

TOXICITY OF ATRAZINE AND RELATED TO TESTICULAR, TISSUE DAMAGING ENZYME LEVELS IN *POECILIA SPHENOPS*

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ABSTRACT

Objective: This study was designed to evaluate the toxic effects of Atrazine (ATZ) on the enzyme levels in *Poecilia sphenops*.

Methods: The cytosolic testicular enzyme and tissue-damaging enzyme activity were measured in *Poecilia sphenops* was exposed to 3 sub-lethal concentrations (1.25, 2.5 and 5 mg/l) of atrazine for 100days and control was also maintained. The toxic effects of ATZ to *Poecilia sphenops* were assessed through testicular marker enzyme, tissue-damaging enzyme level.

Results: The activities of testicular functional enzyme ALP, ACP, SDH, LDH, G6PDH and tissue-damaging the activities of glutamate oxaloacetic transaminase (GOT) and glutamate pyruvic transaminase (GPT) activity levels were altered in treated groups compared with that of the control.

Conclusion: This study demonstrates that atrazine induces tissue damage in terms of enhanced enzyme activity and testicular enzyme activity in *Poecilia sphenops*. Atrazine has toxicity to the reproductive system in *Poecilia sphenops*.

Keywords: Atrazine, *Poecilia sphenops*, Testicular Marker, ALP, ACP, SDH, LDH, G6PDH, GPT GOT

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INTRODUCTION

Pesticides affect all members of an ecosystem from the smallest invertebrates to birds and humans. Toxicities of pesticides in both urban and agricultural settings are responsible for the death of many birds, fishes, and smaller aquatic animals on which fishes depend on for food [1].

Herbicides control or kill plants through a variety of mechanisms, including the inhibition of biological processes, such as photosynthesis, mitosis, cell division, enzyme function, root growth, or leaf formation, interference with the synthesis of pigments, protein or DNA, destruction of cell membranes; or the promotion of uncontrolled growth [2]. Atrazine is a triazine herbicide that is used as a selective pre-emergence and post-emergence herbicide for the control of weeds in asparagus, maize, sorghum, sugarcane, pineapple and paddy fields. It is also used in forestry for non-selective weed control on non-crop areas. It has been employed extensively in agriculture in the US and worldwide for over 40 y [3-5]. Because of its widespread use, atrazine residues have contaminated not only plants, soil, water and cultivated ground but also agricultural products like fruits, milk, butter, grains and sugar beet [6].

Acid [EC3.1.3.2] and alkaline phosphatase [EC3.1.3.1] hydrolyse phosphoric esters of various phosphate-containing compounds at acid and alkaline pH, respectively. Acid phosphatases which are ubiquitous in plants, animals, fungi and bacteria are often detected as marker enzymes of lysosomes in cellular soluble fractions, while alkaline phosphatases are found on the plasma membrane [7, 8]. Both enzymes have been isolated from various sources and their enzymatic properties have been investigated [9, 10]. These enzymatic activities are involved in a variety of metabolic processes such as production, transport and recycling of inorganic phosphate, which are prerequisite for cell growth and cell differentiation.

Therefore enzyme assay is one of the important methods to evaluate the toxicity of xenobiotic. At present, no report is available on the effect of atrazine on testicular enzyme activities. This study focus light on the effects of atrazine on tissue damaging enzyme and testicular enzymes ALP, ACP, LDH, SDH, and G6PDH in *Poecilia Sphenops*.

MATERIALS AND METHODS

Test species: *Poecilia sphenops*

The newly hatched juveniles/fries were separated from their respective mother and maintained in 100 L tank. A total of 200 fry (0 d old fry) were separated and used in four equal treatment groups. Fifty individuals' fries in each group were exposed to three different concentrations (1/10th of LC 50, 2.5 ppm, 1/20th of LC 50, 1.25 ppm and 1/30th of LC 50, 0.83 ppm) of atrazine and control also maintained simultaneously. The aquarium system was static and the bathing medium was changed once in a week with the same concentration of atrazine was renewed. Periodically one or two experimental fishes were sacrificed and dissected out the required organ for analysis. The experiment was repeated thrice to minimize the error.

Sample preparation

At the end of 60, 80 and 100 d of atrazine exposure, *Poecilia sphenops* were dissected and liver, gonads and gill tissue samples were obtained and homogenized separately with 0.01M Tris-HCl buffer (pH 7.2) then centrifuged at 12,000 rpm at 4 °C for 15 min. The supernatant obtained was used as an enzyme extract for determination of the enzymes. The protein content of tissue was quantified with the method of Bradford [11].

Testicular enzyme assay

Testicular enzymes such as Sorbitol dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities were assayed by Gerlach [12] and Vassault [13], Acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were assayed by Estiarte [14] and Michell [15], Glucose-6-phosphate dehydrogenase (G6PDH) activity was determined according to the method of Balinsky and Bernstein [16].

Tissue damaging enzymes

The activities of glutamate oxaloacetic transaminase (GOT) and glutamate pyruvic transaminase (GPT) activity was assayed by King [17].

Statistical analysis

The obtained values are expressed as mean±SE. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software package for Windows (version 16.0). Post-hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test (p<0.05) was considered statistically significant.

RESULTS

Testicular enzyme activity

Acid phosphatase activity

Fig. 1 represents the specific activity of ACP in the testis of *Poecilia sphenops* exposed to atrazine. The ACP activity in testis of *Poecilia sphenops* is low in all tested concentration of atrazine when compared to that of control testis. The activity is decreased with increasing concentration. When compared with control a significant

(p<0.05) reduction in ACP activity is found in testis of *Poecilia sphenops* exposed to atrazine.

Alkaline phosphatase activity

The content of ALP activity is decreased in a higher concentration of atrazine exposed *Poecilia sphenops* which is recorded in fig. (2). the activity shows a noticeable change at 1.25 ppm and 2.5 ppm than those of other exposure to 0.83 ppm. Decreased ALP activity in testis of *Poecilia sphenops* exposed to atrazine is statistically significant (p<0.05) when compared to control.

Lactate dehydrogenase activity

The activity of LDH in testis of *Poecilia sphenops* is depicted in fig. (3). LDH activity observed in the atrazine-exposed fish testis is low when compared to control. A significantly (p<0.05) decreased LDH activity was found in 100 d exposed *Poecilia sphenops*. However, the activity is significantly (p<0.05) very low in testis of *Poecilia sphenops* exposed to higher concentration of atrazine. A dose-dependent decrease and duration dependent increase was observed.

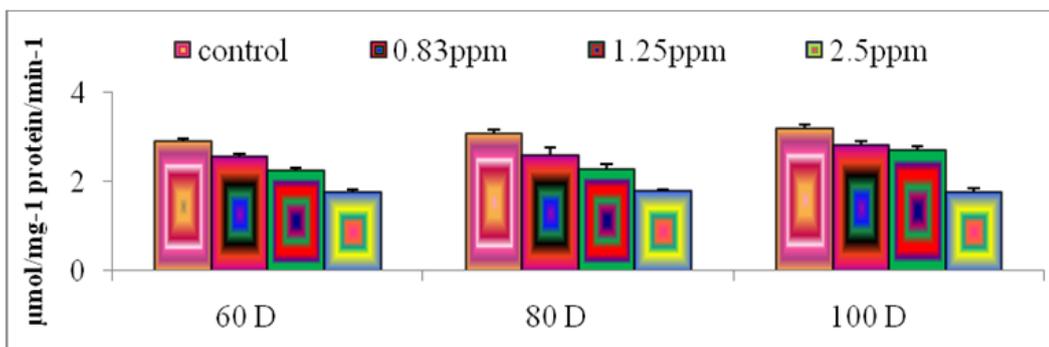


Fig. 1: ACP activity in testis of *Poecilia sphenops* treated with sub-lethal doses of atrazine

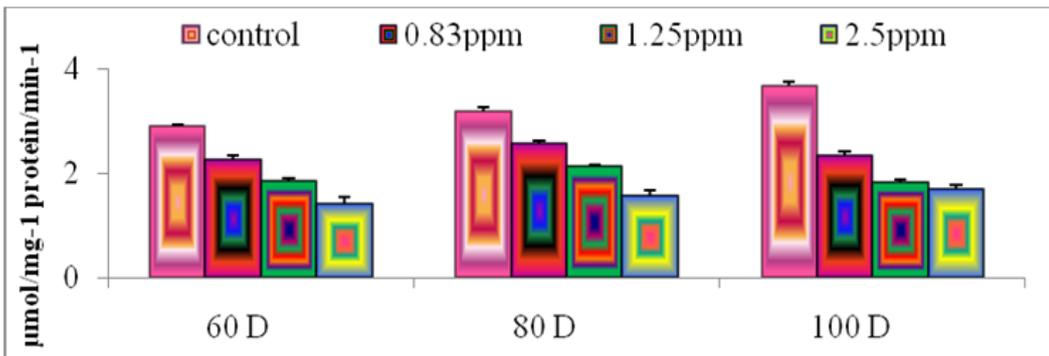


Fig. 2: ALP activity in testis of *Poecilia sphenops* treated with sub-lethal doses of atrazine

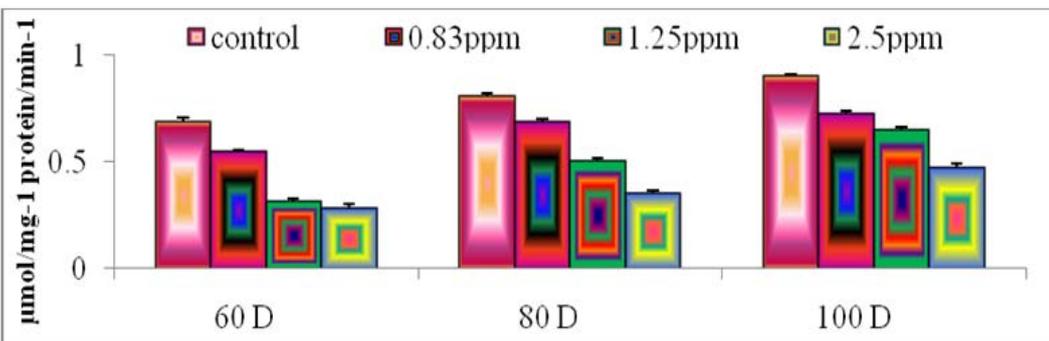


Fig. 3: LDH activity in testis of *Poecilia sphenops* treated with sub-lethal doses of atrazine

Sorbitol dehydrogenase activity

The activity of SDH is decreased at a lower concentration of atrazine and its decrease was significant ($p < 0.05$) at a higher concentration. A prominent reduction in SDH enzyme activity is noticed in 80 days and 100 d when compared to 60 d exposure of

atrazine. A decreased SDH enzyme activity is found in testis in all the concentrations when compared to control.

Significant ($p < 0.05$) decrease in SDH activity over increasing dose and duration dependent increase in testis of *Poecilia sphenops* exposed to three different concentration of atrazine (fig. 4).

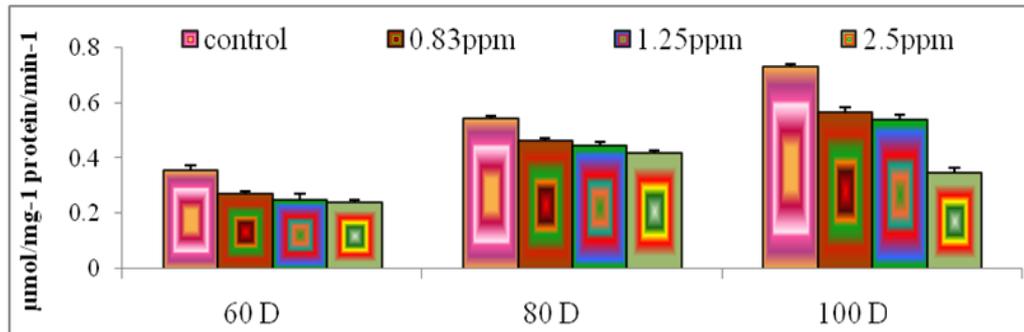


Fig. 4: SDH activity in testis of *Poecilia sphenops* treated with sub-lethal doses of atrazine

Glucose 6 phosphate dehydrogenase activity

Fig. 5 represents the activity of G6PDH of atrazine in testis of *Poecilia sphenops*. The activity of G6PDH remains unchanged at a lower concentration of atrazine and decreases significantly ($p < 0.05$) at a higher concentration. Decreased G6PDH enzyme activity is

notable in all the sampling time, however in 80 days and the activity was high 100 d the activity was higher when compared to 60 d exposure of atrazine. From the data, it is clearly evident that atrazine administration resulted in significant decrease in the activity of the enzyme in *Poecilia sphenops* after all the treatment when compared to that of control.

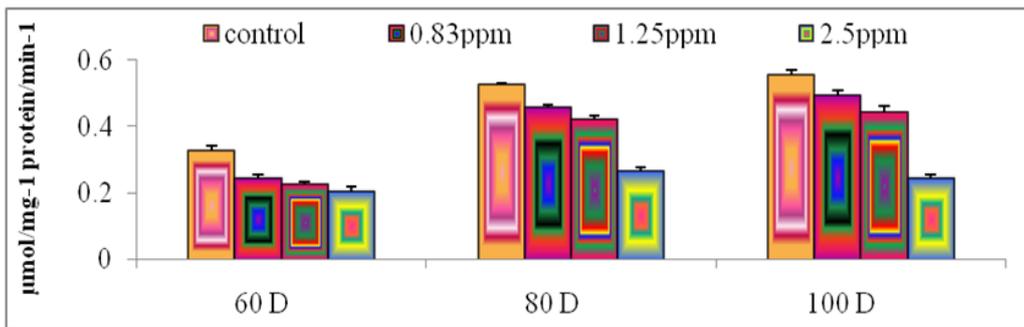


Fig. 5: G6PDH activity in testis of *Poecilia sphenops* treated with sub-lethal doses of atrazine

Impact of atrazine on tissue damaging enzyme activity

Increased GPT and GOT activities are observed in *Poecilia sphenops* exposed to atrazine (fig. 6 to fig. 9). Our data revealed that the administration of atrazine induces a significant increase

($p < 0.05$) in GPT and GOT activity in the liver as compared to control and it is more in male than female. Meantime, activities of the enzyme in the gonads and gills are significantly increased ($p < 0.05$) while compared to control and followed the trend of the liver.

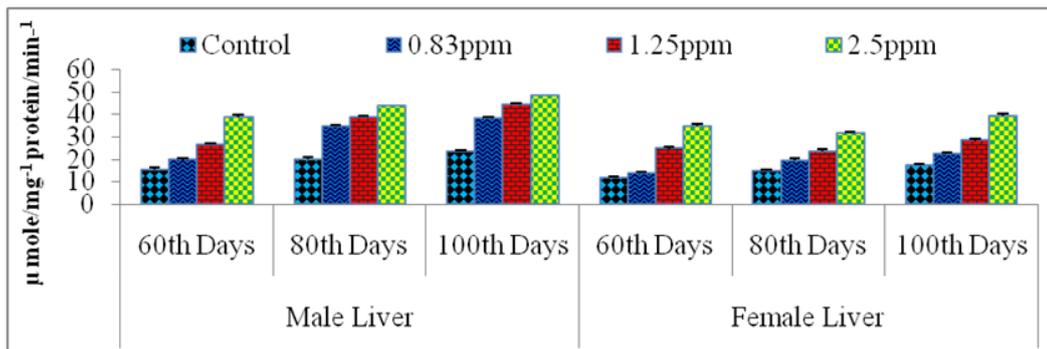


Fig. 6: GPT activity in liver of *Poecilia sphenops* exposed to sub-lethal doses of atrazine

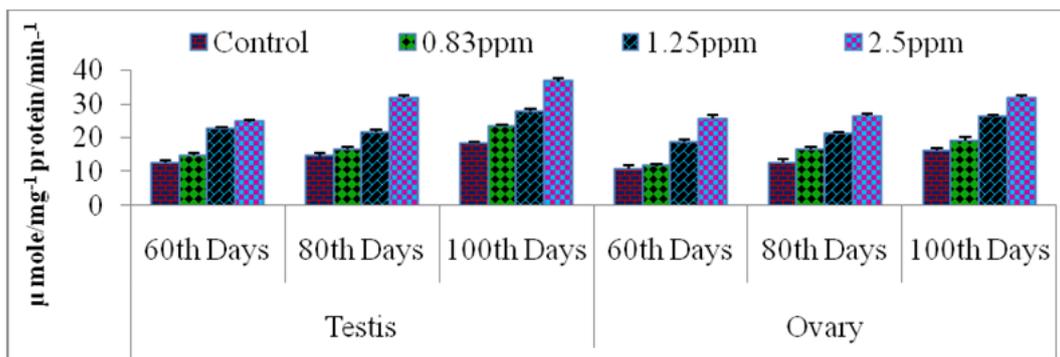


Fig. 7: GPT activity in gonads of *Poecilia spheonops* exposed to sub-lethal doses of atrazine

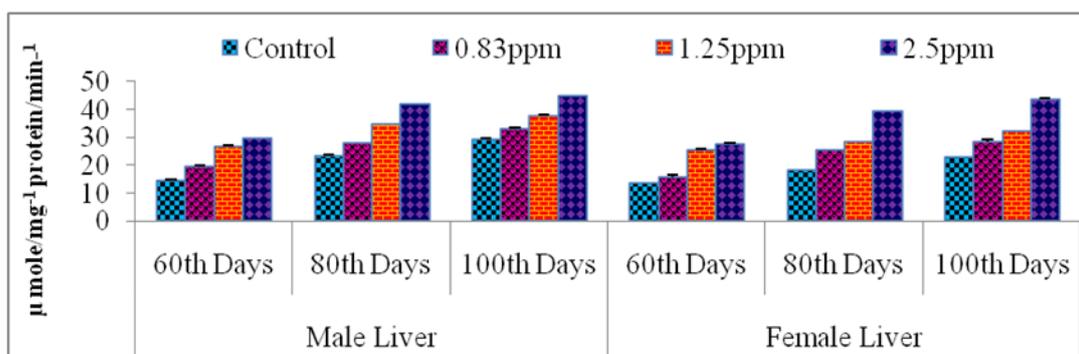


Fig. 8: GOT activity in liver of *Poecilia spheonops* exposed to sub-lethal doses of atrazine

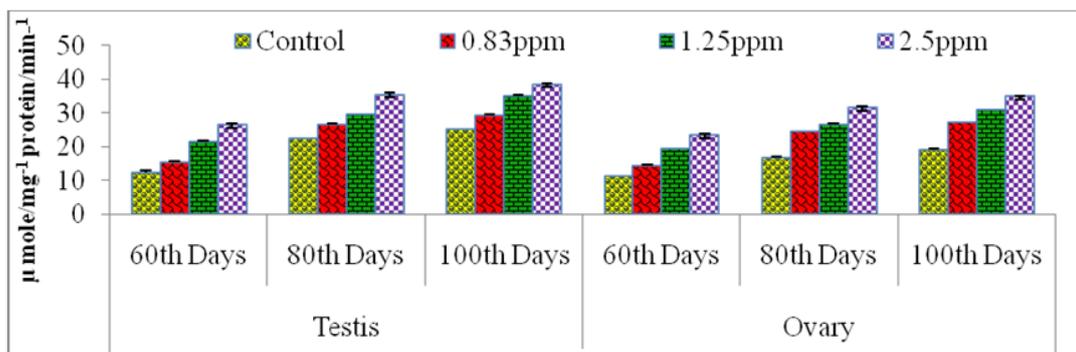


Fig. 9: GOT activity in gonads of *Poecilia spheonops* exposed to sub-lethal doses of atrazine

DISCUSSION

The activities of a testicular enzyme such as ACP, ALP, SDH, LDH and G6PDH are considered as functional enzymatic indicators of male reproduction. The function of the male reproductive system in the rat was impaired by Tetramethylthiuram disulphide (Thiram) fungicide. Administration tetramethyl thiuram disulphide changes the activity of ACP, ALP, and SDH, LDH and G6PDH and such change can be used as a biomarker for testicular toxicity [18].

Poecilia spheonops exposed to atrazine at different sub-lethal concentrations have changed the activities of testicular enzymes like ACP, ALP, SDH, LDH and G6PDH. The ALP activity is related to the spermatogenic mitosis cell division and also the transport of nutrient glucose. ACP is located in the subcellular organs like lysosome of the Leydig cells. It performs the synthesis of protein by carrying sex hormones. Alteration in the activity of ALP and ACP may be the useful tool in determining the spermatogenic function [19]. In this research ALP and ACP enzyme activity in the testis of *Poecilia spheonops* exposed to all concentration of atrazine

is decreased when compared to control. The activities of ACP and ALP enzyme are decreased gradually in low concentration to higher concentration.

A statistically significant decreased in ALP enzyme activity is found in the testis of *Poecilia spheonops* exposed to all atrazine concentrations. In this study, decreased ACP enzyme activity in testis is observed in treated groups when compared with control.

A decrease in the ACP and ALP activity in atrazine-treated *Poecilia spheonops* indicated that atrazine administration produced a state of decreased steroid genesis where inter and intracellular transport is reduced as a result in decreased steroidogenesis. Zhang and Lin [20] observed the almost equal level of ACP and ALP enzymes activity in the rat. In earlier reports decreased ACP and ALP enzymes activity show decrease in the activity of phosphatase in the nucleus of the spermatocytes during spermatogenesis [21]. Decreased enzyme activity of testicular ALP and ACP of 3, 4-DCA-treated rats also reflect testicular degeneration, which may be a consequence of suppressed testosterone and indicative of lytic activity [22].

The steroidogenesis in testis is under physiological control of two dehydrogenases LDH and SDH. LDH and SDH are widely distributed and situated in the seminiferous tubules and germ cells, which are associated with the maturation of spermatogenic cells, testis and spermatozoa. They are involved in the energy metabolism of spermatozoa [23]. SDH activity increases during the entire period of maturation of germ cells and during the depletion of germ cells, SDH activity decreased [24].

LDH present widely in spermatogenic cells and Sertoli cells and play an important role in energy production and biotransformation. Inhibition of LDH may cause damage to spermatogenic cells [25]. In this study, there is a decrease in LDH and SDH activity. The increased enzyme activity in testis and liver under normal condition, a statistically significant decrease in SDH and LDH activity ($p < 0.05$), was found in the testis of *Poecilia sphenops* exposed to all atrazine concentrations. The decreased in SDH and LDH enzyme activity is statistically differ from control. The decreased activity of LDH and SDH will affect the process of spermatogenesis and may induce infertility.

The greatest intensity of enzymatic reactions occurs in Leydig cells. The G6PDH is most commonly found in Leydig, Sertoli, and spermatogenic cells. G6PDH is more active in Leydig cells [26]. In this study, a continuous decrease in G6PDH activity ($p < 0.05$) is found in the testis in all concentrations of atrazine exposure. The decreased activity of G6PDH in the treated *Poecilia sphenops* suggested that atrazine can impair the function of Leydig cells.

Enzymes are considered as sensitive biochemical indicators of toxicity in organs of the fish [27]. The enzyme GOT and GPT are transaminases, which are basically intercellular enzymes found in most organs of fish [28]. The increase in the activities of these enzymes in the organs of *Poecilia sphenops* exposed to atrazine may be due to damage to these organs, which leads to the liberation of these intercellular enzymes and its raised levels in plasma and muscles. This result was supported by earlier findings and serve as an indicator of tissue damage [29].

In the present study, the activities of Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) of the atrazine treated *Poecilia sphenops* are significantly increased compared with control fish ($p < 0.05$). The alteration in the activity of aminotransferase enzymes may be due to cellular leakage caused by chemical induced injury of the tissue. The increase in GOT and GPT activity suggests that atrazine causes tissue damage in *Poecilia sphenops* and that damage occurred probably through a free radical mechanism. Atrazine produced moderate cytotoxic effects in liver, gonads and gill of *Poecilia sphenops*. The pathological changes are correlated with the altered enzyme activities. A similar result was also found in rat exposed to cypermethrin [30].

In this study, activities of all the enzymes is increased, as the concentration of atrazine increased in all organs tested, hence the elevation observed is dose-dependent. This result was supported, who noted that variation in metabolic enzyme activities in fish is directly proportional to the concentration of the lindane [31]. GOT and GPT are frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle, and gills [32]. It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of even minor cellular damage [33]. Harvey stated that blood levels of GOT and GPT may increase because of cellular damage in the liver and the high levels of these enzymes in serum are usually an indicative of disease and necrosis in the liver of animals [34].

Therefore, increased GOT and GPT activity in the all the organs of *Poecilia sphenops* is caused mainly by leakage of these enzymes from the liver cytosol into the bloodstream as a result of liver damage caused by atrazine. The activity of GOT and GPT increases correspondingly, with the increase in concentrations of the atrazine. Increase in the transaminases is an immune mechanism, which occurs at the initial stages of the diseased condition [35]. On the other hand, Neskovic found an increase in ALT activity in carp (*Cyprinus carpio L.*) after exposure to atrazine [36].

The present results are in agreement with the findings of Jee, who found that increase in activities of serum GOT and GPT in Korean

rockfish (*Sebastes schlegeli*) is observed when exposed to cypermethrin [37]. A significant increase in the activities of serum ALT in fish *Rhamdia quelen* [38] and *Labeo rohita* [39], insecticides exposure, correspondingly.

Toxicant can disrupt the cell membrane permeability replacing the structural or electro-chemically important element in the cell which causes functional failure [40]. Changes in these enzymes activities disrupt normal physiological and biochemical processes of *Poecilia sphenops*. However, in this study the decreased testicular enzyme activities of ALP, ACP, LDH, SDH, and G6PDH, are significantly altered after treatment with atrazine. These findings have shown that atrazine disrupts the steroidogenesis functions. The atrazine toxicity may impair the liver by making edema or swelling thus the liver weight was increased besides vitellogenin syntheses which are reflected in elevated tissue damage. Therefore, these parameters can be used as indicators of atrazine toxicity.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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