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Malathion induced histopathological effects on goat oviduct

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Abstract
The present *in vitro* study was conducted during 2013-2014 to observe Malathion induced toxicity on oviduct of goat (*Capra hircus*) with an aim to provide help in understanding the cases of early abortion, fertility complications and sterility. Malathion exposure was given at two concentrations- 1nM and 100nM; both exposed for 4 hours and 6 hours; alongwith zero hour control for each treatment. Maximum damage in oviduct tissue was observed after 6 hours of exposure to 100 nM Malathion. In isthmus the mucosal folds were disrupted after pesticide exposure. Width of mucosal folds was also found to be reduced alongwith erosion of apices portion of mucosal cells. Higher damage to ciliated and secretory cells was noticed in 100 nM treatment as compared to 1nM. Appearance of gaps alongwith separation of submucosa and mucosa was also prominent after 6 hours of 100 nM Malathion treatment. Structure of mucosal and sub mucosal lining was also remarkably distorted following pesticide treatment. Serosa layer also revealed high damage following Malathion exposure.

Keywords: Malathion, *Capra hircus*, oviduct, mucosa, *in vitro*

1. Introduction
Capra hircus, commonly known as goat is a seasonal polyestrous animal having estrous cycle of about 18 to 22 days. With 15% of World's goat population, India is among the highest livestock holding countries in the world. Most of India's goat population is found in West Bengal, Rajasthan, Uttar Pradesh, Maharashtra, Tamil Nadu, Bihar and Madhya Pradesh. Goat rearing is a major source of income mainly in arid, semi arid and mountainous areas. Goats also act as a source of meat and milk.

In recent years, pesticides are commonly used to menace pest attack which may enter the animal's body through food chain. These compounds have been shown to be capable of interfering with the synthesis and action of natural hormones, acting as endocrine disruptors affecting reproductive system [1]. Organochlorines affect mammal female and male reproductive systems, decreasing reproduction probably due to alterations in folliculogenesis, fertilization, embryo and sperm mortility [2-5]. Organophosphate (OP) insecticides such as Malathion are commonly used in agriculture, industry, and as an ectoparasiticide agent in veterinary medicine [6-8]. Exposure to low doses of OP can produce intoxications in vertebrates, causing neurotoxicity by inhibition of acetylcholinesterase (AChE) and inducing muscular weakness or paralysis [9]. OP insecticides may cause damage to digestive, immune and nervous systems, as well as to the urinary-reproductive system. In the latter case, it has been demonstrated that they can induce infertility, abortions, and physical malformations in human and experimental animals [10]. Even when pesticides have been designed to offer high specificity against insects, their use produces several effects on other organisms. The oviduct provides the necessary nutrition and environment that facilitates sperm transport and facilitation, fertilization and embryo cleavage. Considering the above, the present investigation was carried out to study the influence of nanomolar concentrations and exposure durations of Malathion on oviduct of goat.

2. Material and Methods
2.1 Collection of material and pesticide exposure
The present study was conducted during the year 2013-14 and female genitalia (goat oviduct) was procured from the slaughter house near Kurukshetra city. The material was brought to the Reproductive Biology and Ageing Laboratory in Department of Zoology, Kurukshetra University, Kurukshetra in ice

buckets in normal saline at 4 °C. The oviduct so brought was dissected out, cleared off adhering adipose tissue and processed for *in vitro* experimental protocol as described in Table 1. The concentration of Malathion obtained from market was 50%. The stock concentration of 10^{-6}M Malathion was prepared in Dimethyl Sulfoxide. The required final concentration that is 1nM and 100 nM were made in the medium itself (TCM-199). After washing with normal saline, the oviduct was placed in culture medium (TCM-199) containing Malathion.

Table 1: Experimental layout to study the effect of exposure time and dose of Malathion on goat oviduct

S. No.	Group A (Zero hour Control)	Duration of exposure	Group B (1 nM Malathion)	Group C (100 nM Malathion)
1.	Group A1	4 hours	Group B1 (1 nM Malathion)	Group C1 (100nM malathion)
2.	Group A2	6 hours	Group B2 (1 nM Malathion)	Group C2 (100nM Malathion)

Overall, the experiment was divided into three broad groups (Table 1). Group A was the zero hour control, in which group A1 for 4 hour control and group A2 for 6 hour control. These were kept in Bouins fixative. Group B was exposed to 1 nm concentration of pesticide where group B1 for exposed for 4 hours while group B2 for 6 hours. Likewise, Group C was exposed to 100 nm concentration of Malathion where group C1 for exposed for 4 hours while group C2 for 6 hours.

2.2 Histopathological study

The tissue was harvested after stipulated time and processed for histological slide preparation. For histological slides the oviduct was fixed in aqueous Bouin's fixative (picric acid, formalin and acetic acid in the ratio 75:20:5) for 24 hours. The tissue was washed in running tap water for 6 hours and then processed through various grades of alcohol for dehydration. After proper dehydration, the tissue was kept in oven at 58 – 60 °C in xylene and wax (1:1) for about 12 hours, followed by pure wax at the same temperature for upto 24 hours. Thereafter the tissue was embedded in the wax to form blocks. The pesticide treated oviduct at different doses of exposure and exposure time was sectioned serially at 5 µm thickness and stained with haematoxylin and eosin. The stained slides were examined under microscope and photographed.

3. Results

During the present investigation, the adverse effects of Malathion exposure on goat oviduct were observed in concentration and dose dependent manner. Nanomolar concentration of Malathion caused damage to mucosal, submucosal and muscular layers of oviduct alongwith disorganization of oviducal lining. Following treatments, various morphological observations included mucosal folds disruption, reduction in width of mucosal folds, dislodging of mucosal lining, damage to secretory cells and vacuolization (Fig. 3-8) as compared to control (Fig. 1-2). The thickness of isthmus was found to be reduced after pesticide exposure over control. Maximum damage was observed in the tissue at 6 hours exposure of 100 nM Malathion. The mucosal folds were disrupted with a reduction in their width. Erosion of apices portion of mucosal cells was also clearly visible. There was sloughing off mucosal cells and dislodging of mucosal lining subjected to Malathion treatment. The damage to ciliated and secretory cells became more prominent in 100 nM treatment as compared to 1nM. Gaps could be easily seen and separation of submucosa and mucosa occurred after 6 hours exposure to 100 nM Malthion. Disorganization of mucosal lining also increased with increase in duration of exposure.

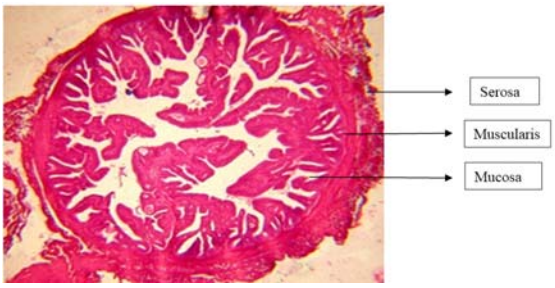

Fig 1: Microphotograph of T.S. of oviduct showing normal structure (treated, 100X, H& E)

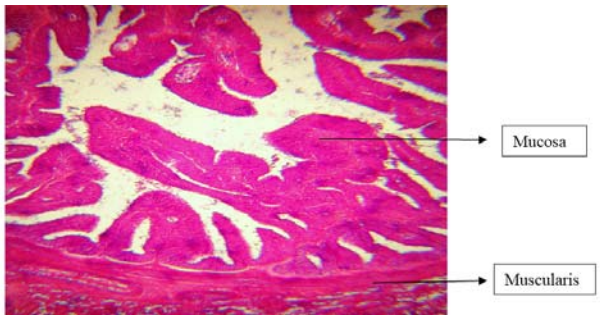

Fig 2: Microphotograph of T.S. of oviduct showing normal structure (treated, 400X, H&E)

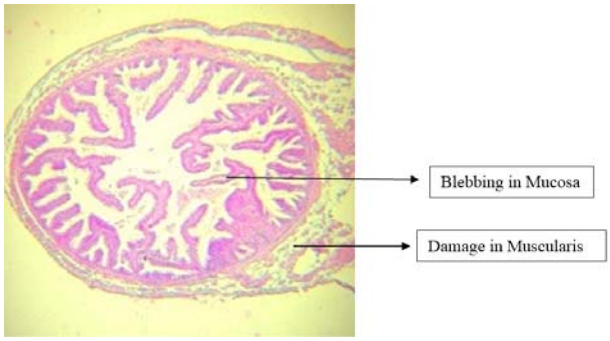

Fig 3: Microphotograph of T.S. of oviduct showing changes in serosa, muscularis and mucosa after 4 hour treatment with 1 nM/ml concentration of malathion (100X, H & E)

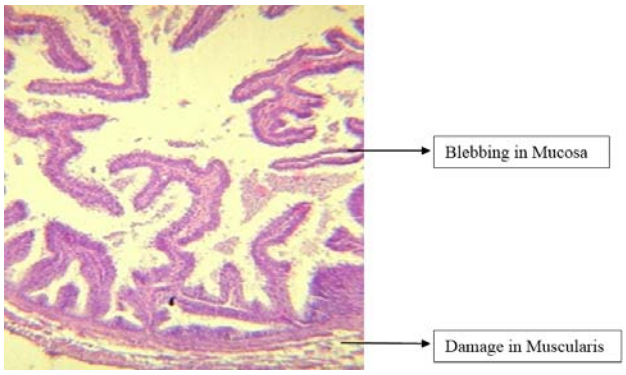

Fig 4: Microphotograph of T.S. of oviduct showing changes in serosa, muscularis and Mucosa after 4 hour treatment with 1 nM/ml concentration of malathion (400X, H & E)

Fig 5: Microphotograph of T.S. of oviduct showing changes in serosa, muscularis and Mucosa after 4 hour treatment with 100 nM/ml concentration of malathion (100X, H & E)

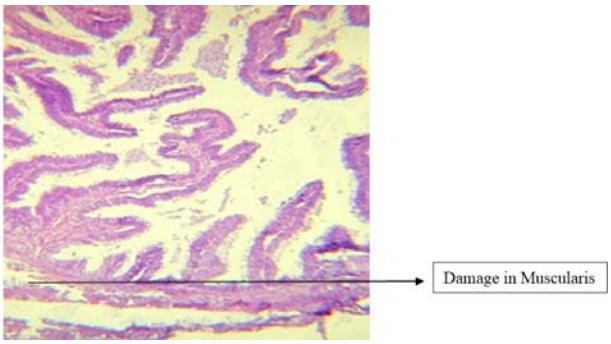

Fig 6: Microphotograph of T.S. of oviduct showing changes in serosa, muscularis and Mucosa after 4 hour treatment with 100 nM/ml concentration of Malathion (400X, H & E)

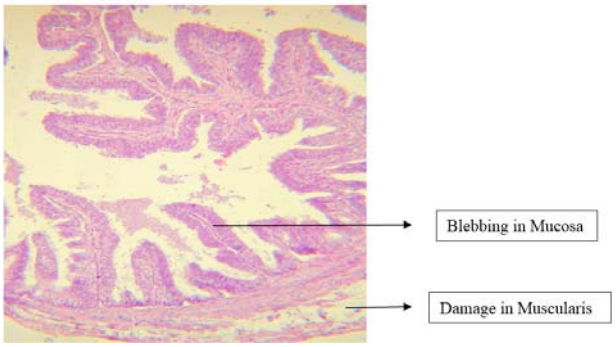

Fig 7: Microphotograph of T.S. of oviduct showing changes in serosa, muscularis and Mucosa after 6 hour treatment with 1 nM/ml concentration of Malathion (100X, H & E)

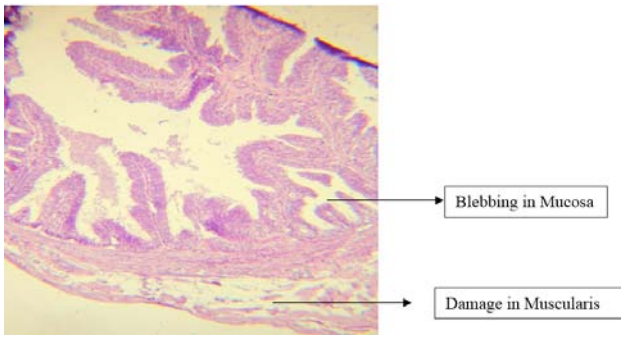

Fig 8: Microphotograph of T.S. of oviduct showing changes in serosa, muscularis and Mucosa after 6 hour treatment with 1 nM/ml concentration of Malathion (400X, H & E)

4. Discussion

In vitro study on Malathion induced cytotoxicity in goat isthmus brought to light the anti- fertility effect of this organophosphate that might cause various histopathological and cytopathological changes in the reproductive system of female mammals adversely affecting their fertility. In our study, Malathion was found to damage mucosal lining of isthmus, disrupt mucosal folds, damages secretory cells, increased vacuolization that can highly affect ova movement in oviduct and impair the function of acting as spermatozoa reservoir thereby influencing fertilization of gametes. It may also affect the secretion of secretory granules in lumen of oviduct which are supposed to be the nutrients for ova. Insecticide chlordecone (Kepone) in oviducts of birds and mammals has been reported to exhibit accelerated growth, cytodifferentiation, cellular hypertrophy, secretory activity, and maturation. Ultrastructure of the oviduct surface showed increased growth of microvilli and profuse ciliation with increased endoplasmic reticulum and Golgi development in estrogen-sensitive quail oviduct cells [11]. Some pesticides are estrogenic that lead to receptor mediated changes in endocrinology of oviduct. DDT was found to inhibit 17β-estradiol secretion by binding to Estrogen Receptor α (ERα) along with progesterone receptor (PR) in alligator oviduct [12]. There are several reports on diazinon and malathion cytotoxicity [13], genotoxicity [14-15] and teratogenic damage in several animals [16], including humans [17]. Studies in human populations exposed for long periods to OP have shown alterations in the endocrine functions, specifically in regulation or secretion of gonadotropic hormones [6]. Several mechanisms have been proposed to explain the OP effect in reproductive systems. OP insecticides are hydrophobic and lipophilic molecules that facilitate the insecticide incorporation in the plasmatic membrane, especially the lipid bilayer. Studies using malathion, methyl parathion, and parathion indicate that this interaction with membrane lipids may alter their physicochemical properties, affecting the membrane structure and its organization, which allows the toxic agents to penetrate the cell [18-20]. Organophosphates inhibit AChE activity through the irreversible union with the catalytic site of the enzyme. This enzyme is related with the modulation of the neuromuscular impulse transmission. Besides, it can be related to other non-neuromuscular processes as cell interactions mediated by electrical events and changes in ion concentrations, which occur during the interaction of gametes and embryo development [21]. Since germinal cell proliferation during the foetal stage is highly sensitive to different environmental pollutants, alterations in reproductive development are observed at lower pesticide concentrations than those required to have a toxic effect in adult animals [22].

Oviduct is no less important in the entire female reproductive system. It is the place where fertilization and developmental cleavages occur. Studies on the direct effect of any pesticide or other toxicant on its morphology and function is scarce focusing on the need of recent research. Insecticides that target the female reproductive system can cause a wide variety of adverse effects. Changes in sexual behavior, onset of puberty, cyclicity, fertility, gestation time, pregnancy outcome and lactation as well as premature menopause are among the potential manifestations of female reproductive toxicity; all capable of disrupting the female reproduction.

5. Conclusion

As evident from this study, damage in histomorphology of oviduct by malathion exposure could be one of the reasons leading to various fertility and sterility problems in domesticated animals. This study has focussed on the effect of

pesticides on fallopian tube which could be responsible for decline in population of domesticated animals and can help in understanding the increasing infertility and abortions in mammals including humans.

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