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Research Article

Amygdalin Mitigates Benzo[a]pyrene-Induced Stress and Hepatic Dysfunction in Stellate Sturgeon Fry (*Acipenser stellatus*): Insights Into Immune and Metabolic Modulation

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This study evaluated the protective role of amygdalin against benzo[a]pyrene (BaP) toxicity in stellate sturgeon fry (*Acipenser stellatus*). Fish were pre-exposed to amygdalin (5 g/kg) and subsequently challenged with sublethal BaP concentrations (50% and 75% LC50). Exposure to BaP elevated cortisol and liver enzymes (ALT, AST, ALP; $p < 0.05$), indicating hepatotoxic stress, whereas pretreatment with amygdalin significantly reduced ALT and AST activities ($p < 0.05$). Immune parameters (C3, IgM, and lysozyme) improved with amygdalin supplementation ($p < 0.05$) but showed only partial recovery under BaP exposure alone. Total antioxidative capacity (TAC) increased with amygdalin yet declined when combined with BaP, reflecting oxidative overload. In conclusion, the findings suggest that amygdalin may provide protective effects against BaP-induced stress and liver damage in sturgeon fry.

Keywords: amygdalin; antioxidant capacity; hepatic biomarkers; immunomodulation; sturgeon

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BaP) represent a pervasive threat to aquatic ecosystems worldwide. Derived primarily from incomplete fossil fuel combustion and industrial effluents [1], these compounds induce oxidative stress and hepatic dysfunction in aquatic organisms, compromising their survival [2]. In fish, BaP exposure triggers reactive oxygen species (ROS) generation, DNA damage, and immunosuppression [3], posing significant risks to aquatic biodiversity and aquaculture productivity.

The animal's attempt to maintain homeostasis in a disrupted state is known as coping with stress [4]. Stress factors and responses in fish are more diverse and critical than in other vertebrates, and some of these responses can be indirectly fatal. Stressors such as salinity, temperature, or the presence of chemicals in the water that disrupt the hydromineral system can induce stress in fish [5]. Stress disrupts body systems and can have detrimental effects on behavior, growth, reproduction, immune system performance, and disease resistance [6].

Some studies investigate the impact of antistress compounds on the growth and well-being of fish. These investigations also explore potential therapeutic applications for animals. Furthermore, it is discovered that nano selenium, vitamin C, and vitamin E can enhance immunoglobulin levels, growth rates, and antioxidant capabilities in fish exposed to ammonia stress [7]. It is demonstrated that amygdalin can alleviate inflammation and pain by inhibiting prostaglandins and nitrite oxide synthesis [8]. Moreover, it is revealed that amygdalin can impede the proliferation of colon cancer cells by suppressing gene expression associated with cell cycle progression [9].

While the toxic effects of BaP on hepatic enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP]) and immune parameters (e.g., IgM, lysozyme) have been documented in various fish species [10], studies on endangered sturgeons (e.g., *Acipenser stellatus*) remain scarce. This is particularly concerning given sturgeons' unique biological traits (late maturation, slow growth) and benthic habitat preferences, which heighten their susceptibility to PAH accumulation [11].

Natural compounds with antioxidant and immunomodulatory properties have emerged as promising tools to mitigate pollutant-induced stress in aquaculture [12]. Amygdalin, a cyanogenic glycoside found in almonds and stone fruit seeds, exhibits multimechanistic actions, including ROS scavenging and inflammatory cytokine regulation [13]. Although mammalian studies demonstrate its hepatoprotective effects against toxins [14], its efficacy in aquatic species—particularly under PAH exposure—remains unexplored.

This study aimed to evaluate amygdalin's protective effects against BaP-induced hepatic damage in *A. stellatus* fry through ALT, AST, and ALP profiling; assess its ability to modulate innate (complement C3, lysozyme) and adaptive (IgM) immune responses; and quantify total antioxidant capacity (TAC) to elucidate potential mechanisms. As the first investigation of amygdalin's dual role in sturgeon stress mitigation, our findings provide a foundation for sustainable aquaculture practices and conservation strategies.

2. Materials and Methods

2.1. Fish. A total of 300 stellate sturgeon fry (*Acipenser stellatus*) with an average weight of 20 ± 1.6 g and length of 18 ± 2.8 cm were obtained from the Shahid Beheshti Sturgeon Center in Rasht, Iran. The fish were maintained in 500-L fiberglass tanks for 14 days prior to experimentation. Water quality parameters were maintained at pH 7.6 ± 0.2, temperature 19 ± 1°C, and dissolved oxygen 8.4 ± 0.1 mg/L throughout the study. Commercial feed pellets were provided twice daily at 3% of body weight [15].

2.2. Experimental Treatments. In the initial stages, an empirical investigation was conducted to determine the optimal dose of amygdalin (purchased from Sigma-Aldrich co., USA). Based on Zarei and colleagues, various concentrations ranging from 2.5 g/kg body weight to 40 g/kg were tested, with increments of 2.5 g/kg. After careful evaluation, it was concluded that the precise dosage for optimal effects was 5 g/kg body weight [16, 17]. Once this crucial information was obtained, the next step involved administering the stellar sturgeon fry with the optimal dose of amygdalin through subcutaneous injection beneath their pectoral fins (day 0). After a lapse of 24 h period, the fry were relocated to tanks where they would be exposed to BaP-induced stressors for further assessment and observation (day 1). To determine the LC50 values of BaP (Sigma-Aldrich Co., USA), an acute toxicity bioassay procedure was employed using standard methods [18]. Lethality was tested using a geometric series of five concentrations of BaP ranging from 500 to 3000 μg/L biased on previous studies, along with a control group consisting of acetone only. The experiment took place in 60-L tanks (three replication per each concentration and the control group, three fish per tank and nine fish per treatment) for 96 h for determine acute toxicity (LC50 96 h). To preserve optimal water conditions, fish were deliberately withheld from feeding for a consecutive span of 24 hours prior to the commencement of the acute toxicity investigation, as well as during the actual experiment itself. The LC50 value of the BaP was calculated using PROBIT analysis with a 95% confidence interval, using the probit analysis program (Version 19). Based on the 96-h LC50 value ($1.75\ mg{\cdot}L^{-1}$), the fish fry were exposed to two sublethal concentrations of BaP, which were 50% (B50) and 75% (B75) of the LC50 values. The experiment involved five groups of fish: 1—control fish that did not receive any chemicals (Control), 2—fish exposed to B50, 3—fish pretreated with amygdalin (A) and then exposed to B50 (A + B50), 4—fish exposed to B75, and 5—fish pretreated with A and then exposed to B75 (A + B75). Eight days after amygdalin injection (day 7), blood samples were collected from the caudal vein. For this procedure, fish were first anesthetized in a clove oil bath (30 mg/L) until opercular movement slowed significantly and the fish lost equilibrium, and the fish was placed in a wet cotton cloth, then blood was drawn with 2-mL heparinized disposable syringes and then centrifuged at 3000 rpm for 10 min at 4°C, and finally the samples were stored in a −70°C. After blood collection, the fish were allowed to recover in clean, aerated water. For terminal sampling (euthanasia), fish from all groups were immersed in a benzocaine bath (100 mg/L) until the cessation of opercular movement and a complete loss of response to a tail pinch stimulus, ensuring a deep, irreversible plane of anesthesia. While irreversibly anesthetized, fish were subsequently euthanized by rapid freezing to facilitate subsequent tissue analysis. Liver tissue samples were then obtained [19].

2.3. ALT and AST. The levels of ALT and AST were measured using Pars-Azmoon kits (Iran) following the method described by Prashanth and Manjunatha [20]. Briefly, the solution was analyzed colorimetrically at a temperature of 37°C and a wavelength of 340 nm. ALT and AST were measured in liver tissue, and the measurement unit was u/kg.

2.4. ALP. ALP activity was determined using a Pars-Azmoon kit (Iran) following the method described by Rafiee, Mortazavi, and Asghari [21]. The conversion of nitrophenyl phosphate to nitrophenol and phosphate was used for the measurement, which was performed at 37°C and a wavelength of 405 nm. ALP was measured in liver tissue, and the measurement unit was u/kg.

2.5. TAC. The ferric reducing antioxidant power (FRAP) methodology constitutes a procedure employed to evaluate the antioxidant capacity of various substances by quantifying their ability to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+})

within an acidic milieu, thereby facilitating the development of a measurable chromatic complex. To implement this methodology, it is imperative to initially prepare tissue or cellular samples in phosphate-buffered saline (PBS), akin to the procedure utilized in the ABTS assay. The requisite reagents for this analysis comprise the FRAP reagent, which incorporates ferric chloride. Upon the preparation of samples and the consolidation of reagents, these components are combined and permitted to incubate for a designated duration. Subsequent to the incubation phase, the absorbance of the resultant solution is quantified at 593 nm utilizing a spectrophotometer. The degree of colorimetric development is directly proportional to the TAC of the sample under examination. To precisely determine the values of TAC, absorbance readings are compared with a standard curve derived from established concentrations of Trolox, thereby facilitating accurate computations and dependable outcomes in the assessment of antioxidant characteristics through this complex methodological approach [22, 23].

2.6. Complement Component C3. The concentration of fish complement component C3 was measured using the Fish ELISA kit (Hangzhou Eastbiopharm Co., Ltd.) and a sandwich enzyme-linked immunosorbent assay (ELISA) method described by Nash et al. [24]. Briefly, 40 μL of the sample was added to a precoated well containing a monoclonal antibody against fish complement component C3. Next, 10 μL of biotin-labeled C3 antibodies was added, and the mixture was incubated at 37°C for 1 h. After washing to remove unbound enzyme, 50 μL of streptavidin-HRP was added to the well to form immune complexes, which were incubated at 37°C for 10 min. The resulting color change from blue to yellow was stopped by adding 50 μL of sulfuric acid. The optical density (OD) was measured at 450 nm using an ELISA reader (ELX800 Absorbance Reader, BioTek, USA). The standard concentration and corresponding OD values were used to calculate the standard curve linear regression equation, which was then applied to the OD values of the samples to calculate the corresponding sample concentration. Negative and positive controls were included by using a blank well (containing only Chromogen solution A and B and the stop solution) and a standard well (containing only Streptavidin-HRP), respectively. The inter- and intra-assay coefficients of variation (CV) were < 12% and < 10%, respectively. The C3 values were expressed as mg/dL.

2.7. Immunoglobulin M (IgM) and Cortisol. IgM and cortisol, like complement component C3, were measured using a sandwich ELISA with separate ELISA kits from Eastbiopharm (Hangzhou Eastbiopharm Co., Ltd.). The method was based on the approach described by Nash et al. [24].

2.8. Lysozyme Activity. Lysozyme activity was determined using a modified turbidimetric method. Specifically, 25 μL of the sample was added to a cuvette with 175 μL of a suspension of *Micrococcus lysodeikticus* bacteria (0.2 μg/mL PBS). The absorbance of the samples was measured at a wavelength of 450 nm in the first and fifth minutes using a spectrophotometer (Ultaspect 3000, Pharmacia Biotech). Every 0.001 absorbance reduction per minute was considered a unit of lysozyme activity. The experiment was repeated three times, and egg white lysozyme (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard [25].

2.9. Statistical Analysis. The statistical analysis was performed using SPSS version 19 on a Windows 10 operating system, and the graphs were created using Excel 2013 software. The normality and homogeneity of variance were assessed using Kolmogorov–Smirnov and Levene's tests, respectively. Differences between treatment groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences among the means of the parameters at a confidence level of 95%. All data are presented as mean ± standard deviation (SD).

3. Results

3.1. ALT. ALT activity in the serum of stellate sturgeon fry exhibited significant alterations under different treatment conditions (Figure 1) ($p = 0.001$, $df = 5$, $F = 18.34$). The control group showed the lowest ALT activity (approximately 2000 U/kg), while the highest level was observed in the B50 group, with values reaching nearly 8000 U/kg ($p = 0.001$). Pretreatment with amygdalin prior to 50% BaP exposure (A + B50 group) significantly mitigated this elevation, although ALT activity in this group remained higher than in the control ($p = 0.001$). A comparable pattern was observed in the B75 group, where ALT activity remained high and was not significantly different from the A + B50 group ($p = 0.001$). Interestingly, the A + B75 group, which received amygdalin before 75% BaP exposure, exhibited a further reduction in ALT activity relative to B75 ($p = 0.002$). Fish administered only amygdalin, without BaP exposure, showed moderate ALT activity, higher than the control but significantly lower than all BaP-exposed groups ($p = 0.001$) (Figure 1).

3.2. AST. AST levels in stellate sturgeon fry serum significantly differed among experimental groups (Figure 2) ($p = 0.001$, $df = 5$, $F = 27.42$). The maximum AST level was recorded in the B50 group ($p = 0.001$). The group receiving amygdalin only exhibited a low but significant elevation of AST levels relative to the control ($p = 0.002$). The A + B50 group exhibited significantly lower AST activity in relation to the B50 group ($p = 0.001$), even below the control levels (Figure 2). The B75 and A + B75 groups significantly elevated their AST activity relative to the control ($p = 0.001$) but lower than the B50 peak levels. There was no significant difference between B75 and A + B75 groups ($p = 0.09$) (Figure 2).

3.3. ALP. The ALP activity in stellate sturgeon fry serum presented significant differences among the treatment groups (Figure 3) ($p = 0.001$, $df = 5$, $F = 43.45$). The amygdalin-only group presented the highest ALP activity

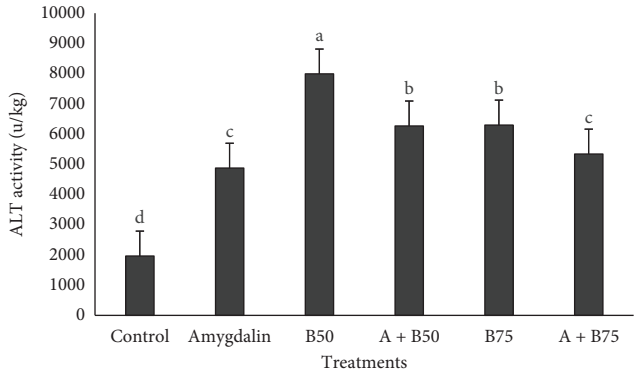

Figure 1: Changes in ALT activity in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

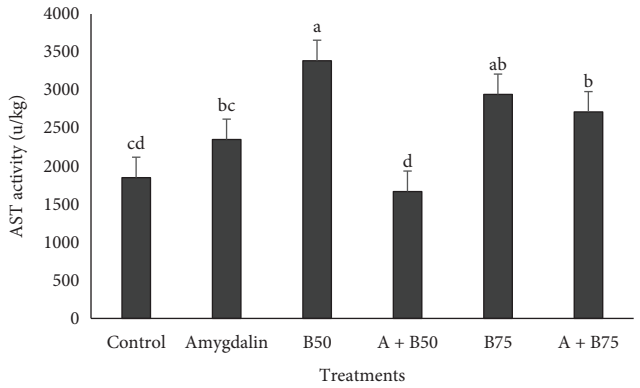

Figure 2: Changes in AST activity in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

(~4200 U/kg), significantly greater than all other groups ($p = 0.001$). The B50 group registered moderately high ALP levels (~3700 U/kg) significantly greater than the control but lower than the amygdalin group ($p = 0.001$). The A + B50 group showed a significant reduction in ALP activity relative to the B50 group ($p = 0.001$), with levels approaching the control group ($p = 0.13$). In the B75 and A + B75 groups, the ALP activity registered moderate elevation (~2800 U/kg) significantly greater than the control group ($p = 0.001$) (Figure 3).

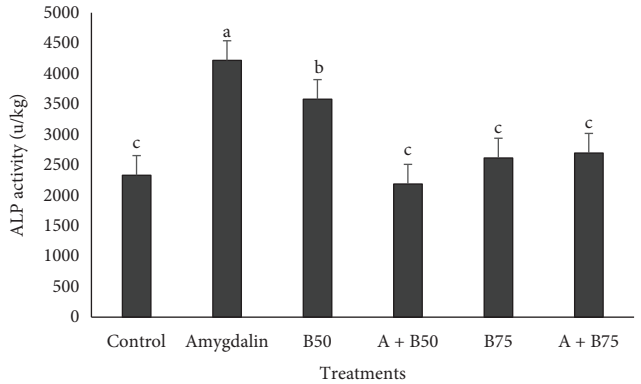

Figure 3: Changes in ALP activity in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

3.4. Cortisol. The cortisol levels of stellate sturgeon (*Acipenser stellatus*) fry significantly increased after exposure to BaP. The fry of the B50 and B75 groups showed significantly increased cortisol concentrations in comparison to the control group and the amygdalin-only group ($p = 0.001$, df = 5, $F = 25.54$) (Figure 4). There was no significant difference in cortisol levels between the B50 and B75 groups ($p = 0.58$). Pretreatment with amygdalin alone did not significantly change cortisol levels in comparison to the control group, neither ($p = 0.23$). In addition, amygdalin pretreatment in the BaP-exposed group (A + B50 and A + B75) did not significantly decrease cortisol levels in comparison to their respective BaP-only group (B50 and B75). The cortisol levels of A + B50 and A + B75 statistically unchanged in comparison to the B50 and B75 groups, respectively, ($p = 0.12$) (Figure 4).

3.5. TAC. Exposure to BaP significantly influenced the TAC in stellate sturgeon (*Acipenser stellatus*) fry (Figure 5). TAC levels in fish treated with BaP at 50% (B50) and 75% (B75) concentrations showed no significant difference compared to the control group ($p = 0.08$). Pretreatment with amygdalin alone significantly enhanced TAC values compared to the control ($p = 0.001$, df = 5, $F = 47.21$). However, when amygdalin was combined with BaP exposure (A + B50 and A + B75 groups), the TAC levels decreased markedly compared to the amygdalin-only group. In fact, the A + B50 and A + B75 groups showed significantly lower TAC than even the B50 and B75 groups, respectively ($p = 0.001$) (Figure 5).

3.6. Complement Component C3. Complement component C3 concentration in stellate sturgeon (*Acipenser stellatus*) fry was significantly altered after exposure to BaP and after

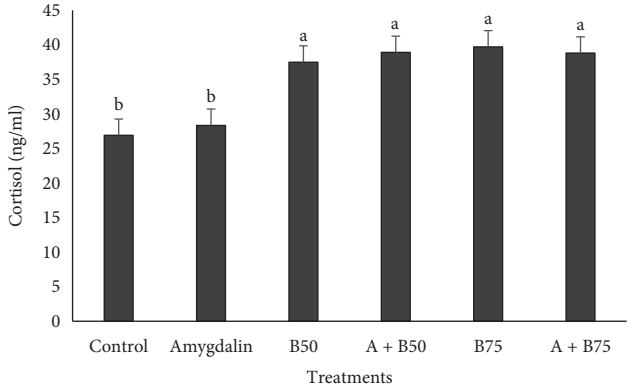

Figure 4: Changes in cortisol concentration in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

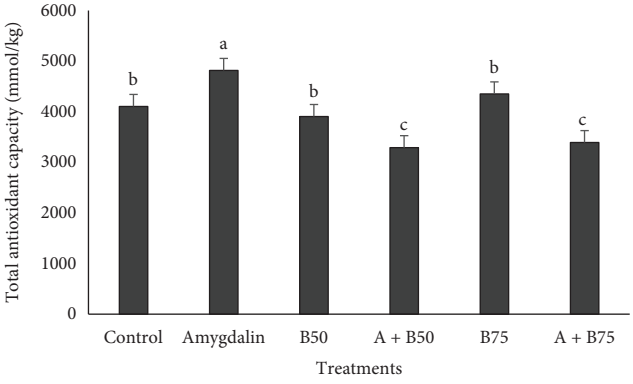

Figure 5: Changes in total antioxidant capacity in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

amygdalin pretreatment ($p = 0.001$, df = 5, $F = 47.21$) (Figure 6). The amygdalin-only group had the greatest levels of C3, significantly elevated when compared to all other groups, including the control group ($p = 0.001$). The 50% and 75% BaP-exposed fry, in the absence of amygdalin pretreatment (B50 and B75), had very low levels of C3 when compared to the control group ($p = 0.001$), and the B75 group showed no significant difference in the levels of C3 when compared to the B50 group ($p = 0.14$). Amygdalin

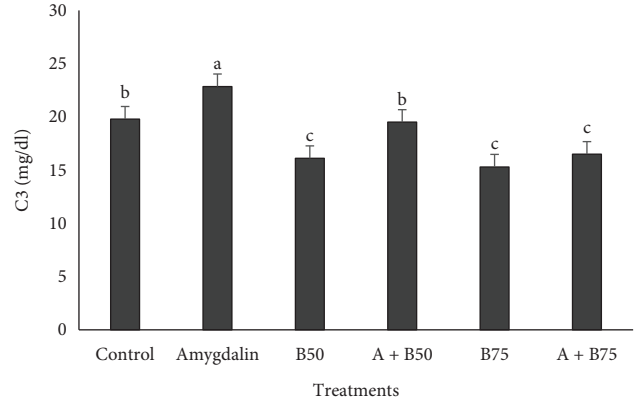

Figure 6: Changes in C3 level in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

pretreatment in BaP-exposed groups, A + B50 and A + B75, resulted in the partial restoration of C3 compared to their respective BaP-only exposure groups. The A + B50 group, for instance, had significantly elevated levels of C3 when compared to B50 group ($p = 0.002$) but showed no significant difference in the levels of C3 when compared to the control group ($p = 0.27$) (Figure 6).

3.7. IgM. The assessment of IgM levels in stellate sturgeon fry subjected to varying concentrations of BaP, with or without amygdalin pretreatment, revealed significant immunological alterations ($p = 0.001$, df = 5, $F = 36.21$) (Figure 7). Fry pretreated with amygdalin alone exhibited a statistically significant elevation in IgM levels, reaching approximately 60 mg/dL, compared to all other experimental groups ($p = 0.001$). In contrast, exposure to 50% BaP (B50 group) did not result in a statistically significant difference from the control group ($p = 0.12$). However, in the A + B50 group, IgM levels moderately increased to approximately 55 mg/dL, which was statistically significant compared to the control ($p = 0.003$). Exposure to 75% BaP alone (B75 group) led to a significant decline in IgM levels, reducing them to approximately 45 mg/dL ($p = 0.002$). The A + B75 group, which received amygdalin pretreatment prior to 75% BaP exposure, showed partial restoration of IgM levels to approximately 50 mg/dL; this change was not statistically significant compared to the control ($p = 0.31$).

3.8. Lysozyme Activity. The results of the stellate sturgeon fry's lysozyme activity in the various treatment of BaP and the amygdalin pretreatment reveal the significant differences ($p = 0.001$, df = 5, $F = 15.72$) (Figure 8). Similar to the results

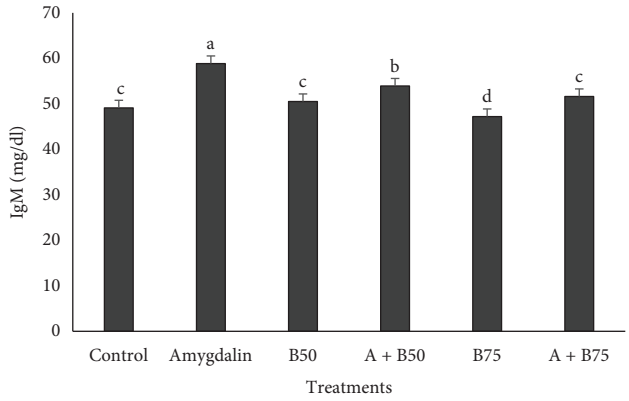

FIGURE 7: Changes in IgM level in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

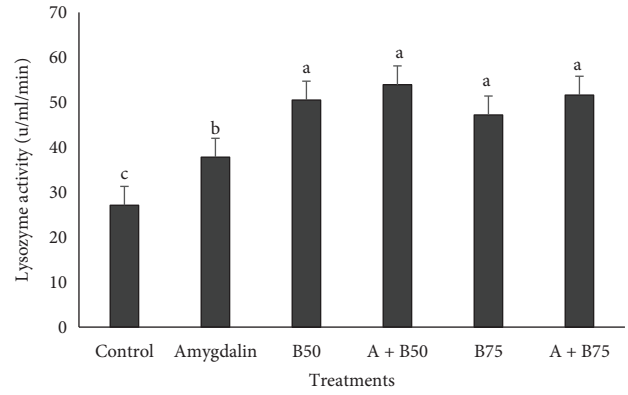

FIGURE 8: Changes in lysozyme activity in stellate sturgeon fry (*Acipenser stellatus*) treated at different concentrations of benzo alpha pyrene (BaP). In group (B50), fish were in 50% BaP without amygdalin pretreatment, while in group (A + B50), the fish received amygdalin pretreatment along with 50% BaP. Similarly, in the (B75) group, the fish were exposed to 75% BaP without amygdalin pretreatment, and in the (A + B75) group, the fish received amygdalin pretreatment along with 75% BaP. Data are presented as mean ± SD ($n = 9$). Different lowercase letters above bars denote statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

of the IgM, the amygdalin-only group exhibited the highest lysozyme activity ($p = 0.001$). The exposure of 50% and 75% of the LC_{50} concentration of BaP exhibited the increase of somewhat the control group's lysozyme activity, but the difference has not been significantly determined ($p = 0.15$). However, in group A + B50, in which the amygdalin pretreatment was done before the exposure of BaP, the lysozyme activity significantly increased to about 35.5 U/mL/min ($p = 0.001$). Conversely, the exposure of 75% of BaP (B75) exhibited the decrease in the lysozyme activity in such a way that the mean value reached the down of about 29.5 U/mL/min. Surprisingly, the amygdalin pretreatment in the exposure of 75% of BaP in group A + B75 exhibited to restore the lysozyme activity to about 33.5 U/mL/min. This value significantly increased than in group B50 ($p = 0.001$).

4. Discussion

In the current research, we examined the physiological effects of BaP, a strong PAH, on stellate sturgeon fry (*Acipenser stellatus*) and determined the potential restorative abilities of amygdalin pretreatment. Amygdalin, a plant glucoside, is a biologically active substance in the vitamin B17 family and is found highly in bitter almonds (Corson & Crews, 2007). The findings reveal remarkable alterations in liver enzyme activities (ALT, AST, ALP), stress hormone levels (cortisol), and cumulative antioxidant power (TAC), with different levels of amygdalin modulation at varying concentrations of BaP exposure (50% and 75%).

4.1. Liver Enzyme Activities (ALT, AST, and ALP). The reduction of ALT and AST in the A + B50 group ($p < 0.05$) suggests that amygdalin pretreatment offers liver protection. Beyond a general antioxidant effect, this hepatoprotection may be mediated through the modulation of specific cell signaling pathways. For instance, the Nrf2 (nuclear factor erythroid 2–related factor 2) pathway is a key regulator of the cellular antioxidant response. It is plausible that amygdalin activates Nrf2, leading to the transcription of cytoprotective genes encoding for enzymes like NAD(P)H quinone dehydrogenase 1 (NQO1) and heme oxygenase-1 (HO-1), thereby enhancing the liver's resilience to BaP-induced oxidative insult [26]. Concurrently, amygdalin may protect hepatocytes by suppressing the NF-κB (nuclear Factor kappa-light-chain-enhancer of activated B cells) pathway, a primary regulator of inflammation. By inhibiting NF-κB activation, amygdalin could reduce the production of pro-inflammatory cytokines triggered by BaP, thus mitigating inflammatory liver damage.

4.2. Cortisol Levels. BaP, composed of multiple fused rings, disrupts cellular processes like lipid metabolism and DNA repair. In flounder, BaP administration raises cortisol, a stress marker [2]. Though cortisol regulation mechanisms in fish remain partly unclear [27], our findings show significant cortisol elevation in B50 and B75, highlighting BaP as a strong stressor activating the hypothalamic–pituitary–inter-renal (HPI) axis [28]. Cortisol, crucial for stress response, mediates metabolism and osmoregulation [27]. The absence of significant cortisol reduction in A + B50 and A + B75 ($p > 0.05$) implies amygdalin is ineffective in modulating this axis under BaP-induced stress. This contrasts with antioxidants like vitamin C, which have reduced cortisol in pollutant-exposed fish [29]. Amygdalin's action may primarily target oxidative pathways, not neuroendocrine regulation, or BaP's stress burden may be too severe for amygdalin to counteract.

4.3. TAC. The results for TAC revealed a complex interaction between amygdalin and BaP-induced stress. As expected, the amygdalin-only group showed a significant TAC increase ($p < 0.05$), supporting its intrinsic antioxidant potential, likely attributable to its phenolic compounds and secondary metabolites [13]. However, the sharp decline in TAC in the A + B50 and A + B75 groups, to levels below those of the BaP-only groups, was unexpected. This suggests that under the severe oxidative burden imposed by BaP, the role of amygdalin may shift or become more complex. There are two non-mutually exclusive hypotheses to explain this phenomenon. First, it is plausible that amygdalin, or its metabolites, exhibits a pro-oxidant effect under these specific conditions of high oxidative stress. Some antioxidant compounds can act as pro-oxidants in the presence of high metal ion concentrations or specific redox environments, potentially generating additional ROS and thereby depleting the overall antioxidant capacity [30]. Second, the metabolism of both BaP (via the cytochrome P450 system) and amygdalin itself may generate significant oxidative intermediates. The organism's defense systems, including glutathione and NADPH, might be recruited to detoxify these compounds, thereby depleting the very antioxidant resources that the TAC assay measures. In this scenario, amygdalin pretreatment could inadvertently increase the metabolic load, leading to a net decrease in measurable antioxidant capacity compared to facing BaP stress alone. This complex interaction warrants further investigation, including direct measurement of ROS levels, specific antioxidant enzyme activities (e.g., SOD, CAT, GPx), and phase I/II metabolism gene expression to elucidate the precise mechanisms [31, 32].

4.4. Immunological Parameters: C3, IgM, and Lysozyme. The highest lysozyme levels (~37.5 U/mL/min) were found in the amygdalin-only group, pointing to enhanced innate immune defense. The immunostimulatory effect of amygdalin, observed for both innate (C3, lysozyme) and adaptive (IgM) parameters, may also be linked to its interaction with key signaling pathways. The NF-κB pathway is not only pro-inflammatory but also pivotal for the expression of many immune genes, including those encoding lysozyme and complement factors. A modulated activation of NF-κB could potentially explain the boosted innate immunity. Furthermore, amygdalin may influence the mitogen-activated protein kinase (MAPK) pathways, which are involved in cell proliferation, stress responses, and immunomodulation in fish, potentially leading to enhanced lymphocyte activation and immunoglobulin production [33, 34].

5. Conclusion

This study provides the first evidence that amygdalin pretreatment can offer significant but selective protection against BaP-induced stress in stellate sturgeon (*Acipenser stellatus*) fry. The protective effects were parameter-specific and influenced by the level of toxicant exposure. Amygdalin effectively mitigated hepatotoxicity, as indicated by the significant reduction in ALT and AST activities, and provided substantial immunomodulatory benefits, particularly by restoring complement C3 and IgM levels and enhancing lysozyme activity. However, amygdalin did not significantly alter the cortisol response to BaP, and its effect on the TAC was complex, showing a beneficial effect alone but a potential pro-oxidant interaction under high BaP co-exposure. These findings suggest that amygdalin holds promise as a prophylactic agent in aquaculture for enhancing liver resilience and immune function in PAH-contaminated environments, but its efficacy is contingent on the specific physiological system and the intensity of the stressor. Further research is needed to refine the dosage, understand the underlying mechanisms for its limited effects on the stress axis and redox balance under high load, and assess long-term outcomes.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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