

## Pentoxifylline attenuates malathion-induced oxidative damage in rat

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### ABSTRACT

**Introduction:** Toxic effects of pesticides are commonly associated with reactive oxygen species damage and pentoxifylline a phosphodiesterase inhibitor is a drug well known for antioxidant properties. The purpose of this study was to evaluate the oxidative damages following a subacute exposure to malathion, an organophosphorus insecticide and pentoxifylline's ability to counteract these effects.

**Material and Methods:** Malathion (200 mg/kg/day) and pentoxifylline (50 mg/kg/day) alone or in combination were administered intraperitoneally to rats and after one week, total antioxidant capacity (TAC) and total thiol groups (TTG) were measured in their blood.

**Results:** Pentoxifylline increased total antioxidant capacity (TAC) and total thiol groups (TTG) in blood significantly compared to malathion which decreased them.

**Conclusion:** Our findings suggest that oxidative stress occurs in exposure to malathion and oxidative damage may be a contributory factor in complications associated with malathion. The results indicate that malathion exposure decreases TAC and TTG, a process involved in oxidative stress and pentoxifylline could prevent this damage.

**Keywords:** Malathion, Oxidative Stress, Pentoxifylline, Antioxidants Analysis, Rats

### INTRODUCTION

Malathion [S-1,2 (bis-ethoxycarbonyl) ethyl O, O-dimethyl phosphorodithioate], an organophosphorus (OP) compound is commonly used as insecticide and acaricide in agricultural, veterinary, medical and public health practices. Its main toxicity is inhibition of acetylcholine-esterase (AChE) resulting in accumulation of acetylcholine (ACh). In recent years, studies have indicated that OP compounds especially malathion can induce oxidative stress by altering the status of oxidant-antioxidant balance of the body (1, 2) which

induces toxicity in many organs such as blood (3, 4), liver (5, 6) and muscles (7). Any imbalance between the production of reactive oxygen species (ROS) and cellular antioxidant capacity leads to oxidative stress, a condition that has been associated with a number of physiological and pathological events (8-12).

Pentoxifylline (PTX), a methylxanthine derivative and nonspecific type 5 phosphodiesterase inhibitor, is a drug widely used in the management of peripheral arterial disease and, in particular, for intermittent claudication (13-15). PTX can enhance the chemotactic

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response of neutrophils but may inhibit phagocytosis and superoxide production by neutrophils and monocytes (16). Previous studies showed additional therapeutic potentials for PTX as an anti-inflammatory and immunomodulator agent. In recent years, in vitro and in vivo experiments indicated that it has antioxidant properties too (17-18).

Therefore, the goal of the present study was to investigate the effects of pentoxifylline on oxidative stress induced by malathion in rats.

## MATERIAL AND METHODS

### Chemicals:

Dithionitrobenzoic acid (DTNB), Tris base, 2,4,6-tripyridyl-s-triazine (TPTZ), malathion, ethylenediamine tetraacetic acid (EDTA) and pentoxifylline were used in this study.

### Animals and reagents:

Adult male Wistar rats weighing 180-250 g maintained on a 12-hr light/dark cycle with free access to tap water and standard laboratory chow were used. Animals were randomly divided into four groups of 6 animals. Three groups received malathion (200 mg/kg/day) and pentoxifylline (50 mg/kg/day) alone or in combination intraperitoneally (IP) on daily basis for one week. One group only received normal saline (0.9% NaCl) as control. At the end of treatment, 24 hours after the last dose of, animals were killed, their plasma were separated and their mitochondria were isolated quickly and kept frozen at -80°C.

### Assay of total antioxidant capacity (TAC):

Antioxidant capacity was determined by measuring the amount of reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  using FRAP test. Briefly, in this test, the medium is exposed to  $\text{Fe}^{3+}$  and the antioxidants present in medium reduce it to  $\text{Fe}^{2+}$ . The reagent contains TPTZ dissolved in acetate buffer (pH 3.6) and  $\text{FeCl}_3$ . The complex between  $\text{Fe}^{2+}$  and TPTZ gives a blue color with absorbance at 593 nm that is measured on the basis of a calibration curve obtained by different

concentrations of  $\text{FeCl}_3$ . Results were reported as nmol/mg Pr (3).

### Assay of Total Thiol Molecules:

Total sulfhydryl content was determined in plasma. 0.20 ml of plasma was mixed in a 10 ml test tube with 0.6 ml of Tris-EDTA buffer (Tris base 0.25 M, EDTA 20 mM, pH 8.2) followed by addition of 40 ml of 10 mM of DTNB in methanol. The final volume of the reaction mixture was made up to 4.0 ml by adding 3.16 ml of methanol. The test tube was capped and set aside for 15-20 min until the color developed then it was centrifuged at 3000 g for 10 min at ambient temperature. The absorbance of the supernatant was measured at 412 nm (3).

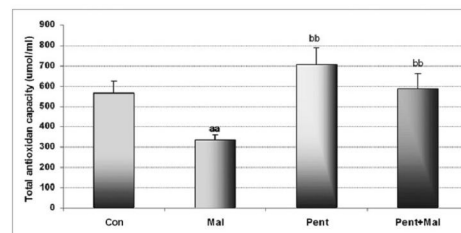
### Statistical analysis:

Mean and standard error values were determined for all the parameters and the results were expressed as mean $\pm$ SEM. All data were analyzed employing analysis of variance ANOVA followed by Tukey's post hoc test using SPSS version 16. Differences between groups were considered significant when  $p < 0.05$ .

## RESULTS

Figure 1 shows total antioxidant capacity in the plasma of rats one week after the treatment. There was a significant decrease in TAC in malathion group compared to control and PTX groups. Furthermore, TAC increased in PTX treated rats as compared to controls.

Fig 1: Total antioxidant capacity in plasma of rats after one week



intraperitoneal injections

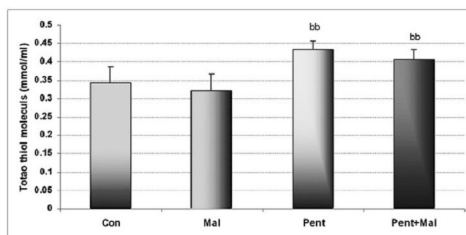
aa: significantly different from control group at  $P < 0.05$

bb: significantly different from Mal group at  $P < 0.05$

Mal: malathion, PTX: pentoxifylline, Con: control

Figure 2 demonstrates total thiol molecules in the plasma of rats. There was a significant decrease in total thiol groups (TTG) in malathion group when compared to PTX group.

Fig 2: Total thiol molecules in plasma of rats after one week of intraperitoneal injections



bb: significantly different from Mal group at  $P < 0.05$

Mal: malathion, PTX: pentoxifylline, Con: control

## DISCUSSION

The results of this study indicate that PTX is capable of ameliorating malathion-induced toxicity in rats. In this experiment, PTX noticeably improved biomarkers of oxidative stress including TAC and TTG.

Organophosphorus compounds are a group of pesticides widely used in agriculture and their biodegradability has made them good candidates for this purpose (19); however, OP-induced poisoning is also very common and unavoidable. As mentioned before, their main mechanism of toxicity is inhibition of AChE; furthermore, oxidative stress has been proven to be another major mechanism in OPs' acute and chronic toxicity (20, 21). The potential of malathion to induce oxidative stress in blood has been previously reported and confirmed by several studies (1, 2). Also, it has been established that OPs induce lipoperoxides LPO and decrease TAC which confirm their oxidative stress-inducing ability (3, 22).

Malathion, like other OPs, is detoxified via conjugation reactions with glutathione peroxidase

(GSH-px) (23). GSH depletion is associated with oxidative stress and cytotoxicity (24). Previous studies confirmed that malathion exposure causes a reduction of the GSH-px content in blood (25-26). In the present study a reduction in intracellular thiols such as GSH-px and TTG has been correlated with increased malathion toxicity (Fig. 2).

Several studies supporting our findings have shown that acute oral administration of OPs altered the thiol groups in blood (3, 22, 27). Supporting the present findings, malathion has also been proved to provoke alterations in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) following subchronic exposure in animals (1, 4).

In our previous studies, we demonstrated that OPs exposure significantly changes oxidative parameters such as TAC, TTG and thiobarbituric reactive substances (TBARS) in blood (2, 22) and impairs the enzymatic activity measured as CAT, SOD and GPx in acute exposure to malathion (4). Several studies indicate that malathion exposure increases TBARS levels in the erythrocytes, liver (4) and brain of rats (28). Our findings were in agreement with our previous studies in regard to alteration of TAC in response to malathion subacute toxicity (Fig. 1).

There is some evidence that increased intracellular concentrations of cAMP and cGMP following the employment of specific phosphodiesterase inhibitors can ameliorate cellular oxidative stress (29, 30). Our results revealed that PTX could increase nonenzymatic antioxidative parameters such as TAC and TTG in blood. This finding shows that PTX is effective in enhancing the antioxidant defense system versus reactive oxygen species harmful activities.

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