



AMELIORATING ROLE OF CHLOROPHYLLIN ON OXIDATIVE STRESS INDUCED BY PIRIMIPHOS METHYL IN ERYTHROCYTES AND BRAIN OF RATS

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ABSTRACT

For the present study the harmful effects of organophosphorous pesticides chronic toxicity on several biochemical parameters and evaluation of the possible protective effects of chlorophyllin as antioxidant in rats exposed to Pirimiphos methyl (POM), one of organophosphorous pesticides widely used in the middle east, were investigated. Sixty adult white male albino rats weighting 150 - 200 gm were used in this study. The rats were divided into four equal groups. 1) Normal group (N): received no drugs. 2) POM group (P): received single dose of POM (50 mg/ Kg b.w) daily for 3 months. 3) Chlorophyllin group (Chl): received single oral dose of Chlorophyllin (27 mg/ Kg b.w) daily for 3 months. 4) Chlorophyllin + POM group (Chl + P): received single dose of POM (50 mg/ Kg b.w) + Chlorophyllin (27 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. Plasma was separated for determination of Ceruloplasmin, NO and γ GT. Moreover, after plasma separation, erythrocytes were washed for determination of AChE, GSH, MDA, CAT, SOD, GPx, GR and GST. Brain AChE, GSH, MDA, CAT, were also determined. The obtained results revealed that, administration of Pirimiphos methyl exhibited a significant decrease in erythrocytes and brain AChE, CAT, GSH, erythrocytes GPx, GR and GST activities. CHL administration in POM intoxicated rats exhibited significant increase in all mentioned parameters. On the other hand, a marked increase in erythrocytes SOD, serum GGT activities, erythrocytes and brain MDA and serum NO level were observed. CHL administration in POM intoxicated rats exhibited significant decrease in all mentioned parameters. From the obtained results, it could be concluded that, the potential of CHL as natural antioxidant act as a powerful agent against the toxic effects of POM.

KEY WORDS: Antioxidants, brain, Chlorophyllin, erythrocytes, organophosphorous pesticides, oxidative stress, Pirimiphos methyl.

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1. INTRODUCTION

The widespread use of organophosphate pesticides by public health and agricultural programs has led to severe environmental pollution that constitutes a significant potential health hazard because of the possibility of the acute or chronic poisoning of humans [24]. Indeed, residual amounts of organophosphate (OP) pesticides have been detected in the soil, water bodies,

vegetables, grains, and other food products. The acute neurotoxic effects of organophosphorus insecticides are well documented. In recent years, there is widespread concern over exposure to low levels of organophosphates in the diet over a longer period. There are reports, which suggest that organophosphorus insecticides manifest their toxic effects by enhanced production of reactive oxygen species

(ROS) which is a major cellular source of oxidative stress. ROS can damage every major cellular component including membrane, lipids, carbohydrates and DNA. The pathophysiological consequence of such uncontrolled injury is widespread tissue damage. Oxidative damage occurs when forces that favour oxidation outweighs antioxidant protection within cells [28].

Pirimiphos-methyl (O-2-diethylamino-6-methylpyrimidin-4-yl O, O-dimethyl phosphorothioate: POM) is an OP insecticide widely used to treat grains to protect against insect attack during storage. POM shows activity against a wide spectrum of insect pests, including ants, aphids, beetles, caterpillars, and cockroaches. Commercial uses of POM are now employed in a wide variety of areas, including growing crops, public health and stored products. POM as a organophosphate grain protectant has been registered in the USA since 1986 for treating stored corn and sorghum at 8 mg/kg to control insects [8].

Nowadays, there is considerable emphasis on identifying the potential of natural plant products as chemopreventive agents present in food consumed by the human population. Many plant products exert antioxidative effects and some of these are widely used in food in different parts of the world [21].

Chlorophyllin (CHL) is a water-soluble mixture of sodium-copper salts of the green plant pigment chlorophyll. It has been used as a coloring agent and shown to be a potent antimutagenic agent against a variety of mutagens *in vitro* and *in vivo* [12]. It has also been shown to exhibit anticarcinogenic activity in animal models. It is marketed for controlling body, fecal and urinary odor in geriatric patients and accelerating wound healing. Its antimutagenic and anticarcinogenic activities have been attributed to formation of complexes with the mutagens and carcinogens and diminishing their bioavailability by faster excretion. CHL is

also a potent inhibitor of cytochrome P450 enzymes involved in the bioactivation of several environmental carcinogens. The radical-scavenging mechanism of CHL is fundamentally derived from the structure of porphyrin compound. Porphyrins contain a chelated metal in the center of the molecules. The structure of these compounds has been demonstrated to capture electrons so that, CHL and its related compounds can then scavenge radicals or suppress metabolic activation [32].

Accordingly, the aim of the present study was to evaluate the antioxidant effect of chlorophyllin in pirimiphos methyl intoxicated rats.

2. MATERIALS AND METHODS

2.1. Animals

Sixty adult white male albino rats weighting 150 - 200 gm 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:

Control normal group (C): Consisted of 15 rats, received no drugs, fed on normal diet for 3 months. POM group (P): Composed of 15 rats, were fed on normal diet and administered Pirimiphos methyl orally for 3 months at a dose level of 50 mg/ Kg b.w/day (1/40 LD₅₀). Chlorophyllin group (Chl): Included 15 rats were fed on normal diet and received single oral dose of Chlorophyllin at a dose of 27 mg/ Kg b.w. dissolved in distilled water daily for 3 months. Chlorophyllin + POM group (Chl + P): Comprised 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 Chlorophyllin at a dose of 27 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 rpm 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 analyses.

2.4. Biochemical analysis:

Erythrocytes and brain acetyl cholinesterase (AChE) (Henry 1974), Reduced glutathione (GSH) (Beutler et al., 1963), Lipid peroxidation (MDA) Esterbauer et al., (1982), Catalase activity (CAT) (Sinha, 1972), serum Ceruloplasmin (Schoslnsky et al., 1974), Nitric oxide (NO) (Montgomery and Dymock 1961), Gamma glutamyl transferase (γ GT) (Lee 2003), erythrocytes Super oxide dismutase activity (SOD) (Nishikimi et al. 1972), Glutathione peroxidase (GPx) (Paglia and Valentine 1967), Glutathione reductase (GR) (Goldberg and Spooner 1983), glutathione-S-transferase (GST), (Habig et al., 1974) were determined according to the methods described previously.

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 all over 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 [15].

However, administration of chlorophyllin to POM intoxicated rats exhibited a significant increase after 2 and 3 months as compared with P group. The recorded increase in AChE activity may be related to the antioxidant capacity of chlorophyllin, which inhibit or decrease formation of free radicals resulted from POM administration. This suggestion was supported by the findings of [32] who mentioned that, chelated metal in the center of the Porphyrins molecules of chlorophyllin capture electrons enabling CHL and its related compounds to scavenge radicals.

The obtained data in table (1) revealed that, administration of POM to normal rats

Table (1): Effect of Chlorophyllin 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 (μ mol/L)	Eryth. GSH (mg/g Hb)	Brain GSH mg/g tissue	Serum Cerulo. (mg/dl)	
1st Month	C	2.71 \pm 0.05 ^a	58.48 \pm 0.54 ^a	7.59 \pm 0.17 ^c	108.00 \pm 2.00 ^c	24.60 \pm 1.50 ^c	52.20 \pm 0.97 ^b	75.60 \pm 1.69 ^a	18.28 \pm 0.75 ^c
	P	2.33 \pm 0.02 ^b	47.44 \pm 0.59 ^b	14.21 \pm 0.24 ^a	379.60 \pm 12.80 ^a	50.80 \pm 1.28 ^a	32.40 \pm 0.68 ^c	34.60 \pm 1.50 ^c	32.06 \pm 0.69 ^b
	CHL	2.69 \pm 0.03 ^a	58.72 \pm 0.58 ^a	7.27 \pm 0.13 ^c	116.00 \pm 2.88 ^c	23.60 \pm 0.87 ^c	52.80 \pm 1.88 ^b	77.40 \pm 1.03 ^a	17.26 \pm 0.32 ^c
	CHL +P	2.24 \pm 0.05 ^b	47.46 \pm 0.49 ^b	11.77 \pm 0.28 ^b	306.20 \pm 4.87 ^b	43.20 \pm 1.98 ^b	75.20 \pm 3.68 ^a	48.40 \pm 1.2 ^b	52.34 \pm 1.06 ^a
2nd Month	C	2.71 \pm 0.07 ^a	58.48 \pm 0.50 ^a	7.72 \pm 0.08 ^c	110.40 \pm 4.17 ^c	21.80 \pm 1.32 ^c	55.00 \pm 1.67 ^b	73.80 \pm 1.83 ^a	17.64 \pm 0.26 ^c
	P	2.30 \pm 0.02 ^c	47.66 \pm 0.54 ^b	14.84 \pm 0.27 ^a	350.20 \pm 18.14 ^a	52.00 \pm 2.35 ^a	33.60 \pm 1.57 ^c	34.40 \pm 1.33 ^c	36.52 \pm 1.46 ^b
	CHL	2.63 \pm 0.04 ^a	56.84 \pm 0.90 ^a	7.05 \pm 0.18 ^d	114.00 \pm 6.64 ^c	24.00 \pm 1.18 ^c	51.20 \pm 1.24 ^b	74.00 \pm 1.22 ^a	16.96 \pm 0.51 ^c
	CHL +P	2.40 \pm 0.02 ^b	49.54 \pm 0.42 ^b	11.09 \pm 0.12 ^b	297.80 \pm 0.97 ^b	41.80 \pm 1.07 ^b	81.60 \pm 2.44 ^a	57.40 \pm 2.94 ^b	52.20 \pm 1.00 ^a
3rd Month	C	2.74 \pm 0.10 ^a	58.10 \pm 0.47 ^a	7.74 \pm 0.09 ^c	112.00 \pm 4.34 ^c	23.80 \pm 1.46 ^c	51.80 \pm 3.6 ^b	76.60 \pm 1.33 ^a	17.90 \pm 0.42 ^c
	P	2.11 \pm 0.03 ^b	42.10 \pm 0.36 ^c	16.66 \pm 0.31 ^a	377.00 \pm 11.32 ^a	51.40 \pm 2.16 ^a	33.00 \pm 1.5 ^c	34.80 \pm 1.62 ^c	23.04 \pm 1.36 ^b
	CHL	2.74 \pm 0.02 ^a	58.68 \pm 1.10 ^a	7.34 \pm 0.14 ^c	121.60 \pm 6.08 ^c	22.80 \pm 1.53 ^c	54.80 \pm 1.56 ^b	75.00 \pm 1.58 ^a	18.54 \pm 0.22 ^c
	CHL +P	2.50 \pm 0.02 ^b	47.46 \pm 0.71 ^b	11.03 \pm 0.21 ^b	268.60 \pm 6.48 ^b	35.80 \pm 2.56 ^b	80.20 \pm 2.67 ^a	66.80 \pm 1.83 ^b	36.78 \pm 0.68 ^a

(C: Control Normal group, P: POM group, Chl: Chlorophyllin group, Chl + P: Chlorophyllin + 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).

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

Parameter	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)	
Groups								
1st Month	C	33.54± 0.55 ^c	2.74± 0.07 ^a	13.22± 0.28 ^a	16.82± 0.44 ^a	6.68± 0.13 ^a	5.30± 0.05 ^a	3.31± 0.16 ^c
	P	59.74 ± 0.57 ^a	0.98± 0.04 ^b	7.36± 0.18 ^c	10.70±0.18 ^b	3.10± 0.11 ^c	4.28± 0.04 ^b	11.01± 0.15 ^a
	CHL	34.20± 0.23 ^b	2.92± 0.06 ^a	13.72± 0.24 ^a	16.84± 0.37 ^a	6.79±0.11 ^a	5.19± 0.06 ^a	3.66± 0.21 ^c
	CHL +P	58.68± 0.28 ^a	1.08± 0.12 ^b	10.70± 0.18 ^b	11.36± 0.28 ^b	5.22± 0.14 ^b	5.16± 0.02 ^a	9.78± 0.14 ^b
2nd Month	C	33.54± 0.53 ^c	2.80± 0.08 ^a	13.62± 0.26 ^a	17.72± 0.27 ^a	6.40± 0.231 ^a	5.31± 0.02 ^a	3.68± 0.22 ^c
	P	64.32± 0.51 ^a	0.90± 0.07 ^c	6.96± 0.05 ^c	10.20± 0.27 ^c	2.73± 0.17 ^c	4.42± 0.15 ^b	10.62± 0.15 ^a
	CHL	34.66± 0.39 ^c	2.86± 0.10 ^a	13.98± 0.35 ^a	17.80± 0.25 ^a	6.42± 0.16 ^a	5.52± 0.06 ^a	3.56± 0.18 ^c
	CHL +P	58.02± 0.43 ^b	1.22± 0.18 ^b	10.56± 0.21 ^b	10.14± 0.39 ^b	4.58± 0.20 ^b	5.34± 0.13 ^a	5.88± 0.41 ^b
3rd Month	C	31.62± 0.57 ^c	2.88± 0.07 ^a	13.34± 0.72 ^a	18.19± 0.30 ^a	6.64± 0.19 ^a	5.50± 0.13 ^a	3.84± 0.29 ^b
	P	66.56± 2.11 ^a	0.86± 0.05 ^c	7.12± 0.10 ^c	8.91± 0.35 ^c	2.71± 0.24 ^c	4.47± 0.05 ^b	10.60± 0.22 ^a
	CHL	34.92± 0.09 ^c	2.72± 0.14 ^a	13.60± 0.25 ^a	18.24± 0.33 ^a	6.32± 0.19 ^a	5.40± 0.01 ^a	3.42± 0.18 ^b
	CHL +P	56.12± 0.40 ^b	1.74± 0.15 ^b	10.56± 0.18 ^b	15.38± 0.33 ^b	5.77± 0.05 ^b	5.48± 0.14 ^a	3.42± 0.20 ^b

(C: Control Normal group, P: POM group, Chl: Chlorophyllin group, Chl + P: Chlorophyllin + 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 ($P<0.05$).

exhibited a significant increase in erythrocytes and brain (MDA) and serum NO levels when compared to C group all over 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 [11]. 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 [38] 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 [5]. 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 [39].

However, administration of chlorophyllin to POM intoxicated rats exhibited a significant decrease in erythrocytes and brain (MDA) and serum NO levels all over the experimental period as compared with P group. The recorded results may be related to radical scavenging mechanism of porphyrin ring in chlorophyllin structure, which contain a chelated metal in the center of the molecules that capture electrons so that CHL and its related compounds can then scavenge radicals or suppress metabolic activation [32].

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 all over 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 chlorophyllin to POM intoxicated rats exhibited a significant increase in erythrocytes and brain reduced glutathione (GSH) and serum Ceruloplasmin level all over the experimental period as compared with P group. It was reported that, conversion of SH groups into disulphides and other

oxidized species (e.g. oxyacids) is one of the earliest events observed during the radical-mediated oxidation of proteins [40]. It is likely that CHL prevents one or more of these forms of damage thus restore level of GSH [20].

The obtained data in table (2) revealed that, administration of POM to normal rats exhibited a significant increase in erythrocytes (SOD) activity all over 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 chlorophyllin to POM intoxicated rats exhibited a significant decrease all over the experimental period as compared with P group. The recorded results may be related to the antioxidant activity of chlorophyllin and its free radical scavenging power through the chelated metal present in the center of the molecules of Porphyrin ring [10].

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 all over 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 [23]. The present results

confirm the previous reports of [13], 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 [18]. Generally, oxidative stress results in reduction in tissue antioxidants because these agents are utilized in terminating the lipid peroxidation chain reactions.

However, administration of chlorophyllin 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 all over the experimental period. Also, Daily dose of chlorophyllin to rats which received oral dose of POM exhibited a significant increase in erythrocytes GPx after 3 months. Moreover, Daily dose of chlorophyllin to rats which received oral dose of POM exhibited a significant increase in erythrocytes GR activities all over the experimental period when compared to P group. It has been hypothesized that one of the principal mechanisms by which antioxidants alleviate oxidative stress either by scavenging ROS or induce/enhance antioxidant enzymes. This suggestion was supported by the findings of [22] who mentioned that, CHL may be protecting cells from oxidative stress by scavenging ROS generated inside the cells. These results may be also attributed to the radical-scavenging mechanism of CHL which is fundamentally derived from the structure of porphyrin compound [32]. Porphyrins contain a chelated metal in the center of the molecules. The structure of these compounds has been demonstrated to capture electrons so that CHL and its related compounds can then scavenge

radicals or suppress metabolic activation [10].

The obtained data in table (2) revealed that, administration of POM to normal rats exhibited a significant increase in serum (γ GT) activity all over 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 [26], it has an inverse relationship with the levels of many other antioxidants. [4] 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 [26] and humans [23]. Biological significance of γ -GT-dependent lipid peroxidation in vivo might be multifold. Varying levels of γ -GT activity can be detected in erythrocytes and lymphocytes. It is conceivable that the pro-oxidant effects of γ -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 [7]. The increased serum (γ GT) activity has been attributed to the significant tissue injury provoked by pesticides, even at low doses employed in this study as stated by [6].

However, administration of chlorophyllin to POM intoxicated rats exhibited a significant decrease all over 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 [22]. 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 [38]. These radicals increase the rate of lipid peroxidation, increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced cellular survival, and membrane fluidity [11]. Administration of antioxidants restores the imbalance in antioxidant defense mechanism and preserves the structural integrity of the hepatocellular membrane against free radicals [27].

4. Conclusion

From the obtained results, it could be concluded that, chronic toxicity induced by organophosphorous pesticides extensively alters and induced disturbances in enzymatic and non-enzymatic antioxidant system in erythrocytes and brain tissues of rats. However, chlorophyllin administration as a natural antioxidant efficiently protects erythrocytes and brain from deleterious effect of oxidative stress induced by organophosphorous pesticides. This study suggests that, natural antioxidants may be effective in controlling oxidative damage produced by several chemicals and pollutants found extensively in our environment.

5. REFERENCES

1. Abdollahi, M., Mostafalou, S., Pournourmohammadi, S., Shadnia, S., (2004). Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to Malathion. Comparative

- Biochemistry and Physiology Part C 137: 29–34.
2. Akhgari, M., Abdollahi, M., Kebryaezadeh, A., Hosseini, R., Sabzevari, O. (2003). Biochemical evidence for free radical induced lipid peroxidation as a mechanism for subchronic toxicity of Malathion in blood and liver of rats. *Hum. Exp. Toxicol.* 22: 205–211.
 3. Almeida, M.G., Fanini, F., Davino, S.C., Aznar, A.E., Koch, O.R., Barros, S.B.M. (1997). Pro- and anti-oxidant parameters in rat liver after short-term exposure to hexachlorobenzene. *Hum. Exp. Toxicol.* 16: 257–261.
 4. Arnal, N., Astiz, M., deAlaniz, M. J. T., Marra, C. A. (2011). Clinical parameters and biomarkers of oxidative stress in agricultural workers who applied copper-based pesticides. *Ecotoxicology and Environmental Safety* 74: 1779–1786
 5. Aslan, A., Cemek, M., Eser, O., Altunbas, K., Buyukokurog˘lu, M. E., Cosar, M., Bas, O., Ela, Y., Fidan, H., (2009). Does dexmedetomidine reduce secondary damage after spinal cord injury? An experimental study, *Eur. Spine J.* 18: 336–344.
 6. Astiz, M., Maria, J.T., Carlos, A. M. (2009 a). The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat. *Pesticide Biochemistry and Physiology* 94: 93–99.
 7. Banerjee, B.D., Seth, V., Bhattacharya, A., Pasha, S.T., Chakraborty, A.K. (1999). Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicology Letters* 107: 33–47
 8. Beeman, R.W., and Wright, V.F. (1990). Monitoring for resistance to chlorpyrifos methyl, pirimiphos methyl and Malathion in Kansas populations of stored-product insects. *J Kansas Entomol Soc.* 63:385–392.
 9. Beutler, E., Duron, O., and Kelly, B. M. (1963). Improved method for determination for liver glutathione. *J. Lab. Clin. Med* 61: 882-888.
 10. Cho, K.J., Han, S.H., Kim, B.Y., Hwang, S.G., Park K. K., Yang K.H., Chung, A.S., (2000). Chlorophyllin Suppression of Lipopolysaccharide-Induced Nitric Oxide Production in RAW 264.7 Cells. *Toxicology and Applied Pharmacology* 166: 120–127.
 11. Comporti, M., (1985). Lipid peroxidation and cellular damage in toxic liver injury, *Lab. Invest.* 53: 599–603.
 12. Egner, P.A., Wang, J.B., Zhu, Y.R., Zhang, B.C., Wu, Y., Zhang, Q.N., Qian, Q. S., Kuang, S. Y., Gange, S.J., Jacobson, L.P., Helzlsouer, K.J., Bailey, G.S., Groopman, J.D., Kensler, T.W. (2001). Chlorophyllin intervention reduces aflatoxin–DNA adducts in individuals at high risk for liver cancer, *Proc. Natl. Acad. Sci. U. S. A.* 98: 14601–14606.
 13. El-Sharkawy, A. M., Abdel-Rahman, S. Z., Hassan, A. A., Gabr, M. H., El-Zoghby, S. M., El-Sewedy, S. M., (1994): Biochemical effects of some insecticides on the metabolic enzymes regulating glutathione metabolism. *Bull. Environ. Contam. Toxicol.* 52: 505–510.
 14. Esterbauer, H., Cheeseman, K.H., Danzani, M.U., Poli, G., and Slater, T.F. (1982). Separation and characterization of the aldehyde products of ADP/Fe²⁺ C stimulated lipid peroxidation in rat liver microsomes. *Biochem. J.* 208: 129-140.
 15. Giri, S., Prasad, S.B., Giri, A., Sharma, G.D. (2002). Genotoxic effects of Malathion: an organophosphorus insecticide, using three mammalian bioassays in vivo. *Mutat. Res.* 514: 223–231.
 16. Goldberg, D. M., and Spooner, R. J., (1983). UV method for determination of glutathione reductase activity. *Methods of enzymatic analysis*

- (Bergmeyer, H.V. Ed.) third Edn 3: 258-265.
17. Habig, W., Pabst, M., and Jakoby, W. (1974). Colorimetric determination of Glutathion S Transferase. *J. Biol. Chem* 249: 7130-7139.
 18. Hazarika, A., Sarkar, S. N., Hajare, S., Kataria, M., Malik, J.K. (2003). Influence of Malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. *Toxicology* 185: 1-8
 19. Henry, R.J. (1974). Colorimetric assay for the determination of Cholinesterase in serum and plasma. *Clin. Chem., Principle and Tech.* Harber u. Row Pulpishers Inc., II edition, pp. 917.
 20. Kamat, J.P., Boloor, K.K., Devasagayam, T.P.A. (2000). Chlorophyllin as an effective antioxidant against membrane damage in vitro and ex vivo. *Biochimica et Biophysica Acta*, 1487: 113–127.
 21. Khan, S.M., Sobti, R.C., Kataria, L. (2005). Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract, *Clin. Chim. Acta*, 358: 131–138.
 22. Kumar, S.S., Shankar, B., Sainis, K.B. (2004). Effect of chlorophyllin against oxidative stress in splenic lymphocytes in vitro and in vivo. *Biochimica et Biophysica Acta*, 1672: 100–111
 23. L´opez, O.A.F., Hernandez, L., Rodrigo, F., Gil, G., Pena, J.L., Serrano, T., Parr´on, E., Villanueva, A. (2007). Changes in antioxidant enzymes in humans with long-term exposure to pesticides. *Toxicology Letters* 171: 146–153
 24. Lasram, M. M., Annabi, A.B., Elj, N., Selmi, S., Kamoun, A., El-Fazaa, S., Gharbi, N. (2009). Metabolic disorders of acute exposure to Malathion in adult Wistar rats. *J. Hazard. Mater.* 163: 1052–1055.
 25. Lee, D.H. (2003). kinetic colorimetric method for determination of γ -GT activity. *Clin. Chem.* 49: 1358-1366.
 26. Lim, J.S., Yang, J.H., Chun, B.Y., Kam, S., Jacobs, D.R., Lee, D.H., (2004). Is serum g glutamyl transferase inversely associated with serum antioxidants as a marker of oxidative stress? *Free Radic. Biol. Med.* 37:1018–1023.
 27. Mahmoud, A.M. (2011). Influence of rutin on biochemical alterations in hyperammonemia in rats. *Experimental and Toxicologic Pathology.* 12: 55-65
 28. Marczin, N., Elhabashi, N., Hoare, G. S., Bundy, R.E., Yacoub, M. (2003). Antioxidants in myocardial ischemia reperfusion injury therapeutic potential and basic mechanisms, *Arch. Biochem. Biophys.* 5: 222–236.
 29. Montgomery, H.A.C., and Dymock, J.F. (1961). Colorimetric determination of nitrite. *Analyst* 86: 414.
 30. Nishikimi, M., Rao, N.A., and Yogi, K. (1972). Colorimetric determination of super oxide dismutase. *Biochem. Bioph. Common.* 46: 849-854.
 31. O`zer, S., Ayfer, T., G`ulden, O., Sule, C., Gazi, C., Nursal, G., G`oksel, S., (2008). Protective effect of resveratrol against naphthalene-induced oxidative stress in mice. *Ecotoxicol. Environ. Safety* 71: 301–308.
 32. Ong, T.M., Whong, W.Z., Stewart, J., and Brockman, H.E. (1986). Chlorophyllin: A potent antimutagen against environmental and dietary complex mixtures. *Mutat. Res.* 173: 111–115.
 33. Paglia, D.E., and Valetine W.N. (1967). UV method for determination of glutathione peroxidase activity. *J. Lab. Clin. Med.* 70: 158-169.
 34. Roy, S., Roy, S., Sharma, C.B., (2004). Fenitrothion-induced changes in lipids of rats, *Biomed. Chromatogr.* 18: 648–654.
 35. Schoslnsky, K.H., Lehmann, H. P., and Beeler, M. F. (1974). Measurement of ceruloplasmin from its oxidase activity in Serum by Use of

- O-Dianisidine Dihydrochloride.
Clinical Chemistry, 20: 1556-1563.
36. Silva, A.P., Meotti, F.C., Santos, A. R. S., Farina, M. (2006). Lactational exposure to malathion inhibits brain acetylcholinesterase in mice. *NeuroToxicology* 27: 1101–1105
 37. Sinha, A.K., (1972). Colorimetric assay of catalase. *Analytical Biochemistry* 47: 389.
 38. Thampi, H.B.S., Manoj, G., Leelamma, S., Menon, V. P. (1991). Dietary fiber and lipid peroxidation: effect of dietary fiber on levels of lipids and lipid peroxides in high fat diet, *Indian J. Exp. Biol.* 29: 563–567.
 39. Thannickal, V.J., Fanburg, B.L. (2000). Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 279: 1005–1028.
 40. Uchida, K., Shiraishi, M., Naito, Y., Torii, Y., Nakamura, Y., Osawa, T. (1999). Activation of stress signalling pathways by the end product of lipid peroxidation-4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production, *J. Biol. Chem.* 274: 2234-2242.
 41. Vallance, P., Leiper, J. (2002). Blocking NO synthesis: how, where and why? *Nat Rev Drug Discov* 1: 939–950.



التأثير الوقائي لمادة الكلوروفيلين على الإجهاد التأكسدي المحدث باستخدام البيريميفوس ميثيل في دم ومخ "الفئران"

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الملخص العربي

أصبحت المركبات الفسفورية العضوية هي أكثر المبيدات الحشرية انتشاراً اليوم على الإطلاق 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 للكلوروفيلين 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 وتتراوح أوزانها بين 150-200 جرام هذا وقد تم تقسيم الفئران إلى أربعة مجموعات متساوية اشتملت كل مجموعة على عدد 15 فأر وتم توزيعها كالاتي: مجموعته (1): تعتبر المجموعة الضابطة لم تتناول أي دواء. مجموعته (2): تجرعت البيريميفوس ميثيل يوماً لثلاثة أشهر. مجموعته (3): تجرعت مستخلص الكلوروفيلين يوماً لثلاثة أشهر. مجموعته (4): تجرعت البيريميفوس ميثيل ومستخلص الكلوروفيلين يوماً لثلاثة 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 والرابعة. مما سبق نستنتج أن الكلوروفيلين له تأثير وقائي واضح في حماية الفئران من التأثير الضار لمادة البيريميفوس ميثيل ولذلك ننصح بضرورة استخدامه كمادة فعالة في العقاقير المستخدمة للوقاية من الإجهاد التأكسدي الناتج عن التعرض للعديد من ملوثات البيئة ومن ضمنها المبيدات الحشرية.

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