E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2023; 11(3): 94-102

www.fisheriesjournal.com
Received: 15-02-2023
Accepted: 22-03-2023

Abiha Tarique
Department of Zoology, Isabella Thoburn College, Lucknow, Uttar Pradesh, India

Zainab khatoon
Department of Zoology, Isabella Thoburn College, Lucknow, Uttar Pradesh, India

Sunil P Trivedi
Department of Zoology, University of Lucknow, Lucknow, Uttar Pradesh, India

Vivek Kumar
Department of Zoology, Isabella Thoburn College, Lucknow, Uttar Pradesh, India

Corresponding Author:
Vivek Kumar
Department of Zoology, Isabella Thoburn College, Lucknow, Uttar Pradesh, India

Effect of synthetic hormone, 17α-ethinylestradiol on hematological and biochemical parameters in fish *Channa punctatus* (Bloch.1793)

Abiha Tarique, Zainab khatoon, Sunil P Trivedi and Vivek Kumar

DOI: https://doi.org/10.22271/fish.2023.v11.i3b.2814

Abstract
Environmental Protection includes programs that are aimed to reducing risks to the environment from hazardous materials and chemical wastes. Nowadays, 17α-ethinylestradiol, a synthetic estrogen, has become one of the most commonly used active ingredients for oral contraception, interfere with the normal functioning of endocrine systems, thus affecting reproduction and development in wildlife. The fresh water food fish, *Channa punctatus*, was selected as the test animal to perform the desired experimental work. The physio-chemical parameters (Temperature, pH, DO, CO_2, and Hardness) of test medium, hematological (WBC, RBC, Hb, PCV, MCV, MCH, and Platelet) and biochemical (Total protein, AST, ALT, ALP, Urea and Creatinine) parameters of the fish were analysed. *Channa punctatus* was exposed to three different concentrations of 17α-ethinylestradiol (5, 10 and 20 ng/L), along with a control group for 28 days and their samples were collected at an interval of 7, 14, 21 and 28 days. Result shows that hematological parameters- RBC, Hb, PCV, MCV, MCH and Platelet were found significantly decreased and WBC significantly increased and the biochemical parameters- AST, ALT, ALP and Urea were found significantly increased while, Total protein and Creatinine showed insignificant differences on exposure to 17α-ethinylestradiol. Therefore, the study stated that 17α-ethinylestradiol produce an adverse effect in hematological and biochemical parameter which might affect normal physiology and immunity of fish.

Keywords: 17α-Ethinylestradiol, *Channa punctatus*, hematological parameters, biochemical parameters

1. Introduction
A group of physiologically active chemicals known as steroid hormones share a cyclopentan-o-perhydrophenanthrene ring. Natural steroids produced by the adrenal cortex, testis, ovary, and placenta in both humans and animals include progesterone, glucocorticoids, mineralocorticoids, androgens, and estrogens (Porcu *et al*., 2016) [2]. Estrogens, which mostly impact women and are made up of estradiol, estrone, and estriol, are crucial for preserving the health of the brain, breasts, and reproductive systems. The two main sex hormones used in fish aquaculture are androgens and estrogens, which may either be natural (Found in nature) or synthetic (produced in a lab). Additionally, it has been shown that synthetic estrogens, which are extensively used in contraceptive and other pharmaceutical purposes, infiltrate the aquatic environment via effluent discharges from sewage treatment facilities (Blackwell *et al*., 2014; Patel *et al*., 2019; Kiyama & Wada-Kiyama, 2015; Yu *et al*., 2019) [3-6]. These might change how hormone systems normally operate, which would have an impact on how animals reproduce and grow (U.S. Department of Labour, Maniquue, 1998). The primary estrogens and contraceptives that are dangerous to aquatic life are 17-estradiol (E2), estrone (E1), estriol (E3), 17α-ethynylestradiol (EE2), and mestranol (MeEE2) because of their propensity to alter the endocrine system. It is anticipated that the effects of EE2, which was created particularly to interfere with human reproduction through an estrogen receptor-mediated mode of action (MOA), would be similar to those shown in mammalian systems on aquatic species. According to a 2007 study by Notch *et al*., EE2 is a potent inducer of the growth of hepatic tumours and may have co-carcinogenic effects by hindering an organism's capacity to repair DNA adducts via NER mechanisms.

The main DNA repair route for eradicating various damages brought on by bulky adduct-forming mutagens is nucleotide excision repair (NER) (Notch *et al*., 2007) [9]. The quantitative study verified that male zebrafish exposed to 100 ng/L EE2 saw a considerable reduction in the transcript levels of multiple hepatic NER genes. Observable biochemical and histological abnormalities of the liver, gonads, and kidneys, adjustments to the reproductive process and development, and behavioural changes are only a few effects of this endocrine system destabilisation (da Cunha *et al*. 2016) [10].

Fish are particularly prone to physical and chemical changes, which could be seen in their blood components. Fish exposed to chemicals may have an increase or reduction in their hemological levels. The significant role that hemato-biochemistry plays in clinical disease is one of the reasons why fish physiological research in this field is promising. Hemato-biochemical analyses provide useful diagnostics to assess the health condition of species of cultivated fish (Fazio, 2019) [11]. In particular, blood indices are thought to be the most important physiological indicators for analysing fish stress reactions (Seibel *et al*., 2021) [12]. Understanding the hemato-biochemical alterations that occur in fish raised in intensive environments might enhance production, fish welfare, and the standard of aquaculture practises (Fazio, 2019; Uiuiu *et al*., 2021) [11, 13].

Fish hematological characteristics are crucial for determining the state of their health. However, the primary causes of the change in fish's hematological parameters include food composition, metabolic adaptability, and fluctuations in fish activity (Alhadi Ighwela *et al*., 2012) [14]. Hematological indices may tell you a lot about a fish's ability to transport oxygen, immune system, illness risk, stress level, nutritional state, and other factors. The levels of the red blood parameters (Ht, Hb, RBC, and MCV) might change during stress. Studies on fish blood parameters are crucial for determining characteristics related to their physiologic capability (Wells *et al*., 2005) [15], and they are also an essential tool for assessing their immune systems (Tavares-Dias & Moraes, 2007) [16].

Fish biochemical markers are vulnerable to possible accumulation-related negative consequences. The activity of several enzymes is thought to be sensitive biochemical markers that may be used to detect toxicants in water before they have a chance to harm fish (Gül *et al*., 2004) [17]. It is thought that measuring certain enzymes, such as lactate dehydrogenase (LDH) and aspartate alanine aminotransferase (AST, ALT), might help assess pollution levels during long-term exposure (Younis *et al*., 2012) [18].

Determining the degree to which drugs evaluated at ecologically relevant doses interact with the endocrine system and consequently have negative consequences is a key aim of endocrine disruption research. In this investigation, test fish were used to investigate how 17α-ethinylestradiol affected their health using biochemical and hematological markers.

2. Materials and Methods

2.1 Test Chemical

Compounds with a purity of >98% were employed to produce 17α-ethinylestradiol (EE2) (manufactured by SIGMA-ALDRICH, Co., 3050 Spruce Street, St. Louis, MO 63103, USA; 314-771-5765). On the basis of prior work by Kidd *et al*., 2007, test solutions were prepared by adding EE2 to ethanol (purity of 99.9%) to create defined stock solutions at each test concentration (0, 5, 10, and 20 ng/L).

2.2 Test specimen

A total of 120 healthy *Channa punctatus* fish, with a mean initial weight of 30±5 g and a length of 14±2 cm, were obtained from the local lentic aquatic habitat of Lucknow, India. They were randomly placed into four aquaria, each holding 100 litres of water, and allowed to acclimatise to the laboratory environment for fifteen days prior to the experiment.

2.3 A test-based setup

The experiment was set up at in department of Zoology, University of Lucknow, India. Four (4) groups were used in the experiment, with group 1 acting as the control and group 2, 3, and 4 being exposed to EE2 of 5, 10, and 20 ng/L, respectively. Samples were taken at intervals of 7, 14, 21, and 28 days during the course of the exposure, which lasted 28 days. After the required exposure period had elapsed, three fish from each group were sampled; they were then anaesthetized with 0.1% (v/w) diethyl ether and had blood drawn. With the help of a 21-gauge needle and a 2-ml disposable syringe, blood was drawn from the caudal peduncle. Heparinized tubes were used to collect the blood samples (Wintrobe, 1934) [20] (HO *et al*., 1947) [21]. Two drops of heparin were added to every 5 millilitres of blood (Hesser, 1960). The sample was then thoroughly and gently mixed.

2.4 Hematological Examination

2.4.1 *Total RBC and WBC count*

Using an updated Neubaur's hemocytometer, the total number of RBC, total red blood cells, and total white blood cells were counted (Shah & Altindag, 2004) [23].

2.4.2 Hematocrit and haemoglobin estimation

The cyanmethemoglobin method was used to measure haemoglobin using the haemoglobin test kit from DIAGNOVA, Ranbaxy, India. The methods developed by Blaxhall & Daisley, in 1973 [24] were used to calculate hematocrit.

2.4.3 Corpuscular values

A common formula was used to calculate the MCV, MCH, and MCHC.

MCV (fl) = Hematocrit/Red Blood Cell x 10;
MCH (pg) = Hemoglobin(g/dl)/ Red Blood Cell × 10

2.4.4 Platelets

Platelet tests can be ordered on their own, but they are usually done as part of a larger blood test called a complete blood count (CBC), which checks the number and size of WBC, RBC, and PLT. A vein was sampled for blood for both tests. A PLT test requires no fasting or additional preparation.

2.5 Test for Liver Function

The health of the liver of *Channa puntatus* was evaluated using the following liver function assays:

2.5.1 Estimation of total protein

The technique of Lowry *et al*. (1951) was used to estimate the total protein content. As a benchmark, bovine serum albumin was employed.

2.5.2 Determination of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline

Phosphatase (ALP)
Bergmeyer *et al*. (1986) [26] assessed the activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The ALT, AST, and ALP enzyme activities were measured in IU/L of serum.

2.6 Test for Kidney Function
To assess the renal health of *Channa puntatus*, the following tests of kidney function were performed:

2.6.1 Determination of Urea
Utilising the Nesselerization approach, which was discussed in Aitken *et al*. (2003) [27], urea was determined. In the presence of Fe3+ ions and thiosemicarbazide at 100 °C, urea contained in blood immediately interacts with diacetyl monoxide to generate a red-coloured salt complex in an acidic solution, which is detectable at blue-green wavelengths (520–540 nm).

2.6.2 Determination of Creatinine
The Jaffe spectrophotometric approach, which was reported in Aitken *et al*. (2003) [27], was used to measure creatinine. At a wavelength of 340 nm, serum creatinine was measured and expressed in mg dL^{-1}.

2.7 Statistical Analysis
The Duncan Multiple Range Test (DMRT) (Duncan, 1955) [28] was used to assess differences between individual means, and the data were analysed using SPSS 26 window version. Data gathered from the experiment were subjected to an analysis of variance (ANOVA) test. A difference was deemed significant at a ($P > 0.05$). The physiochemical parameters were examined in accordance with APHA *et al*.'s (2017) [1] research.

3. Results
3.1 Physiochemical Conditions
During this study, the following physico-chemical properties of the water in the aquariums that had been treated with 17α-ethinylestradiol were found with in limit and suitable for fish survival (APHA *et al*. 2017) [1]. Water temperature was 25.5 to 27.5 °C; dissolved oxygen was 5.97 to 6.98 mg/L; pH was 6.9 to 7.4; CO_2 was 23.6 to 29.4 mg/L; and hardness was 170 to 180 mg/L.

3.2 Hematological variables
The packed cell volume (PCV) values for groups 2, 3, and 4 were substantially ($P < 0.05$) decreased from group 1 (control), with values ranging from 6.2±0.15 to 2.1±0.14, according to the hematological results of this study. Haemoglobin estimation for the control, group 1, and group 2 showed a substantial decrease, with values ranging from 11.3±0.53 to 9.1±0.54. Red Blood Cell (RBC) levels in groups 2 and 3 indicated a significant difference ($P < 0.05$), with group 1 obtaining the highest values (2.23±0.16 to 2.23±0.15) and group 4 obtaining the lowest values (0.76±0.15 to 0.67±0.14).
White blood cells (WBC) in groups 2, 3, and 4 differed significantly ($P < 0.05$) from those in group 1 (the control). The highest value was achieved in group 4 (20 ng/L) following a 28-day exposure period, ranging from 60.40±1.5 to 71.20±1.4, and the lowest value was obtained in group 1 (30.90±1.5) which differed significantly ($P < 0.05$). Groups 2, 3, and 4 had significantly different platelet (PLT) contents from group 1 ($P < 0.05$). (control). Compared to other treatments, the blood platelets in the control experiment (Group 1) were higher. When compared to group 1, the mean corpuscular haemoglobin (MCH) of groups 2 and 3 significantly differs ($P < 0.05$) from the control. The MCH results from the other treatments differed significantly ($P < 0.05$) and ranged from 50.5±1.66 (group 1) to 39.8±1.56 to 36.4±1.45 (group 4), respectively. Group 1 had the highest mean corpuscular volume (MCV) (97.5±3.64), while group 4 had the lowest MCV (48.4±2.56 to 37.6±2.45). Table 1 shows the mean values recorded for each parameter.

3.3 Biochemical parameters
3.3.1 LFT: Liver Function Test
In order to assist the metabolism of xenobiotics and other macromolecules, the transaminases (AST and ALT) and ALP control physiological activities. As a result, changes in their activities serve as markers to show the integrity of these organs in organisms and enable direct detection of impairments to the functioning of the liver and kidneys. As a result, we also looked at the enzyme activity (IU/L) of ALT, AST, and ALP in serum samples from *Channa punctatus* fish exposed to malathion at 7, 14, 21, and 28 days. In fish serum exposed to EE2, it was shown that the transaminases' and the ALP enzymes' activity decreased with time. Additionally, the enzyme activity in the malathion-exposed fish serum decreased considerably ($P > 0.05$ DMRT) in the following order: AST > ALT > ALP compared to the controls. Compared to controls, the AST activities in fish serum are increased. Groups 2, 3, and 4's AST were substantially different from group 1's (the control group) ($P < 0.05$). Following a 7-day exposure period, the maximum value was obtained in group 2 (05 ng/L EE2), ranging from 394.78±1.82 to 266.01±1.79, while the lowest value was obtained in group 1 (211.75±1.85). The ALT also varied considerably ($P < 0.05$) from those in group 1 (the control) in groups 2, 3, and 4. Following a 7-day exposure period, group 2 acquired the highest value (05 ng/L), ranging from 34.47±1.78 to 17.93±1.73, while group 1 received the lowest value, 9.79±1.75. Similar to this, after a 28-day exposure period, ALP in group 2 (05 ng/L EE2) was measured at 9.80±0.38, the highest value, and at 3.02±0.36, the lowest. Following a 28-day exposure period, total protein activity levels in fish serum likewise increase by 4.74±0.24 in group 2 (05 ng/L EE2) compared to the control of 3.20±0.25. The lowest value was reached in group 3 by exposure, which was 2.74±0.24 compared to the unexposed (control) fish.

3.3.2 KFT: Kidney Function Test
After 7, 14, 21, and 28 days of EE2 exposure, the renal function test was performed on blood samples of *Channa punctatus* fish to ascertain the physiological harm done to the kidneys. After 7 days of exposure, there was a decrease in urea (mg/dL), from 15.27±0.72 (group 1) to 11.84±0.68 (group 4), which was the greatest, and an increase in creatinine (mg/dL), from 0.31±0.02 (group 1), which was the lowest, to 0.37±0.02 (group 4), which was the highest. Following a 14-day exposure, there was a significant ($P < 0.05$, DMRT) decrease in urea, from 15.35±0.75 (group 1) to 8.86 0.75, and although an increase in creatinine, from 0.36±0.04 (group 2) to 0.47±0.03 (group 4), which was insignificant compared to the controls, who were at 0.31±0.02 ($P < 0.05$, DMRT). Urea levels decreased from 8.03±0.68

(group 2) to 5.11±0.63 (group 4) during the exposure of 21 days, compared to 15.27±0.66 in the control group, but creatinine levels increased from 0.48±0.03 (group 2) to 0.53±0.04 (group 4). Nevertheless, after 28 days of exposure, their levels remained lowered, with urea levels decreased from 5.51±0.73 in group 2 to 4.35±0.79 in group 4 as compared to control values of 15.27±0.75 and creatinine from 0.57±0.05 in group 4 as compared to 0.31±0.04 in group 2.

4. Discussion

Toxins that affect how blood is formed can be tracked using hematological indicators in the blood. Fish exposed to 17α-ethinylestradiol showed a substantial decrease in their hematological markers ($P > 0.05$). Anemia and a decline in RBC values have been noted in fish like Salvalinus fontinalis (Holcombe *et al*., 1976) [29]. Even though there is enough oxygen in the water, fish will not be able to take in much of it because of their low erythrocyte counts. Fish will consequently get anoxia (a lack of oxygen). Fish with anaemia may have lower values for all red blood cell markers (Witeska *et al*., 2015) [30]. The condition of the fish has been evaluated using hematological parameters. Nonetheless, hematological indicators of the fish's nutritional condition and general health have been said to be reliable (Katalay & Parlak, 2004) [31], as they indicate the nutritional status and overall health of the fish (Akinrotimi *et al*., 2012) [32]. The fall in Ht and Hb of the fish after MT delivery may indicate that their condition has worsened as a result of the androgen therapy (AS & J, 2013) [33]. According to Joshi *et al*., 2002 [34], fish may be less able to increase their activity to fulfil sporadic needs if their haemoglobin levels are lower.

Granulocytes, monocytes, lymphocytes, and thrombocytes are the main types of white blood cells (WBCs), which have the main roles in immunological response, defence against infection, and defence against invading species. The differential WBC count in the current study showed a marginal increase in WBCs but a marked increase in neutrophils and a marked decrease in lymphocytes. This increase in WBCs in the circulatory system led to a higher survival rate for the experimental fish, and these findings are consistent with (Davis *et al*., 2008) [35] findings. The study's decreased hematological parameter values for the treated fish indicated that the treated fish's physiological functions were impacted.

The function of the liver was examined using the biomarkers AST, ALP, and ALT. According to Hadi *et al*., 2009 [36], those enzymes are plasma non-functional enzymes that are often found in the cells of the liver, heart, gills, kidneys, muscle, and other organs. According to Verma *et al*., 1981 [37], these enzymes are also thought to be significant in determining the health of the liver and several other organs. In this research, 17α-ethinylestradiol treatment in *Channa punctatus* led to a significant reduction in AST, ALT, and protein levels as well as an increase in ALP when compared to the control group. By regulating the transfer of amino group function from alpha-amino acids to alpha-keto acids, the ALT and AST enzymes are considered markers of liver health and function by Kumar Rupesh *et al*. (2011) [38] and Hassaan *et al*. (2014) [39]. Animal blood contains significant levels of ALT and AST, which are mostly generated when liver cells are damaged. The ALT and AST enzyme activities in the present research were considerably elevated by the EE2 therapy, notably in group 2 concentrations of 394.78±1.82 and 34.47±1.78, respectively. The movement of various substances, the body's defence against pathogenic agents, osmotic control, and other processes all depend on serum proteins (Rudneva & Kovyrshina, 2011) [40]. In many illness states, the ability for protein synthesis is reduced, which also lowers protein absorption or protein loss by haemodilution (Patriche *et al*., 2009; Patriche *et al*., 2011) [41, 45]. The highest observed total protein levels in the present investigation were 4.74±0.24 (group 2), and the lowest were 2.96±0.25 (group 4).

Creatinine and urea are nitrogenous metabolic byproducts. The main byproduct of dietary protein and tissue protein turnover is urea. According to Borges *et al*. (2005) [43], a high blood urea content is likely an indication of stress linked to a rise in cholesterol. A normal urea level is greater than 7.1 mg/dL or less than 1.8 mg/dL. An abnormal urea level indicates renal illness, gastrointestinal haemorrhage, and congestive heart failure. Utilising a variety of *in vivo* and *in vitro* techniques, urea and creatinine have been used as significant indicators for the assessment of chemical effects on the kidney (M. E. Davis & Berndt, 1994) [44]. Muscle creatine catabolism results in creatinine. A dramatic change in the normal creatinine level may signal a urinary tract blockage, kidney failure, dystrophy, decreased blood supply to the kidneys, or prerenal azotemia, among other disorders that affect the kidneys or muscles.

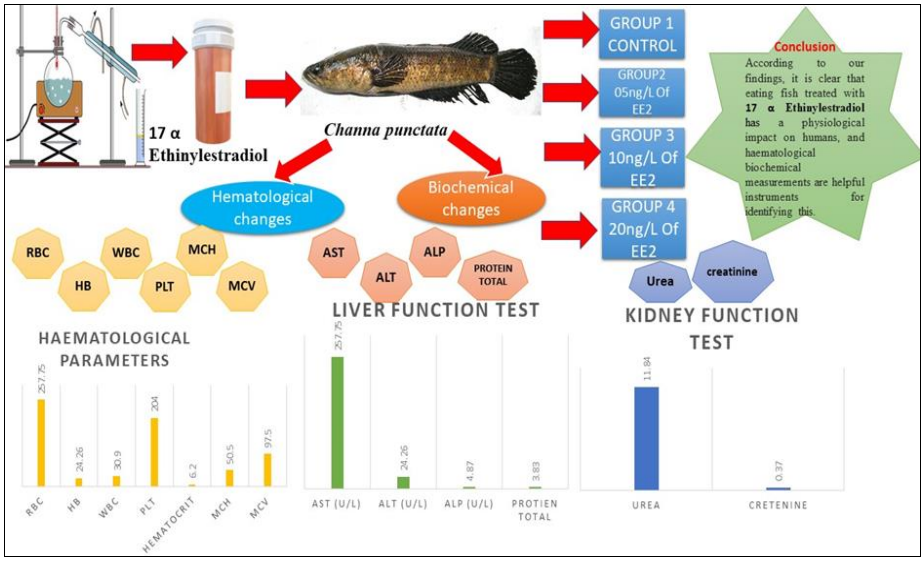
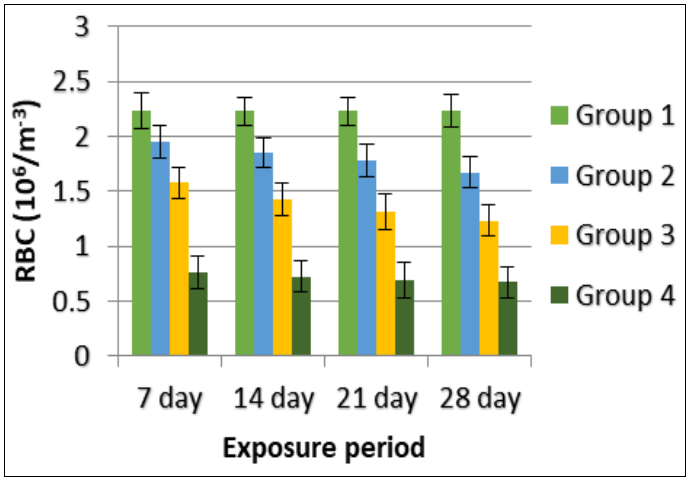
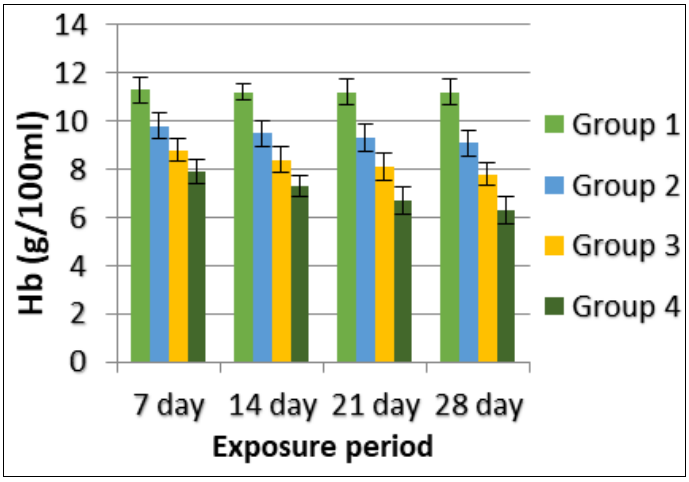
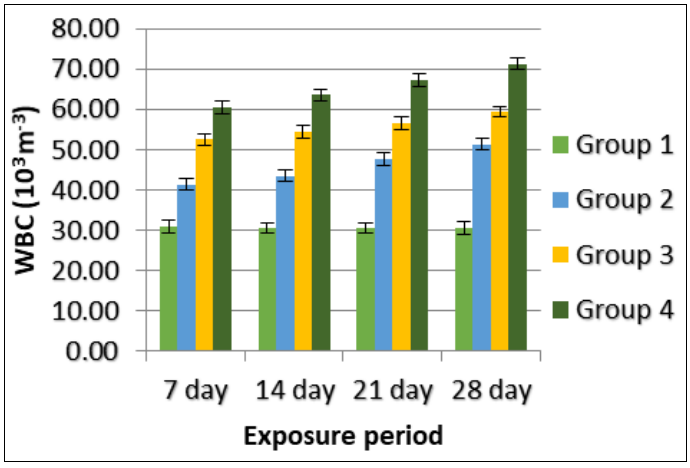


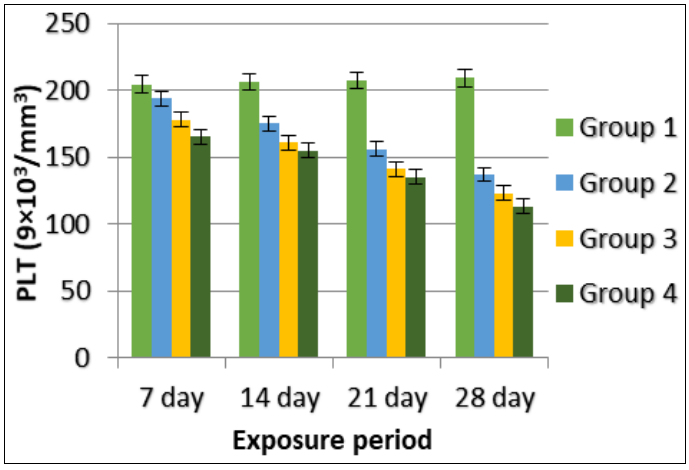
Table 1: Mean value of Hematological Parameters of *Channa punctata* exposed to 17α-ethinylestradiol for 28 days

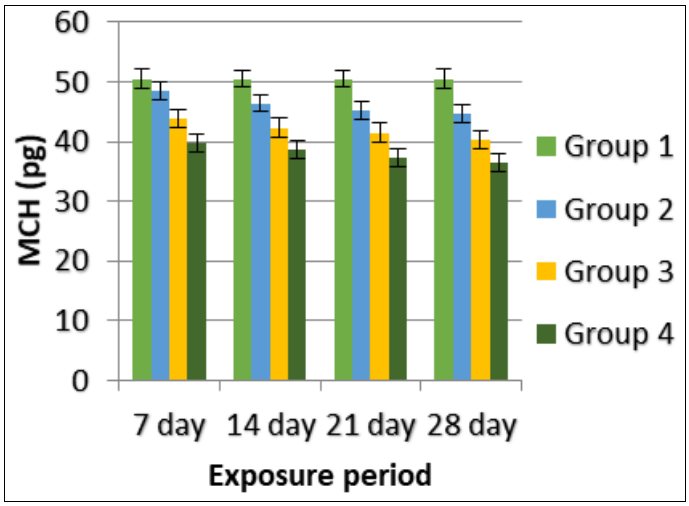
Parameters	Exposure period (days)	Group 1 Control	Group 2 5 ng/l	Group 3 10 ng/L	Group 4 20 ng/L
RBC ($10^6/m^{-3}$)	7 days	2.23±0.16	1.95±0.15	1.58±0.14	0.76±0.15
	14 days	2.23±0.13	1.85±0.14	1.43±0.15	0.72±0.14
	21 days	2.23±0.13	1.78±0.15	1.31±0.16	0.69±0.16
	28 days	2.23±0.15	1.67±0.14	1.23±0.14	0.67±0.14
Hb (g/100ml)	7 days	11.3±0.53	9.8±0.55	8.8±0.45	7.9±0.53
	14 days	11.2±0.33	9.5±0.54	8.4±0.53	7.3±0.45
	21 days	11.2±0.53	9.3±0.55	8.1±0.56	6.7±0.55
	28 days	11.2±0.54	9.1±0.54	7.8±0.45	6.3±0.54
WBC (10^3 m^{-3})	7 days	30.90±1.5	41.40±1.5	52.60±1.4	60.40±1.5
	14 days	30.60±1.3	43.40±1.4	54.40±1.5	63.60±1.4
	21 days	30.60±1.3	47.70±1.5	56.40±1.6	67.30±1.6
	28 days	30.60±1.5	51.30±1.4	59.40±1.4	71.20±1.4
PLT ($9\times10^3/mm^3$)	7 days	204±6.6	194±5.5	178±5.4	165±5.5
	14 days	206±6.3	175±5.4	161±5.5	155±5.4
	21 days	207±6.3	156±5.5	141±5.6	135±5.6
	28 days	209±6.5	137±5.4	123±5.4	113±5.4
Hematocrit (PCV) (%)	7 days	6.2±0.15	5.3±0.14	4.2±0.12	3.1±0.16
	14 days	6.1±0.13	5.1±0.14	3.9±0.15	2.8±0.14
	21 days	6.1±0.13	4.8±0.15	3.7±0.16	2.6±0.16
	28 days	6.1±0.15	4.6±0.14	3.1±0.14	2.1±0.14
MCH (pg)	7 days	50.5±1.66	48.5±1.55	43.8±1.45	39.8±1.56
	14 days	50.5±1.33	46.4±1.45	42.2±1.65	38.6±1.45
	21 days	50.5±1.33	45.2±1.56	41.5±1.66	37.2±1.56
	28 days	50.5±1.55	44.7±1.54	40.2±1.45	36.4±1.45
MCV (fl)	7 days	97.5±3.64	75.1±2.55	57.7±2.45	48.4±2.56
	14 days	97.5±3.33	71.3±2.45	53.8±2.55	42.3±2.45
	21 days	97.5±3.33	66.9±2.54	51.1±2.66	39.6±2.36
	28 days	97.5±3.55	64.2±2.54	48.2±2.54	37.6±2.45

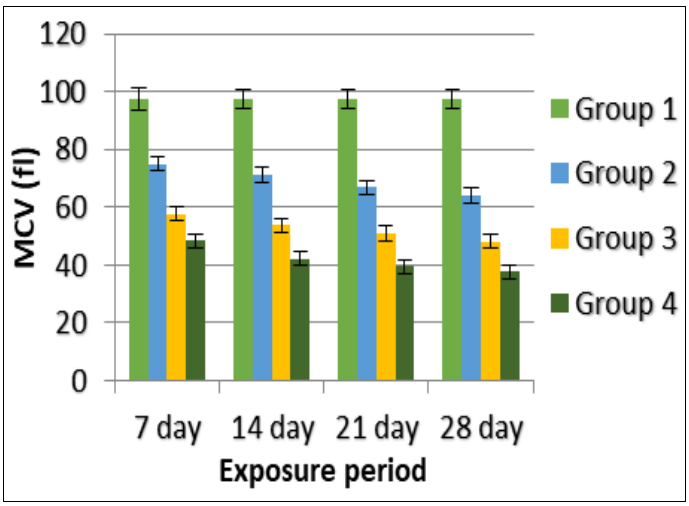


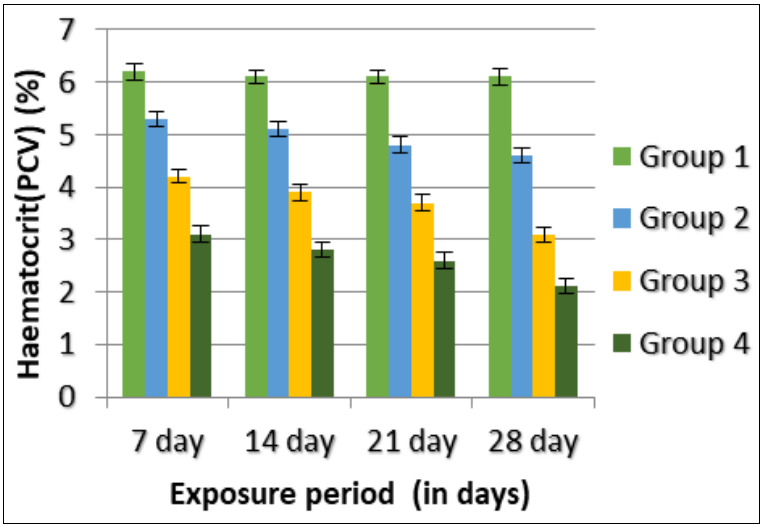








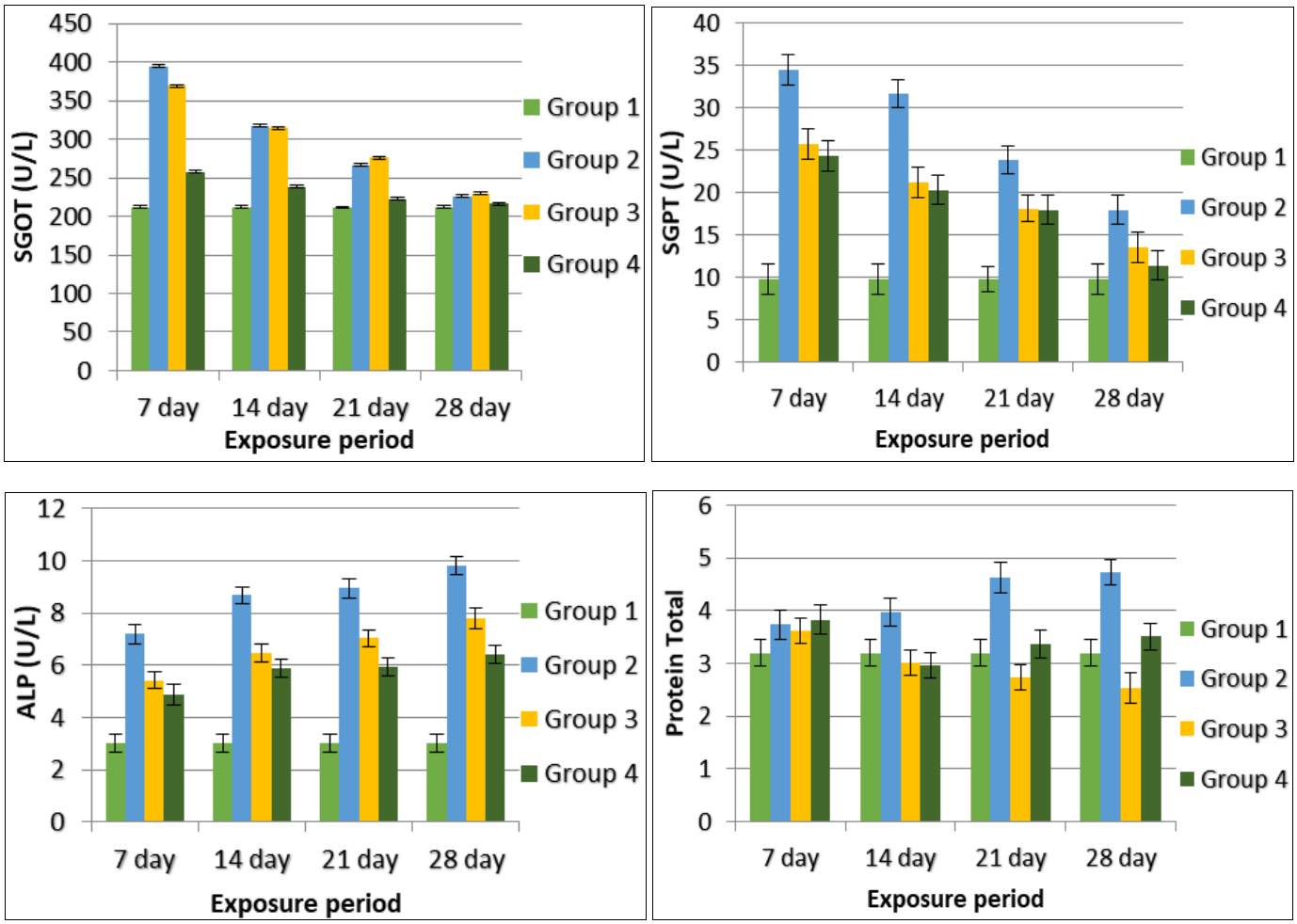




Graphs 1: Shows changes in Hematological Parameters of *Channa punctata* exposed to 17α-ethinylestradiol for 28 days

Table 2: Mean value of Biochemical Parameters (LFT) of *Channa punctata* exposed to 17α-ethinylestradiol for 28 days

Parameters	Exposure period (days)	Group 1 Control	Group 2 5 ng/l	Group 3 10 ng/L	Group 4 20 ng/L
AST (U/L)	7 days	211.75±1.85	394.78±1.82	369.25±1.82	257.75±1.78
	14 days	211.75±1.75	317.82±1.73	314.73±1.73	238.07±1.75
	21 days	211.75±1.46	266.31±1.63	276.15±1.63	222.26±1.73
	28 days	211.75±1.75	266.01±1.79	229.60±1.79	216.18±1.75
ALT (U/L)	7 days	9.79±1.75	34.47±1.78	25.66±1.82	24.26±1.78
	14 days	9.79±1.76	31.68±1.62	21.15±1.73	20.27±1.75
	21 days	9.79±1.46	23.81±1.58	18.11±1.63	17.9±1.73
	28 days	9.79±1.75	17.93±1.73	13.50±1.79	11.36±1.75
ALP (U/L)	7 days	3.02±0.36	7.19±0.38	5.42±0.32	4.87±0.38
	14 days	3.02±0.35	8.69±0.32	6.47±0.33	5.88±0.35
	21 days	3.02±0.36	8.94±0.33	7.03±0.33	5.94±0.33
	28 days	3.02±0.35	9.80±0.38	7.79±0.39	6.43±0.35
Total Proteins	7 days	3.20±0.25	3.74±0.28	3.61±0.24	3.83±028
	14 days	3.20±0.25	3.97±0.26	3.01±0.23	2.96±0.25
	21 days	3.20±0.26	4.63±0.28	2.74±0.24	3.37±0.27
	28 days	3.20±0.25	4.74±0.24	2.53±0.29	3.51±0.25



Graphs 2: Shows changes in Biochemical Parameters (LFT) of *Channa punctata* exposed to 17α-ethinylestradiol for 28 days

Table 3: Mean value of Biochemical Parameters (KFT) of *Channa punctata* exposed to 17α-ethinylestradiol for 28 days.

Parameters	Exposure period (days)	Group 1 Control	Group 2 5 ng/l	Group 3 10 ng/L	Group 4 20 ng/L
Urea	7 days	15.27±0.72	13.21±0.78	12.20±0.72	11.84±0.68
	14 days	15.35±0.75	11.43±0.72	10.23±0.73	8.86±0.75
	21 days	15.27±0.66	8.03±0.68	07.96±0.63	5.11±0.63
	28 days	15.27±0.75	5.51±0.73	04.04±0.79	4.35±0.79
Creatinine	7 days	0.31±0.02	0.31±0.03	0.35±0.02	0.37±0.02
	14 days	0.31±0.03	0.36±0.04	0.42±0.04	0.47±0.03
	21 days	0.31±0.04	0.39±0.03	0.48±0.03	0.53±0.04
	28 days	0.31±0.04	0.43±0.03	0.52±0.05	0.57±0.05

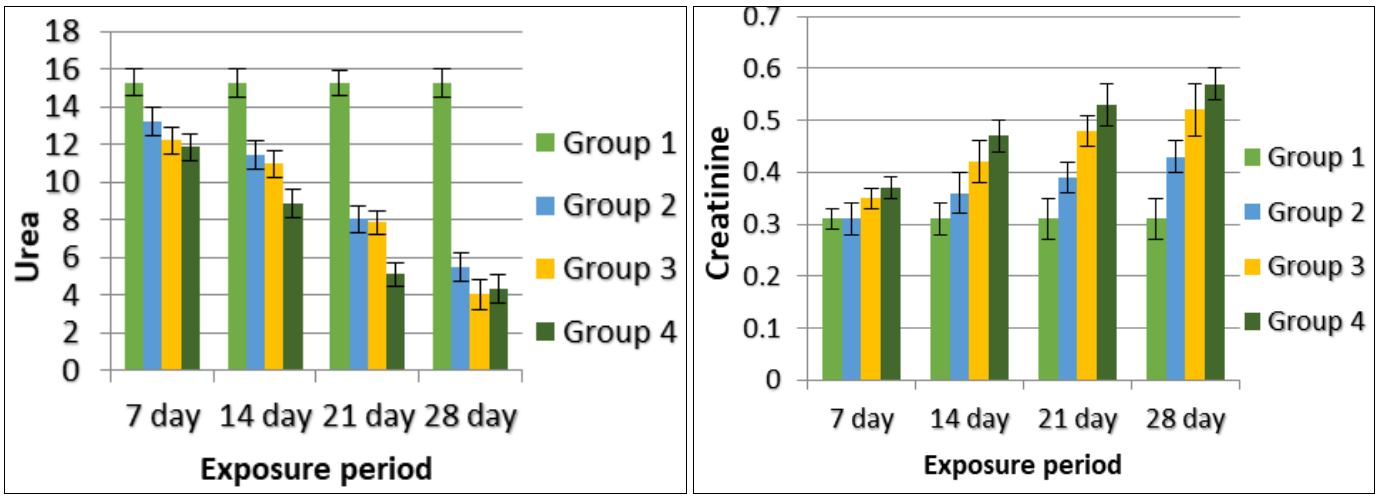


Fig 3: Graphs shows changes in Biochemical Parameters (KFT) of *Channa punctata* exposed to 17α-ethinylestradiol for 28 days

5. Conclusion

Chemicals used to improve commerce, agriculture, medicine, and even the most basic home comforts could have a negative effect on human and environmental health when they are made, used, and thrown away. Because using steroid hormones pollutes water supplies in a big way, it has become a controversial topic in pharmaceutical and personal care products (PPCPs), animal husbandry, and raising cattle. But studies have shown that the technology most wastewater treatment plants use to get rid of steroids and hormones is not good enough. As a result, these potentially dangerous substances may infiltrate groundwater and occasionally surface water. Our findings contribute to a better understanding of the physiochemical, hematological and biochemical characteristics of the fish *Channa punctatus*. Hematological and biochemical characteristics are acknowledged as a crucial tool for observing changes in water quality as well as fish health. According to our findings, it is clear that eating fish has a physiological impact on humans, and hematological and biochemical measurements are helpful instruments for identifying this.

6. Acknowledgement

The Authors are thankful to Department of Zoology, University of Lucknow, is to provide space and tools to perform experiment.

7. Authors' contributions

All writers contributed to the completion of this work. The experimental planning, statistical analysis of the data, graph production, and text writing were all done by author Vivek Kumar. The selection of test animals and the experiment's execution were handled by author Abiha Tarique. The work was edited in its entirety by author Zainab Khatoon. Sunil P. Trivedi, the author, oversaw the experiment and provided advice on article development. The final text was reviewed and approved by all writers.

8. Compliance with ethical standards

According to the guidelines set out by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the University of Lucknow in Lucknow established an Institutional Animal Ethics Committee (IAEC) with registration number 1861/GO/Re/S/16/CPCSEA. The authors carried out the experiment in accordance with the CPCSEA's specified procedures.

9. Reference

1. APHA, Standard Methods for the Examination of Water and Wastewater 23rd ed.). Washington DC: American Public Health Association; c2017.
2. Porcu P, Barron AM, Frye CA, *et al*. Neurosteroidogenesis Today: Novel Targets for Neuroactive Steroid Synthesis and Action and Their Relevance for Translational Research. J Neuroendocrinol. 2016;28(2):1-19. doi:10.1111/jne.12351
3. Blackwell BR, Brown TR, Broadway PR, *et al*. Characterization of trenbolone acetate and estradiol metabolite excretion profiles in implanted steers. Environ Toxicol Chem. 2014;33(12):2850-2858. doi:10.1002/etc.2757
4. Patel M, Kumar R, Kishor K, Mlsna T, Pittman CU, Mohan D. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. Chem Rev. 2019;119(6):3510-3673. doi:10.1021/acs.chemrev.8b00299
5. Kiyama R, Wada-Kiyama Y. Estrogenic endocrine disruptors: Molecular mechanisms of action. Environ Int. 2015;83:11-40. doi:10.1016/j.envint.2015.05.012
6. Yu W, Du B, Yang L, *et al*. Occurrence, sorption, and transformation of free and conjugated natural steroid estrogens in the environment. Environmental Science and Pollution Research. 2019;26(10):9443-9468. doi:10.1007/s11356-019-04402-z
7. Usanjobling, † Moniques. Widespread Sexual Disruption in Wild Fish.; c1998.
8. Gunnarsson L, Jauhiainen A, Kristiansson E, Nerman O, Larsson DGJ. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ Sci Technol. 2008;42(15):5807-5813. doi:10.1021/es8005173
9. Notch EG, Miniutti DM, Mayer GD. 17α-Ethinylestradiol decreases expression of multiple hepatic nucleotide excision repair genes in zebrafish (Danio rerio). Aquatic Toxicology. 2007;84(3):301-309.
10. da Cunha DL, Camargo da Silva SM, Bila DM, da Mota Oliveira JL, de Novaes Sarcinelli P, Larentis AL. Regulamentação do estrogênio sintético 17α-etinilestradiol em matrizes aquáticas na Europa, Estados Unidos e Brasil. Cad Saude Publica. 2016, 32(3). doi:10.1590/0102-311X00056715
11. Fazio F. Fish hematology analysis as an important tool of aquaculture: A review. Aquaculture. 2019;500:237-242. doi:10.1016/j.aquaculture.2018.10.030
12. Seibel H, Baßmann B, Rebl A. Blood Will Tell: What Hematological Analyses Can Reveal About Fish Welfare. Front Vet Sci. 2021, 8. doi:10.3389/fvets.2021.616955
13. Uiuiu P, Lațiu C, Păpuc T, *et al*. Multi-approach assessment for stress evaluation in rainbow trout females, Oncorhynchus mykiss (Walbaum, 1792) from three different farms during the summer season. Animals. 2021, 11(6). doi:10.3390/ani11061810
14. Alhadi Ighwela K, Ahmad A, Bolong Abol-Munafi A, Bin Ahmad A, Abol-Munafi A. Hematological Changes in Nile Tilapia (*Oreochromis niloticus*) Fed with Varying Dietary Maltose Levels Utilization of dietary nutrients in Malaysian mahseer (Tor tambroides). View project Phytohormone in plant cell differentiation and organ development View project Hematological Changes in Nile Tilapia (*Oreochromis niloticus*) Fed with Varying Dietary Maltose Levels. World Journal of Fish and Marine Sciences. 2012;4(4):376-381. https://www.researchgate.net/publication/235656432
15. Wells RMG, Baldwin J, Seymour RS, Christian K, Brittain T. Red blood cell function and hematology in two tropical freshwater fishes from Australia. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology. 2005;141(1):87-93. doi:10.1016/j.cbpb.2005.04.005
16. Tavares-Dias M, Moraes FR de. Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf.), with an assessment of morphologic, cytochemical, and ultra-structural features. Vet Clin Pathol. 2007, 36.
17. Gül Ş, Belge-Kurutaş E, Yıldız E, Şahan A, Doran F. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey. Environ Int. 2004;30(5):605-

609.
18. Younis EM, Abdel-Warith AA, Al-Asgah NA. Hematological and enzymatic responses of Nile tilapia *Oreochromis niloticus* during short and long term sublethal exposure to zinc. Afr. J Biotechnol. 2012;11(19):4442-4446.
19. Kidd KA, Blanchfield PJ, Mills KH, *et al*. Collapse of a fish population after exposure to a synthetic estrogen. Proceedings of the National Academy of Sciences. 2007;104(21):8897-8901.
20. Wintrobe MM. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia haematologica. 1934;51(32):32-49.
21. HO Y, AD H, NM B. Studies on the origin, development, and seasonal variations in the blood cells of the perch, Perca flavescens. Anat Rec. 1947;99(4):680.
22. Hesser EF. Methods for routine fish hematology. The Progressive Fish-Culturist. 1960;22(4):164-171.
23. Shah SL, Altindag A. Hematological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull Environ Contam Toxicol. 2004;73(5):911-918. doi:10.1007/s00128-004-0513-y
24. Blaxhall PC, Daisley KW. Routine hematological methods for use with fish blood. J Fish Biol. 1973;5(6):771-781.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry. 1951;193:265-275.
26. Bergmeyer HU, Herder M, Ref R. International federation of clinical chemistry (IFCC). J clin. Chem. Clin. Biochem. 1986;24(7):497-510.
27. Aitken MM, Hall E, Scott L, Davot JL, Allen WM. Liver-related biochemical changes in the serum of dogs being treated with phenobarbitone. Veterinary record. 2003;153(1):13-16.
28. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955;11(1):1-42.
29. Holcombe GW, Benoit DA, Leonard EN, McKim JM. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Board of Canada. 1976;33(8):1731-1741.
30. Witeska M, Dudyk J, Jarkiewicz N. Hematological effects of 2-phenoxyethanol and etomidate in carp (*Cyprinus carpio* L.). Vet Anaesth Analg. 2015;42(5):537-546.
31. Katalay S, Parlak H. The Effects of Pollution on Hematological Parameters of Black Goby (*Gobius Niger* L., 1758) in Foça and Aliağa Bays*. 2004, 21. http://jfas.ege.edu.tr/
32. Akinrotimi OA, Agokei EO, Aranyo AA. Changes in blood parameters of Tilapia guineensis exposed to different salinity levels. Journal of Environmental Engineering and Technology. 2012;1(2):4-12.
33. AS K, JK. Effect of 17 a-Methyl Testosterone on Hematology and Histology of Liver and Heart of Oreochromis andersonii (Castelnau, 1861). Journal of Marine Science: Research & Development. 2013, 03(03). doi:10.4172/2155-9910.1000130
34. Joshi PK, Bose M, Harish D. Hematological changes in the blood of *Clarias batrachus* exposed to mercuric chloride. Journal of Ecotoxicology & Environmental Monitoring. 2002;12(2):119-122.
35. Davis AK, Maney DL, Maerz JC. The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. Funct Ecol. 2008;22(5):760-772. doi:10.1111/j.1365-2435.2008.01467.x
36. Hadi A, Shokr A, Alwan S. Effects of aluminum on the biochemical parameters of fresh water fish *Tilapia zillii*. J Sci Appl. 2009;3(1):33-41.
37. Verma SR, Rani S, Dalela RC. Isolated and combined effects of pesticides on serum transaminases in *Mystus vittatus* (African catfish). Toxicol Lett. 1981;8(1-2):67-71.
38. Kumar Rupesh M, Rahiman Fasalu OM, Mani Tamizh T, *et al*. Evaluation of hepatoprotective activity of Asparagus racemosus root against paracetamol induced acute liver injury in rats. Pharmacol. Online. 2011;1:1059-1066.
39. Hassaan MS, Soltan MA, Ghonemy MMR. Effect of synbiotics between Bacillus licheniformis and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*). The Egyptian Journal of Aquatic Research. 2014;40(2):199-208.
40. Rudneva II, Kovyrshina TB. Comparative study of electrophoretic characteristics of serum albumin of round goby *Neogobius melanostomus* from Black Sea and Azov Sea. Int J Sci Nat. 2011;1(1):131-136.
41. Patriche T, Patriche N, Tenciu M. Cyprinids total blood proteins determination. Scientific Papers Animal Science and Biotechnologies. Published online; c2009.
42. Stosik M, Deptula W, Travnicek M. Resistance in carps (*Cyprinus carpio* L.) affected by a natural bacterial infection. Veterinarni medicina-praha-. 2001;46(1):6-15.
43. Borges A, Siqueira DR, Jurinitz DF, *et al*. Biochemical composition of seminal plasma and annual variations in semen characteristics of Jundia Rhamdia quelen (Quoy and Gaimard, Pimelodidae). Fish Physiol Biochem. 2005;31:45-53.
44. Davis ME, Berndt WD. Renal methods for toxicology. Principles and methods of toxicology, 3rd Ed New York Raven. Published online. 1994:871-894.
45. Patriche T, Patriche N, Bocioc E, Coada MT. Serum biochemical parameters of farmed carp (*Cyprinus carpio*). Aquaculture, Aquarium, Conservation & Legislation. 2011 Apr 1;4(2):137-40.