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Original Research

Quantitative Determination of Lethal Concentration Lc_{50} of Atrazine on Biochemical Parameters; Total Protein and Serum Albumin of Freshwater Fish Grass Carp (*Ctenopharyngodon idella*)

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Abstract

The indiscriminate use of herbicides has led to the contamination of water bodies, possibly affecting the health of aquatic biota, especially fish. Atrazine is considered as toxicants for aquatic fauna, due to its high persistence in soil, high half-life and high mobility toward aquatic bodies as well as high solubility in water. The objective of the present study was to determine (LC_{50}) and to evaluate the acute and chronic toxicity of atrazine on the biochemical parameters; total protein and serum albumin of freshwater grass carp (*Ctenopharyngodon idella*). Above 15 μlL^{-1}, the LC_{50} was recorded revealing sensitivity of grass carp to atrazine. Grass carp was exposed to atrazine for 01 (15 μlL^{-1}), 02 (13 μlL^{-1}), 03 (10 μlL^{-1}), and 04 (08 μlL^{-1}) days/concentration for scrutinizing acute toxicity. Likewise, fish were exposed to atrazine for 10 (06 μlL^{-1}), 15 (04 μlL^{-1}), and 25 (02 μlL^{-1}) days/concentration for scrutinizing chronic toxicity. Control group concentration was 8.3 gL^{-1} and 3.5 gL^{-1}. Total protein concentration observed for acute toxicity was 7.5 gL^{-1}, 6.5 gL^{-1}, 4.6 gL^{-1}, and 3.2 g/L and serum albumin concentration was 2.7 gL^{-1}, 1.6 gL^{-1}, 1.4 gL^{-1}, and 1.1 gL^{-1} respectively. Similarly total protein concentration observed for chronic toxicity was 8.2 gL^{-1}, 6.8 gL^{-1}, and 4.3 gL^{-1} and serum albumin concentration was 2.1 gL^{-1}, 1.7 gL^{-1}, and 1.4 gL^{-1} respectively. Markedly decline (denoted by $P<0.05$, $P\leq0.01$ and $P\leq0.001$) was noticed

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in both the parameters concentration during acute as well as chronic toxicity, when compared with control group concentration, indicating negatively impinge of atrazine on grass carp as well as atrazine present in aquatic bodies must jeopardize the health of other aquatic fauna.

Keywords: acute toxicity, grass carp, total protein, serum albumin, atrazine

Introduction

The presence of pesticides in the environment has caused significant social and scientific development anxiety worldwide, as their extensive usage globally can create potential risks to the environment, especially aquatic bodies, resulting in extensive damage to non-target species, particularly fish [1]. The aquatic ecosystems are known to receive a wide spectrum of pollutants, which may be introduced to it directly or indirectly and herbicide are one of these pollutants representing a serious environmental problem [2-4]. The major source of contamination by these herbicides is the deposits resulting from their application to control agriculture pests and harmful aquatic herbs [5]. In modern farm practices chemicals play the most important roles and are considered as integral part of modern agriculture systems [6-7]. But it has been found that chemicals like agricultural herbicides used against undesirable herbs also have adverse side-effects on the environment [8]. It has been confirmed by environmental toxicology studies that herbicides affect non-target species in the environment [9]. Agricultural production can be improved by the use of herbicides, but unfortunately it may reach non-targeted areas and can impact non-target organisms, especially aquatic species and their environment [10]. During application their particles can volatilize and effect different areas like; adhering to wind contributing to wind pollution, effect wildlife, birds, domesticated animals via grazing, soil microorganisms, aquatic organisms particularly fish via; reducing their biodiversity, alteration in reproductive and behavioral responses, increased disease susceptibility and accumulation of toxic substances that can reach human via food chain thus affecting human being as well [11]. Specially they can effects the physical and chemical composition of aquatic bodies and can affect all the fauna present in aquatic bodies, especially fish [12]. They can disturb enzymatic and hormonal activities of fish. High doses of herbicides can kill fish while low doses can affect fish behavior, physiology, growth, and reproduction [13]. Fish exposed to any medium have toxicant showed, restlessness, rapid body movement, convulsions, difficulty in respiration, excess mucous secretion, change in color, and loss of balance [14].

Atrazine (2-chloro-4-thylamino-6-isopropylamino-striazine, ATZ) is one of the most commonly used herbicides. Its utilization is controversial worldwide, as it is currently banned in Europe but not in the U.S. and other countries [15]. Different studies have been conducted on atrazine's effects on fish, as in ATZ affecting hematological parameters, locomotor activity, immune response, metabolism, oxidative stress, osmoregulatory disturbance and reproduction [16-22]. So in accordance with these effects, the present study was conducted having aims and objectives, to scrutinized the toxicological impinge of atrazine on grass carp (Ctenopharyngodon idella) following acute and chronic toxicity via undertaking the evaluation of biochemical parameters; total protein and serum albumin.

Methodology

Experimental Fish

The healthy and active specimens of grass carp (*Ctenopharyngodon idella*) were selected as a model for the present investigation and were procured from Sherabad Hatchery, Peshawar, Khyber Pakhtunkhwa, Pakistan.

Maintenance of Experimental Fish

After procuring, all fishes were carefully analyzed and treated with 0.2% KMno4 solution for two minutes before stocking to get rid of any dermal infection. Fish were transferred to acclimatization tanks containing tap water for two weeks for acclimatization purpose. After acclimatization fishes were transferred to experimental tanks containing tap water for experimentation. Fish were fed properly with commercial food on every alternate day in both acclimatization and experimentation tanks. The pH and temperature of the water in tanks were kept constant and checked every other day. Other parameters were additionally checked in both acclimatization and experimental tanks, including total hardness, calcium hardness, magnesium hardness, water conductivity, dissolved oxygen, total solids, total dissolved solids, total suspended solids, total alkalinity and chloride having concentrations like 95 mg/l, 61.6 mg/l, 35 mg/l, 431 µS/cm, 7.37 ppm, 321 mg/l, 221 mg/l, 100 mg/l, 163.3 mg/l and 20.3 mg/l, respectively indicating that all of these parameter concentrations were in normal range.

Experimental Design

For the research purpose, one group was considered as the control group consisted of 05 fishes and kept without treatment. For acute toxicity analysis, 26 fishes were taken and made up of four groups. Group (1 and 2) consisted of 05 fishes each which was treated under a dose of 15 µl/L and 13 µl/L for 01 and 02 days respectively. Group (3 and 4) consisted of 08 fishes each which was processed under a dose of 10 µl/L and 08 µl/L for 03 and 04 days respectively. For chronic toxicity analysis 40 fishes were

taken and divided into three groups. Group 1st, 2nd and 3rd consisted of 10, 15 and 15 fishes and was treated under dose of 06, 04 and 02 µl/L for 10, 15 and 25 days respectively.

Blood Collection and Preservation

Blood samples were collected from the freshly anesthetized fish on the spot. For anesthetizing of fish, chemical MS 222 was used. Blood samples were collected from the caudal vein of fish and sometimes from direct puncturing of heart of fish. Blood samples were collected with the help of hypodermic syringes which were heparinized with the help of a few drops of heparin to avoid blood clotting [23, 24].

After collection, blood samples were stored in EDTA tubes having ethylenediaminetetraacetic acid and were rotated gently for mixing of blood with ethylenediaminetetraacetic acid for preventing blood clotting. Gel tubes were also utilized for storing blood samples and were found more beneficial as compared to EDTA tubes because blood biochemical parameters are determined from the serum of the blood and gel tube have a special layer of gel which is helpful in isolation of blood cells from the serum of the blood via centrifugation (3000 RPM). After assembling, all blood sample tubes were shifted to the ice box and conveyed to the lab for analysis of blood total protein and serum albumin.

Estimation of Total Protein and Serum Albumin

For analysis of total protein and serum albumin the biochemical analyzer set (Merck micro lab 300 biochemistry analyzer) protocol was followed.

Statistical Calculations

Results were reported in mean, standard deviation and standard error of mean. SPSS software was used to calculate paired T test to detect the significant (P value) difference between control and experimental means, and represented as $P<0.05$ = significant, $P\leq0.01$= highly significant and $P\leq0.001$ = maximum highly significant and result reported above these values are represented as nonsignificant.

Results

Concentrations of Control Group

Total protein concentration for the control group was; for fish 1 = 8.2 g/L, fish 2 = 8.8 g/L and fish 3 = 8.0 g/L, with a mean value of 8.2 g/L, standard deviation value 0.4 and standard error of mean = 0.2. Likewise serum albumin concentration for the control group was; for fish 1 = 3.6 g/L, fish 2 = 3.4 g/L and fish 3 = 3.5 g/L, with a mean value of 3.5 g/L, standard deviation value 0.1 and standard error of mean = 0.05.

Concentration Obtained After Treatment

Acute Toxicity (Figs1 and 2)

Total protein

Total protein concentration obtained after treatment of fish for 01 day was: fish 1 = 7.8 g/L, fish 2 = 7.0 g/L and fish 3 = 7.8 g/L, with a mean ± S.D value 7.5 g/L ± 0.46, standard error of mean = 0.26 and paired t test value = 1.58 with a significant (p) value = 0.25 (Non-Significant). Concentration obtained after treatment of fish for two days were: fish 1 = 6.5 g/L, fish 2 = 6.3 g/L and fish 3 = 6.8 g/L, with a mean ± S.D value = 6.5 g/L ± 0.25, standard error of mean = 0.14 and paired t test value = 4.75 with a significant (p) value = 0.04 (Significant, $p<0.05$). Concentration obtained after treatment of fish for three days were: fish 1 = 4.0 g/L, fish 2 = 5.0 g/L and fish 3 = 4.8 g/L, with a mean ± S.D value = 4.6 g/L ± 0.52, standard error of mean = 0.30 and paired t test value = 12.48 with a significant (p) value = 0.006 (highly significant, $P\leq0.01$). Concentration obtained after treatment of fish for four

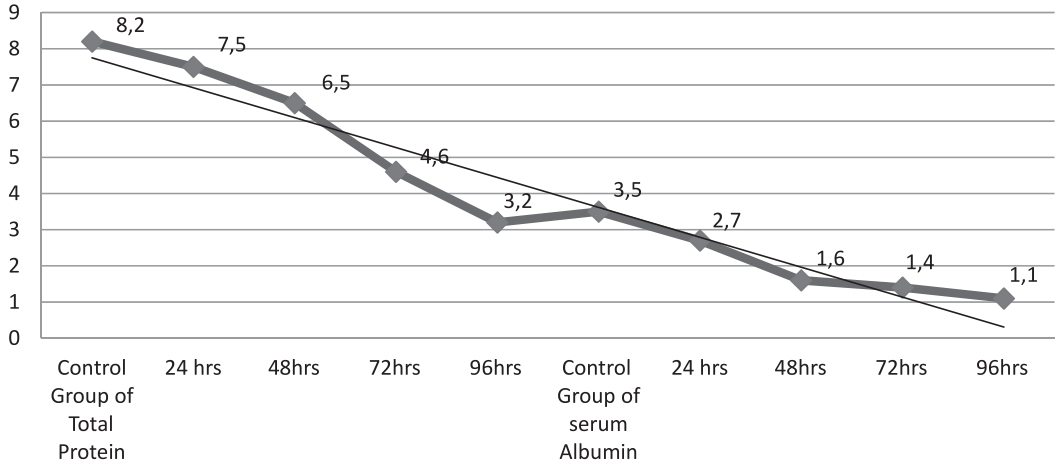

Fig. 1. Comparison of control group and treated group (Acute toxicity) of biochemical parameters: total protein and serum albumin (g/L).

days were: fish 1 = 3.6 g/L, fish 2 = 3.0 g/L and fish 3 = 3.2 g/L, with a mean ± S.D value = 3.2 g/L ± 0.30, standard error of mean = 0.17, and paired t-test value = 13.65 with a significant (p) value = 0.005 (highly significant, P≤0.01).

Serum Albumin

Serum albumin concentration obtained after treatment of fish for one day was: fish 1 = 2.8 g/L, fish 2 = 2.6 g/L and fish 3 = 2.9 g/L, with a mean ± S.D value = 2.7 g/L ± 0.15, standard error of mean = 0.08 and paired t-test value = 8.69 with a significant (p) value = 0.01 (highly significant, P≤0.01). Concentration obtained after treatment of fish for two days were: fish 1 = 1.6 g/L, fish 2 = 1.6 g/L and fish 3 = 1.6 g/L, with a mean ± S.D value = 1.6 g/L ± 0.00, standard error of mean = 0.00 and paired t-test value = 32.90 with a significant (p) value = 0.0009 (Maximum highly significant, P≤0.001). Concentration obtained after treatment of fish for three days were: fish 1 = 1.4 g/L, fish 2 = 1.3 g/L and fish 3 = 1.3 g/L, with a mean ± S.D value = 1.4 g/L ± 0.05, standard error of mean = 0.03 and paired t-test value = 65.00 with a significant (p) value = 65.00 (Maximum highly significant, P ≤ 0.001) Concentration obtained after treatment of fish for four days were: fish 1 = 1.3 g/L, fish 2 = 1.2 g/L and fish 3 = 1.2 g/L, with a mean ± S.D value = 1.1 g/L ± 0.15, standard error of mean = 0.08 and paired t-test value = 68.00 with a significant (p) value = 68.00 (Maximum highly significant, P≤0.001

Chronic Toxicity (Figs 1 and 2)

Total protein concentration obtained after treatment of fish for 10 days were: fish 1 = 8.8 g/L, fish 2 = 7.0 g/L and fish 3 = 9.0 g/L, with a mean ± S.D value = 8.2 g/L ± 1.10, standard error of mean = 0.63, and paired t test value = 0.07 with significant (p) value = 0.94 (Non-Significant). Concentration obtained after treatment of fish for 15 days were: fish 1 = 7.0 g/L, fish 2 = 6.7 g/L and fish 3 = 6.8g/L, with a mean ± S.D value = 6.8g/L ± 0.15, standard error of mean = 0.08, and paired t-test value = 5.00 with a significant (p) value = 0.03 (Significant, P<0.05).

Concentration obtained after treatment of fish for 25 days were: fish 1 = 4.0 g/L, fish 2 = 4.0 g/L and fish 3 = 5.0 g/L, with a mean ± S.D value = 4.3 g/L ± 0.5, standard error of mean = 0.33, and paired t-test value = 7.55 with a significant (p) value = 0.01 (Significant, P<0.05).

Serum Albumin

Serum albumin concentration obtained after treatment of fish for 10 days were: fish 1 = 2.3 g/L, fish 2 = 2.3 g/L and fish 3 = 1.8 g/L, with a mean ± S.D value = 2.7 g/L ± 0.28, standard error of mean = 0.16 and paired t-test value = 11.73 with a significant (p) value = 0.007 (highly significant, P<0.01). Concentration obtained after treatment of fish for 15 days were: fish 1 = 1.9 g/L, fish 2 = 2.0 g/L and fish 3 = 1.4 g/L, with a mean ± S.D value = 1.6 g/L ± 0.32, standard error of mean = 0.18 and paired t-test value = 11.92 with a significant (p) value = 0.007 (highly significant, P<0.01). Concentration obtained after treatment of fish for 25 days were: fish 1 = 1.5 g/L, fish 2 = 1.5 g/L and fish 3 = 1.2 g/L, with a mean ± S.D value = 1.4g/L ± 0.17, standard error of mean = 0.1 and student t test value = 36.37 with a significant (p) value 0.0008 (Maximum highly significant, P<0.001)

Discussion

Atrazine herbicides are used to control broad-leaf weeds in crops [27]. Atrazine has high mobility so that after application it can move to untargeted areas, especially to aquatic bodies [28]. Different concentrations

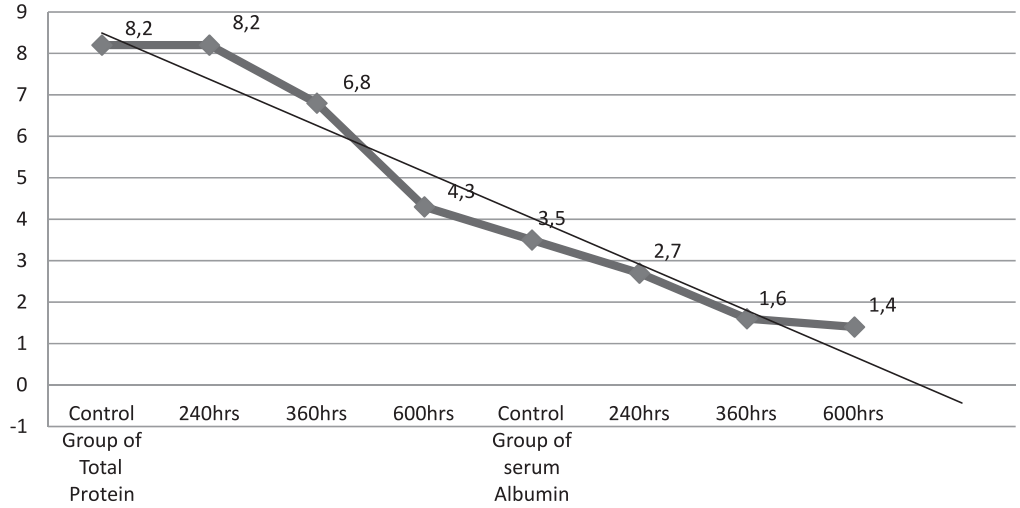

Fig. 2. Comparison of control group and treated group (Chronic toxicity) of biochemical parameters: total protein and serum albumin (g/L).

of atrazine have shown different alterations in biochemical parameters of the fish [29]. The presence of 1000 µ of atrazine in a pool has caused the death of rainbow trout (*Onchorhynchus mykiss*) [30]. Blood biochemical parameter concentrations of common carp are altered after exposure to different doses of herbicides [31]. Atrazine affected the fat oxidation and antioxidant enzymes, kidneys (including endoplasmic softening), and the egg sac of *Channa punctatus* [10, 32, 33]. Bioaccumulation of atrazine in different organs of fish have been noted and thus we concluded that environmental pollutants can contaminate fish bodies and pollutants can enter fish bodies through the gills and skin [34]. Exposing fish to different concentrations of atrazine can also bring changes in fish behavior, such as irregular movement, increased opercular movement, floating on the side, vertical movement, fast swimming, coming toward the water surface, etc., and such abnormal behavior indicates that herbicides affect the CNS of the fish [35]. Atrazine is toxic to aquatic animals and most studies have noted that exposure of fish to atrazine results in biochemical parameter alteration, behavioral abnormality, and structural deformality, plus stress on reproduction and the immune system by quantifying white blood cells [22, 36-40]. Histopathological changes in kidney, liver, gills, and other organs due to exposure to different atrazine concentrations can lead to death [41, 42, 36].

Protein is also one of the important biochemical parameters used to understand the general state of fish health and biological mechanisms of metabolism under pollutant stress [43]. In the present study the total protein concentration starts depletion from the first day, and such depletions of total protein were also observed continuously on remaining days of exposure to atrazine for acute toxicity. A decline in total protein concentration of Oreochromis niloticus and Chrysichthyes auratus against atrazine herbicide have been observed [44]. Carp exposed to 10 µl/L atrazine for 72 hrs had significantly lower plasma protein concentrations [45]. In addition, our results showed an agreement with the results obtained by different researchers [46-50].

The reduction in protein could be attributed to adjustment of the fish to its new environmental conditions as a result of stress response [51]. Unlike mammals, fish consume protein and do not store it in the body tissue for muscle energy when a carbohydrate source is absent, hence the exposed fish meet their extra energy requirements from body proteins, which are used to produce glucose for fish by the process of gluconeogenesis [52, 53]. A decreased protein level may be attributed to stress-mediated immobilization of these compounds to fulfill an increased element for energy by the fish to cope with environmental conditions exposed by the toxicant [54]. So from this we concluded that in the present study when fish were exposed to atrazine doses for acute toxicity we observed a decrease in protein concentrations compared to the control group because the protein was converted to glucose by the process of gluconeogenesis. On the other hand, in silver catfish *Rhamdia quelen* were observed to have increased levels of protein, which is in disagreement with our results [55].

Serum albumin plays an important role in maintaining the osmotic balance between the circulating blood and the tissue membrane [56]. A significant decrease in serum albumin observed in the present investigation was in agreement with the work of Ravichandran [57]. This is also supported by a study in which it was observed that when Nuvan toxicant exposure increased we saw further decrease in serum albumin [58]. A similar trend of serum albumin decrement was also observed in Indian catfish, *Mystus vittatus*, after Nuvan toxicant exposure [59]. The finding in *Cyprinus carpio* after exposure to monocrotphos pesticides showed similar results [60].

These findings are also supported by results observed in Monosex tilapia treated with cabofuran [61]. Similar results were recorded in *Cyprinus carpio* subjected to Chlorpyrifos [61]. The effect of indofil toxicity was noted on the serum albumin content of *Channa punctatus* (Bloch) [62]. Recently similar findings were interpreted in *Clarias gariepinus* on paraquat dichloride toxicity [63]. The reduced level of albumin is observed in rock fish exposed to cypermethrin [63]. Similar findings were reported in *Mystus vittatus* exposed to metasystox and sevin regarding serum albumin [64]. However, similar findings were estimated in sheet fish after exposure to 2-phenoxy ethanol [48]. Depletion of serum albumin was found in *Nile Tilapia*, *Oreochromis mossambicus* exposed to benomyl [65]. Recently, similar results of decreasing serum albumin were observed in *Channa punctatus* under indofil toxicity, and the same was witnessed in *Channa punctatus* and *Clarias gariepinus* exposed to sub-lethal concentrations of Nuvan toxicant [66].

Conclusion

The result obtained for acute toxicity and chronic toxicity in the case of total protein and serum albumin lead to the conclusion that both total protein and serum albumin show continuous decline against the different doses of the herbicide atrazine.

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