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Research Article

IN VITRO STUDIES ON THE PROTECTIVE EFFECT OF VITAMIN C AGAINST MALATHION INDUCED TOXICITY IN THE GOAT (*CAPRA HIRCUS*) OVIDUCT

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ABSTRACT

During the present investigations protective effect of vitamin C on Malathion induced toxicity has been analysed in ampulla region of goat. *In vitro* cultured oviduct tissue treated with two doses of Malathion concentration (1nm, 100 nm) shows a significant disruption of mucosa and submucosal layers, damage of mucosal folds, degeneration of microvilli, blebbing in mucosa and increase in the width of lumen of oviduct. In contrast, supplementation with Vitamin C reduces oviduct damage by Malathion. The importance of carrying out *in vitro* reproductive toxicology assays lies on the need of knowing alteration which these insecticides may cause at cellular level, since they are endocrine disruptors that interfere with reproductive function.

Keywords: Ampulla, Malathion, Vitamin C, Reproductive toxicity.

INTRODUCTION

Pesticides are used worldwide in agriculture to control, destroy or inhibit weeds, insects, fungi, and other pests. Malathion is a pesticide that is widely used in agriculture, residential landscaping and in public health pest control programs such as mosquito eradication (Ferrer *et al*., 2003). It is one of the organophosphate insecticides which are used as an ectoparasiticide agent in veterinary medicine(USEPA, 2000). Muscular weakness, paralysis and neurotoxicity have been reported in vertebrates by exposure to low dose of organophosphates (Błasiak, *et al*., 1999). Goat is an important livestock species in developing countries. Goat is a polyestrous animal having oestrous cycle of 19-22 days. The breeding season in goat extends over seven month from late August to March. The reproductive success of female depends on the proper functioning of the reproductive tract, which provides nutrition and specific environment to the gametes for fertilization and early development. The oviducts are derived embryologically from the cranial region of the primitive Mullarian duct and Ampulla is the site where fertilization occurs. So, the influence of nanomolar concentrations of Malathion has been studied in goat oviduct in the present study. Vitamin C and E have been reported to divulge protective effects against free radical generation to enhance spermatogenesis in rats (Gabbianelli, *et al.*, 2004). Ascorbic acid also called Vitamin C is a puissant antioxidant. Its protective effect against testicular damage induced by Atrazine has been reported earlier (Sharma, *et al.*, 2010). Since Malathion has been reported to induce infertility, abortions and physical malformation in experimental animals (Cavieres, 2004), Vitamin C has been selected in the present study to investigate its protective effect against malathion induced toxicity in the goat *Capra hircus* oviduct.

MATERIAL AND METHODS

Collection of Material

During the present investigations, female genitalia of mature goats were procured from the slaughter houses of Kurukshetra. The material was brought to the laboratory in the Department of Zoology in ice bucket in the normal saline. On the basis of morphology of the oviduct, they

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were separated with the help of forceps, cut into small pieces and processed for *in vitro* experimental protocol as described in (Table 1).

Preparation of Malathion

The concentration of Malathion obtained from market was 50%. 330.4 gram of malathion was dissolved in 1000 ml dimithyl sulfoxide solution to get 1 molar solution. The stock concentration of 10^{-6} M was thus prepared in DMSO solution. Finally the required concentration that is 1 nM and 100 nM were made in the medium itself (TCM-99). After washing with normal saline the oviduct was placed in culture medium containing Malathion and the following experimental design was followed (Table 1).

Table 1. Experimental Design to study the effect of exposure time and dose of Malathion and Malathion + Vitamin C on goat oviduct.

Exposure Hours	Control Group A	Treated Group B	Treated GroupC	Ameliorating Group D	Ameliorating Group E
4	Group A1	Group B1 (1nm Malathion)	Group C1 (100nM Malathion)	Group D1 (1nm Malathion+100ug/ml Vit C)	Group E1 (100nM Malathion+200ug/ml Vit C)
8	Group A2	Group B2 (1nm Malathion)	Group C2 (100nM Malathion)	Group D2 (1nm Malathion+100ug/ml Vit C)	Group E2 (100nM Malathaion+200ug/ml Vit C)

The experiment was divided into 5 groups (Table 1). Group A was the zero hour control, group A1 for 4 hours control and group A2 for 8 hours control. Group B was exposed to 1nM Malathion where as group B1 was exposed for 4 hours while group B2 for 8 hours. Likewise group C1 was exposed to 100 nM pesticide concentration for 4 hours while group C2 was exposed to 100 nM Malathion for 8 hours. Group D (protective group) was administered with 1nM Malathion along with 100ug/ml Vitamin C where group D1 was exposed for 4 hours and group D2 for 8 hours. Lastly Group E was exposed to 100 nM Malathion plus 200 ug/ml Vit C. with exposure of 4 and 8 hours to groups E1 and E2 respectively. All the groups were further preceded for histopathalogical studies.

Histological Study

The tissue was harvested after stipulated time and processed for histological slide preparations. For histological slides, the oviduct was fixed in aqueous Bouins fixative for 24 hours. Then the tissue was washed in running tap water for six hours. It was dehydrated in various grades of alcohol. After proper dehydration it was cleared in xylene and embedded in paraffin wax at 58° to 60° C. The tissue was sectioned serially at 5 µm thickness with rotatory microtome followed by streching and dewaxing by xylene. After downgrading in different grades of alcohol, the sections were stained with the haemotoxylene for 10 minutes and allowed to develop for 5 to 15 minutes in tap water. After dehydration in 70% ethanol, the sections were stained with eosin for 1 to 2 minutes. The slides were washed in 70% ethanol and dehydrated in 90 % and absolute alcohol and cleared in xylene and were mounted in DPX. Each section was examined under Olympus microscope CX41 having Magnus digital Adaptor to study the morphological characteristics of tissue. Photomicrographs were captured at 40X and 400X.

RESULTS

The control groups showed the normal morphology of the oviducts (Figure 1 to 4). The present study revealed the deteriorating effects of 1nM (Figure 5 to 8) and 100 nM (Figure 9 to 12) Malathion exposure on goat oviduct in B and C groups respectively as compared to the control group of the same time durations (figs 1 to 4). Nanomolar concentration of Malathion caused disruption of mucosal folds, damage of submucosa and muscular layers and disorganization of mucosal lining (Figure 5 to 8). Further exposure for longer duration with 100 nM Malathion revealed severe disruptive observations which include degeneration of microvilli, blebbing in mucosa, vacuole formation, and damage to secretory cells and increase in the width of lumen of oviduct (Figure 9 to 12). Maximum damage was observed in the tissue at 8 hrs. Exposure of 100 nM Malathion. Pycnosis, vacuolation , disruption of mucosa and submucosa layers were frequently observed in ampulla of oviduct. All the degenerating changes increased as the duration of exposure was increased from 0 to 4 hours and 0 to 8 hours. Experiment group D was administered with 1nM dose of Malathion along with100ug/ml Vitamin C for two different time durations (Figure 13, 14, 15, 16). Significant restoring consequences were obtained in the histomorphological study of Vitamin C treated oviducts. There was recovery in the reduction of size of lumen of oviduct. Shape of mucosal folds and microvilli were reestablished up to a remarkable extent. Luminal diameter showed decreasing trend with increase in the duration of exposure time i.e. 8 hrs. (Figure 15) as compared to 4 hrs

(Figure 13). Group E administered with 100 nM Malathion plus Vit C had significant restoring consequences at higher doses and longer duration of exposures (Figure 16-20). Decrease in vacuolization and pycnosis was prominent revealing aphrodisiac property that helps in reestablishment of the structure of oviduct.

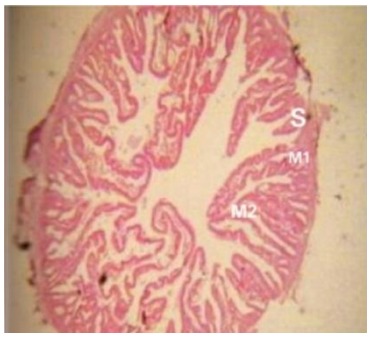

Figure 1. Control 4 hours (40X).

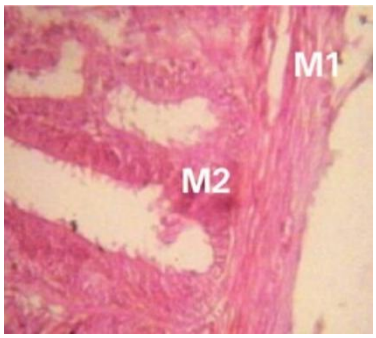

Figure 2. Control 4 hours (400X).

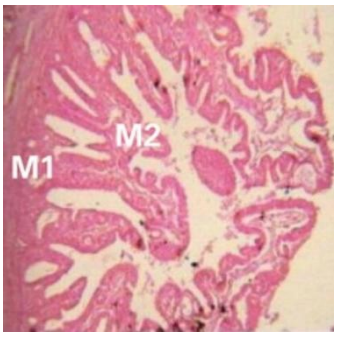

Figure 3. Control 8 hours (40X).

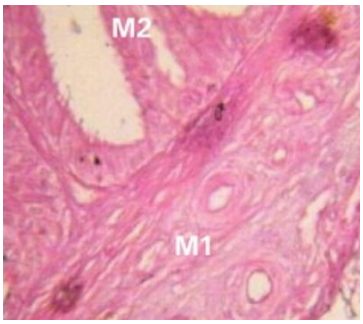

Figure 4. Control 8 hours (400X).

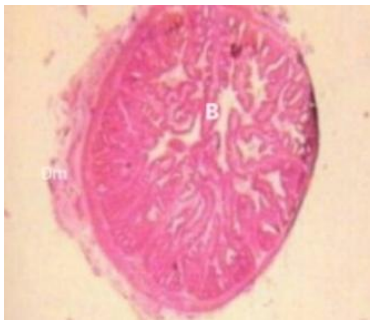

Figure 5. 1 nm 4 hours (40X).

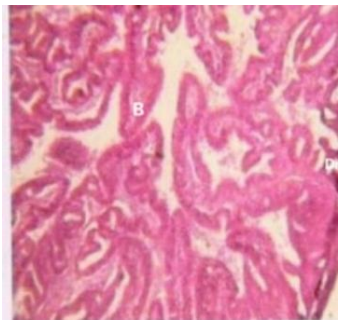

Figure 6. 1 nm 4 hours (400X).

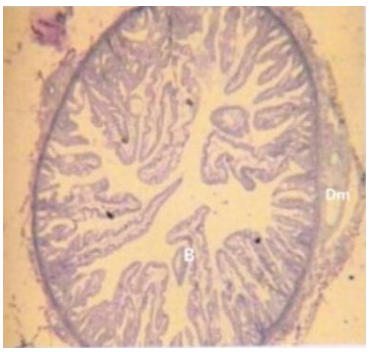

Figure 7. 1 nm 8 hours (40X).

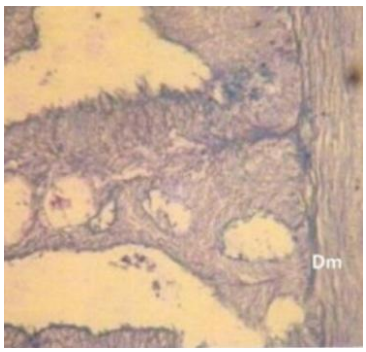

Figure 8. 1 nm 8 hours (400X).

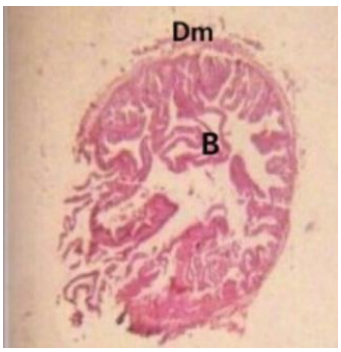

Figure 9. 100 nm 4 hours (40X).

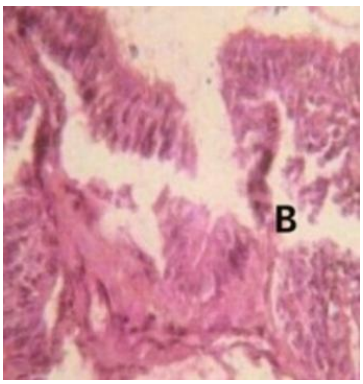

Figure 10. 100 nm 4 hours (400X).

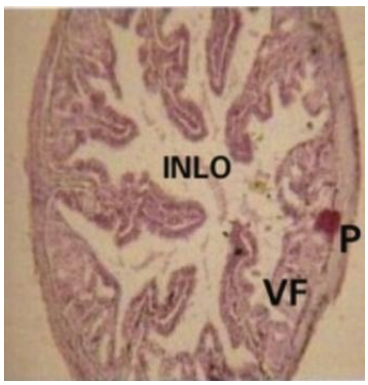

Figure 11. 100 nm 8 hours (40X).

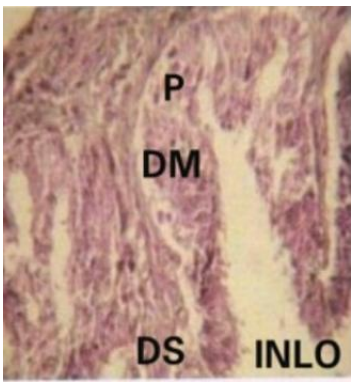

Figure 12. 100 nm 8 hours (400X).

S-Serosa, M1-Muscularis, M2- Mucosa, B-Blebbing in Mucosa, DM-Damage in Muscularis, Dm-Degenration in microvilli, DS-Damage to Secretory cells, VF-Vacuole Formation, P-Pycnosis, INLO-Increase in Lumen of Oviduct.

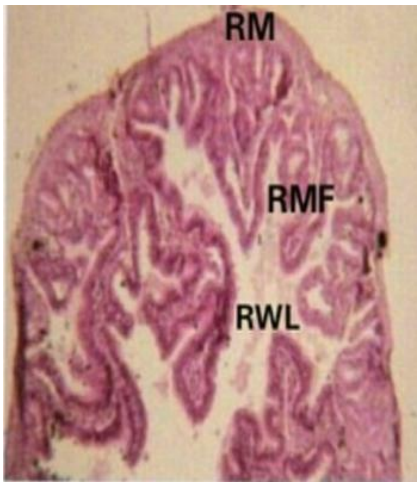

Figure 13. 100 nm + Vit C 4 hours (40X).

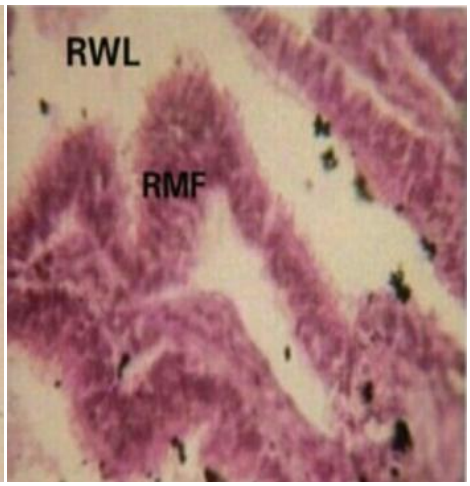

Figure 14. 100 nm + Vit C 4 hours (400X).

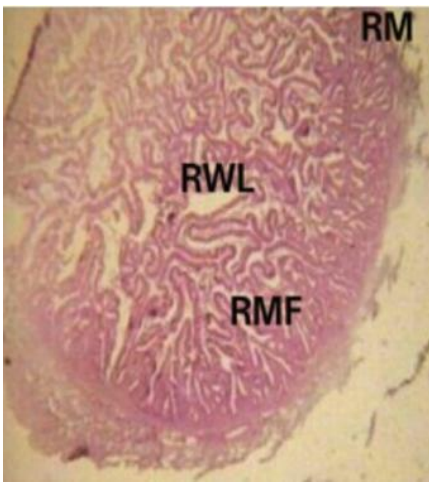

Figure 15. 100 nm + Vit C 8 hours (40X).

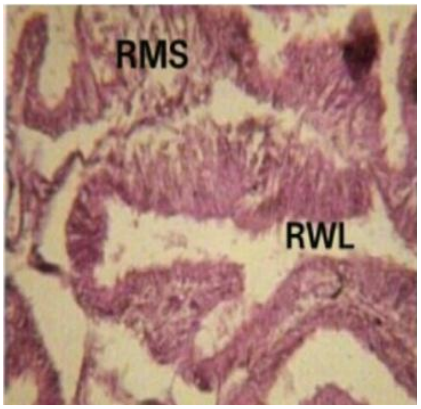

Figure 16. 100 nm + Vit C 8 hours (400X).

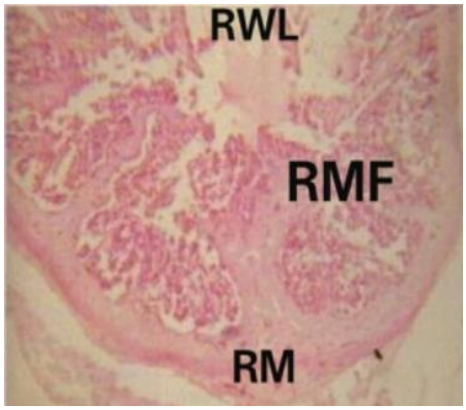

Figure 17. 100 nm + Vit C 4 hours (40X).

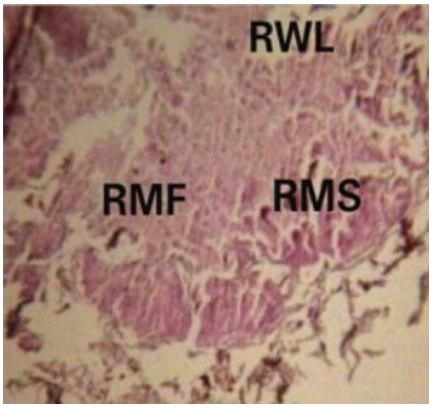

Figure 18. 100 nm + Vit C 4 hours (400X).

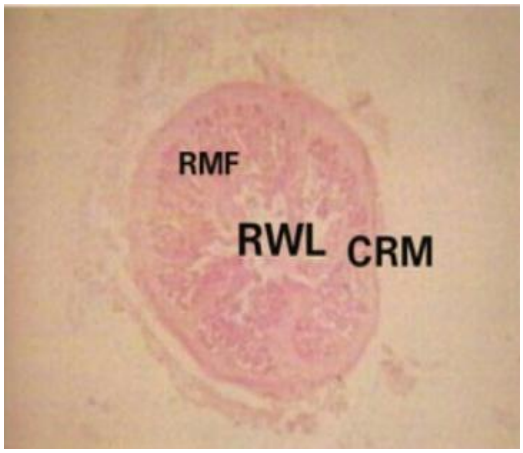

Figure 19. 100 nm + Vit C 8 hours (400X).

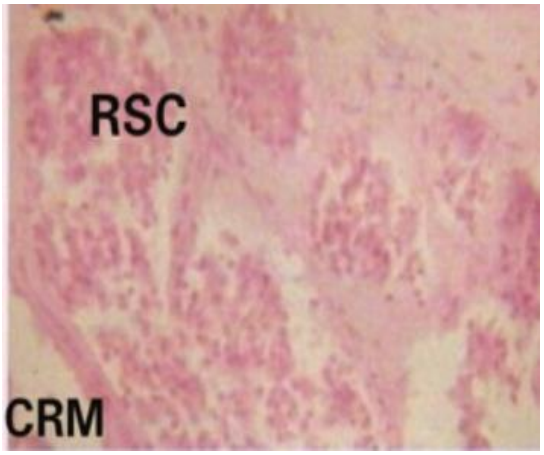

Figure 20. 100 nm + Vit C 8 hours (400X).

RWL-Reduced Width of Lumen, RMF- Recovery of Mucosal Folds, RM- Restored Muscularis, CRM-Complete Restoration of Muscularis, RSC-Recovered Secretory Cells.

DISCUSSION

Our present report of *in vitro* study on Malathion induced cytotoxicity in goat oviduct are in conformity with earlier the reports on Diazinon and Chlorpyrifos cytotoxicity (Giordano, *et al.,* 2007), genotoxicity (Giri, *et al.*, 2002) and toratogenic damage in several animals. The anti-fertility effects of this organophosphate cause various histopathological and cytopathological changes in the reproductive system of female mammals. Present observations of disruption of mucosal folds, damaged mucosal lining of isthmus, increased vacuolization reveal the effects of ova movement and impaired fertilization of ova. Studies on the toxic effects of various organophosphates on oviducts of various farm and domestic animals are scarce focusing on the need of recent research. The present study revealing the protective effect of Vitamin C on deteriorating effects of Malathion on goat oviduct are in consonance with the reports of (Giri *et al.*, 2002), who described that natural agents such as garlic extract or Vitamin C have ability to reduce deleterious effect of Permethrin. As Vitamin C supplemented group in the present study showed less abnormality and recovery of deteriorating effects, it is suggested that biological alternatives should be explored as an alternative to these toxic agents especially pesticides and parasiticides. Thus the present study suggests the role of vitamin C as an antitoxicological drug against commonly used organophosphates is worth further investigation.

CONCLUSION

Present study shows that Malathion is a potent organophosphate insecticide which damages the histomorphology of oviduct in goats that could be the serious cause of infertility in domestic animals. Present studies opine that Malathion disturbed normal oviduct functions either via enhancing oxidative stress or interfering with underlying endocrine regulation. Frequent exposure as well as acute toxicity of Malathion may lead to infertility problem. In protective groups, significant restoring effects were observed by Vitamin C which should be considered as an alternative dietary supplement that can reverse the basic effect of Malathion though the effect is both dose and time dependent.

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