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AMELIORATIVE ACTION OF *Tribulus terrestris* ON ATRAZINE EXPOSED FRESH WATER FISH *Oreochromis mossambicus* (W. K. H. Peters, 1852)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation is designed to compare to study of responses when conducted in freshwater fish *Oreochromis mossambicus* exposed at sublethal concentration of atrazine (16.5 mg/L) in 120 hours. The results revealed that treatment of atrazine enhanced the level of the hepatic enzyme activity of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Subsequently the treatment of *Tribulus terrestrisis* active constituents at a dose of 1.2 g/L drastically restored to the normal levels of all hepatic enzymes (AST, ALT, ALP and LDH). Therefore our experimental studies suggest that the administration of *Tribulus terrestris* is proved to be the best ameliorative agent for atrazine herbicide which induced the damage in hepatic.

Keywords: AST; ALT; ALP; LDH; herbicide; *Oreochromis mossembicus*; *Tribulus terrestris*.

1. INTRODUCTION

Fishes can be used to as a biomarker of environmental health and can be assume noteworthy roles in to the evaluating potential hazard related to pollution in the aquatic environment since they are directly exposed to the chemicals either directly from surface run-off waters or indirectly through the food chain of the ecosystem [1,2]. Carps have been effectively used in experimental laboratory and field studies to assess toxic effects of exposition to the several types of pesticides [3,4,5].

Atrazine is a chloro-triazine herbicide with a chemical structure based on a central carbon/nitrogen ring. The structure of this herbicide makes it highly water soluble. Atrazine also has the potential to absorb to soil or become airborne, however, the physical properties of this chemical make it more prone to dissolving in water [6]. Atrazine treatment was shown

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to negatively affect acetylcholinesterase activity, mRNA level in muscles, brain, kidney, liver and gill of common carp [7,8]. Atrazine has been found to induce oxidative stress and disturb the swimming of larval zebra fish [9]. Oxidative stress occurs when the level of ROS exceeds the antioxidant capacity of a cell, acting as a mediator in tissue injury, leading to pathophysiological alterations and apoptosis [10,1]. In the production of free radicals and ROS it leads to oxidative damage, DNA affecting, proteins, lipids, and nucleic acids also. Thus, the cellular physiological processes are seriously disrupted in diverse living organisms (Yoon, et al., 2019; Slaninova et al. [11]; Zhang et al. [12]. The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, location and blood supply [13].

This herbicide is associated with relatively high chronic toxicity. Atrazine is a carcinogen that affects the central nervous system, reproductive system, immune system and cardiovascular function. Exposure to atrazine in animals is associated with some types of NHL (Non-Hodgkin Lymphoma) in adult humans. In 2007, the USEPA began reviewing several epidemiological cancer studies concerning atrazine and its possible association with carcinogenic effect in humans. Atrazine runoff and leaching from application sites into rivers, streams, lakes, reservoirs and groundwater is an ecological concern. In some aquatic ecosystems contaminated by atrazine, photosynthesis of algae, a primary producer in food chain, may be inhibited [14].

The use of herbal medicines can be traced back thousands of years ago. There is an increased to hunt for drugs and nutraceuticals from medicinal and nutritional plants in recent years [15]. However, with the high demand of bio-actives from traditional knowledge for therapeutic use proper scientific evaluation of therapeutic potential and validation by modern science is highly warranted. *Tribulus terrestris* is extremely rich in saponins, flavonoids, alkaloids, glycosides, phytosteroids and other constituents [16]. Many pharmaceutical preparations and dietary supplements that include the saponin fraction of *Tribulus terrestris* are currently on sale worldwide [17,18,19], (Jagadeesan and Kavitha, 2006) Pharmacological analysis has revealed that there are several active components of *Tribulus terrestris* [20]. It has been reported that *Tribulus terrestris* exhibits its effects in particularly its saponin-containing steroidal compounds that are structurally in glycoside form [21]. Despite the various steroidal saponins that have been isolated [22,23,24], their mechanisms of action remain obscure. It has been demonstrated that the hypertensive effects of *Tribulus terrestris* are exerted via decreasing levels of activity of angiotensin converting enzyme (ACE), especially in the kidneys [25]. It has been proposed that *Tribulus terrestris* may have a hypolipidemic effect, although it is still not clear how it affects lipid metabolism [26]. The mechanism of action of the apparent beneficial effects of *Tribulus terrestris* on the cardiovascular system and hyperlipidemia remain to be elucidated.

2. MATERIALS AND METHODS

2.1 Chemicals

Atrazine and other necessary reagents of analytical grade were bought form Hi-Media laboratories Ltd, Mumbai, India.

2.2 Fish Maintenance and Acclimatization

The freshwater fish *Oreochromis mossambicus* were collected from around the Cheyyar surroundings, Thiruvannamalai District. The collected fish was acclimated to laboratory condition for 15 days. They were checked thoroughly for injury and disease conditions, and only healthy fishes were used for this study. After washing with 0.01% KMnO4 solution for 15 min, they were placed in nine plastic pools (500 L) containing non-chlorinated water. Prior to the start of the experiment, the fishes were acclimatized to food and laboratory conditions with 12h dark and 12h light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24°C for 15 days. The fishes measuring 11-14cm in length and 40-60g weight were selected irrespective of their sex for the experiments. Solutions were renewed once daily after exposure period, animals were sacrificed and isolated for their liver tissues, homogenized and stored at −80 °C for further biochemical analyses.

2.3 Supplement Feed

Healthy plants of *Tribulus terrestris* were collected from the areas around Cheyyar. The plants were washed in running tap water for 10 minutes and were dried, 1kg of *Tribulus terrestris* were macerated thrice at room temperature and prepared in powdered form and equal amount of rice bran was mixed well and small amount of water were added and small pellet were made.

2.4 Experimental Design

Fishes were divided into four equal groups each comprising of 25 fishes.

Group I	Fish exposed to freshwater for 120 hours (Control)
Group II	Fish exposed to sublethal concentration of atrazine (16.5 mg/L) for 120 hours
Group III	Fish exposed to atrazine (16.5 mg/L) and *Tribulus terrestris* (1.2g/L) for 120 hours
Group IV	*Tribulus terrestris* alone (1.2 g/L) for 120 hours

2.5 Biochemical Analysis

The activity of alkaline phosphatase was assayed by the method of King and Armstrong, [27]. The activity of AST, ALT and LDH was determined by adopting the method of King (1965).

2.6 Statistical Analysis

Values are given as mean ± S.D. for 25 fish in each group. The data for various biochemical parameters were analyzed using analysis of 't'-test and group means was compared by Duncan's multiple range test (DMRT) 1957. Values were considered statistically significant when $p<0.05$ and the values sharing a common superscript did not differ significantly.

3. RESULTS

After 120 h of atrazine exposure significant increase in ALT, ALP, AST and LDH activity was observed in liver tissues of *Oreochromis mossambicus* when compared to their corresponding control. Mostly all the biochemical parameters were found to be restored near normal level at the time of recovery period of *Tribulus terrestris* treatment.

4. DISCUSSION

The activity of ALT, AST and ALP in the liver tissues of fish is tested as hepatic functions indicators. In the present study the activities of ALT, ALP and AST in the liver tissue atrazine exposed fish were drastically increased. Many studies have examined that atrazine causes a disturbance in liver function manifested by an increase in activity of ALT, ALP and AST. Therefore, the increase in ALT, ALP and AST activity noted in this study may be explained by the leakage of these enzymes from the liver cytosol into the blood stream. On the other hand, the significantly elevated aminotransferase's (ALT and AST) activities in the blood of fish during short-term exposure, with concomitant decrease in liver during prolonged exposure, could be due to cellular degradation of these tissues, resulting in interference with the normal physiological and biochemical processes such as Kreb's and tricaboxylic cycles and subsequent leakage into the bloodstream [28], (Banaee et al., 2014). When cell membrane of hepatocytes is damaged variety of enzymes such as ALT, AST, and ALP are released into the blood stream from the cytosol.

The hepatic injury caused by atrazine may be attributed to the enhanced oxidative stress. The induced hepatic damage mainly related to the lipid peroxidation causes rapid breakdown of the structure and function of the membranes such as those of the endoplasmic reticulum, mitochondria, and lysosomes, leading to plasma membrane damage [1]. Furthermore, the dynamics of ALT and AST activities during short-term and prolonged exposures appear to be related to the reduction of protein in the tissues of atrazine exposed fishes. This seems obvious as increased levels of these enzymes in stress conditions have stimulatory effects on gluconeogenic process to generate energy for detoxification process, homeostatic maintenance and tissue repairs. Thus, increased levels of these enzymes in the liver of atrazine-exposed fishes is in response to oxidative stress and could be an attempt to supply enough energy needed to survive the toxic stress. During the recovery period *Tribulus terrestris* exposed time reach the near normal level.

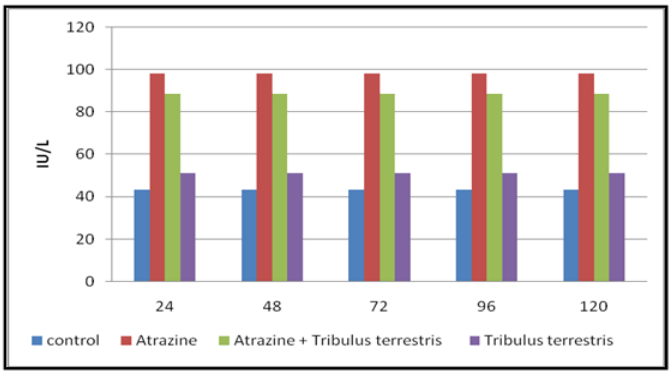

Fig. 1. Alanine amino transferase (ALT) activity in liver tissues of *Oreochromis mossambicus* exposed for 120 hours

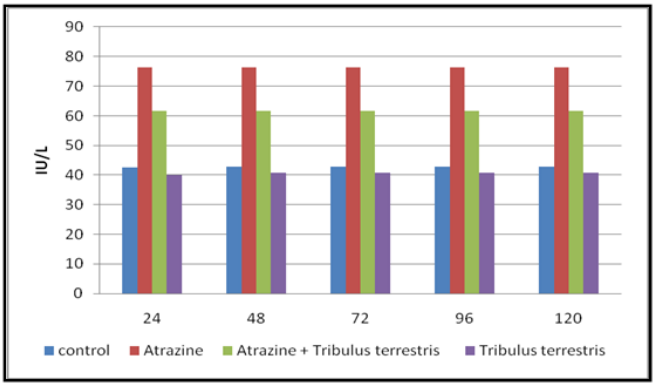

Fig. 2. Alkaline phosphatase (ALP) activity in the liver tissues of *Oreochromis mossambicus* exposed for 120 hours

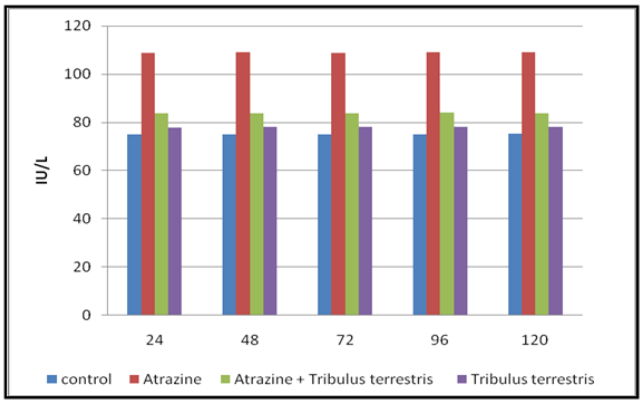

Fig. 3. Aspartate aminotransferase (AST) activity in liver tissues of *Oreochromis mossambicus* exposed for 120 hours

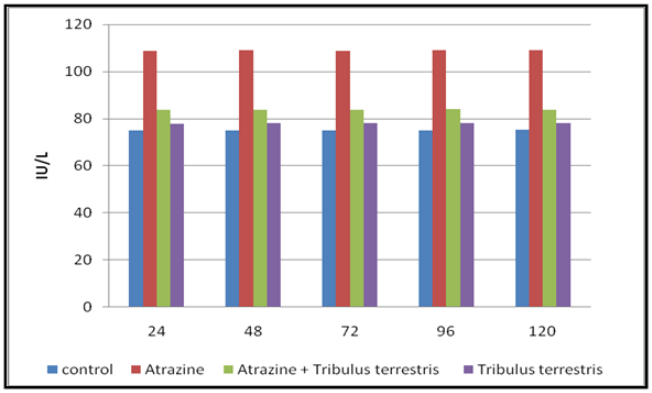

Fig. 4. Lactate Dehydrogenase (LDH) activities in liver tissues of *Oreochromis mossambicus* exposed for 120 hours

Lactate dehydrogenase is a bifunctional enzyme of the glycolytic pathway which is involved in the inter conversion of lactate and pyruvate. LDH is located on strategic point between glycolysis and citric acid cycle, which catalyzes the reversible oxidation of lactate and malate to pyruvate, serving in the terminal step of glycolysis [29]. LDH is an important glycolytic enzyme in anaerobic pathway of carbohydrate metabolism. In this study, the trend in LDH activity is in contrast to that noticed in aminotransferase enzymes. LDH activities significantly increased in all the tissues during both exposures, except the slight increase observed in the liver of fish at subleathal acute exposure. During

prolonged exposure, the impact of atrazine became overwhelming as evidenced in decreased activities of LDH in tissues that also corroborated the reduction of protein in these tissues. Significant increase in LDH may favor the anaerobic respiration to meet up the energy demand when aerobic oxidations are lowered [30]. Furthermore, in case of anoxic condition the body also tries to shift respiratory metabolism to anaerobiosis and hence conversion of lactate to pyruvate take place in order to mitigate the energy crisis [31] and thus LDH reflects the metabolic capacity of tissues after long term exposure to contaminated water bodies [32]. Liver tissue is the richest source of LDH and any deformities in the liver organ might increase the level of LDH [30]. During the treatment the stabilization of these marker enzymes were most effectively done by *T. terrestris* is clearly indicates that the improvement of the functional status of the liver cells by the way of restoration of these enzymes activities in the liver tissues and it may protect against acute organ dysfunction and cellular injury depending upon the antioxidative effect.

5. CONCLUSION

In the present experimental studies suggest that *T. terrestris* is having more patented against the atrazine toxicity. Further research is needed to supporting these results.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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