



Contemplating Toxicity of Atrazine on Lipid Profile of Fresh Water Fish (*Ctenopharyngodon idella*): An Experimental Approach

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Abstract: Productivity of agriculture is dependent upon the utilization of herbicides but they find avenues to get into water bodies, affecting aquatic fauna, particularly fish. The current effort accordingly focused to contemplate toxicity of sub lethal concentration of herbicide i.e. atrazine by estimating the lipid profile that included Cholesterol (CH), Triglyceride (TG), High density lipid (HDL) and Low density lipid (LDL) of freshwater grass carp, (*Ctenopharyngodon idella*) for (24, 48, 72, 96, 240, 360 and 600 hrs) under the dose (15, 13, 10, 08, 06, 04 and 02 $\mu\text{l L}^{-1}$) respectively. Merck micro lab 300 biochemistry analyzer was used for analyzing lipid profile. Acute toxicity exposure explored that all components of total lipids documented decline in concentration against each time period and maximum decrease was observed against 72h exposure respectively. Similarly chronic toxicity exposure also indicated the decline against each time period and maximum decline in CH, TG and LDL was observed against 600h while HDL showed reduction in concentration against 360h exposure respectively. In all component of the lipid profile, significant decline in concentration was observed as; $P < 0.05$, $P \leq 0.01$ and $P \leq 0.001$, specifically in acute toxicity groups as compared to chronic toxicity groups thus showing undesirable effects of on aquatic fauna present inside water bodies.

Keywords: Acute toxicity, Grass Carp, Cholesterol, Triglyceride.

1. INTRODUCTION

The water pollution has become the growing concern in the recent years mainly the toxic pollutants that have altered the ecological balance by accumulating in the aquatic environment, decreasing the productivity and fecundity of aquatic life thus, effecting the humans that rely on those organisms as a major source of protein [1]. All animals are affected by such toxicant but fish the most vulnerable among these organisms is heavily exposed to different toxicants with no escape from the pollution and serves as the most important bio monitors for estimation of metal pollution level [2].

Herbicide plays an important role in agriculture production and considered as an important part of agronomy [3]. The agriculture products can be increased by implanting the herbicide which ends in killing the undesired plants and leaving the desired

crop [4]. But the implementation of this herbicide also disturbed the ecosystem, i.e. adherence of herbicide particles to wind ends in wind pollution thus effecting wildlife and birds. Adherence to herbs thus affecting the domesticated animals viz grazing as food chain and adherence of particles to soil can affects the soil microorganism. Furthermore, these particles viz wind, soil erosion and surface runoff these particles finds the ways to aquatic bodies thus effecting the aquatic organisms particularly fish biodiversity and has been threatened by producing variation in its physiological system and similarly accumulating of these toxic substances in tissues finds it end in human bodies through food chain. [5].

Herbicide alters the ecosystem of every fauna present inside aquatic bodies, particularly fish [6]. Alteration in the ecosystem effects the enzymatic and hormonal activities of fish thus influences its physiology, behavior, growth as well as

reproduction [7]. The high toxic media also ends in excess amount of the mucus which in combination with water produces unpleasant smell, rapid movement of the body, degenerating of fin rays that led to losing of the balance, suffocation that effect the respiration, changes of body color etc [8].

Atrazine was formulated in 1958 as the second series of 1, 3, 5-triazines. It was the succeeding mostly used herbicide after glyphosate in U.S during 2014 and used to avoid the pre-and post-emergence broadleaf weeds or grassy weeds in different crops for example, maize (corn), sorghum, sugarcane, pine, lupins, eucalyptus plantations and triazine tolerant (TT) canola [9]. Atrazine the most important endocrine disruptors are banned in the US and other countries while some countries used it to reduced the pre and post growth of the weed in different crops [10]. Atrazine after being applied to soils it does not break down within a few weeks because of its half-life that ranges from 13 to 261 days in soil. Because of high mobility i.e. about 600 miles from the point of application it has been spotted inside aquatic bodies [11]. Different analysis have proved the harmful effects of atrazine on fish hematological parameters, locomotor activities, metabolism, immune responses, osmoregulatory disturbance, oxidative stress and reproduction of fishes [12-18]. In this regard the present study was design to scrutinize the toxicity of atrazine to grass carp (*Ctenopharyngodon idella*) by subsequently finding out its acute (24, 48, 72 and 96hrs) and chronic toxicity (240, 350 and 600 hrs) via undertaking the evaluation of lipid profile including cholesterol, triglyceride, high density lipid and low density lipid.

2. MATERIAL AND METHODS

2.1 Maintenance of Experimental Fish

Grass carp (*Ctenopharyngodon idella*: 8.5 ± 5.5 cm; 9.5 ± 6.5 g) were procured from carp hatchery of Mardan and Peshawar and acclimatized for two weeks in aquarium having tap water and then were shifted to experimental tanks. Both in acclimatization and experimentation tanks fish were fed with commercial carp pellet diet (Oryza Organics, Pakistan) on each alternate day. Physiochemical parameters of water were also recorded on every alternate day during acclimation and exposure period and were found in permissible limits as per the recommended values of APHA and

American Public Health. Different water quality parameter was also checked on every alternate day including pH (normal), temperature (normal), total hardness; 95mg/l, calcium hardness; 61.6mg/l, magnesium hardness; 35mg/l, water conductivity; 431 μ S/cm, DO; 7.37ppm, TS; 321mg/l, TDS; 221mg/l, TSS; 100mg/l, total alkalinity; 163.3mg/l and chloride concentration; 20.3mg/l and all these values indicated normal parameter concentrations.

2.2 Experimental Design

Fish were divided in to four groups of 10 fish per group for acute toxicity analysis and exposed to herbicide (atrazine) for 24, 48, 72 and 96 hours respectively. Group1 to 4 were treated against dose of 15 μ L⁻¹ for 24h, 13 μ L⁻¹ for 48h, 10 μ L⁻¹ for 72h and 08 μ L⁻¹ for 96h respectively. Similarly for chronic toxicity fish were divided in to three groups of 10 fish per group and each group was exposed against dose of 6 μ L⁻¹ for 240h, 4 μ L⁻¹ for 360 and 02 μ L⁻¹ for 600h respectively.

The experiment was conducted in semi-static conditions, following OECD guideline number 203 [19]. After the stipulated time, three fish were randomly selected and anesthetized using clove oil [20]. The anesthetic was prepared fresh by dissolving clove oil into absolute alcohol (Merck, Germany) in a ratio of 1:2.

2.3 Blood Collection and Preservation

Samples of blood were collected from caudal vein of fish and sometimes from direct puncturing of fish heart. The blood was obtained with the help of heparinized hypodermic syringes that contains heparin for avoiding blood clot [21-22]. After collection of blood, the tubes were kept in ice box and then were shifted to lab for further analysis. EDTA tubes and gel tube were utilized for storage of blood samples and serum was obtained from blood viz centrifugation at 3000rpm.

2.4 Estimation of Lipid Profile and Statistical Analysis

Merck micro lab 300 biochemistry analyzer was used for analyzing lipid profile. Results were statistically reported by SPSS software.

3. RESULTS

Cholesterol concentration during acute and chronic toxicity evaluation against atrazine declined. Maximum highly declined in concentration ($P \leq 0.001$) was noted after exposing fish for 48 and 72h. However, a slight rise in concentration was noted with exposure for 96h, but the decline in concentration was highly significant ($P \leq 0.01$) as shown in the table 2 and fig.1. Likewise, during chronic toxicity highly significant decline ($P \leq 0.01$) in concentration was noted after exposure for 360h while maximum highly significant decline ($P \leq 0.001$) in concentration was observed after 600h exposure as evidenced in the table 3 and fig. 1. TG concentration was a highly significant decline ($P \leq 0.01$) after exposure of fish for 72hrs during

acute toxicity as noted in table 2 and fig.2 while during chronic toxicity TG concentration was highly significant decline ($P \leq 0.01$) after exposure for 600h as shown in the table3 and fig. 2.

Highly significant decline ($P \leq 0.01$) was observed after exposure for 48hrs while maximum highly declined ($P \leq 0.001$) was observed in HDL after exposure for 72hrs and 96hrs during acute toxicity, as indicated in the table 2 and fig. 3. During chronic toxicity HDL concentration was a highly significant decline ($P \leq 0.01$) after exposure for 360 and 600h respectively as indicated in the table3 and fig. 3. Similarly LDL concentration was highly significant declined ($P \leq 0.01$) after exposure of fish for 24 and 48h respectively, while maximum highly significant decline ($P \leq 0.001$) was noticed

Table 1. Lipid Profile concentration of control group

S.No.	Lipid Profile	Fish 1	Fish 2	Fish 3	Mean \pm SD	Standard error of mean
1	Cholesterol (mmol/L)	180	186	184	183.3 \pm 3.0	1.7
2	Triglyceride (mmol/L)	65	60	63	62.3 \pm 2.5	1.4
3	HDL (mmol/L)	148	140	144	144 \pm 4.0	2.3
4	LDL (mmol/L)	35	40	44	39.6 \pm 2.6	1.5

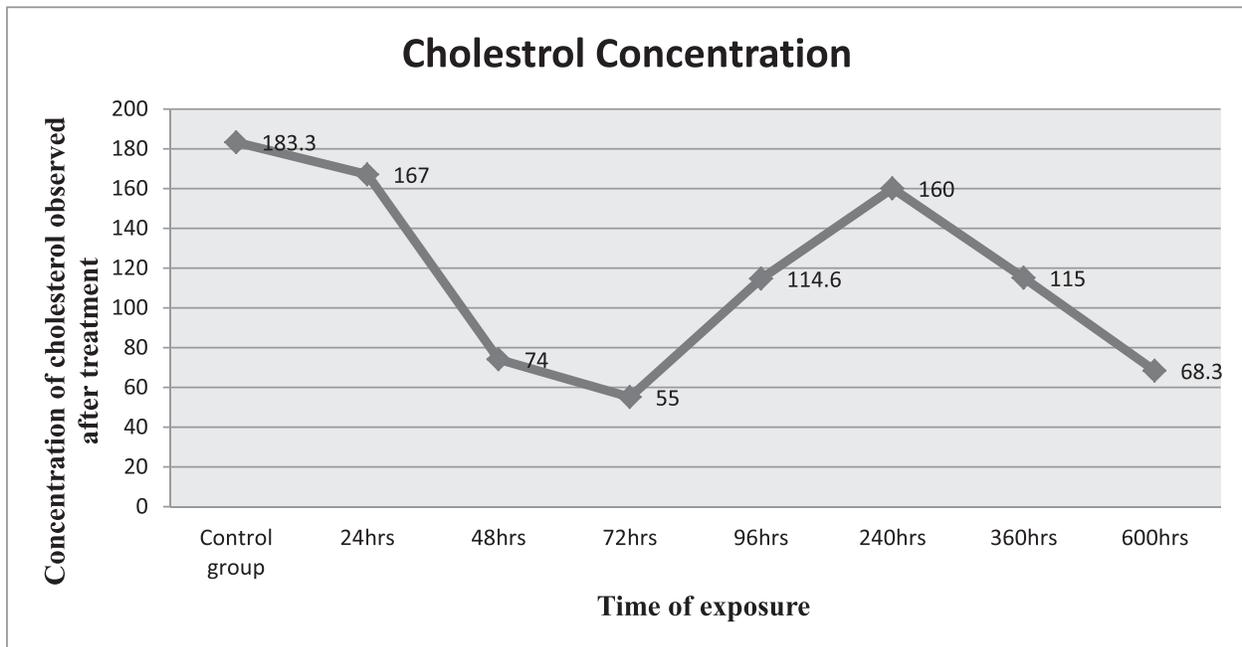


Fig. 1. Comparison of cholesterol concentration between control group and treated groups (acute and chronic toxicity)

Table 2. Lipid Profile concentration of control group

S.No.	Lipid Profile	Hours of treatment	Fish 1	Fish 2	Fish 3	Mean \pm SD	Standard error of mean	Paired T test value	Significant Value (P)
1	Cholesterol (mmol/L)	24hrs	178	160	165	167.6 \pm 9.2	5.3	2.19	0.15NS
		48hrs	77	78	67	74 \pm 6.0	3.5	26.69	0.001***
		72hrs	52	58	55	55 \pm 3.0	1.73	385.00	0.000***
		96hrs	114	110	120	114.6 \pm 5.0	2.90	18.49	0.003**
2	Triglyceride (mmol/L)	24hrs	60	54	50	54.6 \pm 5.0	2.90	3.17	0.086NS
		48hrs	45	50	50	48.3 \pm 2.88	1.66	4.83	0.04 NS
		72hrs	26	28	32	28.6 \pm 3.6	1.76	13.52	0.005**
		96hrs	60	62	52	58 \pm 5.2	3.0	1.24	0.34 NS
3	HDL (mmol/L)	24hrs	131	140	136	135.6 \pm 4.5	2.60	1.69	0.23 NS
		48hrs	80	90	92	87.3 \pm 6.4	3.71	9.94	0.01**
		72hrs	30	35	36	33.6 \pm 6.80	3.92	28.07	0.001***
		96hrs	53	40	56	49.6 \pm 8.5	4.91	27.10	0.001***
4	LDL (mmol/L)	24hrs	35	34	36	35 \pm 4.16	2.40	1.94	0.019**
		48hrs	18	18	24	20 \pm 3.4	2.0	13.53	0.005**
		72hrs	15	20	22	19 \pm 3.6	2.08	31.00	0.001***
		96hrs	35	40	40	38 \pm 2.3	1.33	1.0	0.423 NS

[Significant Value =P<0.05] [High Significant value=P \leq 0.01] {Maximum highly significant value= P \leq 0.001}

[Non Significant Value= P>0.05]

[Significant=*] [Highly Significant =**] [Maximum highly significant value=***] [Non Significant= NS]

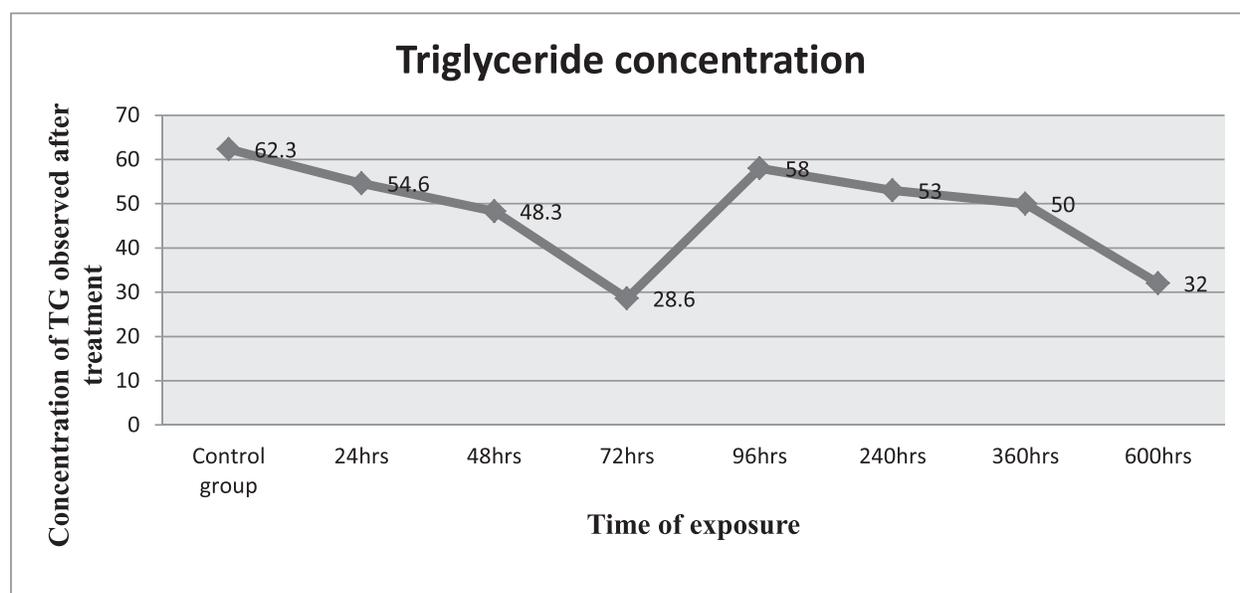


Fig. 2. Comparison of TG concentration between control group and treated groups (acute and chronic toxicity)

Table 3. Lipid Profile concentration of control group

S.No.	Lipid Profile	Hours of treatment	Fish 1	Fish 2	Fish 3	Mean ± SD	Standard error of mean	Paired T test value	Significant Value (P)
1	Cholesterol (mmol/L)	240hrs	160	155	165	160±6.65	3.84	6.07	0.26NS
		360hrs	120	115	110	115±7.37	4.25	16.05	0.004**
		600hrs	60	70	75	68.3±5.56	3.21	35.77	0.001***
2	Triglyceride (mmol/L)	240hrs	55	54	50	53±2.6	1.52	4.76	0.41 NS
		360hrs	50	52	48	50.0±4.04	2.33	5.42	0.032NS
		600hrs	30	28	38	32±5.2	3.05	10.31	0.009**
3	HDL (mmol/L)	240hrs	108	110	100	106±5.2	3.0	9.12	0.012*
		360hrs	80	92	83	85±6.2	3.60	10.06	0.01**
		600hrs	75	74	80	76.3±3.2	1.85	24.80	0.002**
4	LDL (mmol/L)	240hrs	36	34	38	36±2.0	1.15	1.57	0.25NS
		360hrs	28	18	22	22.6±5.0	2.90	3.40	0.07NS
		600hrs	20	20	22	20.6±1.15	0.66	9.12	0.01**

[Significant Value =P<0.05] [High Significant value=P≤0.01] {Maximum highly significant value= P≤0.001}
 [Non Significant Value= P>0.05]
 [Significant=*] [Highly Significant =**] [Maximum highly significant value=***] [Non Significant= NS]

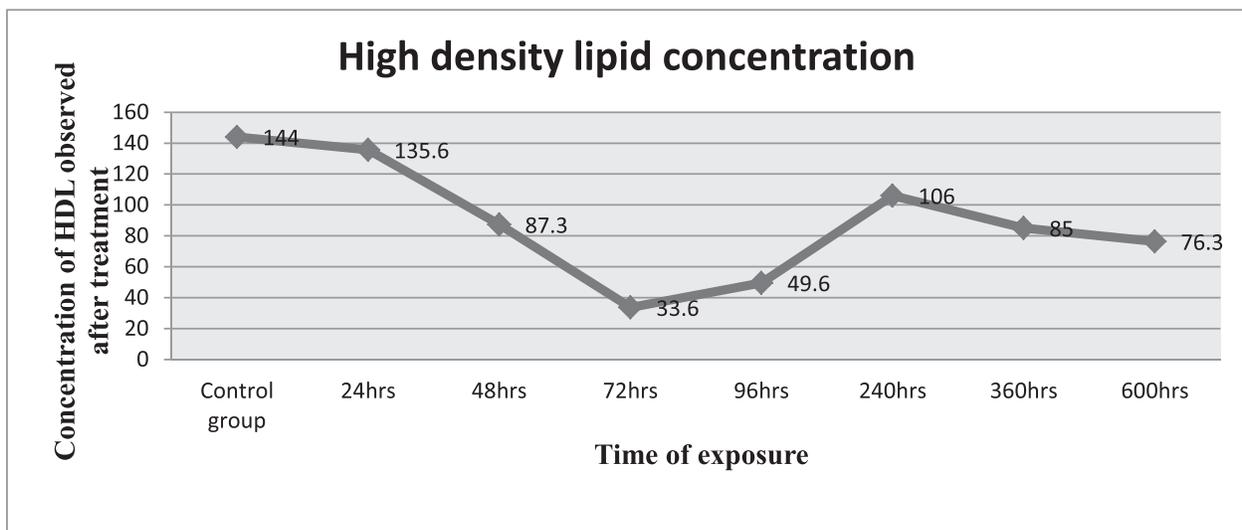


Fig. 3. Comparison of HDL concentration between control group and treated groups (acute and chronic toxicity)

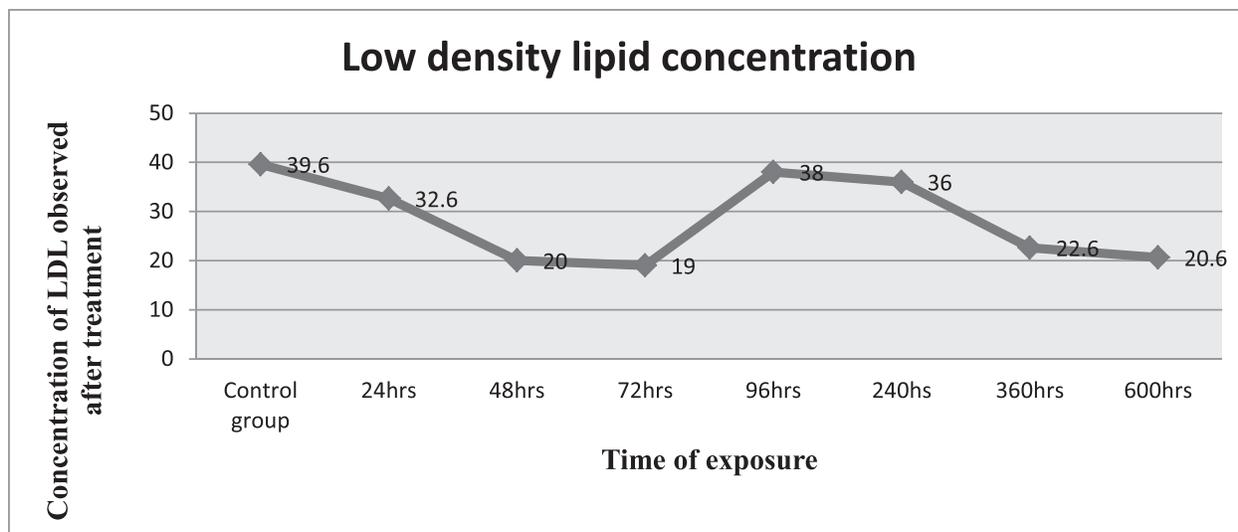


Fig. 4. Comparison of LDL concentration between control group and treated groups (acute and chronic toxicity)

after exposure for 72hrs during acute toxicity, as shown in the table 2 and fig. 4. During chronic toxicity highly significant decline ($P \leq 0.01$) was observed after exposure for 600h as shown in the table 3 and fig. 4.

Present findings indicated that lipid profile of grass carp was declined against various doses of atrazine in both acute and chronic toxicity and difference was observed in significant ($P < 0.05$), highly significant ($P \leq 0.01$), and maximum highly significant ($P \leq 0.001$) when herbicide treated group was compared with the control group and comparatively effect was more pronounced in the acute toxicity group as compared to chronic toxicity groups.

4. DISCUSSION

The most common cause of water pollution is runoff of domestic and industrial waste that is directly released into streams or ponds without treatment. Waste is consisted of various pollutants including; heavy metals, radioactive substances, herbicides and corrosive substances like acids and bases [23]. However, in Pakistan, another prominent source of aquatic pollution is agriculture industry; where growers use herbicides to manage herbs (unwanted plants) which obstruct the growth of undesired plants. But on the rear end, these herbicides find avenues to water bodies thereby affecting aquatic fauna. In response to a stressor such as herbicide exposure, the fish undergo a series of biochemical

and physiological alteration in an effort to cancel the challenge imposed on them. Therefore, blood parameters such as hematological and biochemical indices can serve as important markers for diagnosing the structural and functional status of fish exposed to herbicide [24].

Atrazine affects the fish in different ways like alteration in blood parameters of *Cyprinus carpio*, fat oxidation and antioxidant enzyme of *Channa punctatus*, after exposing to different concentration of atrazine herbicide [25]. Atrazine is toxic to aquatic animals and exposure of fish to atrazine result in biochemical parameters alteration, behaviorally abnormality, structurally deformation, causing stress, on reproduction, on the immune system by quantifying white blood cells etc [26-29].

Lipids serve as vital source of energy by providing structural components for reproductive growth [30]. In the present study, grass carp (*Ctenopharyngodon idella*) was exposed to various doses of atrazine for short term (acute toxicity) as well for the long term (chronic toxicity) to scrutinize lipid profile concentration. The results indicated markedly decline (denoted by $P < 0.05$, $P \leq 0.01$ and $P \leq 0.001$) in a concentration of lipid profile, including cholesterol, triglyceride, high density lipids and low density lipids. For justification of the present study, reduced level of lipid content in the organs of the fish after 96 hours exposure to atrazine were found to be 1.56 ± 0.13 mg/100mg wet tissue, 1.90 ± 0.04 mg/100mg wet tissue and 1.23 ± 0.19 mg/100mg wet tissue in the gills, liver

and kidney respectively. It might be ascribable to the diminution in the absorption of carbohydrate and protein, resulting in the depletion of energy during toxic stress, which contributes to the degradation of lipid to combat the required energy.

As the level of the protein and carbohydrate absorption decreases the lipid level also decreases due to lipid metabolism to match the needed energy during the stress condition [31]. The present study was in agreement with El-Sayed et al [32] who illustrated that the decrease in body lipid in appropriate habitat was a direct of utilization of body fat as an energy supply to meet the increase in physiology demands. So, to manage with stress situation the fish utilized the fats in the body to raise energy to overcome such stress situation.

Khan et al [33] observed effects of cadmium on biochemical contents in liver and ovary of *Garramalaya* and found that a substantial decrease in cholesterol and stated that this may be due to general damage. Shakooret al [34] examined the result of sub lethal doses of fenvalerate on the blood, liver and muscles of fish *Ctenopharyngodon idella* and observed decreased level of cholesterol. Virk and Sharma [35] studied biochemical changes induced by nickel and chromium in the liver of *Cyprinus carpio* and observed a significant diminution in the cholesterol content of the liver.

Triglyceride is the storage form of fats and major resources of oils and fat, which are flowing into the blood. The decrease in the quantity of cholesterol may be connected to its utilization in the manufacture of cortisol arising from stress created by the toxin atrazine [29]. The reduction of triglyceride volumes in blood plasma at high concentrations of the toxin atrazine could be due to the imbalance created by the higher concentrations of the toxin, affecting the digestive system, liver and related enzymes as well as hormonal and natural metabolic imbalance in fish studied.

In conformity with present study cholesterol, triglyceride, high density lipid and low density lipid have been discovered to be declined after famous treatment (200, 400, 800rpm) at different time interval (1, 7, 14, 21 and 28 days). It may be caused by utilization of cholesterol and other lipid fraction of treated fish to counteract toxic stress and stabilized the molecules of toxicants [36]. Further, this may be attributed to hindrance

in lipid metabolism [36]. In accordance with the present finding, the similar decreased lipid profile has been reported by Ghosh 1988 [37] who reported the decline concentration of cholesterol in *Channa punctatus* against the chromium. Similar in *Clarias batrachus* same findings were observed by Khareet al [38] which was exposed to malathione. Furthermore, Sehgal and Goswami [39] Rani et al [40] Shankar and Kulkarani [41] observed the same findings in *Channa punctatus*, *Tilapamos sambuca*, *Notopterus notopterus* and *Cirrhinus mrigala* respectively. Therefore, all of the above studies were in accord with the present findings and therefore indicate the present resolutions.

Against stress, lipid profile concentration was also increased as indicated by different studies which are not in agreement with the present study. Intensity of hyper-lipemic state may reflect the degree of stress imposed on the animal under the influence of toxic reagents and environmental pollutants [42]. The increase of total plasma lipids may be due to the increase of lipid peroxides formation induced by the effect of butataf herbicide as previously reported by Mousa 2004 [43]. Otherwise, the destruction of the liver cells and other organs due to the effect of the butataf herbicide increase the levels of total lipids in the plasma [43-44].

5. CONCLUSION

Biochemical indices (lipid profile) of grass carp revealed that effects of atrazine herbicide. These fluctuations in biochemical indices can be considered as sensitive biomarkers i.e. for evaluating animal's health, especially in herbicides effecting region that causes stress to fish on exposure. The present results have proved the hazardous effects of the herbicide on the natural ecosystem with an alarming increase in pollution over the years. The current findings proved to clarify the risk of atrazine herbicide on lipid profile of grass carp fish. Decrease in lipid profile concentration [denoted by $P < 0.05$ (significant), $P \leq 0.01$ (highly significant) and $P \leq 0.001$ (maximum highly significant)] was seen in all components of the lipid profile against various doses of atrazine, thus showing the contrary effects of atrazine on aquatic fauna.

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