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Research report

Cypermethrin induced damage in genomic DNA and histopathological changes in brain and haematotoxicity in rats: The protective effect of sesame oil

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ABSTRACT

The protective effect of sesame oil against cypermethrin-induced brain toxicity was studied. Female rats were orally treated with cypermethrin, sesame oil and their combination for 30 consecutive days. The results showed that cypermethrin increased thiobarbituric acid-reactive substances (TBARS), and decreased glutathione (GSH) and the activities of the antioxidant enzymes. Brain injury was confirmed by histopathological changes and DNA damage. Also, the reduction in the activities of acetylcholinesterase and monoamine oxidase (AChE & MAO), total protein, albumin and body weight, and the induction in triacylglycerol and cholesterol have been observed due to cypermethrin toxicity. Animals treated with sesame oil and cypermethrin together showed that brain TBARS and plasma triacylglycerol and cholesterol returned to the control level which indicating a protective effect of sesame oil. Also, sesame oil was able to attenuate the decrease in total protein, albumin, triacylglycerol and cholesterol, GSH, AChE and antioxidant enzymes induced by cypermethrin. In addition, sesame oil protected the brain histological changes and fragmentation of genomic DNA in animals treated with cypermethrin. The present results showed a protective effect of sesame oil against the cypermethrin induced brain toxicity and this could be associated mainly with the attenuation of the oxidative stress and the preservation in antioxidant enzymes.

1. Introduction

The widespread use of pesticides in public health protection and agricultural programs has caused severe environmental pollution and health hazards, particularly in developing countries, including cases of severe acute and chronic human and animal poisoning as well as damage to other non-targeted organisms [2]. Pesticides have been considered potential chemical mutagens and some studies have shown that various agrochemical ingredients possess genotoxic properties, leading to mutations, chromosomal alterations or DNA damage [7].

The toxicity of pyrethroid insecticides to mammals has received much attention in recent years because animals exposed to these insecticides showed changes in their physiological activities besides other pathological features [17]. Cypermethrin, a synthetic pyrethroids insecticide has been extensively used in the last two decades in many of the developing countries, especially in Egypt, for combating agricultural pests and insects of veterinary as well as human concern [2]. Human exposure to cypermethrin is reported to occur mainly occupationally during application or through pyrethroids residues such as those detected in cow's milk, bread, fruits and vegetables [48].

In an epidemiological study, population exposed to cypermethrin in cotton fields showed ill health effects such as severe giddiness, nervous, skin and eye disorders, neonatal deaths and congenital defects. Cypermethrin can also elicit a range of neurotoxic, immunotoxic and genotoxic effects and reproductive toxicity in various experimental systems [50,60]. Cypermethrin, a class II pyrethroid insecticide, is involved in the pathogenesis of various neurological disorders. Cypermethrin can cross the blood-brain barrier and exert its effect on nigrostriatal system. Cypermethrin is hypothesized to increase the incidences of Parkinson's disease (PD) in exposed individuals *via* an elusive mechanism, as it acts on the nervous system in a non-selective manner [51]. Also, cypermethrin caused changes in biochemical and haematological changes in rabbits [62]. Cypermethrin has been classified by the US Environmental Protection Agency [56] as a possible carcinogen.

Abbreviations: TBARS, thiobarbituric acid-reactive substances; TBA, thiobarbituric acid; GST, glutathione S-transferase; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GSH, reduced glutathione; ROS, reactive oxygen species; MAO, monoamine oxidase.

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Cypermethrin exerts its neurotoxic effect through voltage-dependent sodium channel and integral protein ATPase in the neuronal membrane [25]. Histologically, some deformation areas due to ischemia and pyknosis of the cytoplasm of the neurons were observed in brain tissues of rats treated with cypermethrin [45]. Cypermethrin could form DNA monoadducts and DNA interstrand crosslinks [9]. Cypermethrin also causes induction of DNA damage and micronucleus *in vitro* in human lymphocytes [55]. Both *in vitro* and *in vivo* experiments with rat peripheral blood lymphocytes showed that cypermethrin severely damages DNA and causes imbalance in the prooxidant/antioxidant status in lymphocytes [15,52]. *In vivo* studies have also shown that cypermethrin causes free radical-mediated tissue damages [16,59,60].

The toxicity of pyrethroid insecticides to mammalian animals has received much attention in recent years because animals exposed to these insecticides exhibited changes in their physiological activities beside other pathological features [43]. Although extensive research work is under way in different laboratories on various aspect of synthetic pyrethroid, including metabolism, pharmacological characteristics, ecotoxicity and detection of residues, little attention has been paid to the protective effects of antioxidants against the toxicity of pyrethroid.

Sesame oil is extract from the plant Sesamum indicum (HS), family: Pedaliaceae. Sesame oil offers protection over blood pressure, lipid profiles and lipid peroxidation in hypertensive patients. Sesame oil attenuates oxidative stress and multiple organ failure triggered by endotoxin lipopolysaccharide in rats. Sesamin and sesaminol are the major phenolic constituents of sesame oil which have been reported to possess a broad spectrum of pharmacological effects including antimutagenic, antioxidant, antihypertensive, anti-inflammatory and antithrombotic [47]. Sesame oil decreases lipid peroxidation by inhibiting the generation of reactive oxygen free radicals [20].

Cypermethrin is widely used as insecticide in developing countries controlling pests. It is widely used in Egypt in pest-control programs in agriculture and in public health as well. Cypermethrin have become the dominant insecticides used by the farmers in Egypt. Considering that the involvement of reactive oxygen species (ROS) has been implicated in the toxicity of various pesticides. Cypermethrin is one of the most common contaminants in the ecosystem. Recently, Jin et al. studied the effects of cypermethrin exposure on the induction of oxidative stress [24]. There no enough data for the protection against the toxicity of cypermethrin. Therefore, this study was designed to investigate: (1) the possibility of oxidative stress induction by cypermethrin and (2) the possibility of sesame oil to minimize the toxic effects by cypermethrin.

2. Materials and methods

2.1. Chemicals

Cypermethrin 25% E.C [(RS)-cyano-(3-phenoxyphenyl) methyl-(IRS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate] was purchased from Mitchell Cotts Chemicals, West Uorkshyer, UK. The pure sesame oil was obtained from local commercial suppliers. The reagent kit for plasma protein, albumin, cholesterol and triacylglycerol concentrations was purchased from SENTINEL CH. (*via* principle Eugenio 5-20155 Milan, Italy). All other chemicals used in this experiment were of analytical grade. The dose selection, the time of exposure and oral treatment for cypermethrin was based on previously published studies [22,48]. Sesame oil was given orally by gavages at a volume of 5 ml/kg body weight according to the previous study of Shakoori et al. [47].

2.2. Animals and experimental design

Forty female Wistar rats weighting 180–200 g were used in the present experiments. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were caged in groups of five and given food and water *ad libitum*. After 2 weeks of acclimation, animals were divided into four equal groups (10 for each treatment group). The first group served as control. Groups 2, 3 and 4 were treated with sesame oil (5 ml/kg b.w.), cypermethrin (12 mg/kg b.w.) and the combination of both sesame oil (5 ml/kg b.w.) plus cypermethrin (12 mg/kg b.w.). Rats were orally administered their respective doses every day for 30 days. There are no mortality animals during the experimental period.

2.3. Blood collection and tissue preparation

Blood samples were collected from the sacrificed animals and placed immediately on ice. Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 860 × *g* for 20 min and stored at −60 °C. Stored plasma samples were analyzed for the activities of antioxidant enzymes (glutathione *S*-transferase, GST; superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPX) and the marker of lipid per oxidation (thiobarbituric acid-reactive substances, TBARS).

Brain was immediately removed; washed using chilled saline solution. Tissues were minced and homogenized (10%, w/v), separately, in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 × *g* for 20 min at 4 °C, and the resultant supernatant was used for the determination of enzyme activities, and the levels of TBARS, reduced glutathione (GSH) and protein content.

2.4. Tissue and plasma thiobarbituric acid reactive substances and antioxidant enzymes

Plasma and tissue supernatant thiobarbituric acid-reactive substances (TBARS) were measured at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol, TBA). An extinction coefficient of 156,000 M^{-1} cm^{-1} was used for calculation [12]. Reduced glutathione content was assayed by the method of [6]. The method utilized metaphosphoric acid for protein precipitation and 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) for color development and its density was measured at 412 nm. Glutathione S-transferase (GST; EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of [18]. P-nitrobenzylchloride was used as substrate. The absorbance was measured spectrophotometrically at 310 nm using UV-Double Beam Spectrophotometer. The catalase enzyme (CAT; EC 1.11.1.6) converts H_2O_2 into water. The CAT activity in plasma and tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme [59].

Superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to Misra and Fridovich [35]. The assay procedure involves the inhibition of epinephrine autooxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant and the change in extinction coefficient was followed at 480 nm in a spectrophotometer. Glutathione peroxidase (GPX) activity assayed using the method of Chiu et al. in plasma and brain extracts [8].

2.5. Protein estimation

The protein content of the tissue extracts mentioned earlier was determined by the method of Lowry et al. using bovine serum albumin as a standard [32].

2.6. Biochemical parameters

Plasma cholesterol and triacylglycerol (TG) were determined with kits from SENTINEL CH. (*via* principle Eugenio 5-20155 Milan, Italy). Stored plasma samples were analyzed for total protein and albumin and concentrations were measured with kits from SENTINEL CH. (*via* principle Eugenio 5-20155 Milan, Italy).

2.7. Plasma and brain acetylcholinesterase and monoamine oxidase estimation

Acetylcholinesterase (AChE; EC 3.1.1.7) activity was estimated in plasma and tissue supernatant using acetylcholine iodide as a substrate according to the method of Ellman et al. [11]. The activity of monoamine oxidase activity was estimated in plasma and tissue supernatant according the method of Sandler et al. [40].

2.8. Histological section preparation

Brain specimens were obtained from rats, and immediately fixed in 10% formalin, and then treated with conventional grade of alcohol and xylol, embedded in paraffin and sectioned at 4–6 μm thickness. The sections were stained with Haematoxylin and Eosin (H&E) stain for studying the histopathological changes [31].

2.9. Haematological parameters

Blood samples were collected from the sacrificed animals 24 h after the last dose and placed immediately on ice. Heparin was used as an anticoagulant and noncoagulated blood was tested, shortly after collection, for hemoglobin (Hb), total erythrocyte count (TEC), packed cells volume (PCV) and total leukocyte counts (TLC) by Particle counter (ERMA Inc., Tokyo. Model PCE-210).