

# “Histopathological studies on male and female follicle of *Laevicaulis alte*.”

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## ABSTRACT

In the present study an attempt has been made to study the effect of one of pesticides, endosulfan, which is found to be most toxic amongst all pesticides. Fresh specimens of *Laevicaulis alte* were collected from the cultivated fields in and around Hinganwadi, Pale and Kalwan area of Nashik District and maintained under laboratory conditions in troughs with sufficient amount of moist soil. Slugs were treated with lethal concentration (LC<sub>50</sub> concentration) of pesticide, endosulfan for 96 hr period during pre-reproductive, reproductive and post-reproductive period. After 96 hr acute toxicity of endosulfan, tissues belonging to control and experimental groups (LC<sub>50</sub> concentrations) were removed and taken for histopathological studies and it was observe that there is distortion of structures of follicle.

**KEYWORDS :** Endosulfan, Toxic, Slug.

## INTRODUCTION:

The toxicity of any chemical alters the physiological state of the animal, there by impairing the various metabolic activities. However, to have a clear understanding as to how these chemicals cause injury to the tissues, it is essential to have an insight into the histopathological analysis of the tissues. There is voluminous literature available on the effect of different pesticides. Control of pest molluscs, slugs and snails, has been a world wide problem which is now a days being seriously thoughtover by large number of ecologists and malacologists. Several pest control methods and molluscicides have been suggested.

A number of studies have been carried out on the pesticide toxicity in aquatic gastropods. (Ramana Rao and Ramamurthi, 1978; Muley and Mane, 1989; Magare, 1991; Chaudhari and Lomte, 1992, Jadhav et. al., 1995; Lomte and Waykar, 2000; Ahirrao et al., 2004; Ahirrao and Kulkarni, 2005; Ahirrao and Khedkar, 2012; Ahirrao and Borale, 2013 And Borale and Ahirrao, 2013. Little attention however, has been paid to the mode of action of the pesticides on the body of pest molluscs and the changes brought about by these pesticides in the histology and the physiology of different organs of these molluscs. Effects of organophosphorous pesticides on the liver of carp and catfishes have been studied by Konar (1975). Considerable work has been carried out in *Channa* species in this connection (Dubale and Shah, 1979; Hanumanteet al., 1981; Kulshrestha and Arora, 1984).

Effect of sumithion on the histopathology of *Clarius betrachus* was studied by Mandal and Kulshrestha (1980) while Rao et al. (1980) worked out the impact of endosulfan on *Labeorohita*. Thus, there are relatively very few reports on the impact of pesticides on the histopathological changes in molluscs. Most of the work in this connection has been dealt in vertebrates, especially on the fishes. Since little work is carried out on slugs, except on neurosecretion and reproductive system of *Laeviculis alte* (Bodhankar, 1984; Vyawahare, 1988; and Jawalikar, 1989). In the present study an attempt has been made to study the effect of one of the tested pesticides, endosulfan, which is found to be most toxic amongst all pesticides on pre-reproductive, period of land slug, *Laevicaulis alte*.

## MATERIAL AND METHODS:

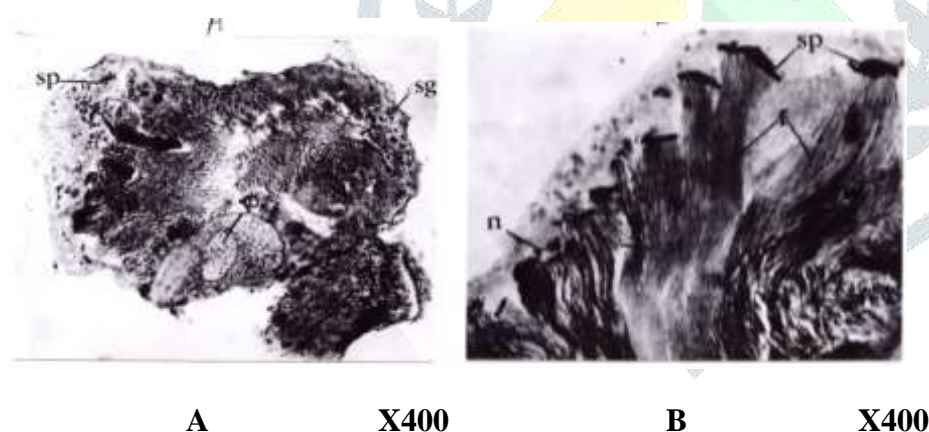
Fresh specimens of *Laevicaulis alte* were collected from the cultivated fields in and around Hinganwadi, Pale and Kalwan area of Nashik District and maintained under laboratory conditions in troughs with sufficient amount of moist soil. They were fed once in a day on carrot, potato or calotropis leaves. They were kept in laboratory for three days before subjected to experimentations. Healthy and mature animals of more or less equal size were selected and were starved during experimental period. Slugs were treated with lethal concentration (LC<sub>50</sub> concentration) of pesticide, endosulfan for 96 h during pre-reproductive,. After 96 hour acute toxicity of endosulfan, gonad, foot belonging to control and experimental groups (LC<sub>50</sub> concentrations) were removed and fixed in aqueous Boiun's fluid fixative. The different tissues were then dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin wax (58-60°C). The sections were cut at 6-7 µ thickness on a rotary microtome. The sections of gonad were stained with Mallory's triple stain. All the observations were made under light microscope to evaluate the changes at cellular level due to pesticide, endosulfan and photomicrographed.

## RESULTS:

### Histological changes:

#### Male follicle:

**Control:** In pre-reproductive period, testicular follicle showed many sperm morulae, nutritive cells and lipid globules were also abundant (Fig. 7-A). In reproductive period, the follicles elaborated, each showed presence of lumen and sperm morulae decreased in this season. Inter-follicular connective tissue reduced. Lipid globules and nutritive cells were less than pre-reproductive period (Fig. 8-A). The inter-follicular connective tissue expanded (Fig. 8-A). Lipid globules and nutritive cells were slightly more than those seen in pre-reproductive but less than reproductive period.

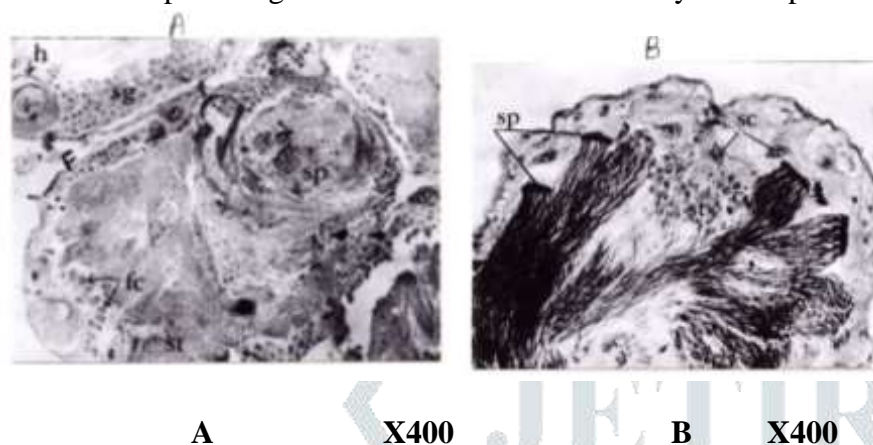


**Fig. 7** – Histopathological changes in the male follicles from the gonad of *Laevicaulisalte* due to pesticide Endosulfan during pre – reproductive period. A – control ; B – Experimental group (x 100)(mo – releasing oocyte; n – nucleus; nu – nucleolus; ro – releasing oocytes; s – sperms; f – follicle; sp- spermatozoa; nc- nutritive cells)

#### Endosulfan exposed groups :

In pre-reproductive period, follicle wall ruptured at many places and follicular content much scattered and the sperm morulae increased in size. Their close association with lipid globules and nutritive cell was not visible. The sperm appeared in almost every follicle. The spermatids lost their integrity in

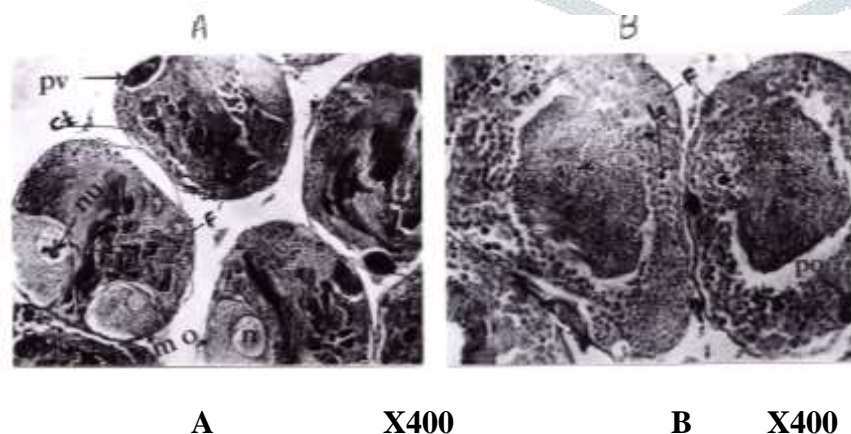
development and probably led to dissolution (Fig. 7-B). In reproductive period, the follicles distorted and sperm morulae and spermatocytes clumped at many places. Lipid globules and nutritive cells increased in size giving a swollen shape (Fig. 8-B). In post-reproductive period, the follicle wall distorted, spermatocytes and spermatids clumped. At a few places sperms were also seen. The study showed that in pre-reproductive period, the follicles were much affected. On the other hand, in reproductive and post-reproductive period enhanced spermatogenesis to give rise to sperms, which were also released but in both the seasons spermatogenesis was diminished and only a few sperms were produced.



**Fig. 8** – Histopathological changes in the male follicles from the gonad of *Laevecaulisalte* due to pesticide Endosulfan during reproductive period. A – control ; B – Experimental group (x 100) (h- hull formed; f- follicles; sp – spermatozoa; sg – spermatogonia; st – spermatids; fc – follicle cell)

#### Female follicle:

**Control :** In pre-reproductive period, compact follicles contained many oogonia and small and a few large sized oocytes with prominent nuclei and nucleoli-lipid globules and nutritive cells were abundant (Fig. 9-A). In reproductive period, compact follicles showed few oogonia and many small and large previtellogenic and vitellogenic oocytes. A few degenerating pre-vitellogenic oocytes were also seen. Lipid globules and nutritive cells were comparatively more than monsoon.



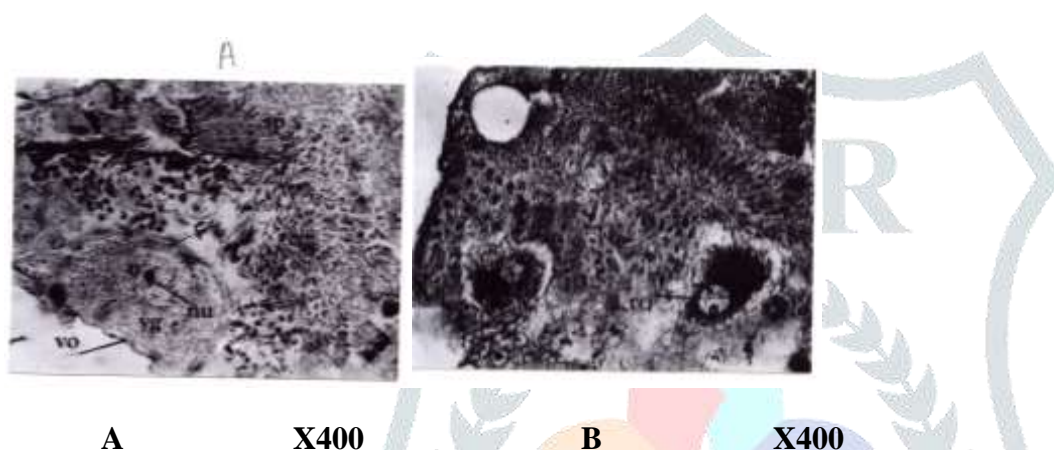
**Fig. 9** –Histopathological changes in the female follicles from the gonad of *Laevecaulisalte* due to pesticide Endosulfan during pre -reproductive period. A – control ; B – Experimental group (x 100) (pv – previtellogenic oocytes; n – nucleus; nu – nucleolus; mo – maturing oocytes; f – follicle; ct – connective tissue; lg- lipid globules; nc- nutritive cells)



### Endosulfan exposed groups :

In pre-reproductive period in lethal concentration, at places the follicle wall distorted but mostly showed continuity. The follicle wall was shrunken and only a few nutritive cells and lipid globules remained restricted along the periphery of the follicle wall. The oogonia showed prominent nuclei and nucleoli but cytoplasm was opaque. These are likely to undergo degeneration. Small sized pre-vitellogenic oocytes nuclei were under karyolytic condition. The vitellogenic oocytes showed fragmentation of cytoplasm. In reproductive period, in a few vitellogenic oocytes nucleoli stained dark. Follicles distorted at many places and shrank. Lipid globules and nutritive cells reduced in size. Previtellogenic oocytes reduced in size. Vitellogenic oocytes showed dense and coagulated cytoplasm

Thus, there was severe effect on the follicle and also the follicular content in post-reproductive period and pre-reproductive period. In reproductive period, the growth of the gametes were enhanced but many of them could not reach to fully mature condition for their release from the follicles.



**Fig. 10** – Histopathological changes in the female follicles from the gonad of *Laevicaulisalte* due to pesticide Endosulfan during reproductive period. A – control ; B – Experimental group (x 100) (vo – vitellogenic oocytes; ro – releasing oocytes; n- nucleus; nu – nucleolus; f – follicles)

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