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RESEARCH ARTICLE

Attenuation of Propoxur-induced Oxidative Stress and Immunotoxicity in Rats Treated with Dietary Ginger (*Zingiber officinales Rosc*)

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Abstract

Propoxur (2-isopropoxyl phenyl *N*-methyl carbamate) is the cabamate pesticide widely used in public health programmes and household in many countries. In the current study we evaluate the protective effect of dietary feeding of *Zingiber officinales* Rosc (ginger) against propoxur-induced oxidative stress and immune dysfunctions in the adult male albino Wistar rats. Oxidative stress was determined by estimating the extent of malondialdehyde (MDA) which is lipid peroxidtion marker and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) and activity of reduced glutathione (GSH). Immunotoxicity was monitored by evaluating antibody (Ab) titre as humoral immune response and leucocyte migration inhibition (LMI); macrophage migration inhibition (MMI) as a cell mediated immune response. Propoxur treated orally (10 mg/kg body weight for 28 days) was associated with increased MDA level and compromised antioxidant defenses in rats fed a normal diet. Concomitant dietary feeding of ginger (1% w/w) significantly protects propoxur-induced lipid peroxidation, accompanied by modulating antioxidant enzymes as well as GSH. However, ginger also attenuate serum Ab titire, LMI and MMI in the propoxur exposed animals. These finding suggest that a dietary ginger exert protective effect against free radical injury and immunotoxicity caused subchronic propoxur exposure.

Keywords

Propoxur, Ginger, Antioxidant, Lipid peroxidation, Immunotoxicity

Introduction

Propoxur (2-isopropoxyl phenyl *N*-methyl carbamate) is a well known carbmate pesticide. Widespread use of propoxur in household and public health programmes (Qian *et al,* 2010). This pesticide act by inhibitory reversibly acetyl choline esterase (AChE) enzyme activity resulting in a cholinergic stimulation. Several cases of suicidal and occupational poisoning have been reported (Sosan and Akingbohungbe, 2009). Hence, propoxur has generated considerable concern regarding its subtle health effects. Possible effect of persistent exposure to propoxur on oxidative stress and functional integrity of the immune system has heightened interest in these paparameters as additional indices to analyse potential long term health hazards (Yadav *et al*, 2010). Carbamate insecticide may induce oxidative stress leading to generation of free radicals and alterations in antioxidant enzyme system (Banerjee *et al,* 1999; Seth *et al*, 2000). Previous report shows that both, humoral and cell mediated immune responses were suppressed in rats exposed to subchronic exposure of propoxur in male Wistar rats (Seth *et al*, 2002).

Nowadays many botanicals natural plant products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. In traditional Indian medicine or Ayurveda ginger have been used as medicine. Ginger rhizome (*Zingiber officinale* Rosce), commonly known as ginger, is consumed worldwide as a spice and a flavoring agent (Ahmed *et al*, 2000). Experimental studies have shown that ginger possesses antiemetic, anti-inflammatory, anticancer, anxyolitic, antithrombotic and cardiovascular effects (Kaul and Joshi, 2001). With regards to the digestive tract, it has been shown that ginger extract counteracted the cisplatin-induced delay in gastric emptying in rats and also it reduced tachygastric activity induced by circular vection in humans, an effect which is believed to contribute to its efficacy in the treatment of nausea associated with motion sickness (Sharma and Gupta, 1998; Lien *et al*, 2003). Gingerols (i.e. 6-gingerol, 8-gingerol and zingerone) have been identified as the main active ingredients of ginger and also responsible for its characteristic pungent taste (Onyenekwe, 2000). Other constituents include volatile oil, aryl alkanes, shogaols, diarylheptanoids and starch (Sekiwa *et al*, 2000). Ginger

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exerts an antioxidative effect by decreasing lipid peroxidation, increasing GSH content and maintaining normal levels of antioxidant enzymes, higher levels of antioxidant enzymes have been well correlated with increased lipid peroxidation after lindane exposure (Ahmed *et al*, 2008). The present work was conducted to study the effect of ginger on the modulation of oxidative stress and immune dysfunctions induced by subchronic exposure of propoxur in albino rats.

Materials and Method

Adult male albino Wistar rats weighing 200-250 g were placed in individual raised-bottom, galvanized wire cages and kept under standard laboratory conditions of light-dark cycle (12 - 12 hr) and temperature (25° ± 2°C). They were maintained on a standard diet and water was available *ad libitum.* The experimental diet (1 % ginger) was prepared as follows. Fresh ginger was purchased from the local market, peeled, washed, coarsely minced, air dried and pulverized with a blender to fine powder. This was added w/w to already pulverized feed and thoroughly mixed so as to get a diet containing 1% ginger. Propoxur was dissolved in groundnut oil of pharmaceutical grade and administered orally, once daily. Control rats received same volume of vehicle in identical manner. The animals were randomly divided into four groups of 8 animals each and treated for 28 days as follows: Group 1: Control: rats fed on normal diet; Group 2: Ginger; rats fed on 1 % w/w ginger diet; Group 3: Propoxur: rats received 1/10 LD_{50} (10 mg/kg, b.w., orally) along with normal diet. Group 4: Propoxur+ ginger: rats received propoxur (10 mg/kg, b.w. orally) along with 1% w/w ginger diet. All rats were given free access to respective diets and water. Food consumption, general condition and any other symptoms were observed daily and body weights were recorded weekly. After overnight fasting, animals were sacrificed by decapitation and heparinized blood samples were collected and processed for erythrocyte isolation. Whole blood samples were also collected and serum was separated for various biochemical investigations. Hemoglobin (Hb) concentration in hemolysate was estimated spectrophotometrically at 540 nm with Drabkin's reagent (Drabkin, 1949). Serum protein was estimated by the method of Lowry *et al* (1951). Tsuchihashi extract was prepared as described by Banerjee *et al* (2000). Malondialdehyde (MDA) level in serum was determined by Satoh (1978). The activity of superoxide dismutase (SOD) in Tsuchihashi extract was measured by the method of Nandi and Chatterjee (1988). Catalase (CAT) activity in Tsuchihashi extract was determined as described by Sinha (1972). Reduced glutathione (GSH) content in blood was measured by the method of Beutler *et al* (1963). For immunological tests rats were immunized with ovalbumin (*sc*, 3 mg dissolved in 0.2 ml normal saline) mixed with an equal volume of Freund's complete adjuvant. Sterile liquid paraffin (5 ml) was injected (*ip*) in rats immunized with ovalbumin 48 h before terminating the exposure. Humoral immune response was assayed by estimating the serum antibody titer to ovalbumin using hemagglutination technique (Seth *et al*, 2000). Leucocyte migration inhibition (LMI) and macrophage migration inhibition (MMI) assays were carried out as described by Seth *et al* (2002). The study was ethically approved from the institutional ethical committee of University College of Medical Science, Delhi. The results were analyzed statistically using Student's *t*-test.

Results and Discussion

Oxidative stress results

As shown in Table 1, propoxur treatment enhanced lipid peroxidation in rats fed normal diet. However, levels of MDA were significantly lower in rats fed the ginger supplemented diet as compared to controls or propoxur treated group. The SOD activity remained unchanged in rats fed the ginger diet whereas propoxur caused a nearly three-fold increase in activity. However, dietary ginger caused a significant decrease ($P< 0.01$) in SOD activity in propoxur treated rats. Similarly, propoxur treatment significantly enhanced the erythrocyte. The CAT activity in animals fed basal diet. The treatment with dietary ginger significantly prevented the increase in CAT activity.

Immunotoxicity results

Humoral and cell mediated immune response parameters shown in the Table 2. Rats exposed to propoxur showed a significant decrease in primary antibody titer as well as decreased LMI and MMI compared to control indicating that propoxur exposure suppresses both humoral- and cell-mediated immunity. The dietary ginger feeding with propoxur restored antibody response as well as LMI and MMI to control levels.

Free radicals play an important role in toxicity of pesticides and environmental chemicals, However, oxidative stress, generated by xenobiotic induces disturbances in antioxidant enzymes system (Banerjee *et al*, 2001; 2008). Therapy using free radical scavengers or antioxidants has potential to prevent, delay or ameliorate many of these disorders (Sharma and Singh, 2010; Muthaiyah *et al*, 2011). At present there is considerable interest in free radical mediated damage in biological system due to pesticide exposure and search for herbal drugs with antioxidant activity has gained importance as dietary intake of antioxidants obtained from natural

Table 1. Effect of propoxur alone or in combination with ginger on oxidative stress parameters in albino rats (n=8).

Parameters	Treatment Groups			
	Control	Ginger	Propoxure	Propoxure+Ginger
MDA (nmol/ml)	2.29 ± 0.05	1.82 ± 0.07	4.0.5 ± 0.28*	3.21 ± 0.18
SOD (U/gHb)	587.78 ± 23.39	598.78±87.81	1231.91± 45.92*	903.95±20.06
CAT (U/gHb)	2.32 ± 0.02	2.02 ± 0.05	3.97 ± 0.10*	2.83 ± 0.13
GSH (mg/gHb)	2.22 ± 0.03	2.46 ± 0.05	1.19 ± 0.10*	2.16 ± 0.09

Each value represents mean ± standard deviation. *Significant at P <0.05.

Table 2. Effect of propoxur alone or in combination with ginger on immunotoxicity parameters in albino rats (n=8)

Parameters	Treatment Groups			
	Control	Ginger	Propoxure	Propoxure+Ginger
Ab titre (-log_2)	8.04 ± 0.20	8.07 ± 0.11	5.13 ± 0.14*	7.50 ± 0.32
LMI (%)	72.00 ± 0.79	69.50 ± 0.93	24.04 ± 1.11*	61.22 ± 3.64
MMI (%)	64.50 ± 0.79	61.44 ± 2.06	22.30 ± 0.61*	63.04 ± 1.83

Each value represents mean ± standard deviation. *Significant at P <0.05

sources are considered to be relatively safe and without undesirable side effects (Xavier *et al*, 2004). The effect of propoxur and dietary ginger on lipid peroxidation, antioxidant enzymes and glutathione content are depicted in Table 1. These results are consistent with our previous reports of increased oxidative stress parameters following propoxur exposure (Suke *et al*, 2006). The treatment with dietary ginger, reduced the level of oxidative stress and normalized antibody titre level, LMI and MMI response to ovalbumin, indicating a reversal of propoxur-induced effect on humoral and cell mediated immune response. Over the past two decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole or their identified ingredients with antioxidant properties have substantial protective effects on a variety of human disease states (Halvorsen *et al*, 2002; Atawodi, 2005) . Spices and herbs are recognized as sources of natural antioxidants that can protect from oxidative stress and thus play an important role in chemo-prevention of diseases (Surh, 2002). Ginger has been used extensively in folklore medicine to treat common ailments and new scientific evidences in favour of some of these beneficial properties are emerging which support its consumption and use to ameliorate different human disorders (Karna *et al*, 2011; Shanmugam *et al*, 2010; Khanom *et al*, 2003). In rats fed with high fat diet, supplementation with ginger provided significant antioxidant effects, raising tissue concentrations of superoxide dismutase, catalase and reduced glutathione (Ahmed *et al*, 2008). However, studies on protective effect of plant products on attenuation of pesticide induced toxic effects is scarce and to our knowledge there is no information concerning the protective effect of dietary ginger on oxidative stress induced by propoxur. Study of protective effect of ginger on free radical mediated toxicity induced by carbamate pesticides like propoxur therefore appeared to be of interest.

The present results clearly showed that significant increase in MDA, SOD, CAT levels as well as concomitant decrease in GSH concentration elicited by propoxur were recovered by dietary ginger treatment. The hypothesis that the effect on humoral and cell mediated immune response may be a manifestation of increased oxidative stress is supported by the finding that propoxur intoxication is accompanied by increase in serum MDA level (indicative of increased lipid peroxidation) and a decrease in total GSH content in erythrocytes. Ginger, per se did not have any effect on antibody production or GSH content in the above study (Table 1 and 2), nevertheless, ginger attenuated both immunotoxic and oxidative stress effect of propoxur, which further suggested a possible nexus between propoxur, free radical generation and immunotoxicity. The immune system is regulated by an intricate role in immune regulation (Institóris *et al*, 2001). Increased oxygen free radical generation (OFR) due to pesticide exposure can exert deleterious effect on immune function as OFR has many molecular and cellular targets in the immune system (Seth *et al*, 2001). Natural antioxidants strengthen the endogenous antioxidant defense mechanism and restore the optimal balance by neutralizing the reactive species and thus search for crude drugs of plant origin with antioxidant activity has become a central focus of study in recent years (Moongkarndi *et al*, 2010). Such studies on oxidative/antioxidant status during a free radical challenge can be used as an index of protection against development of lipid peroxidation in experimental animals for assessing dietary and therapeutic measures. Curcumin and zingerone – compounds abundant in ginger rhizomes are known to have antioxidant activity (Wang *et al,* 2003). Hence, ginger may attenuate free radical mediated toxic effects by lowering lipid peroxidation and maintaining the activities of antioxidants and this may be attributed to curcumin and zingerone components present in ginger. Thus, attenuation of propoxur-induced oxidative stress and immunotoxicity by ginger, an antioxidant with potent hydroxyl and peroxyl radical scavenging activity, supported our hypothesis that oxidative stress contributed to propoxur-induced immune dysfunction.

The results of the present study indicated that ginger exerts significant protective effects against propoxur-induced oxidative stress and immune dysfunction by its potent antioxidant activities.

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