EFFECTS OF ZINGIBER OFFICINALE ROSCOE ON METHYL PARATHION INTOXICATION IN RATS

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Abstract

This research aimed to investigate the effect of Zingiber officinale Roscoe on Methyl parathion intoxication in rats. Methyl parathion (MP) is an organophosphate insecticide. Inhibition of acetylcholinesterase is regarded as a direct toxic, whereas, its indirect role also induces oxidative stress, generates free radicals and alters the lipid membrane, resulting in cell damage or cell death. Ginger (Zingiber officinale Roscoe) is a traditional herbal medicine in Thailand, and it potentially reduces oxidative stress with antioxidant activity. In this study, rats were divided into 4 groups: control (normal diet), ginger diet, MP and MP with ginger diet. The study demonstrated that feeding the rats with normal and ginger diet did not affect the AChE levels of red blood cells, which declined when receiving are MP and MP with ginger diet. This result correlated with the AChE activity in muscles, which was found to decrease in the number of motor endplates giving positive AChE activity, and those motor endplates showed features of distortion and edema. The status of oxidative stress, as indicated by a lipid peroxidation marker (TBARS), showed that the highest TBARS was observed in the MP group. The ABTS radical cation decolorization assay found that ginger possessed antioxidant activity. In conclusion, oxidative stress was caused by low dose MP exposure and can be prevented by a ginger diet supplement.

Keyword: methyl parathion, organophosphate, oxidative stress, antioxidant activity, free radical, Ginger, AChE, motor endplate, distortion, edema, peroxidation, TBARS, ABTS, cation decolorization assay

Chemical insecticides are used most widely in agriculture for increasing agricultural production in Thailand. However, many people do not understand the correct way of using them, as they contaminate the environment and affect health. The World Health Organization (WHO) reported that 1 million serious accidents occur worldwide.(1) Organophosphate compound (OP) toxicity is an important health problem. Between 1992 and 1994 the Division of Epidemiology, Ministry of Public Health, Thailand showed that the organophosphate insecticide, Methyl parathion (MP), was the most common cause of death in Thailand and it is an acetylcholinesterase (AChE) inhibitor. Metabolism of MP converts to methyl-paraoxon through an oxidative desulfuration reaction, which is immediated by cytochrome P450 in the liver. Methyl-paraoxon is a reactive metabolite, which binds tightly to the hydroxyl group of the serine residue present in the “esteratic” region of...
active cholinesterase site located on the post-synaptic membrane.

Inhibition of AChE results in the accumulation of acetylcholine, the neurotransmitter acting at the cholinergic synapses and neuroeffector junctions in the central and peripheral nervous systems. Furthermore, some reported studies have revealed that oxidative stress could be an important component in the mechanism of OP poisoning. As a result, changes occur in molecules and damage to cells such as blood cells, etc. Dietary ginger is of interest in studying oxidative stress induced by insecticide exposure. The rhizome of ginger has also been used in traditional herbal medicine for treatment of symptoms such as common cold, digestive disorder, rheumatism, neuralgia, colic and motion sickness. The antioxidative properties of gingerol and other constituents of ginger have been confirmed by in vitro and in vivo test systems. Mansour and Khalil reported that gingerol has been found to possess substantial antioxidant activity, as determined by inhibition of lipid peroxidation induced by the FeCl3 ascorbate. Stoliova et al found that ginger extract showed a higher antioxidant capacity, especially at high temperature. Ginger contains pungent ingredients such as 6-gingerol and 6-paradol, which also have anti-tumor promotional and anti-proliferative effects. Dietary ginger significantly attenuated malathion-induced lipid peroxidation and oxidative stress in rats, with the possible involvement of free radicals in organophosphate-induced toxicity and protective action on the morphology of skeletal muscle. This study was conducted to evaluate the effect of Zingiber officinale Roscoe on cholinesterase activity, and its antioxidant activity on MP intoxication.

Materials and methods
Preparation of experimental diets
Fresh ginger was purchased from a market, peeled, washed, coarsely minced, air dried and pulverized with a blender to fine powder. This was added to feed that had been already, pulverized and mixed so as to obtain a diet containing 0.5%, 1.0% and 1.5% w/w ginger. The ingredients were mixed and baked in an oven at 60 °C until the final weight was one kilogram.

Administration of MP
Commercially available 50% MP was used in this study. The animals were treated with the vehicle and MP (0.5, 1.0, 1.5 mg/kg) by intraperitoneal injection (i.p.).

Animals
Male and female Sprague-Dawley rats (180-200 g) were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. They were divided into 12 groups, (3 sets of 4) with six animals in each one, and housed individually. The temperature (20-22 °C), humidity (50+10%) and lighting (12 h day/night cycle) were constantly controlled. They were acclimatized one week before beginning the experiment. Water ad lib, food consumption and general conditions were given to all of the animals. Symptoms were observed daily and body weight was recorded weekly by using the standard worksheet of the Hippocratic screening test.

Selection of the optimal MP dose
Male and female rats were divided, six/group, into 4 groups. They were treated with MP 0.5 and 1.0 at 1.5 mg/kg/day for 1, 2, 3 and 4 weeks, and fed a normal diet with water ad lib.

Selection of the optimal ginger diet dose
Male and female rats were divided, six/group, into 4 groups. They were treated with a 0.5%, 1.0% and 1.5% w/w ginger diet for 1, 2, 3 and 4 weeks and fed water ad lib.

Treatment of animals with the optimal MP dose and ginger diet
Male and female rats were divided, six/group, into 4 groups. They were treated for 1 month and fed water ad lib.

Sample collection
After overnight fasting, the rats were sacrificed and whole blood with ethylenediamine tetra acetate acid (EDTA) was collected. Sera were separated for biochemical investigations. The liver was immediately removed and kept at -70 °C. The muscle was removed and fixed in 0.4% formalin at 4 °C.

ABTS radical cation decolorization assay.
The original ABTS assay was based on the reaction between ABTS and potassium persulfate. The generation of ABTS•+ involved the direct
production of blue/green ABTS+ radical cation. The addition of antioxidants to the pre-formed radical cation reduced ABTS to an extent, and on time-scale, depending on the antioxidant activity. Thus, the extent of decolorization as percentage inhibition of the ABTS+ radical cation was determined as a function of concentration and time and calculation related to the relativity of Trolox as a standard, under the same condition. This method was modified the protocol from Re et al. Briefly, ABTS was dissolved in water to a 7 mM concentration, and radical cation was produced by reacting ABTS stock solution with 7 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 hrs before use. The ABTS+ solution was diluted with distilled water and measured at an absorbency of 0.7-0.8 at 734 nm at room temperature. Stock powder of ginger was dissolved in distilled water (20 mg/mL) and centrifuged at 12,000 rpm for 10 minutes. The supernatant was separated. After the addition of 1,300 μl of diluted ABTS+ solution (A 734 nm=0.7-0.8) to 300 μL of distilled water and 100 μL of sample, an absorbency reading took place until the absorbency was stable. The data representing the percentage of reduction (A412 nm), was collected from the Trolox (standard reference) and samples in triplicate out at least three times.

**Thiobarbituric acid reactive substances (TBARS) assay**

**Serum preparation**

Blood samples were centrifuged for 15 minutes at 3,000 rpm at room temperature and the serum was the separated and stored at -20 °C until analyzed. The lipid peroxidation levels in serum were measured following a modification of the methodology described by Santos et al. Sample (100 μL) was mixed with 450 μL of normal saline and 200 μL of thiobarbituric acid (TBA) and added to 1,000 μL of trichloroacetic acid (TCA). After heating for 30 minutes at 95-100 °C and cooling, TBARS solution was added to 2,000 μL of distilled water and centrifuged at 3,000 rpm for 10 minutes. The absorbance of the solution was measured at 532 nm. The amount of TBARS was calculated as malondialdehyde (MDA) equivalents using 1, 1, 3, 3-tetramethoxy-propane as standard.

**Determination of AChE (true cholinesterase) in red blood cells**

AChE in red blood cells was measured with the method described by Ellman. Packed red blood cells, 20 μL, were diluted in 200 μL of distilled water, then, 20 μL of diluted sample was mixed in 3,000 μL of 5, 5-dithiobisnitrobenzoic acid solution for setting to zero and mixed with 50 μL of 5% acetylthiocholine iodide (AI). The absorbance of the solution was measured at 405 nm. The AChE level was calculated and reported in U/L.

**Histochemical methods for demonstrating AChE activity in skeletal muscles of rats**

The method described by El-Badawi et al. was modified as follows. The techniques were applied on rat tissue with equally good results. Gastrocnemius muscle was fixed in ice-cold (-4-4 °C) 4% neutral formalin. Sections (60 μm) were cut in a sliding microtome at -30°C, mounted on slides and dried rapidly at room temperature (18-25 °C) by a current of air. Sections were incubated in medium at 4 °C for 2 hours. The medium was prepared no earlier than 30 minutes before use by adding to the substrate (AI). Sections were dehydrated and cleared in 95% alcohol (30 sec), absolute alcohol (30 sec) and three times xylene (30 sec), and then mounted in Permount. The sections were examined by ordinary light microscopy. Sites of AChE activity were marked by a sharply defined reddish brown granular precipitate, which developed as a result of reduced ferricyanide to ferricyanide ion with subsequent precipitation of Cu++ as cupric ferrocyanide (Hatchett’s brown). AChE activity depended upon the number of motor endplates and intensity of the reddish brown precipitate. Strong and weak positive AChE activity appeared as a bright sharply defined and dull reddish brown granular precipitate, respectively.

**Statistical analysis**

The level of AChE, TBARS and number of motor endplates for positive AChE activity were evaluated for statistical significance by ANOVA. The Independent-Samples T-test was used to compare the difference of rat body weight and the mean was expressed as ± SD (Post Hoc: Tukey,
Descriptive statistics was used for the morphology of motor endplates and the ABTS radical cation decolorization assay.

**Results**

The body weight of the animals increased, in each group but with no statistical significance between the groups at the end of the experiment. Several effects such as tremors, fasciculation, lacrimation, salivation, abdominal cramp, motor weakness or paralysis, and very aggressive behavior were observed in the rats exposed to MP. The effects were recorded on a standard working sheet of the Hippocratic screening test. No deaths occurred among the rats tested.

**Selection of the optimal MP dose**

The animal were treated with 1.0 mg/kg of MP as a selected optimal dose for 4 weeks, and they showed signs of acute toxicity, significantly decreasing AChE activity and enhanced lipid peroxidation.

**Selection of the optimal ginger diet dose**

The animals were treated with a 1.5%W/W ginger diet as a selected optimal dose for 4 weeks. The AChE activity in male rats give this treatment showed no significant increase from that of the controls, but a small increase was seen in female rats.

**Treatment of animals given the optimal MP and ginger diet dose**

The animals treated with 1.0 mg/kg of MP showed little sign of acute toxicity or enhanced lipid peroxidation with increase MDA levels when compared to those treated with both 1.0 mg/kg of MP and a 1.5%W/W ginger diet (Fig. 1). The AChE activity in red blood cells declined in animals treated with 1.0 mg/kg of MP (Fig. 2).

**ABTS radical cation decolorization assay**

The percentage of reduced ABTS of ginger powder (20 mg/mL) and 1.5%W/W ginger diet, was found to be 80% and equal to 0.24 mM of Trolox, 4.41 mg/mL of 1.5%W/W ginger diet and 7.7 x 10-4 mg/mL of ginger powder.

**AChE activity in muscle**

The number of motor endplates for positive AChE activity was unchanged in animals treated with a 1.5%W/W ginger diet and control diet, but it decreased significantly in both groups of animals treated with 1.0 mg/kg of MP and a 1.5%W/W ginger diet plus 1.0 mg/kg of MP when compared to the control (Table 1).

The control and ginger diet group showed unchanged staining intensity of motor endplates and sharply defined reddish brown granular precipitate. The morphology of motor endplates treated with 1.0 mg/kg of MP and a 1.5%W/W ginger diet showed little sign of acute toxicity or enhanced lipid peroxidation with increase MDA levels when compared to those treated with both 1.0 mg/kg of MP and a 1.5%W/W ginger diet (Fig. 1).

**Figure 1.** Serum TBARS concentration (MDA) in male and female rats treated with 1.0 mg/kg of MP and a 1.5%W/W ginger diet for 4 weeks (values are expressed as mean ± SD, n=6).

**Figure 2.** AChE level of red blood cells in male and female rats treated with 1.0 mg/kg MP of and a 1.5%W/W ginger diet for 4 weeks (values are expressed as mean ± SD, n=6).
with MP and ginger diet plus MP showed distortion and edema (Fig. 3). Ginger diet supplement could not improve AChE activity of the motor endplates.

Discussion

Free radicals do not only generate from oxidation reactions in the human body, but also after organophosphate (OP) insecticide or MP exposure. They might alter antioxidants or oxygen-free radical scavenging enzymes and change molecules in cells such as blood or tissue cells, resulting in cell damage or cell death. However, AChE inhibition does not explain all the symptoms of OP intoxication, such as renal dysfunction secondary to OP exposure in humans, and was found not to correlate with the degree of cholinesterase suppression. More recently, it has been postulated that

Table 1. The number of motor endplates for positive AChE activity in the muscle of male and female rats treated with 1.0 mg/kg of MP and a 1.5% W/W ginger diet for 4 weeks (mean ± SD, n=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>No. of motor endplates for positive AChE activity</th>
<th>Sex</th>
<th>No. of motor endplates for positive AChE activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Male</td>
<td>140.50±7.39</td>
<td>Female</td>
<td>143.66±4.32</td>
</tr>
<tr>
<td>1.5%W/W Ginger diet</td>
<td>Male</td>
<td>141.33±4.41</td>
<td>Female</td>
<td>147.50±4.80</td>
</tr>
<tr>
<td>1.0 mg/kg of MP</td>
<td>Male</td>
<td>71.83±7.46*</td>
<td>Female</td>
<td>73.33±7.63*</td>
</tr>
<tr>
<td>1.5%W/W Ginger diet + 1.0 mg/kg MP</td>
<td>Male</td>
<td>77.66±6.47*</td>
<td>Female</td>
<td>81.50±4.50*</td>
</tr>
</tbody>
</table>

* Significantly different from the control (p<0.05)

Figure 3. Normal motor endplate showing positive AChE activity in the muscle of a rat treated with a normal and 1.5%W/W ginger diet. The positive AChE activity is a bright sharply defined reddish brown granular precipitate (A): (x40). The endplate is intact with sharply defined granular precipitate with an irregular and a blurred endplate border (B): (x40). Low AChE activity of the motor endplate in the muscle of a rat treated with 1.0 mg/kg of MP and a 1.5%W/W ginger diet with 1.0 mg/kg of MP. The staining pattern is distorted with a blurred endplate border (C): (x40). The motor endplate is distorted with a thick linear staining pattern, with no granular precipitate (D): (x40).
OPs produce oxidative stress in different tissues through the formation of reactive oxygen species (ROS)(6,20) and damage to the lipid bilayer of the cell membrane, resulting in cell destruction and promotion of MDA content.(21) In subchronic exposure to OPs, in addition to cholinesterase inhibition, induction of oxidative stress has been reported as the mechanism of toxicity.(20)

Many natural plant products are an antioxidant against xenobiotic-induced oxidative stress and are therefore interesting. Gingerol and zingerone are pungent principles of fresh ginger that have been reported as antioxidants and can inhibit lipid peroxidation.(22-24) Active components of ginger rhizomes may affect lipid peroxidation by scavenging reactive oxygen species or chelating metal ions needed for initiation of lipid peroxidation.(12) Further study showed sub-chronic exposure of malathion induced oxidative stress and liver damage in rats.(25) This study investigated the effects of a dietary supplement of ginger on lipid peroxidation induced by MP, which has never been reported before. The results obtained from this study suggested that crude ginger and a boiled ginger diet had a powerful antioxidative effect by decreasing lipid peroxidation.(12,26) However, crude ginger might contain more than one antioxidant ingredient and could be responsible for significant inhibition of lipid peroxidation.

The clinical manifestations and pathophysiology of MP intoxication in relation to AChE activity has been well established. A progressive decrease in AChE activity correlated with increasing toxic symptoms. MP is suggested as a stronger AChE inhibitor. Ginger supplement in the diet did not prevent the inhibition of AChE by MP that was shown by AChE activity in muscle, which illustrated low AChE activity in animals exposed to MP. The morphology of motor endplates was observed with some degree of changes after MP exposure. Ginger supplement could not be preserved from/by the morphology of motor endplates after MP exposure. Distortion and edema of motor endplates may be due to the altered permeability of cell membrane, as reflected by oxidative damage. Furthermore, the number of motor endplates in animals treated with MP significantly decreased. The result indicated that prolonged exposure to methyl parathion could cause motor endplate damaged and loss of AChE activity. This finding has never been reported before. However, the number of motor endplates should be confirmed by electron microscopy.

This study demonstrated that feeding ginger to rats can prevent the formation of unwanted free radicals when exposed to OP insecticide. For the health of workers and the general population, handling and application of MP should be trusted and carefully managed concurrently with ginger diet supplementation. In human consumption, fresh ginger or ginger commercial products such as ginger jam, ginger beer, ginger refresher mixer, sucrose free ginger and ginger tablets are beneficial for health as they are excellent antioxidants, thus, preventing oxidation of lipids and adverse effects of lipid peroxidation. Ginger consumption might be one of many ways for providing safer human health when compared to synthetic antioxidants. However, the clear mechanism of ginger in preventing oxidative stress from pesticides should be investigated further in order to validate this herb for human health and the treatment of diseases.

In conclusion, this study considered the effects of ginger on MP intoxication. There was a considerable increase in lipid peroxidation. Levels indicated enormous oxidative stress in MP poisoned animals. Prolonged exposure to MP inhibited AChE in red blood cells and muscles, and abnormal morphology of motor endplates was found. Ginger supplementation prevented lipid peroxidation in the case of prolonged MP exposure.

References
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Effect of *Zingiber officinale*

ผลของจิตต์การแก้พิษที่เกิดจากสารเคมีกำจัดแมลงชนิดเมทธิลพาราไธออนในหนู

เบญจพล มหัศจรรย์, วท.ม., 1 ไพฑูรย์ สมบัติ, วท.ม., 2 สมิทัศน์ ภู่ชัย, วท.ม., 2 อัทธิ์ ปัทมา, วท.ม., 3 รัมย์ เลิศประเสริฐสุข, วท.ม., 4 ชุชิต ประยุทธ์ภิรมย์, วท.ม., 5

1 ภาควิชาวิทยาศาสตร์สุขภาพ มหาวิทยาลัยนเรศวร จังหวัดพะเยา, 2 ภาควิชานิติเวชศาสตร์, 3 ภาควิชาเภสัชศาสตร์, 4 ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

บทคัดย่อ เมทธิลพาราไธออน เป็นสารเคมีกำจัดแมลงในกลุ่มออร์แกนฟอสเฟท ซึ่งทำให้เกิดสารค้างคาวในเซลล์ของร่างกาย โดยกลไกปฏิกิริยาสร้างอนุมูลอิสระออกซิเจนและทำให้เกิดการเปลี่ยนแปลงในเซลล์ ทำให้ร่างกายมีผลทำให้เซลล์ลูกทราย ในคนที่ได้รับปริมาณต่ำเช่นนี้จะเกิดปฏิกิริยาล้างเชื้อerging ไร้คัดลอกต่ำขึ้นได้ขึ้นมาก จึงต้องเป็นยาสมุนไพรของไทยชนิดหนึ่งซึ่งมีคุณสมบัติเกี่ยวกับปฏิกิริยาค่าลดอนุมูลอิสระออกซิเจนที่ทำให้ลดการต่อต้านสารเคมีของร่างกาย ดังนั้นได้มี

จินต์การศึกษาทดลองในหนูเพื่อศึกษาปัญหาเกี่ยวกับปฏิกิริยาต่อต้านพิษของเมทธิลพาราไธออนโดยทำการวิเคราะห์สารเคมีในกลุ่มเนื้อเยื่อต่างๆ กลูตาโธอน และ สาระภูมิคุ้มกันที่เกิดขึ้นในเซลล์ ปรากฏว่าเมทธิลพาราไธออนทำให้สารเคมีในกลุ่มเนื้อเยื่อต่างๆ กลูตาโธอน และ สาระภูมิคุ้มกันที่เกิดขึ้นในเซลล์ ทำให้ลดลง แต่ขิงสามารถป้องกันการเกิดปฏิกิริยากันที่เกิดจากการได้รับเมทธิลพาราไธออนในขนาดต่ำได้ เชียงใหม่เวชสาร 2553;49(3):81-88.

คำสำคัญ เมทธิลพาราไธออน สารเคมีกำจัดแมลง สารเคมีค้างคาว ยาสมุนไพร ขิง ต่อต้านพิษ ผลการทดลอง เอกสาร