



## ANTIOXIDANT EFFECT OF THYME ON PIRIMIPHOS METHYL INDUCED TOXICITY IN RATS

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### ABSTRACT

In the present study, the effects of thyme supplementation on lipid peroxidation and antioxidant enzymes in chronic intoxicated rats with organophosphorous pesticides have been evaluated. This study was carried out on 60 male rats. The rats were divided into four equal groups. 1) Normal group (N): received no drugs. 2) POM group (P): received single oral dose of POM (50 mg/ Kg b.w) daily for 3 months. 3) Thyme group (Thy): received single dose of Thyme per os (45 mg/ Kg b.w) daily for 3 months. 4) Thyme + POM group (Thy + P): received single dose of POM (50 mg/ Kg b.w) + Thyme (45 mg/ Kg b.w) per os daily for 3 months. Blood and brain samples were collected from all animal groups three times at one, two and three months from the onset of experiment and used for determination of antioxidant enzyme activities, non-enzymatic antioxidant (GSH and Ceruloplasmin) and free radicals (MDA and NO). Moreover, AChE activity in erythrocytes and brain were also determined. Pirimiphos methyl induced a significant decrease in antioxidant enzyme activities and erythrocytes and brain AChE. In addition, marked depletion in erythrocytes and brain GSH, were observed. Moreover, administration of Pirimiphos methyl exhibited a significant increase in serum ceruloplasmin and erythrocytes SOD and serum GGT activities in addition to a marked increase in erythrocytes and brain MDA and serum NO levels. While administration of thyme to rats received, oral dose of POM exhibited a significant increase in antioxidant activities, GSH, ceruloplasmin and a marked decrease in erythrocytes and brain MDA and serum NO. These results concluded that, thyme constitutes a powerful antioxidant property that was able to improve the adverse biochemical changes induced by organophosphorous compounds that are widely in our country.

**Key Words:** Antioxidants, brain, erythrocytes, organophosphorous pesticides, oxidative stress, Pirimiphos methyl, Thyme.

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### 1. INTRODUCTION

Pesticides are used extensively in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors [33]. The unregulated use and its aerial application over large agricultural and urban areas has caused severe environmental pollution and potential health hazards. Organophosphate (OP) compounds are widely used and include some

of the most toxic chemical agents. Recently, more than 100 different OP compounds have been synthesized and are extensively used worldwide in agriculture and public health programs as insecticides, acaricides and nematicides, in veterinary medicine as ectoparasiticides, and in commerce as lubricants, plasticizers, and flame-retardants [8]. Exposure to low-level of pesticides is

known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in human and experimental studies. Conversely, some biochemical alterations may not necessarily lead to clinically recognizable symptoms, although all the biochemical responses can be used as markers of exposure or effect. The biochemical changes induced after exposure to pesticides or their active metabolites include target cell/receptor binding, protein and DNA adduct formation, and induction or inhibition of enzymes. Oxidative stress can also be induced by pesticides, either by overproduction of free radicals or by alteration in antioxidant defense mechanisms, including detoxification and scavenging enzymes [1]. There have been great efforts to find safe and potent natural antioxidants from various plant sources. As harmless sources of antioxidants, wild herbs, spices, fruits, nuts, and leafy vegetables have been investigated, for their antioxidant properties. The increasing interest in natural dietary components has focused attention on plants used as food or spices which are a rich source of bionutrients or bio-active phytochemicals. Thyme (*Thymus vulgaris L.*) are aromatic herbs that are used extensively to add a distinctive aroma and flavour to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics [22]. *Thymus vulgaris L* is a perennial herb indigenous in central and southern Europe, Africa and Asia that are rich in essential oils and antioxidative phenolic substances [42]. It is widely used in folk medicine for the treatment of a variety of diseases including gastroenteric and bronchopulmonary disorders, anthelmintic, antispasmodic, carminative, sedative, diaphoretic [35]. It has been reported that thyme possesses numerous biological activities including antispasmodic, antimicrobial, antioxidant and antifungal

[38]. Phenolic phytochemicals (phenylpropanoids) serve as effective antioxidants (phenolic antioxidants) due to their ability to donate hydrogen from hydroxyl groups positioned along the aromatic ring to terminate free radical oxidation of lipids and other biomolecules [16]. Phenolic antioxidants therefore short-circuit a destructive chain reaction that ultimately degrades cellular membranes. Accordingly, the aim of the present study was to evaluate the antioxidant effect of rutin in pirimiphos methyl intoxicated rats.

## 2. MATERIALS AND METHODS

### 2.1. Animals

Sixty adult white male albino rats weighting 150 - 200 g were used for the study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were allowed free access to standard dry rat diet and tap water ad libitum.

### 2.2. Experimental design:

The rats were divided into four groups:

- 1) Control normal group (C): Comprised 15 rats, received no drugs, fed on normal diet for 3 months.
- 2) POM group (P): Consisted of 15 rats, were fed on normal diet and administrated Pirimiphos methyl orally for 3 months at a dose level of 50 mg/ Kg b.w/day (1/40 LD50).
- 3) Thyme group (Thy): Composed of 15 rats, were fed on normal diet and received single oral dose of Thyme at a dose of 45 mg/ Kg b.w/day dissolved in distilled water for 3 months.
- 4) Thyme + POM group (Thy + P): Included 15 rats, were fed on normal diet and received single dose of Pirimiphos methyl per os daily for 3 months at a dose of 50 mg/ Kg b.w (1/40 LD50) followed by oral administration of Thyme at a dose level of 45 mg/ Kg b.w.

### 2.3. Sampling

#### 1- Blood samples:

Blood samples were collected by ocular vein puncture from all animal groups 3 times along the duration of experiment in dry, clean and screw capped heparinized tubes and plasma were separated by centrifugation at 2500 r.p.m. for 15 minutes. The clean clear plasma was separated by Pasteur pipette and kept in a deep freeze at -20°C until used for subsequent biochemical analysis Moreover, after plasma separation, erythrocytes were washed for subsequent biochemical analysis.

#### 2- Brain specimens:

Rats were killed by decapitation. The brain specimen quickly removed, cleaned by rinsing with cold saline and stored at -20°C. Briefly, brain tissues were minced into small pieces, homogenized in normal saline 0.9%. The homogenates were centrifuged at 10,000 for 15 minute at 4°C. The supernatant was used for subsequent biochemical analysis.

### 2.4. Biochemical analysis

Biochemical analysis were determined according to the methods described previously. Erythrocytes and brain acetyl cholinesterase (AChE) [21], Reduced glutathione (GSH) [11], Lipid peroxidation (MDA) [15], Catalase activity (CAT) [38], serum Ceruloplasmin [36], Nitric oxide (NO) [29], Gamma glutamyl transferase ( $\gamma$ GT) [26], erythrocytes Super oxide dismutase activity (SOD) [30], Glutathione peroxidase (GPx) [32], Glutathione reductase (GR) [18], glutathione-S-transferase (GST) [19].

### 2.5. Statistical analysis

The results were expressed as mean  $\pm$  SE and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when  $p < 0.05$ .

## 3. RESULTS & DISCUSSION

The obtained data in table (1 & 2) revealed that, administration of POM to normal rats exhibited a significant decrease in erythrocytes and brain AChE activities as compared with C group allover the experimental period. AChE activity is known as biomarker of chronic toxicity in human following pesticide exposure. It was recorded that, in acute exposure, the main mechanism of toxicity of (OP) is irreversibly binding to the enzyme acetylcholinesterase and inhibiting its activity that results in accumulation and prolonged effect of acetylcholine and consequently followed with acute muscarinic and nicotinic effects. [1]. Moreover, the recorded decrease in AChE activity might be due to the inhibition of the enzyme by the toxic metabolites of OPI. It was recorded that, phosphorothioate insecticides converted to their corresponding oxygen analogs by called mixed-function oxidases (MFO), a microsomal system of enzymes among which the enzyme cytochrome P450 (CYP450) plays a major role. The oxons are direct inhibitors of AChE [17].

However, administration of thyme to rats exposed to POM exhibited a significant increase in erythrocytes AChE activity after 2 and 3 months and in brain AChE activity after 3 months as compared with P group. The recorded increase in AChE activity may be related to the antioxidant capacity of thyme which inhibit or decrease formation of free radiacls resulted from POM administration. This suggestion was supported by the findings of [16] who mentioned that, hydroxyl groups positioned along the aromatic ring of thyme have the ability to donate hydrogen thus terminate free radical oxidation of lipids and other biomolecules

Table (1): Effect of Thyme administration on erythrocytes and brain AChE activity, MDA, GSH levels and serum NO, Ceruloplasmin concentration in normal and Pirimiphos Methyl (POM) intoxicated rats:

Parameter Groups	Eryth. AChE (U/g Hb)	Brain AChE (U/g tissue)	Eryth. MDA (nmol/g Hg)	Brain MDA (nmol/g tissue)	Serum NO ( $\mu\text{mol/L}$ )	Eryth. GSH (mg/g Hb)	Brain GSH (mg/g tissue)	Serum Cerulo. (mg/dl)
1st Month	C	2.71 $\pm$ 0.05 <sup>a</sup>	58.48 $\pm$ 0.54 <sup>a</sup>	7.59 $\pm$ 0.17 <sup>c</sup>	108.00 $\pm$ 2.00 <sup>c</sup>	24.60 $\pm$ 1.50 <sup>c</sup>	52.20 $\pm$ 0.97 <sup>b</sup>	75.60 $\pm$ 1.69 <sup>a</sup>
	P	2.33 $\pm$ 0.02 <sup>b</sup>	47.44 $\pm$ 0.59 <sup>b</sup>	14.21 $\pm$ 0.24 <sup>a</sup>	379.60 $\pm$ 12.80 <sup>a</sup>	50.80 $\pm$ 1.28 <sup>a</sup>	32.40 $\pm$ 0.68 <sup>c</sup>	34.60 $\pm$ 1.50 <sup>c</sup>
	Thy	2.75 $\pm$ 0.04 <sup>a</sup>	58.00 $\pm$ 0.91 <sup>a</sup>	7.41 $\pm$ 0.14 <sup>c</sup>	123.40 $\pm$ 3.14 <sup>c</sup>	24.80 $\pm$ 1.77 <sup>c</sup>	55.60 $\pm$ 1.44 <sup>b</sup>	75.00 $\pm$ 1.48 <sup>a</sup>
	Thy +P	2.32 $\pm$ 0.02 <sup>b</sup>	48.56 $\pm$ 0.83 <sup>b</sup>	12.15 $\pm$ 0.16 <sup>b</sup>	301.40 $\pm$ 5.70 <sup>b</sup>	42.20 $\pm$ 1.24 <sup>b</sup>	82.20 $\pm$ 3.72 <sup>a</sup>	54.40 $\pm$ 1.66 <sup>b</sup>
2nd Month	C	2.71 $\pm$ 0.07 <sup>a</sup>	58.48 $\pm$ 0.50 <sup>a</sup>	7.72 $\pm$ 0.08 <sup>c</sup>	110.40 $\pm$ 4.17 <sup>c</sup>	21.80 $\pm$ 1.32 <sup>c</sup>	55.00 $\pm$ 1.67 <sup>b</sup>	73.80 $\pm$ 1.83 <sup>a</sup>
	P	2.30 $\pm$ 0.02 <sup>c</sup>	47.66 $\pm$ 0.54 <sup>b</sup>	14.84 $\pm$ 0.27 <sup>a</sup>	350.20 $\pm$ 18.14 <sup>a</sup>	52.00 $\pm$ 2.35 <sup>a</sup>	33.60 $\pm$ 1.57 <sup>c</sup>	34.40 $\pm$ 1.33 <sup>c</sup>
	Thy	2.75 $\pm$ 0.03 <sup>a</sup>	58.52 $\pm$ 0.44 <sup>a</sup>	7.41 $\pm$ 0.19 <sup>c</sup>	114.60 $\pm$ 6.55 <sup>c</sup>	23.80 $\pm$ 1.83 <sup>c</sup>	54.40 $\pm$ 1.44 <sup>b</sup>	75.00 $\pm$ 1.70 <sup>a</sup>
	Thy +P	2.54 $\pm$ 0.02 <sup>b</sup>	46.50 $\pm$ 0.29 <sup>b</sup>	11.52 $\pm$ 0.16 <sup>b</sup>	292.20 $\pm$ 4.26 <sup>b</sup>	41.20 $\pm$ 0.80 <sup>b</sup>	85.20 $\pm$ 2.31 <sup>a</sup>	57.40 $\pm$ 1.50 <sup>b</sup>
3rd Month	C	2.74 $\pm$ 0.10 <sup>a</sup>	58.10 $\pm$ 0.47 <sup>a</sup>	7.74 $\pm$ 0.09 <sup>c</sup>	112.00 $\pm$ 4.34 <sup>c</sup>	23.80 $\pm$ 1.46 <sup>c</sup>	51.80 $\pm$ 3.6 <sup>b</sup>	76.60 $\pm$ 1.33 <sup>a</sup>
	P	2.11 $\pm$ 0.03 <sup>b</sup>	42.10 $\pm$ 0.36 <sup>c</sup>	16.66 $\pm$ 0.31 <sup>a</sup>	377.00 $\pm$ 11.32 <sup>a</sup>	51.40 $\pm$ 2.16 <sup>a</sup>	33.00 $\pm$ 1.5 <sup>c</sup>	34.80 $\pm$ 1.62 <sup>c</sup>
	Thy	2.74 $\pm$ 0.04 <sup>a</sup>	58.10 $\pm$ 0.71 <sup>a</sup>	7.72 $\pm$ 0.11 <sup>c</sup>	110.60 $\pm$ 3.14 <sup>c</sup>	25.20 $\pm$ 1.98 <sup>c</sup>	54.60 $\pm$ 2.80 <sup>b</sup>	76.80 $\pm$ 1.24 <sup>a</sup>
	Thy +P	2.77 $\pm$ 0.03 <sup>a</sup>	46.24 $\pm$ 0.87 <sup>b</sup>	10.88 $\pm$ 0.25 <sup>b</sup>	245.60 $\pm$ 6.73 <sup>b</sup>	33.00 $\pm$ 1.76 <sup>b</sup>	75.80 $\pm$ 2.41 <sup>a</sup>	65.80 $\pm$ 1.77 <sup>b</sup>

(C: Control Normal group, P: POM group, Thy: Thyme group, Thy + P: Thyme + POM group), Data are presented as (Mean  $\pm$  S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ( $P<0.05$ ).

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Table (2): Effect of Thyme administration on erythrocytes and brain antioxidant enzymes in normal and Pirimiphos Methyl (POM) intoxicated rats:

Parameter Groups	Eryth. SOD (U/g Hb)	Eryth. CAT (U/g Hg)	Brain CAT (U/g tissue)	Eryth. GPx (mU/g Hb)	Eryth. GR (U/g Hb)	Eryth. GST (U/g Hb)	Serum GGT (U/L)
1st Month	C            33.54± 0.55 <sup>c</sup>	2.74± 0.07 <sup>a</sup>	13.22± 0.28 <sup>a</sup>	16.82± 0.44 <sup>a</sup>	6.68± 0.13 <sup>a</sup>	5.30± 0.05 <sup>a</sup>	3.31± 0.16 <sup>c</sup>
	P            59.74 ± 0.57 <sup>a</sup>	0.98± 0.04 <sup>b</sup>	7.36± 0.18 <sup>c</sup>	10.70±0.18 <sup>b</sup>	3.10± 0.11 <sup>c</sup>	4.28± 0.04 <sup>b</sup>	11.01± 0.15 <sup>a</sup>
	Thy          34.30± 0.36 <sup>c</sup>	2.84± 0.05 <sup>a</sup>	13.62± 0.20 <sup>a</sup>	17.07± 0.52 <sup>a</sup>	6.52± 0.19 <sup>a</sup>	5.35± 0.17 <sup>a</sup>	3.68± 0.29 <sup>c</sup>
	Thy +P      42.14± 1.19 <sup>b</sup>	1.04± 0.09 <sup>b</sup>	9.88± 0.07 <sup>b</sup>	12.01± 0.60 <sup>b</sup>	4.34± 0.18 <sup>b</sup>	5.25± 0.05 <sup>a</sup>	9.91± 0.50 <sup>b</sup>
2nd Month	C            33.54± 0.53 <sup>c</sup>	2.80± 0.08 <sup>a</sup>	13.62± 0.26 <sup>a</sup>	17.72± 0.27 <sup>a</sup>	6.40± 0.231 <sup>a</sup>	5.31± 0.02 <sup>a</sup>	3.68± 0.22 <sup>c</sup>
	P            64.32± 0.51 <sup>a</sup>	0.90± 0.07 <sup>c</sup>	6.96± 0.05 <sup>c</sup>	10.20± 0.27 <sup>c</sup>	2.73± 0.17 <sup>c</sup>	4.42± 0.15 <sup>b</sup>	10.62± 0.15 <sup>a</sup>
	Thy          34.16± 0.28 <sup>c</sup>	2.72± 0.06 <sup>a</sup>	13.52± 0.22 <sup>a</sup>	18.01± 0.28 <sup>a</sup>	6.85± 0.23 <sup>a</sup>	5.47± 0.15 <sup>a</sup>	4.02± 0.18 <sup>c</sup>
	Thy +P      44.30± 1.05 <sup>b</sup>	1.72± 0.15 <sup>b</sup>	9.80± 0.07 <sup>b</sup>	13.11± 0.33 <sup>b</sup>	4.77± 0.24 <sup>b</sup>	5.38± 0.11 <sup>a</sup>	6.03± 0.58 <sup>b</sup>
3rd Month	C            31.62± 0.57 <sup>c</sup>	2.88± 0.07 <sup>a</sup>	13.34± 0.72 <sup>a</sup>	18.19± 0.30 <sup>a</sup>	6.64± 0.19 <sup>a</sup>	5.50± 0.13 <sup>a</sup>	3.84± 0.29 <sup>b</sup>
	P            66.56± 2.11 <sup>a</sup>	0.86± 0.05 <sup>c</sup>	7.12± 0.10 <sup>c</sup>	8.91± 0.35 <sup>c</sup>	2.71± 0.24 <sup>c</sup>	4.47± 0.05 <sup>b</sup>	10.60± 0.22 <sup>a</sup>
	Thy          34.40± 0.90 <sup>c</sup>	2.52± 0.16 <sup>a</sup>	13.68± 0.17 <sup>a</sup>	17.89± 0.27 <sup>a</sup>	6.64± 0.17 <sup>a</sup>	5.42± 0.14 <sup>a</sup>	4.10± 0.27 <sup>b</sup>
	Thy +P      41.16± 0.83 <sup>b</sup>	1.76± 0.18 <sup>b</sup>	9.88± 0.07 <sup>b</sup>	14.60± 0.25 <sup>b</sup>	5.74± 0.10 <sup>b</sup>	5.17± 0.11 <sup>a</sup>	4.20± 0.23 <sup>b</sup>

(C: Control Normal group, P: POM group, Thy: Thyme group, Thy + P: Thyme + POM group). Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

The obtained data in table (1) revealed that, administration of POM to normal rats exhibited a significant increase in erythrocytes and brain (MDA) and serum NO levels when compared to C group allover the experimental period. LPO has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocyte survival, and membrane fluidity. Increase in the levels of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals [12]. The recorded results might be due to induction of cytochrome 450, inhibition of AChE and disturbance in activities of GSH and GST enzymes causing lipid peroxidation as reported by [34]. Another suggestion for the recorded results stated by [39] who suggested that, dimethoate induced LPO in liver could possibly result from an enhanced microsomal oxidative capacity induced by the insecticide. Thus, elevated levels of cytochrome P450 lead to high rates of radicals production, which, in turn, increase the rate of lipid peroxidation.

Nitrate and nitrite [a marker of endogenous nitric oxide (NO) production], possesses both antioxidant and pro-oxidant properties. An antioxidative property of NO has been reported in the many studies [6]. NO is an effective chain-breaking antioxidant in free radical-mediated LPO and reacts rapidly with peroxy radicals as a sacrificial chain-terminating antioxidant. It is well documented that iNOS produces NO and NO-derived reactive nitrogen species such as peroxynitrite. In healthy neuronal tissue, iNOS is not commonly present, but it can be expressed by astrocyte, neurons, and endothelial cells after brain offence where it can initiate the production of high amounts of NO. Overproduction of NO may lead to neuronal damage and death. The reaction between NO and super-oxide anion generates

the cytotoxic compound, peroxynitrite, that leads to neuronal toxicity [41]. Under normal physiological conditions, antioxidant enzymes are responsible to eliminate the highly reactive molecules. However, under unphysiological conditions, the excessive accumulation of reactive species induces several cellular dysfunctions [40]. However, administration of thyme to POM intoxicated rats exhibited a significant decrease in erythrocytes and brain (MDA) and serum NO levels allover the experimental period as compared with P group. The recorded results may be related to the antioxidant properties of phenolic compounds found in thyme [10]. It is well documented that the leaves and flowers of plants containing numerous aroma chemicals. Phenolic phytochemicals are thought to promote optimal health partly via their antioxidant and free radical scavenging effects thereby protecting cellular components against free radical induced damage [13].

The obtained data in table (1) revealed that, administration of POM to normal rats exhibited a significant decrease in erythrocytes and brain (GSH) level and significant increase in serum Ceruloplasmin level when compared to C group allover the experimental period. The recorded results may be attributed to the utilization of GSH in the metabolism of pesticides through GST. The study of [36] revealed that, lindane, malathion and propoxur increased the activity of GST by conjugation of GSH to pesticides *in vivo*. This could be understood in view of the fact that some pesticides (organochlorine and organophosphate) consume GSH through GST catalyzed reaction as a major way of detoxification of these chemicals. In contrast, other classes of pesticides such as carbamate may utilize GSH in conjugation reaction but only in minor amounts compared to organophosphate or organochlorine [3].

However, administration of thyme to POM intoxicated rats exhibited a significant

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increase in erythrocytes and brain reduced glutathione (GSH) and serum Ceruloplasmin level allover the experimental period as compared with P group. These results may be attributed to the antioxidant activity of thyme. This suggestion was supported by the findings of [4] who mentioned that, TOH could optimally antagonize radiation-induced toxicity, which may be due to its free radical scavenging potential, by normalizing the intracellular antioxidant levels, also by its anti-lipid peroxidative potential.

The obtained data in table (2) revealed that, administration of POM to normal rats exhibited a significant increase in erythrocytes (SOD) activity allover the experimental period when compared to C group. The increased in SOD activity has been attributed to activation of the compensatory mechanism through the effect of OP on progenitor cells with its extent depending on the magnitude of the oxidative stress and hence, on the dose of the stressor. Supporting this idea, there is evidence that administration of malathion for 4 weeks increases the (SOD) activity in erythrocytes and liver [2].

However, administration of thyme to POM intoxicated rats exhibited a significant decrease allover the experimental period as compared with P group. The recorded results may be related to the antioxidant activity of thyme and its free radical scavenging power through its phenolic hydroxyl group as reported by [4].

The obtained data in table (2) revealed that, administration of POM to normal rats exhibited a significant decrease in erythrocytes and brain CAT, erythrocytes GPx, GR and GST activities allover the experimental period as compared with C group . The decrease in antioxidant enzymes has been interpreted as an indirect inhibition of the enzymes resulting from the binding of oxidative molecules produced during pesticide metabolism. Three enzyme systems (GST, esterases and monooxygenases) are

involved in the detoxification of organophosphate insecticide class. These enzymes act by rapidly metabolizing the insecticide to non-toxic products or by rapidly binding and very slowly turning over the insecticide [24]. The present results confirm the previous reports of [14], who showed that repeated administration of dimethoate induced disturbances in the activities of the enzyme regulating GSH metabolism. Glutathione S-transferases are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms. As demonstrated in other studies, the activities of antioxidant enzymes can be altered in a variety of animal tissues poisoned with OPI [20]. Generally, oxidative stress results in reduction in tissue antioxidants because these agents are utilized in terminating the lipid peroxidation chain reactions.

However, administration of thyme to POM intoxicated rats exhibited a significant increase in erythrocytes CAT activity after 2 and 3 months and a significant increase in brain CAT activity allover the experimental period. Also, daily dose of thyme to rats which received oral dose of POM exhibited a significant increase in erythrocytes GPx after 2 and 3 months. Moreover, daily dose of thyme to rats which received oral dose of POM exhibited a significant increase in erythrocytes GR and GST activities allover the experimental period when compared to P group. The recorded results might be due to the capacity for free radical trapping due to the presence of the phenolic hydroxyl group in thyme as reported by [4] who observed that, pretreatment of TOH increased the activity of SOD, GST and CAT in gamma irradiated V79 cells. The radioprotective effect of TOH can thus be explained by the scavenging of free radicals generated by radiation before they cause damage to cellular macromolecules. Moreover, [26] attributed the antioxidant activity of thyme to the

presence of phenolic compounds thymol and carvacrol. Another suggestion for the recorded results stated by [43] who reported that, the antioxidant properties of thyme oil are being utilised by the cells, thus sparing the intracellular antioxidant systems such as SOD and GPx. It is also possible that thyme oil is influencing other cellular systems suggesting more detailed examination of further antioxidant parameters is required.

The obtained data in table (2) revealed that, administration of POM to normal rats exhibited a significant increase in serum ( $\gamma$ GT) activity allover the experimental period when compared to C group. Among the enzymes usually determined to evaluate hepatic function, GGT is considered by many authors to be a reliable biomarker closely involved in the establishment of oxidative stress damage [31]. This enzyme has a central role in glutathione hepatic re-synthesis. Moreover, as suggested by [27], it has an inverse relationship with the levels of many other antioxidants. [5] observed that GGT is more sensitive than other enzymes (AST, ALT, and ALP), changing by almost 90 percent compared to control values. In addition, this enzyme is positively correlated with LDH, total copper and NCBC and is negatively correlated with the production of albumin. GGT has been used as a biomarker of pesticide-induced liver damage, and other researchers have demonstrated an association between increased activity of this enzyme and reduced antioxidant ability in rats [27] and humans [24]. Biological significance of  $\gamma$ -GT-dependent lipid peroxidation *in vivo* might be multifold. Varying levels of  $\gamma$ -GT activity can be detected in erythrocytes and lymphocytes. It is conceivable that the pro-oxidant effects of  $\gamma$ -GT activity are normally balanced by its established role in favoring the cellular uptake of precursors for GSH resynthesis, thus allowing the reconstitution of cellular antioxidant defense [9]. The increased serum ( $\gamma$ GT) activity has been attributed to the significant tissue injury

provoked by pesticides, even at low doses employed in this study as stated by [7].

However, administration of thyme to POM intoxicated rats exhibited a significant decrease allover the experimental period as compared with P group. It has been hypothesized that one of the principal causes of leakage of cellular enzyme into plasma is hepatic injury as reported by [23]. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. The recorded results may be related to antioxidant activity of chlorophyllin and its radical scavenging capacity to free radicals produced due to metabolism of organophosphorous pesticides by cytochrome P450 [39]. These radicals increase the rate of lipid peroxidation, increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced cellular survival, and membrane fluidity [12]. Administration of antioxidants restores the imbalance in antioxidant defense mechanism and preserves the structural integrity of the hepatocellular membrane against free radicals [28].

From the obtained results it could be concluded that, chronic toxicity experimentally induced with POM in rats extensively alters and induced disturbances in enzymatic and non enzymatic antioxidant system in erythrocytes and brain tissues. Moreover, thyme administration efficiently protects erythrocytes and brain from deleterious effect of oxidative stress induced by POM. This study suggested that, thyme may be effective in controlling oxidative damage through its powerful antioxidant capacity.

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## مجلة بها للعلوم الطبية البيطرية

**التأثير المضاد للأكسدة للزرعتر على التسمم المحدث باستخدام البيريميفوس ميثيل في فئران التجارب**  
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### الملخص العربي

أصبحت المركبات الفسفورية العضوية هي أكثر المبيدات الحشرية انتشاراً اليوم على الاطلاق على المستوى العالمي. وقد يسبب التعرض المزمن لهذه المركبات أشد الضرر على الصحة، حيث أنها قد تسبب السرطان، واضطراب في وظائف الأيض والهرمونات والمناعة. ولذلك يهدف هذا البحث إلى دراسة التأثير الكيميائي الحيوي للزرعتر على الإجهاد التأكسدي في دم ومخ الفئران المحدث فيها التسمم المزمن تجريبياً باستخدام مادة البيريميفوس ميثيل. وقد أجريت هذه الدراسة على عدد 60 من ذكور الفئران البيضاء وتترواح أوزانها بين 150-200 جرام هذا وقد تم تقسيم الفئران إلى أربعة مجموعات متساوية اشتملت كل مجموعة على عدد 15 فأر وتم توزيعها كالتالي: مجموعة (1): تعتبر المجموعة الضابطة لم تتناول أي دواء. مجموعة (2): تجرعت البيريميفوس ميثيل يومياً لمدة ثلاثة أشهر. مجموعة (3): تجرعت مستخلص الزرعتر يومياً لمدة ثلاثة أشهر. مجموعة (4): تجرعت البيريميفوس ميثيل ومستخلص الزرعتر يومياً لمدة ثلاثة أشهر. هذا وقد أظهرت النتائج وجود نقص واضح في نشاط الانزيمات المضادة للأكسدة في المجموعة الثانية وعلى العكس ظهر تحسن واضح في النتائج في المجموعة الرابعة. كذلك مستوى مضادات الأكسدة الغير انزيمية في دم ومخ الفئران أظهرت النتائج وجود نقص واضح في المجموعة الثانية وعلى العكس ظهر تحسن واضح في نتائج المجموعة الرابعة.

مما سبق نستنتج أن الزرعتر له تأثير وقائي واضح في حماية الفئران من التأثير الضار لمادة البيريميفوس ميثيل ولذلك ننصح بضرورة استخدامه كمواد فعالة في العقاقير المستخدمة ل الوقاية من الإجهاد التأكسدي الناتج عن التعرض للعديد من ملوثات البيئة ومن ضمنها المبيدات الحشرية.

(مجلة بها للعلوم الطبية البيطرية: عدد 24 (1)، يونيو 2013: 195-184)