CHAPTER I

INTRODUCTION

1.1 Statement and significance of the problem

Nowaday many insecticide chemicals are the most widely used for agriculture to increase agricultural products in Thailand. But many people do not understand correctly the way of using the insecticides, these pesticide chemicals may deposit in environment and resulting in health and environmental problems.

In 1992-1994 Division of Epidemiology, Ministry of Public Health, Thailand showed that organophosphate pesticide caused death of people mostly in Thailand.

Methyl parathion (MP), an organophosphate insecticide is a acetylcholinesterase (AChE), inhibitor. Its toxicity acts through inhibition of cholinesterase and cause accumulation of acetylcholine (ACh) at peripheral and central cholinergic synapses and resulting in over stimulation of the cholinergic system (Howard and Pope, 2002).

Organophosphate pesticides induce oxidative stress, generation of free radical and alterations in antioxidants or oxygen free radical (OFR) scavenging enzymes. As a result, free radicals change molecules in cells such as blood cells or tissue cell, resulting in cell damage or cells death (Ortiz *et al.*, 1995; Lopes *et al.*, 1997; Videira *et al.*, 2001).

Dietary ginger is the one that much interest to study on oxidative stress induced by pesticide exposure.Dietary feeding of ginger significantly attenuated malathion-induced lipid peroxidation and oxidative stress in rats and the result indicated the possible involement of free radicals in organophosphate-induced toxicity and highlight the protective action of ginger (Ahmed *et al.*, 2000).

The aim of the present study was to investigate the effects of ginger (*Zingiber officinale* Roscoe) on methyl parathion intoxication in rats and histological technique for examination of morphology of skeletal muscle was also included.

1.2 Methyl parathion

Methyl parathion (0,0-Dimethyl 0-4-nitrophenylphosphorothioate) is one of the most widely used pesticides for agricultural crops. Cotton, vegetables and soybean agricultures have been indicated of spraying of methyl parathion. Human can be exposed to methyl parathion by breathing, eating, drinking or touching objects contaminated with this the chemicals (Abu-Qare and Abou-Donia, 2000; Castillo *et al.*, 2002).

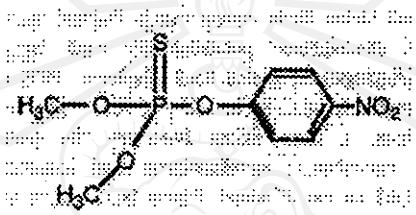

Figure 1 Structure of Methyl parathion (Lopes *et al.*, 1997)

Oral LD50 value of methyl parathion in rodents ranges from 3-35 mg/kg body weight, and dermal LD50 value, from 44 to 67 mg/kg body weight. Methyl Parathion is a very dangerous poison. It rapidly enters the body on contact with all skin surface and eyes. Clothing contaminated with material must be removed immediately and all skin washed thoroughly. Methyl parathion is rapidly absorbed via all routes of exposure and rapidly distributed to the tissue of the body. The liver is the primary organ of metabolism and detoxification.

Metabolism of methyl parathion was converted to methyl-paraoxon through an oxidative desulfuration reaction which immediated by cytochrome P450 in liver (Albores *et al.*,2001). Methyl-paraoxon is a reactive metabolite, which binds tightly to the hydroxyl group of the serine residue present in the "esteratic" region of cholinesterase's active site located on the post-synaptic membrane (Liu *et al*, 1999: Zhu *et al.*, 2001). Inhibition of AChE results in the accumulation of acetylcholine, the neurotransmitter acting at cholinergic synapses and neuroeffector junctions in the central and peripheral nervous systems.

Toxicity of methyl parathion depends on route of exposure, toxication of the chemical and the ACh level of accumulation. Acute poisoning symptoms when touching MP directly include eye and skin irritation, vomiting, gastrointestinal tract damage (Zhu *et al.*, 2001).

If methyl parathion is absorbed into the body, it can cause poisoning in many systems in the body such as respiratory system, thereby stimulating muscarinic receptor at bronchial and parasympathetic nervous system that can cause bronchoconstriction, increased bronchial secretion, excessive sweating, salivation, lacrimation and pinpoint pupils.

The effects on gastrointestinal system include vomiting, abdominal cramps and diarrhea. Furthermore , methyl parathion stimulates nicotinic receptor at the ganglion of sympathetic and parasympathetic autonomic nervous system including neuromuscular junction of skeletal muscle. Effects on central nervous system include anxiety, dizziness, mental confusion, convulsion, restlessness, coma, depression of the respiratory center and death. Methyl parathion and methyl-paraoxon are mainly detoxified in liver by oxidation, hydrolysis, and demethylation (Huang and Sultatos, 1993). The metabolic products are 0-methyl-0-p-nitrophenyl phosphorothioate, dimethyl phosphorothioic, dimethylphosphoric acid and p-nitrophenol (Barr *et al.*, 2002).The elimination of methyl parathion and metabolic product occurs primarily 75% via the urine and 10% in the faces (IPCS, 1996).

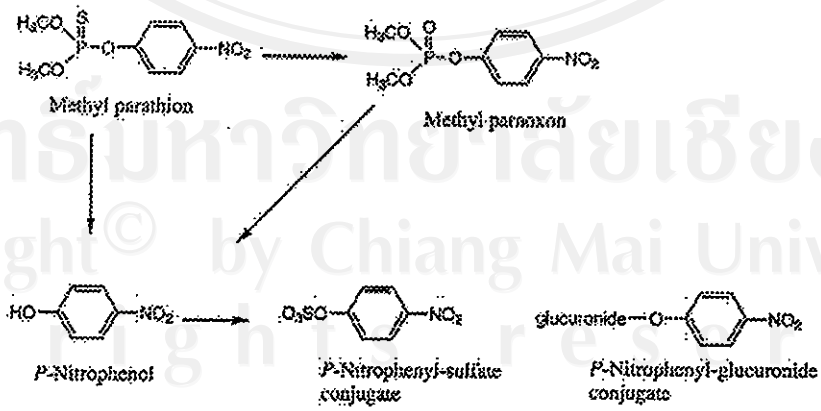

Figure 2 Metabolic pathways of methyl parathion (Abu-Qare *et al.*, 2001)

1.3 Free radicals

Reactive oxygen metabolites are generated by the excitation of the electrons secondary to addition of the energy or interaction with the transition elements. The reactive oxygen metabolites produced are thus more highly reactive than original oxygen molecule and are called active oxygen species. Active oxygen species were listed in Figure 3.

$O_2^{\bullet -}$	Superoxide radical
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
$^{\bullet}O_2$	Singlet oxygen
$HOO^{\bullet}$	Hydroperoxyl radical
LOOH	Alkylhydroperoxide
$LOO^{\bullet}$	Alkylperoxyl radical
$LO^{\bullet}$	Alkoxyl radical
ClO^{-}	Hypochlorite ion
$Fe^{2+}O$	Ferryl ion
$Fe^{3+}O$	Periferry ion
$NO^{\bullet}$	Nitric oxide

Figure 3 Active oxygen species (Yoshikawa and Naito, 2002)

However, if active oxygen species or free radicals are generated excessively or at abnormal sites, the balance between formation and removal is lost, resulting in oxidative stress (Pollack and Leeuwenburgh, 2000).

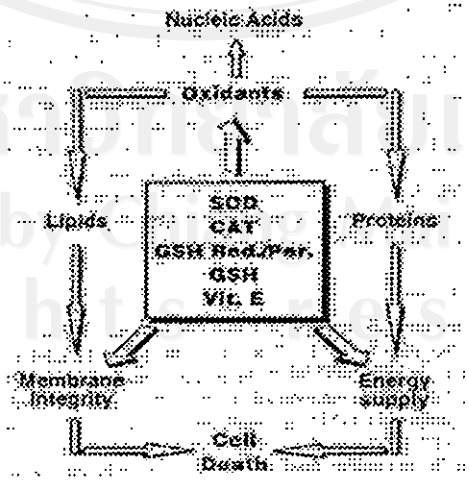

Figure 4 Oxidative injury can cause depletion of free radical scavenging antioxidants

Consequently, active oxygen species and free radicals can attack molecules in biological membranes and tissues, thus can cause many diseases (Asmus and Bonifacic, 2000).

Reactive oxygen species trigger peroxidation of the lipids membrane leading to some changes in structure and function. These changes may be modulated the cells ability to respond to the microenvironment (Gerber, 2000; Ternay *et al.*, 1997).

1.4 Lipid peroxidation

Lipids are generally hydrophobic, and act as fuel for metabolism, support for membrane structure, and selective cell membrane transport. Unsaturated fatty acids are vulnerable to free radical attack(Marks *et al.*, 1996). Many markers have been proposed for oxidative damage to lipids, including lipid peroxides malondialdehyde (MDA) and 4-hydroxynonenal. Lipid peroxidation is a very important mechanism of cell membrane destruction (Yoshikawa and Naito, 2002). Lipid peroxidation is a chain reaction by which unsaturated fatty acids are oxidized in various pathological conditions. When hydrogen atom is removed from a fatty acid molecule for some reason , the free radical chain reaction proceeds as shown in Figure1-5. Thus, radicals that can be involved in the extraction of hydrogen atoms from lipids include the hydroxyl radical ($HO^{\bullet}$), the hydroperoxyl radical ($HOO^{\bullet}$), the lipid peroxyl radical ($LOO^{\bullet}$) and the alkoxyl radical ($LO^{\bullet}$). The peroxidation chain reaction propagates itself once it has started. The process by which lipid radicals ($L^{\bullet}$) are generated from lipids (LH) is called the chain initiation reaction. Lipid radicals ($L^{\bullet}$) thus generated react immediately with oxygen, resulting in the formation of $LOO^{\bullet}$, which attacks another lipid and remove a hydrogen atom from it, resulting in the formation of lipid hydroperoxide (Lipid peroxide; LOOH) and another $L^{\bullet}$. This new $L^{\bullet}$ also reacts with oxygen and forms $LOO^{\bullet}$, which attacks another lipid to generate lipid peroxide, so lipid peroxide accumulates as the chain reaction proceeds (Alessio, 2000).

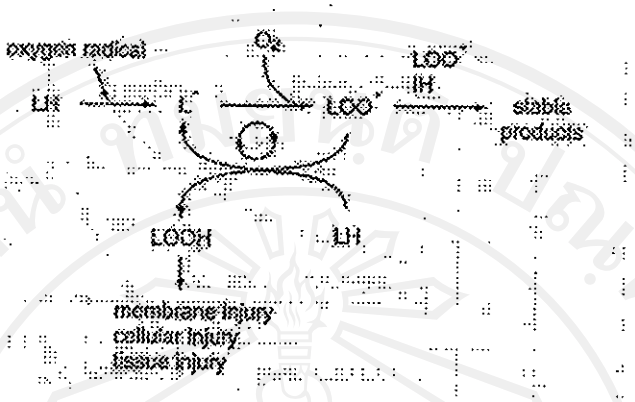

Figure 5 The chain reaction causing lipid peroxidation (Yoshikawa and Naito, 2002)

The free radical peroxidation of lipid is an important factor in local injury to cell membranes an impairment of the activity of enzymes and receptors bound to the membrane, and the lipid peroxide thus produced can affect even remote organs.

1.5 Glutathione

Glutathione (r-glutamylcysteinylglycine, GSH) is a tripeptide (Castillo *et al.*, 1999; Oqus *et al.*, 1998). It is composed of cysteine, glutamic acid and glycine and its active group is represented by the thiol of cysteine residue (Cotgreare and Gerdes, 1998). It is one of most important molecules in the cellular defense against toxic compound (Sen, 1997: Pastore *et al.*, 2003).

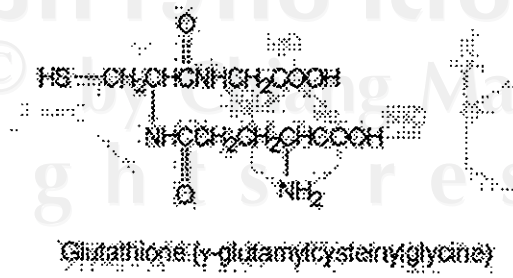

Figure 6 Structure of glutathione (Timbrell, 2000)

GSH is found in most cells of many organ such as liver, spleen, kidney, erythrocytes and leucoeytes, but most concentration in liver. It is present in virtually all mammalian cells between 1 and 10 mM. The synthesis and degradation of GSH take place by reactions of the r-glutamyl cycle (Luperchio *et al.*, 1996; Vella, 2003).

In cells, total GSH can be found in free form or bound to proteins. The redox status depends on the relative of the reduced GSH (GSH) and oxidized forms of GSH (GSSG).

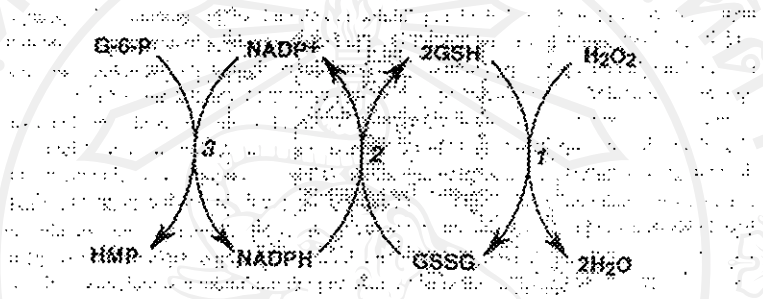

Figure 7 Glutathione redox cycle ; 1: Glutathione peroxidase, 2: Glutathione reductase, 3: G-6-PD (Sciuto, 1997)

The major functions of GSH is to preserve the thiol group of proteins in the reduced state and detoxification of xenobiotics by conjugation is catalyzed by glutathione-S-transferases (GSTs) (Oqus *et al.*, 2003).

1.6 Glutathione-S-tranferase

Glutathion-S-transferase (GST; EC 2.5.1.18) plays a central role in detoxification of potential alkylating agent (Habig *et al.*, 1974; Ilio *et al.*, 1995),as pharmacologically active compound in protecting cells against toxic effect of xenobiotics and endogenous compounds. GST is cytosolic enzyme that can detectable in the microsomal fraction and found in many tissues such as liver, kidney, gut, testis and adrenal gland (Timbrell, 2000).

1.7 Glutathione reductase

Glutathione reductase (GR; EC 1.6.4.2) is the enzyme that maintains GSH in its reduced form. It plays a role in converting the disulfide form of GSH to the sulfhydryl form by using NADPH produced in the pentose phosphate pathway as and electron donor.

$$GSSG + NADPH + H^{+} \rightleftharpoons 2\,GSH + NADP^{+}$$

GR persents in the cell and localizes in the cytosol as well as in the mitochondrial matrix. It requires adequate supply of NADPH as a cofactor (Sen, 1997).

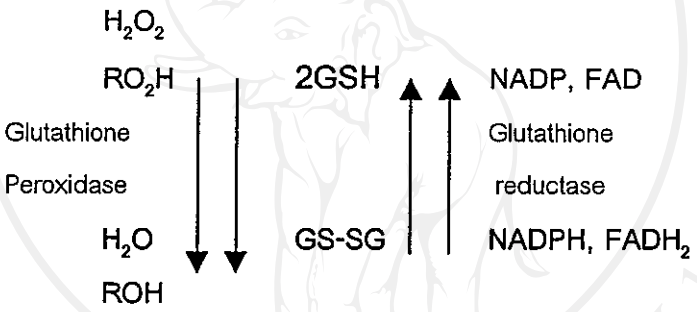

Figure 8 Role of glutathione reductase (Somani, *et al.*, 1997; Parke, 1999)

1.8 Acetylcholinesterase

Acetylcholinesterase is the enzyme that hydrolyze acethylcholine (ACh) to choline and acetate after its release (Richard, 2003).

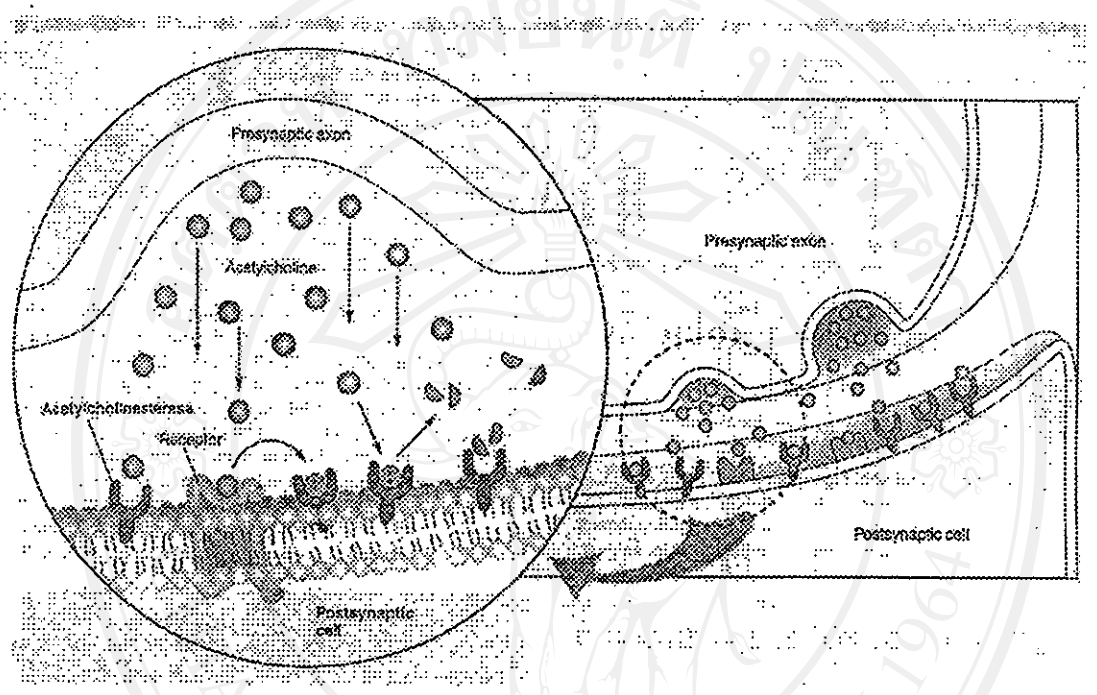

Figure 9 The action of acetylcholinesterase (AChE) (Wheatley, 2002)

Methyl paraoxon is a main toxic metabolite of methyl parathion that can irreversible inhibit acetylcholinesterase (AChE) function, there by causing accumulation of ACh at peripheral and central cholinergic synapses and resulting in over stimulation of the cholinergic system. The contraction of skeletal muscle occurs in response to action potentials that travel down somatic motor axons originating in the central nervous system. The transfer of the signal from nerve to muscle takes place at the neuromuscular junction, called the myoneural junction or motor endplate. Methyl parathion toxicity can occure at neuromuscular junction of skeletal muscle and fasciculation of muscles fibrils including muscle twitching and weakness may be result.

1.8.1 The Structure of the Neuromuscular junction.

On a muscular cell, the axon of a motor neuron typically branches into several terminals, which constitute the presynaptic portion of the neuromuscular junction. The terminals lie in grooves in the surface of the muscular cell, outside the muscle cell membrane, and a Schwann cell covers them all. The nerve terminal is located numerous membrane-enclosed vesicles containing ACh.

The postsynaptic portion of the junction or endplate membrane is the part of the muscle cell membrane lying immediately beneath the axon terminals. Here the membrane is formed into postjunctional folds, at the mouths of which are located many nicotinic ACh receptor molecules. Between the nerve and muscle is a narrow space called the synaptic cleft. ACh must diffuse across this gap to reach the receptors in the postsynaptic membrane. Also located in the synaptic cleft is the enzyme cholinesterase.

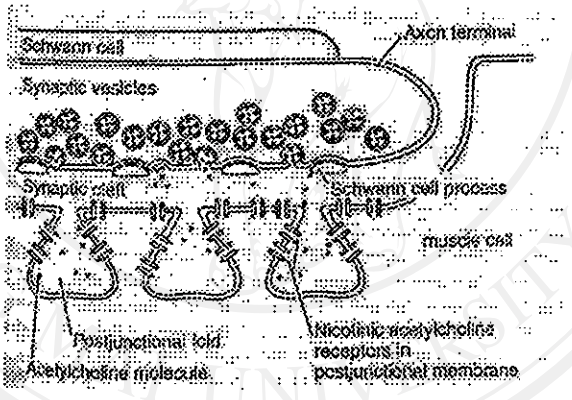

Figure 10 Structure features of the neuromuscular junction (Richard, 2003)

1.9 Ginger

Ginger is technically a rhizome or underground stem of the plant *Zingiber officinale* Roscoe. It is grown commercially in most tropical regions for its rhizomes. Ginger is among the most frequently and heavily consumed dietary condiments throughout the world (Newall and Anderson, 1996). 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 (Sharma *et al.*, 1997; Bhandari *et al.*, 1998)

Ginger has many constituents such as carbohydrate, lipid 6-8%, oleo-resin about 33% including derivatives with a methyl side chain, shogaol homologues, zingerone, dehydrogingerdione, 6-gingesulfonic acid and volatile oils (Chen *et al.*, 1986; Aeschbach *et al.*, 1994; Barnes and Anderson, 2002; Zancan *et al.*, 2002).

The antioxidative properties of gingerol and other constituents of ginger have been confirmed in various 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 phospholipid peroxidation induced by the $FeCl_3$ ascorbate system (Mansour and Khalil, 2000). It was demonstrated that ginger contains such pungent ingredients as [6]-gingerol and [6]-paradol, which also have anti-tumor promotional and antiproliferative effects (Surh, 1999).

The total oleoresin extracted from ginger rhizomes was in the ratio of 20:1:2 (fresh ginger: solar dried: solar dried/steam distilled ginger rhizomes) with respect to the [6]-gingerol content (Balladin *et al.*, 1998).

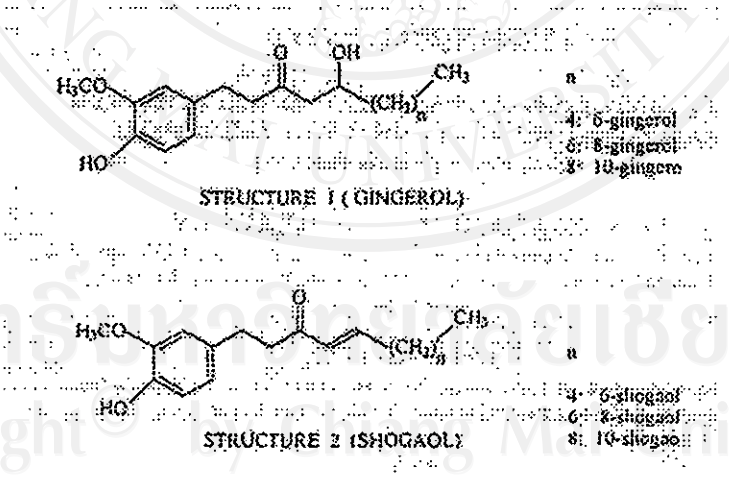

Figure 11 Structure of Gingerol and Shogaol (Balladin, 1998)

Shobana and Naidu, 1995 investigated spice mix namely ginger, onion and garlic and found that cumulative inhibition of lipid peroxidation and the antioxidant activity of spice extracts were retained even after boiling, indicating that the spice constituents were resistant to thermal denaturation. The aqueous extracts of ginger inhibited lipid peroxidation by 72% (Sujatha and Srinivas, 1995) and the ginger rhizome extract exhibited the highest antioxidant activity and had activity comparable to commercial antioxidants, sustane 20 and sustane HW-4 (Mansour and Khalil, 2000). The ginger preparations were powerful scavengers of hydroxyl radicals ($OH^{\bullet}$) and also exerted pro-oxidant action in the bleomycin assay, accelerated damage to DNA in the presence of a bleomycin-ferric iron complex (Aruoma *et al.*, 1997).

1.10 Objective of the study

1. To investigate the effects of ginger (*Zingiber officinale* Roscoe) on methyl parathion intoxication in rats.

2. To investigate the antioxidative mechanism (GSH,GST,GR) of ginger (*Zingiber officinale* Roscoe) on methyl parathion intoxication in rats.