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HISTOMORPHOLOGICAL CHANGES IN THE TESTIS OF FISH CYPRINION WATSONI, EXPOSED TO ENDOSULFAN

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy

In

BIOLOGY

(Reproductive Physiology)

By

Ommia Kalsoom

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QUAID-I-AZAM UNIVERSITY
ISLAMABAD

Department of Biological Sciences
Quaid-i-Azam University
Islamabad
2003

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CERTIFICATE

This thesis, submitted by **Miss. Ommia Kalsoom** is accepted in its present form by the Department of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy in Biology (Reproductive Physiology).

SUPERVISOR: Samina Jalali

Dr. Samina Jalali

EXTERNAL EXAMINER: _____

CHAIRMAN: H.S. Qureshi

Dated: 22nd May 2003

All the efforts, the entire honor and all the achievements are dedicated with love and respect to my loving parents and grandmother.

CONTENTS

In the name of Allah, the Beneficent, the Merciful.

Praise be to Allah, Lord of the Worlds,

The Beneficent, the Merciful:

Owner of the day of Judgement,

Thee (alone) we worship; thee (alone) we ask for help.

Show us the straight path,

The path of those whom thou hast favoured;

Not (the path) of those who earn thine anger, nor of those who go astray.

AL FATEHA

ACKNOWLEDGEMENTS

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In the end I am thankful to all those who helped me.

Ommia Kalsoom

LIST OF ABBREVIATIONS

POPs	Persistent organic pollutants
OCLs	Organochlorine insecticides.
DDT	Dichlorodiphenyl trichloroethane.
PCBs	Polychlorinated biphenyls.
GSI	Gonadosomatic index.
K	Faulton's condition factor.
ppb	Parts per billion
HCH	Hexachlorocyclohexane.
VTG	Vitellogenin.

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ABSTRACT

Endosulfan is an organochlorine insecticide. The current study is aimed at assessing whether endosulfan had any potential to cause negative effects on reproductive and developmental parameters of male fresh water Cyprinid fish, Cyprinion watsoni. The fish was exposed to .75 ppb and 1ppb endosulfan on alternate day for 30 days during early spawning season (March).

The body length and body weight of .75ppb treated group increased significantly ($p<0.05$) compared to control. The body weight of 1ppb group increased non significantly ($p>0.05$) while the body length increased significantly ($p<0.05$) compared to control. The condition factor (K) and GSI of endosulfan treated (.75ppb and 1ppb) groups showed non significant ($p>0.05$) difference compared to control. Testicular weight, length and breadth of (both right and left testis) fish treated with .75ppb endosulfan increased significantly ($p<0.05$) compared to control. A non significant ($p>0.05$) increased is also observed in testicular weight and breadth (both right and left) of 1ppb group compared to control. 1ppb endosulfan treatment showed significant ($p<0.05$) increase in length of left testis whereas non significant ($p>0.05$) increase in length of right testis was observed compared to control. A significant ($p<0.05$) decrease in the mean diameter of spermatogonia was observed in 1ppb group compared to control. While .75ppb endosulfan treatment showed non significant ($p>0.05$) difference. The mean diameter of primary spermatocytes, secondary spermatocytes, spermatids and nuclei of spermatogonia showed non significant ($p>0.05$) difference after treatment with endosulfan (1ppb and .75ppb) compared to control. Histomorphological studies showed that endosulfan treatment caused various changes i.e. lobules were loosely arranged, spermatogonia had irregular nuclear and cell membrane. spermatocytes and spermatids showed clumping and sperm count was reduced. The result of this study indicates that Cyprinion watsoni exposed to endosulfan may show reproductive impairment.

INTRODUCTION

INTRODUCTION

A range of contaminants, including heavy metals and organic pollutants, which are often present in the sewagei may cause developmental abnormalities and deformities in fish. These deformities are usually non specific and their occurrence may depend on the type of toxicant, condition of exposure, fish species under investigation and prevailing environmental factors (Nowak, 1993).

Toxicology is the study of poisons. It includes the identification of poisons, their chemical properties, and their biological effect as well as the treatment of disease conditions that they caused.

Toxicologists deals with many different chemicals, feed additives, environmental contaminants, pesticides, and natural toxins of plant and animal origin which may adversely affect the health of animal (Gary, 1996).

Endocrine disrupting compounds are natural or man made agents present in the environment that interfere with normal endocrine function. This diverse group of chemical includes environmental estrogens, and a number of industrial, municipal, agriculture and natural compounds (Bothman et al., 1999).

Water is one the most essential item needed by man, plants and other living beings for their survival. Water maintains an ecological balance between various groups of living organisms and their environment. It is the most precious resources on the earth covering almost 70% of earth (Kumar et al.,1999).

The physical properties of water in any aquatic system are largely regulated by the existing meteorological conditions and chemical properties. The effects of physical forces such as light and heat are of great significance as they are solely responsible for certain phenomena, such as thermal stratification, chemical stratification, diurnal and seasonal qualitative and quantitative variations in the plankton, micro and macro organisms, and also in the quality of water. The even increasing pollution and rapid industrial growth in the present era are contributing to a maximum extent influencing the physio-chemical properties of most water bodies (Kaushik and Saksena, 1999).

Water pollution by persistent organic pollutants (POPs) necessitates the establishment of water quality criteria and the estimation of "safe" level for fish (Tarzwell, 1966). Fish has long been used as indicator of aquatic pollution (Johnson, 1968; Holden, 1972; Johnson et al., 1988).

Fish are valuable sources of high-grade protein and other organic products. They occupy a significant position in the socio-economic fabrics of South Asian countries by providing the population not only the nutritious food but also income and employment opportunities. Of the 21,723 fish species known to science, over 20% live in fresh waters and majority of them live in tropics between latitudes 23° 5´ N and 23°5´ S. Now here in the world is zoogeographic region so blessed as Indian subcontinents (India, Pakistan, Nepal, Burma, Srilanka and Bangladesh) in respect to the diversity of fish wild like that dwells the inland waters (Talwar and Jhingran, 1992).

Cyprinion watsoni belongs to Cyprinid group found in the streams of hilly areas of Northern Pakistan and extends upto Afghanistan, Iran, and some parts of Syria and Eastern corner of Arabian penisila (Jaya Ram, 1981).

Wide spread contamination of aquatic ecosystem by a whole battery of persistent organic pollutants has been responsible for massive "fish kills" (Saunders, 1969).

A number of chemicals have been shown to accumulate in fish aside from its obvious significance from ecological stand point, the accumulation of xenobiotics by aquatic species have been recognized as a potential human health hazard as well (Huckel and Milburn, 1990).

The organochlorine insecticides (OCLs), beginning with DDT (1,1-bis (4-chlorophenyl)-2,2,2-trichloroethane), were introduced over a period of about 5-10 years after the end of world war II.Their use expanded rapidly there after, particularly in the United States and Europe, between their introduction and the early 1960s. Although there were no major concerns as to their environmental effects for at least ten years, it was soon realized that the OCLs were not broadly toxic to nonpest invertebrates but were very persistent in soils and aquatic sediments. Moreover there were increasing number of reports in the literature of significant quantities of OCLs

residues, particularly of DDT and dieldrin (1,2,3,4,10,10-hexachloro-6, 7-epoxy-1, 4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonephthalene), in various plants and animal tissues (Wiktelius and Edwards, 1997).

High levels of OCLs have also been found in African fish. DDT and dieldrin residues in wide range of species of fish, and those of HCH, endosulfan, and endrin differed considerably between species. Some of these residues were probably high enough, particularly in the genera Barbus, Clarias, Hydrocynus, and Sarotherodan, to significantly influence the behavior of the fish used in studies in the U.S and Europe (Edwards, 1973a,b).

Residues level of xenobiotics in aquatic organisms have been commonly used to estimate the degree of pollution of aquatic environment (Phillips, 1980). Monitoring and residues analysis programs must however, take into consideration biotransformation of xenobitics, which occur in fish in vivo (Lech and Bend, 1980).

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-mthano-2, 4, 3-benzo-dioxathiepin-3-oxide) is an organochlorine insecticide, which is less persistent in the environment than other organochlorines. Technical endosulfan consists of the mixture of two isomers-alpha and beta endosulfan. Residues of endosulfan in fish from target areas have been frequently reported (Moulten, 1973; Pic et al., 1981; Matthiessen et al., 1982; Nowak and Ahmad, 1989; Nowak, 1990; Nowak and Julli, 1991).

Over the past 20 years artificial chemicals and substances such as pesticides are suspected causing 8% fish kill. Fish population can display the effect of exposure the reduced viability sperm, egg and larvae increased incidence of abnormalities and reduced life expectancy. Reproductive dysfunction in aquatic species as a result of contaminant exposure is a considerable current interest (Collier, 1992).

Toxicity of endosulfan to four species of fishes (Barbus stigma, Ophiocephalus punctatus, Channa gachua and Clarias batrachus) at various conditions of temperature, water hardness and PH was determined. The 96-hour LC50 ranged from 0.0001936 to 0.002775 ppm. Toxicity of endosulfan to the four species of fish increased at higher PH levels (Khillare and Wagh, 1987).

Distribution of organochlorine in different organs is often due to differences in lipid content (Phillips, 1980). The relative contribution of various organs to biotransformation of xenobiotics depends on the blood perfusion, the weight of organ, and their metabolic activity (Lindrostroemseppa et al., 1981).

A one step extraction and clean up procedure for determining endosulfan in fish was investigated. Three species of fresh water fish exposed to 0.7-16mu g/liter technical grade endosulfan in tanks for various period of time, were found to concentrate both alpha and beta endosulfan and metabolize them to sulfate, diol, ether and lactone. Fish collected from Gwydir river in cotton growing area in summer (Dec.1986-Feb.1987) were found to contain endosulfan residues suggesting endosulfan is quite stable in the environment and can cause residues (Nowak and Ahmad, 1989).

Due to its high toxicity to fish, endosulfan is often suspected as the cause of fish kills in cotton growing regions of New South Wales and Queens land and other areas where it is used. Since it is not very persistent in water, analysis of water samples rarely provides sufficient cause and effect evidence. Residues in dead fish would seem to be better indicators of exposure (Nowak et al., 1994).

Polychlorinated biphenyls (PCBs) are a class of synthetic organochlorine lipophilic haloginated aromatics (Fielden et al., 1997). PCBs have been found to impair both reproduction and development in fish (Olsson, 1999). It is becoming increasingly evident that a large number of anthropogenic substances, released into the aquatic environment, may interfere with fish reproduction and development. Disturbed reproduction and early life stage mortality have been observed in Salmonids from the Baltic sea and the Great Lakes of North America and in Perch (Perca fluviatilis) downstream of pulp-mill effluents (Howell et al., 1980; Mac et al., 1985; Munkittric et al., 1992; Borjesson et al., 1994; Sandstrom, 1994). Several investigators (Von Westernhagen et al., 1981; Hansen et al., 1985; Cross and Hose, 1988; Spies and Rice, 1988) have found adverse reproductive effect in fish populations inhabiting PCB contaminated environments. Reproductive dysfunction in aquatic species as a result of contaminant exposure is of considerable current interest (Mattison and Thomford 1989; Collier, 1992). Numerous laboratory studies have shown that the potentials exist for chemical contaminants such as PCBs to adversely affect the

reproductive process of several fish species (Weis and Weis, 1989). The presence of PCB and other organochlorines can affect reproductive success in animals (Wasternhagen et al., 1987; Addison, 1989; Elliot et al., 1988; Casillas et al., 1991). Reproductive impairment among wild life may have disastrous effects on the sustainability of animal populations (Asplund et al., 1999). It is becoming increasingly evident that a large number of anthropogenic substances, released into the aquatic environment may interfere with fish reproduction and development. For example PCBs have played a central role in the poor reproductive outcome of the three seal species in the Baltic as well as in otter (Lutra lutra) in Sweden (Saundergren et al., 1980; Olsson and Saundergren, 1991a; Olsson and Saundergren 1991b). Endosulfan is one of the most toxic chemicals to fish with LC50 value reported as low as 0.014 μg^{1} for harlequin fish, Rosbora hetromorpha, 48h LC50 in a flow through system (Alabaster, 1969). For fish present in Australian waters, such as rainbow fish, Malanotaenia sp., Firetail gudeon, Hypseleotris galii, Bony bream, Nematolosa erebi, European carp, Cyprinus carpio, Golden perches, Macquaria ambiqua, 96h LC50 ranges from 0.1 to 5.0μg^{-1} (Saunderam et al., 1992). There is no information on the lethal toxicity of endosulfan to Catfish (Tandanus tandanus).

The recent concern about a possible decline in sperm count and increased incidence of male genital abnormalities of humans (Sharpe and shakkebak, 1993); of alligators with abnormal male genitalia (Guillette et al., 1994); and of male fish in rivers which show female characteristics (Purdam et al., 1994) has emphasized the fact that many of these chemicals can be harmful at levels well below those that are lethal such concern, which has been well documented (Colbom and Clement, 1992); have focused over-whelmingly on those chemicals that mimic the normal hormone estradiol. These include organo-chlorine pesticides, such as DDT, PCBs and breakdown products of the alkyl-phenoxy detergents (Sumpter et al., 1996) as well as estradiol of human and animal origin (Routledge et al., 1998).

Adult male rats were exposed to 0, 2.5, 5, 10 mg endosulfan per kg body weight, decreased spermatozoa counts in the cauda epididymis and reduced intratesticular spermatid counts associated with an elevation in the activities of specific testicular marker enzymes (Sorbital dehydrogenase, lactic dehydrogenase, gamma glutamyl transpeptidase and glucose-6-phospate dehydrogenase) were seen in all the

endosulfan treated groups. Endosulfan caused impairment in testicular functions by altering activities of the enzymes responsible for the spermatogenesis, there by influencing intratesticular sprematid count and causing low sperm production and sperm deformity (Sinha et al., 1995).

There is increasing evidence that normal male reproductive function can be disrupted by exposure to pollutants in the environment that can exogenously mimic, antagonized or block sex hormone function. One possible consequence of exposure to these xenobiotics is disruption to spermatogenesis. Adults male guppies (poeciliidae: teleostei) were exposed to environmentally relevant levels of the common xenobiotics tributyltin and bisphenol A in experimental aquaria. After 21 days of exposure there was found significant decline (by 40-75%) in total sperm counts for male fishes exposed to tributyltin and bisphenol A compared to control, no change in the testis size or sperm length was found. However Sertoli cells which facilitate the transport of maturing sperms in to the testicular deferent duct (where they are stored prior to ejaculation), are directly sensitive to xenobiotic action and it is therefore possible that spermatogenesis was inhibited through in vivo interference with normal Sertoli cell function (Haubruge et al., 2000). In recent years hierarchy of techniques has become available for detecting chemicals, which may cause endocrine disruption in the aquatic environment. The molecular structure of a chemical provides a first indication about estrogenic activity, i.e. there is likely hood of interfering with the female hormone receptor. In vitro competitive binding assays for this receptor and specific cell cultures are also used to demonstrate an estrogenic response, but this does not adequately indicate whether the substance will cause adverse reproductive effects in an entire organism. An elevated level of vitellogenin, a typical female lipoprotein in the plasma of male fish is an in vivo estrogen-mediated response (Gimeno et al., 1998).

Temporal and dose response relationships of vitellogenin (VTG) mRNA induction and subsequent plasma VTG accumulation were established for sheep shead minnows (Cyperinus varigatus) treated with p-nonyl phenol (an alkyl phenol) and organo chlorine pesticides methoxychlor and endosulfan. 32 adult male fish per treatment were exposed to measured concentrations of p-nonyl phenol, methoxychlor and endosulfan. Separate triethylene glycol and 17 beta-estradiol treatments served as the

negative and positive controls respectively. Both of the chemical i.e. p-nonyl phenol and methoxychlor showed a dose dependent increase in the plasma VTG over the entire time course of exposure. Exposure with endosulfan failed to induce measurable levels of either hepatic VTG mRNA or serum VTG at the chemical concentration tested (Hemmer et al., 2001).

Endosulfan is a chlorinated hydrocarbon insecticide and acts as contact poison for wide variety of insects and mites on cereals, coffee, cotton, fruit, oil seeds, potato, tea, vegetables and other crops. It can also be used as wood preservative. Endosulfan is a highly toxic substance, because it is easily absorbed by the stomach, by the lungs, through the skin, meaning that all routes of exposure can pose a hazard. Acute toxicity stimulation of the central nervous system is the main characteristics of endosulfan poisoning. It is also a part of the cause for the decrease in the quality of semen, in increase in defects in male sex organs, and increased incidence of breast cancer, which has been observed in the last 50 year. Endosulfan has also been found to cause mutations. Finally there is some good news about long-term reproductive future of fish. The endocrine system of the fish is disturbed but the genetic blue print is not damaged. This means that cleaning up endocrine disrupters will get to the root of the problem. The regulatory status of endosulfan differs from one country to another, but a lot of countries have found it relevant to put in place specific regulations on endosulfan use, by banning, restricting, or severely restricting it. Campaigns have been going on worldwide for several years to ban endosulfan completely to save future generations of humans and animals.

MATERIALS AND METHODS

MATERIALS AND METHODS

Cyprinion watsoni is a small fish (max size 12cm) and is commonly found in the hilly streams of Islamabad. The temperature of water ranges between 14°C in the coldest months and 29°C in the warmest months. The size of the fish used for the experiment ranges between 6-8 cm.

Procurement and maintenance of fish:

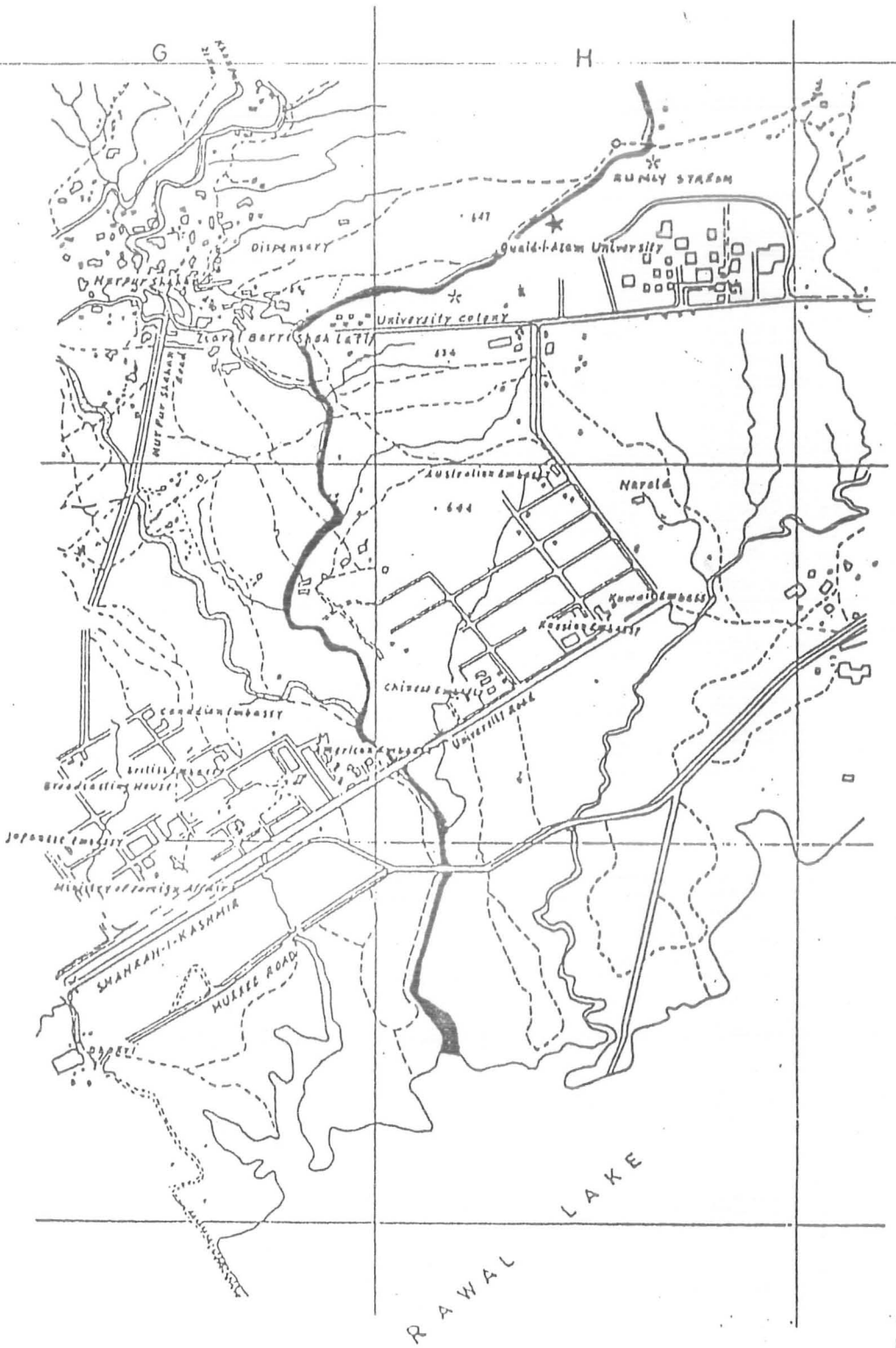
Live specimens of cyprinion watsoni were collected with nets from Ramly stream near Quaid-i-Azam University, Islamabad in early spawning period (March to May) (Shaikh and Jalali, 1986). These fish were then kept in the stocking tanks in the experimental fish laboratory of the Department of Biology (Quaid-i-Azam University Islamabad). The tanks had total capacity of 75 liters of water. The fish were then acclimatized according to the environmental conditions of the laboratory for at least one week prior to the initiation of the experiment. The fish were fed daily on the tropical fish food. The water was renewed after every two days. The water temperature was not controlled so it varied with the ambient laboratory conditions.

Chemical used and route of administration:

The chemical used for the experiment was endosulfan. Pure endosulfan is in the form of colorless crystals. The technical grade is 99.9% pure but commercial sample used for the experiment is 32.9%w/wgms/lit. Endosulfan is semisoluble in water so it is administered through water of aquaria.

Experimental design:

The fish were divided into three groups, in each group there were 30 fish, kept in three different aquaria. The aquaria had total capacity of 75 liters water, containing 40 liters of water. One group was exposed to ultra low concentration of .75ppb, the other to 1ppb and third group was maintained in parallel as control for 30 days. The body weight and body length was taken two times before and after experiment. For body weight normal electron balance was used whereas for body length verneir caliper was used. The aquaria were cleaned and the doses were restored after every two days. The fish fed on tropical fish food throughout the experimental period.



Part map of Islamabad showing Ramli Stream **(Bold Line) and Sites of** Sampling (*).

Gonadosomatic index (GSI):

Record of the body weight and testicular weight were used to determine gonadosomatic index, which was calculated according to the following formula

GSI = total testicular weight (gm) /body weight (gm) x 100

Faulton's condition factor:

Faulton's condition factor was calculated according to the following formula

Condition factor (K) = body weight (gm) /(standard body length) ^{3} cm.

Histological procedure:

The testes of the fish were dissected out, weighed to the nearest (mg) and their length and breadth (cm) was measured. The testis were immersed in fixative sera, dehydrated in the ethanol series as

80%:	overnight
90%:	2-3hours
100%:	2-3 hours.

Were cleared in the cedar wood oil and embedded as

Benzene 1:	10-15 minutes
Benzene 2:	10-15 minutes
Benzene+Paraplast:	20 minutes at 60°C
Paraplast 1:	10-12 hours at 60°C
Paraplast 2:	10-12 hours at 60°C
Paraplast 3:	10-12 hours at 60°C

Tissue blocks were then made and were sectioned with a Cambridge microtome at the thickness of 5-6 μ. The sections were affixed to precleaned albuminized glass slides and stained with hematoxyline and eosin

Xylene 1 :	5-10 minutes
Xylene 2 :	5-10 minutes
100% alcohol :	2-5 minutes
90% alcohol :	2-5 minutes
70% alcohol :	2-5 minutes
50% alcohol :	2-5 minutes
30% alcohol :	2-5 minutes

Hematoxyline:		1- 2 dips
Water	:	5-10 minutes
30% alcohol	:	2-5 minutes
50% alcohol	:	2-5 minutes
70% alcohol	:	2-5 minutes
90% alcohol	:	2-5 minutes
Eosin	:	2-3 dips
90% alcohol	:	2-5 minutes
100% alcohol	:	2-5 minutes
Xylene	:	5-10 minutes

The slides were mounted with canada balsam. Microscopic examination was carried out under a Nikon Optiphot research microscope.

The diameter of various types of spermatogenic cells i.e. spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids were observed with the help of ocular micrometer. The diameter of the nuclei of the spermatogonia was also noted down to see the effect of endosulfan.

RESULTS

RESULTS

Overall Behavior and Mortality:

Overall behavior of fish (both control and endosulfan treated) was observed during the entire experimental period. No abnormal behavior was observed, however, treated fish (.75ppb and 1ppb endosulfan) were observed to display anxietic behavior for about an hour after ingesting endosulfan. No dose dependent mortality was observed during the course of experiment.

Gross Morphology of Testes:

The testes of Cyprinion watsoni are a pair of elongated organs suspended by lengthwise mesenteries in the upper section of the body, and are found alongside the swimbladder. The sperm ducts of both the testes unite to form a common duct that opens to the exterior by a gonopore. The size and color of testes varied according to the stage of sexual maturity and ripeness.

Gross Histology of Testes:

A thin layer of connective tissue surrounds the testis, the tunica albuginea which is continuous with the connective tissue fibers of the wall of the seminiferous lobules. Seminiferous lobules are separated by the narrow spaces that are filled by the interstitial tissues containing Leydig cells, connective tissue and blood vessels. Spermatogonia lie on the inner side of the lobule wall. As the spermatogenesis progresses, each lobule gives rise to a number of the cysts which possess different stages of spermatogenic cells. Spermatogenesis takes place within the cysts of lobules and the spermatozoa are released into the spermatic duct.

Body weight (gm):

Mean body weight of control and endosulfan treated (.75 ppb and 1ppb) groups is given in table 1. Fig. 1a. The body weight of .75 ppb endosulfan treated group showed a significant ($P<0.05$) increase compared to control. There was non significant ($P>0.05$) difference between 1ppb group and control. Comparison of low and high dose treatment also showed non significant ($P>0.05$) difference.

Standard body length (cm):

Mean body length of control and endosulfan treated (.75 ppb and 1ppb) groups is given in the table 1. Fig. 1b. The body length of endosulfan treated (.75 ppb and 1ppb) groups increased significantly ($P<0.05$) compared to control. High dose (1ppb) endosulfan caused a significant ($P<0.05$) decrease in standard body length compared to low dose.

Condition factor (K):

Mean condition factor of control and endosulfan treated (.75 ppb and 1ppb) groups is given in table 1. Fig. 1c. The condition factor of both endosulfan treated (.75 ppb and 1ppb) groups showed non-significant ($P>0.05$) difference when compared to control. A significant ($P<0.05$) difference was noticed between .75ppb and 1ppb endosulfan treated groups.

Testicular weight (mg):

Mean testicular weight of control and endosulfan treated (.75 ppb and 1ppb) groups is given in table 2. Fig.2a. Testicular weight (both right and left) of fish treated with .75 ppb endosulfan increased significantly ($P<0.05$) compared to control. Weight of right testis of fish treated with low dose (.75 ppb endosulfan) increased significantly ($P<0.05$) compared to high dose (1ppb endosulfan) treated group whereas weight of left testis of .75 ppb group increased non-significantly ($P>0.05$) compared to 1ppb endosulfan treated group. A non significant ($P>0.05$) increase was also observed in testicular weight (both right and left) of 1ppb group compared to control.

Gonadosomatic Index (GSI):

Mean Gonadosomatic Index (GSI) of control and endosulfan treated (.75 ppb and 1ppb) fish is given in table 2. Fig.2b. Effect of endosulfan on the GSI of treated groups was compared with control. A non significant ($P>0.05$) difference was noticed among the control and endosulfan treated (.75 ppb and 1ppb) groups. The GSI of 1ppb endosulfan treated group decreased compared to .75 ppb endosulfan treated group but this was statistically non significant ($P>0.05$).

Table No 1: Effect of Endosulfan on body weight, standard body length and faulton's condition factor (K) of Cyprinion watsoni:

Experimental Group	Body weight (g)	Std. Body Length (cm)	Faulton,s Condition Factor (K)
Control	4.33 ± 0.44	6.01 ± 0.19	0.02 ± 0.0007
.75 ppb	7.27 ± 1.11a*	7.8 ± 0.39 a**c*	0.02 ± 0.00026 c*
1 ppb	5.56 ± 0.40	6.68 ± 0.22 b*	0.02 ± 0.00029

Values (Mean ± S.E), student "t" test

a,b = treated groups Vs control

c = low dose Vs high dose treatement

P < 0.05*

P < 0.01**

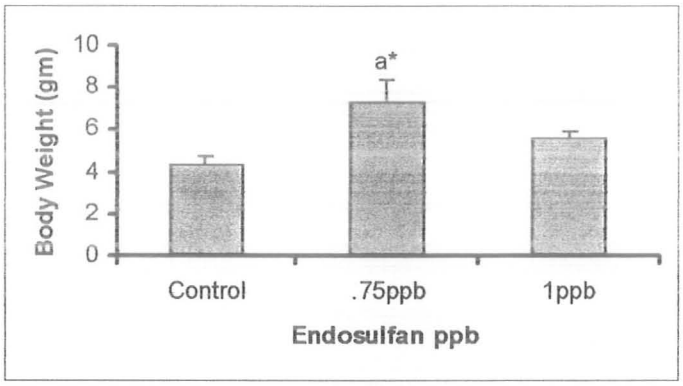


Fig 1a:

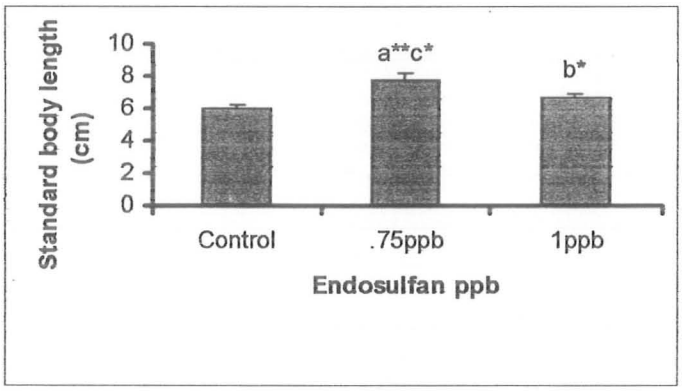


Fig 1b:

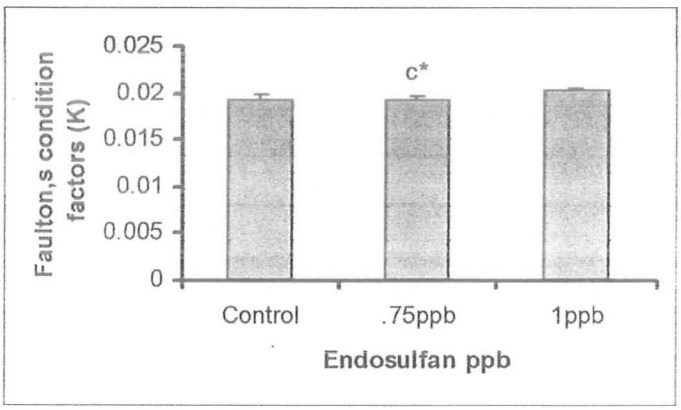


Fig 1c:

Fig 1: Effect of Endosulfan on body weight, standard body length and Faulton's condition factor of fish Cyprinion watsoni.

Values (Mean ± S.E), student "t" test

a , b = treated groups Vs Control

c = low dose Vs High dose treatment

$P < 0.05$*, $P < 0.01$**

Table No 2: Effect of Endosulfan on testicular weight and gonadosomatic index (GSI) of Cyprinion watsoni:

Experimental Group	Testicular Weight (mg)		Gonadosomatic index (GSI)
	Right	Left	
Control	15.61±3.60	17.57±4.33	0.66±0.12
.75ppb	63.9±13.80a**c**	53.89±10.93a**	1.30±0.34
1ppb	24.38±4.28	28.7±6.30	0.80±0.08

Values (Mean ± S.E) Student "t" test

a, b = treated groups Vs control

c = low dose Vs high dose treatment

P < 0.01**

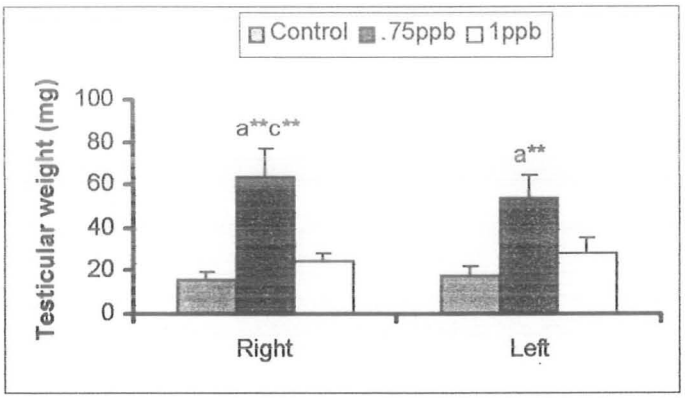


Fig 2a:

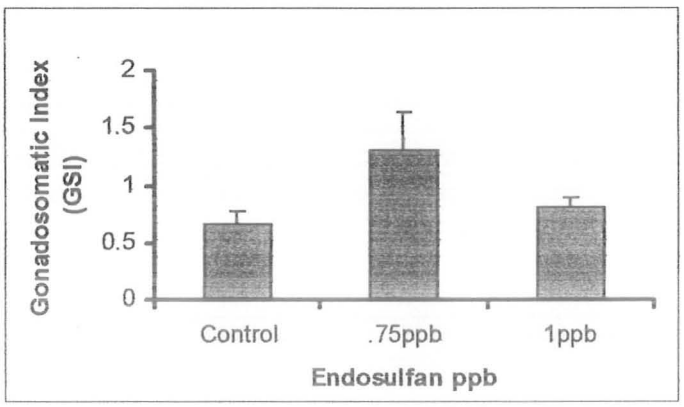


Fig 2b:

Fig 2: Effect of Endosulfan on testicular weight and gonadosomatic index (GSI) of Cyprinion watsoni:

Values (Mean ± S.E) Student "t" test

a , b = treated groups Vs control

c = low dose Vs high dose treatment

$P < 0.01$**

Testicular length (cm):

Mean length of right and left testes of control and endosulfan treated (.75 ppb and 1 ppb) groups is given in table 3. Fig. 3a . A significant ($P<0.05$) increase in length of both right and left testes of .75 ppb group was observed compared to that of 1ppb group and control. High dose (1ppb) endosulfan treatment showed significant ($P<0.05$) increase in length of left testis whereas non significant ($P>0.05$) increase in length of right testis was observed compared to control.

Testicular breadth (cm):

Mean breadth of right and left testes of control and endosulfan treated (.75 ppb and 1 ppb) groups is given in Table 3. Fig. 3b. The breadth of both right and left testes of .75 ppb endosulfan treated group showed a significant ($P<0.05$) increase compared to control. Right and left testicular breadth of the group treated with 1ppb endosulfan increased non-significantly ($P>0.05$) compared to control. A non significant ($P>0.05$) decrease was observed in the breadth of right testes whereas the breadth of left testis of high dose (1ppb) treated group decreased significantly ($P<0.05$) compared to low dose (.75 ppb) treated group.

Morphometry of spermatogenic cells:

Spermatogonia:

Mean diameter of spermatogonia of control and endosulfan treated (.75 ppb and 1ppb) groups is given in table 4. Fig. 4a . The diameter of spermatogonia of the group treated with .75 ppb decreased compared to control but this was statistically non-significant ($P>0.05$). A significant ($P<0.05$) decrease was observed in 1ppb group compared to control. Comparison of low (.75 ppb) and high (1ppb) dose treatment showed no significant ($P>0.05$) difference.

Primary spermatocytes:

Mean diameter of control and treated (.75 ppb and 1ppb) groups is given in the table 4. Fig. 4b. No significant increase or decrease was noticed in the diameter of primary spermatocytes of .75 ppb group compared to control and 1ppb group. Diameter of the group treated with 1ppb endosulfan decreased non-significantly ($P>0.05$) compared to control.

Table No 3: Effect of Endosulfan on testicular length and testicular breadth of Cyprinion watsoni:

Experimental Group	Testicular length (cm)		Testicular breadth (cm)	
	Right	Left	Right	Left
Control	1.23±0.10	1.15±0.13	0.16±0.02	0.16±0.02
.75 ppb	2.34±0.19a***c***	2.57±0.15a***c***	0.29±0.04a**	0.24±0.02a**c*
1ppb	1.58±0.14	1.65±0.104b**	0.22±0.02	0.18±0.02

Values (Mean ± S.E) Student "t" test

a , b = treated groups Vs control

c = low dose Vs high dose treatment

P < 0.05*

P < 0.01**

P < 0.001***

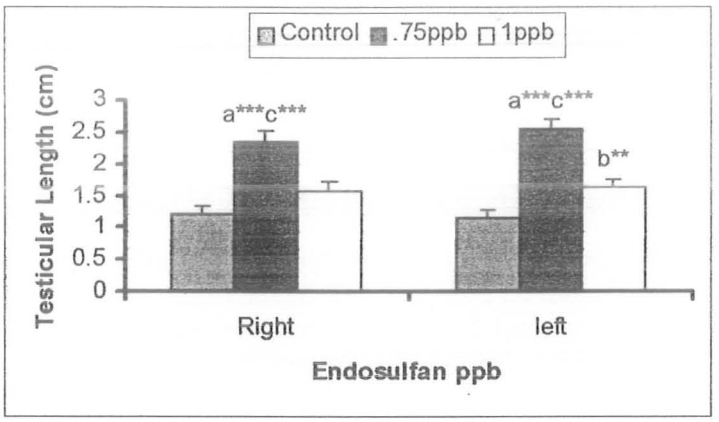


Fig 3a:

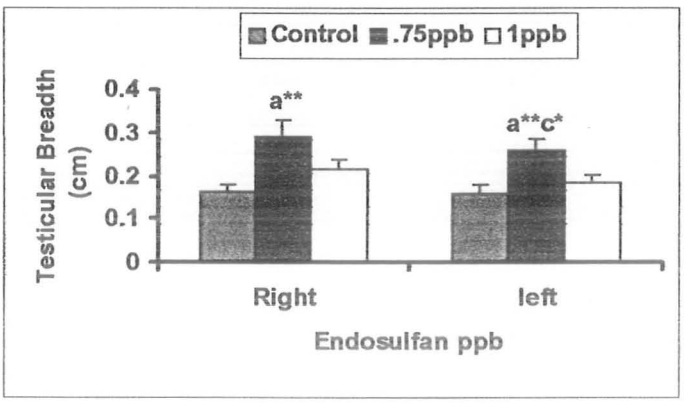


Fig 3b:

Fig 3: Effect of Endosulfan on testicular length and testicular breadth of Cyprinion watsoni:

Values (Mean ± S.E) Student "t" test

a , b = treated groups Vs control

c = low dose Vs high dose treatment

P < 0.05*

P < 0.01**

P < 0.001***

Secondary spermatocyte:

Mean diameter of secondary spermatocytes of control and endosulfan treated groups is given in table 4. Fig. 4c. There was no significant ($P>0.05$) increase in the diameter of secondary spermatocytes of .75 ppb group compared to 1ppb group and control. The high dose (1ppb) treated group also showed non significant ($P>0.05$) difference compared to control.

Spermatids:

Mean diameter of spermatids of control and treated (.75 ppb and 1ppb) groups is given in table 4. Fig. 4d. No significant ($P>0.05$) difference was noticed in the diameter of spermatids of groups treated with .75 ppb and 1ppb endosulfan compared to control. The comparison of low (.75 ppb) and high (1ppb) dose treatment also showed no significant ($P<0.05$) difference in the diameter of spermatids.

Nuclear Diameter of Spermatogonia:

Mean nuclear diameter of spermatogonia of control and endosulfan treated (.75 ppb and 1ppb) groups is given in the table 4. Fig. 4e. The nuclear diameter of spermatogonia of endosulfan treated (.75 ppb and 1ppb) groups decreased non-significantly ($P>0.05$) compared to control. The difference between the nuclear diameter of low (.75 ppb) and high dose (1ppb) treated groups was also non-significant ($P>0.05$).

Table No 4: Effect of Endosulfan on diameter of various types of spermatogenic cells and diameter of the nucleus of spermatogonia in the testis of Cyprinion watsoni:

Experi-mental Group	Spermatogonia (µm)	Pr. Spermatocyte (µm)	Sec. spermatocyte (µm)	Spermatid (µm)	Spermatogonia nucleus(µm)
Control	9.98±0.07	3.91±0.03	2.73±0.05	1.81±0.02	6.45±0.06
.75 ppb	9.57±0.06	3.94±0.07	2.91±0.11	1.89±0.05	6.27±0.12
1 ppb	9.54±0.07b***	3.81±0.05	2.67±0.08	1.79±0.02	6.33±0.08

Values (Mean±S.E), Student "t" test

b = 1ppb Endosulfan treated group Vs control.

P < 0.001***

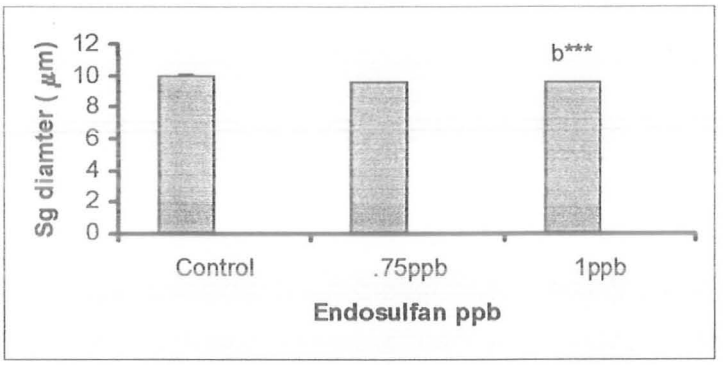


Fig 4a:

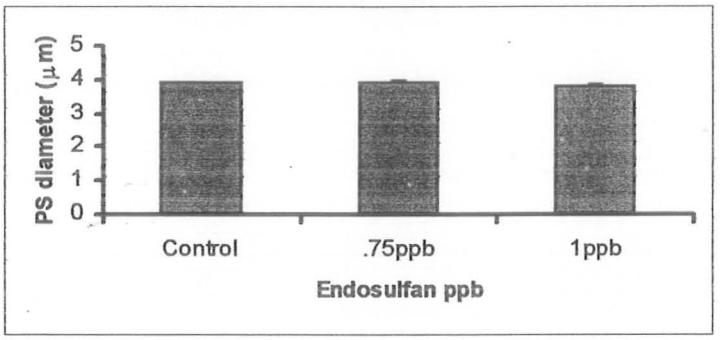


Fig 4b:

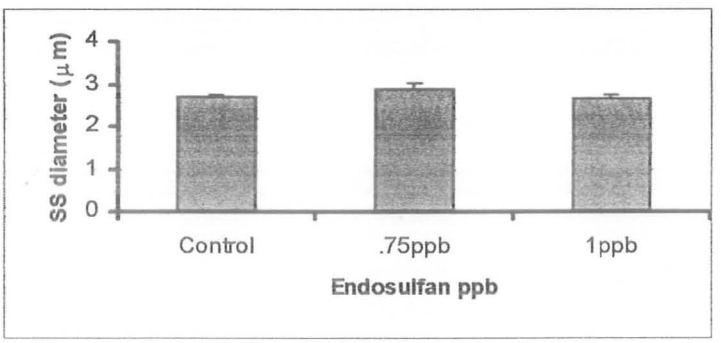


Fig 4c:

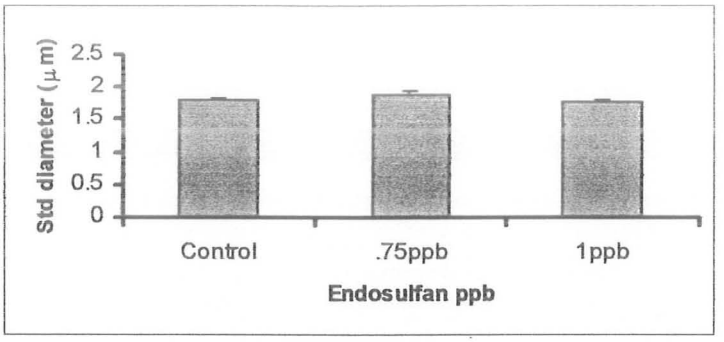


Fig 4d:

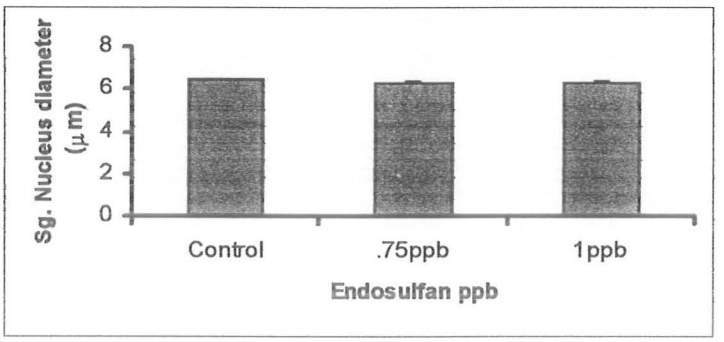


Fig 4e:

Fig 4: Effect of Endosulfan on diameter of various types of spermatogenic cells and diameter of the nucleus of spermatogonia in the testis of Cyprinion watsoni:

Values (Mean±S.E), Student "t" test

b = 1ppb Endosulfan treated group Vs control.

P < 0.001***

HISTOMORPHOLOGY

Control:

Histomorphological examination of the testicular section of Cyprinion watsoni showed many spermatogenic lobules (Fig. 5a, 7a, 8a). The lobules occupy a larger area of the testis. Inter-lobular walls separated the lobules. The inter-lobular walls are distended and thin. Testis is covered by tunica albugenia on its outer surface (Fig. 6a). Compactly arranged cysts are present in the lobules. The cysts containing spermatogonia are mostly visible in the peripheral area. Each spermatogonium is large and spherical compared with other cells and possess large, lightly stained spherical nucleus with distinct nucleolus (Fig. 6a, 7a, 8a). The nuclear membrane is very conspicous. The chromatin is attached to the inner side of the nuclear membrane (Fig. 6a, 8a). Other cysts contain primary and secondary spermatocytes. Primary spermatocytes are smaller than spermatogonia, their nuclei are darkly stained, and they have scanty cytoplasm. Secondary spermatocytes are even smaller than primary spermatocytes, their nuclei are darkly stained (Fig. 7a). Round spermatids are also observed in the some of the cysts (Fig. 5a, 7a, 8a, 9a). All the stages of spermatogenic cells are present in the normal state.

The interlobular area is filled with interstium. The interstium consists of Leydig cells, connective tissue, lymphatic vessels, blood vessels and capillaries (Fig. 8a).

.75ppb group:

Histomorphological examination of the testicular section of the .75ppb treated group showed following changes. The lobules have changed their organization, they are loosely arranged. The cysts are also loosely arranged as compared to control. The interlobular space has increased significantly compared to control (Fig. 5b, 7b, 8b). The cyst containing spermatogonia are fewer and loosely arranged. Their cytoplasm is thin and cell membrane is irregular in shape. The chromatin material is interspersed in the nucleus (Fig. 6b, 7b, 8b). Number of primary and secondary spermatocytes has increased due to arrest of spermatogenic cycle, so there are found fewer cysts containing spermatozoa (Fig. 7b). The primary and secondary spermatocytes show clumping, evenly distributed through out the testicular tissue as compared to control (Fig. 5b, 6b, 7b, 9b). The cell membrane and nuclear membrane of primary and secondary spermatocytes is not intact (Fig. 9b).

The cysts containing spermatids have also increased due to arrest of spermatogenic cycle. The spermatids show clumping like primary and secondary spermatocytes (Fig. 5b, 7b, 8b, 9b).

The interstium consists of blood cells and Leydig cells (Fig. 8b).

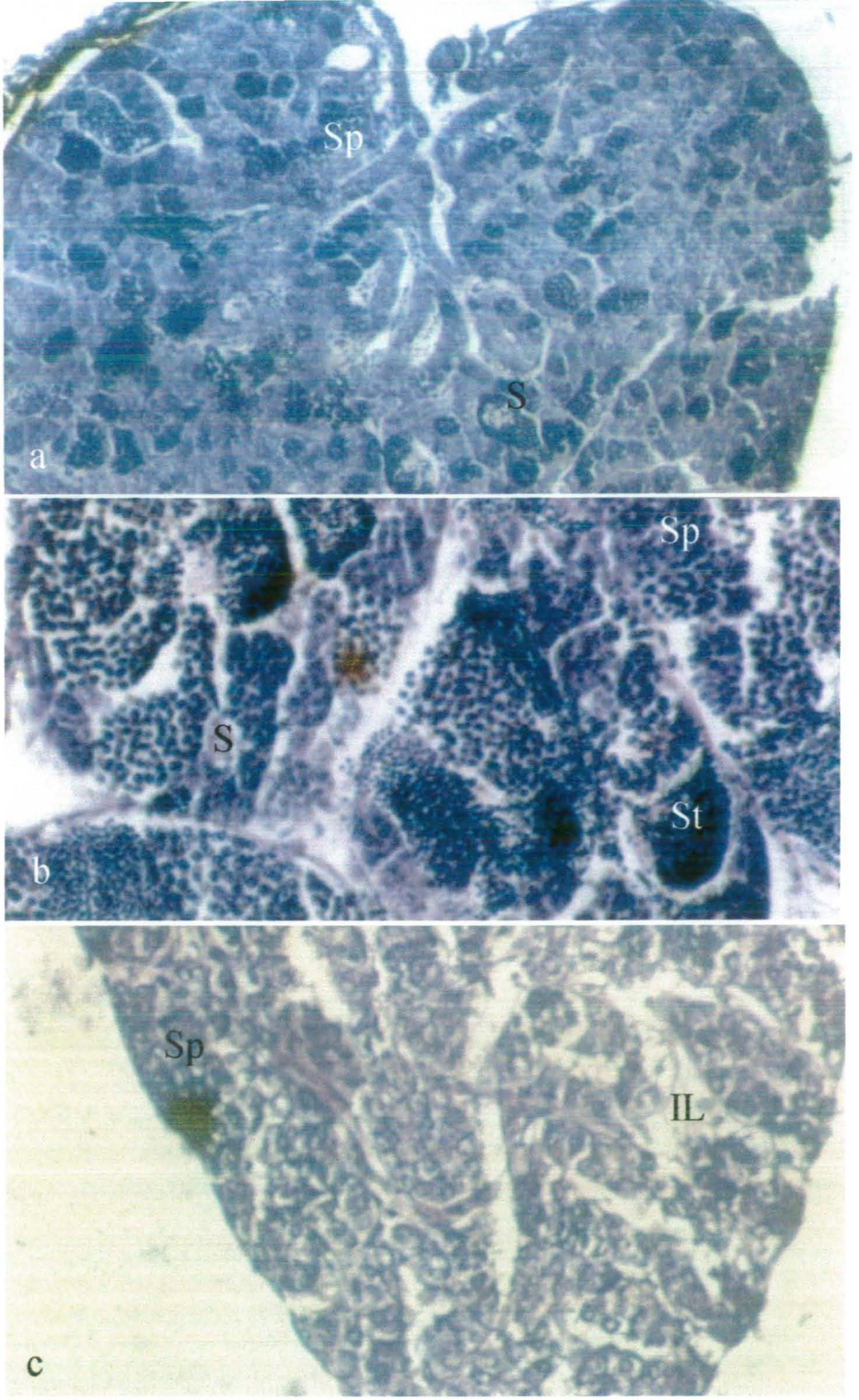


Fig.5. Photomicrograph of Cyprinion watsoni testis showing gross morphology (**a**) Of control group with compact lobules of spermatogonia (S), spermatocytes (Sp) and spermatids (St) X220.87. (**b**) Testis treated with .75ppb endosulfan has increased interlobular (IL) areas, clumping of spermatocytes and spermatids. X 441.75. (**c**) Testis treated with high dose endosulfan showing increased interlobular areas and clumping of spermatocytes X 220.87. (H.E).

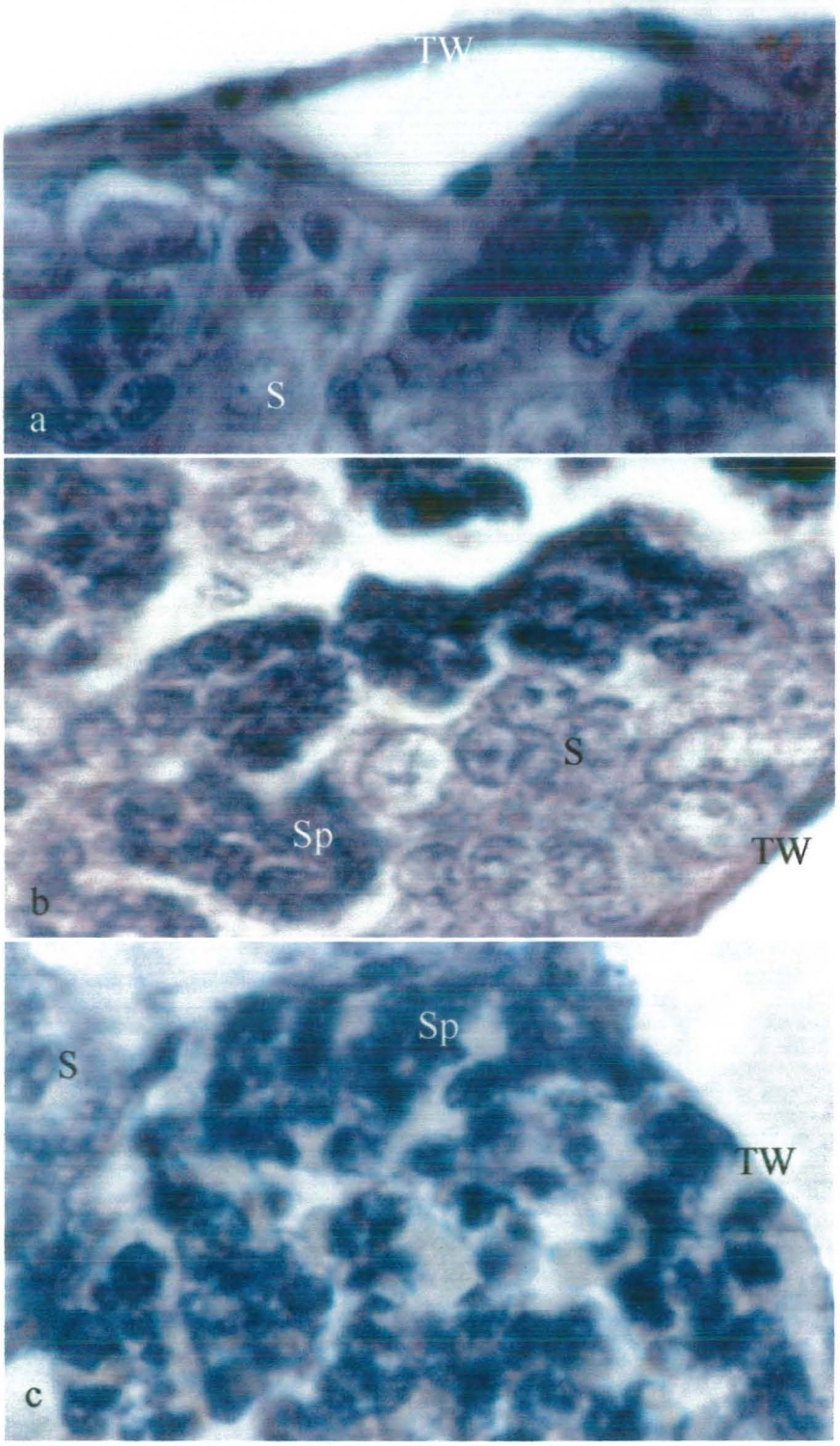


Fig.6. Photomicrograph of the testicular section of Cyprinion watsoni showing (**a**) Normal morphology of the testicular wall (TW) and spermatogonia (S).(**b,c**) section of testis of Cyprinion watsoni treated with .75ppb and 1ppb endosulfan showing thinning of testicular wall, disintegration of spermatogonia and clumping of spermatocytes (Sp) X 2208.77. (H.E).

Ippb group:

Histomorphological examination of the testicular section of 1ppb endosulfan treated group showed following changes. The testicular wall shows decrease in thickness compared to control and low dose treated group (Fig. 6c). The lobules and cysts located both towards the periphery and hilus are loosely arranged (Fig. 5c, 7c, 8c). The interlobular space increases more towards the hilus (Fig. 5c). Spermatogonia containing cysts are fewer and are loosely arranged compared to the control and low dose treated group. They possess irregular cell membrane and lightly stained cytoplasm. The nuclear membrane is also irregular (Fig. 8c, 9c). Number of primary and secondary spermatocytes increase significantly due to arrest of spermatogenic cycle therefore there are found few cysts containing spermatozoa (Fig. 7c). The primary and secondary spermatocytes show clumping like .75ppb endosulfan treated group (Fig. 6c, 7c, 8c, 9c). Their cell membrane and nuclear membrane are irregular in shape (Fig. 6c, 9c).

The cysts containing round spermatids have also increased, they show clumping like spermatocytes (Fig. 7c).

Lymphatic vessels and blood capillaries are visible in the interstium (Fig. 8c).

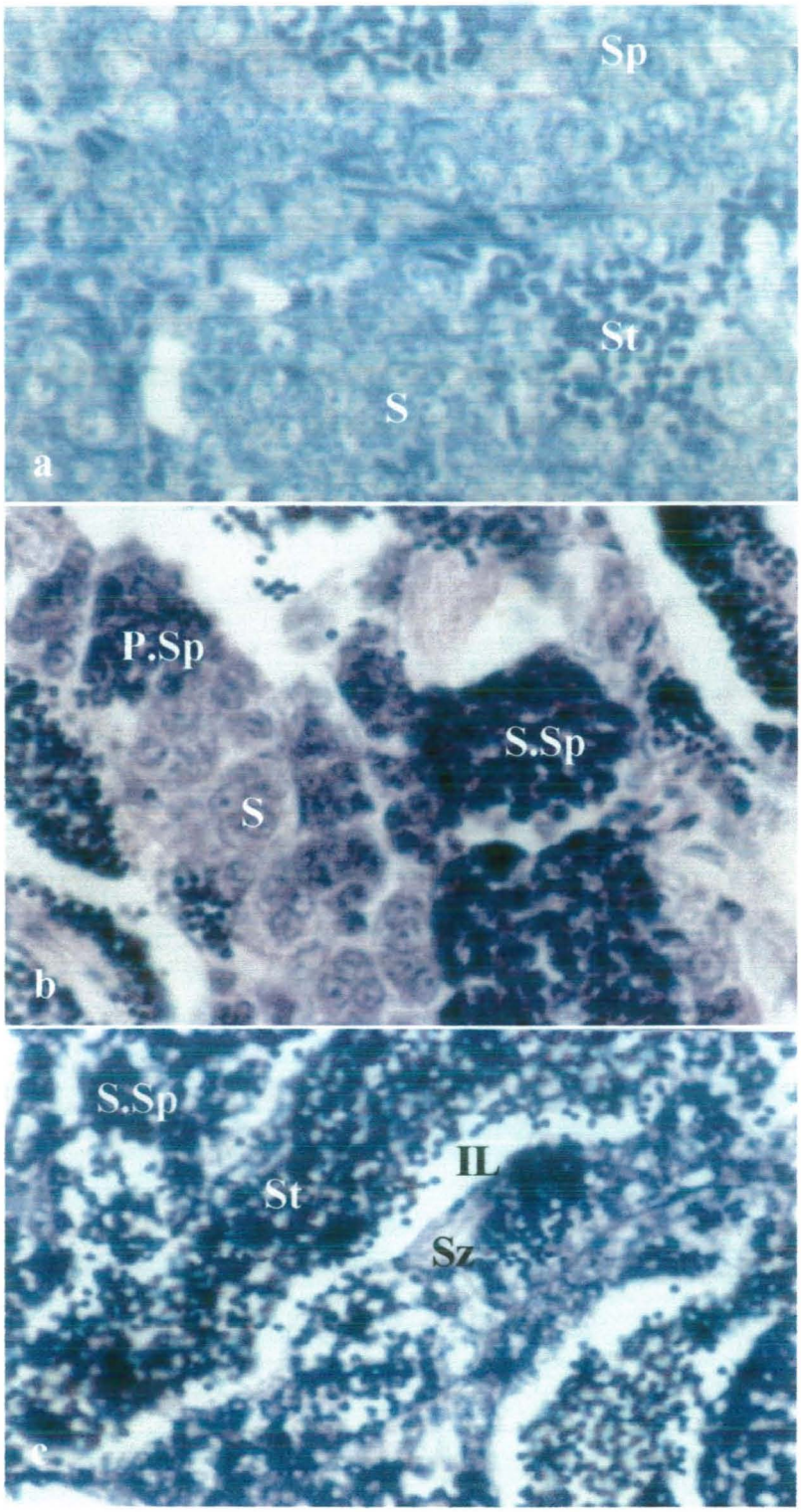


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Fig.7. Photomicrograph of the testis of fish Cyprinion watsoni **(a)** Showing normal and compact lobules of spermatogonia (S), spermatocytes (Sp) and spermatids (St). **(b)** testis treated with low dose endosulfan has increased interlobular areas (IL), some spermatozoa (Sz), disintegration of spermatogonia, clumping of primary spermatocytes (P.Sp), secondary spermatocytes (S.Sp) and spermatids. **(c)** Testis treated with high dose endosulfan showing increased interlobular areas, some spermatozoa, clumping of secondary spermatocytes and spermatids X 883.5. (H.E).

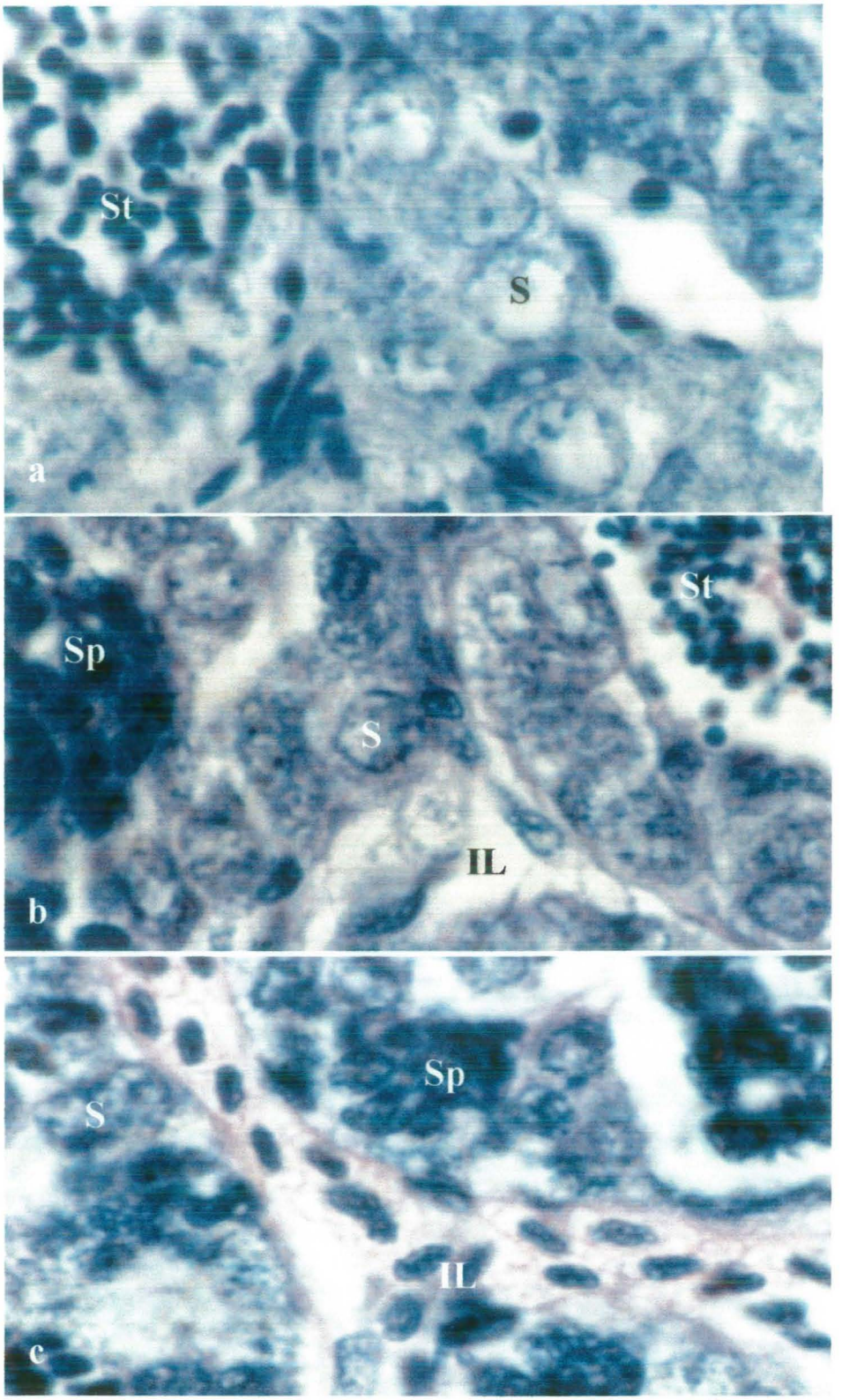

Fig.8. Photomicrograph of the testicular section of fish Cyprinion watsoni (**a**) control group with compact lobules of normal spermatogonia (S) and spermatids (St). (**b**) Testis treated with .75ppb endosulfan has increased interlobular areas (IL), disintegration of spermatogonia, clumping of spermatocytes (Sp) and spermatids. (**c**) Fish testis treated with high dose endosulfan showing increased interlobular areas, disintegration of spermatogonia and spermatocytes and clumping of spermatocytes X 2208.77. (H.E).

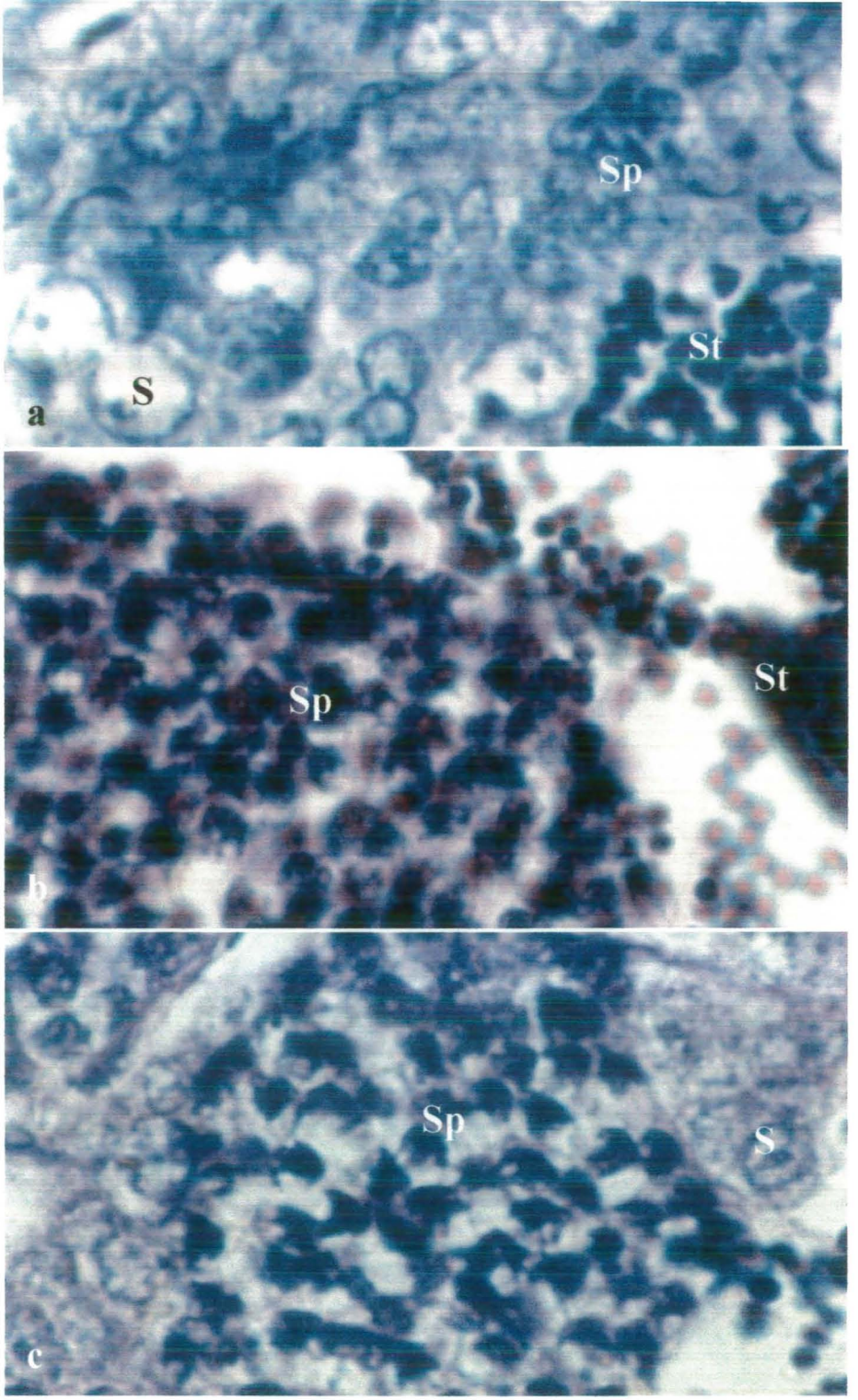

Fig.9. Photomicrograph of Cyprinion watsoni testis (**a**) Showing normal morphology of spermatogonia (S), spermatocytes (Sp) and spermatids (St). (**b**) Group treated with .75ppb endosulfan showing disintegration and clumping of spermatocytes and spermatids. (**c**) Fish testis treated with high dose caused disintegration of spermatogonia and spermatocytes and clumping of spermatocytes X 2208.77. (H.E).

DISCUSSION

DISCUSSION

The current study was conducted to evaluate the effect of endosulfan on testicular histology of fish Cyprinion watsoni because no information is available with reference on the adverse effect of endosulfan on the testicular histology of fish Cyprinion watsoni.

Cyprinion watsoni was selected as a test organism due to their abundance in the Ramly, fresh water stream of Islamabad. Moreover these are smaller in size, can easily be handled and maintained in laboratories for experimental purposes.

Endosulfan (6,7,8,9,10,10hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide) is an organochlorine insectiside,which is less persistent in the environment but more toxic to the fish (Moulton, 1973; Pic et al., 1981; Matthiessen et al., 1982; Nowak and Ahmad, 1989; Nowak, 1990; Nowak and Julli 1991; Geobel et al., 1982). The US Environmental protection Agency (USEPA) classifies endosulfan as a category 1b (highly hazardous) pesticide. That is why it was decided to use endosulfan as the chemical for exposure.

The exposure route is of major importance for the uptake of xenobiotics in fish (Ekelund, 1989). Under laboratory conditions fish has been shown to accumulate endosulfan from surrounding water (Schoettger, 1970; Herzberg, 1986; Nowak, 1991; Nowak and Sinderam, 1991). Therefore it was decided to administer endosulfan through water. Distribution of organochlorine in different organs is often due to differences in lipid content (Phillips, 1980). The relative contribution of various organs to biotransformation of xenobiotics depends on the blood perfusion, the weight of organ, and their metabolic activity (Lindrostroemseppa et al., 1981).

The fish size is an important determinant of reproductive success in fish (Collier et al., 1992). The present study showed that the mean body size (length) of the fish treated with .75ppb and 1ppb endosulfan increased significantly ($p<0.05$) compared to control.

Mean body weight showed significant increase ($p<0.05$) in fish treated with .75ppb endosulfan compared to control, however fish treated with 1ppb endosulfan showed non significant ($p>0.05$) increase in body weight compared to control.

Condition factor is a generalized indicator of the overall health or "plumpness" of a fish and can reflect the integrated effect of nutritional status and metabolic stress (Adams and Mclean, 1985). In the present study there was no significant ($p>0.05$) difference in the condition factor (K) of low dose (.75ppb) and high (1ppb) dose treated groups compared to control. Johnson et al. (1996) observed no significant difference in either condition factor or length – weight relationship in English sole (Plironectes vetuluts) from the area contaminated with aromatic and chlorinated hydrocarbons.

Mean testicular weight of the fish treated with .75ppb endosulfan showed a significant increase ($p<0.05$) compared to control. There was no significant ($p>0.05$) increase in the testis of the fish treated with 1ppb endosulfan compared to control. Mean testicular lengths and breadths of treated and control groups were compared. The fish treated with .75ppb endosulfan showed a significant ($p<0.05$) increase in the length and breadth of both right and left testis. However high dose (1ppb) endosulfan treatment showed significant ($p<0.05$) increase in the length and breadth of left testis where as length and breadth of right testis increased non significantly ($p>0.05$) upon high (1ppb) dose treatment. Unlike the results of present study, Sinha et al. (2001) while studying effect of endosulfan on spermatogenesis in rats, investigated that testis showed significant decrease in weight in treated groups. Singh and Pandey (1990) observed that endosulfan did not alter the body weight and testicular weights of the treated animals (rats). They also suggested an altered endocrine functioning of the testicular tissue even after short-term treatment. Aromatic hydrocarbons and PCBs have been associated with reproductive failure in fish in control laboratory exposures (Roweet et al., 1983; Chen and Soustegart, 1984). Deleterious effects of PCBs on various aspects of male reproduction in rats have been found (Sager, 1983; Sager et al., 1987, 1991). The effects include hypothyroidism in treated animals (Gray et al., 1993; Ness et al., 1993; Li et al., 1994), and also increase in adult testis size (Cooke and Meisami, 1991; Cooke et al., 1991). In vitro and in vivo studies has shown that selected PCBs and their mixture are capable of mimicking some of the biological activities of estrogen (Gierthy et al., 1995).

The simplest measure of gonadal dysfunction is to measure the gonadosomatic index (GSI) in control and treated fish (Kime, 1995). In the present study no significant

($p>0.05$) difference was noticed in the control and endosulfan treated groups. Some authors as an objective have used the gonadosomatic index (GSI), sensitive and reliable indicators of the gonadal state (Clemens and Reed, 1967). Johnson et al., (1998) observed a decrease in the GSI of English sole (Parophrys vetulus) from Dawamish waterway contaminated with aromatic hydrocarbons and PCBs than in the fish from Sinclair Inlet.

Effects on the endocrine system are seen as alteration in hormonal receptors binding and in steroid hormone balance. Direct and indirect evidence for a weak estrogenic activity was observed for PCBs. Changes in steroid levels are associated with reproductive condition of male fish. The decrease in testosterone level during spawning period is due to its utilization for maturation of sperms (Jalali and Haider, 1985). Singh and Pandey (1990) also observed reduction in plasma testosterone and testicular testosterone contents of male rats upon endosulfan treatment.

Morphometry of the spermatogenic cells like cellular diameter of spermatogonia, primary spermatocyte, secondary spermatocytes and spermatids of fish Cyprinion watsoni were observed and measured for the assessment of any potential effect of endosulfan on these parameters. The nuclear diameter of spermatogonia was also measured.

In the present study, diameter of spermatogonia treated with low dose (.75ppb) endosulfan decreased compared to control but this decrease was statistically non-significant ($p>0.05$). However diameter of spermatogonia treated with high dose (1ppb) endosulfan decreased significantly ($p<0.05$) compared to control. Similar results were obtained by Ishaq (2001) while studying the effect of PCBs (Aroclor 1242) on testis of fish Cyprinion watsoni.

Differences were observed in the diameter of primary spermatocyte of control and the treated groups. The diameter of primary spermatocytes increased non-significantly ($p>0.05$) compared to control, whereas high dose (1ppb) caused non significant decrease in diameter of primary spermatocyte.

In the present study diameter of secondary spermatocyte of Cyprinion watsoni were observed. Diameter of secondary spermatocytes of fish treated with .75ppb endosulfan increased non-significantly ($p>0.05$) compared to control and high dose

treated groups showed a non-significant ($p>0.05$) decrease in the diameter of secondary spermatoctye of Cyprinion watsoni. The same effects of PCB (Aroclor 1242) on primary and secondary spermatocytes were observed by Ishaq (2001) while working on reproductive organs of male Cyprinion watsoni.

The diameter of spermatids of control and treated groups were measured, to observe any effect of endosulfan on its structure. The diameter of spermatid of fish treated with .75ppb endsulfan, increased compared to control but this increase was statistically non-significant ($p>0.05$). The fish treated with 1ppb endosulfan showed non-significant ($p>0.05$) decrease in diameter of spermatid.

Due to scanty information regarding the effect of toxic chemicals including endosulfan, the differences in the diameter of spermatid of Cyprinion watsoni could not be explained. However it can be suggested that the xenobiotics in the environment may have detrimental effects on the normal testicular processes of fish Cyprinion watsoni.

In the present study nuclear diameter of spermatogonia was also measured to evaluate the potential effects of endosulfan. The nuclear diameter of the low dose (.75ppb) and high dose (1ppb) endosulfan treated group decreased non-significantly ($p>0.05$) compared to control. In the pre-spawning period a non significant effect was noted on the nuclear diameter of spermatogonia by PCB (Ishaq, 2001).

Biological consequences of contaminant residues and fish deformities, such as reproductive impairments, should be determined to interpret the significance of these residues. Histological and ultra structural changes need to be studied not only because they are more sensitive indicator of pollution (Rapport, 1984) but also because they may explain, and hopefully predict, changes at higher levels of organization (organism/population).

In the present study the histomorphology of the testis of Cyprinion watsoni was also observed to evaluate the potential effects of endosulfan. The present study was conducted in March and early April so the histological picture of the normal / control testis presented a well-organized structure. All the stages of spermatogenic cells were present within the cysts of lobules. The spermatogenic lobules and interlobular walls were organized.

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Treatment of .75ppb endosulfan caused thinning of testicular walls compared to control. The cysts and lobules were loosely arranged compared to control. Irregular cell membrane and nuclear membrane was observed in spermatogonia. Histological examination also revealed disintegration of some of the spermatogenic cells with in the cysts of some lobules. Similar results (disintegration of spermatogenic cells) were observed by Sangalang et al. (1981) while studying the effect of 2.5ug/g PCB diet on Gadus morhua. In the present study primary and secondary spermatocytes were more affected. Their number has increased and showed clumping and irregular cell membrane. PCB (Aroclor 1242) caused disintegration of spermatogonia and spermatocytes and clumping of spermatocytes in the testis of fish Cyprinion watsoni (Ishaq, 2001). Clumping of early spermatocytes was also observed by Sangalang et al. (1981). High dose treatment has increased number of the spermatids. The spermatids also showed clumping like spermatocytes. The sperm count was reduced. This may be due to arrest of spermatogenic cycle. Sinha et al. (1995) suggested that endosulfan caused impairment in testicular functions by altering activities of the enzymes responsible for spermatogenesis, there by influencing intratesticular spermatid count and causing low sperm production and sperm deformity.

Histological examination of testis of Cyprinion watsoni treated with 1ppb endosulfan revealed that lobules changed their organization and the cysts and lobules are loosely arranged i.e. the interlobular space has increased compared to control. The cysts containing spermatogonia were decreased and they had irregular cell and nuclear membrane. The number of primary and secondary spermatocytes containing cysts increased in number and showed irregular cell membrane and clumping like low (.75ppb) dose treated groups. These results can be correlated with the results of Sangalang et al. (1981) while studying the effects of 5µg/g PCB diet on Gadus morhua. Similar results were obtained by Ishaq (2001) while studying the effect of 10mg Aroclore 1242 (PCB) on the testis of Cyprinion watsoni. High dose (1ppb) treatment increased the number of cysts containing spermatids and they also showed clumping like spermatocytes. Unlike the present study Sinha et al. (1995) and Sinha et al. (2001) observed reduction in the spermatid count in testis while studying the effect of endosulfan on testis of rats. In the present study there was observed reduced sperm counts in the testis upon high dose (1ppb) treatment. According to Dalsenter et al. (1999) the daily sperm production was permanently decreased in the highest dose

group (3.0mg-endosulfan/kg-body weight of rats). Sinha et al. (1995) observed low sperm production and sperm deformity in the testis of rats treated with endosulfan. Sinha et al. (2001) noticed reduced sperm count in the cauda epididymis while studying the effect of endosulfan (1 or 2 mg/kg/day) on spermatogenesis in rats.

In summary the results of this study indicate that Cyprinion watsoni exposed to endosulfan, show histological changes in reproductive organs and testicular atrophy (Naqvi and Vaishnavi, 1993).

Endosulfan effect is needed to be studied genetically and biochemically. Endosulfan is constantly arealy sprayed in cotton growing areas, it is practically insoluble in water, but readily adhere to clay particles and persists in soil and water for several years. This chemical is extremely toxic to most fish and can cause massive mortalities (Naqvi and Vaishnavi, 1993). Its effect on all the species of fresh water fish and flora must be studied. Experimental studies on the toxicological effects of endosulfan are important if we want to understand the potential impact of degradation of fresh water fish population. Such studies will enable us to identify the contaminants/chemicals responsible for reproductive impairment and the wild life species, which are at risk, and may in turn help us in successful conservation and management of commercially important fish species.

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