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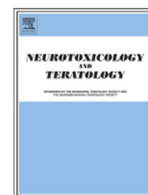

Influence of green tea extract on oxidative damage and apoptosis induced by deltamethrin in rat brain

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ABSTRACT

In the present study, we investigated the protective effect of an aqueous extract of green tea leaves (GTE) against neurotoxicity and oxidative damage induced by deltamethrin (DM) in male rats. Four different groups of rats were used: the 1st group was the vehicle treated control group, the 2nd group received DM (0.6 mg/kg BW), the 3rd group received DM plus GTE, and the 4th received GTE alone (25 mg/kg BW). The brain tissues were collected at the end of the experimental regimen for subsequent investigation. Rats that were given DM had a highly significant elevation in MDA content, nitric oxide concentration, DNA fragmentation and expression level of apoptotic genes, TP53 and COX2. Additionally, a significant reduction in the total antioxidant capacity in the second group was detected. The findings for the 3rd group highlight the efficacy of GTE as a neuro-protectant in DM-induced neurotoxicity through improving the oxidative status and DNA fragmentation as well as suppressing the expression of the TP53 and COX2 genes. In conclusion, GTE, at a concentration of 25 mg/kg/day, protected against DM-induced neurotoxicity through its antioxidant and antiapoptotic influence; therefore, it can be used as a protective natural product against DM-induced neurotoxicity.

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1. Introduction

The use of pesticide is one of the most researched topics in the agro fields of our economy. Although pesticides can significantly elevate crop productivity, they cause serious hazards in non-target organisms (Yonar and Sakin, 2011). Pesticides of the pyrethroid class, such as deltamethrin (DM), a type II synthetic pyrethroid, are widely used as insecticides due to their low environmental persistence and toxicity. DM is successfully substituted for organophosphates in pest-control programs (Shukla et al., 2002). Although DM was initially thought to be the safest available insecticide, a numbers of recent studies have been published on its toxicity (Kim et al., 2010; Hines, 2013; Abdel-Daim et al., 2013). The negative effects of DM on hematological, urinary and respiratory systems have previously been reported. DM exposure leads to the pathophysiology of a broad spectrum of cerebrovascular and neurodegenerative diseases (Mani et al., 2014). The mechanism of DM neurotoxicity arises through calcium overload which in turn is provoked by the delayed opening of the voltage gates of Na channel (VGSC) and inhibition of γ-aminobutyric acid receptors (Hossain and Richardson, 2011). Additionally, other studies demonstrated that a VGSC-independent mechanism attributes to the DM-induced disruption of neuronal activity at higher levels of organization such as hippocampal neurons (Meyer et al., 2008). There is abundant literature reporting that DM increases the generation of reactive oxygen species (ROS) and free radicals, causing extensive oxidative stress and excessive lipid peroxidation (LPO) as well as reducing antioxidant enzyme activity (Abdel-Daim et al., 2013), which could be the main cause of DM toxicity. Under normal conditions, the body is endowed with effective antioxidant systems to combat the overproduction of ROS. However, under some circumstances, the balance between ROS production and its elimination is disturbed, leading to oxidative stress (Lushchak, 2011). The damage to macromolecules, including proteins, lipids, and nucleic acids, is considered to result from ROS overproduction, which is believed to be involved in the etiology of many neurodegenerative disorders (Mani et al., 2014). Herbs and medicinal plants are considered to be a potential therapeutic option for various diseases, including neurodegenerative disease (Khan et al., 2012). Green tea is a widely consumed beverage worldwide. Green tea leaves are reported to be antimutagenic through reducing cancer formation and chromosomal damage (Bushman, 1998). Additionally, the National Cancer Institute selected its extract as a cancer chemopreventive (Steele et al., 1999). The tealeaf contains polyphenols as the main component, comprising approximately 30% to 42% of the dry weight, and most of these are catechins (Graham, 1992). Epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG) are the major catechins present in green tea (Sang et al.,

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2011). Those catechins act directly as radical scavengers for reactive oxygen and nitrogen species. Chemically, they all possess multiple hydroxyl substituents responsible for their antioxidant activity (Sang et al., 2005). They also exert indirect effects through activation of antioxidant enzymes (Mandel and Youdim, 2012). Recent findings indicate that DM may induce toxic manifestations by enhancing the production of ROS and disrupting the balance between pro-oxidants and antioxidants. In addition, there are scarce data about its neurodegenerative toxicity, mechanism and the possible protective role of natural antioxidants. Therefore, the present investigation was designed to evaluate the potency of DM in inducing neurotoxicity and the neuroprotective influence of GTE (25 mg/kg/day) through monitoring its effect on the oxidative status, DNA damage and apoptotic gene expression.

2. Materials and methods

2.1. Animals

Forty-eight male albino rats, weighing 120–140 g, were used in the present study. They were maintained under standard conditions in accordance with the Ethical Principles for the Care and Use of Laboratory Animals (Guide for the care and use of laboratory animals, 1995). The Animal Care and Use Committee of Beni-Suef University approved the study. All efforts were made to minimize animal suffering.

2.2. Chemicals

Deltamethrin (>99% pure) was obtained from the KZ pesticide company (*Egypt*). DM was dissolved in corn oil. The selected dose of DM was based on previous studies in which 1/10 of the LD50 induced biochemical alterations in rats without having morbidity (Oda and El-Maddawy, 2011). The green tea leaves were obtained from Lipton green tea Unilever brand, packed in the United Arab Emirates Unilever Gulf FZE. The GTE was prepared according to Maity et al. (Maity et al., 1998) by soaking 15 g of instant green tea powder in 100 ml of boiling distilled water for 5 min. The GTE contained epigallocatechin gallate (337 mg/l), epigallocatechin (268 mg/l), epicatechin (90 mg/l), epicatechin gallate (60 mg/l), and coffeic acid (35 mg/l) as determined by the HPLC method (Maiani et al., 1977).

2.3. Experimental regimen

The rats were randomly divided into four different groups ($n = 12$). The route of administration selected for the study was oral gavage, which was performed once daily for 30 consecutive days.

- The 1st group was treated with corn oil (0.2 ml\ rat) and acted as the control group.
- The 2nd group received DM (0.6 mg/kg BW).
- The 3rd group was given DM (0.6 mg/kg) plus GTE (25 mg/kg BW).
- The 4th group was given GTE alone (25 mg/kg BW).

Throughout the experimental time, there were no signs of toxicity in the animals treated with DM. The animals were fasted overnight, anesthetized and sacrificed by cervical dislocation at the end of the experiment, and the brain tissue was collected for further analysis.

2.4. Oxidative stress parameter assay

Brain samples were homogenized in cold phosphate buffered saline (pH 7.4) using Teflon homogenizer. The homogenates were centrifuged at 14,000 $\times g$ for 15 min at 4 °C. The supernatant was used to measure the neuronal MDA according to the method described in Mihara and Uchiyama (1978), nitric oxide concentration (NO) (Miranda et al., 2001), total antioxidant capacity (TAC) using a commercial kit (purchased from Bio Diagnostic Company, Egypt) and the total protein concentration according to Bradford (1976).

2.5. DNA damage evaluation

Apoptotic changes in the brain tissue were assessed using three different techniques that included estimation of DNA fragmentation percentage, DNA laddering assay and Comet assay.

2.5.1. DNA fragmentation percentage

DNA fragmentation assay is a quantitative method used for grading the DNA damage (Perandones et al., 1993). The brain tissues were lysed in 0.5 ml of hypotonic lysis buffer containing 10 mM Tris–HCl (pH 8), 1 mM EDTA and 0.2% Triton X-100, and centrifuged at 14,000 $\times g$ for 20 min at 4 °C. The pellets were resuspended in hypotonic lysis buffer. To the resuspended pellets and the supernatants, 0.5 ml of 10% trichloroacetic acid (TCA) was added. The samples were centrifuged for 20 min at 10,000 $\times g$ at 4 °C, and the pellets were suspended in 500 μl of 5% TCA. Subsequently, each sample was treated with a double volume of diphenylamine (DPA) solution [200 mg DPA in 10 ml glacial acetic acid, 150 μl of sulfuric acid and 60 μl acetaldehyde] and incubated at 4 °C for 48 h. The proportion of fragmented DNA was calculated from the absorbance reading at 578 nm using the equation:

DNA fragmentation = OD of fragmented DNA (supernatant)/[OD of fragmented DNA (supernatant) + OD of intact DNA (pellet)] × 100.

2.5.2. DNA laddering assay

The genomic DNA was extracted from brain tissue according to Wu et al. (Wu et al., 2005) to estimate DNA damages. DNA samples (10 μg) were separately loaded into 1.5% agarose gel electrophoresis for 45 min at 80 V. After electrophoresis gel was photographed through a digital camera. The migration of the fragmented DNA on agarose gel results in a characteristic laddering pattern which is considered a distinctive feature of the apoptotic DNA damage.

2.5.3. Comet assay (alkaline single-cell microgel electrophoresis)

Comet assay was performed according to Singh et al. (Singh et al., 1988). Briefly, 100 mg of crushed brain samples was suspended in 1 ml ice cold PBS, stirred for 5 min and filtered. 100 μl of cell suspension was thoroughly mixed with 600 μl of low-melting agarose, followed by spreading of 100 μl of the mixture on agarose pre-coated slides. The slides were left to solidify at 4 °C, and then they were immersed in chilled lysing solution for 1 h at 4 °C. The slides were removed and placed in a horizontal electrophoresis chamber, filled with freshly prepared electrophoretic alkaline buffer for 20 min. After electrophoresis, the slides were washed gently in 0.4 M Tris–HCl buffer and stained with ethidium bromide. The DNA migration patterns of 100 cells for each sample were observed using fluorescence microscope, and images were captured by a Nikon CCD camera. The qualitative and quantitative extent of DNA damage in the cells was estimated using the Comet 5 image analysis software developed by Kinetic Imaging Ltd. (Liverpool, UK).

2.6. Histopathological examination

The brain tissues from the different groups were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding to obtain 4 μm sections. The sections were stained with hematoxylin and eosin and examined under optical microscope (Bancroft and Stevens, 1996).

2.7. Apoptotic gene expression analysis

2.7.1. Immunohistochemistry for P53 and COX2 proteins

Brain tissue sections were deparaffinized in xylene and rehydrated in graded alcohol. Drops of Hydrogen Peroxide Block (Thermo Scientific,

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