in corn oil; ALA group: rats received oral daily dose of ALA (20 mg/kg b.w.) by gavaging dissolved in saline. α-CYP and ALA group: rats received oral daily dose of α-CYP (14.5 mg/kg b.w.) by gavage dissolved in corn oil and ALA (20 mg/kg b.w.) by gavaging dissolved in saline. The duration of the experiment was four weeks and the dose and route of administration of the experimental substances was chosen from previous study of Rotimil et al. (2015).

2.4. Biochemical analysis

2.4.1. Brain tissue homogenate

The brain tissue of different groups was homogenized in ice cold phosphate buffer (50 mM, pH 7.5) using a potter-Elvehjem Homogenizer fitted with a Taflon plunger. Homogenates we centrifuged at 11,000 × *g* for 20 min and the resulting supernatants were divided into aliquots and stored at −80 °C until assay of the selected parameters. For separation of total SOD (cytoplasmic and mitochondrial) centrifugation at 10,000 × *g* from 15 min at 4 °C was done.

For determination of GABA level, tissue was homogenized in PBS and stored overnight at ≤ –20 °C. After two freeze − thaw cycles were performed to break the cell membranes; the homogenate was centrifuged for 5 min at 5000 × *g*. The supernatant was stored at ≤ −20 °C until used for analysis.

The concentration of the protein was determined using the method of bradford (Bradford, 1976) and crystalline bovine serum albumin was used as standard protein.

2.4.2. Determination of acetylcholinesterase activity

The activity of acetylcholinesterase enzyme was performed using AmpliteTM Colorimetric Acetylcholinesterase Assay Kit according to the instructions of the supplier. Briefly, 20 µL of 50 units/mL acetylcholinesterase standard stock solution was added to 980 µL of assay buffer to generate 1000 mU/mL standard. 200 µL was taken of 1000 mU/mL standard to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 mU/mL standard acetylcholinesterase solutions. Acetylcholinesterase standards and acetylcholinesterase-containing test samples were added into a 96-well white/clear bottom microplate. 50 µL of acetylthiocholine reaction mixture, containing 250 µL from 20 x DTNB (5.5'-dithio-bis-(2-nitrobenzoic acid), was added to each well of the acetylcholinesterase standard, blank control, and test samples to make the total acetylcholinesterase assay volume of 100 µL/well. The reaction was incubated for 30 min at room temperature and protected from light. The increase in absorbance was monitored at 405 nm with an absorbance microplate reader. The absorbance in blank wells was used as a control, and subtracted from the values for those wells with the acetylcholinesterase reactions. An acetylcholinesterase standard curve was drawn. The acetylcholinesterase activity was reported as mU/mg protein.

2.4.3. Estimation of GABA level

The level of GABA was determined as described in the manual of Rat Gamma-aminobutyric acid, GABA ELISA Kit. In principle, the provided microtiter plate has been pre-coated with an antibody specific to GABA. Standards or samples were then added to the microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for GABA. Next, avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Then a TMB substrate solution was added to each well. Only those wells that contain GABA, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of GABA in the samples is then determined by comparing the optical density (O.D.) of the samples to the standard curve. GABA level was calculated as mg/ml/g Tissue.

2.4.4. Determination of antioxidant enzymes activities

The activities of superoxide dismutase, catalase, and glutathione peroxidase enzymes were measured in tissue homogenates following the instructions supplied with their relevant kits according to the instructions of the manufacturer. The enzymatic activities were expressed as U/mg protein.

2.4.5. Determination of malondialdehyde (MDA) level

The level of MDA as an index of lipid peroxidation was determined through its reaction with thiobarbituric acid (TBA) using the Lipid Peroxide Kit according to the instructions of the supplier. TBARS values were calculated as nmol MDA per g tissue.

2.5. Histopathological examination

For preparation of samples used in the examination by light microscopy, specimens of cerebellum were collected from all experimental groups and fixed in 10% neutral buffered formalin. After proper fixation, the tissue was rinsed with water and dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin. Tissue blocks were cut into thin section 5 µ and routinely prepared and stained with haematoxylin and eosin stain (H & E) (Bancroft and Gamble, 2002), then sections were examined using light microscopy.