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# MALATHION INDUCED CHANGES IN CATALASE AND SUPEROXIDE DISMUTASE IN TESTICULAR TISSUES OF GOAT *IN VITRO*

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## ABSTRACT

*In the present study alterations in the activity of two antioxidant enzymes, catalase (EC.1.15.1.1) and superoxide dismutase (EC.1.11.1.6) in Malathion exposed testicular tissue of goat in vitro have been analyzed by spectrophotometric method. Malathion exposure significantly decreased catalase activity from 2.29±0.05 to 1.66±0.05 and 1.49±0.03 after 4 hour of exposure at 1nm and 100nm/ml concentration respectively. After 8 hour exposure at 100nm/ml showed significant decline from 2.13±0.16 to 1.33±0.04.At the same dose level, Malathion induced a significantly decline in superoxide dismutase activity from 6.96±0.15 to 4.85±0.20, 4.12±0.12 and 3.84±0.04 after 8 hour of exposure (p<0.05). These results indicate that short-term exposure to Malathion induces oxidative stress in the testis by decreasing antioxidant enzymes.*

## *KEY WORDS*

*Malathion, Testis, Catalase, Superoxide dismutase.*

## INTRODUCTION

Malathion, an organophosphorous compound is extensively used in agriculture, veterinary, medical and public health practices, because it is relatively cheap and possesses low acute toxicity towards mammals [1]. Pesticide hazards to man and environment are mushrooming in the developing countries, but developed nations are also facing problem in certain restricted areas [2].

It has been postulated that organophosphorous induces oxidative stress in different tissues through formation of reactive oxygen species. Oxidative stress is positively correlated to xenobiotic exposure at different levels of environmental contamination [3]. The peroxidation of membrane lipids seems to be an unavoidable process in tissue injury, which can impair antioxidant defenses, result in oxidative damage by tilting the balance between oxidants and antioxidants [4-5].

The prevention of lipid peroxidation is an essential process in aerobic organisms, as lipid and lipid peroxidation products can cause DNA damage and directly inhibit proteins such as $Na^{+}/Cl^{+}$ ATPase and glutamate transporters [6].The main basis of organophosphorous toxicity in production of oxidative stress might be due to their “redox cycling” activity. These compounds readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hydrogen peroxide through dismutation reaction or Reactive Oxygen Species generation via changes in normal antioxidant homeostasis resulting in depletion of antioxidants [7]. Pesticides like Malathion sometimes cause overproduction of ROS in intra and extracellular spaces, and result in decline of sperm count and infertility in wildlife and human [8].The antioxidants system plays an effective role in protecting testes and other biological tissue below a critical threshold of ROS thus preventing testicular dysfunction [9].Antioxidants has a number of biological activities of carcinogens which can also prevent genetic changes by inhibiting DNA damage induced due to reactive oxygen metabolites [10].

## MATERIALS AND METHODS

***Reagents:***The reagents used during the study were of analytical grade and procured from standard laboratory suppliers.

***Experiment Design***: The testis of *Capra hircus* procured from the slaughter houses near Kurukshetra and brought to the laboratory in culture media. The testis was cut into small pieces and processed for in vitro experimental protocol. After washing with normal saline, the tissue was placed in culture medium (TCM-199) which was fortified with antibiotics (200 unit penicillin 10 IU/ml and streptomycin 1µg/ml). The 1 nanomole/ml Methyl parathion in culture medium is employed for toxicity assessment.

Experimental group was treated with different concentration of Malathion ($1\times10^{-3}$nm, 1nm, 100nm) each group was further divided into three subgroups A, B, and C exposed for 1h, 4h, and 8h respectively. The cultures were maintained at $39^0$C, humidity 95% and 5% $CO_2$ concentration and control was run simultaneously.

***Catalase (EC. 1. 11. 1. 6:*** Catalase was assayed by the method of Abbe [11]. 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 240 wavelength nm, against a blank containing all the components except the enzymeat10 seconds interval for 3 minute on a systronics spectrophotometer.

***Superoxide dismutase (EC. 1. 15. 1. 1):*** Superoxide dismutase was assayed by the method of Marklund [12].Briefly, the assay mixture contained 2ml 0.1mM TrisHCl buffer, 500µl of 0.2 mMpyrogallol and 750µl enzyme source. The increase in absorbance was measured immediately at 420nm, against ablank containing all the components except the enzyme and pyrogallol, at 10s intervals for 3 minute on a Systronics Spectrophotometer.

***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

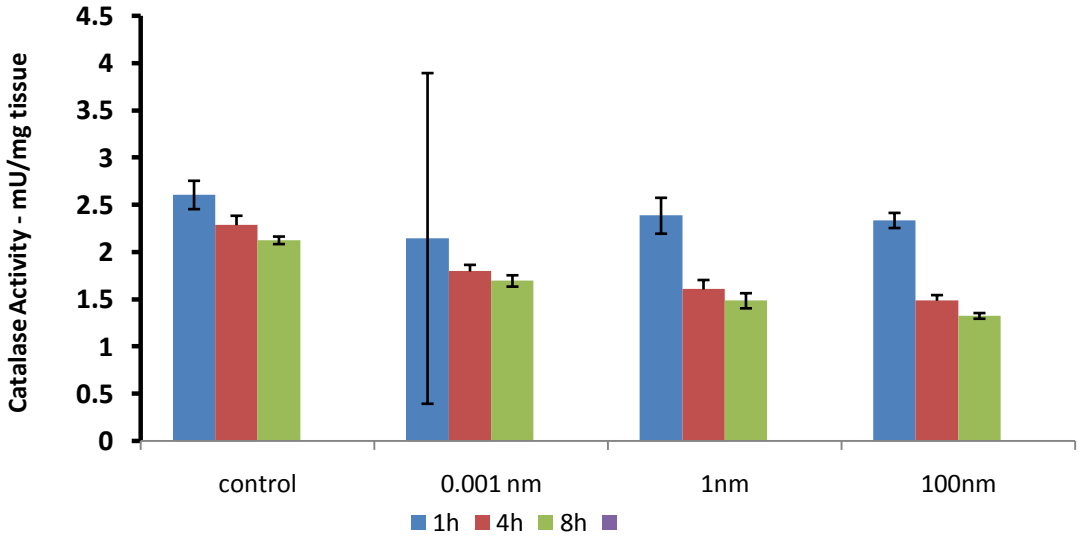
Different concentration of Malathion (1nm, 100nm, and $10^{-3}$nm) for 1h, 4h, and 8h were used to analyze the effect on catalase and superoxide dismutase activity in testicular tissue of goat. Activity of superoxide dismutase and catalase was affected by Malathion and it declined in dose and time depended manner. After 4 hour of exposure duration catalase activity declined from 2.29±0.05mU/mg in control to 1.80±0.04, 1.61±0.05, and 1.49±0.03 after exposure of $1\times10^{-3}$nm, 1nm and 100nm/ml concentration of Malathion respectively. As the exposure duration increased up to 8 hour. This catalase activity further declined from 2.13±0.16 in control group to 1.70±0.05 mU/mg tissue in $1\times10^{-3}$nm/ml, 1.49±0.05 in 1nm and 1.33±0.04mU/mg tissue in 100nm concentration (**Table-1, Figure-1**).

**Table 1: Effect of Malathion on catalase enzyme activity (mU/mg tissue) of control and experimental groups exposed to varied concentrations and time duration.**

| Exposure duration | Control | $1x10^{-3}$nm/ml | 1nm/ml | 100nm/ml |
|---|---|---|---|---|
| 1 Hour | 2.61±0.08 | 2.50±1.01 | 2.39±0.10 | 2.34±0.04 |
| 4 Hour | 2.29±0.05 | 1.80±0.04 | 1.61±0.05* | 1.49±0.03* |
| 8 Hour | 2.13±0.16 | 1.70±0.05 | 1.49±0.05 | 1.33±0.04* |

Values are expressed as mean±SE, *P<0.05

**Figure-1 Malathion induced changes in catalase activity (mU/mg tissue) in testicular tissues of goat.**



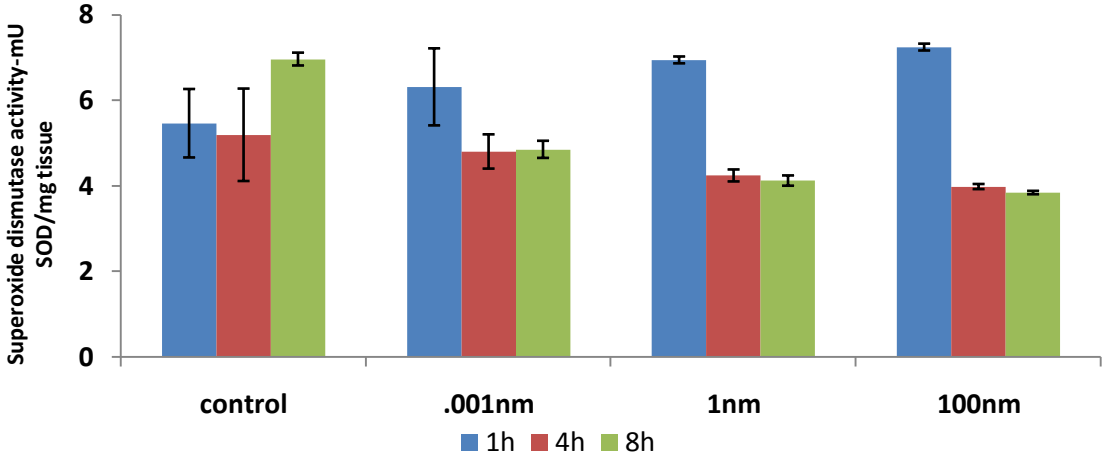

Activity of superoxide dismutase in control was 5.46±0.80mU SOD/ml tissue however it increased up to 6.31±0.90, 6.94±0.05 and 7.24±0.08 after 1 hour in $1\times10^{-3}$nm, 1nm, and 100nm/ml concentration. After 4 hour of exposure decline in the activity was noticed and it was 5.19±1.08 in control tissue. However it was 4.80±0.47 in minimum concentration ($1\times 10^{-3}$) and 3.98±0.06 in highest concentration (100nm).A significant decline was observed after 8 hour of exposure duration superoxide dismutase activity was 6.96±0.15 in control and declined to 4.85±0.20, 4.12±0.12, 3.84±0.04 after 8 hour exposure of 1×10-3nm, 1nm, 100nm/ml concentration of Malathion respectively (**Table-2, Figure-2**)

**Table 2: Effect of Malathion on Superoxide dismutase activity (mU/mg tissue) in goat testicular tissue exposed to different concentrations of Malathion for varied time duration.**

| Exposure duration | Control | $1x10^{-3}$nm/ml | 1nm/ml | 100nm/ml |
|---|---|---|---|---|
| 1 Hour | 5.46±0.80 | 6.31±0.9 | 6.94±0.08 | 7.24±0.08 |
| 4 Hour | 5.19±1.08 | 4.80±0.47 | 4.24±0.14 | 3.98±0.06 |
| 8 Hour | 6.96±0.15 | 4.85±0.20* | 4.12±0.12* | 3.84±0.04* |

Values are expressed as Mean±SD, *P<0.05

**Figure-2 Activity of superoxide dismutase on Malathion induced testicular tissues of goat.**



## DISCUSSION

In the present study the effect of short-term exposure to Malathion on testicular antioxidant system was studied by administering various doses of Malathion 1nmol, 100nmol, 10-3nmol/ml for 1, 4, 8 hour in order to identify whether Malathion induces oxidative stress in the testis. Enzyme activity of the Malathion treated testicular tissue of goat not showed no significant change after 1 hour of exposure duration compared with those of control group but after 4 and 8 hour exposure showed a significant decline the catalase and superoxide dismutase activity($p<0.05$) in 1nmol, 100nmol/ml concentration. The results of present study strongly support the earlier findingsthat the antioxidant enzymes, mainly Superoxide dismutase, Catalase was the first line of defense against free radical induced oxidative stress [13]. SOD was responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide. In our study we have observed that when the concentration of Malathion and exposure duration increased, the activity of enzyme significantly declined. It was also reported that subchronic treatment with Malathion gradually decreased the activities of Superoxide dismutase and Catalase in rat liver [14]. Our data showed that depletion of Superoxide dismutase, Catalase was directly mediated by Malathion, proved the role of enzymes as, antioxidant, converting superoxide radicals to hydrogen peroxide, then decomposition of hydrogen peroxide to molecular oxygen and water. The reduction in the activity of catalase is an index of an inability of the testes to eliminate hydrogen peroxide produced by activation of methoxychlor and its metabolites or inactivation of enzyme caused by excess ROS production in testis as already established [15]. The present study further advocates the findings reported earlier that the effect of short-term exposure to methoxychlor on testicular antioxidant system was studied by various dose (50, 100, 200mg/kg body weight per day) for 1, 4, 7 days in order to induced oxidative stress in testes [16]. The present study suggest that Malathion induced modest oxidative stress in testicular tissue of goat in vitro after 4 and 8 hour exposure and high concentration activity of enzymes decline significantly ($P<0.05$).

## CONCLUSION

It becomes evident from the present study that exposure to Malathion influences in physiology of the testicular tissue thus influence fertility.

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