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Sub Lethal Effects of Copper and Arsenic on Some Biochemical and Hematological Parameters of the Blood in an Air Breathing Fish *Heteropneustes fossilis*

Satyendra Singh* and A. K. Srivastava

Department of Zoology, DAV College Kanpur–208001, U.P. India

Abstract: *Study was undertaken to evaluate biochemical and hematological alterations resulting from the exposure of Heteropneustes fossilis to both sub-lethal concentration of copper (1.84 ppm and 1.10ppm) and arsenic (1.10 ppm and 0.78ppm) on various tissues for 15 days, 30 days and 60 days. Three groups of 14 fish were taken as control group, arsenic exposed group and copper exposed group. At the end of experiment periodically blood sample were taken from the control and experimental group of fish. Blood was assayed for selected biochemical and hematological parameters (hematocrit, haemoglobin per cent red blood cell counts, white blood cell counts, MCV, MCH, MCHC, clotting time, plasma protein, plasma glucose, blood plasma GOT, GPT, alkaline phosphatase, cholesterol, triglycerides). The hematological indices of both sub-lethal concentration of both test metal indicated a dose-dependent decrease in hemoglobin values, hematocrit values, red blood counts, MCH, MCHC, which are indication of anemia of the normal chronic type. Clotting time significantly increased in copper exposed fish. A dose-duration dependent increase was observed in MCV and WBC count. Biochemical analysis demonstrated the blood plasma level of protein and glucose was lowered in the exposed fish as compared to the control group, but a significant dose dependent elevation was recorded in all other bio chemical parameters. During exposure time a relative decrease in blood glucose level and an increase in blood serum cholesterol level and SGOT, SGPT, alkaline phosphatase, triglycerides level was increased. The result of present study demonstrates the toxic effect of both test metal in test fish Heteropneustes fossilis and recorded hematological and biochemical parameters can be used as an indicator of copper and arsenic related toxicological stress in fish on been exposed to sub-lethal concentration of both metal.*

Keywords: Copper, Arsenic, Biochemistry, Hematology, *Hetropneustes fossilis,* Toxicity.

Introduction

Heavy metals in the environment have a long biological half-life and therefore a major threat to aqua fauna, particularly fishes are found to be more sensitive comparatively. Higher concentration of heavy metals kills the aquatic organisms, at sub-lethal concentration, they are gradually intensified in various aquatic organisms, when they reach higher trophic levels in a food chain, and health hazards caused by heavy metals have become a great cause of concern only when they affected human life via the food chain. Among various toxic pollutants, heavy metals are particularly severe in their action due to tendency of bio-magnification in the food chain. The global heavy metal pollution of aquatic bodies is a major environmental problem. As pollution may induce certain biochemical and hematological changes in fishes before the drastic cellular and systemic dysfunction manifest themselves, appropriate, parameters could use effectively as sensitive indicators (Aldridge, 1983) thus fish in turn could serve as a biological indicator to monitor the aquatic pollution caused by copper and arsenic concentrations. Keeping these facts in view the present investigation was under taken to study the Sub-lethal effect of copper sulphate and sodium arsenate on

*Email: ssrathore0535@gmail.com

some important biochemical and hematological parameters of the blood, in an air breathing fresh water fish *Heteropneustes fossilis.*

Materials and Methods

The test fishes *Heteropneustes fossilis* of almost same size were purchased alive in healthy conditions from local fish market, procured from nearby water ponds of city Kanpur. The fishes were screened for any physical damage, disease and mortality. The live specimen of adult fresh fish *Heteropneustes fossilis* were acclimatized under natural photoperiod and standard laboratory conditions for 15 days in fiber glass aquaria containing non-chlorinated tap water of 20 liters volume to recover from stress and fed every day twice with wheat flour pellets, boiled egg protein and ground dried shrimps purchased from local fish market. Before stocking, the aquaria were washed with 0.1% $KMnO_4$ to free the walls from any possible fungal infections if any and acclimatization was judged satisfactory when the incidence of fish mortality were less than 10% of total fish during one week prior to the commencement of the experiment. The fishes were also treated with 0.1% $KMnO_4$ solutions to check any possible bacterial infections. The media in aquaria both control and experimental was renewed on alternate days to prevent accumulations of metabolites. Apart from one control aquaria four more aquaria with test concentration were maintained for acute toxicity test, while two sub-lethal concentrations in two aquaria with one control group were maintained for both heavy metals during chronic exposures. For chronic toxicity test, the two test concentrations $1/10^{th}$ and $1/6^{th}$ ppm to the Lc_{50} value at 96 hours are considered as sub-lethal concentration which are 1.10 ppm, 1.84 ppm for copper and 0.78 ppm, 1.31ppm for arsenic.

The bioassay system was described in standard methods (APHA, AWWA and WPCF, 1998). The LC_{50} and 95% confidence limit were calculated by the Computer-Programmed. The group of 14-14 fishes expose to sub-lethal concentration of 1.84 ppm and 1.10 ppm of copper sulphate and 1.31 ppm and 0.78ppm of sodium arsenate for 15 days, 30 days and 60 days along with control. The aquaria water was changed at every 24hrs and fresh solution added. Fish were fed dried shrimps and boiled eggs at every alternate during chronic experimental period. For biochemical parameters we collected blood in citrated tuberculin syringes. For the quantitative estimation of total protein in tissue *H. fossilis* we adapted the method of Lowry *et al.* (1951). After exposure of 15 day's fish is immediately dissected after removing from test solution, which was preceded by anesthesia by M.S.-222 (1gm/L) tricaine methane sulfonate (Sigma Chemical Co., St. Louis, USA). Liver, Kidney, Spleen, Gills and Muscle are removed immediately and weight is measured. Samples are placed in crushed ice in an icebox. Blood was drawn from the dorsal aorta in direct eppendorf tube containing 0.1 ml. of EDTA for biochemical parameter. The blood was gently mixed with the oxalate mixture to avoid coagulation.

Estimation of Triglycerides

Blood plasma triglyceride was estimated by the kit method (M/s Span Diagnostics, Udhana (Gujarat) India).

Estimation of Cholesterol

Blood plasma Cholesterol was estimated by the kit method (Span Diagnostics).

Estimation of Alkaline Phosphatase

Blood plasma alkaline phosphatase was estimated by the kit method (Span Diagnostics).

Estimation of Glutamate Oxalacetate Transminase (GOT)

Blood plasma GOT was estimated by the kit method (Span Diagnostics).

Estimation of Glutamate Pyruvate Transaminase (GPT)

Blood plasma GPT was estimated by the kit method (Span Diagnostics).

Estimation of Glucose

Blood plasma glucose was estimated by the kit method by (Glucose based on GOD – POD), Span Diagnostics.

Hematological Estimation

After completion of exposure period of 30 and 60 days fish were sacrificed with gentle below on head. The fresh blood was collected in micropipettes by caudal vein in vials coated with EDTA (Ethylene diamine tetra acetic acid), as anticoagulant. Hematological parameters were estimated by standard methods. Red Blood Cells (RBCs) and White Blood Cells (WBC) were counted by Neubauer's improved hematocytometer using Hymen and Turk's solution as diluting fluid. Mean Corpuscular Hemoglobin (MCH) and mean cell volume (MCV) were calculated by using formula by Dacie and lewis, (1977). Hemoglobin content (g/100 ml) were determined by Sahlis-Hellige method using 0.1N HCl by Hemoglobinometer, packed cell volume PCV was measured by capillary method, while clotting time by the tube method as used in clinical hematology. All parameters were done for four replicates.

Results and Discussion

Biochemical Parameter

Various Observed Biochemical Parameters are given in Table 1-4

Hematology

Results of sublethal exposures to both test

Table 1 Alteration in protein level (mg protein/1 gm body weight) in different tissue of *Heteropneustes fossilis* to 1.31 and 0.78 ppm of Arsenic for 15 days.

Tissues level (µg/L)	Control	As 1.31 ppm	As 0.78 ppm
Liver	28.43 ± 0.06	21.45 ± 0.5	18.31 ± 0.2
Kidney	27.34 ± 0.2	24.06 ± 0.5	20.18 ± 0.1
Gills	31.08 ± 0.7	24.94 ± 0.2	21.34 ± 0.5
Spleen	29.28 ± 0.8	23.23 ± 0.06	21.43 ± 0.6
Muscle	33.53 ± 0.7	26.44 ± 0.5	22.98 ± 0.2

Result are mean ±SE of three determinants at significance response P<0.05

Table 2 Alteration in protein level (mg protein/1 gm body weight) in different tissue of *Heteropneustes fossilis* to 1.84 and 1.10ppm of Copper for 15 days

Tissues level (µg/L)	Control	Cu 1.10 ppm	Cu 1.84 ppm
Liver	28.43 ± 0.06	18.98 ± 0.4	13.02 ± 0.5
Kidney	27.34 ± 0.2	23.86 ± 0.3	18.12 ± 0.4
Gills	31.08 ± 0.7	18.15 ± 0.3	13.98 ± 0.6
Spleen	29.28 ± 0.8	15.61 ± 0.4	7.53 ± 0.08
Muscle	33.53 ± 0.7	21.01 ± 0.06	17.08 ± 0.5

Result are mean ± SE of three determinants at significance response P<0.05

Table 3 Enzyme activities (µg/l) in the blood plasma of *Heteropneustes fossils* exposed to 1.31 and 0.78ppm. of Arsenic for 15 days.

Tissues level (µg/L)	Control	As. 1.28 ppm	As 0.78 ppm
Glucose	395.67 ± 2.6	149.95 ± 3.4	124.94 ± 1.6
Cholesterol	3.92 ± 0.5	7.45 ± 0.6	5.20 ± 0.01
Triglyceride	98.21 ± 2.8	131.43 ± 1.7	105.50 ± 2.9
GOT	66.72 ± 2.3	94.05 ± 2.8	78.84 ± 2.6
GPT	90.20 ± 1.7	135.20 ± 1.4	110.65 ± 3.2
Alkaline Phosphatase	798.31 ± 0.5	1406.69 ± 1.2	1265.20 ± 1.0

Values are mean±SE of three determinants at significance level. P<0.05

Table 4 Enzyme activities (µg/l) in the blood plasma of *Heteropneustes fossilis* exposed to 1.84ppm and 1.10ppm of Copper for 15 days.

Tissues (µg/l)	Control	Cu 1.84 ppm	Cu 1.10 ppm
Glucose	395.67 ± 2.6	235.18 ± 3.3	179.21 ± 2.4
Cholesterol	3.92 ± 0.5	5.98 ± 0.23	5.12 ± 0.03
Triglyceride	98.21 ± 2.8	118.31 ± 3.8	106.40 ± 1.5
GOT	66.72 ± 2.3	75.64 ± 2.9	69.15 ± 1.9
GPT	90.20 ± 1.7	124.34 ± 3.3	114.79 ± 3.8
Alkaline P.	798.31 ± 0.5	1104.05 ± 1.22	995.55 ± 0.8

Values are mean±SE of three determinants at significance level. P<0.05

Table 5 Alteration in Hematological parameter in *Heteropneustes fossilis* exposed to sub-lethal concentrations (1.10ppm and 1.84ppm) of copper for 30 days.

Parameter	Control	Cu 1.10ppm	Cu 1.84ppm
RBC ($x10^6/mm^3$)	3.20 ± 0.06	2.46 ± 0.2	2.31 ± 0.38
Hb (gm/100ml.)	13.4 ± 0.26	12 ± .64	10.15 ± 0.4
WBC ($10^3/mm^3$)	26.5 ± 0.2	37.43 ± 0.2	39.63 ± 0.08
PCV (mm^3)	32.45 ± 0.3	29.23± 0.2	26.40 ± 0.54
MCH (Pg)	44.02 ± 2.0	48.83± 0.4	42.26 ± 1.4
MCV(mm^3)	98.09 ± 3.8	116.43 ± 0.4	114 .35 ± 3.7
MCHC(g/dl)	43.18 ±0.4	41.7 ± 2.14	36.4 ± 2.6
Clotting time (sec)	72.24±0.5	148 ±1.53	156 ±1.88

Values are mean ± SE (N= 4) at significance level. P < 0.05

metal are given in Table 5-8.

In the present investigation chronic exposure of fish to two sub-lethal concentrations 1.31ppm and 0.78 ppm of sodium arsenate and 1.84 ppm and 1.10ppm of copper sulphate for 30 days and 60 days are estimated. The both sub-lethal concentrations induced changes in hematological parameters in exposed fishes against the control group. In sodium arsenate

Table 6 Alteration in Hematological parameter in *Heteropneustes fossilis* exposed to sub-lethal concentrations (0.78ppm and 1.31ppm) to arsenic for 30 days.

Parameter	Control	As 0.78 ppm	As 1.31 ppm
RBC (10^6/mm^3)	3.20 ± 0.06	2.25 ± 0.6	2.15 ± 0.6
Hb (gm/100ml.)	13.4 ± 0.26	9.6 ± 0.2	8.8 ± 0.2
WBC (10^3/mm^3)	26.5 ± 0.2	28.4 ± 0.2	25.50 ± 0.5
PCV (mm^3)	32.45 ± 0.3	27.4 ± 0.2	24.43± 0.2
MCH (Pg)	44.02 ± 2.0	44.27 ± 0.58	38.8 ± 1.0
MCV (mm^3)	98.09 ± 3.8	114.62 ± 3.24	117.44 ± 2.0
MCHC(g/dl)	43.18 ±0.4	34.18 ± 0.1	36.25 ± 0.8
Clotting time (sec)	72.24±0.5	94.62 ± 0.4	124 ± 2.6

Values are mean ± SE (N= 4) at significance level. $P < 0.05$

Table 7 Alteration in Hematological parameter in *Heteropneustes fossilis* exposed to sub-lethal copper concentration for 60 days.

Parameter	Control	Cu 1.10ppm	Cu 1.84ppm
RBC (10^6/mm^3)	3.04 ± 0.06	2.40 ± 0.03	2.10 ± 0.1
Hb (gm/100ml.)	13.6 ± 0.1	10.8 ± 0.4	8.14± 0.8
WBC (10^3/mm^3)	23.54 ± 0.2	24.45 ± 0.2	27.45 ± 0.2
PCV (mm^3)	31.11 ± 0.1	24.66 ± 0.2	22.8 ± 0.2
MCH (Pg)	45.34 ± 0.8	48.0 ± 1.3	42.34 ± 0.4
MCV (mm^3)	103 ± 0.8	103.62 ± 1.3	104.4 ± 2.3
MCHC (g/dl)	44.4 ± 1.6	48.44 ± 0.6	39.03± 2.95
Clotting time (sec)	86 ± 1.64	138.5 ± 1.8	144.8 ± 1.8

Values are mean ± SE (N= 4) at significance level. $P < 0.05$

Table 8 Alteration in Hematological parameter in *Heteropneustes fossilis* exposed to sub-lethal concentration to arsenic for 60 days.

Parameter	Control	As 0.78 ppm	As 1.31 ppm
RBC (10^6/mm^3)	3.04 ± 0.06	2.10 ± 0.02	1.96 ± 0.04
Hb (gm/100ml.)	13.6 ± 0.1	7.47 ± 0.4	4.6 ± 0.5
WBC (10^3/mm^3)	23.54 ± 0.2	21.8 ± 0.5	24.98 ± 0.2
PCV (mm^3)	31.11 ± 0.1	22.63 ± 0.6	18.60 ± 0.4
MCH (Pg)	45.34 ± 0.8	34.62 ± 1.8	23.82 ± 1.5
MCV (mm^3)	103 ± 0.8	101.8 ± 5.0	82.2 ± 0.8
MCHC (g/dl)	44.4 ± 1.6	33.6 ± 2.6	27.5 ± 1.5
Clotting time (sec.)	86 ± 1.6	148 ± 1.6	156 ± 3.0

Values are mean ± SE (N= 4) at significance level. $P < 0.05$

exposure Hb percentage, RBCs, PCV, MCH, and MCHC decrease significantly in 30days while MCV, WBC and clotting time significantly increase in both concentration of arsenic. In 60 days exposure of sodium arsenate the RBCs count, Hb percentage PCV, MCH, MCV and MCHC also decrease in both concentrations, but clotting time and WBC count significantly increased in both concentration. In copper sulphate exposure RBC, PCV, MCHC are significantly decreased. Hemoglobin (%) in lower concentration is almost equal to control value, but decrease in higher concentration was noticed for 30 days, WBC, MCV clotting time are significantly increase for 30 days. In 60 days exposure of copper sulphate RBC, Hb%, PCV, are significantly decreases. MCH value in higher concentration decrease but increase in lower concentration MCV value is almost equal to the control value in both concentrations for the 60 days duration of exposure. The clotting time significantly increase in copper sulphate solutions. A dose and duration dependent increase was observed in WBC count.

Protein

Studies on biochemical constituents of blood help to understand the physiological status of fish with changing ecophysiological conditions. In present study change in protein content in stress response to Arsenic and Copper lead to irreversible and reversible disturbances of integrated functions such as physiological behavior and reproduction, which are also supported by (Collivin, 1984) in *Perca fluviatilis* exposed to copper and *M. vittatus* to Chromium and Mercury (Sivakami *et al.*, 1994; Kasthuri *et al.*, 1997). Decrease in protein content also affects the other biochemical parameter. Depletion in tissue protein in *H. fossilis* due to Copper and arsenic toxicity may be attributed to either rapid utilization of body protein or poor intake of dietary protein by fish, which support to view of Syverson (1981) who opined that the heavy metal in general interfere with protein synthesis. Further under stress conditions the dietary protein consumed by the fish is not utilized by the body tissue as also suggested by Bhaskaran and Palanichamy (1990) hence the treated fish meet out their extra energy demands from protein metabolism, which is mobilized to produce glucose, the instant energy, which is made available for fish by the process of gluconeogenesis, also reported by Vasanthi *et al.*, (1990).

The reduced level of protein content in present study may be attributed to metabolic utilization of keto acids to gluconeogenesis pathway for the synthesis of glucose or for the maintenance of osmoregulation and ionic balance. The decrease in protein content also suggested an increase in proteolytic activity and possible utilization of its products for metabolic purpose. Depletion of tissue protein in fishes exposed to various toxicants have already been reported by Ram and Sathyanesan (1984) suggested that protein also being energy source is to spare during chronic period of stress. The decreased level of protein in different tissue like gill, liver, kidney, spleen and muscle after 15 day exposure to copper and arsenic may be attributed to lack of protein biosynthesis or inhibition of translation or may also be due to poor rate of absorption of amino acid glycine due to damage of intestinal villi induced by these metals a similar opinion with Pradhan and Hota (1993).

Glucose

Glucose is considered as instant and immediate source of energy in stressful condition. Reduction in carbohydrate content in blood plasma of the exposed fish to both metal at both sub-lethal concentrations in comparison to control in present investigation seems to be due to rapid utilization of blood glucose and depletion of stored glycogen to provide energy for *Heteropneustes fossilis* under toxicity of both metal which is similar with the observation of Kasthuri and Chandran (1997) in *M. gulio* under lead toxicity Reduced glucose level or hypoglycemia were also observed by Subhadra and Sastry (1985); in *H. fossilis* exposed to Zinc. The hypoglycemic

response in the exposed fish after 15 days was found to be at higher level in low sub-lethal concentration in both metal primarily, probably due to abnormal behavioral activities which were needed more energy. The high level of glucose at higher concentration may be attributed to slow operculum movement and relatively slow other abnormal behavioral activities against control values.

Cholesterol

The hypercholesterolemia in blood plasma registered in the cat-fish exposed to both copper and arsenic for 15 days exposure at both sub-lethal concentrations, the hypercholesterolemia in present study may be attributed to liver damage of exposed fish *H. fossilis* as liver plays a central role in biodegradation of toxic agents like metals. Hypercholesterolemia is thought to be a fatal factor for heart and blood vessel disease the similar results are with the finding of Srivastava and Srivastava (1995) who observed hypercholesterolemia in *H. fossilis* after exposure to organochlorine and malachite green in *Clarias batrachus* and *H. fossilis* (Srivastava and Singh, 1995; Desai *et al.*, 2002) discussed the hypercholesterolemia in brains, liver due to the movement of cholesterol into brain and liver mostly due to hepatic dysfunction or blocking of synthesized cholesterol in the liver. The hypercholesterolemia in present study is direct indication of various tissues damage which could not utilize the cholesterol during cell repair and growth due to metal toxicity. The essential phospholipids tend to restore the normal glucose level during Zinc toxicity to fish *H. fossilis* (Kothari and Soni, 2002), which may also be another reason of hypercholesterolemia in present stud due chronic exposure of copper and arsenic respectively.

Glutamate Oxalacetate Transminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Alkaline Phosphatase

Enzyme histochemical data may prove an additional dimension to biochemical results as enzymes are now understood to have broader scope in all physiological function due to nature to serve as stress areas of the metal toxicity to fish. Transaminases are concerned with energy metabolism which allow interplay among carbohydrate, fat and protein metabolism to serve the changing demands of the organism Bell (1968). Glutomic oxalacetic transaminase is most active, widely distributed and well-studied transaminase in various tissues of different fishes (Kothari *et al.*, 2003). The gradual rise of serum GOT level in *Clarias batrachus* during stress led to vigorous activity and metabolism to ward-off the stressful situation (Tandon and Chandra, 1978). The fish possesses some mechanisms to regulate the intake of food and balance the energy demand in different stressful situation faced by fish during its life. In present study the elevated level of GPT and GOT are also due to adjustment of the stressed fish to cope up with chemical stress caused by arsenic and copper exposure. Tissue damage of various exposed organs also a reasonable cause of their elevated level in blood plasma of *H. fossilis*.

Alkaline Phosphatase

Alkaline phosphatase is an enzyme found in every organ but primarily in bones and liver (Gautam and Thakur, 2003). The activities of alkaline phosphate inhibited significantly in liver, gills, kidney, spleen and muscle in sub-lethal exposure of both copper and arsenic in present study as advocated by their elevated level in blood plasma of exposed fish. Our findings are in agreement with the findings of Bhatnagar *et al.*, (1995) who discussed the inhibition alkaline phophatases in various tissues of fish exposed chronically to endosulphan and pyrethroid induced toxicity in *Clarias batrachus.*

Hematology

Blood is a pathophysiological reflector of whole body and therefore blood parameters are important in diagnosing the functional status of

the animal exposed to toxicants. Hematological analysis therefore can serve as a rapid and economical method for assessing the metal toxicity on fishes. Anemia is one of the most sensitive pathological situations developed as a result of metals poisoning.

Arsenic Induced Hematology

Arsenic is a well-known accumulative poison in animals' it has a great ability to accumulate in kidney, gill, liver and other tissue (Qladimeji, *et al.*, 1984). Kidney and liver is the major target organ of arsenic poisoning, as these are the hemopoietic organs. In present study after 15 days of exposure to the 1.31ppm and 0.78ppm of sodium arsenate, there was a significant decrease of the erythrocyte count, Hb%, and PCV. Kidney damage usually cause a decrease in erythropoietin level, which in turns decreases RBCs production and hemoglobin synthesis even under hypotoxic condition (Reddy *et al.*, 1998). The decrease in hemoglobin on exposure of sodium arsenate was due to decrease of RBCs count, which in turn might be due to sodium arsenate effect on erythropoietic organs primarily like kidney and spleen which lead into inhibited erythropoiesis and thus inadequate hemoglobin synthesis a similar view with Srivastava and Srivastava (2001). Anemia can also be caused by other number of pathological conditions such as lysis of RBC, erythropenia, increased ESR or hemodilution. It has been reported leucopenia caused by toxicant was among the relevant reasons of fish kills. The above discussion led us to conclude that sodium arsenate even in sub-lethal concentration alters the normal hematological value. Such response would be result to toxicity of chemical substance which induces tissue damage.

Copper Induced Hematology

The erythrocyte counts decreased more in high concentration of both toxicants at 30 and 60 days exposure and when compared with each other. The values of reduced RBC were more marked in arsenic exposure than copper. The Hb% also depleted more significantly in arsenic exposure, a clear indication of comparatively more toxic nature of arsenic than copper. The total erythrocyte, Packed Cell Volume (PCV) and MCV indicated the RBC destruction thus manifestation of anemia associated with erythropenia. Damaged intestinal villi have been observed in *Heteropneustes fossilis* following exposure of fish to Zinc and Chromium (Shandilya and Banergee, 1989) thus resulting into poor iron absorption which is solely essential for erythropoisis, may be another reason of decreased level of hemoglobin. The decreased RBC count in present study can also be correlated with the reduction in oxygen carrying capacity due to gill damage by metal a parallel finding with Reddy *et al.* (1998). Gradual decrease in total RBC count, hematocrit and Hb% reflected the anemic condition of fish and disruption of young blood cells due to toxicity of heavy metals copper and arsenic, in present study is in conformity with the result of Van varent *et al.*, (1994); Singh *et al.*, (1992) in cat-fish *Clarias batrachus* exposed to copper. Copper and arsenic exposure to *H. fossilis* in present study induced leucocytosis as advocated by increased WBC count. The number of WBC increased regularly in the acute period of $CuSO_4$ exposure, which seems to be associated to malfunctioning of hemopoietic system stressed by copper intoxication. This correlates the findings of Sharma *et al*. (1982). During recovery of number of WBC began to normalize but still remained above the control value, this might be due to gradual automatics repair of damaged hemopoietic tissue to tide over stressful condition caused by copper and arsenic exposure. White blood corpuscles play a major role in the defense mechanism of fish which may be directly proportional to the severity of the causative stress condition and may be attributed to an increase in leucocytes mobilization. The leucocytosis is for the removal of cellular debris of necrosed tissue at a quicker rate, also evidenced by Garg *et al.* (1989) in *H. fossilis* exposed to manganese,

Singh (1995) to copper and chromium and Tyagi and Srivastava (2005) Zinc exposed fish *Channa punctatus*. In this study the WBC count increased from normal values. Thrombocytosis and decreased blood clotting time was observed in *C. fasciatus* following exposure to copper and cobalt (Srivastava and Agarwal, 1979).

Significant increased value of the MCV to *H. fossilis* after exposure to copper and arsenic in present study also supported by Shastry and Gupta (1994) in fresh water teleost fish against various toxicants. Increase in MCV values in both sub-lethal concentration of both copper and arsenic in this study may be considered as good index of RBC destruction, swelling of RBC due to endosmosis and thus disturbed osmoregulation (Butlar, 1978) and also to increased number of lymphocytes. The above reasons are in co-relation with study of Srivastava and Srivastava (2002) in *H. fossilis* exposed to cadmium Decreased MCHC values in present study observed after exposure to both copper and arsenic exposed fish *H. fossilis*. Study is parallel to other researchers (Verma *et al.*, 1998) observed in *Oreochromis mossambicus* and in *H. fossilis* exposed to Cadmium by Srivastava and Srivastava (2002). Decreased values of MCHC in this study are suggested to due to much loss of hemoglobin content or megaloblostic anemia also is parallel to study of Wepner (1992), in *Tilapia sperrmamii* exposed to Zinc and Iron. Reduced MCH values are direct indication of hemoglobin destruction caused by copper and arsenic exposure. The present study at fish *H. fossilis* exposed to both sub-lethal concentration of copper and arsenic revealed a marked reduction time in blood coagulation which is also estimated by Srivastava and Sinha (1996) in *H. fossilis* exposed to pesticides. In present study thrombocytopenia seems to lead into the increased clotting time of blood. The above altered hematological parameter in *H. fossilis* against control value concludes that test fish is under chemical stress thus fish faced disturbance in other physiological parameter. In conclusion the Changes in the hematological and biochemical parameters indicate that they can be used as indicators of copper and arsenic related stress in fish on exposure to elevated levels of them in the water.

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