TOXIC EFFECTS OF PESTICIDES (CHLORPYRIFOS 50% + CYPERMETHRIN 5% EC) ON OVARY AND TESTIS OF AN AIR BREATHING TELEOST - *ANABAS TESTUDINEUS*

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ABSTRACT

Anabas testudineus was exposed for 24hrs to 96hrs with pesticide (chlorpyrifos 50% + cypermethrin 5% EC) at a concentration at 0.1, 0.15. 0.2 and 0.25 ppm under laboratory condition. The fish shows several histological changes in ovary and testis tissues. The histological changes observed in ovary like oocytes shrinkage, oocyte degeneration and fragmentation of ova were recorded in an increasing order of doses. Changes in testis included degeneration of germinal epithelium, interstitial cells and induce impairments of the structure of Semniferous tubules and spermatogenesis at increasing doses.

Keywords: Toxicity, Histology, Pesticides. *Anabas testudineus*, Ovary, Testis.

INTRODUCTION

India is an agricultural country. It is totally dependent on agriculture and agricultural products to feed people. So a large amount of agro-chemicals and insecticides are used to increase the agricultural production to meet the demand of food grains for country's ever increasing production.

Pesticides are useful tools in agriculture and forestry, but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored (Konar 1975; Basak and Konar 1976, 1977). The aquatic ecosystem as a greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity.

Pesticides at higher concentrations are known to reduce the survival, growth and reproduction of fish (MCkim *et al*,1975) and produce many visible effects on fish (Johnson 1968). Due to effect of pesticides (chlorpyrifos 50%+cypermethrin 5% EC), important organs like, ovary and testis are damaged. Until the use of pesticides in crop farming is replaced by other means of pest control such as integrated pest management, use of toxic pesticides at lowest possible doses need to be recommended.

Cypermethrin produces drastic effects on both the invertebrates & vertebrate species (Gowlan *et al.*, 2002) and vertebrates (Das and Mukherjee 2003). Cypermethrin is highly toxic to fish, cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural application as well as in consumer products for domestic purposes. A recent study at Xuzhou Medical College in China showed that, in male rats, cypermethrin can exibit a toxic effect on the reproductive system. After 15 days of continual dosing, both androgen receptor levels and serum testosterone levels were significantly reduced.

Chlorpyrifos is a crystalline organophasphate insecticide. It was introduced in 1965 by Dow Chemical Company and is known by trade names, including Durshan and Lorsban. It acts on the nervous system if insects by inhibiting acetylcholinesterase.

Chloropyrifos is moderately toxic to human and chronic exposure has been linked to neurological effects, developmental disorders, and Specially fishes. Cypermethrin is highly toxic to fish, cypermethrin is a synthetic pyrethroid use as an insecticide in large scale commercial agriculture application as well as in consumer products for domestic purposes. A recent study at Xuzhou Medical college in china showed that, in male rate, cypermethrin can exibit toxic effect on the reproductive system. After 15 days of continual dosing, both androgen receptor levels and serum testosterone levels were significantly reduced.

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Pyrethroids are more toxic to fish than the organophosphate pesticides (Oros and Werner, 2005). They are potent neurotoxicants that interfere with nervecell functions by interacting with voltage- dependent sodium channel, resulting in repetitive firing of neurons and eventually causing paralysis (Shafer and meyer, 2004).

The objective of this research work is to study the histopathological changes in the combined toxicity of both the classes of pesticides e.g chlorpyrifos 50%+cypermethrin 5% EC, in the ovary and testis of an airbreathing fish-Anabas testudineus, commonly called Kabbai.

MATERIAL AND METHODS

Healthy specimen of Anabas testudineus of uniform size (7.5 cm + 11 cm) and weight (13 + 47 gm) were collected from local pond at Muzaffarpur District of North Bihar. The healthy fishes were acclimatised in laboratory aquaria for one month.

Fish were disinfected by subjecting them to a bath of 0.1% aqueous potassium permagnate (Kmno4) solution for 12-15 min to remove any dermal infection and fish were transferred to a large tank known as control tank, having adequate amount of water.

During the period of acclimatization fishes were feed alternatively different type of food like, bread, piece of soya bean, piece of snail and sliced goat and chicken liver, No mortality was recorded among the fish during this period.

The average Physiochemical condition were maintained during this period Water of the tank was renewed every day or alternate to minimize contamination as well as subjected to a gut evacuation period before the experiment.

In present study pesticides used as a toxicant, the stock solution of chlorpyrifos 50% and cypermethrin 5% EC was prepared according to the method prescribed in the standard method (APHA – AWWA, WFF, 1998) for experimental purpose. It is filtered first through cotton cloth and later through filter paper. The filtrate was a saturated solution of mixture of chlorpyrifos 50% + cypermethrin 5% EC, and sub-dilute solutions were prepared from this stock solution.

The different concentration of cypermethrin 5% EC + chlorpyrifos 50% for Anabas testudineus were 0.05, 0.1, 0.2 and 0.25 ppm respectively with their control condition. Ten acclimated fish were released in each aquarium containing different concentrations of pesticide. All tests were done at room temperature and physiochemical conditions were maintened. The behaviour and other exeternal changes in the body of fishes were observed. Dead fishes were removed and mortality was recorded at 24, 48, 72 and 96 hrs of exposure time, temperature, dissolved oxygen and PH were recorded daily. The LC50 Value of Anabas testudineus recorded time to time.

For histopathological investigation the fish were exposed to various sublethal concentrations. At the end of 96 hrs exposure period testis and ovary samples were collected in small plastic vails with ten (10%) percentage neutral buffer formalin and Bouin's solution fixatives.

The numbers of section of samples were prepared using a microtom, stained and studied under a microscope.

RESULTS AND DISCUSSION

Physico-chemical characteristics of test water

The physico-chemical characteristics of the water were observed throughout during the present study, using procedures described by APHA (2005). Recorded parameters as below –

Temperature 250 + 20 C, PH 7.10 + 0.15 Dissolved O_2 7.50 + 1.50 ppm. Total hardness of CaCO3 165 + 5

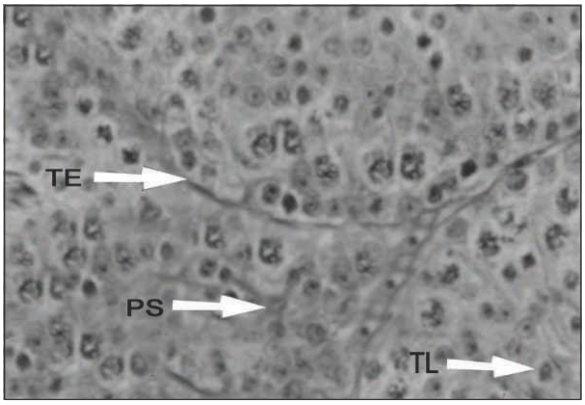
After exposure to pesticides (Chlorpyrifos 50% + Cypermethrin 5% EC) fishes showed abonormal behavior such as restlessness sudden quick movements, rolling movements swimming on the back because cypermethrin is very toxic in nature.

Histopathological observations

Fish exposed to sublethal concentration of pesticides (Chlorpyrifos 50% + Cypermethrin 5% EC) for various exposure periods showed considerable degree

ppm, total alkalinity of $CaCO_3$ 150.20+7.77 ppm and of changes in the histoanatomy of the testis and ovaries

chlorides 14.42 + 1.05 ppm. All the above mentioned values are average values with standard error after observations.



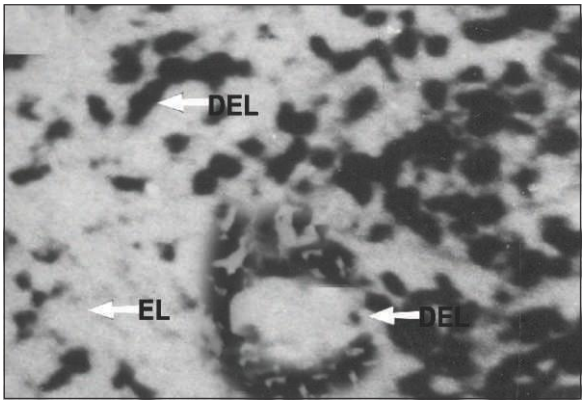


Fig 1: Histological section of the normal testis of the fish showing well developed testicular lobules (TL) with full of sperms, the testicular epithelium (TE) is well maintained (H&E) (200X).

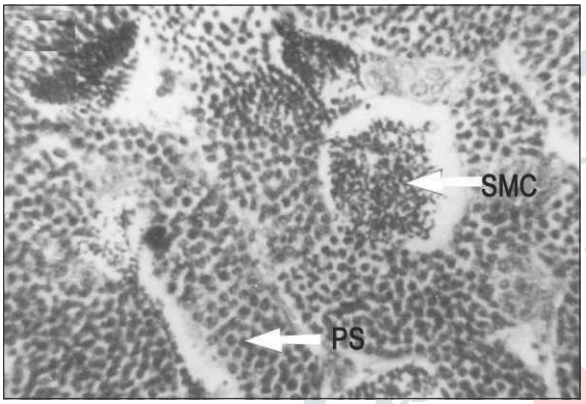


Fig 2: Histological section of the normal testis of the fish showing lobular epithelium as well as spermatocyte in their different stages of development viz. sperm mother cell (SMC) and primary spermatocyte (PS) (H&E) (400x)

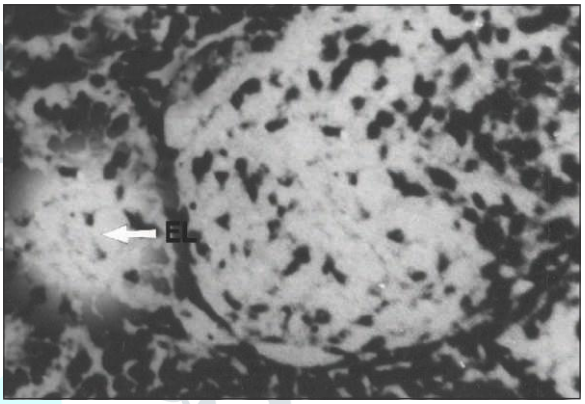
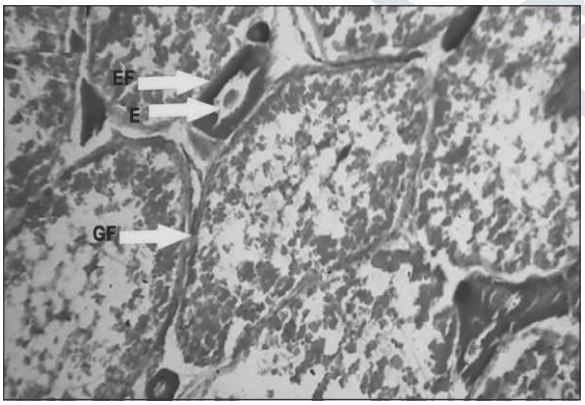
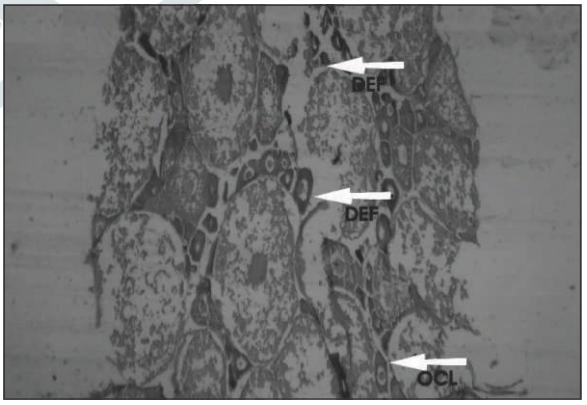


Fig 3-4: Histological section of pesticide exposed testis showing degenerated lobuler epithelium (DLE) as well as empty lobules (EL). The number of spermatids and sperms and greatly reduced. The intermediate connective tissues between the lobule also undergone degeneration. (H&E) (200X)





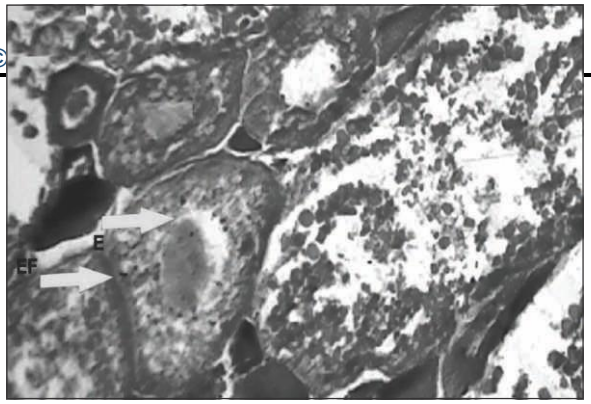


Fig 5-6: Histological sections of normal overay of the fish showing egg foliicfe (EF), egg (E), grafian foliicle (GF) area normally arranged.

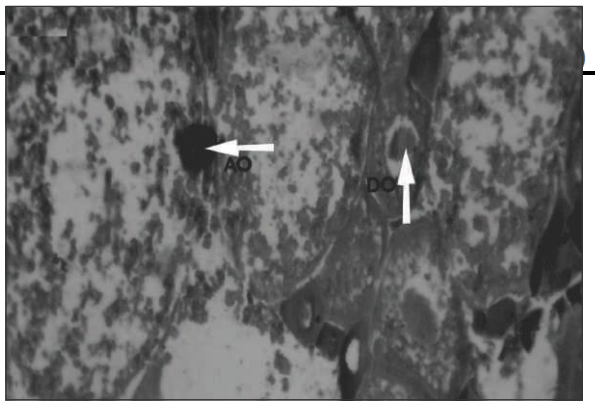


Fig 7-8: Histological sections of ovary of the fish exposed of pesticides showing degenerated egg (DE), degenerated follicular epithellium (DEF) and shrinkage in oocytes.

The seminiferous tubules are generally of varying shapes and sizes.Well developed testicular lobues (TL) with full of sperms can be seen. The testicular epithelium is well defined and well showing lobular epithelium as well as spermatocyts in their different stages of development viz, sperm mother cell (SMC) and primary spermatocytes. Fig(1&2).

Testis of Anabas testudineus showed significant changes when exposed to combination of chloropyrifos 50% + cypermethrin 5% EC. Extensive cytotoxic damage such as general inflammatory response, degeneration of germinal epithelium interstitial cells and induces impairment of the structure of semniferous tubules and spermatogenesis can be seen at increasing doses. Exposed testis showed generated lobular epithelium as well as empty lobules, the number of spermatids and sperms are greatly reduced. Fig(3&4).

Normal histology of the ovary of Anabas testudineus reveals that it is surrounded by an ovarian wall which is differentiated into an outer thin peritoneum, a thicker tunica albuginea made up of connective tissue, muscle fibers and blood capillaries. The inner most layer is the germinal epithelium which joins with the tunica albugnea at several places and projects into the central lumen the ovocoel, in the form of finger like projections called ovigeroves lamellae Fig (5&6).

The ovaries of A. testudineus have shown significant alteration on exposure to test pesticides (Chlorpyrifos 50% + Cypermethrin 5% EC). Marked Changes were observed in histological structure of ovary. Oocyte with decreased atretic oocytes, cytoplasmic retraction and cytoplasmic degeneration were also observed along with clumping of oocytes.

The cytoplasm showed vacuolization at the perip hery of oocytes which gradually extends towards the centre and some extent of damage can be seen in the architecture of tunica albuginea and inner germinal epithelium, degenerated egg (DE) follicular epithelium(DEF) and shrinking oocytes after 96 hrs of exposure periods. fig (7&8).
In present investigations gross inflammation was found in the gonadal tissue of treated fish with exposure of pesticides (Chlorpyrifos 50% + cypermethrin 5% EC). Testicular inflammation was documented as one of the common responses in both aquatic animals exposed to environmental toxicants (Sokel *et al*, 1985; Ruby *et al*, 1987).

Testes in fishes represent the most dynamic organs. These are especially vulnerable to a wide variety of chemical toxicants among which the pesticides like organochlorine, organophosphorus, carbonates and synthetic pyrethroids play a very crucial role. Pesticides that cause testicular atrophy either damage the seminiferous tubules or destroy the intercellular leyding cells or both, thereby culminating in the partial or total arrest of spermatogenesis in a variety of fishes. Studies of spermatogenesis in fishes have been well described by many workers (Tumer, 1919 Mathews,1939, Swarup and Srivastava, 1979). According to Hoar (1965), spermatozoa are formed from the sperm mother cell (SMC) or spermatogonia through a series of cytological stages collectively referred to as spermatogenesis. The gradual reduction in the size is carried up to spermatid formation which metamorphosis into spermatozoa by reduction in its nuclear size. Long term Static bio-assays conducted in the laboratory clearly indicate that exposure of combination of cypermethrin 5% EC + Chlorpyrifos 50% is responsible for histoanatomical damage of fish tedtis in terms of condensation of spermatogonic cells, vacuolization of tubular cells distortion of seminiferous cells along with inflammatory lesions. Shrinkage of interstitial cells and vacuolisation of tubular cells which resulted in peculiar starry sky appearance of the testicular tissue after longer duration of exposure even with low concentration is a strong evidence for testicular atrophy.

In case of ovary, nucleus showed several spherical structures formed by the fragmentation of nucleus lying randomly in the chromatin network, Yolk vesicles are arranged at the periphery, vacuolization in the nucleus, stromal hemorrhage and damage of germinal epithelium. Histoanatomical abnormalities in ovaries may be caused by several factors viz, ionizing radiations, electric current parasitic infections mechanical injuries xenobiotic toxicants (Sarojini and Victor, 1985) any by a variety of effluents and aquatic pollutants (Shukla *et al*, 1984; Saxena and Garg 1978; Johnson *et al*, 1988 Mc Comic *et al*,1989, Davis and Cook,1993; Garmer *et al*. 1995; Kumar *et al*, 2000). Almost similar histopthological findings were reported by Hossain *et al*. (2000) in the ovaries of *Anabas testudineus* and *C. Punctalus* after the exposure 0.5 and 530 ppm concentration of pesticide, dimecron 100smc. Giri *et al.* (2000) reported the effects of insecticide basathrin induced histoanatomical result of ovarian tissue of cat fish, H. Foossilis Katti and Sathyanesan (1985) observed evposure dependent and

concentration-mediated changes in testis of *Clarias batrchus* treated with lead. Hilderbrand *et al*, 1973, Shankar and Mandal (1973), have reported similar observation in lead treated rats.

The results of the present study indicate that the pesticides such as (Chlorpyrifos 50% + cypermethrin 5% EC) also cause far reaching consequences in the aquatic system. Even the sublethal concentration of pesticides (Chlorpyrifos 50% + cypermethrin 5% EC) is enough to cause significant changes in gonads of fishes like *Anabas testudineus*. Pesticides are the most hazardous substance that not only affects the target organism but also the non-target organism. This can be said that the toxic chemicals enter the food chain and causes biomagnifications in different strata of food chain. Although the pesticides are frequently used in the paddy fields to yield a higher production of crops, perhaps it acts as a silent killer that have detrimental effect on environment, damaging and causing that to non-target organism. This type of study can suggest that the use of pesticide in the paddy field should be in a control rate which does not affect the non-target organism. Government has for mutated several action plans but the need of the hour is that the people should realize by themselves about the negative effects of the pesticides.

Thus it can be concluded that gonads of the fishes, undergo severe histopathological alteration when exposed to the sub-lethal concentration of pesticides (Chlorpyrifos 50% + cypermethrin 5% EC) it ultimately produces a negative impact on population and production of etable fishes. If this process continues, one day this group of fish may face danger of extinction.

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