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Protective effects of *Nigella sativa* oil on propoxur-induced toxicity and oxidative stress in rat brain regions

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ABSTRACT

Propoxur (PPr) is a widely used broad spectrum carbamate insecticide mainly used to control household pests. Because of the widespread use of pesticides for domestic and industrial applications, evaluation of their neurotoxic effects is of major concern to public health. The aim of the present study was to evaluate the possible protective effects of *Nigella sativa* oil (NSO), an antioxidant agent, against PPr-induced toxicity and oxidative stress in different brain regions of rats including cerebellum, cortex and hippocampus. In the present study, 32 male Sprague–Dawley rats were used and divided into four equal groups. Group 1 was allocated as the control group. Groups 2–4 were orally administered 1 ml/kg/bw/day NSO, 8.51 mg/kg/bw/day PPr or NSO plus PPr, respectively, for 30 days. Lipid peroxidation (LPO), protein carbonyl content (PCC) and acetylcholine esterase activity (AChE) were determined. Enzymatic antioxidant activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-*S*-transferase (GST)] and non-enzymatic antioxidants [reduced glutathione (GSH)] were determined. PPr treatment significantly increased the levels of LPO, PCC and oxidized glutathione (GSSG) in brain regions. On the contrary, levels of GSH and the activities of SOD, CAT, GSH-Px, GST and AChE were significantly decreased. NSO treatment to PPr intoxicated rats restored such biochemical parameters to within control levels except GST activity, emphasizing its antioxidant role. We conclude that NSO significantly reduces PPr-induced toxicity and oxidative stress in rat brain regions via a free radicals scavenging mechanism.

1. Introduction

Propoxur (2-(1-methylethoxy) phenol methyl carbamate; Fig. 1) is a carbamate insecticide developed by Bayer AG, Germany, and registered for use against ants, cockroaches, crickets, fleas, flies, mosquitoes, wasps, and ticks in many countries [1]. Propoxur (PPr) was found to be neurotoxic, with massive reversible inhibition of acetylcholine esterase (AChE) as the main mechanism of action [2]. As a consequence, the risk of human exposure by repeated low doses of these substances is relatively high [1,3]. The exact mechanism(s) of PPr-induced toxicity have yet to be fully defined. Involvement of reactive oxygen species (ROS) has been posited as a likely mechanism of PPr-induced toxicity [4,5]. ROS are closely implicated in several diseases of nervous system including Parkinson's disease, schizophrenia and Alzheimer's disease [6]. Carbamate insecticides may induce oxidative stress leading to generation of free radicals with alteration in enzymatic and non-enzymatic antioxidant systems [7,8]. Lipid peroxidation (LPO) has been suggested as one of the molecular mechanisms involved in carbamate-induced toxicity [4]. Enzymatic antioxidants and glutathione metabolism-regulating enzymes may protect the cellular system against various deleterious effects of free radicals induced by pesticides [9,4].

The brain exhibits distinct variations in cellular as well as regional distribution of antioxidant biochemical defenses [10]. Thus, neural cells and/or brain regions are likely to differentially respond to changes in metabolic rates associated with the generation of ROS [11]. Indeed, there is abundant evidence invoking regional sensitivity to oxidative stress that is dependent on cellular and regional redox status [12].

More attentions have been paid to the protective effects of natural antioxidants against chemicals-induced toxicities especially whenever free radical generations are involved. *Nigella sativa* L. (*N. sativa*) is a plant of Ranunculaceae family that grows spontaneously and widely in several southern Mediterranean and Middle

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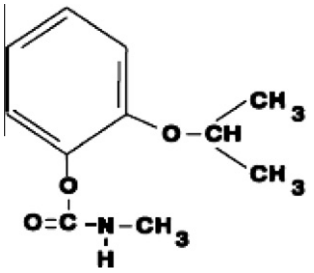

Fig. 1. Propoxur; chemical name: 2-(1-methylethoxy) phenol methyl carbamate; trade name: BAYGON®; CAS No. 114-26-1; empirical formula: $C_{11}H_{15}NO_3$.

Eastern countries [13]. *N.* sativa seed has over 100 different chemical constituents, including abundant sources of all the essential fatty acids. Although it is the oil that most often used medicinally, the seeds are a bit spicy and are often used whole in cooking curries, pastries and Mediterranean cheeses [13]. The seeds of *N. sativa,* also known as black seed or black cumin, are often used as a spice but are also used extensively in the traditional medicine of many countries [14]. *N. sativa* has been used traditionally for the treatment of many diseases owing to the reported antiviral [15], anti-schistosomiasis [16], anti-inflammatory [17], and immunomodulatory [18] activities. Furthermore, it was found that *N. sativa* extract has anti-tumor properties [19], attenuates toxic side effects caused by several chemotherapeutic agents [20] and protects against gentamicin-induced nephrotoxicity [21]. In addition, it prevents hippocampal neurodegeneration after chronic toluene exposure in rats [22].

The high lipid content of the brain makes it particularly susceptible to free radicals mediated insult [23]. Antioxidant enzymes are involved in the defense system against free radical mediated tissue or cellular damage [24]. Therefore, the present study was planned to evaluate the role of NSO as a protective agent against propoxur-induced toxicity and oxidative stress in rat brain regions.

2. Materials and methods

2.1. Chemicals

Propoxur (isopropoxiphenylmethylcarbamate) of 99.4% purity was purchased from ChemService (West Chester, PA, USA). Thiobarbituric acid (TBA), reduced glutathione (GSH), 5,5-dithio bis (2-nitrobenzoic acid), 1-chloro 2,4 dinitrobenzene (CDNB), glutathione reductase (GR), were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). *N. sativa* seeds were purchased locally, and identity was confirmed by professional botanists (Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia). All the other chemicals used were of analytical grade.

2.2. Preparation of the decoction

The dried seeds of *N. sativa* were purchased from a local market and authenticated in the Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia and voucher specimens are deposited at the Pharmacognosy Department. The seeds were crushed and cold macerated in petroleum ether (40–60 °C) for three days. The extract was taken out, petroleum ether evaporated and the oil was filtered. Yield was 17.50% v/w with reference to dried seeds. The extracted oils were kept in screw-capped tubes in the dark at −20 °C until use. This dose corresponds to normal therapeutic dose administered to adult humans as calculated based on relative surface areas of human and rat [25].

2.3. Experimental groups

Thirty-two adult male Sprague–Dawley rats (250–300 g) were randomly assigned into four groups. The first group was administered groundnut oil as a vehicle (1 ml/kg/day), the second group was administered *N. sativa* oil (NSO), the third group was administered PPr and the fourth group was administered a combination of NSO and PPr. The selected dose of the insecticide was based on previous studies in which 1/10 LD50 of propoxuor induced biochemical alterations in rats without morbidity [26]. Propoxur was dissolved in groundnut oil of pharmaceutical quality and was administered orally in a dose of 8.51 mg/kg body weight/day for 30 days with intragastric tube. NSO was administered (1 ml/kg/day) orally at least 1 h before the administration of PPr.

2.4. Tissue preparation

Twenty-four hours after last treatment, the animals were euthanized using carbon dioxide asphyxiation and brains were immediately removed and washed in ice-cold physiological saline repeatedly and dissected over ice-cold glass slides to the following regions: cerebellum, cerebral cortex and hippocampus [27]. Pooled regions from each of the brain tissue were blotted, weighed accurately, and placed in chilled 0.01 mol/l Tris–HCl buffer (pH 7.4). The samples were homogenized using a Potter–Elvehjem homogenizer (Wheaten Science Products, Milliville, NJ, USA) to produce 10% homogenates and used for determining the biochemical parameters described below. Protein concentrations of the tissue homogenates were determined by the standard method of Lowry et al. [28], using bovine serum albumin as the standard.

2.5. Biochemical assays

2.5.1. Acetylcholine esterase (AChE) assay

AChE activity was determined by the method of Ellman et al. [29]. Total assay volume of 355 μl, consisted of 5 μl of tissue, 300 μL of chromogen/buffer (0.3 mM 5,5′-dithio-bis (2-nitrobenzoic acid); final DTNB concentration in assay 0.25 mM) and 50 μl of substrate (8.45 mM acetylthiocholine iodide; final concentration in assay 1.2 mM). There was a 5-min pre-incubation period and the reaction was conducted at 37 °C. Specific activity of AChE was expressed as nmoles of substrates hydrolyzed/min/mg protein.

2.5.2. Reduced and oxidized glutathione (GSH and GSSG) assay

GSH level in the selected brain regions was determined by the method of Ellman [30]. Briefly, an aliquot of 1 ml of suspension precipitated with 10% trichloroacetic acid (TCA). The precipitate was removed by centrifugation. The supernatant was then divided into two aliquots. One was directly used for total GSH assay according to the modified method of Ellman, 1959, and the other for GSSG. One hundred microliters of supernatant fractions with 2 μl vinyl pyridine were incubated at room temperature for 1 h to scavenge GSH for the GSSG determination. The GSSG was then subtracted from the total glutathione to evaluate the GSH levels. GSH was determined by the DTNB-glutathione reductase recycling mechanism.

2.5.3. Lipid peroxidation (LPO) assay

LPO was determined in brain tissues using the method of Devasagayam and Tarachand [31]. Briefly, the reaction mixture consisted of 1 ml 0.15 mol/l Tris–HCl buffer (pH 7.4), 0.3 ml 10 mmol