ORIGINAL RESEARCH PAPER

Zoology

EFFECT OF PARATHION ON GOAT'S OVARY

KEY WORDS:

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ABSTRACT

the food chain shows at higher trophic level the concentration of pesticide or toxin is higher successively such phenomenon is known as biomagnification. Pesticides shows various kinds of effects in different organisms at higher trophic level in the food chain. The most precious effect of pesticide is on the fertility of an organism. Present study reveals the effect of parathion on Goat's ovary such as effect on nuclear membrane, cytoplasmic membrane, follicular fluid, theca cells, follicles, ovarian wall of Goat's ovary after treated with 10-9 molar concentration solution of parathion for 1, & 2 hours.

INTRODUCTION:-

pesticides occupy a rather unique position among the many chemicals that men encounter daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form of life. Toxicological evaluation of the hazard of handling and use of pesticides have for more years focused primarily on preventing injury to men and common laboratory animals have served as the experimental models for man's biochemical, physiologic and pathologic responses to these chemicals. Environmental contaminants have been linked to adverse effect on reproductive functions raising concerned about human health. Which can be affected adversely by exposure to these chemicals [1]. Several excellent reviews showed that ovarian follicle as a target for xenobiotics and hence resulted in female in fertility [2]. Basal granulosa cells, parietal granulosa cells, gap junctions, cumulous cells, gonadotropins and other membrane or intracellular hormone receptors may serve as loci for ovarian toxicants. Keeping in view the effect of pesticides on female infertility, present study on the effect of parathion on Goat's ovary *in vitro* has been undertaken to assess the hazardous impact of different exposure of single concentration of parathion on Goat's ovary cells. The parathion causes morphological alterations in granulosa cells, oocytes, theca cells, and also affect the follicular fluid. The damage is more in high exposure period. Parathion is one of the most acutely toxic pesticides registered by the EPA. Because of its high toxic nature, parathion is classified as a restricted use pesticide (RUP) [3]. RUPs may be purchased and used only by certified applicators. Products containing parathion must bear the signal word " danger" [4].

MATERIALS AND METHODS:-

the mature Goat's ovaries were procured from slaughter houses around the Kurukshetra city. The material brought to laboratory at 4°C in normal saline solution. Parathion pesticide solution of 1 nano mol concentration, antibiotic solution, TCM-199 medium.

METHOD:-

Antibiotic Solution:-

dissolve 0.033 gm, antibiotic (Penicillin & Streptomycin) in 10ml of distilled water.

Medium Propagation:-

dissolve 0.932gm of TCM 199 medium in 100ml of water. Add 0.1ml of antibiotic solution to 99.9ml of above medium prepared. This is 100ml of medium prepared.

Treatment Medium Preparation:-

.291 + 100ml distilled water
↓
10^{-2} molar solution
1ml of 10^{-2} molar solution + 100ml distilled water
↓
10^{-4} molar solution
1ml of 10^{-4} molar solution + 10ml distilled water
↓
10^{-5} molar solution
1ml of 10^{-5} molar solution + 100ml distilled water
↓
10^{-7} molar solution
1ml of 10^{-7} molar solution + 99ml of medium
↓
10^{-9} molar treatment solution

Tissue Culture:-

- ovarian tissue was procured from the slaughter houses after the immediate slaughter of the animal.
- Washed the tissue 3 to 5 times in normal saline.
- Transferred the tissue into the laboratory in a thermos flask within 2-3 hours.
- Again, washed the tissue with normal saline.
- Separated out the ovary and clean it.
- Cut the tissue into small pieces & put into culture plates of 35mm cavity in medium & pesticide solution.
- Then these culture plates were kept in a CO_2 incubator (38°C, 5% CO_2 & 92-95% humidity) & treated for 1 and 2 hours.
- After respective time, fixed the tissue in Buin's fixative for overnight.
- Wash the tissue under running tap water for 2-3 hours.
- Dehydration of tissue was done as following:-
  Made blocks with the help of L- pieces in pure wax.
  Fixed the blocks on block holder after trimming.
  Do section cutting with the help of microtome at 6-micron thickness.

30% alcohol (20minutes)
↓
50% alcohol (20minutes)
↓
70% alcohol (overnight)
↓
90% alcohol (15minutes)
↓
100% alcohol (15minutes)
↓
100% alcohol + Xylene (15minutes) (1:1)
↓
Xylene (15minutes)
↓
Xylene + wax (12hours) (1:1)
↓
Pure wax (24hours)

Histological Staining:-

Toluidine Blue Staining:- stretched slides were dewaxed in xylene (15 minutes), passed through alcoholic grades from 100% to 30% alcohol for 5 minutes each. After being kept in distilled water for 4-5 minutes, slides were stained in toluidine blue for 3-5 minutes. The stained slides were then blot dried & excess stain was removed by giving two dips in n- butanol. The slides were finally mounted in DPX after keeping them in xylene for 15 minutes.

RESULT:

Microphotographs

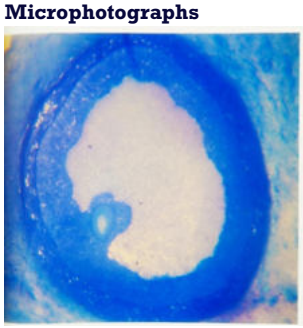

Fig. 1: Microphotograph of goat's ovary 10X showing the antral follicle with theca cells, granulosa cells and oocyte after 1 hr. of culture duration stained with toluidine blue. (control)

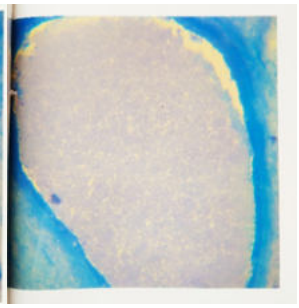

Fig. 3: Microphotograph of goat's ovary10X showing thicker ovarian wall, atriatic follicle and slightly hypertrophied theca cells after 1 hr. of cultured duration stained with toluidine blue (treated with parathion solution)

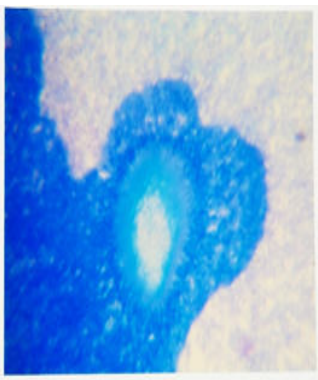

Fig. 2: Microphotograph of goat's ovary 40X showing the oocyte with cumulus oophorous within the antral follicle after 1 hr. of culture duration stained with toluidine blue. (control)

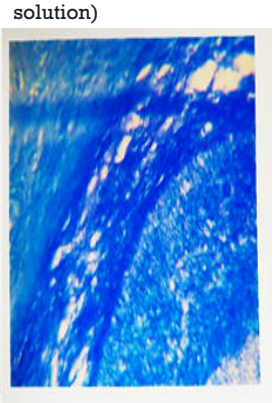

Fig. 4: Microphotograph of goat's ovary 40X showing the disruption in basement membrane of the follicle after 1hour of culture duration stained with toluidine blue. (treated with parathion solution).

Fig.5: microphotograph of Goat's ovary 10X Showing the secondary follicle with granulosa Cell layer after 2hours of culture duration stained With toluidine blue (control).

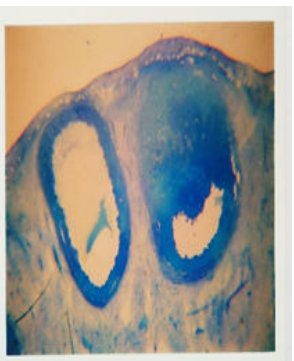

Fig.7: microphotograph of Goat's ovary 10X showing the atretic follicles with degeneration in oocyte and oolemma with unclear Cytoplasmic sap after 2hours of culture Duration stained with toluidine blue (treated with parathion solution).

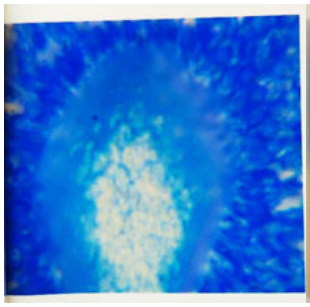

Fig.6: microphotograph of Goat's ovary showing The antral follicle with granulosa cells, theca interna, Theca externa with oocytes after 2hours of culture Duration stained with toluidine blue (control).

Fig.8: microphotograph of Goat's ovary 40X showing the loosened granulosa cells and degeneration in basement membrane after 2hours of culture duration stained with Toluidine blue (treated with parathion solution).

Observation Table:-

Sr. no.	Effect	Control (1hour)	Treated (2hour)	Control (2hour)	Treated (2hour)
1	Granulosa cells a) nuclear membrane	Normal, fully extended	Wrinkled, wavy, irregular	Normal, fully extended	Disrupted, wavy
	b) cytoplasmic membrane	Normal, fully extended	Wrinkled & irregular	Normal, fully extended	Disrupted, irregular
	c) nucleus	Spherical & nuclear sap was clear	Condensation of nuclei of cells	normal	Condensation & fragmentation occurred
	d) cytoplasm	Dense	Cytoplasm became hyalinized	No change	Highly condensed & highly pycnotic cells
2	Follicular fluid	Normal	Hazy with dead floating cells	normal	Pycnotic nuclei with fragmented & dead cells
3	Theca cells	With distinct nucleus normally spindle stayed cells	Slightly hypertrophied oval cells	Normal	Oval hypertrophic cells with large nucleus
4	follicles	Normal	Atriatic follicle observed	Normal	Atriatic follicles increased
5	Ovarian wall	normal	Thick	Normal	Thicker

CONCLUSION:-

from the above discussion of the parathion on Goat's ovary we may conclude that ovary as a vital part of female reproductive system serve as a locus for ovarian toxicants and endocrine disruptors. This highly toxic compound cause alterations in ovarian cells & follicles & thus knows to elicit diverse effect on reproductive prosses & regulation.

By studying the effect of these toxicants on female reproductive system, we are able to know their adverse effects on female fertility & reproduction. We can also analyze the methods to counter these damages in domestic species & in human being also. These studies act as a model to imply techniques to assess the damage in these species.

REFERENCES:-

1. Colborn et al., 1993, Kavlok et. Al., 1996.
2. Richards, 1986; Mattison and Thomford, 1989.
3. Meister, R.T. (ed.) 1987, 1992.
4. Meister, R.T. (ed.) 1992.