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Introduction:

Teleost fish comprise more than 21,000 species. the largest group of vertebrates, inhabiting a wide variety of marine and freshwater environments from the abysses of the deep sea to high mountain lakes [1]. Many chemicals produced by human activities end up in the aquatic environment. Aquatic animals living in polluted areas, such as fish, might be continuously exposed throughout their life to a multitude of different chemicals originating from industrial, agricultural and domestic sources.

During the last decade the role of substances that can interfere with the endocrine system of an individual has been in focus, the endocrine disrupting chemicals (EDC)[2]. The main increasing indication of many xenobiotic chemicals (called as endocrine disruptor chemicals, EDCs) through interfering with the endocrine system, which have the ability to induce reproductive and developmental abnormalities in humans and animals.[3].

The last two decades have perceived growing scientific disquiets and public argument over the potential contrary facts that may result from exposure to a group of chemicals that have the potential to alter the normal functioning of the endocrine system in wildlife and humans[4]. Contamination of the aquatic environment by chemicals that can mimic the effects of estrogens and the consequences of this contamination is a very recent one. It has, of course, been known for a long time [5] that some environmentally persistent man-made chemicals can act as weak estrogens [6].

The effects of potential endocrine disruptors can be examined in both sexes. Valuation of pollution requires a simple biomarker and vitellogenin can be the easiest select. [7]. They can cause difficulties in cell, organ, individual, and population levels(8).To examine endocrine disrupting chemicals (EDCs)and their interactions with functioning and the development of various systems in animals and humans a number of studies have been performed worldwide .

Therefore different screening and testing systems for EDCs have been established by the Organization for Economic Cooperation and Development and the U.S Environmental Protection Agency [9]. There has been a marked increase in scientific regulatory and public awareness of the presence of endocrine disrupting chemicals (EDCs) in the environment since the 1980s[10],which highlighted the potential adverse effects of exposure to EDCs. This increased awareness led to many studies of the effects of EDCs on wildlife.[11].

A common principle for all fish, however, is the production of large yolky eggs through the development of the oocyte. The formation, development and maturation of the female gamete and ovum (oogenesis) are intricate processes that require hormonal co-ordination. Oocyte growth is normally divided into four main stages, primary growth, formation of cortical alveoli, the vitellogenic period, and final maturation[12].

Developing offspring of oviparous animals are entirely dependent on stored egg yolk for nutritional sustenance. The nutrients stored in the ovulated egg must be sufficient to sustain progeny development from the time of fertilization to the onset of exogenous feeding. Vitellogenesis is the process whereby the required yolk nutrients are deposited into growing oocytes, which eventually give rise to eggs.

These nutrients consist of maternally derived substances, including proteins, carbohydrates, lipids, vitamins, minerals, and ions that are collectively transported from the liver to the ovary in the form of circulating yolk precursors called vitellogenins [13]. Vitellogenin is produced in the liver in response to estrogens from the ovaries. Under normal circumstances, Vitellogenin(vtg) levels in male fish are either undetectable or low (approximately 10,000 to 100,000 times lower than typical female concentrations [5,14].

The vitellogenin gene is normally silent, it can be induced if male fish are treated with

estrogens. Exposure of male fish to various concentrations of both natural and synthetic estrogens has shown very pronounced dose-response effects[15] and has also shown that male fish are very sensitive to estrogens present in the water of suspected estrogenic chemicals [16].

Vitellogenin concentrations may be abnormal and could have arisen due to estrogenic contamination of either the food or the water, or both. Contamination of water could occur via exposure to xenoestrogens (e.g., from plastic pipe work and tubing [17]) or via exposure to natural estrogens released by female fish also present in the same water as the males [18].The taxonomic hierarchy and taxonomic standing of carp fish is:

Kingdom : Animalia
Subkingdom: Bilateria
Infrakingdom: Deuterostomia
Phylum: Chordata
Subphylum: Vertebrata
Infraphylum: Gnathostomata
Superclass: Osteichthyes
Class: Actinopterygii
Subclass: Neopterygii
Infraclass: Teleostei
Superorder: Ostariophysi
Order: Cypriniformes
Superfamily: Cyprinoidea
Family: Cyprinidae
Genus: *Cyprinus*
Species: *Cyprinus carpio*, Linnaeus, 1758[19].

The aims of study

1-To determine vitellogenin levels and its effects in the carp fish that living in river and commercial basin carp fish.

2-To evaluate the physiological changes and endocrine disrupting between carp fish in river and commercial basin.

3-Focusing the light on the effect of vitellogenin on the histological structures of some organs in carp fish.

2.0 Literature Review

2.1 Vitellogenin (VTG)

Vitellogenin (VTG) is an egg-yolk protein precursor,[20,21].It is synthesized by the liver during the period of reproductive females[22]. Estrogenic substances induced vitellogenin synthesis , such as the natural estrogens, estrone (E1) and 17-β estradiol (E2), the synthetic estrogen, and 17-α-ethinyl-estradiol (EE2) [23,24].

Vitellogenin is homologous among a large variety of animals, from insects to chickens [25,26]. Vitellogenin, is a phospholipoprotein complex required for oocyte growth and use for larval energy supplies [27]. Vitellogenin is transported to the ovary through the blood, incorporated into the growing oocytes and cleaved into the proteins phosphovitin and lipovitellin [28].

It is a large serum phospholipoglycoprotein which serves as the major precursor to the egg-yolk proteins of oviparous vertebrates. In maturing females the liver synthesized and secreted vitellogenin in response to circulating estrogens [29]. In the simple term, vitellogenesis involves stimulating the liver by ovarian estradiol to produce Vtg. Estrogenic xenobiotics can act on the hepatic receptors to induce synthesis of Vtg[5].

Male and female fish, as well as immature juveniles have hepatic estrogen receptors, but only the livers of female fish can normally exposed to estrogens (30). Production of Vtg by males and juveniles provide a bioindicator of exposure to environmental estrogens[5,31].

2.2 Characterization of fish vitellogenin

The term ‘‘vitellogenin’’ was described a female specific protein in the hemolymph of the Cecropia moth. In teleost vertebrate fish, was founded an iron-binding female-specific serum protein (FSSP) [32]. Commonly, Vitellogenin proteins have the following characteristics; they are:-

(1)-Female-specific plasma or serum proteins, (2)-Induced by estrogen,(3)-Has high molecular weight phospholipoglycoproteins and molecular masses ranging from 300 to 600 kDa,(4)-Carrier proteins with both a lipid and ionic component (e.g., calcium, zinc, iron, etc.) and (5)-Precursors to yolk proteins [33]. In teleost species, the proximate composition of vitellogenin is approximately 80% protein ,20% lipid, with phospholipids being the dominant lipid class [34].

The phospholipoglycoprotein nature of teleost vitellogenins is conclusively demonstrated by several researchers [35]. Among most teleost the amino acid composition of Vtg is similar with the nonpolar amino acid residues constituting more than one-third of the total amino acid present. In many cases alanine was found to be the most abundant [36].

Vitellogenin of many fish species have few (0.1–2% of total amino acids) cysteine residues . The lipid contents of fish Vtgs is generally higher than other vertebrate Vtgs and found about 20% for various teleost [32].

Phospholipids constitute about 60-70% of the total lipid content of Vtgs and the rest composed of mainly triacylglycerols and cholesterol [37]. Vtg is an important carrier of lipids into fish eggs [38]. Lipids have a key role in nutrition, since they constitute the most efficient energy storage form. The phosphorus component of fish Vtgs is often lower than in other vertebrate Vtgs. The carbohydrate component of Vtgs have been found to vary considerably in different fish species [32].

2.3 Vitellogenesis

Vitellogenesis, is the process of vitellogenin(yolk protein) synthesis, transport, and uptake into the oocyte[12]. Vitellogenesis(Figure 2.1)is the process of provisioning eggs with yolk [39]. The process of vitellogenesis in females begins under the effect of environmental factors like temperature and photoperiod. Hypothalamus stimulated by these factors to produce neurohormones (gonadotropin-releasing hormone(GnRH)), these hormone stimulates the pituitary gland to secrete gonadotropins.

Then gonadotropins are released into the bloodstream and carried to the ovaries to stimulate the follicle cells to synthesize estrogens (primarily estradiol)[32]. Ovarian follicles secrete estradiol then it was delivered to the liver and it binds to estrogen receptors in the hepatocyte cytoplasm. This stimulates the synthesis of Vtg [40].

Vitellogenin undergoes lipidation, phosphorylation and glycosylation within the liver. Calcium is attached to the phosphorylated portions of Vtg to be delivered for skeletal development [41]. Then parenchymal liver cells secreted vitellogenin and Vtg transported to the ovary where it is taken up by the growing oocytes via receptor-mediated endocytosis.

Vtg In the ovary is cleaved into the two main yolk proteins, lipovitellin (Lv) and phosvitin (Pv), and other small β-components (β') [25]. The yolk proteins, Lv is the major nutritional resource for the growing embryo [42,43]. Cathepsin D is the enzyme which responsible for processing Vg although noteworthy the levels of cathepsin B have also been detected [44]. Vitellogenin is a large glyco-phospholipoprotein. It occurs as a dimer with two equal subunits of about 200 kDa [45].

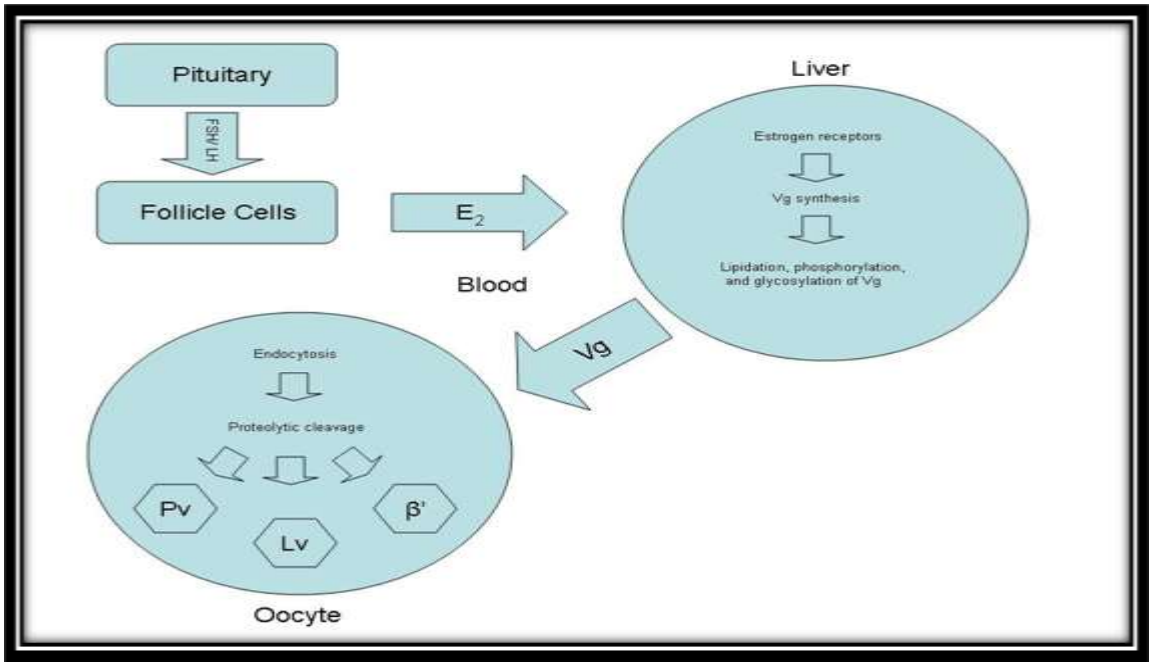


Fig.2.1: Diagram of vitellogenesis.[45]

2.4 Hormonal regulation of vitellogenesis

Vitellogenesis is the longest phase of oocyte development. The primary event during oogenesis is the synthesis vitellogenin by liver and uptake by oocytes [46]. Endocrine regulation of the induction of Vtg synthesis by the liver has been extensively studied both in vivo and in vitro. The brain stimulates the pituitary gland to secrete gonadotropins (peptide hormones).

The brain modulates the release of gonadotropins from the pituitary by the stimulatory effect of the hypothalamic gonadotropin releasing hormone (GnRH) and the inhibitory effect of dopamine. These neurohormones control the release of gonadotropins from the anterior pituitary . Gonadotrpins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)) are the gonadal function-regulating hormones in vertebrates . They also promote meiotic maturation and ovulation [32]. Early vitellogenesis is characterized by further increases in plasma FSH and 17β-estradiol (E2), and increased expression of ovarian FSH receptor [47,48].

In teleosts increased levels of follicle stimulating hormone (FSH) in the blood induce follicular production of E2 oocyte growth and ultimately ovulation [32].Both hormones present in similar levels in asynchronous spawners[49].Both gonadotropins bind to membrane receptors in the ovarian follicle and activate steroid synthesizing enzymes [40].

The steroid synthesizing enzymes create among other things 17β-estradiol which is the primary hormone responsible for vitellogenesis[50], the estradiol is then released into the serum where it is bound by steroid-binding proteins or albumins.

Estradiol stimulate the liver to synthesize vitellogenin, estradiol enters the liver cells by diffusion and is retained in target cells by high affinity binding to a specific

receptor protein (ER, estrogen receptor). Binding affinity of ER for estradiol increases with higher doses of estradiol . Estradiol stimulation of hepatic cells also induces proliferation of rough endoplasmic reticulum and Golgi apparatus where modifications of vitellogenin precursor take place [32].

2.5Vitellogeninsis in male fish

Vitellogenin in male fish produces when this fish exposed to estrogenic endocrine disruptor chemicals(EDCs) (Fig,2.2). The exogenous estrogen enters the male body and reaches the liver through the blood stream, then the exogenous estrogen binds the estrogen receptor (ER) to form the hormone estrogen receptor complex (Fig,2.3)[51]. This complex must dimerize and bind the DNA at the estrogen response element.

This binding activates transcription of mRNA, which is translated into protein. Natural estrogens (e.g. E2), synthetic estrogens (e.g. EE2) and estrogen mimics (e.g. BPA) bind the ER causing the cascade of events that lead to translation of the VTG protein[52].

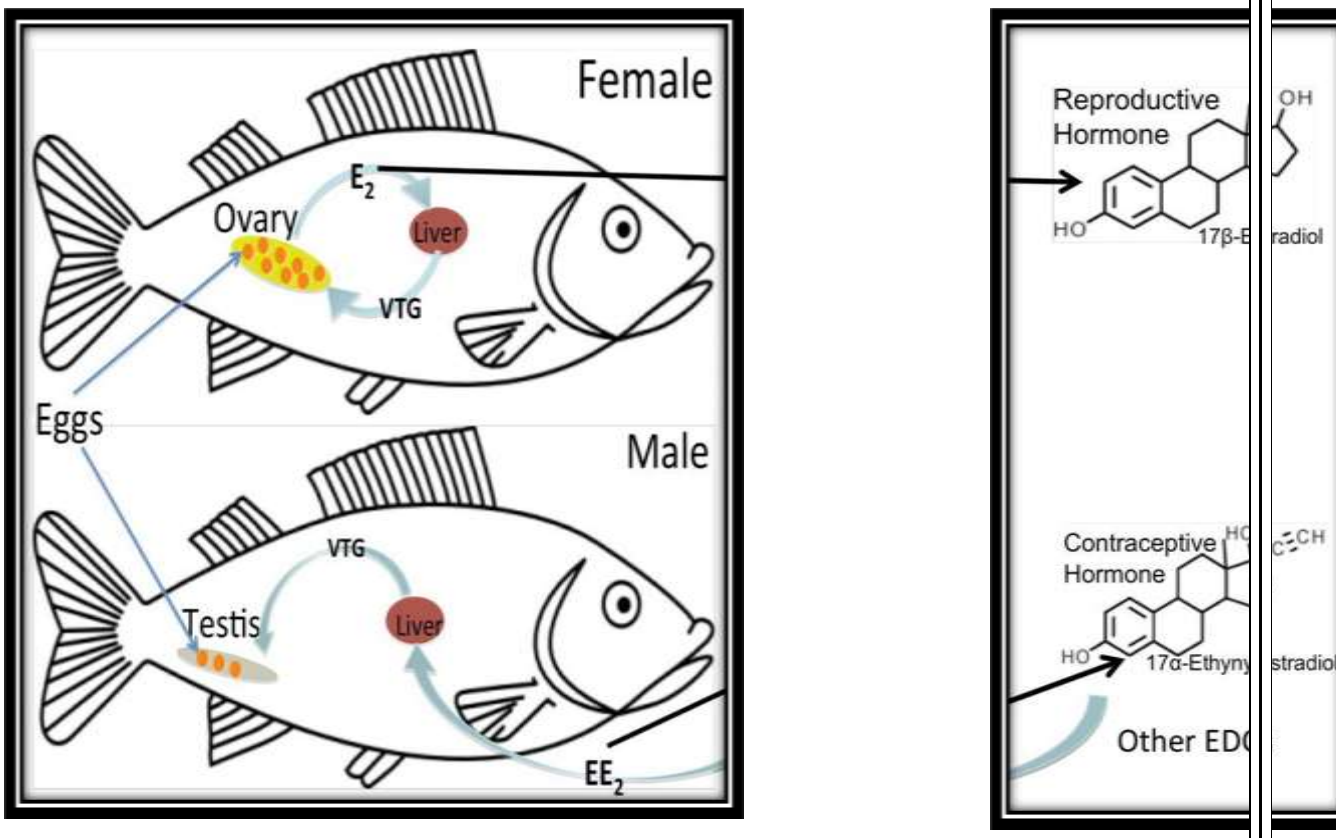


Figure 2.2 The pathway of endogenous and exogenous estrogens in male and female fish[51].

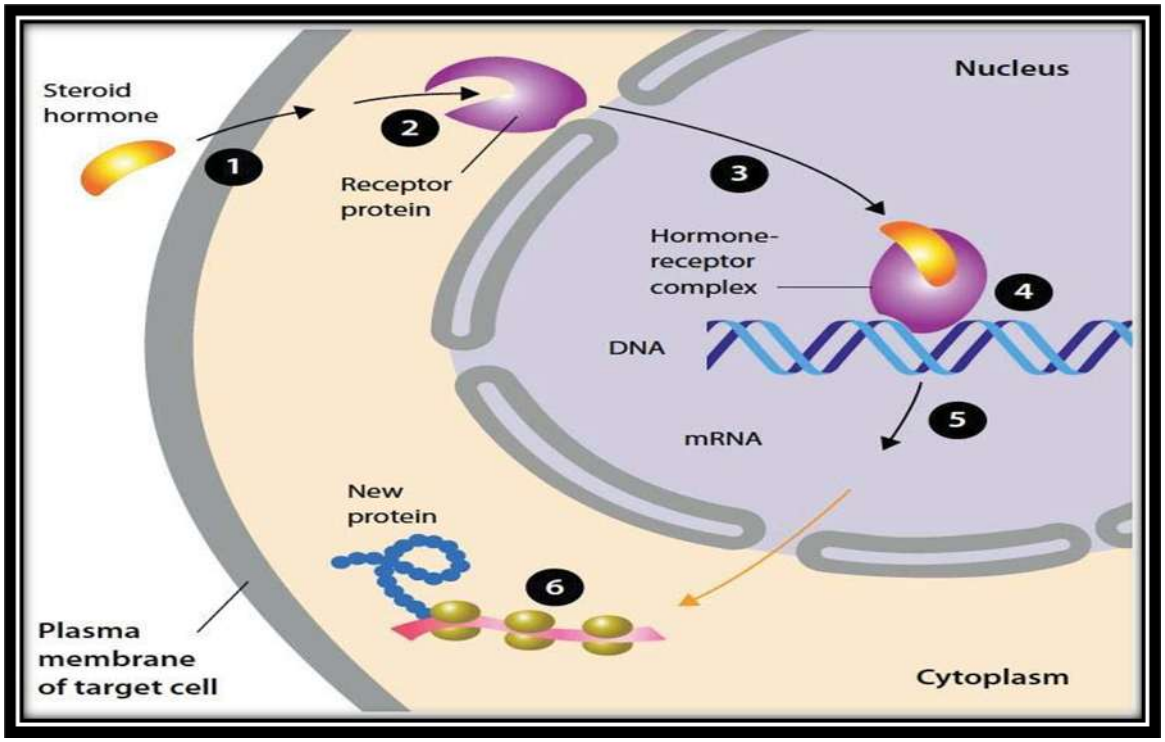


Figure 2.3 The protein activation pathway by steroid hormones[51].

2.6Vitellogenin as a biomarker of EDCs

Many chemical compounds released into the environment have the potential to affect on the vertebrate neuroendocrine system, which regulates vital processes including development, growth, metabolism and reproduction. Generally these chemicals that either mimic or antagonize the actions of naturally occurring hormones known as endocrine disrupting chemicals (EDCs).

EDCs are exogenous chemical agents that interfere with the synthesis, transporting, secretion, binding, action, metabolism or elimination of natural hormones. These include estrogenic EDCs (environmental estrogens), substances that elicit an estrogenic response by mimicking the action of endogenous 17β-estradiol (E2) [53].

Some environmentally persistent man-made chemicals can act as weak estrogens. However, some serious concerns about whether exposure of aquatic organisms to estrogenic chemicals contaminating the water might be leading to subtle, but potentially very serious, effects. Wide range of widely used chemicals, and sometimes their major degradation products, can act as weak estrogens [5].

One of the most documented effects of xenoestrogens is the induction of the egg-yolk precursor protein vitellogenin (Vtg), which used as a biomarker of endocrine disruption in laboratory and field studies on fish [54]. Vitellogenin is found naturally in the blood of maturing female fish, however, one biological response to

xenoestrogens is the production of Vtg in male and immature fish, Therefore, Vtg serves as a biomarker for xenoestrogens in aquatic environments [55,56].

VTG induction is often measured in males because its expression is naturally low or no existent. Measuring VTG induction in females less sensitive than in males because it increases in expression above their normally high levels is often only seen when females are acutely exposed to extremely high concentrations of estrogens and anti-androgens or chronically exposed to low concentrations[52].

The induction of this biomarker in teleosts has been used for EDCs screening and environmental monitoring [57].

2.7 Estrogens

Estrogens are a group of steroid hormones which important in regulating the female reproductive cycle. They are the primary female sex hormones and they important for female sexual differentiation in many species; they comprise three classes, namely estrone (El), estradiol (E2), and estriol (E3) [58]. Among the three estrogens, estradiol is the most potent and the most broadly studied form of estrogen [59]. estrogens were first discovered in the 1920s and 1930s [60]. Estrogen, produced particularly in the ovaries, It is generally considered a female hormone, but produced in both males and females, although the quantities produced in females are much greater than those in males[32].

Steroidal estrogens are potent endocrine-disrupting chemicals (EDCs), designed to be biologically active and known to disrupt the normal growth and development of aquatic organisms at sub-ng L-1 concentrations [61,62]. Estrogens have measured in rivers, estuaries, sewage effluents, septic system discharges, groundwater, and drinking water, with concentrations ranging up to μg L -1 levels [63,64]. There are a variety of ways that estrogens can enter aquatic environments. Terrestrial and aquatic vertebrates produce and excrete estrogens naturally [65,66].

2.8 Estrogenic and anti-estrogenic effects

A wide variety of structurally diverse anthropogenic compounds have been shown to exhibit estrogenic or anti-estrogenic effects [67]. Estrogenic effects refer to compounds mimicking the action of estrogens through binding to the estrogen receptor (ER) and activation of responsive genes. In teleost, two receptor isoforms (α and β and subtypes of these) with differential tissue distribution patterns and binding affinities have been identified [68,69].

Estrogenic effects may also be mediated by elevation of endogenous free plasma estrogen levels caused by competitive binding of xenobiotics to sex steroid binding globulins [70]. Anti-estrogenic effects refer to compounds with weak or no estrogenic activity but the ability to competitively bind to the ER. [71].

2.9 The endocrine system

The endocrine system forms the main regulatory mechanism which controls on different functions in the human or animal body, it conjunction with the nervous and immune system. Hormones are the messengers of the endocrine system that are produced and excreted at low quantities from endocrine glands directly to bloodstream,which transport the hormones to target organ.

Hormones transported in the blood in free or attached state to carrier proteins and bind at the target organs to specialized hormone receptors on the cell surface or within nuclear receptors superfamily. Receptors superfamily is a group of intracellular proteins which play an important role in a wide range of physiological and toxicological processes. This hormone- receptor complex then activates different organ or cell functions.

The binding between hormone and receptor is based on strict complementarities comparable with the 'key and lock' principle. Hormone influence numerous essential regulatory, growth, developmental, and homeostatic mechanisms of organism[72,73].

2.10 Endocrine regulation of reproduction in fish

In fish the sexual differentiation of developing gonads is under the control of hormones of the hypothalamo-pituitary-gonadal axis. Sex steroids play a critical role in early differentiation of the gonads into the two sexual types and subsequent maintenance of the differentiated types[74]. The primary hypothalamic neurohormone is gonadotropin-releasing hormone which stimulates the release of gonadotropins from the anterior pituitary.

Both follicle-stimulating hormones and luteinizing hormone homologues found in vertebrate fish and are referred as to gonadotropin-I (GtH-I) and gonadotropin-II (GtH-II), respectively. Gonadotropins stimulate gametogenesis and synthesis of gonadal sex steroids such as T and E2, which in turn provide feedback to the hypothalamo-pituitary axis to regulate sexual maturation and spawning. Steroid hormones are produced in the gonads; namely the ovaries of females and the testes of males[75].

2.11 Endocine Disruptor Chemicals(EDCs)

Endocrine disruptor chemical (EDCs) is an exogenous substance or mixture that alters function(s) of the endocrine system and therefore causes adversarial health effects in an intact organism, or its posterity or (sub) populations[76], they are substances in our food, environment, and consumer products .These materials interfere with reproduction, metabolism, or action resulting in a deviation from normal homeostatic control or hormone biosynthesis[77].

The effects of EDCs have been reviewed in numerous organisms (vertebrates and invertebrates; primarily teleost[78,79]. There is no endocrine system that is immune to these substances, because of the shared properties of the chemicals and the similarities of the receptors[77] and enzymes involved in the synthesis, release, and degradation of hormones .

Therefore, the role of this Scientific Statement is to provide perspectives on representative outcomes of exposures to endocrine disruptors and evidence for their effects in wildlife, laboratory animals, and humans. These effects included changes in kidney and gill morphology, reproduction abnormalities and induction of vitellogenin (VTG) in males[51,80].

2.11.1Type of endocrine disruptor chemicals(EDCs)

The group of molecules identified as endocrine disruptors is highly heterogeneous and includes:

1-synthetic chemicals:

The substance which used as industrial solvents/lubricants and their byproducts [polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins], plastics [bisphenol A (BPA)], plasticizers (phthalates), pesticides [methoxychlor, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT)], fungicides (vinclozolin), and pharmaceutical agents [diethylstilbestrol (DES)] [77].

2-Natural chemicals:

Human, animal hormones and phyto and mycoestrogens found in sewage, animal husbandry runoff, and intentionally or accidentally as foo[81,82], feed ingredients found in human and animal food (*e.g.*, phytoestrogens, including genistein and coumestrol) can also act as endocrine disruptors. These substances, whereas generally thought to have relatively low binding affinity to ERs[77].

2.11.2Phytoestrogens

Phytoestrogens are a wide group of plant-derived compounds which are structurally and functionally similar to estrogen and have a range of estrogenic activity in animals Estrogenic activity present in soybeans, liquorice, linseed, clover, pomegranate, etc. Plant estrogens are mostly isoflavones which found in the form of glycosides. Soybean phytoestrogens include several isoflavones (glycitein, daidzein, and genistein) and nonisoflavone (coumestrol) compounds with estrogenic activity[83, 22].

The phytoestrogens classes mentioned above have a similar structure to oestradiol and are able to bind the estrogen receptor (ER), although their binding affinity is lower than that of endogenous estradiol, The majority of phytoestrogens belong to a large group of substituted phenolic compounds known as flavonoids. Three classes

of flavonoid, the isoflavones, coumestans and prenylated flavonoids are phytoestrogens [84]. Phytoestrogens are also a family of bioidentical hormones suspected to act as endocrine disruptors in human health[22].

2.12The mechanisms of action of EDCs

There are many ways by EDCs are able to interfere with endogenous endocrine pathways and cause sexsteroid disruption in fish. The type and strength of their effects depend on species, concentration, and the time and duration of exposure [85,86]. The mechanisms of action of EDCs can divide into:

(1) steroid receptor binding agonistic/blocking steroid receptor binding antagonistic effect ("hormone mimics") [87,88].

(2) disruption of production, metabolism orsecretion, transport of natural hormones.

(3) disruption of production and/or function of hormone receptors .

This division indicates discrete and separate routes of action of an EDC, depending on the properties of the molecule in action[82, 88]. However, since the production of, for example, some of the steroid hormone receptors is under autoregulatory control, it is clear that some hormone mimics may act through their agonistic/antagonistic effect on the receptor-regulated activities of target genes, and also by affecting the activity of the receptor gene itself [88].

2-13 Commercial fish diet

Commercial fish diet is a source of oestrogens, the surveyed fish foods for oestrogenic activity by using a yeast oestrogen screen found all the foods tested have activity[89]. Commercial fish diets contain high levels of protein, in the form of fish meal and soybean. Fish meal, which commonly represents 15 to 40% of the diet[18] , is prepared from several sources, including whole fish and fish viscera.

The various animal and plant sources of steroids and "steroid-like" materials found in fish diets are reviewed along with an assessment of physiological mechanisms of action and their potential effects on fish. It is concluded that most, if not all, diets used in fish culture will contain measurable amounts of these compounds.

Depending on the fish species and the sexual stage of the fish available, fish meal may be contain high levels of sex steroid hormones[18], sex steroid levels in fish diets were correlated with the amount of fish by-products incorporated into them[90]. Also fish diets are prepared using soybean (up to 20-30%), that is known to contain several types of phytoestrogens, such as genistein and daidazein, and these have been shown to have oestrogenic activity[91,92]. A variety of animal diets tested by using an in vitro yeast 2 hybrid assay, and found a correlation between the phytoestrogen content and oestrogenic activities of the diets[91].

Both a commercial and a soya-based synthetic diet stimulated significant levels of plasma VTG in Siberian sturgeon compared with a diet based on casein. Other species of fish have also been shown to experience diet-induced increases in the plasma VTG concentration, caused by sex steroids and/or phytoestrogens in the diet, including tilapia [93], goldfish[94] medaka [95], and carp[22].

The estrogenic effects of diet of phytoestrogenic compounds can have a wide range of consequences on various physiological processes in animals[22]. There are numerous studies on the utilization of soybean meal in the diet of various species of fish such as carp, tilapia, catfish, flounder and sea bream[22,96].

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2.14 Effect Endocrine disruption chemicals in fish

There is now unequivocal indication showing that EDCs can have long-term effects on reproduction and subsequent population development in natural fish populations [97]. Fish are considered as one of the primary risk organisms for EDCs, especially those interacting with the reproductive hormones. Firstly, sex determination in teleost fishes is characterised as being very labile, and can be disturbed or even functionally reversed by the external application of hormones at critical developmental stages [98].

Secondly, as the aquatic environment represents the ultimate sink for most anthropogenic contaminants, from industrial, agricultural or municipal effluents, fish populations are directly exposed to a wide variety of EDCs,[97]. Endocrine-disrupting chemicals have harmfully affected a variety of fish species. Effects on reproductive organs are causally linked to exposure mostly in the vicinity of certain sources (such as effluents of water treatment plants,) and in the most contaminated areas.

However, there are recent signs for significant estrogenic exposure of fish not only in freshwater systems[99]. Endocrine disruption in fish has become one of the best researched areas of the field, following the identification of widespread reproductive abnormalities in wild fish populations. [100]. Vitellogenin induction in males, also identified the widespread, abnormal occurrence of intersex in male fish, characterised by mixtures of both ovarian and testicular germ cells in the gonads and by the development of ovarian ducts (ovarian cavities)[101].

Sexual disturbance has a potential effect on the population level because dual sex is associated with other reproductive problems in the males[102,103], The concentrations observed in most of the contaminated rivers have the potential to cause endocrine disturbances and delayed development of the testes in carp fish which living in this river.

A decrease in gonadal-somatic-index ,a ratio of gonad weight against body weight and testis weight of carp living in this river has been observed, which is also an indication of a reduced fertility in this population[104], particularly in the most severe cases. Indeed, reductions in spermiation, milt volume, sperm density and gamete quality are associated with intersex in wild roach. Interestingly, the maturation of ovaries was less obviously effected in female fish although ovarian atresia[102,103].

Other changes to genital structure and secondary sexual characteristics have reported in wild fish, causing concern for potential impacts on the reproductive fitness of these individuals. [105].

2-14.1 Effects of Endocrine disruption chemicals on the testis

Testes are very large bright red organs that demarcated by deep fissures into 5-6 irregular lobes and located between the intestines and swim bladder [106]. In most bony fishes, testes are paired elongated organs which are attached to the dorsal wall of the body by a mesorchium[107]. Testes are either fused along the entire length or completely separated or fused posteriorly[108].

Both testes joint caudally, converging in a central deferent duct system that is open to the exterior throughout the urogenital pore[109]. Histological examination of bony fishes testes explained that these organs are composed of germinal and interstitial compartments that are always separated by a basement membrane; thus, the cells in one compartment do not intermix with the cells of the other compartment[110]. The testes of fish are composed of two compartments: the interstitial and the tubular compartment.

The interstitial compartment has only somatic cells, such as Leydig cells, blood vessels, connective tissue, macrophages and mast cells, lymphatic vessels and neural cells. The tubular compartment is separated from the interstitial compartment by a basement membrane, which is surrounded by myoid cells. In this tubular compartment, the seminiferous epithelium is found, which contains of (somatic) Sertoli cells together with their accompanying developing germ cells[111] .

Sertoli cells rest upon a supporting layer, the basement membrane separates the epithelium from the subjacent interstitium. The germinal cells are between Sertoli cells or surrounded by them, but not in contact with the basement membrane. Germ cells include all the stages of differentiation of the spermatogenic cells [111].The seminiferous epithelium encompasses the tubular lumen, which is filled with fluid.

In this lumen, the spermatozoa are released after completing their developmental process[112].

carp fish has tubular testis, the tubules do not terminate at the testis periphery, but they form loops at the periphery which double back to the efferent ducts, forming a highly branched structure or forming anastomoses of the tubules where they may branch and rejoin . Germ cells develop as synchronous clones around the lobules and spermatozoa are liberated to the lumen of the tubules . This testis type is present in lower fishes, as salmonids, cyprinids and lepisosteids[107].

An in vivo study with fish treated with E2, showed that E2 causes suppression of spermatogenesis. Contrasting results have been obtained by a study conducted by [112]. It has been shown, for example, that both abovementioned xenoestrogens caused a decrease in spermatogenetic cysts and an increase in spermatozeugmata in enlarged sperm ducts. These effects indicate reduced or even fully blocked spermatogonial mitosis.

In the study of the natural female estrogen 17β-estradiol was also tested. Fish exposed to this estrogen showed an increased number of hypertrophied Sertoli cells and efferent duct cells. All stages of spermatogenesis were still present. However, other studies have also indicated depletion of early spermatogenetic stages, similar to the effects of bisphenol A and octylphenol. This suggests that the effects of 17β-estradiol depends on the concentration[113].

In a study comparing three groups of carp fish, one of which lives in an uncontaminated site and the other two groups live in two different rivers in degrees of contamination with estrogen compounds, the testes showed that the fish in the most polluted river contain concentrations of estrogen 3 to 4 times that of the less polluted river . Much smaller than in the less polluted river and the location of control fish [104].

Intersex is another and a much more common condition caused by early exposure of fish larvae to estrogenic substances. The intersex condition in males usually takes the form of ovotestis.[12], Ovotestis can be induced in the laboratory by exposing fish larvae to weak estrogens [12,114].

2-16 Effects of Endocrine disruption chemicals on the Liver

The liver was relatively large and formed of multiple hepatic fragments that surrounded and dispersed between most of the viscera[106]. liver showed polyhedral symmetry of hepatocytes arranged around the central vein. Arrangement of

hepatocytes was in muralium duplex fashion. Hepatocytes showed centrally placed nucleus with eosinophilic cytoplasm[113].

In almost all fish, the structural units known as hepatic lobules were absent from connective tissue septa. The liver was mainly composed of a continuous compact field of hepato- cytes, and scattered with islands of connective tissue enclosing the bile duct and arterial vessels, The hepatic lobule consists of hepatocytes that are the functional center of the liver, and in which the hepatocyte-sinusoidal structures are formed. The sinusoids are capillary networks, and are localized in the space between hepatic plates in which the hepatocytes are arranged[116].

The liver is important in many aspects of nutrition, including lipid and carbohydrates storage and alterations in liver structure may be useful as biomarker that indicate prior exposure to environmental stressors [117]. Stressors associated alterations Of hepatocytes may be found in the nucleus or cytoplasm or both [118]. reported that changes in environmental parameters often damage this delicate vital organ which has direct contact with aquatic environment[119].

As a principle metabolic organ (detoxification organ), the liver plays a major role in the uptake, accumulation , biotransformation , and excretion of xenobiotics. Exposure to toxicants may cause histological changes in the liver, which in turn could be used as a biomarker to indicate prior exposure . The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations of harmful substances, and this could subsequently result in structural damage[115].

Similar studies on various fish species, exposed to various toxicants, showed histopathological changes in the liver of those specimens [120]. significant changes in the liver for mild congestion of blood vessels at 0.35 ppm concentration of hexachlorocyclohexane (HCH).. Alternately, at 1.73 ppm concentration there was marked swelling of the hepatocytes in places with areas of diffuse necrosis .

The normal architecture of liver tissue was markedly disrupted. Sinusoids in most cases were distended and central veins appeared severely damaged due to marked swelling and degeneration of the endothelial lining cells[121].

Highest number of alteration found in liver such as plasma and nuclear alteration, changes inform of structural alterations of hypatocytes ,increased of melanomacrophage centers(MMCs) and focal areas of necrosis[122].

2.17 Effects of Endocrine disruption chemicals on the kidney

As in all vertebrates, the kidney in fish is located retroperitoneal, exterior to the dorsal wall of the body cavity. The kidney is a paired organ that has been described as having various anatomical and functional compartments [123]. Kidneys play the

most important part in the excretion of nitrogenous wastes and in maintaining the water-salt balance (homeostasis) [124]. The kidney is important for retaining salts and excreting water in freshwater fish [125] .

The kidney of all vertebrates is made up of nephrons which are the functional, structural and morphological unit[126]. The kidney of fish receives majority of postbranchial blood and renal lesions may be expected to be good indicators of environmental stress[127].In vertebrates, three kind of kidney are found: the pronephros, mesonephros and metanephros[126].

A kidney consists of large number nephrons which develop from infront backwards. The tubules of the anterior region develop and become functional in early life and constitute the pronephros which called head kidney and those of the more posterior region develop and become functional in later life and form the mesonephros and called tail or trunk kidney[128].

The fresh water teleostean kidneys can be divided into three types :

1.The two kidneys are completely fused and there is no distinction between the trunk and head kidney as in Salmonidae (salmon and trout).

2. Their middle and posterior portions are fused as in the cyprinidae (carps). Their anterior free lobes form the head kidney, while the fused portions constitute the trunk kidney. In many species, the trunk kidney is broad in the middle and gradually narrows in the hind part (e.g. *Cirrhina*, *Labeo* and *Barbus*).

3. Only the posterior portions of kidney are fused. The anterior portion is represented by two slender branches, this type found in fish belonging to Cyprinodontidae, Gasterosteidae and Cottidae. In some species. the head kidney is completely separate from the remaining part .

The trunk kidney is made up of a large number of nephrons, each consisting of a renal corpuscle and the tubule. The head kidney is generally made up of lymphoid, hematopoietic, interrenal and chromaffin tissue and is devoid of renal corpuscles and the tubules[129]. The head of kidney contains endocrine elements, the chromaffin cells and interregnal tissue which are located around the blood vessels[126].

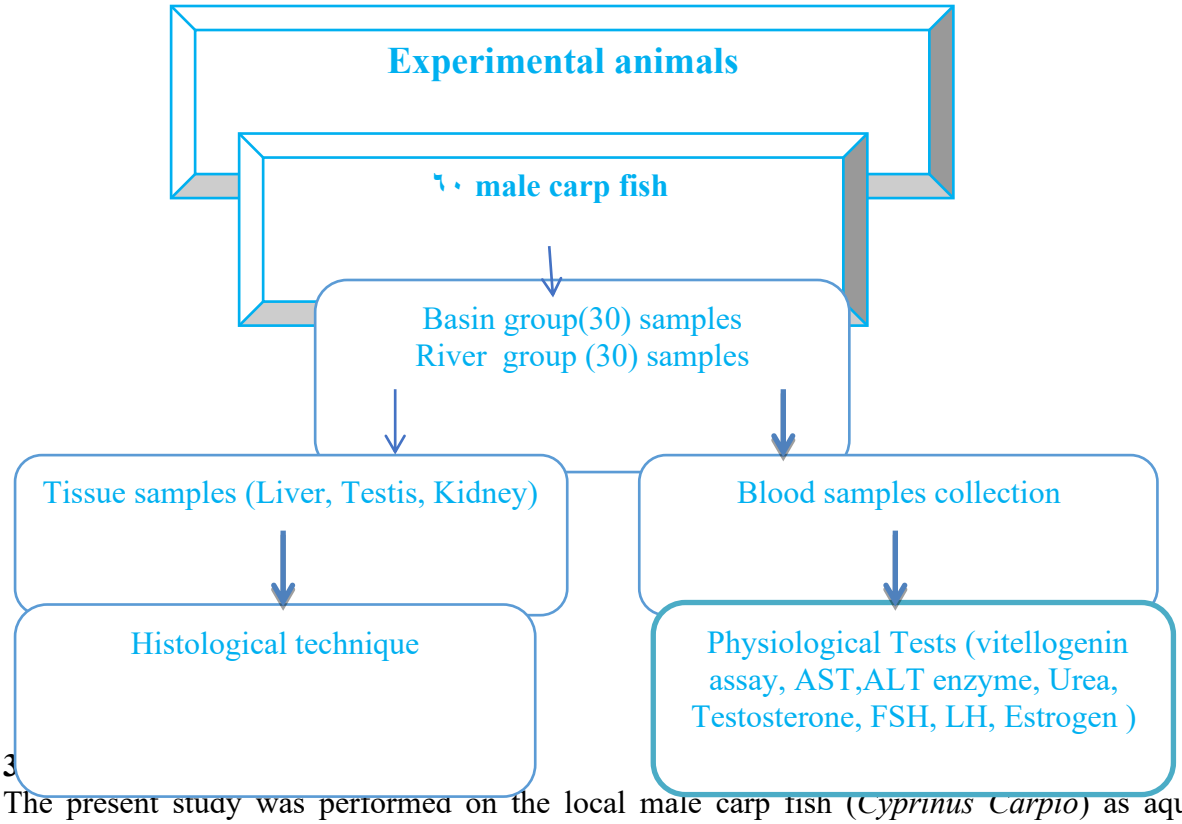
In the common carp the head kidneys were in the form of two oval bodies and separated from each other in the midline and also from the trunk kidneys by wide gap. It may be added that the trunk kidneys were further differentiated into a cranial thick and a caudal thin portion. In between these portions, an expanded three sided ventral lobes were formed[106].

Some changes absorved in the kidney which exposure to EDC such as smild degenerative changes of tubular epithelium could be noticed in a and tubular epithelium showed necrotic changes characterized by karyorrhexis and karyolysis at

the nuclei of the affected cells. Lumen of the tubules were invariably dilated[121]. Although dilation of glomerulus capillaries and intercellular deposit in the interstitial tissue[122].

3.0Materials and Methods

3.1 Experimental design



3

The present study was performed on the local male carp fish (*Cyprinus Carpio*) as aquatic organism and they are obtained from the local river and commercial basin, (30)mature male carp fish weighting(1-1.200kg)obtained from local commercial basin and other (30) similar in sex and weight obtained from Al Samawa river.

3.2.2 Chemicals, stains and Instruments.

Table 3.1 Chemicals with their Productions

NO.	Chemicals	Productions
1	Formalin 37%	Linear chemical ,Barcelona –Spain
2	Chloroform	India
3	Glycerin	England
4	Paraffin wax	England
5	Hematoxylin crystals	England
6	Eosin	England
7	Oil emersion	China

8	Xylene	England
9	Absolute alcohol	Japan
10	Osmium tetroxide	USA Spi supplies-
11	Glutaraldehyde 25%	CDH-India
12	NaH2PO4	Sigma-Aldrich-Germany
13	Na2HPO4	Sigma-Aldrich-Germany
14	AST Kit	Randox
15	ALT Kit	Randox
16	Urea Kit	Randox
18	Carp vitelligenin ELISA Kit	Biosense

Table 3.2 Instruments with their Productions

NO.	Tools	Productions
1	Disposable surgical Gloves	Malaysia
2	Cotton	Jordon
3	Surgical set	England
4	Slides	China
5	Cover slide	China
6	Ependroff tube	Germany
7	Flasks	China
8	Disposable syringe	China
9	Gel tube	Celeco- Jordon
10	Electrical balance	Shimadu company- Japan
11	Centrifuge	GMBH-Germany
12	Processer	Cyan-Germany
13	Bluking	Thermo-England
14	Microtome	Mycrom-Germany
15	Water path	Sakura-Japan

16	Oven	Sakura-Japan
17	Micropipette	Huawei-China
18	Light compound microscope	GENEX-
19	Router (TP-LINK-1C66)	China
20	NanoStation5(5GHz)	China
21	Incubature	Japan
22	Cortex	Japan
23	Biolyzer 300	Germany
24	ELISA washer	USA
25	Elisa reader	USA
26	Microscope camera	OMAX-China
27	Mini vidas	France

Table 3.3 Hematoxylin and eosin stain

Hematoxylin and eosin stain	1.0 gm
Distilled water	1000 ml
Sodium iodate	0.2 gm
Ammonium or potassium alum	50.0 gm
Citric acid	1.0 gm
Chloral hydrate	50.0

3.2.3 Blood samples collection

The collected blood samples is about 2ml of blood from each one of male carp fish directed by caudal vein to detection the level of vitellogenin and other parameter. The blood was left at room temperature for 15 minutes after centrifuged with (2000 rpm) for 15 minutes the serum was collected eppendrof tubes and divided into equal amount (50µl) and frozen until use for laboratory assessments. to purpose examine vitellogenin, testosterone, FSH, LH, estrogen ALT, AST and Urea.

The tissue samples taken with 1cm³ from kidney, testis and liver, the tissue samples were passed through the histological technique which including many stages.



Fig.(3-1) collection of blood sample.

3.3.1 Vitillogenin assay by ELISA kit

Assay Principle

This ELISA utilises specific binding between antibodies and vitellogenin (Vtg) to quantify Vtg in samples from carp and fathead minnow [130]. The wells of microplates have been pre-coated with a specific Capture antibody that binds to Vtg in standard and sample added to the wells. A different Vtg-specific Detecting antibody is added to create a sandwich of Vtg and antibody, which is detected with an enzyme-labelled Secondary antibody. The enzyme activity is determined by adding a substrate that gives a coloured product, and the colour intensity is directly proportional to the amount of Vtg present.

3.3.1.1 Preparation of buffers / Reagents

1.Dilution buffer: PBS (Phosphate buffered saline, pH 7.3), 1% BSA:

It was Dissolved one buffer tablet (bag C) and 1.0 g BSA (vial E) per 100 ml distilled water and Store at 2-8°C (stable for 2-3 days).

2.Washing buffer (PBS, 0.05% Tween-20):

It was Dissolved one buffer tablet (bag D) in 1000 ml distilled water. Store at 2-8°C 3.Substrate solution (*prepare just prior to use*):It was prepared by Dissolved one Urea Hydrogen Peroxide tablet in 20 ml distilled water (dissolved slowly, 10-15 minutes with gentle shaking), then was added one OPD tablet and let it dissolved. The substrate solution should be used within 30 minutes.

3.3.1.2Assay procedure

Preparing dilutions of standard and samples:

1.Dilution of the Vtg standard:

The content of one vial of carp Vtg standard (vial I) was dissolved in 1.0 ml cold Dilution buffer. And was kept the dilutions on ice until use.

2.Dilution of plasma samples:

It was prepared a 1:50 dilution (was added 10 µl sample to 490 µl cold Dilution buffer), a 1:5 000 dilution (was added 10 µl of the 1:50 dilution to 990 µl cold Dilution buffer) and a 1:500 000 dilution (was added 10 µl of the 1:5 000 dilution to 990 µl cold Dilution buffer), and was Kept the dilutions on ice until use.

3.3.1.3Incubation with standard and diluted samples

3. 100 µl Dilution buffer was added to each of the two NSB wells. (NSB wells should be included on every plate. These wells are used to determine Non-Specific Binding) (unspecific background signal).

4. 100 µl of each carp Vtg standard dilution was added in duplicate.

5. 100 µl of each sample dilution was added in duplicate.

6. the plates was sealed and was incubated at 37°C for 1 hour.

3.3.1.4Incubation with Detecting antibody

7.The Detecting antibody (vial F) was diluted 1:500 by added 22 µl to 11 ml Dilution buffer for each plate run in the assay.

8. The plates was washed three times with 300 µl Washing buffer per well.

9. 100 µl of the diluted Detecting antibody was added to all wells.

10.The plates was Sealed and incubated at 37°C for 1 hour.

3.3.1.5 Incubation with Secondary antibody

11.The Secondary antibody (vial G) was diluted 1:2000 by adding 6 µl to 12 ml Dilution buffer for each plate run in the assay.

12.The plates was washed three times with 300 µl Washing buffer per well.

13. 100 µl of the diluted Secondary antibody was added to all wells.

14. The plates was sealed and incubated at 37°C for 1 hour.

3.3.1.6 Development

15. the plates was washed five times with 300 µl Washing buffer per well.

16.100 µl of Substrate solution was added to all wells.

17.The wells was Incubated in the dark (cover the plates with *e.g.* aluminium foil) at room temperature (20-25°C) for 30 minutes.

18.At last was Stopped the reaction by adding 50 µl 2M H2SO to all wells.

19. After five minutes, the absorbance was read at 492 nm with a microplate reader.

3.3.1.7Calculation of results

Subtraction of NSB absorbance values:

On each plate, was calculate the mean of the absorbance values of the two NSB wells and subtract this value from the absorbance values of all other wells on the same plate. This gives the NSB-corrected absorbance values for standard and sample dilutions.



Fig(3-2): Reaction after addition of substrate.

3.3.2 Histological technique

1- Fixation: The samples (liver, kidney, testis) were laid in labeled container contain 10% formalin for 48 hours, and shaky the container gently several times to ensure that the fluid reached all surface and that pieces were not sticky to the bottom or sides (A shank of glass wool placed in container will aid in keeping the tissue free of the bottom).

2- Wishing: The samples were washed by using the tap water for (1 hr.) after fixation to remove the excess of fixative out of the tissue to prevent intervention with subsequent processes, washing is often was done with running water [131].

3- Dehydration: The samples were done to remove all extractable water by dehydrated diffusing through the tissue, alcohol was used. The dehydration procedure was continued by upgrading alcohol from 50% to absolute alcohol (50%, 60%, 70%, 80%, 90%, 100%)

4- Clearing: This stage is considered as a middle step, it is obligatory because the used alcohol for dehydration will not dissolve or mix with molten paraffin. Thus tissue must be submerged in some fluid miscible with both alcohol and paraffin before infiltration can take place. Clearing was too removed or clear opacity from dehydration tissues and making them transparent. Therefore , blocks of tissue seemed almost crystalline and never milky. Xylene was reagents used for clearing[131].

5- Embedding(Blocking): Paraffin wax is considered to be either soft (melting point 50-52°c) or hard (melting point 60-68°c). Hot weather will power the use of harder paraffin. Paraffin must be fully molten to infiltrate the tissue effectively. Embedding with paraffin , paraffin was permitted to solidify around and within the tissue. The tissue was sited in a small container or a

paper box is already filled with malted paraffin. Orientation of the tissue is important in order to limit the proper surface for sectioning [132].

6- Cutting: cutting was done by using the rotary microtome. The thickness is (5 μm). the sample were put in hot water path with (52°c) for extending tissue, then the samples were carried on the slides which had a thin layer of Mayer egg albumin, the slide was put on the hot plate with (40°c) for overnight.

7- Staining: Heamatoxylin and Eosin stain: for demonstrating of the general structure of the tissue [131].

3.4 The measurement

1- Measuring diameters of A-spermatogonia, B-spermatogonia and I-spermatogonia.
2- Measuring diameters of renal corpuscles, proximal and distal convoluted tubules.
3- Measuring diameter of Hepatocyte Nuclei.
4-Measuring the levels of Urea, AST, ALT, Testosterone, Estrogen, LH and FSH.

3.5 Statistical analysis

The data under this study are analyzed using t- test for independent samples.when the value of P less the 0.05 the differences are significant. We used the Minitab v.18 to analysis the results.

Recommendation

1- Immunohistochemical study on the commercial fish to investigated the. Immunochemical reaction.
2-Ultra-structural study on the vital organs in the fish that living in the commercial basin.
3- Embryological study on the fish that living in the commercial basin.
4- Histological study on the fish that living in commercial basin.

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Conclusions

1-The kidney in commercial carp fish, have histological changes and some pathological condition. It appeared sever distraction of glomerular, blood congestion between renal tubules and in different location of kidney and aggregation of inflammatory cells inside of renal tubules, So fish showed significant differences in the diameters of renal corpuscle between both groups with significant decreased in the diameter of proximal convoluted tubules.

2-The liver in commercial carp fish blood hemorrhage in some of location of liver parenchyma and very wide cystic dilation and cellular apoptosis inside the cystic dilation, sever liver degeneration and showed blood clots in different location of liver parenchyma and hepatocyte nuclei have significant decreased compared with histological results of river carp which was normal structure.

3-The testis have prominent histological changes in the seminiferous tubules, aggregation of spermatogenic cells in the spaces between seminiferous tubules because of destruction the wall of seminiferous tubules in comparative between the commercial and river fish and showed blood congestion in different location of testis in the commercial fish.

4-The level of vitellogenin in commercial fish have high significant increased comparing with river fish.

5-The hormonal level of commercial fish have high significant differences between the commercial fish and river fish.

6-The level of ALP and AT were have significant differences between the commercial fish and river fish.

Abbr.	Meaning
ALH	Ascending loop Henle
ALT	alanine transaminase
AST	aspartate transaminase
BPA	bisphenol A
BSA	Bovine serum albumin
BTB	Blood testes barrier
β’	β-components
CT	Collecting duct
DCT	Distal convoluted tubule
DDT	dichlorodiphenyltrichloroethane
DES	diethylstilbestrol
DLH	Descending loop Henle
DNA	deoxyribonucleic acid
E1	Estrone
E2	17-β estradiol
E3	estriol
EE2	17-α-ethinyl-estradiol (EE2)
EDCs	Endocrine Disruptor Chemicals
EM	Electron michrograpgy
ER	estrogen receptor
FSH	Follicle stimulate hormone
GnRH	gonadotropin releasing hormone
GtH-I	gonadotropin-I
GtH-II	gonadotropin-II
HCH	Hexachlorocyclohexane
LFTs	Liver function test
LH	Luteinizing hormone
Lv	lipovitellin
MMCs	Melanomacrophage centers
Mrna	Messenger ribonucleic acid
OPD	peroxidase substrate
PBBs	polybrominated biphenyls
PBS	Phosphate buffered saline
PCBs	polychlorinated biphenyls
PCT	Proximal convoluted tubule
Pi	isoelectric point
Pv	phosvitin
ST	Seminiferous tubule
STWs	sewage-treatment works
T	testosteron
VTG	Vitellogenin

Abstract:

The current study designed to collected sixty male carp fish which divided into two main groups each group contained on thirty male carp fish obtained from the commercial basins and other thirty male carp obtained from Euphrates river in Al-Samawa city on January to February. The study aiming to investigation the variation in the level blood vitellogenin in the commercial and river carp fish, histological and

physiological changes in some organs between commercial basin fish and the river fish.

The result showed the level of vitllogenin in the commercial fish have high significant increased compared with the level of vitellogenin in the river fish. The level of LH and FSH have significant increased in commercial fish compared with river fish. So, the level of AST, ALT. have significant differences between the commercial and river fish in this study. The histological result finding of this work noted there are prominent histological changes in the organs(liver, testis, and kidney) in the fish that obtained from commercial basin.

The histological result of liver tissue section in river carp fish showed the liver parenchyma wide blood sinusoid filled with R.B.C and normal distribution of kupffar cells through the liver parenchyma .The liver showed normal structure in the river fish compared with liver of commercial carp fish. The liver in commercial fish have sever degeneration and many hepatocyte lost their nuclei and have vacuoles in cytoplasmic. The hepatocytes noted randomly distribution through the liver parenchyma. The testis of commercial fish have blood congestion in different location of testis, the most Seminiferous tubules were empty from deferent types of spermatogenic cells in spermatogenesis.

The tissue section of testis in river carp fish showed the seminiferous tubules filled with high aggregation of spermtogenic cells. The kidney of commercial fish have many cystic dilation filled with blood, most renal tubules have sever damage in the epithelial layer with blood congestion between renal tubules. The present result in river carp fish has been showed the glomerular capillaries were filled with blood and normal aggregation of mesangial cells between the glomerular capillaries and Bowman capsule was normal structure and both layers ware very prominent.

4.1 The Histological Result

4.1.1 The Histological Result of Kidney

4.1.1.1 The Histological Result of River Carp Fish

The tissue section of kidney parenchyma was a normal structure without any changes. The current result indicate that the normal distributed of nephrons through the kidney parenchyma. The glomerulus was prominent in normal shape with Proximal convoluted tubule. The parietal and visceral layers of Bowman capsule were prominent structures.

The glomerular capillaries were filled with blood and normal aggregation of mesangial cells between the glomerular capillaries (Fig.4.3). The present result appeared that the Bowman capsule was normal structure and Bowman's space have

normal width and prominent basement membrane. The cortex of kidney was composed mainly of renal corpuscle which was (127.3±3.7µm) in diameter (table1).

The tissue section of kidney showed that most proximal convoluted tubules have high columnar epithelia with prominent basement membrane, the tissue section showed prominent lumen of proximal convoluted tubules. The cells that composed of proximal convoluted tubules wall have oval dark nuclei with acidophilic cytoplasm (Fig.4.4). The myoepithilial cells in the wall of proximal convoluted tubules have prominent elongated dark nuclei.

The measurement of diameter of proximal convoluted tubule (P.C.T.) was(105.3±3.3) (table 2) (Fig 4.4). The histological results of distal convoluted segment appeared the cross section of distal segment have irregular in shape and have very wide lumen. The tissue section of P.C.T showed the internal surface of P.C.T lined by low Cuboidal epithelial without brush border .

The cells that composed of the portion of proximal convoluted wall have dark spherical nuclei with acidophilic cytoplasm. Most tissue section didn't appeared prominent moyepithelial cells in the wall of P.C.T and the measurement of diameter of it(Fig.4.5). Some histological section of distal segments showed exfoliated the epithelial layer and accumulation in the lumen of distal convoluted tubules.

All tissue section showed the lumen of distal segments filled with infiltrate and showed the collecting duct (Fig4.5) (Fig4.6). The tissue section of kidney in carp fish that live in Al Samawa river didn't noted any pathological condition in the kidney parenchyma , no blood hemorrhage, no inflammatory cells aggregation in the parenchyma and no blood congestion compared with kidney section of commercial carp fish living commercial lake.

4.1.1.2 The histological result of kidney of Commercial carp fish:

The histological structure of kidney which have highly vascularized structure .the kidney of carp fish is covered with a very delicate layer of connective tissue capsule. The present result of kidney in carp fish which living in commercial fish in river showed the histological section of kidney have blood congestion in different location of kidney, the blood vesicles are filled with blood between the renal tubules.

The cross section of proximal convoluted tubules which have a wide diameter and showed glomerulus. The measurement of diameter of proximal convoluted tubule (P.C.T.) was (89.6±6.1) (table3) (Fig 4.7). The histological result of kidney in carp fish that living in commercial basin is characterized by distributed many cystic dilation which filled with blood and appeared blood vessels and the glomerulus (Fig.4.8).

The epithelia of proximal convoluted tubules with appeared high columnar epithelia without brush border in some section showed the P.C.T have prominent wide lumen. The present result of kidney in commercial carp fish showed the renal parenchyma have aggregation of inflammatory cells as cluster in shape between the Renal corpuscle and renal tubules and showed high columnar epithelia of proximal convoluted tubule .

The tissue section of kidney finding very wide cystic dilation filled with R.B.C located between the renal corpuscles and vacuolated infiltrated. The renal corpuscles which was (165.1±7.6µm) in diameter have prominent Bowman space, and the glomerular capillary was congested with blood. The misegeal cells between the glomerular capillaries have prominent dark nuclei(Fig 4.9).

The histological finding showed high distribution of R.B.C through the kidney parenchyma with inflammatory cells and wide Bowman space . The tissue section of kidney showed many renal corpuscles have partial destruction of vascular pole more the renal pole and showed the intermediate and distal segments of proximal convoluted tubules, the glomerular capillaries have very wide lumen and showed inflammatory cells in side of renal corpuscles(Fig.4.10).

The present result of kidney showed sever destruction in the wall of renal tubules. The renal tubules showed exfoliated the epithelial layer which aggregation in the lumen of renal tubules. The epithelial cells of renal tubules have prominent voculation in their cytoplasm. Most renal tubules have sever damage in the epithelial layer with blood congestion between renal tubules.

The tissue section showed aggregation of inflammatory cells inside of renal tubules. The present result showed wide glomerular capillaries and appeared basement membrane, columnar cells of proximal convoluted tubules.Most epithelial cells of renal tubules lost the apical position with aggregation cytoplasmic figment in the lunar of renal tubules(Fig.4.11), these changes may be to the effect of endocrine disruptors chemicals(EDCs) or by the high level of vitellogenin in male carp fish.

These results agreed with[121,133]which explained that mild degenerative changes of tubular epithelium in the exposure to estrogenic substance and also reported histological changes in kidney of fish exhibiting degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis following subtle exposure to phenolic compounds. The unusual production of vitellogenin in male fish Can result in kidney damage.

The histological results of kidney in carp fish which lives as commercial fish showed completely distraction of renal corpuscle with some of renal tubules in

addition to blood congestion in kidney parenchyma and showed exfoliated epithelia. The present result showed progressive the glomerular capillaries in renal corpuscle. The tissue section of kidney noted distraction of glomerular capillary and noted R.B.Cs in the Bowman space.

The Bowman capsule which have thick parietal layer with very permanent basement membrane(Fig. 4.12), these changes in the kidney that exposure to EDCs such as estrogen or by the diet of fish in commercial basin.

These results consonant with [134,135,136] explained of many changes in the kidney that exposure to EDCs. These changes were necrosis, degeneration and dissociation of Bowman's capsule and renal tubules, proliferation in the renal tubule , shrinkage of glomerulus, dilated blood vessel, rupture of Bowman's capsule, and obliterated Bowman's space. The present result of kidney showed the main collecting ducts have very thick wall, with wide lumen, aggregation of R.B.C and inflammatory cells in the lumen of collecting ducts, the inner epithelial layer have portial destruction in apical portion of epithelial layer.

Statistical Analysis

Figure(4-1) shows diameters of renal corpuscle in commercial fish compared with river fish.

Table(1) explains there are significant differences (P <0.05) increased in diameter of renal corpuscle between commercial fish which have (156.1 ±7.6) compared with river fish was (127.3 ±3.7).

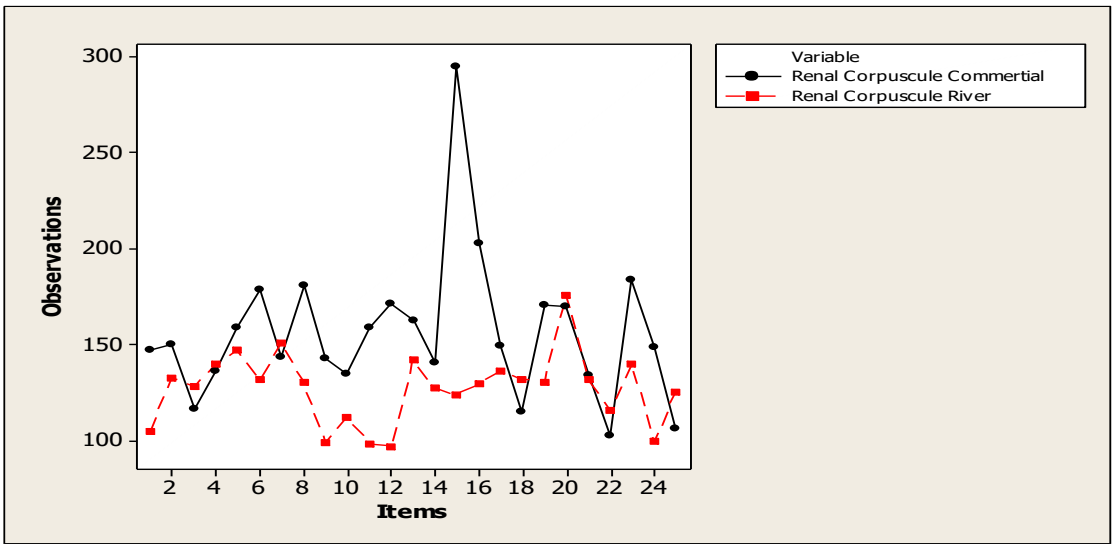


Fig.4-1 Diagram showed diameter of renal corpuscle of commercial and river carp fish.

Table 1 Diameter of renal corpuscle of commercial and river carp fish (µm)

Two-sample T for Renal Corpuscle Commercial vs Renal Corpuscle River				
Group	N	Mean	StDev	SE Mean

Commercial	25	156.1	38.0	7.6
River	25	127.3	18.5	3.7

Difference = µm (Renal Corpuscule Commercial) - um (Renal Corpuscule River)

Estimate for difference: 28.76

95% CI for difference: (11.59; 45.93)

T-Test of difference = 0 (vs not =): T-Value = 3.40 P-Value = 0.002 DF = 34

Figure (4-2) appears the diameters of proximal convoluted tubules in commercial fish compared with river fish.

Table(2) explains there is significant differences (P <0.05) decreased in the diameter of P.C.T between commercial fish which have (89.6±6.1) and with river fish which have (105.3±3.3).

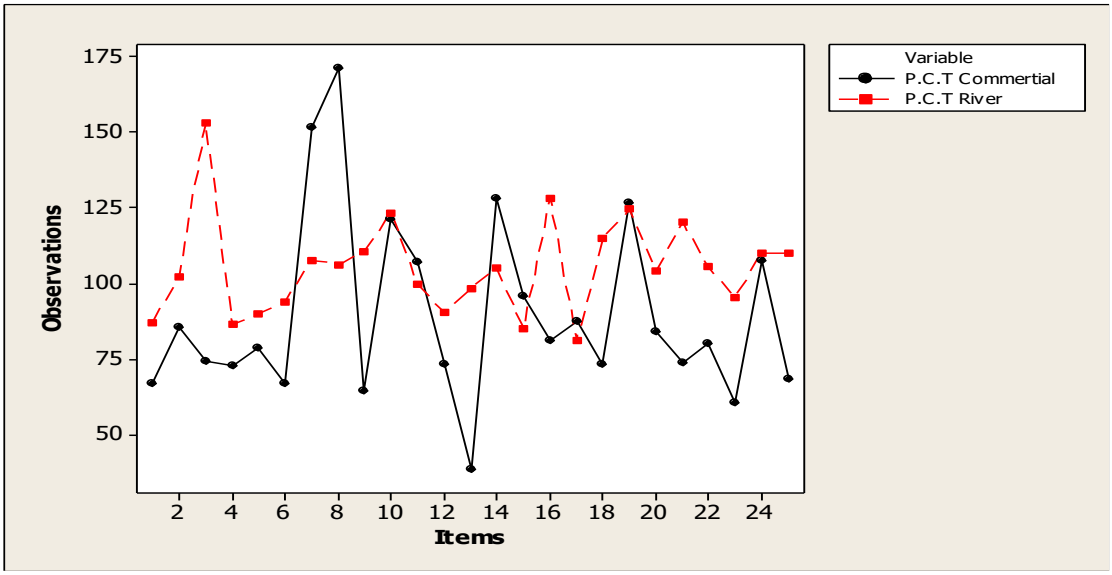


Fig 4-2 Diagram showed diameter of proximal convoluted tubule in commercial and river carp fish.

Table 2 Diameter of proximal convoluted tubule in commercial and river carp fish(µm)

Two-sample T for P.C.T Commercial vs P.C.T River				
Group	N	Mean	StDev	SE Mean
Commercial	25	89.6	30.5	6.1
River	25	105.3	16.3	3.3

Difference = µm (P.C.T Commercial) - um (P.C.T River)

Estimate for difference: -15.75

95% CI for difference: (-29.76; -1.73)

T-Test of difference = 0 (vs not =): T-Value = -2.28 P-Value = 0.029 DF = 36

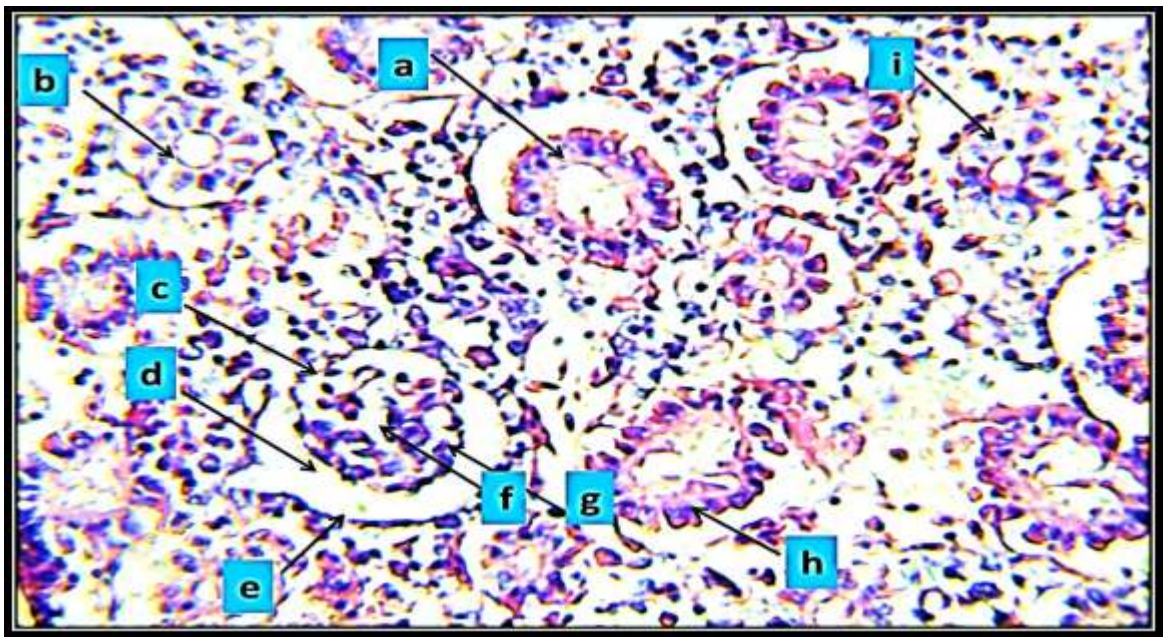


Fig 4.3: Cross section through kidney in river fish which showed a-intermediate Proximal convoluted tubule, b- Proximal convoluted tubule, c- The mesangial cells , d- Bowman's space, e-parietal layer, f- glomerular capillaries, g-visceral layer, h-high columnar epithelia, i- Proximal convoluted tubule 400X, H&E stain.

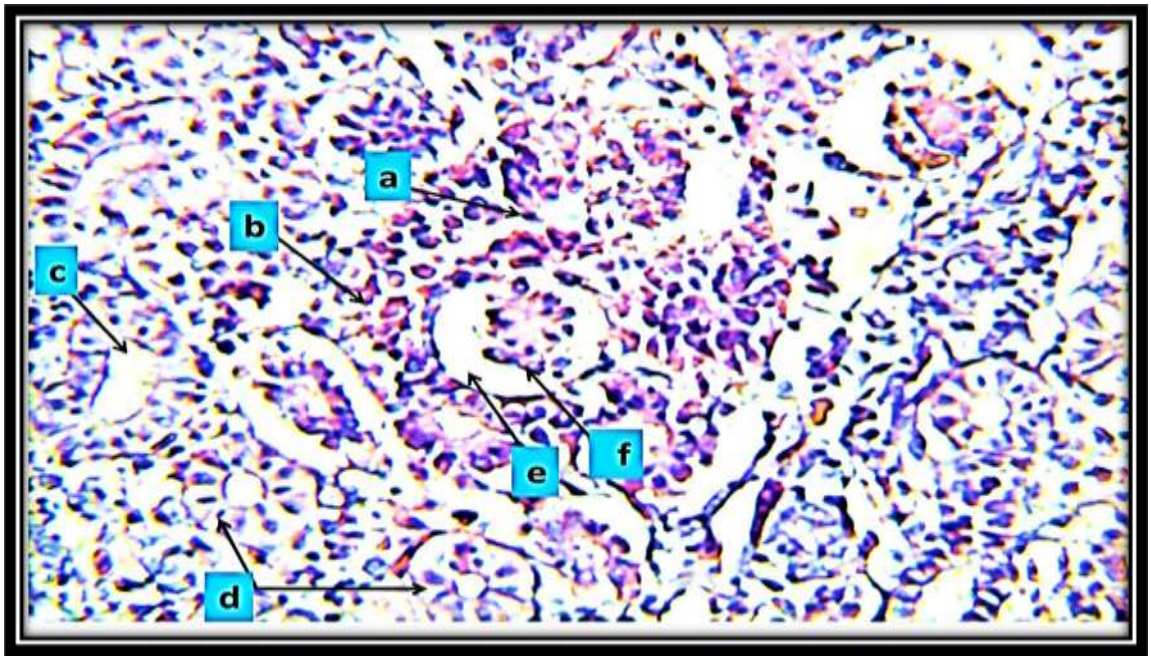


Fig 4.4: Cross section through kidney in river fish which showed a-cystic dilation, b-blood vessel, c- intermediate Proximal convoluted tubule, d-Proximal convoluted tubules, e- Bowman's space, f-Renal corpuscle 400X, H&E stain.

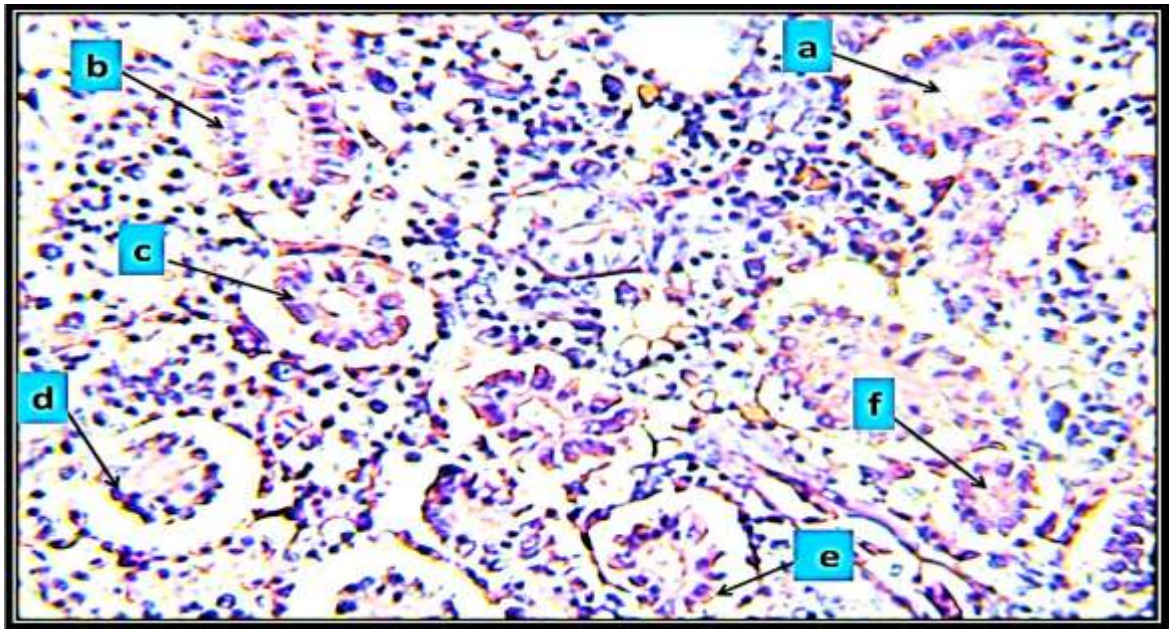


Fig 4.5: Cross section through kidney in river fish which showed a-Lumen of Proximal convoluted tubule, b-intermediate Proximal convoluted tubule, c- Proximal convoluted tubule, d-cuboidal epithelia, e-epithelial layer of Proximal convoluted tubule, f- Proximal convoluted tubule400X, H&E stain.

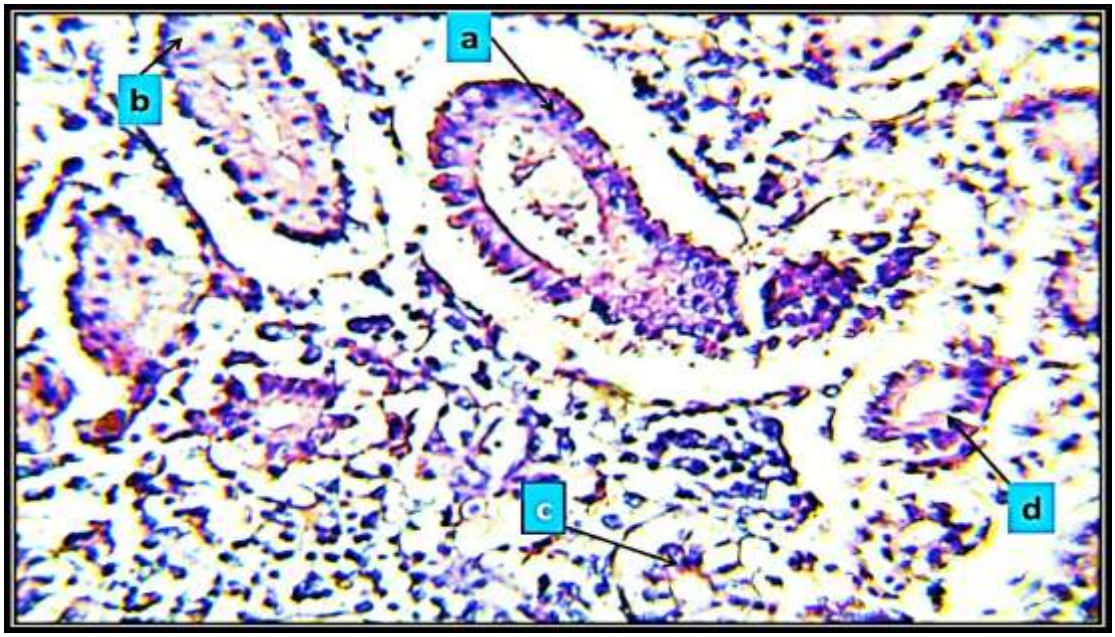


Fig 4.6: Cross section through kidney in river fish which showed a-collecting duct, b-Collecting epithelia, c-Distal segment of Proximal convoluted tubule, d-intermediate Proximal convoluted tubule400X, H&E stain.

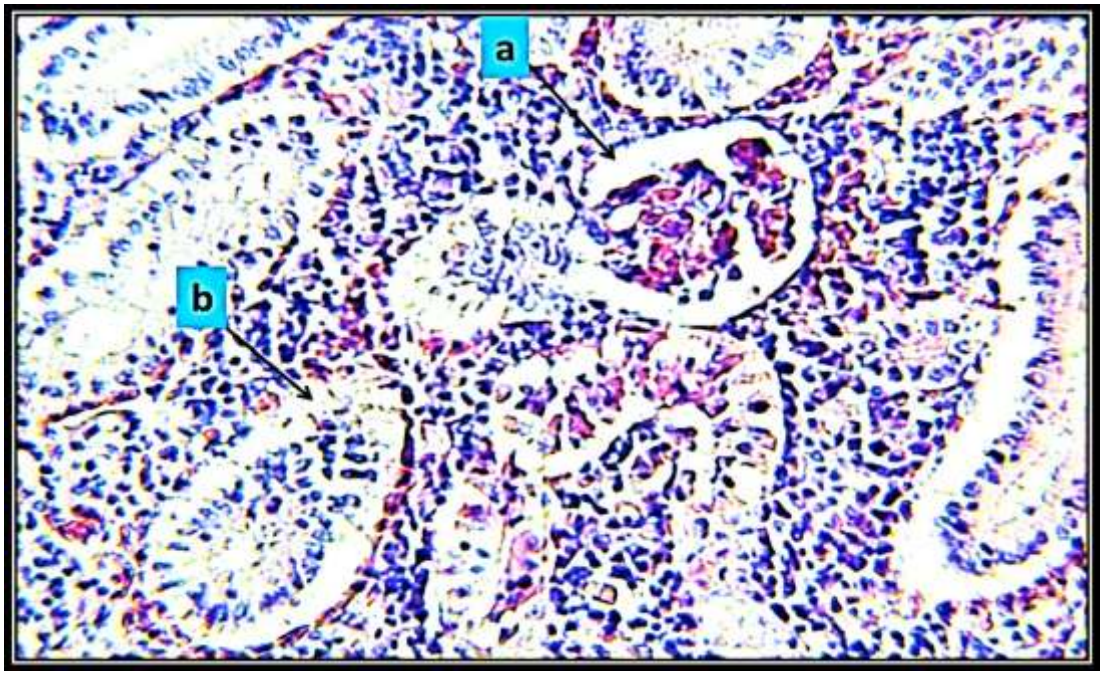


Fig.4.7: Cross section through kidney in commercial fish which showed a- wide proximal convoluted tubule lumen, b- Renal corpuscle400X, H&E stain.

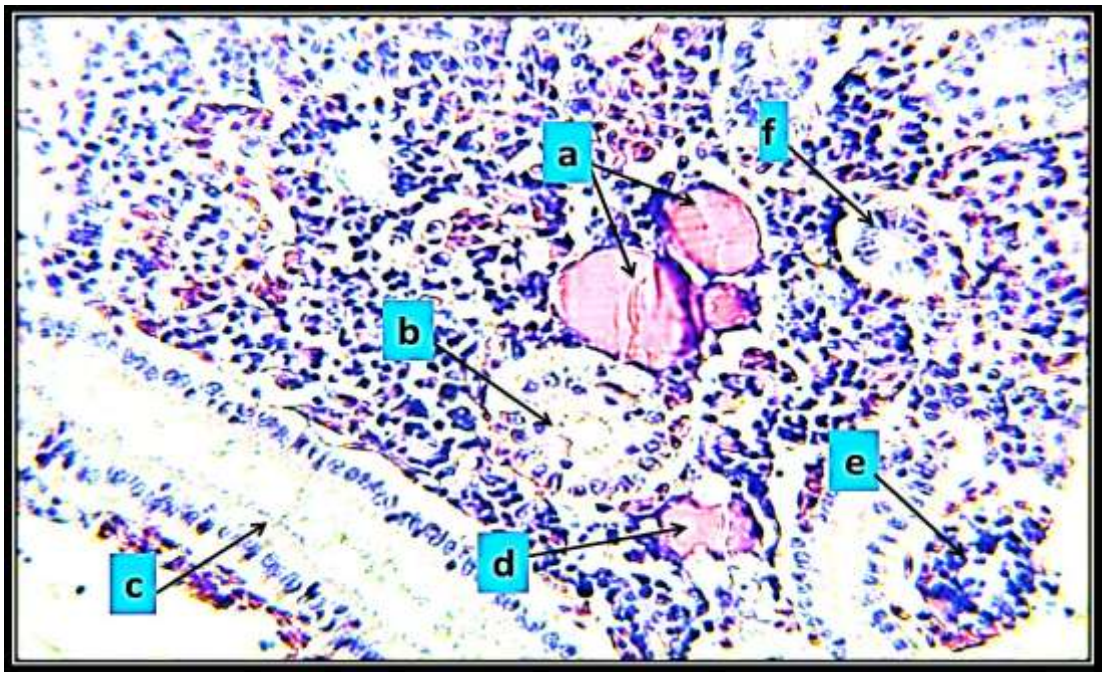


Fig 4.8: Cross section through kidney in commercial fish which showed a-cystic dilation filled with blood, b- Distal portion of Proximal convoluted tubule, c-Collecting duct, d-Blood vessels, e-Renal corpuscle, f-Proximal convoluted tubule400X, H&E stain.

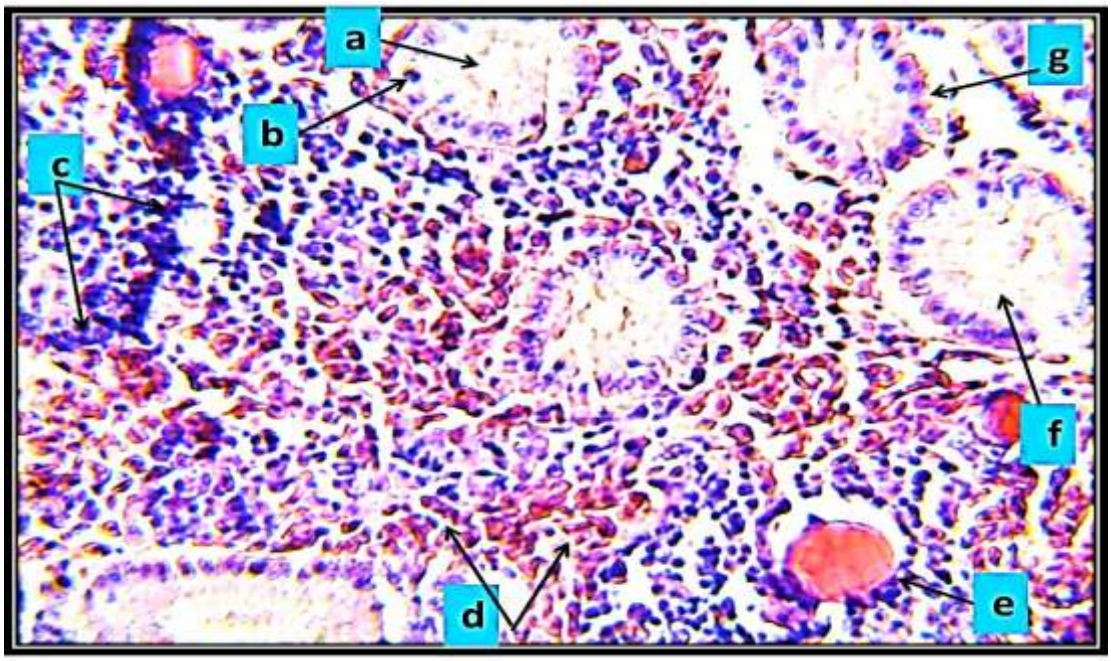


Fig 4.9: Cross section through kidney in commercial fish which showed a-wide Proximal convoluted tubule, b-oval nuclei, c- aggregation of inflammatory cells, d-blood hemorrhage, e- cystic dilation of blood, f- vacuolated infiltrated, g-high columnar epithelia400X, H&E stain.

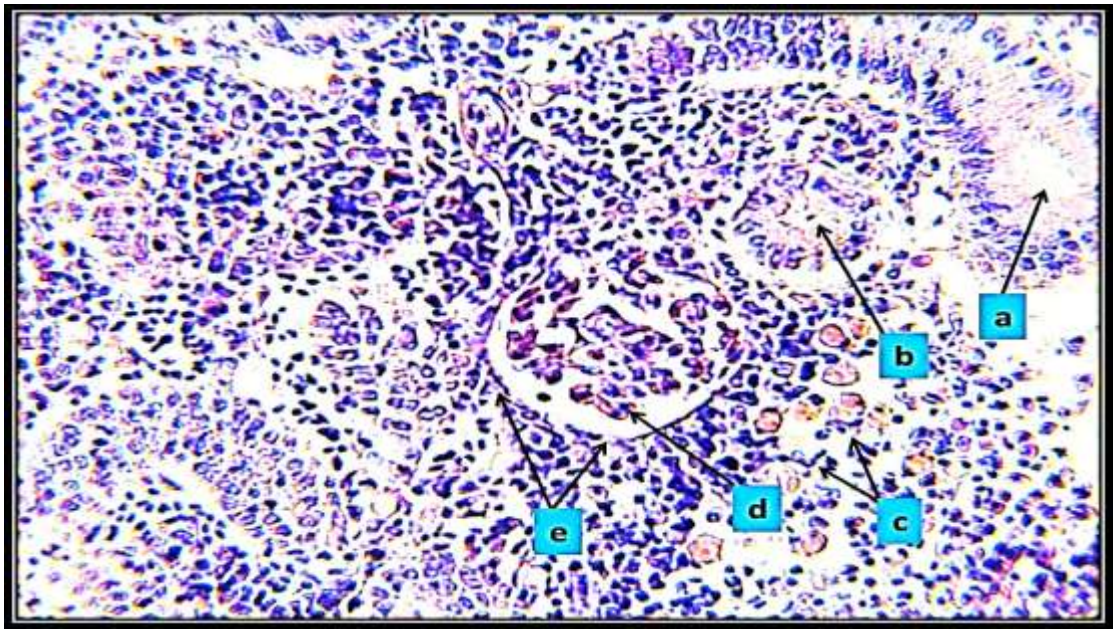


Fig 4.10: Cross section through kidney in commercial fish which showed a-Distal segment of Proximal convoluted tubule, b- intermediate proximal convoluted tubule, c-blood cells, d-wide B.S, e- Renal corpuscle400X, H&E stain.

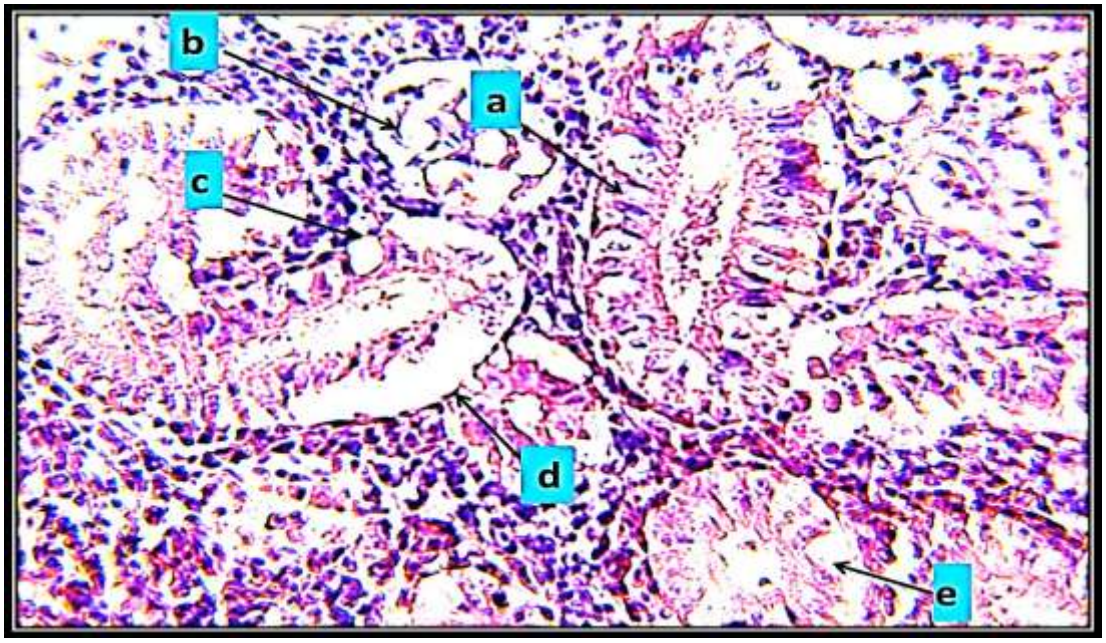


Fig 4.11: Cross section through kidney in commercial fish which showed a-Columnar cells, b-wide glomerular capillaries, c-Vacuolation, d-basement membrane, e- Proximal convoluted tubule400X, H&E stain.

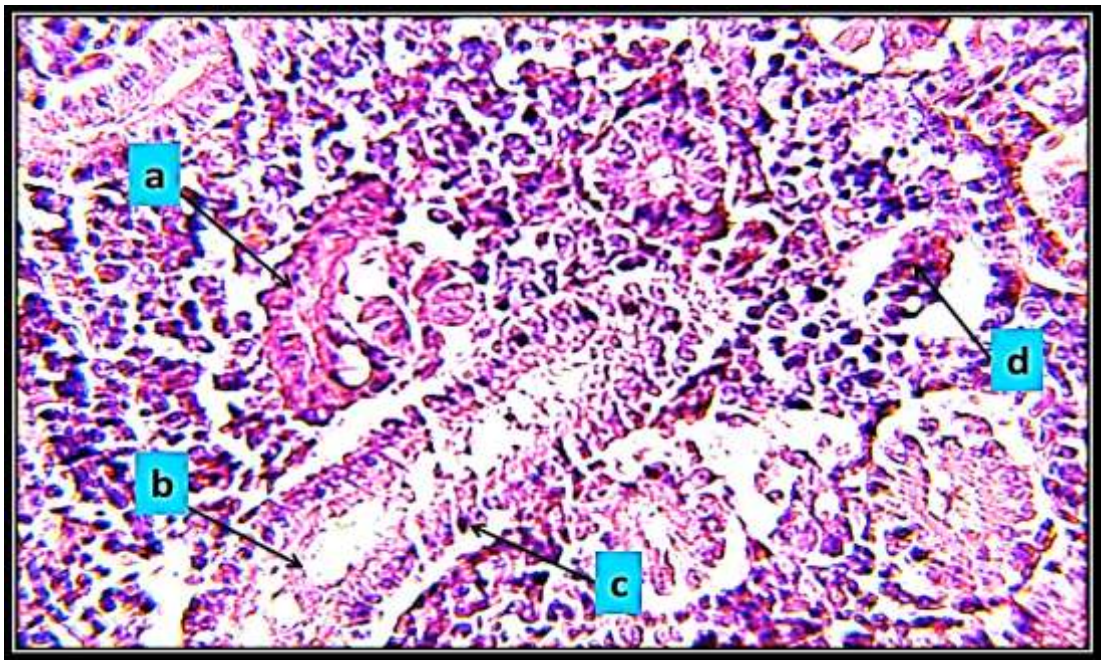


Fig 4.12: Cross section through kidney in commercial fish which showed a-distraction of proximal convoluted tubule, b-lumen of proximal convoluted tubule, c-exfoliated epithelia, d-progressive renal corpuscle400X, H&E stain.

4.1.2 The Histological Result of Liver

4.1.2.1 The Histological Result of Liver of River Fish

The histological result of liver tissue section of river carp fish showed the liver parenchyma have wide blood sinusoid filled with R.B.C .The current result showed normal distribution of kupffar cells through the liver parenchyma .The present of

liver showed normal structure compared with liver of commercial carp fish. The hepatocyte have dark oval nuclei with acidophilic cytoplasm .

This results agreed with [136,115] liver of the control fish appears as a continuous mass of hepatic cells, The hepatocytes are arranged around the central vein. The hepatocytes are large, polygonal in shape with centrally located nuclei. The blood vessel of liver were filled with blood. The current result of liver showed the blood sinusoid lined by normal endothelial layer normal portal area (Fig4.14) .

The current result of liver showed the blood sinusoid lined by normal endothelial layer with normal portal area with normal aggregation of kuffer cells and normal distribution of hypatocyte (Fig4.15). The tissue result of liver in river carp fish showed no pathological lesions in liver parenchyma so, no, abnormal aggregation of inflammatory cells the histological result didn't noted any blood congestion or blood hemorrhage.

4.1.2.2 The Histological Result of Liver of Commercial Fish

The histological result of liver in carp fish which living in commercial basin showed the liver parenchymal have blood hemorrhage in some location of liver parenchyma . The hepatocytes have acidophilic cytoplasm. The current result showed high aggregation of kupffer cells belong the liver parenchyma with inflammatory cells which located as small cluster through the liver parenchyma All tissue section didn't appeared normal Portal area neither central vain in liver parenchyma (Fig. 4.16) .

The present result showed very wide cystic dilation, the tissue section showed cellular apoptosis inside the cystic dilation and inflammatory cells (Fig.4.17) The histological result of liver showed the blood capillary and sinusoid were abnormal in shape and filled with blood, high aggregation of kupffer cells around the abnormal cystic dilation so that inflammatory cells inter in side this dilation these changes may be to the effect of estrogenic substance in the aquatic environment that carp fish found in it .

This results agreed with [115,137,138] increase in the infiltration of lymphocytes and dilation of sinusoids apparent when fish exposure to estrogenic substance, also showed a significant increase in the Kupffer cells after exposure to estrogenic substances and induced of cell death. The histological result of liver in carp fish that living in commercial basin noted irregular portal area and contain inflammatory cells beside the central vein which filled with blood and showed blood congestion, apoptosis and very wide cystic dilation (Fig.4.18).

The current result of liver showed clear parenchymal diastral around of portal area present result showed sever liver degeneration and showed blood clots in different

location of liver parenchyma, cystic dilation, blood congestion and inflammatory cells aggregation (Fig.4.19). This results agreed with [115] which explained of infiltration of lymphocyte around the vena central and severe damage in liver, such as necrotic areas in the fish which exposed to estrogenic substances.

The tissue section of liver showed most blood sinusoids were empty from blood cells. The tissue section noted high proliferation of kupffer cells in different area of liver parenchymal (Fig.4.20). This results similar to[137] which recorded a significant increase in the Kupffer cells after exposure to the estrogenic compound in the fish. The histological result of liver showed that most blood vessels have milled distraction of endothelial layer that lining the inner surface of blood vessel and blood hemorrhage vessels.

The current result of liver of carp fish in commercial basin showed numbers of blood clotting in liver parenchyma as spot shape like structures . The tissue section showed high aggregation of neutrophil cells in many sites of liver parenchyma. So, the tissue section showed many hepatocyte which lost the their nuclei and have cytoplasmic vacuoles and randomly distribution through the liver parenchyma(Fig.4.21).

This results agreed with [136,115] that explained the examination of liver sections after exposure to estrogenic compounds showed degeneration in the form of disintegration in most cytoplasmic contents and Lymphatic aggregations, and vacuolization of cell cytoplasm was appeared after exposure to EDCs. .

Statistical Analysis

Figure(4-13) shows the diameter of hepatocyte nuclei in the liver of commercial fish compared with hepatocyte nuclei diameters of river fish.

Table(3) shows there is significant differences (P <0.05) decreased in the diameter of hepatocyte nuclei between commercial fish have (2.552 ± 0.10) and the hepatocyte nuclei diameters of river fish which have (4.378 ±0.20).

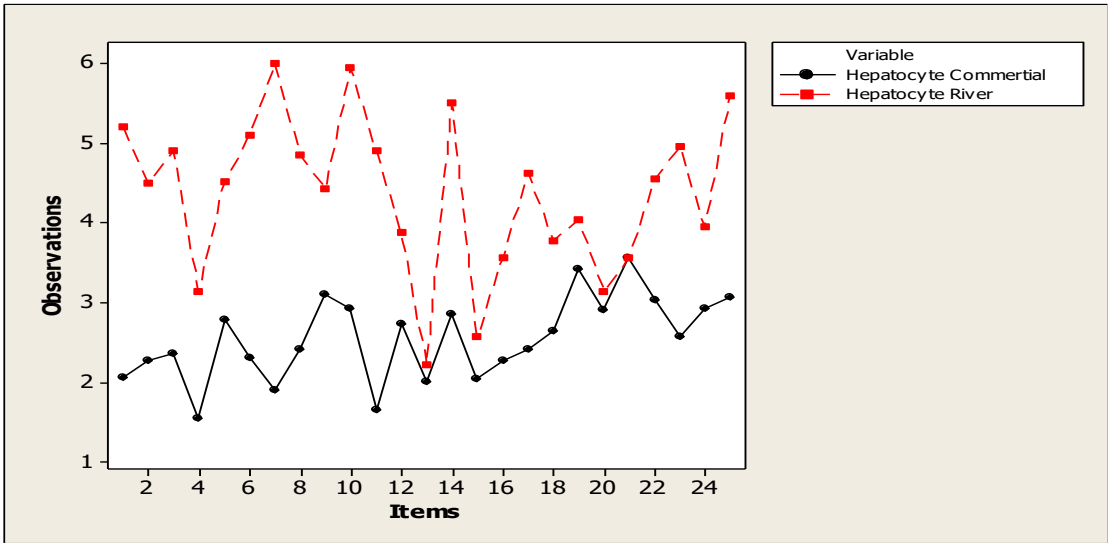
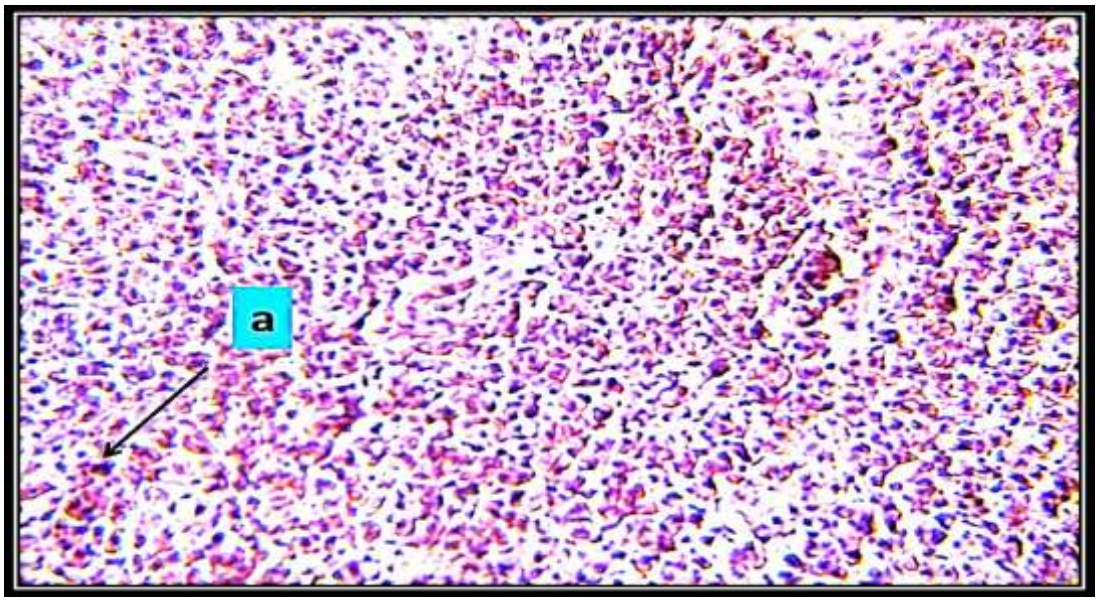


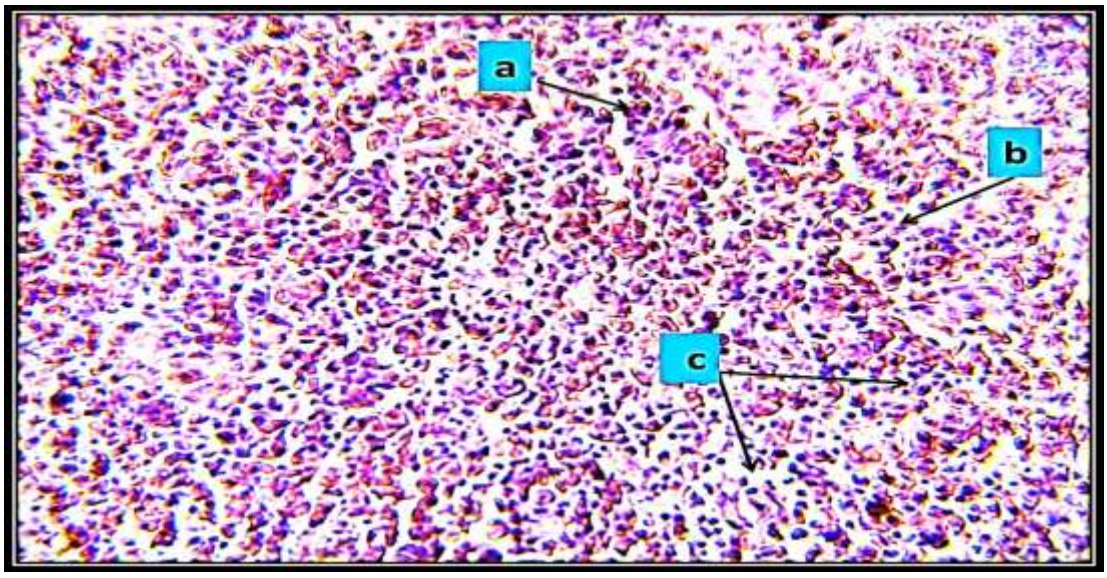
Fig 4.13 Diagram showed diameter of hepatocyte of commercial and river fish

Table 3 Diameter of hepatocyte of commercial and river fish (µm)

Two-sample T for Hepatocyte Commercial vs Hepatocyte River				
Group	N	Mean	StDev	SE Mean
Commercial	25	2.552	0.521	0.10
River	25	4.378	0.990	0.20
Difference = µm (Hepatocyte Commercial) - um(Hepatocyte River)				
Estimate for difference: -1.825				
95% CI for difference: (-2.279; -1.372)				
T-Test of difference = 0 (vs not =): T-Value = -8.16 P-Value = 0.000 DF = 36				

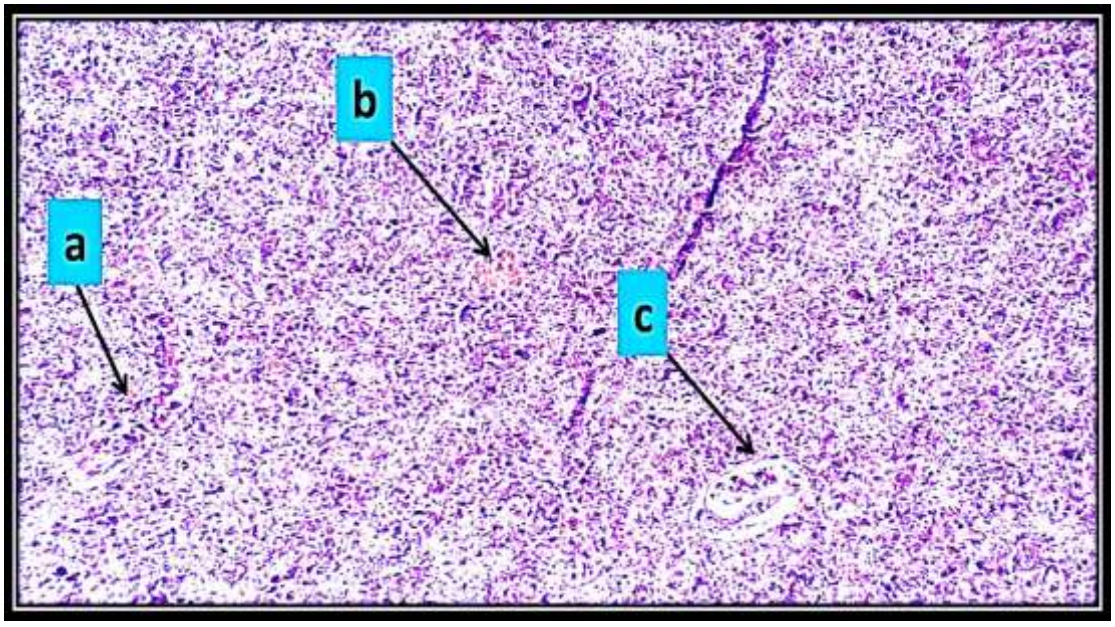


(Fig.4.14): Cross section through liver in river fish which showed a- hepatocyte 400X, H&E stain.

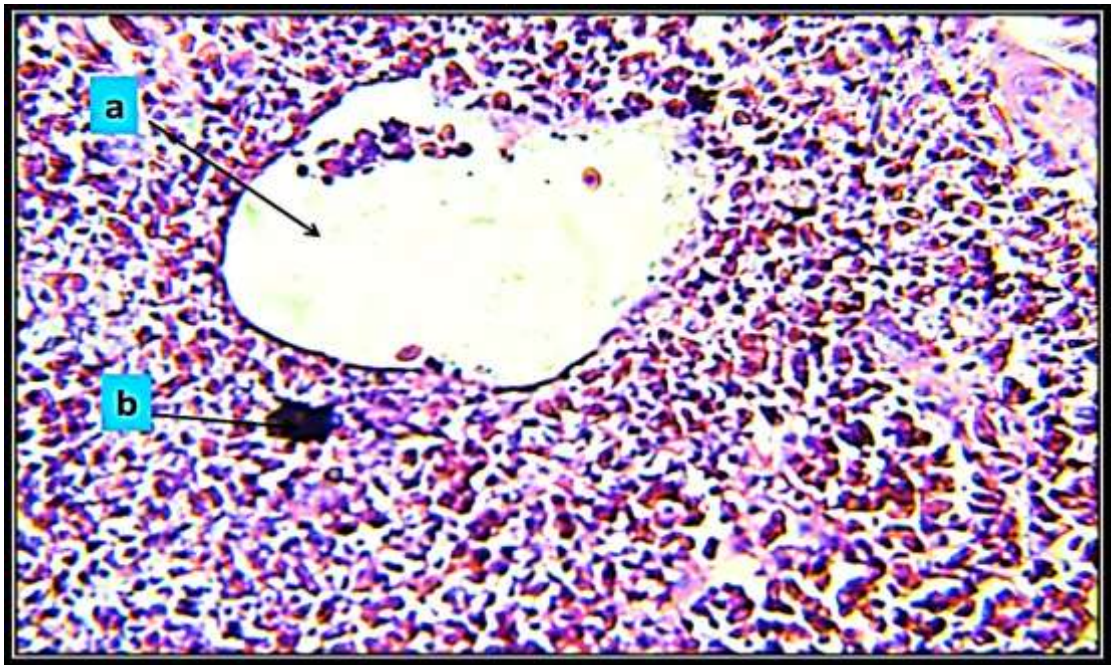


(Fig.4.15): Cross section through liver in river fish which showed a-hepatocyte,

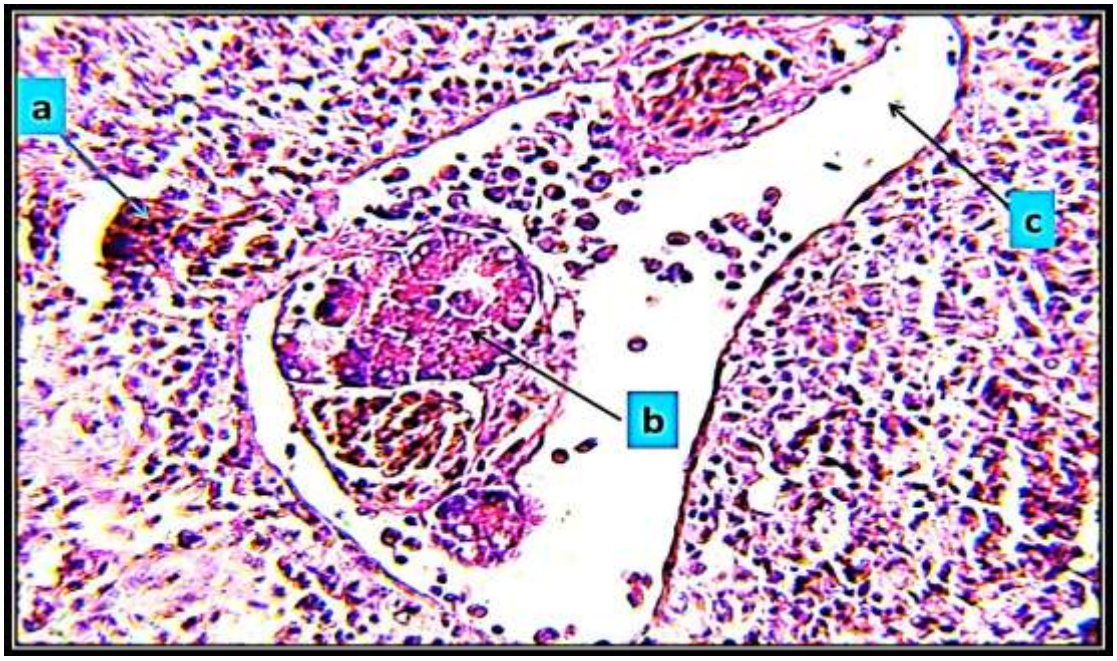
b-normal aggregation of kuffer cells, c- normal distribution of hepatocyte 400X, H&E stain.



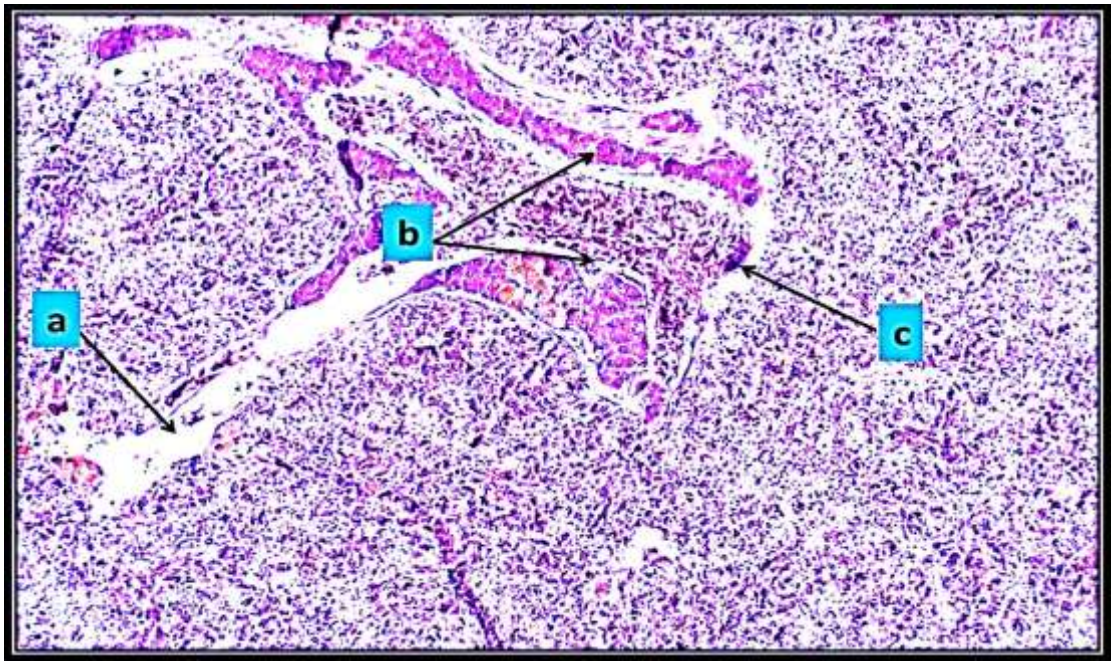
(Fig.4.16): Cross section through liver in commercial fish which showed a-aggregation of inflammatory cells, b-blood hemorrhage, c-abnormal portal area and center vein100X, H&E stain.



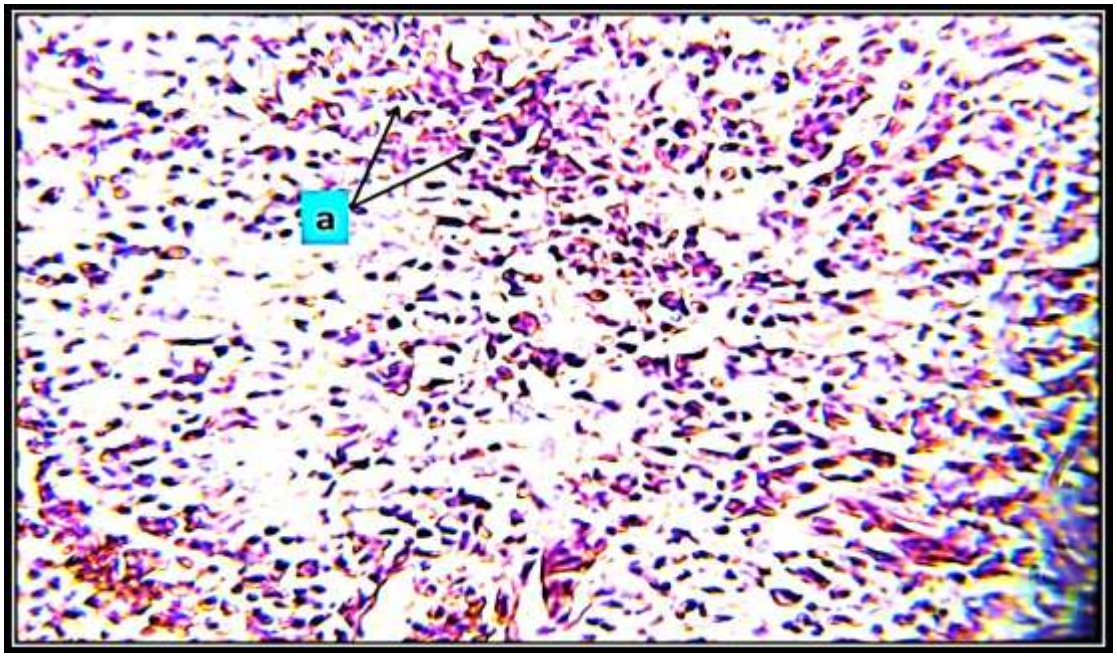
(Fig.4.17): Cross section through liver in commercial fish which showed a-very wide cystic dilation, b- inflammatory cells400X, H&E stain.



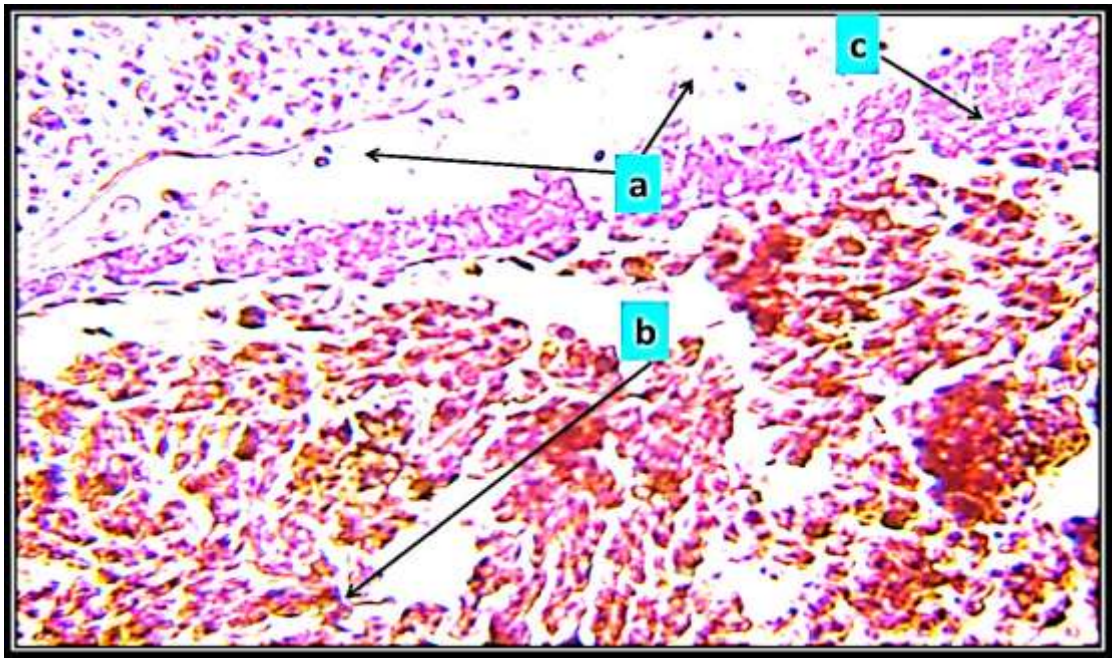
(Fig.4.18): Cross section through liver in commercial fish which showed a- apoptosis, b-congestion blood vessels, c-very wide cystic dilation400X, H&E stain.



(Fig.4.19): Cross section through liver in commercial fish which showed a- cystic dilation, b- blood congestion, c- inflammatory cells aggregation100X, H&E stain.



(Fig.4.20): Cross section through liver in commercial fish which showed a-aggregation of inflammatory cells400X, H&E stain.



(Fig.4.21): Cross section through liver in commercial fish which showed a-inflammatory cells, b-blood hemorrhage vessels, c-blood vessels400X, H&E stain.

4.1.3 The Histological Result of Testis

4.1.3.1 The Histological Result of Testis of River Fish

The tissue section of testis in river carp fish showed the seminiferous tubules filled with high aggregation of spermtogenic cells. This result agreed with [139] that presented of founding of free spermatozoa filled in the lobular lumen. The wall of seminiferous tubules was normal structure with prominent basement membrane

(F.g4.25). The present result of testis in river carp fish was noted normal germinal epithelial that living the basal line of seminiferous tubules wall.

The tissue section of testis parenchyma of river carp fish showed good aggregation of laydig cells in the spaces between the normal seminiferous tubules all leydig cells have dark nuclei and showed germinal epithelia, Leydig cells and founding of spermatogonia cells (Fig.4.25). This results agreed with[140] which explain that leydig cells are located between the tubules in the testes. The present histological result of testis in river carp fish notes normal histological structures ,not found any destruction in wall of seminiferous tubules. The tissue section didn't appeared any inflammatory cells aggregation between the seminiferous tubules .

All tissues section of testis didn't noted any blood hemorrhage or blood congestion in testis parenchyma but in some section showed wide spaces between the seminiferous tubules and this spaces were empty from leydig cells and showed showed germinal epithelia (Fig4.26). The present histological result notes prominent basement membrane, spermatogonia cells, germinal epithelia, and founding of Leydig cells (Fig4.27) (Fig4.28).The histological result of testis in river carp fish showed some of seminiferous tubules were opened between others and mixed the spermetogenic cells in wide lumen because of opining of seminiferous tubules to become as one tubules

4.1.3.2 The Histological Result of Testis of Commercial Fish

The present histological finding of testis in carp fish that living in commercial showed the seminiferous tubules have thin wall with prominent basement membrane , the germinal epithelial that lining the internal surface of seminiferous tubules was separated from the basal line of seminiferous tubules (Fig4.29). All tissue section of testis in male commercial carp showed very wide space between the germinal epithelial and the accumulation of spermatogenic cells in the central lumen of seminiferous tubules and showed Leydig cells and noted exfoliated germinal epithelia (Fig4.29).

The present result of testis showed large aggregation leydig cells as grouping in the space between seminiferous tubules ,the leydig cells which have very prominent dark nuclei which have oval in shape. The tissue section showed prominent the early spermatogenic which were contact with germinal epithelial, blood congestion with distraction in wall of seminiferous tubule also noted abnormal basement membrane and aggregation of inflammatory cells .(Fig4.30).

This result may be to the effect of estrogenic substances in the environment of commercial fish or by effect of increasing of vitellogenin level in the blood of this fish. This result agreed with [139] which present decreased intensity in the mature

free spermatozoe in the lobular lumen in testes of carps exposed to BPA. This reflected a delayed maturation as more number of spermatogonia and spermatocytes were observed in the lobules.

The current result showed clear blood congestion in the space that located between the seminiferous tubules. The tissue section of seminiferous tubules showed distraction of germinal epithelia, aggregation of inflammatory cells and progressive Leydig cells, so this result showed high proliferation of early sepermetogems calls in the germinal epithelial the all tissue section of seminiferous tubules have prominent basement membrane(Fig4.31).

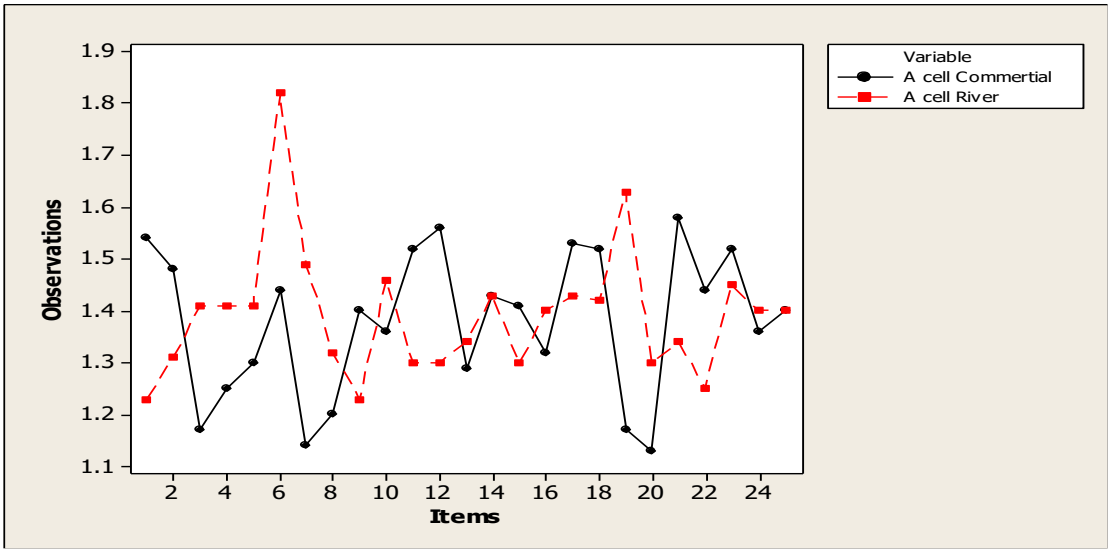
The histological finding of testis showed blood congestion in different location of test and aggregation of inflammatory. The present result showed aggregation of spermatogenic cells in the spaces between seminiferous tubules because of destruction the wall of seminiferous tubules which lead to aggregation of spermatogenic cells in the spaces between the seminiferous tubules (Fig4.32). This result constant with [139] that showed there was increased of interstitial spaces in the testis and a loss of cellular organization in the fish which exposed to estrogenic compound.

All tissue section of seminiferous tubules didn't appear the normal bounders of sertoli cells in side of seminiferous tubule, so the most seminiferous tubules were empty from spermatogenic cells in different stage of development.

Statistical analysis:

Figure(4-22) explains the diameter of A- spermatogonia in commercial fish compared with the diameter of A- spermatogonia in river fish.

Table(4) shows that non- significant differences($P > 0.05$) between diameter of A-spermatogonia of commercial which have (1.378 ± 0.028) and river fish which have (1.391 ± 0.025).



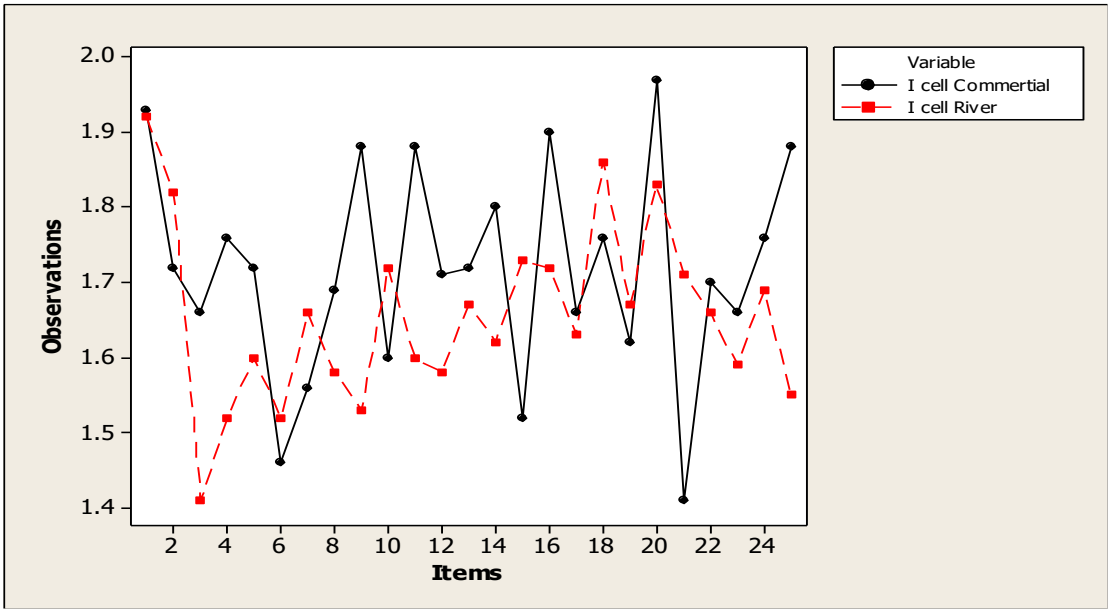
(Fig 4.22) Diagram showed diameter of A-spermatogonia in commercial and river fish.

Table 4 Diameter of A-spermatogonia(µm) in commercial and river fish

Two-sample T for A cell Commercial vs A cell River				
Group	N	Mean	StDev	SE Mean
Commercial	25	1.378	0.141	0.028
River	25	1.391	0.127	0.025
Difference = µm (A cell Commercial) - um (A cell River)				
Estimate for difference: -0.0128				
95% CI for difference: (-0.0891; 0.0635)				
T-Test of difference = 0 (vs not =): T-Value = -0.34 P-Value = 0.737 DF = 47				

Figure(4-23) shows the diameter of I- spermatogonia of commercial fish compared with the diameter of I- spermatogonia in river fish.

Table(5) explains that non-significant differences(P > 0.05) between diameter of I-spermatogonia of commercial(1.717±0.029) and river fish(1.656 ±.0.024).



(Fig 4.23) Diagram showed diameter of I-spermatogonia in commercial and river fish.

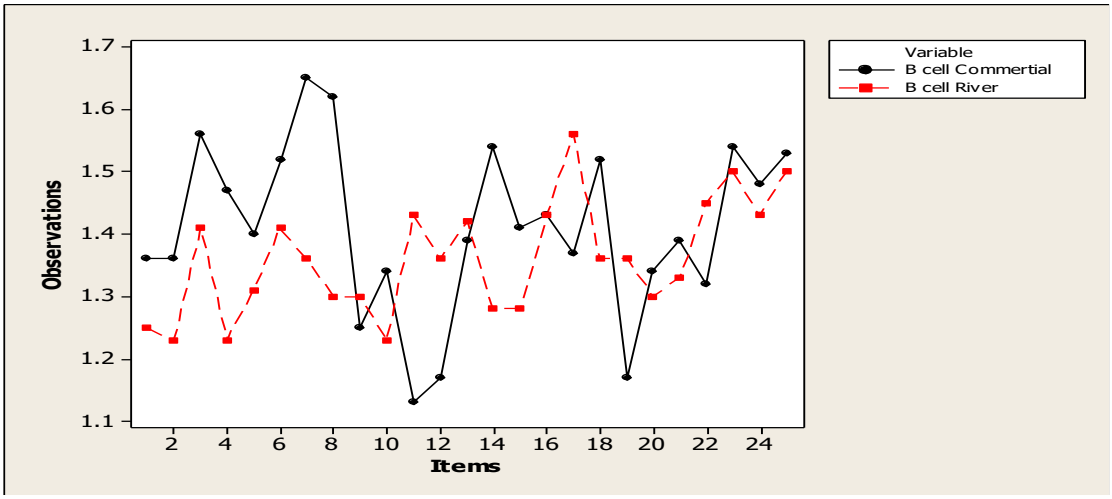
Table 5 Diameter of I-spermatogonia(µm) in commercial and river fish

Two-sample T for I cell Commercial vs I cell River				
Group	N	Mean	StDev	SE Mean
Commercial	25	1.717	0.143	0.029
River	25	1.656	0.118	0.024

Difference = µm (I cell Commercial) - um (I cell River)
Estimate for difference: 0.0616
95% CI for difference: (-0.0132; 0.1364)
T-Test of difference = 0 (vs not =): T-Value = 1.66 P-Value = 0.104 DF = 46

Figure(4-24) explains the diameter of B- spermatogonia of commercial fish compared with river fish.

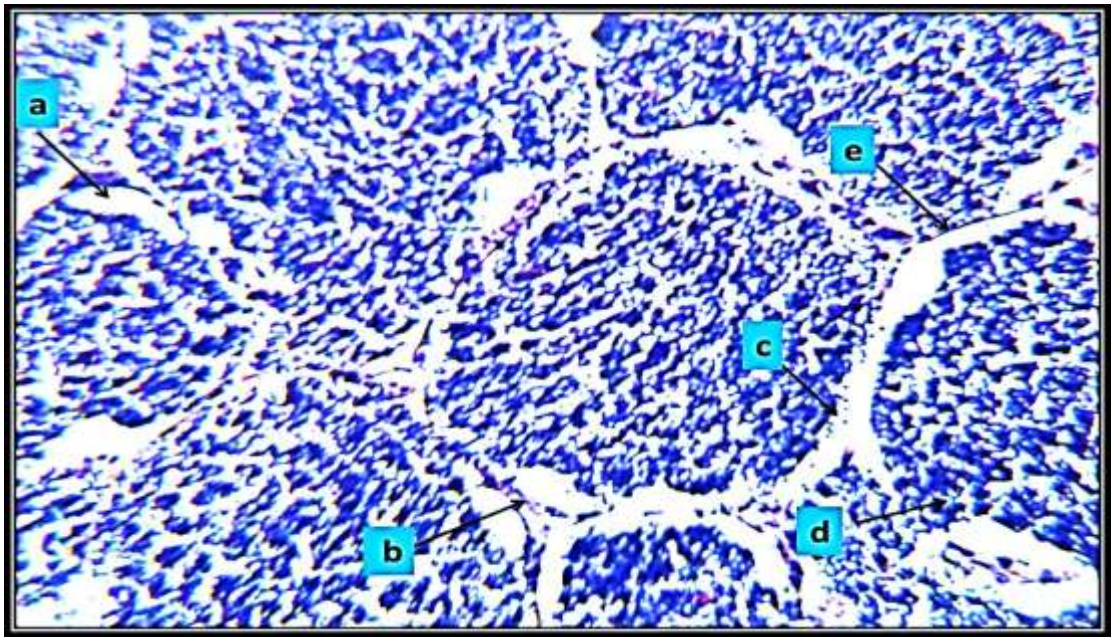
Table(6) shows that non- significant differences(P > 0.05) between diameter of B-spermatogonia of commercial was(1.410± 0.027) and river fish was (1.3608 ±0.018).



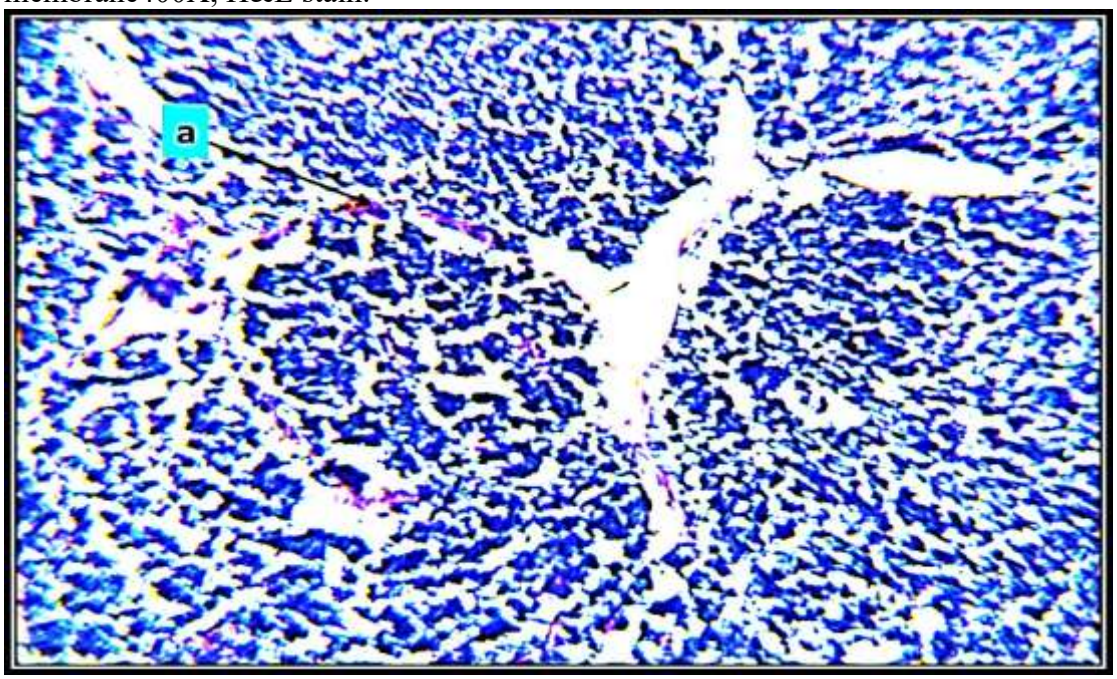
(Fig.4-24) Diagram showed diameter of B-spermatogonia in commercial and river fish.

Table 6 Diameter of B-spermatogonia(µm) in commercial and river fish

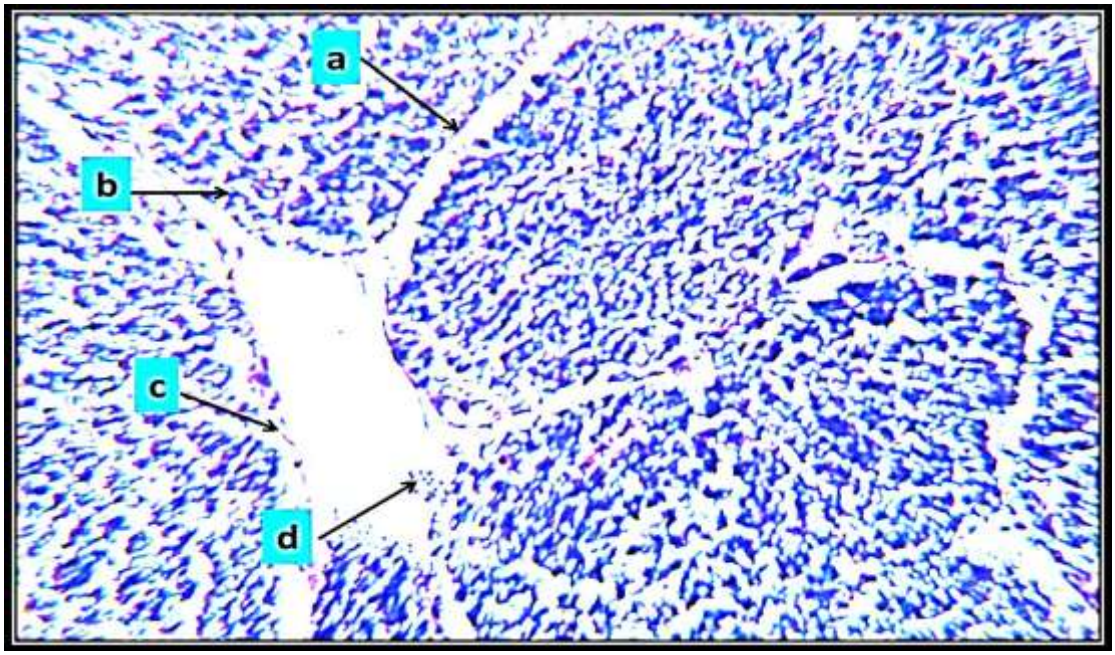
Two-sample T for B cell Commercial vs B cell River				
Group	N	Mean	StDev	SE Mean
Commercial	25	1.410	0.137	0.027
River	25	1.3608	0.0914	0.018
Difference = µm (B cell Commercial) - um (B cell River)				
Estimate for difference: 0.0496				
95% CI for difference: (-0.0169; 0.1161)				
T-Test of difference = 0 (vs not =): T-Value = 1.51 P-Value = 0.140 DF = 41				



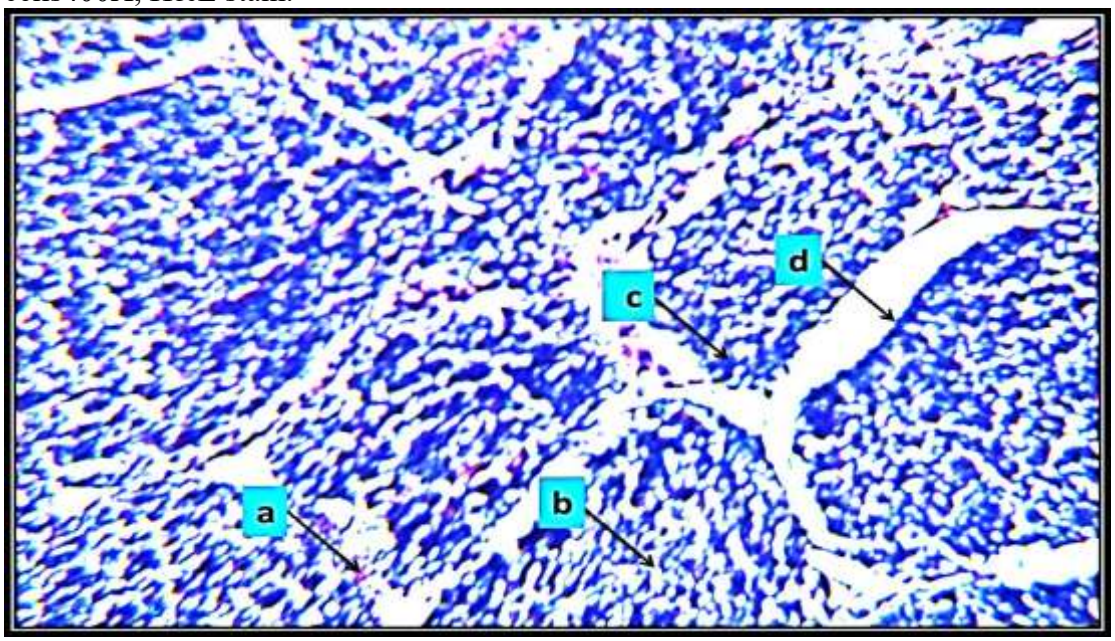
(Fig.4.25): Cross section through testis in river fish which showed a- wide space, b- germinal epithelia, c-Leydig cells, d- spermatogonia cells, e-basement membrane400X, H&E stain.



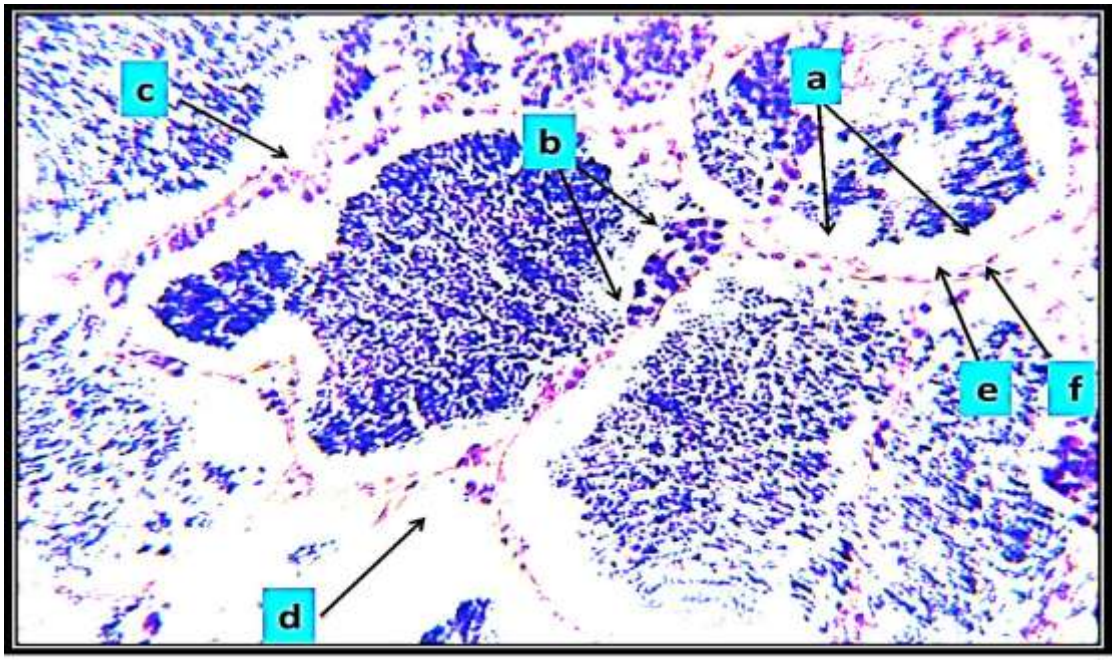
(Fig.4.26): Cross section through testis in river fish which showed a- germinal epithelia 400X, H&E stain.



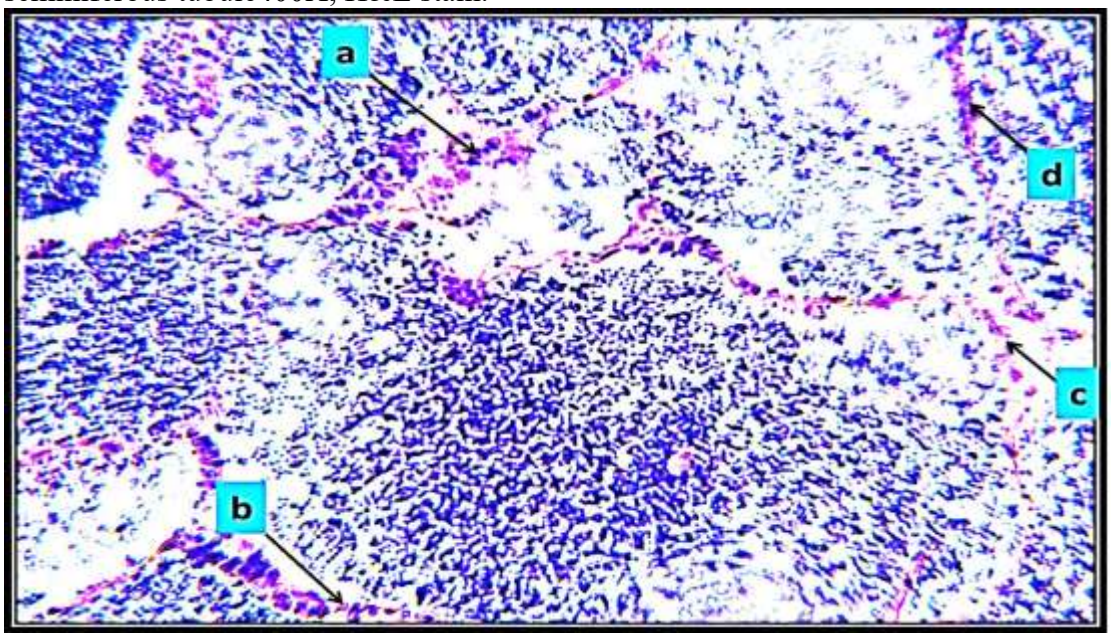
(Fig.4.27): Cross section through testis in river fish which showed a-prominent basement membrane, b- spermatogonia cells, c- germinal epithelia, d- Leydig cells400X, H&E stain.



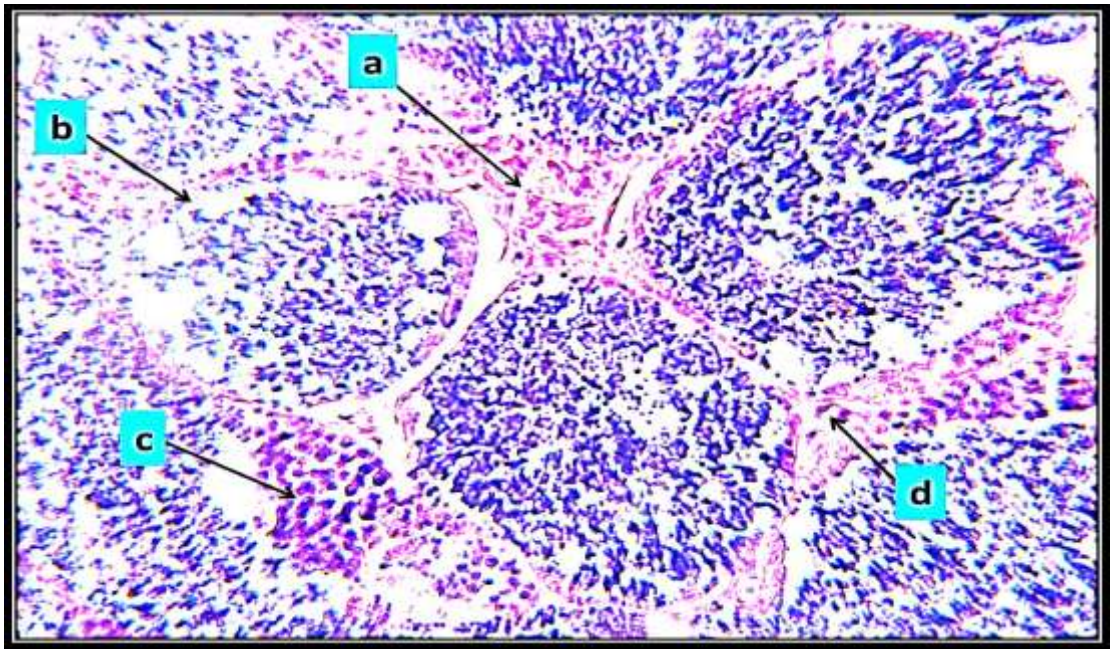
(Fig.4.28): Cross section through testis in river fish which showed a- Leydig cells, b-different stage of spermatogonia cells, c- spermatogonia cells, d- basement membrane400X, H&E stain.



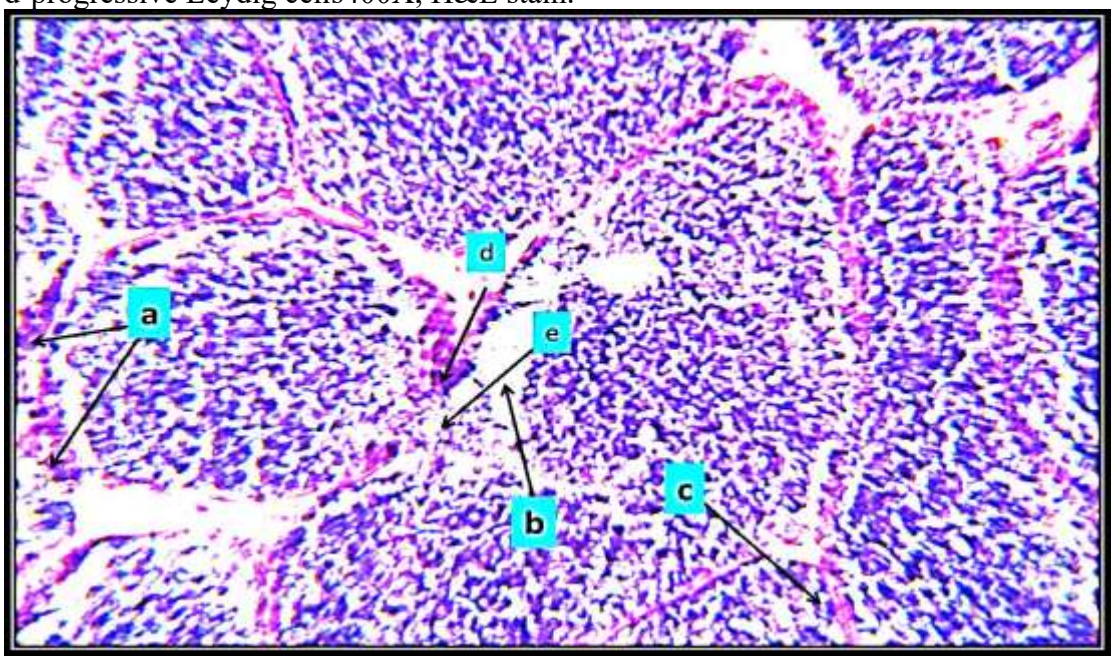
(Fig.4.29): Cross section through testis in commercial fish which showed a-wide space intermediate seminiferous tubule, b- Primary spermatogonia, c- destruction of seminiferous tubule wall, d- Leydig cells, e-exfoliated germinal epithelia, f-wall of seminiferous tubule400X, H&E stain.



(Fig.4.30): Cross section through testis in commercial fish which showed a- blood congestion, b-distraction in wall of seminiferous tubule, c-abnormal basement membrane, d-aggregation of inflammatory cells400X, H&E stain.



(Fig.4.31): Cross section through testis in commercial fish which showed a- blood congestion, b-distraction of germinal epithelia, c- aggregation of inflammatory cells, d-progressive Leydig cells400X, H&E stain.



(Fig.4.32): Cross section through testis in commercial fish which showed a- aggregation of inflammatory cells, b- distraction of spermatogonia cells, c- blood congestion, d-destruction the wall of seminiferous tubules, e-aggregation of spermatogenic 400X, H&E stain.

4.2 The Physiological Result:

4.2.1 The Physiological Results of Vitellogenin

Figure(4-33) shows the physiological results of vitellogenin in the blood of commercial male carp fish compared with the river fish. Table(7) appears that the level of vitellogenin in commercial fish have significant differences($P<0.05$) increased with(0.653 ± 0.026) compared with level of vitellogenin in the river fish which have (0.0403 ± 0.0027). There were significant differences in the levels of vitellogenin between the commercial and river fish.

This results may be to the exposure to the estrogenic substance in the environment or the diet of the commercial carp fish.This results agreed with [22,141] which present high level of plasma Vitellogenin in males and immature females that exposure to soybean phytoestrogens and the highly potent of EE2 in inducing VTG synthesis in various fish species.

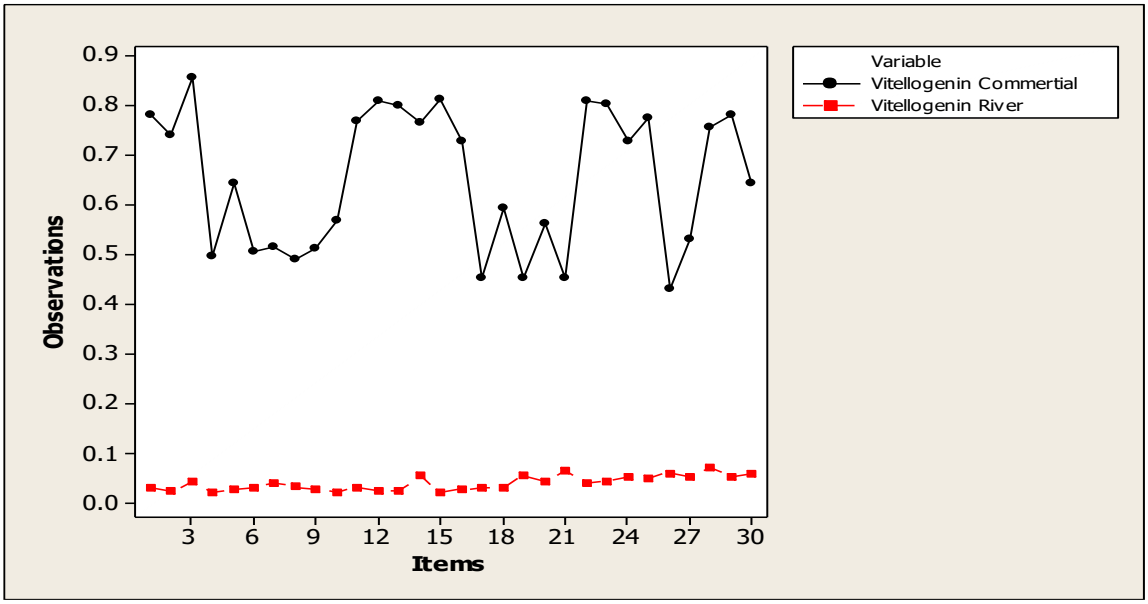


Fig 4.33Digram showed concentration of vitellogenin in commercial and river carp fish.

Table 7 Concentration of vitellogenin (ng/ml) in commercial and river carp fish

Two-sample T for Basin fish Commercial vs Basin fish River				
Group	N	Mean	StDev	SE Mean
Commercial	30	0.653	0.141	0.026
River	30	0.0403	0.0146	0.0027
Difference = mu (Vitellogenin Commertial) - mu (Vitellogenin River) Estimate for difference: 0.6130 95% CI for difference: (0.5601; 0.6658) T-Test of difference = 0 (vs not =): T-Value = 23.71 P-Value = 0.000 DF = 29				

4.2.2 The Physiological Results of Testosterone

Figure(4-34) shows the level of testosterone hormone in the commercial fish compared with the levels of testosterone hormone in the river fish. Table(8) explains there is significant differences($P<0.05$) increased between the levels of testosterone

hormone in commercial fish (0.3205 ± 0.022)(ng/ml) and in river fish was(0.560 ± 0.028).

This result may be because of the destruction of leydeg cells which responsible for the secretion of testosterone due to effect of endocrine disruptors chemicals and increasing of vitellogenin levels in commercial fish. This results constant with [142]that appeared reducing circulating T in male fish caught in the effluent channel and serum T concentrations which reducing by Phytoestrogens such as 3-sitosterol.

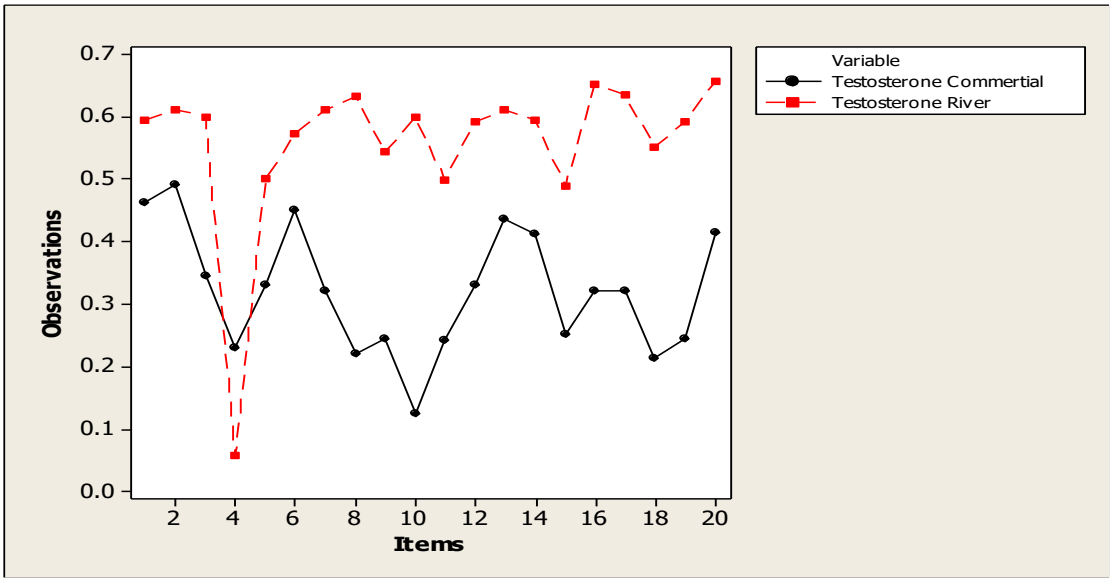


Fig 4.34 Diagram showed concentration of testosterone(ng/ml) in commercial and river carp fish.

Table 8 Concentration of testosterone (ng/ml) in commercial and river carp fish

Two-sample T for Testosterone Commercial vs Testosterone River				
Group	N	Mean	StDev	SE Mean
Commercial	20	0.3205	0.0994	0.022
River	20	0.560	0.127	0.028
Difference = mu (Testosterone Commercial) - mu (Testosterone River) Estimate for difference: -0.2390 95% CI for difference: (-0.3122; -0.1658) T-Test of difference = 0 (vs not =): T-Value = -6.63 P-Value = 0.000 DF = 35				

4.2.3 The Physiological Results of ALT and AST

Figure (4-35) shows the level of GPT in the river compared the level of GPT in commercial fish.

Table (9) explains there are significant differences($P<0.05$) increased between the level of ALT in both groups. The present results appears significant increased in

the level of ALT in the blood of commercial fish with (24.86 ± 0.36) U/L compared with river fish (15.98 ± 0.62) U/L this increasing may be to the increasing of vitellogenin and exposure to the endocrine disrupting chemicals.

Figure(4-36) shows the levels of AST of commercial fish compared with river fish. Table(10) explains there are significant differences($P<0.05$) increased in the levels of AST of commercial fish with (69.55 ±1.0) U/L compared with river fish was (6.86 ±1.0) U/L, this differences of the levels of AST between the two groups. This result may be due to the hepatic damage and the destruction of hepatocytes which caused occurrence of high levels of these enzymes, which may be to the effect of the increasing of vitellogenin and exposure to the endocrine disrupting chemicals.

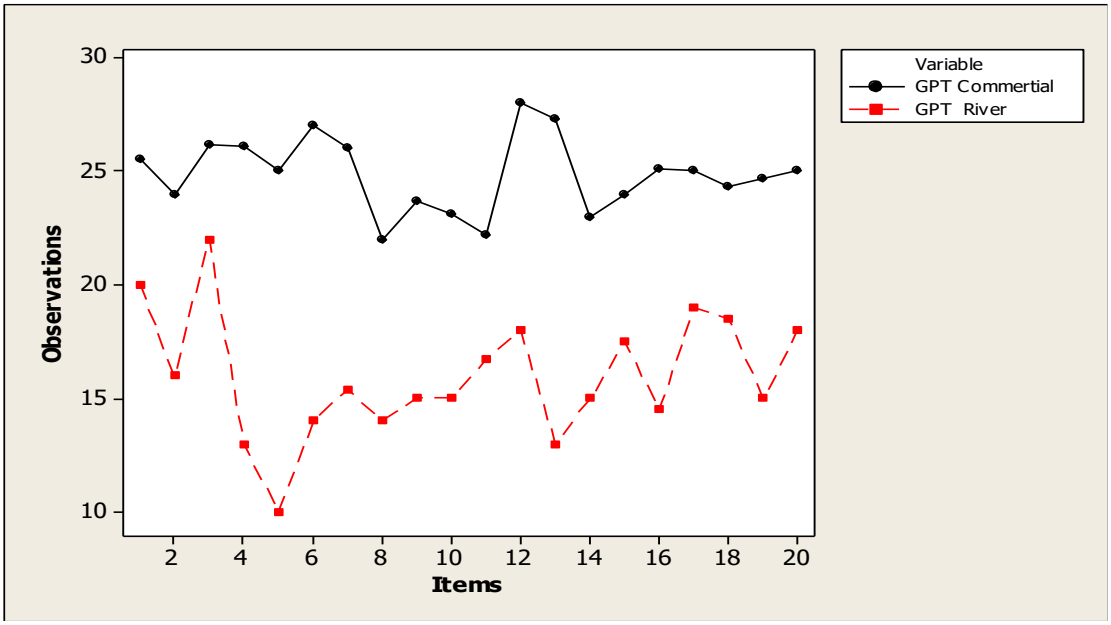


Fig. 4.35 Diagram showed concentration of ALT (GTP) (U/L) in commercial and river carp fish.

Table 9 Concentration of ALT (U/L) in commercial and river carp fish.

Two-sample T for ALT Commercial vs ALT River				
Group	N	Mean	StDev	SE Mean
Commercial	20	24.86	1.63	0.36
River	20	15.98	2.78	0.62
Difference = mu (ALT Commercial) - mu (ALT River)				
Estimate for difference: 8.881				
95% CI for difference: (7.410; 10.351)				
T-Test of difference = 0 (vs not =): T-Value = 12.33 P-Value = 0.000 DF = 30				

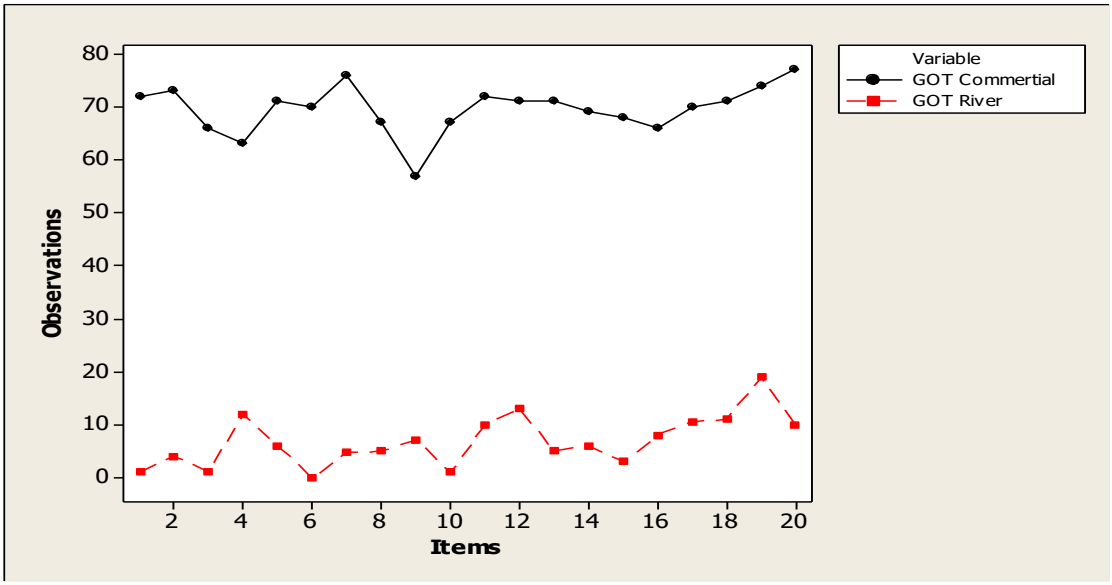


Fig 4.36 Diagram showed concentration of AST(GOT) (U/L) in commercial and river carp fish.

Table 10 Concentration of AST (U/L) in commercial and river carp fish

Two-sample T for AST Commercial vs AST River				
Group	N	Mean	StDev	SE Mean
Commercial	20	69.55	4.54	1.0
River	20	6.86	4.86	1.0
Difference = mu (AST Commercial) - mu (AST River) Estimate for difference: 62.69 95% CI for difference: (59.68; 65.71) T-Test of difference = 0 (vs not =): T-Value = 42.16 P-Value = 0.000 DF = 37				

4.2.4 The Physiological Results of Urea

Figure (4-37) explains the level of urea in blood of commercial and river fish. Table(11) shows there are significant differences($P<0.05$) increased in the levels of the level of urea in commercial fish was (13.285 ± 0.19) mg/dL compared with level of urea in the river fish which was (8.96 ± 0.32) mg/dL, this result may be to the reduction of glomerular filtration rate and renal injuries that may be caused by increasing of vitellogenin and exposure to the endocrine disrupting chemicals.

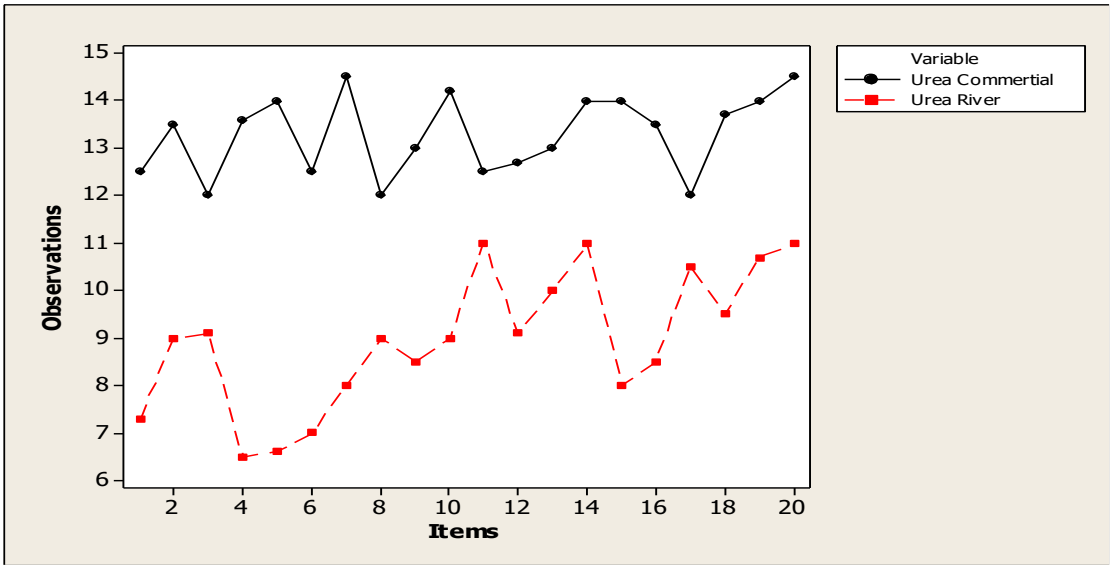


Fig 4.37 Diagram showed concentration of Urea(mg/dL) in commercial and river carp fish.

Table 11 Concentration of Urea (mg/dL) in commercial and river carp fish

Two-sample T for Urea Commercial vs Urea River				
Group	N	Mean	StDev	SE Mean
Commercial	20	13.285	0.843	0.19
River	20	8.96	1.45	0.32
Difference = mu (Urea Commercial) - mu (Urea River)				
Estimate for difference: 4.320				
95% CI for difference: (3.555; 5.085)				
T-Test of difference = 0 (vs not =): T-Value = 11.54 P-Value = 0.000 DF = 30				

4.2.5 The Physiological Results of FSH

Figure (4-38) shows the level of FSH in commercial fish compared with the level of FSH in the river fish. Table(12) explains there are significant differences($P<0.05$) increased in the level of FSH in commercial fish was(7.940 ±0.069) (ng/ml) compared with the level of FSH in the river fish which was(0.5910 ± 0.012) (ng/ml).This increasing in the level of FSH may be to the increasing of vitellogenin and exposure to the endocrine disrupting chemicals

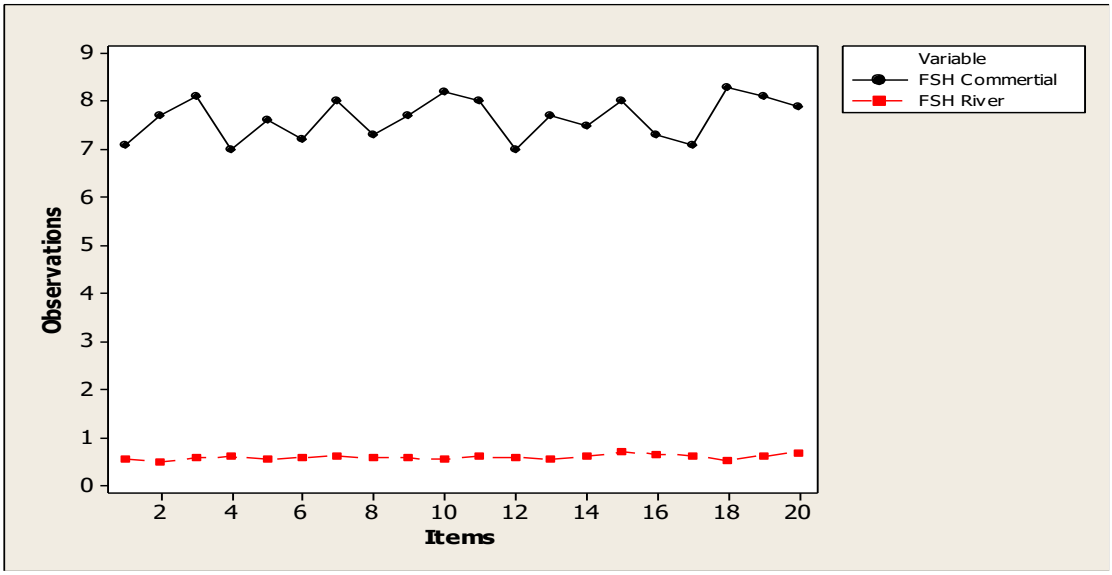


Fig 4.38 Digram showed concentration of FSH (ng/ml) in commercial and river carp fish.

Table 12 Concentration of FSH (ng/ml) in commercial and river carp fish

Two-sample T for **FSH** Commercial vs **FSH** River				
Group	N	Mean	StDev	SE Mean
Commercial	20	7.640	0.428	0.096
River	20	0.5910	0.0516	0.012
Difference = mu (FSH Commercial) - mu (FSH River) Estimate for difference: 7.0490 95% CI for difference: (6.8470; 7.2510) T-Test of difference = 0 (vs not =): T-Value = 73.05 P-Value = 0.000 DF = 19				

4.2.6 The Physiological Results of LH

Figure (4-39) shows the level of LH in commercial fish compared with the level of LH in the river fish. Table(13) explains there are significant differences($P<0.05$) increased in the levels of LH between commercial fish which have(3.350 ± 0.18) (ng/ml) and river fish was(0.7150 ± 0.014) (ng/ml) This increasing in the level of LH may be to the increasing of vitellogenin and exposure to the endocrine disrupting chemicals.

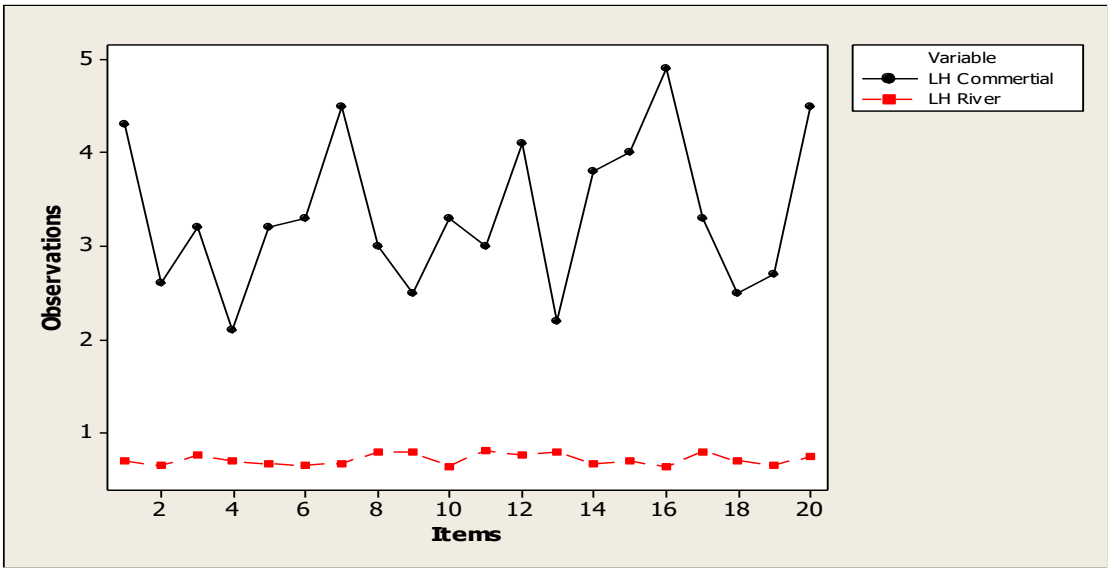


Fig 4.39 Diagram showed concentration of LH(ng/ml) in commercial and river carp fish.

Table 13 Concentration of LH(ng/ml) in commercial and river carp fish

Two-sample T for **LH Commercial** vs **LH** River				
Group	N	Mean	StDev	SE Mean
Commercial	20	3.350	0.819	0.18
River	20	0.7150	0.0611	0.014
Difference = mu (LH Commercial) - mu (LH River)				
Estimate for difference: 2.635				
95% CI for difference: (2.251; 3.019)				
T-Test of difference = 0 (vs not =): T-Value = 14.35 P-Value = 0.000 DF = 19				

4.2.7 The Physiological Results of Estrogen

Figure (4-40) shows the level of estrogen in the blood of commercial and river fish. Table (14) explains there are significant differences($P<0.05$) increased in the level of estrogen in the commercial fish was (292.4±5.6) (pg/mI) and the level of river fish was (194.6 ± 2.8) (pg/mI).These results showed there were significant differences between the commercial and river fish in the level of estrogen. These results may be to the exposure of male carp fish in commercial basin to estrogenic substance. These results agreed with [142] appeared that serum estrogen increased in Male fish collected in the effluent stream of the sewage treatment plant and from the Minnesota River exhibited.

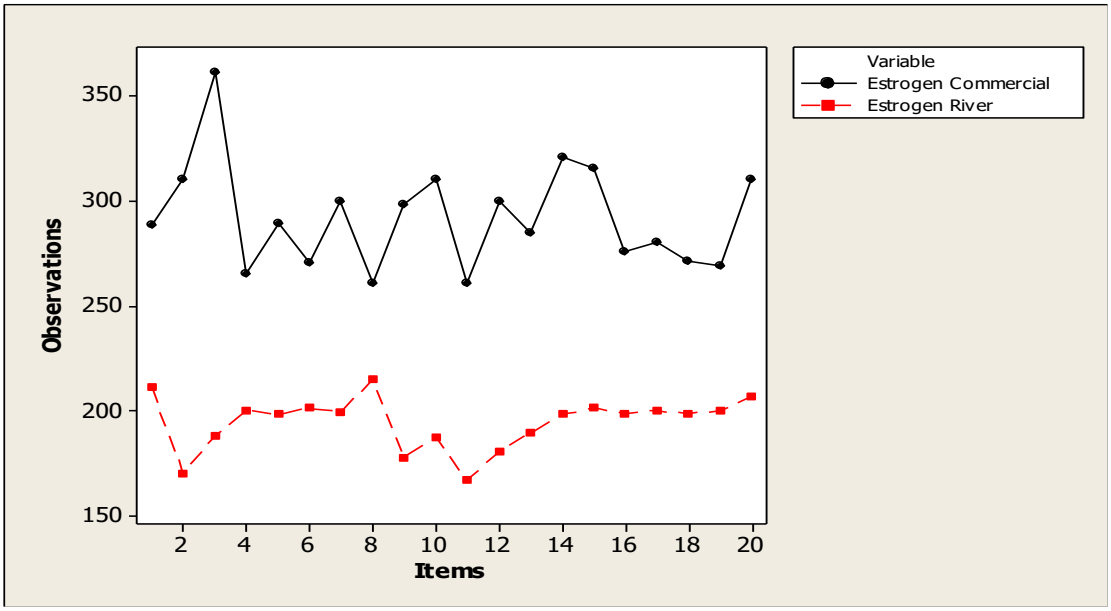


Fig 4.40 Concentration of Estrogen(pg/ml) in commercial and river carp fish.

Table 14 showing concentration of Estrogen(pg/ml) in commercial and river carp fish(pg/ml)

Two-sample T for Estrogen Commercial vs Estrogen River				
Group	N	Mean	StDev	SE Mean
Commercial	20	292.4	25.1	5.6
River	20	194.6	12.7	2.8
Difference = mu (Estrogen Commercial) - mu (Estrogen River) Estimate for difference: 97.79 95% CI for difference: (84.90; 110.68) T-Test of difference = 0 (vs not =): T-Value = 15.54 P-Value = 0.000 DF = 28				

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