DETERMINATION OF AROMATASE ACTIVITY, GONADAL HORMONE AND CELLULAR ACTIVITY IN *LABEO ROHITA* (HAM 1822) EXPERIMENTALLY EXPOSED TO SOME ENDOCRINE DISRUPTION CHEMICALS (EDCS).

SUMMARY OF
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Sexual reproduction in vertebrates is mediated by steroid hormones and numerous endocrine disrupting chemicals (EDCs) discharged into the environment have been shown to interfere with hormone signaling via several mechanisms. In fish, sex determination and differentiation are particularly susceptible to endocrine disruption and exposures to various synthetic and natural endocrine disrupting chemicals have been associated with significant effects on reproductive development and function in wild fish populations. Endocrine disrupting chemicals (EDCs) are chemicals that can have an effect on the endocrine system of animals including aquatic animals (fishes, molluscs, and amphibian), birds and humans. Several EDCs have been detected in water, air, and soil environments. Estrogenic EDCs (e-EDCs) are able to induce an estrogen-like response in organisms The most common e-EDCs listed in literature are Estrone (E1), 17β-Estradiol (E2), Ethinylestradiol (EE2), Estriol (E3), Bisphenol A (BPA), Nonylphenol (NP), Nonylphenol ethoxylates (NPnEO), and Octylphenol, Phthalic acid ester (DMP, DBP, DEHP). In view of the above mentioned facts, the thesis work was conduced focusing on the effects of EDCs on fish reproduction endocrinology and hormone biosynthesis. In particular, we have examined the effect of EDCc on an Indian major carp *Labeo rohita*. Degradation of these compounds leads to a lowering of solubility and increased estrogenicity. EDCs can act at cellular level; induce endocrine disruption via a number of routes that involve steroid receptor binding (agonists), blocking steroid receptor binding (antagonists), or by disrupting the biosynthesis and or metabolism of steroids (Sharpe and Irvine, 2004). Paper mill extracts and municipal wastewater are polluted with a huge number of organic substances containing both natural and xenobiotics compounds (Gehring *et al.,* 2002; Ying *et al.,* 2002).These chemicals mimic endogenous hormone action or inhibit their activity modulating the endocrine system (Guiletter, 2000). Significant

concentrations of EDCs have been detected in sediments and wastewater by several authors in Uttar Pradesh, India. Therefore, monitoring of EDCs in aquatic animals are necessary. On account of above facts,, this study was undertaken to elucidate the impacts of EDCs (phthalic acid ester and organochlorine) under controlled laboratory studies. The experimental animal *L. rohita*, weighing 100g-150g were exposed to phthalic acid ester (DMP, DBP, and DEHP) and γ-HCH in different doses of 0.2mg/L, 0.3mgL, 0.5mg/L. Phthalic acid ester was dissolved in 1% DMSO and γ-HCH was dissolved in 1% Acetone and diluted the stock solution for the required concentration in aquaria water where the fihes were treated for 90 days with renewal of water every third day. This study investigated the effect of these endocrine disrupting chemicals on the gonads (GSI), histopathological changes of gonads, plasma concentrations of Vitellogenin (VTG), 17β-estradiol (E2), Testosterone and Aromatase, and also RNA/DNA ratio.

Methodology used:

Labeo rohita (rohu) was collected from private fish ponds, Lucknow. Identified taxonomically and maintained in laboratory condition. Fishes were fed with healthy diet daily commercially available palette diet (Tyio Pvt. Ltd.) daily. Length and weight of each fish was measured before and after the experiment and the collected data gave the information about growth of fish under controlled and treatment condition. Weight of fish was taken by digital electronic balance and length was measured by a calliper scale. Gonado somatic index (GSI) was calculated according to Singh *et al.* (2013). Estradiol-17β, testosterone and Vitellogenin levels in the serum was measured using kit provided by Enzo -Life Sciences, India, Serum Aromatase was determined by kit (Uscn Life Science Inc). The microscopic anatomy of the cellular changes in gonadal tissues was done using histological techniques

described by Coolidge and Howard (1979). RNA/DNA ratio was measured by using spectrophotometric method by Schinder (1957).

Results:

Effect of Phthalic acid ester (DMP, DBP and DEHP) on *L. rohita*

GSI value in control group ranged from 0.18±0.03-0.32±0.01 and in all DMP treated groups showed insignificant changes compared to control group. GSI in all treatment group ranged 0.19±0.01 - 0.316 ±0.015. Further, there was no change observed in GSI of fish exposed to both DBP and DEHP. In DBP and DEHP exposed groups the testosterone value of decreased significantly ($P<0.05$) and it was 961pg/ml and 463.5 pg/ml respectively in high dose of 0.5mg/L but in control it was 1523.3pg/ml. Serum estradiol and vitellogenin level increased significantly ($P<0.05$) in both groups of treatment DBP and DEHP. Estradiol level range from 438pg/ml to 529pg/ml in DBP and DEHP at different experimental doses and the vitellogenin slightly increased which was highly significant in DEHP treated group where it was 1701 pg/ml but in control group it was 1018pg/ml. Serum Aromatase value was insignificant in DBP treated group but in DEHP treated group it significantly ($P<0.05$) increased compared to control, where it was 79.74 pg/ml and in DEHP treated group it was 213.3pg/ml. The group of fish treated with DBP showed significant decrease of RNA/DNA ratio in high dose of the experimental period after 90 days. RNA/DNA ratio value in control group ranged from 2.2±0.01-2.12±0.09 and in DBP treated group it was not significantly ($P>0.05$) affected in 0.2mg/L, 0.3mg/L and 0.5mg/L after 30 and 60 days. The group of fish treated with DEHP showed also significant decrease of RNA/DNA ratio in high dose 0.5mg/L where it was 1.95±0.05. RNA/DNA ratio also significantly reduced ($P<0.01$)) after 90 days but in low dose and after 60 and 90 days there was insignificant difference between control

and DEHP treated group. In both the groups histological structure of gonad showed intersex condition where many spermatids were surrounded by the ovarian cavity in all group of intersex population. Developing ovary showed perinucleolar oocytes (POC), spermatocytes (ST) delineating imposex condition. There were a lower proportion of spermatozoa in the testes of males exposed with 0.5mg/L DEHP compared to control group. Conversely, there was significantly less count of spermatocytes in the testes of fish exposed with 0.2mg, and 0.3mg/L DBP compared to control. At the high concentration of DEHP, the microphotograph showed degenerating oocytes possibly due to endocrine disruption.

Effect of Organochlorine γ-HCH on *L. rohita*

The experimental fishes were found unhealthy throughout the experimental period. γ-HCH exposed fish had significantly ($P<0.5$) decreased GSI compared to control group. GSI value in control group was 0.17±0.01, 0.26±0.01and 0.35±0.01 after 30, 60 and 90 respectively. However, it reduced to 0.16±0.01, 0.26±0.01 and 0.29±0.01 after 30, 60 and 90 days respectively. HCH treated fish was analysed and found that serum testosterone level significantly ($P<0.5$) increased compared to control group but serum estradiol and vitellogenin level decreased significantly. Serum aromatase level was not significantly ($P>0.05$) different from control thought the experiment period in control group where it was 79.5±4.5, 89.5±2.3 and 87.6±4.5 pg/ml after 30, 60 and 90 days. The fish treated with HCH showed a significant decrease of RNA/DNA ratio in all group of treatments. RNA/DNA ratio value in control group ranged from 2.6±0.09-2.74±0.05 and in HCH treated group it significantly reduced to 1.05±0.09. Gonads of HCH treated *L. rohita* showed intersex condition where developing oocyte, degenerated follicular connective tissue (FTC), atresia, number of spermatids (ST) surrounding the mature oocytes (MOC) were observed.

This study sensitized and created awareness among people to EDCs and their harmful effects on aquatic animals including fish. This study reported effect of endocrine disruption chemicals in *L. rohita*. The level of serum steroid (vitellogenin and testosterone) used as a successful biomarker to check endocrine disruption in fish and aquatic animals. Estrogens and estrogen mimic endocrine disrupting chemicals and are probably responsible for elevated incidence of intersex characteristics among fish and other animals, particularly those exposed during growth and developmental phases of life. Exposure of effluent on fish included development of gonadal abnormalities, changes in sexually dimorphic characteristics, and sex reversal. Many of these effects are mediated through the estrogen mimicking actions of the compounds released into the environment. In the present investigation, it can be concluded that under exposure of endocrine disrupting chemicals affect the reproductive physiology of *L. rohita.* EDCs exposed fishes were intersex (sterile) in reproductive condition.—suggesting that future monitoring should considered important to see if there is an increase in the masculinization or feminization percentage and to determine the limit of dosage of EDCs that can be permeable. The use of house hold waste material should be discarded carefully (not through running waterof natural aquatic bodies) to minimize the effects of EDCs.