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## Research Article

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### EFFECT OF VITAMIN C AND VITAMIN E ON MALATHION INDUCED STRESS ON TESTICULAR TISSUE *IN VITRO*

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(Received: May 14, 2012; Accepted: July 19, 2012)

#### ABSTRACT

During the present investigation ameliorating effect of vitamin C and vitamin E on Malathion induced testicular toxicity has been analyzed in testis tissue of goat *in vitro*. Cultured testis tissue treated with three doses of Malathion concentration (1nm, 100nm, 10-3nm/ml) showed a significant decrease in antioxidants enzyme Catalase and Superoxide dismutase activities. In contrast, supplementation with vitamin C and vitamin E (100µmol/L) reduced testicular oxidative stress. These results indicate that vitamin C and vitamin E have protective roles *in vitro* on the Malathion induced testicular tissue in Catalase and Superoxide dismutase enzyme activities and reduce the testicular damage by Malathion. Thus, the role of vitamins C and E in amelioration Malathion induced oxidative stress has been worked out is not protective in absolute terms.

**Keywords:** Malathion, vitamin C, vitamin E, Testis, enzyme activity, amelioration.

#### INTRODUCTION

Pesticides are toxic chemicals deliberately spread into the environment with the aim of controlling undesired living species. OP is known to inhibit acetylcholinesterase and pseudocholinesterase activity in target tissues (John et al., 2001; Kalenderet al., 2006). A number of systems get affected by OP exposure like the immune system (Handy et al., 2002), pancreas (Gokalpet al., 2005), liver (Kalenderet al., 2005), hematological system (Kalenderet al., 2006) and reproductive system (Faraget al., 2000).

Exposure to Malathion showed decreased testes weight and activity of testicular enzymes (Balasubramaniam, 1987). Malathion exposure has been shown to significantly decrease the sperm count of mice (Bustos Obregón and González-Hormazabal, 2003).

Besides Malathion induces more oxidative stress to testicular tissue are due to the existence more oxidative stress of

polyunsaturated fatty acids testes. (mathuret al., 2011, Turner et al., 2008)

Antioxidants and antioxidative enzymes play an important role in offering protection against oxidative damage. The Ameliorative effects of vitamins C and E, as antioxidants, have been studied with a number of pesticides (Kalenderet al., 2006, 2007; Uzunhisarcikliet al., 2007; Ogutcu et al., 2008). Vitamin C (ascorbic acid) is an effective antioxidant of the hydrophilic phase (Jurczuket al., 2007). Moreover, vitamin C can restore the antioxidant abilities of vitamin E, which suggests that a major function of ascorbic acid is to recycle the tocopheroxyl radical (Serbecic and Beutelspacher, 2005). Vitamin E (α-tocopherol) is the major lipid-soluble antioxidant and is known to protect cellular membranes and lipoproteins from peroxidation (Yavuzet al., 2004). . The antioxidant vitamin E plays a role in the endogenous defence against peroxidation of membrane

lipids (Packer L et al., 2001). These vitamins can also prevent genetic changes by inhibiting the DNA damage induced by reactive oxygen metabolites. (Vermaet al, 2007).

The antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase are the main enzymes that act as defenses and act in concert with non-enzymatic reduced glutathione antioxidants and other antioxidants, such as  $\alpha$ -tocopherol and selenium (Halliwell and Gutteridge, 1999) protect against the adverse effects of ROS  $\alpha$ -tocopherol is the major lipid soluble antioxidant and is known to protect cellular membranes and lipoproteins from peroxidation, (Yavuzet al., 2004). Superoxide dismutase destroys superoxide radical by converting it to peroxide that in turn could be destroyed by glutathione peroxidase or catalase.

This study was made to determine the effect of Malathion exposure goat testis on antioxidative enzymes and to establish whether these effects can be ameliorated by co-treatment with vitamins C and E.

#### Material

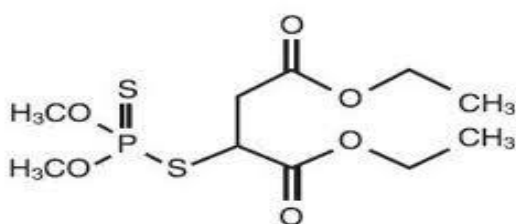
The mature goat (*Capra hircus*) testes were procured from slaughter houses around Kurukshetra (29°6' N, 76°5' E). The material was brought to the Reproductive Physiology Laboratory, Department of Zoology, Kurukshetra University, and Kurukshetra at 4°C in normal saline.

#### Testicular tissue culture

After washing with the normal saline the testis was decapsulated and cut into small pieces for culture. The medium was prepared by mixing TCM199 and antibiotics. The tissues were divided into two groups (Control and Experimental groups). Experimental group was treated with different concentration of malathion (1×10<sup>-3</sup>nm, 1nm, 100nm) and vitamin C(100μmol/L) and vitamin E(100μmol/L). Experiment is divided into five part(10-3nm, 1nm, 100nm, Vitamin C and Vitamin E doses).

#### Malathion (Organophosphate)

Structure:



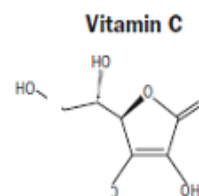
Chemical formula: C<sub>10</sub>H<sub>19</sub>O<sub>6</sub>PS<sub>2</sub>

Molecular mass: 330.36

IUPAC Name: Dimethoxyphosphinothioyl

#### Vitamin C (Ascorbic acid)

Structure

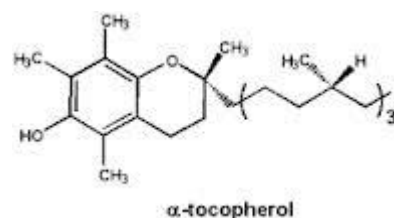


Chemical formula: C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>

Molecular mass: 176.12

#### Vitamin E (α-Tocopherol)

Structure



Chemical formula: C<sub>29</sub>H<sub>50</sub>O<sub>2</sub>

Molecular mass: 430.69g mol<sup>-1</sup>

#### Methods

Harvesting of testicular tissue was carried out after the specified duration.

##### 1) Catalase (EC. 1. 11. 1. 6)

Catalase was assayed by the method of Abbe (1984). Briefly, the assay mixture contained 2.25 ml phosphate buffer, 0.65 ml hydrogen peroxide and 0.1ml enzyme source. The decrease in absorbance was measured immediately at 240wavelength nm, against a blank containing all the components except the enzyme at 10 seconds interval for 3 minute on a systronics spectrophotometer.

##### 2) Superoxide dismutase (EC. 1. 15. 1. 1)

Superoxide dismutase was assayed by the method of Marklund (1974). Briefly, the assay mixture contained 2ml 0.1mM Tris HCl buffer, 500μl of 0.2mM pyrogallol and 750μl enzyme source. The increase in absorbance was measured immediately at 420nm, against a blank containing

all the components except the enzyme and pyrogallol, at 10s intervals for 3 minute on a Systronics Spectrophotometer.

**3) Statistical analysis**

Students t test was used for comparing the level of significance in the results between Malathion treated group and control group. Level were set at P<0.05 by SPSS version 16.

**RESULTS**

The activities of superoxide dismutase and catalase of the testicular tissues showed a significant (P<0.05) decrease in malathion treated testicular tissue for 4h and 8h in comparison with the control. The activity of catalase and superoxide dismutase showed a significant decline in dose

**Table-1:** Effect of varied concentration (1nm, 10-3nm, 100nm) of Malathion on Catalase activity (mU Cat/mg tissue) and ameliorating effect of vit C and vit E (100µg).

Time duration	control	10 <sup>-3</sup> nm				
		10 <sup>-3</sup> nm	Vit C	Vit E		
4hr	3.10±0.02	2.84±0.04	2.92*±0.04	2.80±0.09		
8hr	2.13±0.17	1.44*±0.04	1.55*±0.07	1.50*±0.06		
<b>Contd.</b>						
Time duration	1nm		100nm			
	1nm	Vit C	Vit E	100nm	Vit C	Vit E
4hr	2.67*±0.09	2.98*±0.05	2.82*±0.09	2.25*±0.02	2.35*±0.02	2.30*±0.02
8hr	1.38*±0.06	1.52*±0.54	1.40*±0.11	1.54*±0.06	1.80*±0.05	1.69*±0.06

Values are expressed as mean±SE, \*p<0.05

**Table-2:** Effect of varied concentration (1nm, 10-3nm, 100nm) of Malathion on Superoxide Dismutase activity (mu SOD /mg tissue) and ameliorating effect of vit C and vit E (100µg)

Time duration	control	10 <sup>-3</sup> nm				
		10 <sup>-3</sup> nm	Vit C	Vit E		
4hr	6.62±0.26	6.15*±0.11	6.30*±0.20	6.21*±0.44		
8hr	8.60±0.80	6.26*±0.13	6.40*±0.16	6.45*±0.50		
<b>Contd.</b>						
Time duration	1nm		100nm			
	1nm	Vit C	VitE	100nm	VitC	VitE
4hr	4.25*± 0.17	4.36*±0.33	4.83* ±0.31	3.36*±0.10	3.40* ± 0.20	3.61* ± 0.21
8hr	5.11* ±0.06	5.11*±0.06	5.33* ±0.11	2.86* ±0.02	3.12* ± 0.28	3.15* ± 0.12

Values are expressed as mean ± SE, \*p<0.05

**Table-3:** Pattern of changes in percentage catalase enzyme activity after exposure of malathion (1nm,100nm,10<sup>-3</sup>) and ameliorating effect of vit C & vit E.

Parameter	Time duration	Control	10 <sup>-3</sup> nm			1nm			100nm		
			10 <sup>-3</sup> nm	Vit C	Vit E	1nm	Vit C	Vit E	100nm	Vit C	Vit E
Percentage enzyme activity	4h	100	91.6	94.2	90.33	86.13	96.13	90.97	72.59	75.81	74.2
Decline(%) enzyme activity		8.38	5.80	9.67	13.87	3.87	9.03	27.41	24.19	25.8	
Percentage enzyme activity	8h	100	67.61	72.77	70.43	64.79	71.37	65.73	72.31	84.51	79.35
Decline(%) enzyme activity		32.39	27.23	29.57	35.21	28.63	34.27	27.69	15.49	20.65	

Values are expressed as mean  $\pm$  SE, \*p<0.05

**Table-4:** Pattern of changes in percentage superoxide dismutase enzyme activity after exposure of malathion (1nm,100nm,10<sup>-3</sup>) and ameliorating effect of vit C & vit E.

Parameter	Time duration	Control	10 <sup>-3</sup> nm			1nm			100nm		
			10 <sup>-3</sup> nm	Vit C	Vit E	1nm	Vit C	Vit E	100nm	Vit C	Vit E
Percentage enzyme activity	4h	100	92.91	95.18	93.81	64.2	65.9	72.97	50.76	51.36	54.55
Decline(%) enzyme activity		7.09	4.82	6.11	35.80	34.1	27.03	49.24	48.64	45.45	
Percentage enzyme activity	8h	100	72.8	74.42	74.49	59.42	60.82	61.98	33.26	36.28	36.63
Decline(%) enzyme activity		27.20	25.58	29.57	40.58	39.18	38.02	66.74	63.72	63.37	

Values are expressed as mean  $\pm$  SE, \*p<0.05

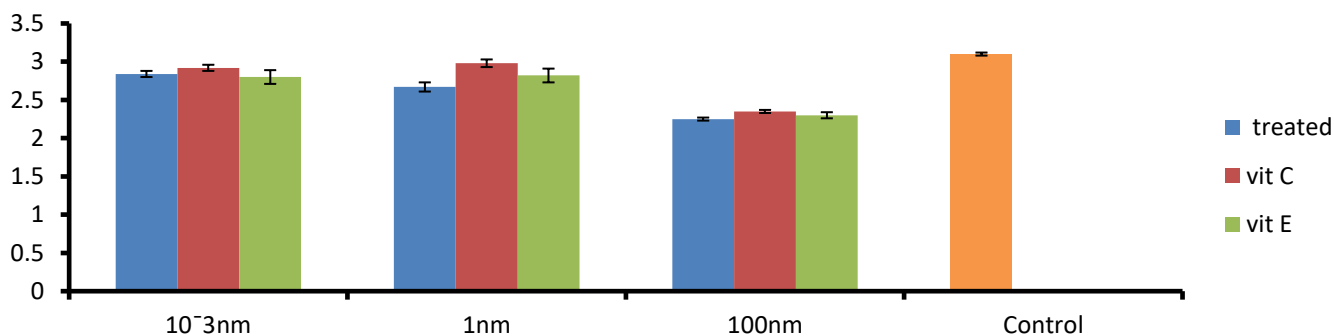


Fig.1 Malathion induced changes in Catalase activity (mU/mg tissue) and amelioration in testicular tissue of goat at 4h duration

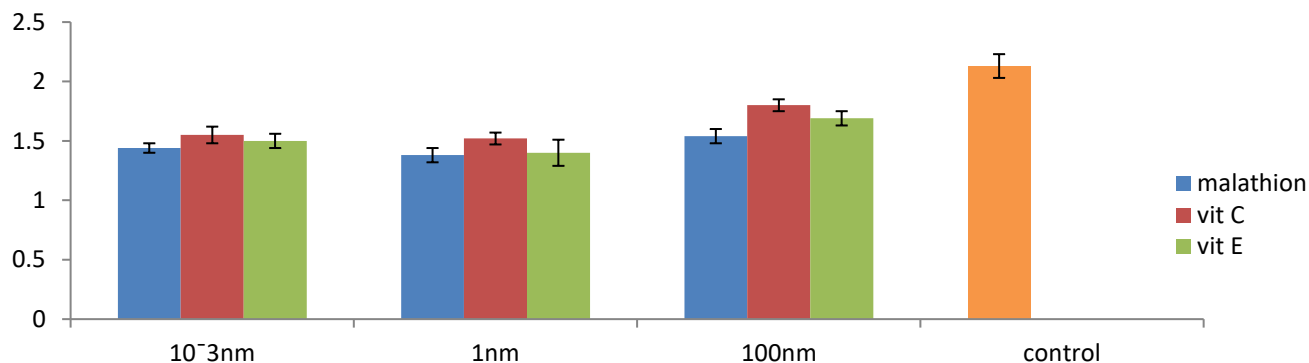


Fig.2 Malathion induced changes in Catalase activity (mU/mg tissue) and amelioration in testicular tissue of goat at 8h duration.

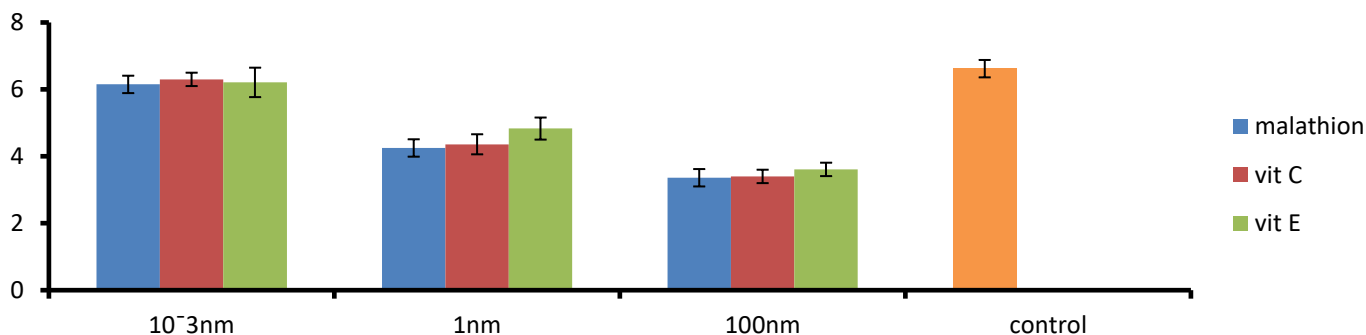


Fig. 3 Malathion induced changes in Superoxide dismutase activity (mU/mg tissue) and amelioration in testicular tissue of goat at 4h duration

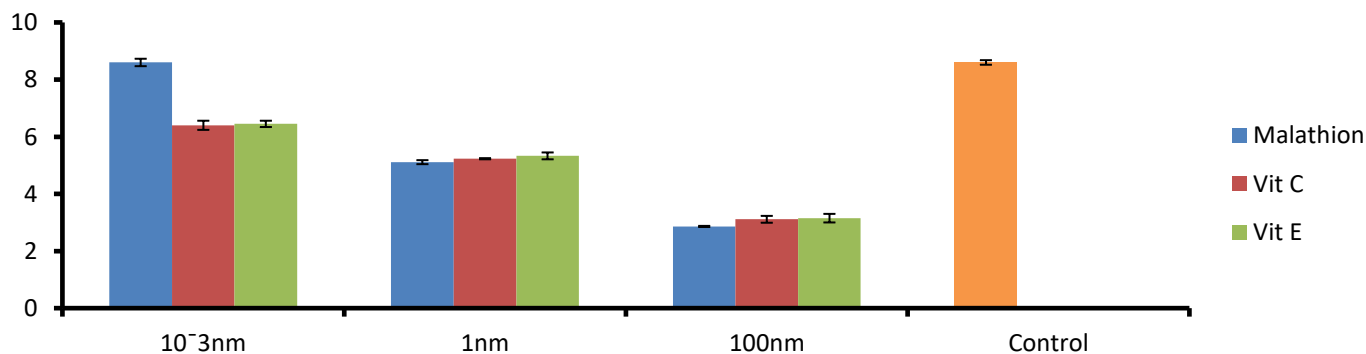


Fig. 4 Malathion induced changes in Superoxide dismutase activity (mU/mg tissue) and amelioration in testicular tissue of goat at 8h duration

dependent manner in testis tissue treated with malathion for 4h and 8h (figure 1 to 4). Both vitamin C and vitamin E significantly mitigated the effects of malathion activities of antioxidants enzymes. The effect of vitamin C and vitamin E on the activity of catalase and superoxide dismutase were significantly ( $P < 0.05$ ) higher than that of malathion (table 1 and table 3). The vitamin C and vitamin E were improved ( $p < 0.05$ ) or ameliorate the effect of malathion (Table 2 and 4). The activity of catalase and superoxide dismutase progressively improved with the increasing vitamin C and vitamin E dose levels.

## DISCUSSION

During the present investigation, we have observed that different doses and time duration of Malathion in testicular tissue in vitro alters the antioxidant system. The testicular tissue treated with malathion in Catalase and superoxide dismutase show a significant changes compared with those of control group. Rezg et al., 2008 reported that subchronic treatment with malathion, gradually decreased the activities of SOD and Catalase in rat liver. In our study,  $100\mu\text{molL}^{-1}$  concentration of vitamin C and vitamin E induced protective role against the oxidative stress induced by malathion at dose level 10-3nm, 1nm and  $100\text{nm L}^{-1}$  shown in table 1 and 3. The protective action of antioxidant may be due to an inhibition of ROS inducing a chain reaction mediated by several antioxidant enzymes including Catalase and superoxide dismutase. The present study further advocates the findings of Latchoumycandaneet al, 2002b who have reported the effect of short term exposure to methoxychlor on testicular antioxidant system was studied by administering various doses of methoxychlor (50, 100, 200mg/kg body weight per day) for 1, 4,7 days in order to induces oxidative stress in the testis. Malathion treated testicular tissues were supplemented with vitamin C and vitamin E showed a significant improvement in testicular tissue of goat in table 2 and 4. Supporting the notion that OPs like Malathion exert their deleterious effects by promoting destructive oxidation of lipids, proteins and DNA within the testes that co-treatment of malathion-exposed rats with vitamins E and C ameliorated the effects of Malathion on the activity of enzyme. These results may be explained that vitamin C and vitamin E attenuate the oxidative stress in testicular tissue of goat after treated with Malathion. It

becomes evident from the present study that exposure to malathion influences in physiology of the testicular tissue thus influence fertility. But after amelioration with vitamin C and vitamin E confirmed showed better protection against the oxidative stress of testicular tissue of goat in vitro.

## Acknowledgement

Author is thankful to Reproductive Physiology Lab, Department of Zoology, Kurukshetra University, Kurukshetra for providing all the facilities throughout the study.

## REFERENCES: