Iran. J. Ichthyol. (2025) 12(1): 22-38
P-ISSN: 2383-1561; E-ISSN: 2383-0964

ORIGINAL ARTICLE

Physiological responses of Starry Sturgeon (*Acipenser stellatus*) juvenile to thermal stress: exploring the effects of Amygdalin in enhancing Sturgeon resilience

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Article history:
Accepted 13 January 2025

Abstract
The Acipenseridae is a crucial inhabitant of aquatic ecosystems, and environmental adversity can have harmful consequences on their health. Stress-relieving compounds and immune system stimulants are currently used to support fish resistance. This study aimed to investigate the metabolic and immune responses to thermal stress in starry sturgeon juvenile previously exposed to amygdalin as a stress-reducing compound. The juveniles were treated at different temperatures (18°C, 22°C, and 26°C) with or without amygdalin, with three replicates. Blood and liver tissue samples were collected, and various parameters were measured. The results revealed significant differences in serum enzymes, cortisol levels, antioxidant capacity, and immune system components among the different groups. It was evident that fish pre-treated with amygdalin and kept at 22 °C (A+T22) and 26 °C (A+T26) experienced a significant decline in ALT (alanine aminotransferase, AST (aspartate aminotransferase) and T-AOC (Total Antioxidant Capacity) levels compared to those who did not undergo such treatment (fish kept at 22 and 26°C). Moreover, when comparing amygdalin-exposed fish at 26°C with their counterparts who were not exposed to amygdalin, there was a noticeable decrease in ALP and cortisol levels. Thermal stress has an adverse effect on the immune parameters of starry sturgeon. However, when amygdalin is administered, it has the potential to enhance fish resilience against thermal stressors by increasing immunity, particularly in C3 and IgM. In conclusion, amygdalin could serve as a beneficial agent for fish by mitigating stress levels and enhancing their resilience against thermal stressors.

Keywords: Metabolic response, Sturgeon, Immunity, Stress

INTRODUCTION

Stressors such as salinity, temperature, or the presence of chemicals in the water that disrupt the hydromineral system can cause stress in fish (Portz et al. 2006). Stress disrupts body systems and can have detrimental effects on behavior, growth, reproduction, immune system performance, and disease resistance (Goos & Consten 2002).

The global aquaculture industry is predicted to be significantly affected by climate change. As we continue to enhance and broaden fish farming operations, the increasing temperatures will increasingly impact fish welfare and give rise to various related problems (Cascarano et al. 2021). The examination of the effects of alterations in temperature on biological organisms is a significant area of interest with regards to worldwide climate fluctuations and variations in seasonal temperatures (Quinn et al. 2017).

Temperature plays a crucial role in the physiology of living organisms in an ecosystem. Deviations from the optimal temperature range can disrupt various homeostatic mechanisms, causing significant impacts (Mitchell et al. 2018). Thermal stress can result in the destruction of cellular components, the release of digestive enzymes that cause widespread tissue damage, longer recovery times, as well as metabolic stress, and serious damage to cold-blooded fish species (Nakano et al. 2014). The impact of heightened water temperatures on the immune systems of fish has been extensively researched. As a general rule, a rise in water temperature leads to a boost in the overall immune parameters of fish, as noted by Bowden (2008). While the stress response caused by temperatures outside the normal range of a fish species can harm immune function (Magnadottir

et al. 1999; Dominguez et al. 2004), the same negative impact can also be seen when fish are exposed to suboptimal temperatures, affecting their immunity and overall health (Quinn et al. 2017).

The physiological responses of fish to stress can be divided into primary and secondary responses. Primary responses include changes in the endocrine glands, such as the release of catecholamines and corticosteroids (Herrera et al. 2019). During stress, the release of catecholamines leads to an increase in cortisol in the blood, which in turn leads to the conversion of stored glycogen into glucose (gluconeogenesis) (Danijela 2015). Increases in cortisol levels and changes in glucose levels can be considered the main indicators of stress in fish (Santos and Pacheco, 1996). Secondary responses include changes in metabolism, hydromineral balance, and cardiovascular, respiratory, and immune function (Barton et al. 2005).

The liver is an organ in animals that contains numerous enzymes, including those capable of converting or eliminating chemicals and harmful pollutants. Aminotransferases, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), are among the most important and commonly used enzymes in this regard and are recognized as stress indicators (Amjad et al. 2018). Stress affects the health status of an organism and induces energy-consuming mechanisms, weakening the immune system and making the animal more susceptible to disease-causing agents. Innate immune markers such as lysozyme, complement system C3, and IgM are used in response to stress (Liu et al. 2022).

Heat shock proteins (HSPs) play an important role in organisms by acting as a defense mechanism against deleterious stress conditions. The induction of HSPs is a vital defensive mechanism that safeguards organisms from pernicious stress conditions (Choresh et al. 2004). Highly HSPs play a pivotal role in facilitating thermotolerance, thereby promoting growth under conditions of moderately elevated temperatures, while concurrently safeguarding the organism against perishing when confronted with more extreme thermal circumstances (Kumar et al., 2022).

Anti-stress and antioxidant compounds are substances used to prevent or reduce the body's response to stressful conditions and protect the body against oxidative reactions. Chemical anti-stress compounds can be used to reduce stress. However, because of their negative effects on the health of fish, humans, and the environment, their use is limited (Boyer et al. 2009).

Amygdalin is a compound derived from almonds that belongs to the nitriloside family and is known by different names, such as B17, mandelonitrile, and laetrile. It is a plant glycoside found in fruits such as apricots, cherries, plums, peaches, and almonds and is also known as vitamin B17 (Song & Xu 2014). Amygdalin is soluble in hot water and alcohol but is completely insoluble in ether and chloroform (Corson & Crews 2007). Its chemical structure consists of two glucose molecules, one benzaldehyde that causes numbness, and one hydrocyanic acid, which is an anticancer compound (Chen et al. 2013). Exposure to amygdalin can have a variety of physiological effects on fish. High concentrations of this compound have been associated with increased levels of stress markers, impaired liver function, and disruption of normal metabolic pathways. Studies indicate that sublethal doses can elevate levels of liver enzymes, suggesting potential hepatic damage, which is critical since the liver plays a crucial role in detoxification and metabolic regulation in fish (Achikanu & Ani 2021). Several studies have investigated the effects of different anti-stress compounds on the growth, survival, and health of fish, as well as their potential therapeutic benefits for animals (Salmani et al. 2024; Vahdatiraad et al. 2024). In the realm of fauna, the compendium known as amygdalin assumes a position of paramount significance as a mighty force in eradicating inflammation and assuaging agony. It accomplishes this formidable feat by curtailing the production of prostaglandins and nitrite oxide synthesis, acting as an inhibitory agent (Zuoqing et al. 2014).

The starry sturgeon (*Acipenser stellatus*) is an

important commercial species found in the Caspian Sea. It belongs to the family Acipenseridae and is characterized by a slender and elongated snout that is compressed from top to bottom. Stellate sturgeon are typically found in the brackish and freshwater environments of the Black Sea and the Caspian Sea basins. These fish are migratory, traveling between rivers and the sea to spawn. They prefer shallow, sandy or gravel bottoms in rivers for spawning, which typically occurs from spring to early summer, depending on water temperature and flow conditions. The species is adapted to a range of salinities, allowing it to inhabit both marine and freshwater systems (Dudu et al. 2008). Due to its precocious nature, this species demonstrates a remarkable ability to yield caviar at a premature stage of development compared to counterparts like Russian and Persian sturgeons. Thus, it stands as an ideal candidate for the production of this luxurious delicacy within controlled breeding environments (Norouzi et al. 2008). This particular species holds a significant role within the scientific community, serving as a model for the intricate evolution of vertebrates. Furthermore, it is esteemed by the food industry for both its delectable meat and prized roe which is essential in caviar production. Unfortunately, the stellate sturgeon has been exploited to such an extent that its population has dwindled drastically, primarily due to its immense economic value (Bacalbașa-Dobrovici 1997).

This species is critically endangered according to IUCN Red List Status. The stellate sturgeon faces numerous threats leading to its declining populations, including habitat loss due to dam construction and pollution, overfishing, and illegal poaching for its roe, which is highly valued in caviar production. Conservation efforts are being implemented to address these challenges, including habitat restoration and breeding programs aimed at augmenting wild populations (Vecsei et al. 2007).

Understanding and enhancing sturgeon resilience is of utmost importance to ensure the survival and sustainable management of these species. Resilience refers to the ability of sturgeons to withstand and recover from stressors, such as thermal changes, which can have detrimental effects on their growth, development, and overall health. Stress-reducing natural compounds can be used to increase the resistance of larval or juvenile sturgeon when released into river mouths, which can be an effective strategy for restoring the stocks of this important fish species. Consequently, understanding the physiological responses of sturgeons to thermal stress and exploring potential strategies to enhance their resilience are crucial in the context of conservation and management of these valuable species. The aim of this study was to investigate the metabolic (ALT, AST, ALP, and cortisol) and immune (lysozyme, C3, and IgM) responses to thermal stress in sturgeon juvenile previously exposed to amygdalin as a stress-reducing compound.

MATERIALS AND METHODS

Fish: A total of 300 starry sturgeon juvenile with an average weight of 20±1.6g and an average length of 18±2.8cm were obtained from the Shahid Beheshti Sturgeon Restoration and Genetic Conservation Center in (Rasht, Iran). The fish were transported to the Marine Biology Laboratory at the University of Guilan for acclimatization. They were kept in a 500-liter tank with a pH of 7.6±0.2, a temperature of 19±1°C, and dissolved oxygen levels of 8.4±0.1mg/L for two weeks. During this two-week period, the fish were fed twice a day at 3% of their body weight with feeding granules purchased from Isfahan Mokammel Co. in Isfahan, Iran. The ingredients of this feed include fish meal, wheat flour, wheat gluten, corn gluten, soybean meal, vegetable protein concentrate, fish oil, vegetable oil, mineral and vitamin supplements for sturgeon, immune stimulants, food absorbents, lecithin, and antioxidants.

Determining the optimal sub-lethal concentration of amygdalin: Before commencing the experiment, obtaining the optimal concentration of amygdalin for injection into the juvenile was necessary. Based on Zarei et al. (2024) Owing to constraints in procuring precious sturgeon samples, the hepatic cells of these majestic creatures were employed in ascertaining the

ideal dosage of amygdalin. With a molar mass of 457.43g/mol, amygdalin presents itself as a naturally occurring chemical compound. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay is a quantitative colorimetric method that relies on the enzymatic regeneration of yellow crystals, which are soluble in water, and the formation of purple, insoluble formazan crystals. MTT reduction occurs solely in living cells through the mitochondrial succinate dehydrogenase enzyme. Formazan crystals can be dissolved in organic solvents such as isopropanol and dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany), and the number of living cells can be determined by measuring the light absorption using a spectrophotometer. This method eliminates the steps of washing and collecting cells, which often result in cell loss and increased test error. All test procedures, from the initiation of cell culture to reading the results with a photometer, are performed in a single microplate, ensuring high repeatability, accuracy, and sensitivity. Consequently, after trypsinizing the adherent cells, a quantity of 5x105 cells/ml was added to the wells of a 96-well plate. After 24 hours, the cells were treated with various concentrations of amygdalin (0, 1.25, 2.5, 5, 10, 20, 40, and 100 mM) and kept in an incubator at 22°C with 5% CO_2. After 24 h, 10% MTT solution (5 mg/ml) was added. After an incubation period of approximately 2.40 to 4 hours, the produced formazan product became soluble by the addition of a solvent such as DMSO (100 microliters). Finally, the solution's optical absorbance was measured at a wavelength of 570 nm, and the survival rate (Formula 1) was calculated (Jamalzadeh et al. 2016). The final volume in each well was 200 µl.

Formula 1:

$$\text{Cell viability} = [\frac{\text{OD (treatment)}}{\text{OD (control)}}] \times 100\%$$

Based on the results of the MTT assay, the optimal dose of amygdalin was selected 80 mM for the experiment (Zarei et al. 2024).

Thermal stress: Sturgeon juvenile were placed into five separate tanks with 10 fish in each tank and three replicates for each treated group. The tanks were equipped with aquarium heaters of 100 Watt and 300 Watt, and the water temperature was increased from 18 to 22 and 26°C. Considering that the water temperature in hatchery facilities hosting sturgeon juvenile was nearly 18°C, this specific temperature was assigned as the control group. The five tanks were: 1) fish kept at 18±1°C (Control), 2) fish kept at 22±1°C (T22), 3) fish pre-treated with amygdalin and kept at 22°C (A+T22), 4) fish kept at 26±1 °C (T26), and 5) fish pre-treated with amygdalin and kept at 26 °C (A+T26). Amygdalin was injected intramuscularly with an insulin syringe at a dose of 80 mM. On the third day, for anesthesia, the fish were transferred to a smaller container filled with water and clove oil (30 mg/L) (Neiffer & Stamper 2009). Subsequently, the fish were placed in a wet cotton cloth, and blood was drawn using 2ml heparinized disposable syringes. The blood samples were then centrifuged at 704 G for 10 minutes at 4°C, and the resulting serum was stored at -70°C. Liver tissue samples were collected from each group. For euthanasia, the fish were first anesthetized with benzocaine and then frozen.

Enzyme Assay: The levels of ALT and AST were measured using Pars-Azmoon kits (Iran) according to the method described by Prashanth & Manjunatha (2022). The solution was analyzed colorimetrically at 37°C and a wavelength of 340 nm, measuring ALT and AST in liver tissue in units of u/kg.

The ALP activity was determined using a Pars-Azmun kit (Iran) based on the method described by Berger (1983). The conversion of nitrophenyl phosphate to nitrophenol and phosphate was used for the measurement, which was performed at 37°C and a wavelength of 405nm.

Total Antioxidant Capacity (T-AOC): The FRAP method is a technique used to assess the antioxidant capacity of substances by measuring their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in an acidic environment, resulting in the formation of a detectable colored complex. To carry out this method, one must first prepare tissue or cell samples in phosphate-buffered saline (PBS), similar to the ABTS

method. The necessary reagents for this process include the FRAP reagent, which contains ferric chloride.

Once the samples are prepared and the reagents assembled, they are mixed together and allowed to incubate for a specified period. Following incubation, the absorbance of the solution is measured at 593 nm using a spectrophotometer. The intensity of color formation directly corresponds to the total antioxidant capacity (T-AOC) of the sample being analyzed.

To determine T-AOC values accurately, absorbance readings are compared against a standard curve created from known concentrations of Trolox, ensuring precise calculations and reliable results in evaluating antioxidant properties through this intricate methodology (Munteanu et al. 2021)

Complement component C3: The concentration of fish complement component C3 was quantified using a Fish ELISA kit (Hangzhou Eastbiopharm Co., Ltd.) and the sandwich enzyme-linked immunosorbent assay (ELISA) method outlined by Nash et al. (2000). In brief, 40µl of the sample was introduced into a pre-coated well containing a monoclonal antibody specific to fish complement component C3. Following this, 10µl of biotin-labeled C3 antibodies were added, and the mixture was incubated at 37°C for one hour. After washing to remove unbound enzymes, 50 µl of streptavidin-HRP was added to form immune complexes, which were further incubated at 37°C for 10 minutes. The resulting blue color shifted to yellow upon appending 50µl of sulfuric acid to halt the reaction. Optical density (OD) was then measured at 450nm using an ELISA reader (ELX800 Absorbance Reader, BioTek, USA). Standard concentrations and corresponding OD values were utilized to generate a standard curve linear regression equation, which was subsequently applied to the sample OD values for concentration calculation. Negative and positive controls were established using a blank well (containing only chromogen solutions 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 found to be <12% and <10%, respectively. The results for C3 concentrations are reported in mg/dL.

Immunoglobulin M and cortisol levels: IgM and cortisol, similar to complement component C3, were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) with separate ELISA kits from Eastbiopharm (Hangzhou Eastbiopharm Co., Ltd.). The method was based on the approach described by Nash et al. (2000).

Lysozyme activity: The modified turbidimetric method was used to determine the activity of lysozyme. A small volume (25µl) of the sample was added to a cuvette containing a suspension of Micrococcus lysodeikticus bacteria (0.2g/ml in phosphate-buffered saline). The total volume in the cuvette was then adjusted to 200µl by adding 175µl of the bacterial suspension.
The absorbance of the samples was measured at a wavelength of 450 nm using a spectrophotometer (Ultaspect 3000, Pharmacia Biotech) in the first and fifth minutes after the addition of the sample. The absorbance provides information about the degree of bacterial lysis caused by the lysozyme activity in the sample. To quantify the lysozyme activity, a decrease in absorbance was considered as an indication of the lysozyme's ability to lyse the bacterial cells. Specifically, every 0.001 absorbance reduction per minute was considered as one unit of lysozyme activity. This metric allows for the comparison of lysozyme activity across different samples. The experiment was performed three times to ensure the reliability of the results. To establish a baseline for comparison, egg white lysozyme obtained from Sigma-Aldrich, St. Louis, MO, USA, was used as the standard (Elsayed et al. 2022).

Statistical analysis: Statistical analysis was conducted using SPSS version 19 on a Windows 11 operating system, with graphs generated using Excel 2013. Normality and variance homogeneity were evaluated using the Kolmogorov-Smirnov test and Levene's test, respectively. Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify significant differences in means at a

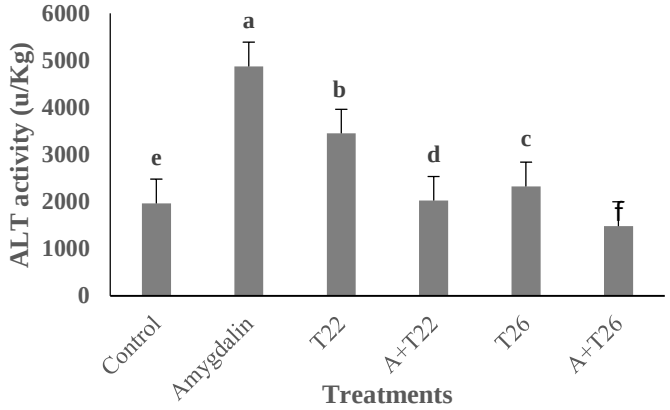

Fig.1. Changes in ALT activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected to thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+T26 group, there were fish at 26°C with amygdalin pretreatment.

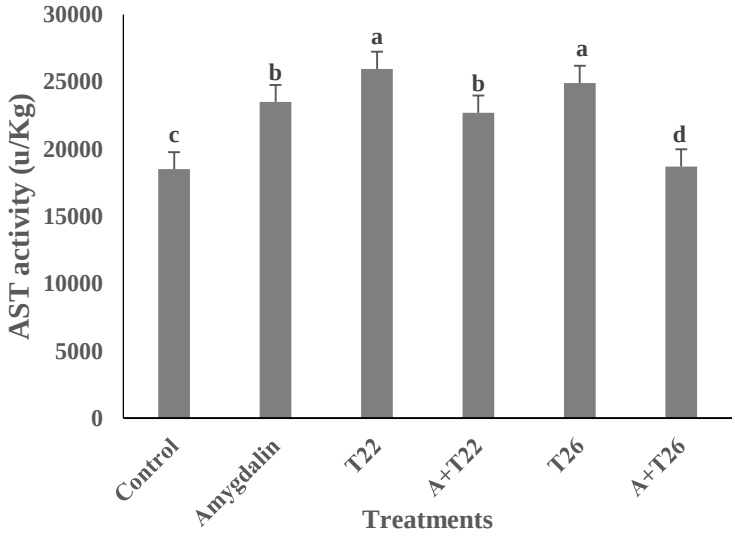

Fig.2. Changes in AST activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected at thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The Amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+T26 group, there were fish at 26°C with amygdalin pretreatment.

95% confidence level. All data are expressed as mean±standard deviation (SD).

RESULTS

Serum Enzymes and Immune Parameters): In comparison with the control and Amygdalin, ALT enzyme activity significantly decreased in all treatment groups (A+T22, T22, A+T26, and T26) (P<0.05; Fig. 1). When compared between the treatment of T22 with A+T22 and the treatment of T26 with A+T26, the activity of the ALT enzyme in fish treated with amygdalin exhibited a significant decrease (P<0.05). Amygdalin treatment yielded the most pronounced elevation of ALT levels.

A significant difference was observed in the level of AST activity between the control and amygdalin and treated groups (Fig. 2). The lowest level of AST activity was found in the T26+A group (P<0.05). Comparing the treatment of T22 with A+T22 and T26 with A+T26 demonstrated a significant reduction in AST activity in fish that were subjected to amygdalin treatment (P<0.05). A marked disparity was observed

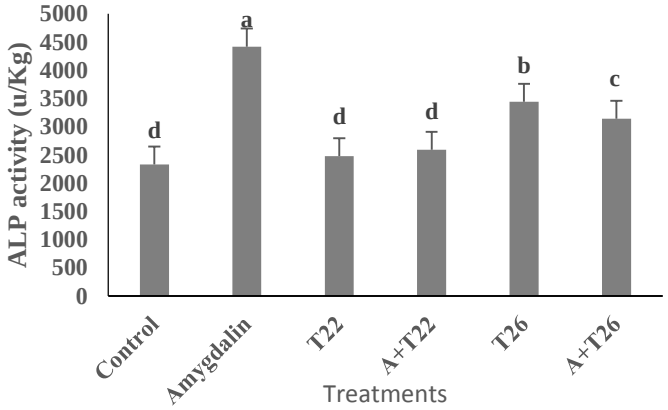

Fig.3. Changes in ALP activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected at thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The Amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+T26 group, there were fish at 26°C with amygdalin pretreatment.

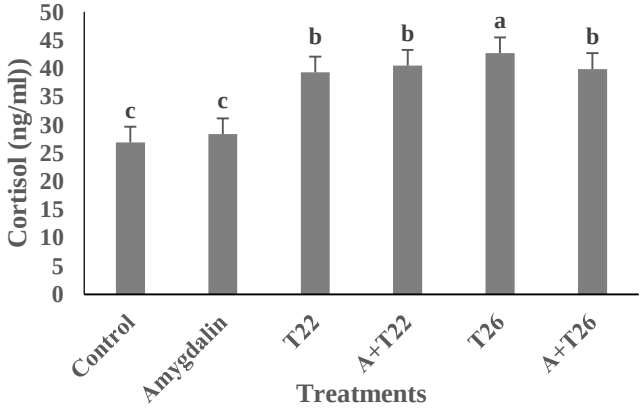

Fig.4. Changes in cortisol activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected at thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The Amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+T26 group, there were fish at 26°C with amygdalin pretreatment.

in the levels of AST between amygdalin treatment and the other treated groups (T22, T26, A+T26, Control) (P<0.05).

A significant difference was observed in the amount of ALP detected between the control, amygdalin group and the treated subjects (P<0.05) (Fig. 3). A remarkable disparity was discerned amidst T22 and A+T22, as well as T26 and A+T26 (P<0.05). The most substantial quantity of ALP was recorded in the treatment involving amygdalin.

During the examination encompassing amygdalin and its control counterparts, it became distinctly evident that the treatments administered exhibited a remarkable elevation in comparison (P<0.05) (Fig. 4). However, there was no significant difference observed between the T22 and T26 groups when compared with the A+T22 and A+T26 (P>0.05). The fish subjected to amygdalin exhibited a noteworthy decrease in their cortisol levels as compared to other treated groups except the control group.

When comparing the control group and Amygdalin group with the thermal treatments, there was a significant difference in the value of T-AOC (P<0.05) (Fig. 5). The groups treated with amygdalin (A+T22 and A+T26) showed a statistically significant decrease in T-AOC activity compared to the T22 and T26 treatments (P<0.05). There was a significant decrease in the amount of T-AOC between amygdalin treatment with A+T22 and A+T26 (P<0.05). There was a substantial decrease in the amount of C3

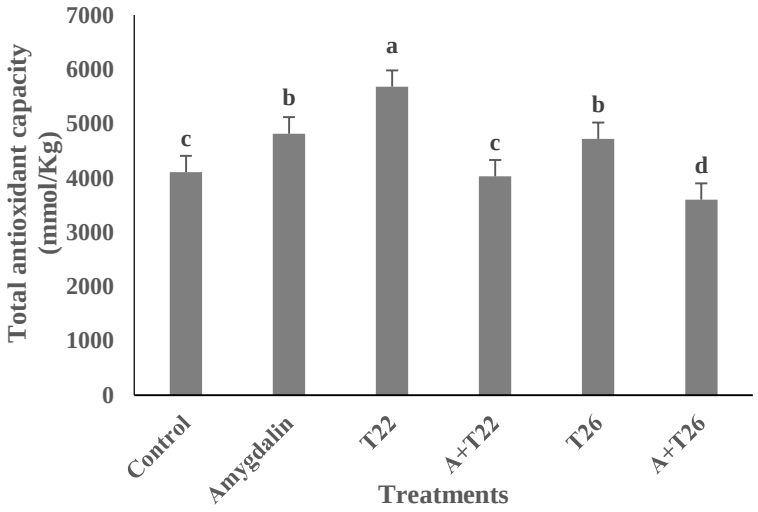

Fig.5. Changes in antioxidant capacity activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected at thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The Amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+T26 group, there were fish at 26°C with amygdalin pretreatment.

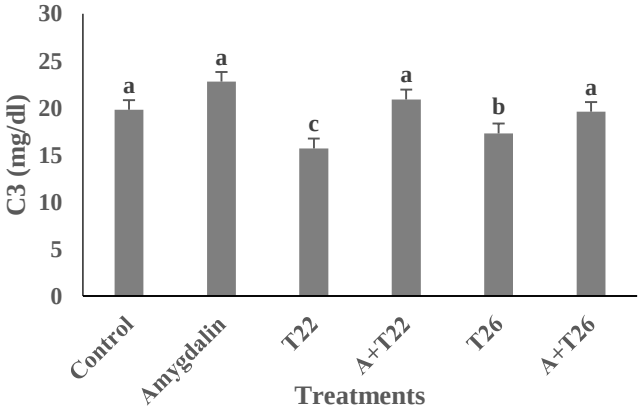

Fig.6. Changes in C3 activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected at thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The Amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+ T26 group, there were fish at 26°C with amygdalin pretreatment.

compared with other treated groups (P<0.05) (Fig. 6). When comparing T22 and A+T22, as well as T26 and A+T26, there was no significant difference observed between the treatments (P>0.05). The highest concentration of C3 was observed following the administration of amygdalin.

In the comparison between the control group and Amygdalin group with the treatments (A+T22, A+T26), the amount of IgM showed significant (P<0.05) (Fig. 7). In the comparison between T22 with A+T22 and T26 with A+T26, no significant difference was observed in the amount of IgM in the treatments (P>0.05). The quantity of IgM exhibited no significant variation among the treatments involving amygdalin (Amygdalin, A+T22, A+T26).

There was a discernible difference in lysozyme between the control and thermal treatments (P<0.05) (Fig. 8). However, no discernible change was observed between treatments T22 and T22+A, as well as between treatments T26 and T26+A (P> 0.05). The fish that received only amygdalin exhibited the highest activity of lysozyme.

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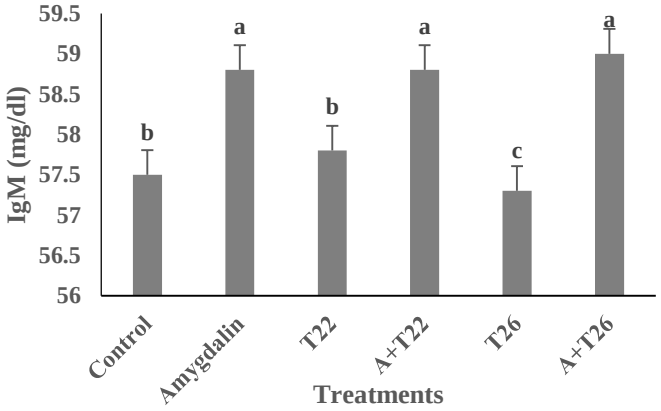

Fig.7. Changes in IgM activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected at thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The Amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+T26 group, there were fish at 26°C with amygdalin pretreatment.

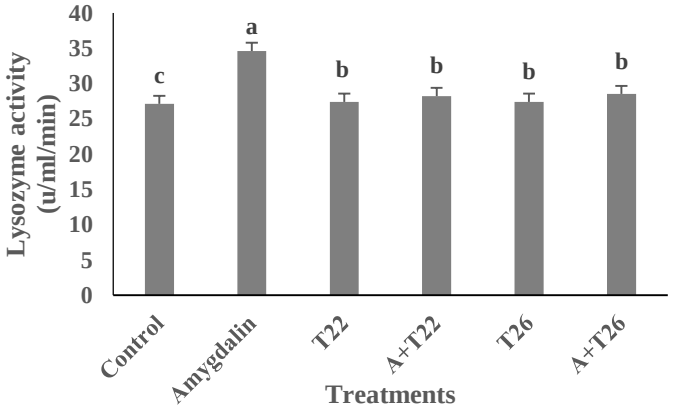

Fig.8. Changes in lysozyme activity in starry sturgeon juvenile (*Acipenser stellatus*) subjected at thermal stress in the presence of amygdalin. The control group was at a temperature of 18°C and without amygdalin pretreatment. The Amygdalin group was at of 18°C just with amygdalin. In the T22 group, there were fish at 22°C without amygdalin pretreatment. In group A+T22, there were fish at 22°C with amygdalin pretreatment. In the T26 group, there were fish at 26°C without amygdalin pretreatment, and in the A+T26 group, there were fish at 26°C with amygdalin pretreatment.

DISCUSSION

Amygdalin finds application in the therapeutics of numerous ailments, aiding in fortifying the immune system and mitigating the burden of stress (Qadir & Fatima 2017). In our examination, the administration of amygdalin has been observed to considerably augment the body's immune system in its resilience against the detrimental effects of heat stress.

The temperature fluctuations resulting from climate change possess the potential to have a profound impact on fish populations. Temperature is the most influential non-living factor that affects the physiological aspects of fish. These changes in temperature have the capacity to modify crucial elements within fish, such as their overall metabolic processes, developmental stages, immune system functionality, and even the operational levels of pivotal enzymes such as ALT and AST (Sun et al. 2022). Thermal stress can lead to cellular damage in fish, including liver damage. As a response, the enzyme AST, which is primarily found in liver cells, may be released into the bloodstream. Elevated ALT levels can indicate tissue damage or organ dysfunction. An investigation conducted on striped catfish provides evidence that as these aquatic creatures engage with higher temperatures intrinsic to their surroundings, they undergo an augmentation in activity concerning particular transaminase enzymes. In practical terms, elevated temperatures act as a catalyst for enhancing AST processes that take place

in the liver and are also carried out by ALT enzyme activities found in both liver and muscle fibers. Indeed, transaminases—specifically AST and ALT—are deemed irreplaceable enzymes integral to amino acid metabolism (Ranjan et al. 2020). The heightened activity of these liver enzymes circulating within the bloodstream serves as evidence that high temperatures induce physiological changes at the hepatic level, specifically in red cusk-eels (Dettleff et al. 2022). Our research indicates that when exposed to increased temperatures, there was an increase observed in both ALT and AST enzyme levels among our samples—an indication that they were experiencing heat-related stress. However, when amygdalin was introduced into the fish under temperature-induced stress conditions, contrasting with those without amygdalin, the levels of ALT and AST enzymes showed a decrease.

Thermal stress can also affect the activity of ALP, an enzyme found in various tissues including the liver, bones, and intestines. The exact effect on ALP levels may vary depending on the specific stress conditions and fish species. In some cases, thermal stress may cause a decrease in ALP activity, while in others it may lead to an increase. In crucian carp, it was observed that ALP activity significantly increased at temperatures of 24°C and 27°C according to a study conducted by Wang et al. (2021). Similarly, in yellowfin tuna, Liu et al. (2022) discovered that acute heat stress resulted in elevated concentrations of ALP. In the study conducted by Ranjan et al. (2020) on striped catfish, it was observed that as the temperature increased, there was a significant increase in ALP activity within the liver. The rise in ALP levels indicates tissue damage within the organism, as stated by Samanta et al. (2014). A similar investigation focusing on *Megalobrama amblycephala* explored the impact of folic acid under temperature stress, ultimately resulting in a decrease in ALP levels. In our study, we found that the administration of amygdalin to starry sturgeon, combined with temperature stress, resulted in a decrease in ALP levels as temperatures increased. Amygdalin possesses pre-hepatic metabolic properties that lead to the production of prunasin in the gut (Cipollone et al., 2008). It seems that the conversion of amygdalin to prunasin (Alwan et al. 2023) can act as an anti-inflammatory compound in the body by controlling metabolic or hepatic enzymes.

The production of cortisol, a hormone associated with stress, is often used as an indicator of stress in various fish species (Madaro et al. 2018). Short-term increases in temperature can act as a stressor for fish, resulting in elevated levels of cortisol (Goikoetxea et al. 2021). Li et al. (2016) conducted a study on trout and discovered that heat stress significantly increased cortisol levels in these fish. To counteract the effects of stress in fish, anti-stress compounds are used. Cortisol is released by fish as a response to stressful conditions (Sadoul et al., 2019). In our investigation involving *Acipenser stellatus*, we found that cortisol levels increased after the fish were subjected to temperature-induced stress. However, upon amygdalin administration, the cortisol level decreased during this period of temperature stress at 26°C. Amygdalin exhibits significant antioxidant activity, which can help mitigate oxidative stress in the body. Oxidative stress is known to trigger cortisol release; therefore, by reducing oxidative stress, amygdalin may indirectly help lower cortisol levels (Jaszczak-Wilke et al. 2021; Barakat et al., 2022).

Enzyme activity is directly impacted by temperature, affecting all enzymes found in fish, including T-AOC enzymes (Volkoff & Rønnestad 2020). As the temperature increases, there is a potential for oxidative stress to occur within the fish tissues. Oxidative stress arises when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system. The role of T-AOC is crucial in combating oxidative stress as it scavenges ROS (Lushchak & Bagnyukova 2006). Thus, it can be inferred that temperature-induced oxidative stress may affect the activity of T-AOC enzyme. This study reveals that an increase in temperature caused a simultaneous increase in T-AOC enzyme activity among *Acipenser stellatus* exposed to higher temperatures; however,

interestingly enough, these levels decreased significantly when the groups were treated with amygdalin. It's important to note that further research is needed to understand the specific mechanisms by which amygdalin affects T-AOC enzyme activity. Overall, this study highlights that amygdalin may have a modulatory effect on T-AOC enzymes activity and antioxidant effects.

Thermal stress, specifically changes in environmental temperature, can have a significant impact on the immune response of fish. Heat stress, caused by rapid temperature fluctuations, can lead to physiological and immunological stress responses in fish, affecting their immune systems and making them more susceptible to pathogens and diseases (Mariana and Badr, 2019). Both natural and artificial environmental stress factors, such as temperature, salinity, pollutants, and handling/confinement, have been shown to suppress immune functions in fish, potentially increasing their susceptibility to infectious diseases (Bowden 2008). Low temperature stress can also affect the immune response in fish, leading to changes in biochemical parameters, oxidative stress, and apoptosis (Cheng et al. 2017).

Additionally, the study conducted by Barbosa et al. (2020) suggests that exposure to temperatures outside the normal physiological range can have negative effects on the immune system, triggering stress responses. A study focusing on *Megalobrama amblycephala* investigated the efficacy of folic acid during thermal stress and discovered a positive correlation between folic acid and C3 levels during augmentation (Sesay et al. 2017). IgM serves as the initial antibody generated within the immune system, thus acting as a primary safeguard (Reddy et al. 1999). Studies conducted on rainbow trout have indicated that IgM levels escalate in response to higher ambient temperatures (Hou et al. 2001). However, it is important to note that not all fish species show consistent effects on IgM when exposed to higher temperatures. A particular study found that temperature did not affect serum IgM levels in channel catfish, but it did influence the development of mucosal IgM antibodies (Lange MD, Webster 2017). According to Saurabh et al. (2008), lysozyme can serve as an indicator of both immune function and stress response. Within the context of Nile tilapia, Mahmoud et al. (2020) found that under natural temperature conditions, serum lysozyme levels remain balanced. However, when exposed to elevated water temperatures, a significant decline in lysozyme activity was observed. Similarly, experiments conducted by Simide et al. (2018) revealed that chronic testing in Nile tilapia led to reduced lysozyme activity. In a separate study involving carp, Dawood et al. (2020) replicated the findings of decreased lysozyme levels in response to higher temperatures, consistent with previous research. These collective observations highlight the impact of temperature-induced stress on lysozyme activity, indicating a potential correlation between elevated temperatures and a decrease in immune function. The current study demonstrates that temperature stress negatively affects immune parameters in fish. However, the presence of amygdalin during thermal stress significantly boosts the immune system, particularly in relation to C3 and IgM levels. Amygdalin plays a crucial role in the immune system by exhibiting anti-inflammatory effects and modulating immune responses. Research indicates its ability to reduce inflammatory cytokines, inhibit nitric oxide production, and suppress the activity of inducible nitric oxide synthase (Wang et al. 2020; Alwan et al. 2023;). Moreover, amygdalin has been observed to activate macrophages and enhance immune responses (Zhang et al. 2022).

CONCLUSION

The study found that amygdalin treatment led to a significant decrease in serum enzyme levels, particularly ALT and ALP, in starry sturgeon juveniles exposed to thermal stress. This suggests that amygdalin may help mitigate tissue damage caused by temperature fluctuations. Fish treated with amygdalin exhibited lower cortisol levels compared to other groups, indicating a potential stress-reducing effect of amygdalin under thermal stress conditions. The research highlighted that thermal stress adversely

affects the immune parameters of starry sturgeon, but the presence of amygdalin can counteract some of these negative effects. Amygdalin appears to be a beneficial agent for improving the resilience of starry sturgeon juveniles against thermal stress, enhancing both metabolic and immune responses.

FUNDING

This research has been financially supported by the Caspian Sea Basin Research Center (NO. 2978321). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Iran. J. Ichthyol. (2025) 12(1): 22-38
P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

doi: 10.22034/iji.v12i1.1039

مقاله کامل

پاسخ‌های فیزیولوژیکی بچه ماهی خاویاری ازون برون (*Acipenser stellatus*) به استرس حرارتی: بررسی اثرات آمیگدالین در افزایش تاب‌آوری ماهیان خاویاری

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چکیده: خانواده Acipenseridae یکی از ساکنان حیاتی اکوسیستم‌های آبی است و ناملایمات زیست‌محیطی می‌تواند عواقب مضری بر سلامت آنها داشته باشد. در حال حاضر از ترکیبات کاهش‌دهنده استرس و محرک‌های سیستم ایمنی برای حمایت از مقاومت ماهی استفاده می‌شود. این مطالعه با هدف بررسی پاسخ‌های متابولیک و ایمنی به استرس حرارتی در بچه ماهیان خاویاری که قبلاً در معرض آمیگدالین به‌عنوان یک ترکیب کاهش‌دهنده استرس قرار گرفته بودند، انجام شد. ماهی‌ها در دماهای مختلف (۱۸ درجه سانتی‌گراد، ۲۲ درجه سانتی‌گراد و ۲۶ درجه سانتی‌گراد) با آمیگدالین یا بدون آمیگدالین، با سه تکرار در معرض قرار گرفتند. نمونه‌های خون و بافت کبد جمع‌آوری شد و پارامترهای مختلف اندازه‌گیری شد. نتایج نشان داد که تفاوت معنی‌داری در آنزیم‌های سرمی، سطوح کورتیزول، ظرفیت آنتی‌اکسیدانی و اجزای سیستم ایمنی در بین گروه‌های مختلف وجود دارد. بدیهی است که ماهیان پیش تیمار شده با آمیگدالین و نگهداری در دمای ۲۲ درجه‌سانتی گراد (A+T22) و ۲۶ درجه سانتی‌گراد (A+T26) کاهش قابل توجهی در ALT (آلانین‌آمینوترانسفراز، AST (آسپارتات‌آمینوترانسفراز) و T-AOC داشتند. ظرفیت آنتی‌اکسیدانی کل در مقایسه با افرادی که تحت چنین درمان قرار نگرفته‌اند (ماهی‌ها در دمای ۲۲ و ۲۶ درجه سانتی‌گراد نگهداری می‌شوند). علاوه بر این، هنگام مقایسه ماهیان در معرض آمیگدالین در دمای ۲۶ درجه سانتی‌گراد با ماهیانی که در معرض آمیگدالین قرار نگرفته بودند، کاهش قابل توجهی در سطح ALP و کورتیزول وجود داشت که تأثیر نامطلوبی بر پارامترهای ایمنی ماهیان خاویاری دارد آمیگدالین تجویز می‌شود، این پتانسیل را دارد که با افزایش ایمنی، به ویژه، انعطاف‌پذیری ماهی را در برابر عوامل استرس زای حرارتی افزایش دهد. در C3 و IgM در نتیجه، آمیگدالین می‌تواند به‌عنوان یک عامل مفید برای ماهی‌ها با کاهش سطح استرس و افزایش انعطاف‌پذیری آنها در برابر عوامل استرس‌زای حرارتی عمل کند.

کلمات‌کلیدی: پاسخ متابولیک، ماهی خاویاری، ایمنی، استرس