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SUB LETHAL EFFECTS OF ATRAZINE ON HEMATOLOGY, HISTOPATHOLOGY AND BIOCHEMISTRY OF CHIRRUH SNOWTROUT (*SCHIZOTHORAX ESOCINUS*)

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ABSTRACT

In this study sub lethal effects of atrazine was studied on chirruh snow trout (*Schizothorax esocinus*), exposed to four different concentrations (1ppm, 2ppm, 3ppm and 4ppm) of atrazine for 96 h. Results of atrazine on fish hematology indicated noteworthy decrease ($p \leq 0.05$) in white blood cells (WBCs), RBCs, Hb, HCT, MCH, Monocytes and Lymphocytes concentrations in treated group compared to control group. Substantial increase in MCV, MCHC, platelets and neutrophils was found in all treated group related to control group ($p \leq 0.05$). Similarly, histopathological findings of gills of show lamellar disorder, disruption of cartilaginous core, epithelial lifting, blood mobbing, damage to secondary lamellae, fusion of secondary gills lamellae, twisting and shortening of secondary gills lamellae and degeneration and atrophy. Liver presented dissolution of cell membrane, Pyknosis, blood congestion, necrosis, hyperplasia and vacuolations. Intestine show necrosis, hemorrhages, over production of goblet cells in villi, disintegration, fusion and shortening of villi. Muscles have blood congestion at various spots, while brain showed mild necrosis, blood congestion, mild pyknosis and necrosis. It is concluded that atrazine is toxic because it can cause alteration in hematology, biochemistry and histopathology of *Schizothorax esocinus*.

Keywords: Atrazine, *Schizothorax esocinus*, Hematology, Biochemistry, Histopathology.

INTRODUCTION

Atrazine (2-chloro4-ethylamino-6-isopropylamino-s-triazine) is frequently used herbicide in rural environments and is widely used on corn, sorghum, sugarcane and pineapples. It is listed among toxic chemicals for aquatic biota. It alters biochemical parameters which result in disturbed metabolism and delay in development and such environment become unsafe for the existence of fish (Sudhasaravanan and Binukumari, 2014).

Atrazine is listed as reasonably lethal to aquatic fauna (Solomon *et al.,* 2008). Lethal effects are reported after exposure to atrazine (Blahova *et al.,* 2013; Mela *et al.,* 2013), deviations of behavior (Saglio and Trijasse 1998; Plhalova *et al.,* 2012), or biochemical changes exposed to acute, sub chronic, or chronic contact. Adverse effects of atrazine are reported on reproduction (Tillitt *et al.,* 2010), immune response (Kreutz *et al.,* 2012), or response system (Fu *et al.,* 2013). Studies have also reported changes in tissues of fishes exposed to atrazine (Plhalova *et al.,* 2012; Paulino *et al.,* 2012).

Environmental effects increased due to the broad usage of atrazine. Toxicity of atrazine is different among species and also the chemicals applied to fishes, but the lethal concentration of atrazine is reported as 3 to 45 mg/L (Elia *et al.,* 2002). Aaronson (1980) has reported mortality of rainbow trout after exposure to1000 µg/L of atrazine. According to Ramesh *et al.* (2009) atrazine exposure can effect common carp (*Cyprinus carpio*) hematology parameters. Nwani *et al.* (2010) reported lethal effects of atrazine on metabolic activities and enzyme action of *Channa punctatus*. Hussein *et al.* (1996) suggested the effect of atrazine on *Oreochromis niloticus* and *Chrysichthys auratus* from Egypt. Warining *et al.* (2004) reported effects of atrazine on Atlantic salmon in fresh and marine water and found substantial change in gill cells. The present study was conducted with the aim to investigate the effects of various concentrations of atrazine on hematological, histopathological and biochemical parameters of chirruh snow trout (*Schizothorax esocinus*).

MATERIALS AND METHODS

Collection and acclimatization of fish: Specimens of *Schizothorax esocinus* were collected from river Swat, and were transported to laboratory in a small glass aquarium having aerated oxygen. Fish specimen were acclimatized for one week prior to experiment and water was changed after every 24 hours. Fishes were feed during acclimatization period and no feed was given to fishes during experimental period. 5 fishes were kept in each aquariums. Each aquarium was filled with 60 liters of water having capacity of 100 liters.

Concentration of herbicide: Five different glass aquaria were used for the experiment. One was used as control and in the remaining four aquariums, four different concentrations of atrazine (1 ppm, 2 ppm, 3 ppm and 4 ppm) diluted with water were used.

Blood collection and analysis: After 96 hours of experiment, fishes were collected from each aquarium and were anesthetized in clove oil to collect blood. Blood was collected by puncturing caudal vein through 1ml disposable syringe.

Hematology: To study hematology, blood was collected in EDTA tubes. Blood was then analyzed using HEMA READER HRG 6300D (auto hematological analyzer Advanced Japanese Technology, China). Hematological parameters studied were; White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (HB), Hematocrit level (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets, Monocytes, Lymphocytes and Neutrophils.

Biochemical Analysis: To study biochemical parameters, blood was collected in gel EDTA tubes. Plasma was collected from blood via centrifugation. Biochemical parameters were analyzed using Chem Reader SBA-733 Plus (Semi auto Chemistry analyzer, Advanced Japanese Technology). Biochemical parameters include in this study were; Electrolytes (Sodium, Potassium, Calcium and Phosphorus), Metabolites (Triglycerides, Cholesterol, Urea, Creatinine, Glucose, and Total Protein) and Enzymes (Alanine Aminotransferase [ALT], Aspartate Aminotransferase [AST], Alkaline Phosphatase [ALP], and Lactate Dehydrogenase [LDH]).

Histopathology: To study histopathology, fish was dissected to obtain specific biomarker organs including brain, gills, intestine, muscles and liver. Every organ was balanced and preserved in 10% neutral buffered formalin (NBF) for 48 h. After fixation, tissues were allowed to dehydrate in ascending succession of alcoholic grades, cleared in xylene and entrenched in paraffin wax. Each tissue was cut at about 4–5 mm thickness and were processed on rotary microtome. After that they were stained with eosin and hematoxylin and were examined under a camera fitted microscope, OPTICA TCB 3.0 Italy.

Statistical analysis: One way ANOVA was used for comparison between control and experimental groups. Data analysis was carried out using SPSS software (Version 24.0). Variables were stated as means and standard deviations. $p \leq 0.05$ was considered as statistically significant.

RESULTS

Hematology: Fishes were divided into 5 different groups. One was kept as control (0 ppm) and the remaining were four experimental groups (1-4ppm). Fishes were exposed to these different concentrations (1 ppm, 2ppm, 3ppm, 4ppm) of atrazine and changes were observed. WBCs (10^9/L), RBCs (10^{12}/L), hemoglobin (g/dl) significantly decreased in all experimental groups. HCT value increased at 1ppm (44.4±0.94) and decreased at 4ppm (27.5±0.92) significantly in all experimental groups. MCV values (fL) increased significantly in all experimental groups of fish. MCH (pg) values decreased significantly in all experimental groups of fish. MCHC (g/dl) increased non-significantly in experimental groups. The number of Platelets (10^9/L) were increased significantly in all experimental groups. Monocytes (10^9/L) and Lymphocytes (10^9/L) level decreased significantly from 1ppm to 4ppm in experiment, whereas the percentage of Neutrophils (10^9/L) increased significantly in all experimental groups. Details analysis of hematological parameters are given in table 1.

Biochemical Analysis: After exposure, significant decrease was observed in all electrolytes of four experimental groups. Significant decrease is observed in all metabolites while cholesterol, creatinine and glucose activity was observed to be increased in all experimental groups. Significant increase was observed in ALT and AST. Increase in activity of ALP was observed in experimental groups (1ppm and 2ppm) and after that decrease is observed in experimental groups (3ppm and 4ppm). Increase in activity of LDH is observed in all experimental groups. Detail of all biochemical parameters are given in table 2.

Histopathology

Gills Tissues: Fishes exposed to atrazine showed disruption of secondary gills lamellae, shortening of secondary gills lamellae and fusion of secondary gills lamellae and mild blood congestion, epithelial lifting, atrophy, disruption of cartilaginous core, blood congestion, lamellar disorganization and curling of gills (Fig. 1).

Liver Tissues: Fishes exposed to atrazine shows pyknosis, lamellar infusion and mild pyknosis, blood congestion, necrosis and vacuolations (Fig. 2).

Muscle Tissues: Fishes exposed to different concentration of atrazine shows blood congestion at various spots (Fig. 3).

Brain Tissues: Fishes exposed to atrazine show mild necrosis and blood congestion, mild pyknosis and necrosis (Fig. 4).

Intestine Tissues: Fishes exposed to atrazine show mild necrosis, mild pyknosis, necrosis, blood congestion and detachment of villi (Fig. 5).

Table 1. Sublethal effects of Atrazine on hematological profile of *Schizothorax esocinus*

Hematological Parameters	Control group	1ppm	2ppm	3ppm	4ppm
WBCs (10^9/L)	2.88 ± 0.017	2.7±0.04**	2.58±0.03**	2.52±0.03**	2.48±0.04**
RBCs (10^{12}/L)	3.7± 0.47	3.4±0.31**	3.0±0.30**	2.8±0.28**	2.7±0.31**
Hb (g/dL)	12.8± 0.50	12.2±0.34**	10.8±0.51**	8.9±0.54**	7.6±0.50**
HCT (%)	36.5± 0.94	44.4±0.98**	41.3±1.23**	32.8±0.98**	27.5±0.92**
MCV (fL)	97.1± 10.5	110.4±13.7**	122.6±18.8**	135.6±15.2**	144.8±14.3
MCH (pg)	36.2± 0.89	28.1±0.74**	22.3±1.38**	22.0±1.27**	22.1±1.35**
MCHC (g/dl)	31.6±2.8	44.5±4.4**	41.0±9.6	38.0±3.87	37.2±2.81
Platelets (10^9/L)	3.8±0.05	6.0±0.08**	7.8±0.12**	8.2±0.05**	11.3±0.02**
Monocytes (10^9/L)	4.5±1.16	4.6±1.2	3.7±0.51	4.5±1.5	2.6±0.58
Lymphocytes (10^9/L)	66.8±1.5	58.5±1.4**	54.2±1.7**	50.1±1.9**	48.6±1.34**
Neutrophils (10^9/L)	25.4± 1.74	38.1±1.52**	49.2±0.88**	42.5±2.3**	48.9±1.15**

Values are expressed as Mean ± SD. Mean with ** expresses significant difference ($p \leq 0.05$).

Table 2. Sublethal effects of Atrazine on biochemical profile of *Schizothorax esocinus*

		Electrolytes			
Parameters	**Control group**	**1ppm**	**2ppm**	**3ppm**	**4ppm**
Sodium (mEq/l)	14.2±0.26	13.1±0.45	12.8±0.31**	12.3±0.15**	12.1±0.22**
Potassium (mEq/l)	13.6±1.1	11.4±2.0	8.4±1.1**	7.0±1.5**	7.8±0.8**
Calcium (mg/dl)	12.8±0.27	12.2±0.45	11.8±0.31**	11.4±0.15**	10.1±0.24**
Phosphorous (mg/dl)	18.5±0.56	14.0±0.32**	12.1±0.32**	10.2±0.14**	8.2±0.14**
Triglycerides (mg/dl)	240.5±3.6	221.5±1.6**	192.4±1.6**	169.4±1.5**	152.1±2.1**
Cholesterol (mg/dl)	225.8±2.9	235.6±3.5	251.4±1.14**	258.2±8.8**	268.6±2.0**
Urea (mg/dl)	12.8±1.5	12.1±2.6	9.6±1.3**	8.4±1.2**	7.9±1.3**
Creatinine (mg/dl)	0.05±0.01	0.04±0.006	0.33±0.01**	0.32±0.01**	0.41±0.04**
Glucose (g/dl)	128.3±2.6	138.2±3.4**	142.1±1.7**	159.4±1.4**	169.5±1.8**
Total Protein (g/dl)	3.3±0.31	3.2±0.13	4.6±0.15**	4.7±0.15**	4.7±0.15**
ALP (U/I)	0.32±0.03	0.38±0.4	0.46±0.03**	0.36±0.8**	0.31±0.04**
ALT (U/I)	0.26±0.2	0.41±0.2	0.51±0.15**	0.56±0.15**	0.58±0.07**
AST (U/I)	2.68±0.04	6.1±0.07**	7.8±0.11**	8.1±0.04**	9.2±0.03**
LDH (U/I)	3.5±0.51	2.9±0.58	4.2±1.12**	6.4±0.92**	5.2±0.62**

Values are expressed as Mean ± SD. Mean with ** expresses significant difference ($p \leq 0.05$).

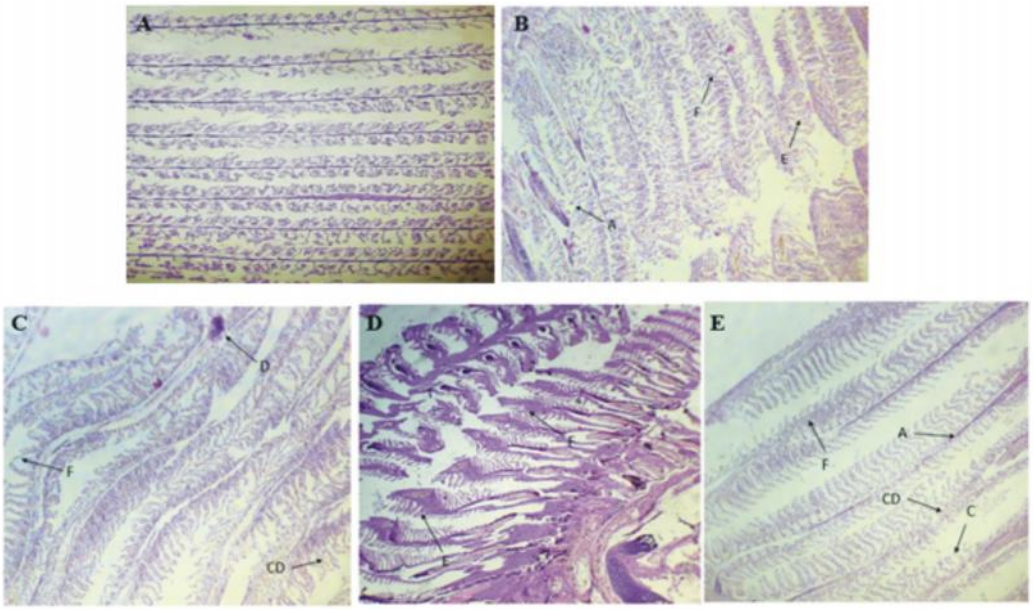

Figure 1. Sublethal effects of atrazine on gills histology of *S. esocinus.* A Shows Gill structure of control fish while Gills tissues of treated fish B to E shows Epithelial lifting €, Fusion of secondary gills lamellae (F), Disruption of cartilaginous core (CD), Disruption of secondary gills lamellae (D), Curling (C) and Atrophy (A).

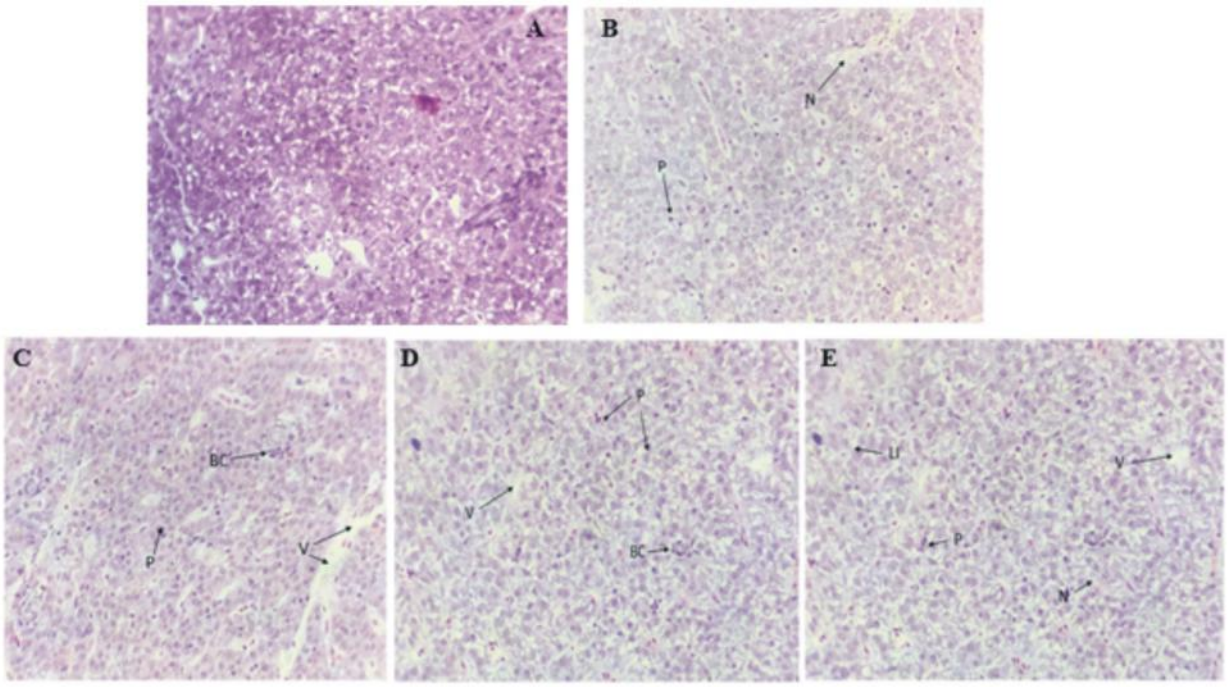

Figure 2. Sublethal effects of atrazine on liver histology of *S. esocinus.* A show Liver structure of control fish. Liver tissues of treated fish B to E Shows Necrosis (N), Pyknosis (P), Blood Congestion (BC), Lymphocyte Infiltration (Li), Vacuolations (V), Necrosis (N) and Blood Congestion (BC).

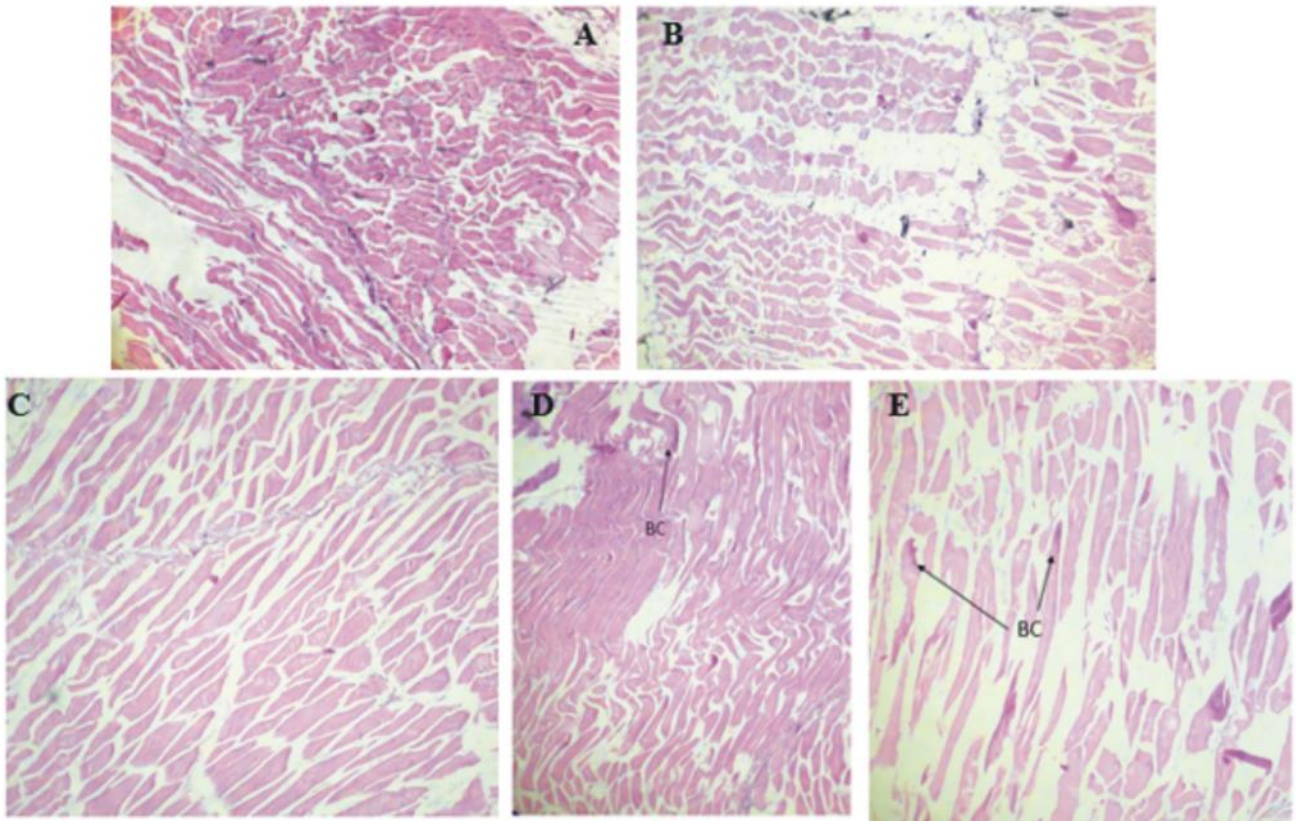

Figure 3. Sublethal effects of atrazine on muscle histology of *S. esocinus.* A shows Muscle structure of control fish. Muscle tissues of treated fish B to E Shows Only Blood Congestion (BC)

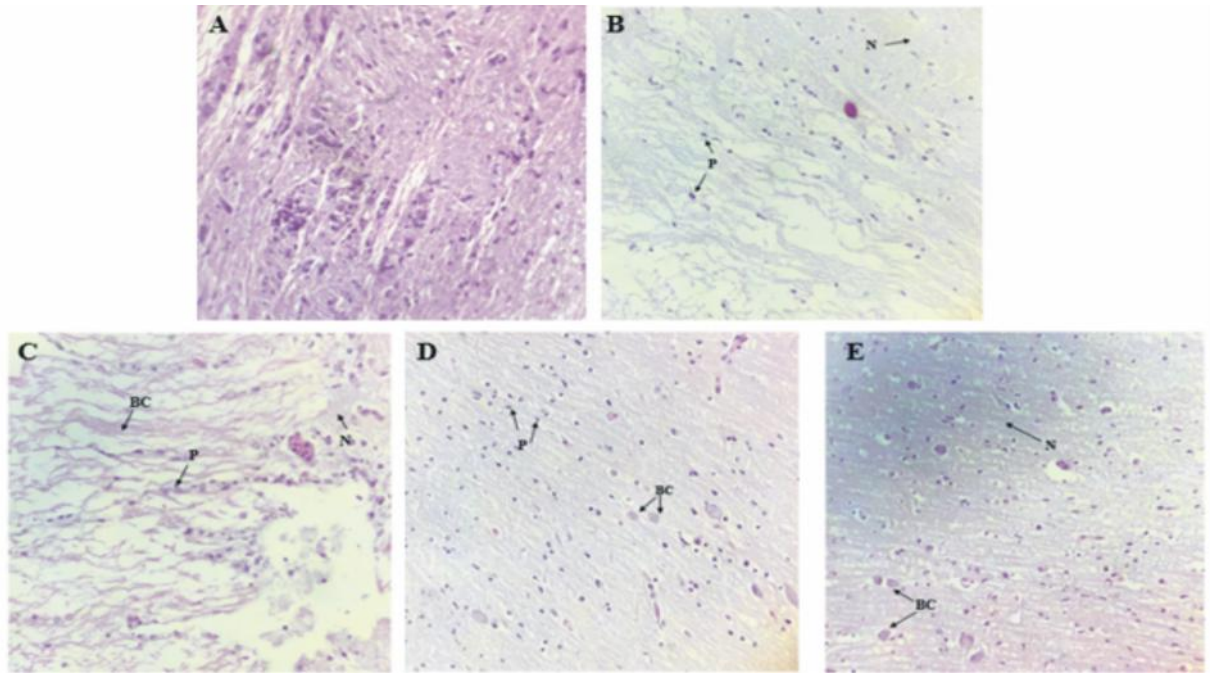

Figure 4. Sublethal effects of atrazine on brain histology of *S. esocinus.* A shows Brain structre of control fish while Brain tissues of treated fish B to E shows Blood congestion (BC), Necrosis (N) and Pyknosis (P).

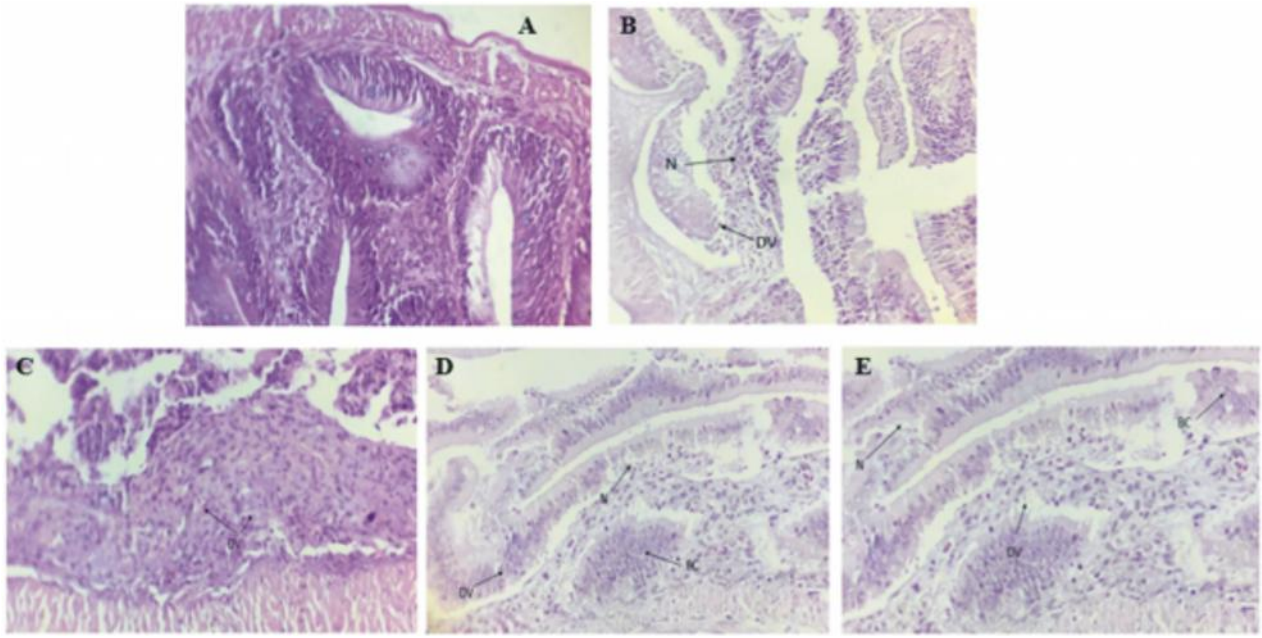

Figure 5. Sublethal effects of atrazine on intestine histology of *S. esocinus.* A shows Intestine structure of control fish. Intestine tissues of treated fish B to E Shows Necrosis (N), Pyknosis (P), Detachement of villi (DV) and Blood Congestion (BC).

DISCUSSION

This study was conducted to understand the adverse effects of atrazine on the hematological, histopathological and biochemical parameters of *schizothorax esocinus*.

Fish hematology has been evidenced as a best technique to perceive changes in fish structure and such changes results in behavior of fish (Authman *et al.,* 2015). Hematological and biochemical keys might be helpful in analyzing the physical and practical grade of fish exposed to chemicals (Adhikari *et al.,* 2004; Evans

and Claiborne 2005). Atrazine is lethal; regularly acquisitive and determined (Fernando *et al.,* 1992). In *C. carpio* changes in blood considerations i.e, RBC count (-63.17%), hemoglobin (-27.35%), plasma glucose (6.78%) and plasma protein (-18.73%) levels were observed low comparing to control group while WBC count (+3.73%) increased (Ramesh *et al.,* 2009). Our results are in comparison with Ramesh *et al.* (2009), as in our study decrease is observed in hemoglobin but increase was observed in WBC level. Increase was observed in biochemical parameters such as cholesterol, creatinine, glucose, protein.

ALT is mainly existing in hepatocytes and its action become increased after which replicate liver mutilation (Mikulikova *et al.,* 2013). Comparable results were reported by Neskovic *et al.,* (1993) after sub-acute (14 days) experience to atrazine in range of 1.5–6mg·L^{-1} in common carp. Activity of ALP proved increased in ranging from 75 to 200% matched to the control group, while ALP actions in tissues (heart, liver, and kidney) revealed significant reduction likened to the control group (Neskovic *et al.,* 1993). Our results are similar with the finding of these authors as in our study increase was also observed in ALT, AST and LDH levels.

According to Ramesh *et al.* (2009) changes in hematology and biochemistry were statistically significant ($p \leq 0.05$) however, WBC count was not significantly altered. Hussein *et al* (1996) described reduced RBCs number, hemoglobin concentration and haematocrit percentage of *Oreochromis nitoticus* and *Chrysichthyes auratus* exposed to 3 and 6 mg/l ATR. Puigdoller *et al.* (2007) described significant rise in hematocrit in Atlantic salmon when exposed to ATR. In our study decrease was observed in RBCs number, haematocrit level and hemoglobin.

Prasad *et al.* (1991) established injury in gill lamellae, reducing breathing ability in Tilapia mosambica exposed to 1.1 mgl^{-1} ATR. Horn and Hanke (1980) establish decline in erythrocytes when *C. carpio* was exposed to 0.1 mg/l atrazine. Ventura *et al* (2008) reported great regularity of micronuclei and nuclear aberrations in *O. niloticus* exposed to atrazine. Erythrocytes were reported low in fishes exposed to traumatic situations. Deviations in erythrocyte level need oxygen requirements in response of gill destruction (Drastichova *et al.,* 2004). Stoppage of erythropoiesis and rise in degree of erythrocyte damage in hematopoietic organs is the reason of reduction in RBC level (Joshi *et al.,* 2002). In our study, decrease is observed in WBC count and Hb level. Our result is supported by Rehwoldt (1978), who established significant reduction of RBCs, Hb and packed cell volume (PCV) in fishes exposed to atrazine and specified lethal effects of atrazine on spleen, liver and anterior kidney.

Atrazine is quickly metabolized in liver and kidney and is evacuated without any significant addition in tissues of fish species. Studies recognized changes in gills tissues because of their direct interaction with water, which permits ingredient into fish body (Graymore *et al.,* 2001; Solomon *et al.,* 2008). So contact with atrazine fish is potently related with deteriorating deviations in kidney and gills and modifications in liver tissues (Fischer-Scherl *et al.,* 1991). In our study fishes exposed to atrazine presented disorder of secondary gills lamellae, shortening of secondary gills lamellae and fusion of secondary gills lamellae and mild blood congestion, epithelial lifting, atrophy, disruption of cartilaginous core, blood congestion, lamellar disorganization and curling of gills, while fishes exposed to atrazine shows pyknosis, lamellar infusion and mild pyknosis, blood congestion, necrosis and vacuolations in liver.

Fischer-Scherl *et al.* (1991) reported changes in many components of renal corpuscles and renal tubules in rainbow trout (*Oncorhynchus mykiss*) when exposed to atrazine 5–40 μgL^{-1} for 28 days. Necrosis was observed in endothelial cells and renal hemopoietic tissue in experimental group exposed at the concentration of 80–2800 μgL^{-1}. Alike results were reported when rare minnow (*Gobiocypris rarus*) was exposed to atrazine for 28 days; histological comment exposed injuries in kidney tissues with wide spread extension in lumen, degenerative and necrotic deviations of tubular epithelia, and contraction of the glomeruli at the concentration of 10 μgL^{-1}. Similar concentration showed clear wounds in gill comprising hyperplasia, necrosis in epithelium region, aneurysm, and lamellar fusion (Yang *et al.,* 2010). Likewise, gills variations were witnessed after severe 6h exposure to atrazine of *Gnathonemus petersii* which results in breaking of epithelium at 0.5$mgL^{-1}$and established into deep pits at 5.0 mgL^{-1} (Alazemi *et al.,* 1996). In present study gills exposed to atrazine indicated disorder of secondary gills lamellae, shortening of secondary gills lamellae and fusion of secondary gills lamellae and mild blood congestion, epithelial lifting, atrophy, disruption of cartilaginous core, blood congestion, lamellar disorganization and curling of gills. Muscles exposed to different concentration of atrazine shows blood congestion at various spots. Brain exposed to atrazine show mild necrosis and blood congestion, mild pyknosis and necrosis, while intestines of *Schizothorax esocinus* exposed to atrazine show mild necrosis, mild pyknosis, necrosis, blood congestion and detachment of villi.

Conclusion: It is concluded from this study that atrazine can impose negative impacts on hematology, histopathology and biochemical parameters of *Schizothorax esocinus*. This study might be useful to access the previously unexpected possible environmental threats of atrazine to aquatic life.

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