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Impacts and Impairments of Atrazine on Male *Poecilia Sphenops*

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ABSTRACT
The effects of atrazine herbicide in male Poecilia sphenops were studied. The testes and liver cytosolic testicular enzyme and antioxidant enzyme activity were measured. Poecilia sphenops was exposed to 3 sub lethal concentrations (1.25, 2.5 and 5mg/L) of atrazine for 30days and control was also maintained. The activities of testicular functional enzyme ALP, ACP, SDH, LDH, and G6PDH and antioxidant enzyme CAT, SOD, GSH, GST and LPO levels were altered in treated groups compared with that of the control. The histopathological changes like cloudy swelling of hepatocytes, hepatocytes with some pyknotic nuclei, lipoid vacuoles and necrosis were observed in liver, whereas in testes alterations like tubular damages, shrinkage, degeneration, necrosis, condensed spermatogonium, damaged seminiferous tubules and damaged tubular wall were noticed. This study demonstrates that atrazine induces oxidative stress in terms of enhanced enzyme activity and testicular enzyme activity in Poecilia sphenops. Histological observations revealed well organized structure in all the sectioned tissues of organs examined and comparison to control. Atrazine has toxicity to the reproductive system in male Poecilia sphenops.

Keywords: *Atrazine, Poecilia sphenops, Testicular Enzyme, Antioxidant Enzyme and Histoarchitexure.*

1. INTRODUCTION

Atrazine is the most commonly used herbicide in the United States of America and is often detected in groundwater and rivers [1].The estimated half-life of atrazine from these short-term tests ranges between a few days to about one year [2, 3], it has relatively persistent in freshwater, with a half-life between 8 and 350 days, depending on the physio-chemical environment [4, 5]. Its intensive use in agriculture has led to the accumulation of atrazine, polluting the soil and the water at levels which exceeds the permissible limits (5ppb). Histology has long been used in health evaluations of wild fishes. Histopathological biomarkers or cellular changes in tissues such as gill, liver, kidney, gonads and spleen received much attention in assessing the effects of environmental stress [6]. Historically, much less attention has been focused on endocrine, neural and gonadal histology. The 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. Some author reported that changes in activity of SDH and LDH may be responsible for the toxic effects of molybdenum on fertility of male rats [7]. It is reported that increased level of testicular enzymes, in fresh water ornamental fish *Poecilia latipinna* is influenced by *Tribulus terrestris* [8]. Atrazine is metabolized by a complex metabolic process involving several groups of xenobiotic metabolizing enzymes such as glutathione-S-transferase (GST). GST is a phase II enzyme with a determinant function in the detoxification processes [9]. In addition, the conjugating enzyme glutathione-S-transferase conjugates potentially harmful electrophilic reactive metabolites with endogenous glutathione and thus protects other nucleophilic centres such as protein and nucleic acids [10]. Antioxidant defences such as catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) are concerned to neutralize the toxicity of reactive oxygen species [11]. The aim of the present study is to assess the toxicity of atrazine herbicide on some biochemical indices and evaluate the histological modifications in testes and liver of *Poecilia sphenops*.

2. EXPERIMENTAL

Healthy *Poecilia sphenops* adult males were procured from the local aquarist. They were acclimatized for a maximum period of 15 days in the laboratory condition. The adult fishes with an average weight of 1.03 ± 0.25gm and average length of 4.66 ± 0.29 cm were used. Based on the 96hrs LC50 values, a set of 20 fish were then exposed to each of the three sub-lethal concentrations of the atrazine (1.25, 2.5 and 5mg/L) for 30days and used for the study. A set of 20 fish were also simultaneously maintained as control (0mg/L). Approximately 0.1gm of testes and liver was extracted and used for the

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determination of enzyme activities. The protein content of tissues was quantified with Bradford method [12]. Acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were assayed by Estiarte and Michell [13,14]. Sorbitol dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities were tested by Gerlach and Vassault [15,16]. Glucose-6-phosphate dehydrogenase (G6PDH) activity was determined according to the method of Balinsky and Bernstein [17].

Antioxidant studies such as catalase (CAT) [18], superoxide dismutase (SOD) [19], glutathione S-transferase (GST) [20], reduced glutathione (GSH) [21], lipid peroxidase (LPO) [22], were estimated. At the end of the experiment, the fishes from each test group as well as the control group were sacrified, the testes and liver were sectioned and fixed in 10% formalin and processed according to standard paraffin procedures of Luna [23].

2.1. Statistical Analysis

The values were expressed as mean ± SE for five animals. 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 carried out for intergroup comparisons using the least significant difference (LSD) test; ($P<0.05$) was considered statistically significant.

3. RESULTS AND DISCUSSION

During the experimental period food intake is decreased with increasing concentration due to increased oxidative stress. About 5% mortalities occurred following the exposure of three sub lethal concentrations of the atrazine. But significant difference between the control and atrazine exposed fish were found for all the enzymes.

The activity of testicular enzyme such as ACP, ALP, SDH, LDH and G6PDH were significantly ($P<0.05$) decreased in atrazine treated fishes when compared to that of control fishes (Fig.1.a,b).The testicular enzyme activity was recorded which decreases up to 2.5mg. The decreased activity was noted in testes when compared to liver. The increased activity was most notable in testes and liver when compared to control. However the activity was lower in higher (5mg/L) concentration of atrazine then other (1.25 and 2.5mg/L) concentration.

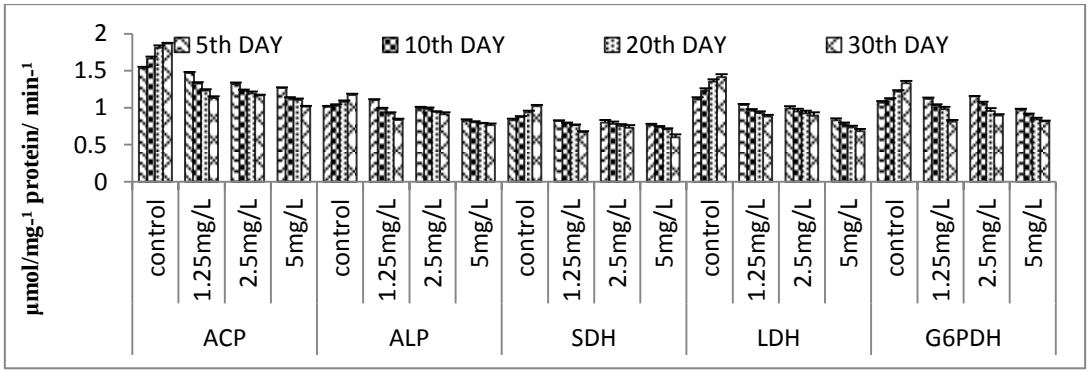

Figure 1 (a). Effects of Atrazine on Testicular Enzyme Activity in Testes of *Poecilia sphenops.*

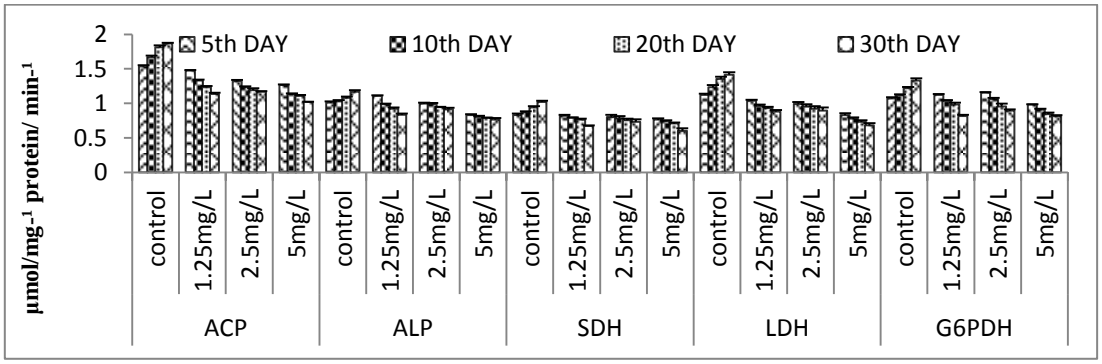

Figure 1 (b). Effects of atrazine on testicular enzyme activity in liver of *Poecilia sphenops.*

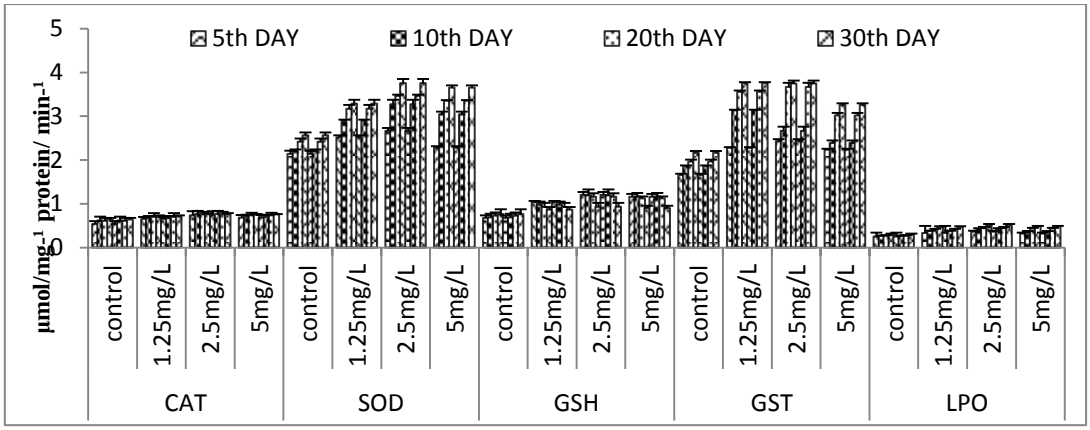

Figure 2 (a): Effects of atrazine on antioxidant enzyme activity in testes of *Poecilia sphenops.*

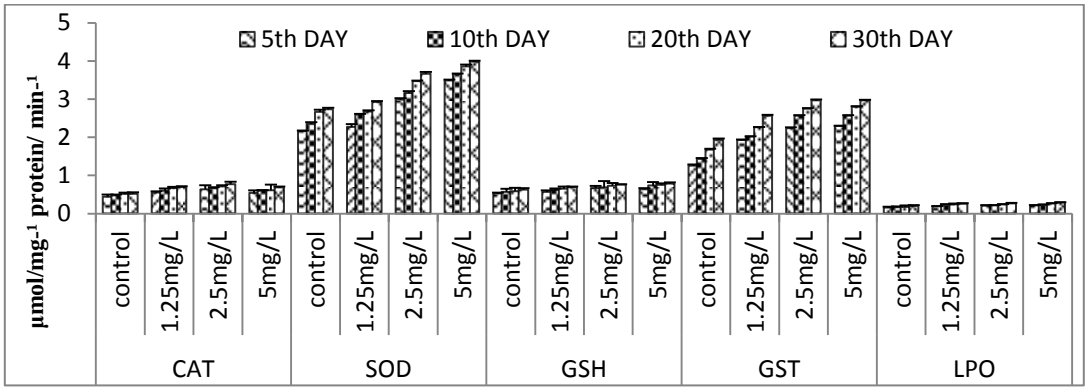

Figure 2 (b): Effects of atrazine on antioxidant enzyme activity in liver of *Poecilia sphenops.*

The changes in the testicular enzymes such as ACP, ALP, SDH, LDH and G6PDH of the *Poecilia sphenops* of various groups are shown in Table 1. A progressive decreased in testicular enzyme activity in *Poecilia sphenops* was observed in atrazine treated groups as compared to control group. Atrazine treated resulted in a decrease in the testicular enzyme at all-time points, though the decrease testicular enzyme activity was statistically significant ($p<0.05$).

The antioxidant enzyme activities were significantly increased ($p<0.05$) in atrazine treated fishes when compared with the control group. However, the levels of the testicular enzymes were significantly distorted upon treatment. Analysis enzymes such as CAT, SOD, GSH, GST and LPO levels showed a significant ($p < 0.05$) increase in the atrazine treated fishes compared with the control (Fig.2.a,b).The enzymes levels were very high in testes compared with liver tissue. The enzyme activity for higher (5mg/L) concentration of atrazine treated fishes ($p<0.05$) is lower than other concentrations. Effects of sub lethal exposure to atrazine on selected antioxidant enzyme are presented in Table 2. An increase in antioxidant enzyme activity was found in fish exposed to atrazine at 1.25, 2.5 and 5mg/L but only in the group exposed to atrazine at 5mg/L the level reached significance ($p< 0.05$) when compared to the control group.

In the cross section of the healthy testes many seminiferous tubules were observed. Each of the tubules was in different stages of spermatogenesis. Some of the tubules contained fully matured sperms, while others were with different stages of development. Testes were characterized by the presence of well-differentiated seminiferous tubules, filled by germ cells and Sertoli cells lining on the basement membrane. The tubules containing spermatogonia spermatid were more in number. Testes morphology was altered by Atrazine exposure, which was mainly evidenced by dilation of seminiferous tubules. Some tubules appeared irregular in form. In most specimens, there was coexistence of dilated tubules with those appearing normal. Few seminiferous tubules were atrophic. On the other hand, the testis of fish treated with atrazine was characterized by necrotic spaces and great reduction in the seminiferous tubules lumen (Fig.3). Giant multinucleated bodies and a large number of apoptotic cells were also frequent within the seminiferous tubules. In treated animals it showed alteration like tubular damages, shrinkage, degeneration and necrosis. The Leydig cells were as closely coupled as in the controls. In fact, the Leydig cell cytoplasmic protrusions were shorter and the intercellular space was larger.

Table: 1: Effects of Atrazine on Testicular Enzyme Activity in *Poecilia sphenops.*

Atrazine mg/L	ACP	ALP	SDH	LDH	G6PDH	ACP	ALP	SDH	LDH	G6PDH
5th Day Liver						**Testes**				
Control	1.533 ± 0.01	1.01 ± 0.01	0.831 ± 0.02	1.12 ± 0.01	1.07 ± 0.02	2.292 ± 0.03	1.61 ± 0.03	1.501 ± 0.02	1.5 ± 0.01	0.95 ± 0.02
1.25	1.472 ± 0.01	1.108 ± 0.01	0.82 ± 0.01	1.04 ± 0.01	1.12 ± 0.01	2.485 ± 0.01	1.65 ± 0.02	1.81 ± 0.01	1.42 ± 0.01	1.27 ± 0.01
2.5	1.323 ± 0.01	0.996 ± 0.01	0.801 ± 0.03	0.992 ± 0.01	1.156 ± 0.01	1.882 ± 0.01	1.664 ± 0.02	1.79 ± 0.03	1.31 ± 0.01	1.34 ± 0.03
5	1.266 ± 0.01	0.827 ± 0.01	0.765 ± 0.01	0.83 ± 0.01	0.982 ± 0.01	1.78 ± 0.02	1.31 ± 0.01	1.41 ± 0.01	1.14 ± 0.01	1.27 ± 0.01
Atrazine mg/L 10th Day Liver						**Testes**				
Control	1.667 ± 0.02	1.03 ± 0.01	0.872 ± 0.01	1.233 ± 0.03	1.12 ± 0.01	2.466 ± 0.01	1.73 ± 0.04	1.69 ± 0.03	1.64 ± 0.03	1.02 ± 0.01
1.25	1.332 ± 0.01	0.987 ± 0.01	0.79 ± 0.01	0.965 ± 0.01	1.04 ± 0.01	2.358 ± 0.01	1.643 ± 0.02	1.56 ± 0.02	1.32 ± 0.01	1.14 ± 0.09
2.5	1.232 ± 0.01	0.986 ± 0.01	0.782 ± 0.03	0.953 ± 0.03	1.06 ± 0.02	2.126 ± 0.0	1.591 ± 0.03	1.71 ± 0.03	1.26 ± 0.08	1.19 ± 0.06
5	1.136 ± 0.01	0.805 ± 0.01	0.739 ± 0.01	0.78 ± 0.01	0.91 ± 0.01	1.667 ± 0.01	1.28 ± 0.01	1.254 ± 0.01	1.02 ± 0.08	1.12 ± 0.02
Atrazine mg/L 20th Day Liver						**Testes**				
Control	1.813 ± 0.02	1.08 ± 0.01	0.94 ± 0.01	1.353 ± 0.03	1.219 ± 0.01	2.476 ± 0.02	1.81 ± 0.01	1.92 ± 0.02	1.68 ± 0.01	1.14 ± 0.01
1.25	1.233 ± 0.01	0.923 ± 0.01	0.765 ± 0.01	0.931 ± 0.01	0.982 ± 0.02	2.353 ±0.01	1.542 ± 0.01	1.501 ± 0.01	1.12 ± 0.03	0.96 ± 0.01
2.5	1.192 ± 0.02	0.946 ± 0.01	0.753 ± 0.02	0.924 ± 0.03	0.956 ± 0.03	2.166 ± 0.02	1.57 ± 0.03	1.53 ± 0.01	1.2 ± 0.01	1.24 ± 0.03
5	1.108 ± 0.01	0.791 ± 0.01	0.71 ± 0.01	0.734 ± 0.02	0.84 ± 0.02	1.658 ±0.03	1.237 ± 0.01	1.216 ± 0.03	0.95 ± 0.01	1.04 ± 0.02
Atrazine mg/L 30th Day Liver						**Testes**				
Control	2.696 ± 0.04	1.853 ± 0.04	1.94 ± 0.04	1.89 ± 0.03	1.34 ± 0.02	2.696 ± 0.01	1.853 ± 0.05	1.94 ± 0.04	1.89 ± 0.02	1.34 ± 0.05
1.25	2.133 ± 0.02	1.53 ± 0.02	1.325 ± 0.02	1.06 ± 0.01	0.895 ± 0.01	2.133 ± 0.01	1.53 ± 0.03	1.325 ± 0.02	1.06 ± 0.01	0.89 ± 0.01
2.5	2.052 ± 0.02	1.32 ±0.04	1.24 ± 0.04	1.1 ± 0.02	1.07 ± 0.01	2.052 ± 0.03	1.32 ± 0.01	1.24 ± 0.09	1.1 ± 0.04	1.07 ± 0.02
5	1.536 ± 0.02	1.18 ± 0.02	1.12 ± 0.02	0.8 ± 0.02	0.98 ± 0.01	1.536 ± 0.02	1.18 ± 0.01	1.12 ± 0.03	0.8 ± 0.02	0.98 ± 0.06

Values are mean ± SEM; n = 6: $p < 0.05$ compared to control.

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Table 2: Effects of Atrazine on Antioxidant Enzyme Activity in *Poecilia sphenops.*

Atrazine mg/L	CAT	SOD	GST	GSH	LPO	CAT	SOD	GST	GSH	LPO
5th Day Liver						**Testes**				
Control	0.439 ± 0.05	2.16 ± 0.07	0.533 ± 0.02	1.256 ±0.03	0.154 ±0.02	0.539 ± 0.06	2.136 ± 0.07	0.676 ± 0.05	1.656 ± 0.01	0.253 ± 0.01
1.25	0.553 ± 0.03	2.267 ± 0.04	0.566 ± 0.04	1.923 ± 0.01	0.185 ± 0.01	0.658 ± 0.03	2.523 ± 0.04	1.033 ± 0.03	2.279 ± 0.01	0.333 ± 0.01
2.5	0.633 ± 0.1	2.985 ± 0.01	0.666 ± 0.05	2.233 ± 0.02	0.206 ± 0.01	0.733 ± 0.09	2.667 ± 0.06	1.206 ± 0.06	2.433 ± 0.04	0.366 ± 0.02
5	0.543 ± 0.01	3.49 ± 0.01	0.633 ± 0.03	2.291 ± 0.01	0.213 ± 0.01	0.686 ± 0.05	2.279 ± 0.03	1.156 ± 0.06	2.233 ± 0.02	0.326 ± 0.01
Atrazine mg/L										
10th Day Liver						**Testes**				
Control	0.476 ± 0.02	2.353 ± 0.04	0.566 ± 0.08	1.433 ± 0.01	0.167 ± 0.01	0.617 ± 0.09	2.216 ± 0.02	0.688 ± 0.08	1.851 ± 0.0	0.267 ± 0.01
1.25	0.596 ± 0.06	2.596 ± 0.01	0.613 ± 0.04	2.018 ± 0.01	0.233 ± 0.01	0.667 ± 0.05	2.907 ± 0.01	0.978 ± 0.07	3.133 ± 0.01	0.383 ± 0.01
2.5	0.67 ± 0.02	3.156 ± 0.05	0.685 ± 0.01	2.567 ± 0.01	0.214 ± 0.01	0.767 ± 0.07	3.276 ± 0.09	1.196 ± 0.01	2.667 ± 0.09	0.423 ± 0.02
5	0.594 ± 0.01	3.653 ± 0.01	0.733 ± 0.09	2.567 ± 0.01	0.235 ± 0.01	0.706 ± 0.04	3.049 ± 0.05	1.167 ± 0.07	2.391 ± 0.05	0.363 ± 0.01
Atrazine mg/L										
20th Day Liver						**Testes**				
Control	0.513 ± 0.03	2.667 ± 0.05	0.583 ± 0.09	1.676 ± 0.02	0.175 ± 0.03	0.613 ± 0.06	2.416 ± 0.07	0.727 ± 0.07	1.966 ± 0.04	0.275 ± 0.01
1.25	0.667 ± 0.03	2.672 ± 0.03	0.653 ± 0.05	2.261 ± 0.01	0.243 ± 0.02	0.699 ± 0.09	3.172 ± 0.08	0.923 ± 0.09	3.561 ± 0.02	0.423 ± 0.01
2.5	0.712 ± 0.02	3.467 ± 0.02	0.726 ± 0.07	2.747 ± 0.01	0.236 ± 0.01	0.76 ± 0.05	3.467 ± 0.02	1.163 ± 0.07	3.667 ± 0.09	0.451 ± 0.02
5	0.606 ± 0.01	3.853 ± 0.02	0.752 ± 0.04	2.796 ± 0.02	0.267 ± 0.01	0.733 ± 0.06	3.353 ± 0.01	1.123 ± 0.04	3.0187 ± 0.05	0.437 ± 0.01
Atrazine mg/L										
30th Day Liver						**Testes**				
Control	0.513 ± 0.01	2.734 ± 0.05	0.626 ± 0.04	1.936 ± 0.02	0.197 ± 0.05	0.643 ± 0.03	2.567 ± 0.05	0.768 ± 0.01	2.166 ± 0.04	0.297 ± 0.02
1.25	0.696 ± 0.01	2.926 ± 0.03	0.693 ± 0.02	2.566 ± 0.01	0.253 ± 0.01	0.716 ± 0.01	3.289 ± 0.03	0.866 ± 0.06	3.756 ± 0.02	0.473 ± 0.01
2.5	0.766 ± 0.03	3.667 ± 0.01	0.756 ± 0.01	2.966 ± 0.04	0.266 ± 0.02	0.764 ± 0.02	3.756 ± 0.04	0.933 ± 0.08	3.747 ± 0.06	0.516 ± 0.01
5	0.683 ± 0.02	3.979 ± 0.01	0.784 ± 0.03	2.967 ± 0.02	0.286 ± 0.016	0.754 ± 0.01	3.651 ± 0.09	0.906 ±0.05	3.256 ± 0.03	0.486 ± 0.01

Values are mean ± SEM; n = 6: $p < 0.05$ compared to control.

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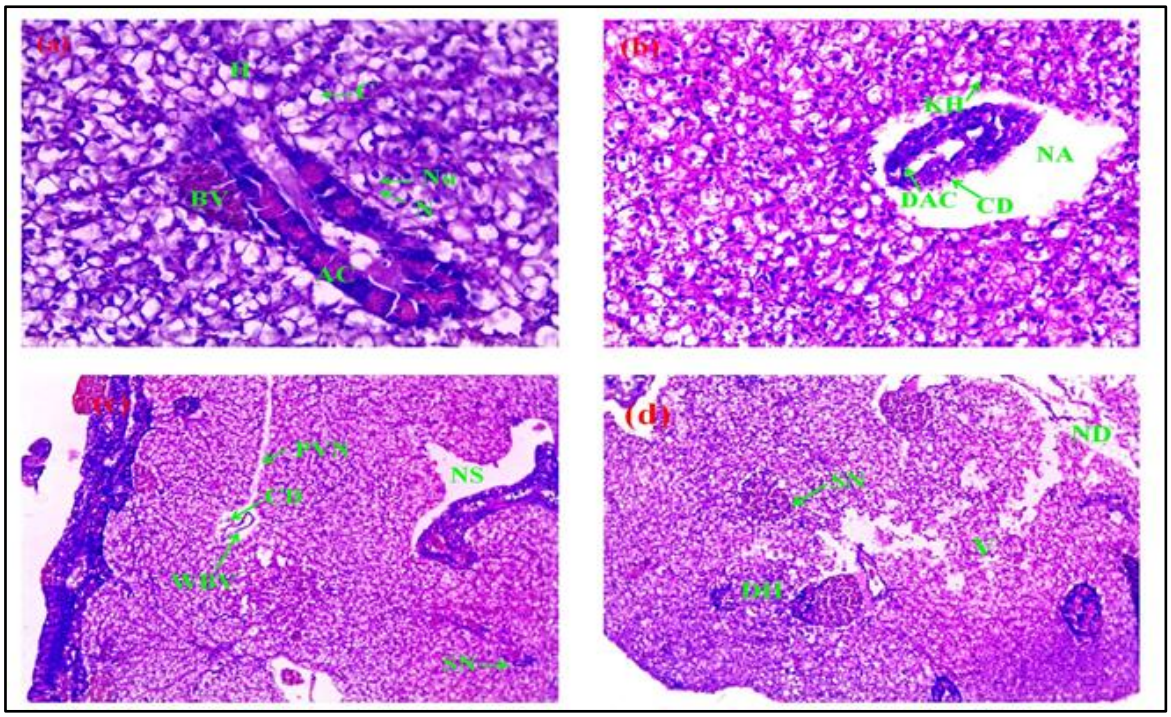

Figure 3: Liver tissue of p.sphenops (a) Control: Hepatocycytes, C-Cytoplasm, AC-Acinar Cell, N-Nucleus, Nu-Nucleolus, (b) liver fish exposed to 1 mg of ATZ: CD-Cell debris, NA-Necrotic area, DAC-Disintegrating Acinar Cell, KH-Karyolsed Hepatocytes: (c) Liver of fish exposed to 2.5 mg of ATZ: PVN-Perivascular necrosis with vascular damage, CD-Cell derbris, NS-Nacrotic spot, SN-Swollen Nucleus, WBV-Wrinkled blood vessel: (d) DH- Degenerating hepatocytes, ND-Necrotic derbris, V-Vacuolation.

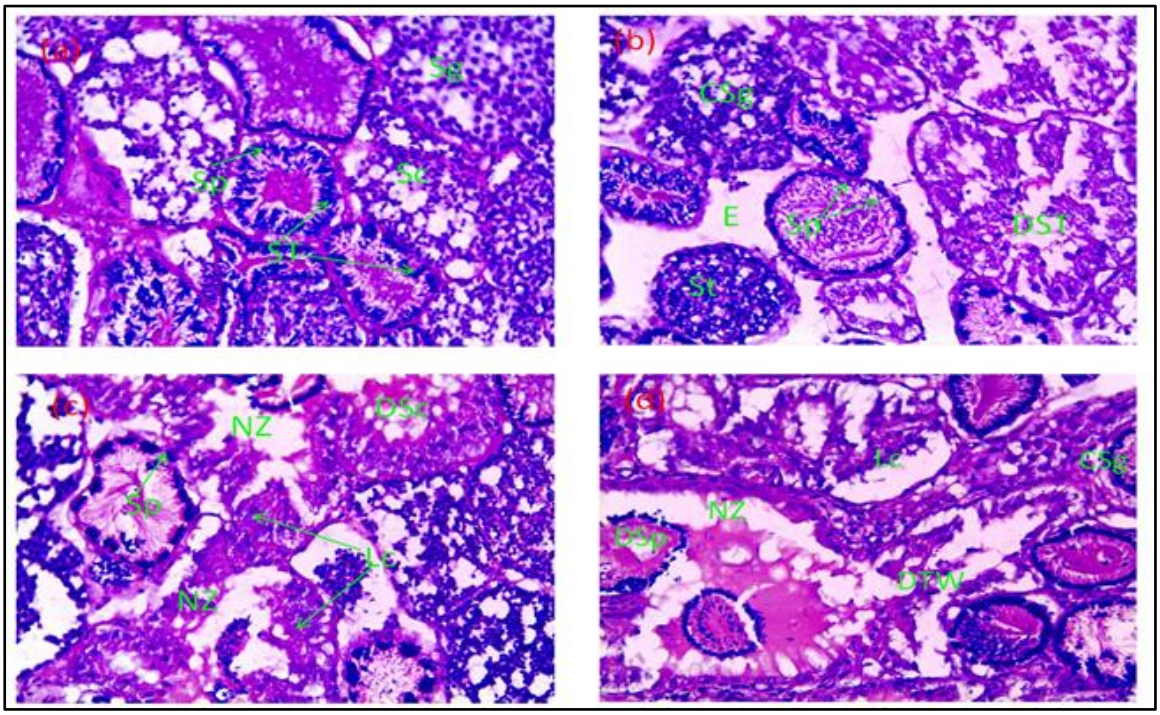

Figure 4: Testis of P. sphenons. (a) Control: Sg-Spermatogonia, St-Spermatid, Sc-spermatocytes: (b) Testis of fish exposed to 1mg of ATZ: St-Spermatid, Sp-Spermatophores, DST-Damaged Seminiferous Tubules, E-Egema, CSg-Condensed sphermatogonium: (c) Testies pf fish exposed to 2.5 mg of ATZ: NZ-Necrotic zone, Le-Ledying Cell, DSc-Damaged Spermatocytes: (d) Testis of fish exposed to 5.0 mg of ATG: DTW-Damaged Tubular Wall, CSg-Condensed sphermatogonium, NZ-Necrotic zone, DSp-Damage Spermatophores.

In the present study no histological changes were observed in control. The structural details of the liver of control *Poecilia sphenops* were shown in

(Fig.4.). The hepatocytes of fish exposed to atrazine show changes like wrinkled blood vessel, necrotic area, disintegrating acinar cell, cell debris, and perivascular necrosis with vascular damage. The liver of fish exposed to atrazine treatments for 30 days showed several histopathological changes, and the frequency of these changes increased with increasing concentration.

Fish are often used as sentinel organisms for ecotoxicological studies because they play number of roles in the trophic web, accumulate toxic substances and respond to low concentration of mutagens [24]. The liver is a main organ responsible for the metabolism of toxic substances like atrazine. In animals, atrazine is readily absorbed from the gastrointestinal tract and is mostly circulated in the liver. Therefore the effect of atrazine on liver-detoxifying enzymes is very important [25]. A period of 30 days exposure to atrazine was examined to study the alterations in antioxidant enzymes such as, SOD, CAT, LPO, GSH, GST and activities of testicular enzymes such as ACP, ALP, G6PHD, LDH and SDH. Increase in these enzyme activities are probably a response towards increased ROS generation in pesticide toxicity [26]. SOD-CAT system provides the first defense line against oxygen toxicity [7]. It has been reported by various researchers that GSH plays an important role in protecting cells from xenobiotic-induced tissues injury [27]. The increase in superoxide dismutase activity after administration of atrazine enhance to increased generation of reactive oxygen species. It has been reported in the literature that exposure of animals to xenobiotic increases SOD activity in various tissues [26, 28]. The elevated level of lipid peroxidation in the testes and liver of *Poecilia sphenops* is the response to exposure of atrazine as observed in the present investigation which suggests that there is an increased production of ROS. Increased ROS production may be related with the metabolism of atrazine herbicide to the peroxidation of membrane lipids of the liver. The liver is noted as site of multiple oxidative reactions and maximal free radical generation [29]. The observed LPO resulting from ROS generated by the atrazine may lead to cell apoptosis. ROS and oxidative stress have been demonstrated to betriggers of apoptosis [30]. Currently, GST is used as a biomarker for the evaluation of the effect of atrazine on *Poecilia sphenops*. GST is also capable of detoxifying ROS as a result of a direct antioxidant action, and its activity in fish cells subjected to oxidative stress [31]. Therefore, it is a potential biomarker not only for pollutants that are detoxified by GST but also for the very large number of pollutants capable of generating oxidative stress. However, an increased LDH and SDH activity was found in the liver of P. sphenops exposed to all the concentrations of atrazine. Hence, increased LDH and SDH concentration in this case is due to dacarbazine-induced damage of seminiferous epithelium as shown in other experimental conditions [32]. So that elevated level of LDH and SDH indicates that cytotoxicity of atrazine on spermatogonia affected their further processes during spermatogenesis. In the present investigation, the activity of ACP, ALP increased in testes in all the concentration of atrazine exposure. The increased ACP, ALP level has a direct effect on testicular functions [8].

Histopathological investigations have proved to be a sensitive tool to identify the direct effects of environmental contamination. In this study, the effects of exposure to atrazine on the testicular and liver histomorphology *Poecilia sphenops* were assessed. Modified testicular histoarchitecture, apoptosis/proliferation imbalance and altered steroidogenesis could have negative effect on sexual maturation. Many recent studies in wildlife have generated increasing concern regarding endocrine disrupting chemicals effects on reproductive functions [33]. There are reports which describes that pesticides can cause various histopathological and cytopathological changes in the reproduction system of male mammals [34]. The histopathological lesions in the liver observed in the present study were cloudy swelling of hepatocytes, hepatocytes with some pycnotic nuclei, lipoid vacuoles and necrosis. Morphological alterations are seen in the fish liver exposed to pesticides [35]. Similar studies have shown cloudy swelling, bile stagnation, necrosis, atropy and vacuolization in the Corydoras paleatus exposed to methyl parathion [36].

A histopathological observation has indicated that exposure to sublethal concentrations of atrazine causes adverse effects in the testes and liver tissues of *Poecilia sphenops*. Histopathological alterations lead to physiological problems, ultimately leading to the death of fish. However, more experiments at lower atrazine concentrations are needed to validate these enzymes activity as biomarkers of oxidative stress in large-scale environmental monitoring programs.

4. CONCLUSIONS

Current histological investigations established a direct correlation between atrazine exposure and histopathological disorders observed in testes and liver tissue. From this study it can be concluded that atrazine exposure results in increased oxidative stress and altered testes and liver antioxidant status. Low dose of atrazine affect the fish. Numerous

histological changes can be observed in fish testes and liver tissue throughout experiment.

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5. REFERENCES

[1]. K.R. Solomon, D.B. Baker, P. Richards, K.R. Dixon, S.J. Klaine, T.W. LaPoint, R.J. Kendall, J.M. Giddings, J.P. Giesy, L.W. Hall, C.P. Weiskopf, W. Williams, (1996) Ecological risk assessment of atrazine in North American surface waters, ***Environmental Toxicology & Chemistry***, **15:**31-74.

[2]. E.L. Kruger, L. Somasundaram, R.S. Kanwar, J.R. Coats, (1993) Persistence and degradation of [14C] atrazine and [14C] deisopropylatrazine as affected by soil depth and moisture conditions, ***Environmental Toxicology and Chemistry*, 12:** 1959-1967.

[3]. C. Accinelli, G. Dinelli, A. Vicari, P. Catizone, (2001) Atrazine and metolachlor degradation in subsoils, ***Biology and Fertility of Soils* 33**: 495-500.

[4]. S.G. Diana, J.R. Restaris, D.J. Schaeffer, K.B. Beckmen, V.R. Beasley, (2000) Effects of atrazine on amphibian growth and survival in artificial aquatic communities, ***Environmental Toxicology and Chemistry***, **19**: 2961-2967.

[5]. L. Tavera-Mendoza, S. Ruby, P. Brousseau, M. Cyr, D. Marcogliese, (2002) Reponse of the amphibian tadpole (Xenopus laevis) to atrazine during sexual differentiation of the testes, ***Environmental Toxicology and Chemistry***, **21**:527-531.

[6]. D.E. Hinton, P.C. Baumann, G.R. Gardner, W.E. Hawkins, J.D. Hendricks, R.A. Murchelano, M.S. Okihiro, (1992) Histopathologic biomarkers. In: Biomarkers Biochemical, Physiological and Histological Markers of Anthropogenic Stress, Arbor London Tokyo, 155-212.

[7]. R. Pandey, S.P. Singh, (2002) Effects of molybdenum on fertility of male rats, ***BioMetals,* 15(1):** 65-72.

[8]. P. Kavitha, P. Subramanian, (2011) Influence of Tribulus terrestris on testicular enzyme in fresh water ornamental fish Poecilia latipinna, ***Fish Physiology and Biochemist***, **37(4)**: 801-807.

[9]. M. Abdollahi, A. Ranjbar, S. Shadnia, S. Nikfar, O.F. Reizaic, (2004) Insecticides and oxidative stress: a review, ***Medical Science Monitor***, **10(6)**: RA141-RA147.

[10]. S.G. George, (1994) Enzymology and molecular biology of phase II xenobiotic conjugating enzymes in fish, In Aquatic toxicology molecular biochemical and cellular perspectives CRC Press, Boca Raton, pp 37-85.

[11]. A. Orbea, M. Ortiz-Zarragoitia, M. Sole, C. Porte, M. Cajaraville, (2002) Antioxidant enzymes and peroxisome proliferation in relation contaminant body burdens of PAHs and PCBs in bivalvia molluscs, crabs and fish from Urdiabai and Plentzia estuaries (Bay of Biscay). ***Aquatic Toxicology***, **58**:75-98.

[12]. M.M. Bradford, (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, ***Analytical Biochemistry,* 72**:248-254.

[13]. M. Estiarte, J. Peuuelas, B.A. Sardans, A. Emmett, C. Soweby, I.K. Beier, A. Schmidt, M.J.M. Tietema, E. Van Mectern, P. Kvacs Lang, P. Mathe, G. De Angelis De Dato, (2008) Root–Surface phosphatase activity in shrublands across a European gradient: Effects of warming, ***Journal of Environmental Biology***, **29**: 25-29.

[14]. R.H. Michell, M.J. Karnovsky, M.L. Karnovsky, (1970) The distributions of some granule-associated enzymes in guinea pig polymorph nuclear leucocytes, ***Biochemical Journal***, **116:** 207-216.

[15]. U. Gerlach, (1983) Sorbitol dehydrogenase. In: Bergmeyer HU (ed) Methods of enzymatic analysis, 3rd edn. Verlag Chemie Weinheim, pp 112-117.

[16]. A. Vassault, (1983) Lactate dehydrogenase UV method with pyruvate and NADH In: Bergmeyer HU (ed) Methods of enzymatic analysis, 3rd edn. Verlag Chemie, Weinheim, pp 118-125.

[17]. D. Balinsky, R.E. Bernstein, (1963) The purification and properties of glucose-6-phosphate dehydrogenase from human erythrocytes***, Biochimica et Biophysica Acta,* 67:**313-315.

[18]. A.L. Calibrone, (1985) Hand book of methods for oxygen radical research, CRC Press Florida pp. 283.

[19]. S. Marklund, G. Marklund, (1974) Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase, ***European Journal of Biochemistry,* 47:** 469-474.

[20]. H.U. Bergmeyer, E. Bernt, (1965) Glutamate-oxaloacetate trans-aminase. In: Bergmeyer H. U. (ed) Methods of enzymatic analysis, Academic Press, New York, 837-851.

[21]. M.J. Moron, J.W. Depierr, B. Mannervik, (1979), Levels of GSH, GR and GST

activities in rat lung and liver, ***Biochimica et Biophysica Acta***, **582**: 67-78.

[22]. H. Ohkawa, N. Ohishi, K. Yagi, (1979) Assay of lipid peroxide in animal tissue by thiobarbituric acid reaction, ***Analytical Biochemistry,*** **95:** 351-358.

[23]. L.G. Luna, (1992). Histopathological methods and color atlas of special stains and artifacts, American HistoLabs Gaithersburg, MD, 767.

[24]. T. Cavas, S. Ergene-Gozukara, (2005) Micronucleus test in fish cells, a bioassay for in situ monitoring of genotoxic pollution in the marine environment, ***Environmental and Molecular Mutagenesis,*** **46**: 64-70.

[25]. M.O. Islam, M. Hara, J. Miyake, (2002) Induction of P-glycoprotein, glutathione-Stransferase and cytochrome P450 in rat liver by atrazine, ***Environmental Toxicology and Pharmacology,*** **12:** 1-6.

[26]. S. John, M. Kale, N. Rathore, D. Bhatnagar, (2001) Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes, ***Journal of Nutritional Biochemistry,*** **12**: 500-4.

[27]. A.C. Elia, W.T. Waller, S.J. Norton, (2002) Biochemical response of bluegill sunfish (Lepomis macrochirus,Rafinesque) to atrazine induced oxidative stress, ***Environmental Contamination and Toxicology,*** **68**: 809-16

[28]. K. Jayachandran, K. Pugazhendy, M. Susiladevi, C. Jayanthi, (2010) Protective role of atropine against atrazine toxicity on antioxidant enzymes in the fingerlings of Labeo rohita (hamilton), ***International Journal of Recent Scientific Research***, **1**: 015-020.

[29]. A. Avci, M. Kacmaz, I. Durak, (2005) Peroxidation in muscle and liver tissues from fish in a contaminated river due to petroleum refinery industry, ***Ecotoxicology and Environmental Safety,*** **6:** 101-105.

[30]. H.M. Shen, Z.G. Liu, (2006) JNK signaling pathway is a key modification in cell death mediated by reactive oxygen and nitrogen species, ***Free Radical Biology and Medicine,*** **40:** 928-939.

[31]. D.A. Monteiro, F.T. Rantin, A.L. Kalinin (2009) The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxa, Brycon cephalus (Gunther, 1869) exposed to organophosphate insecticide Folisuper 600 BR1 (methyl parathion), ***Comparative Biochemistry and Physiology C,*** **149**:40-49.

[32]. A. Buonaguidi, M. Grasso, C. Lania, M. Castelli, F. Francesca, P. Rigatti, (1993) Experience with the determination of LDH-X in seminal plasma as diagnostic and prognostic factor in varicocele, ***Arch Esp Urol,*** **46 (1):** 35-39.

[33]. R.B. Bringolf, J.B. Belden, R.C. Summerfelt, (2004) Effects of atrazine on fathead minnow in a short-term reproductionn assay, ***Environmental Toxicology and Chemistry,*** **23:** 1019-1025.

[34]. M. Uzunhisarcikli, Y. Kalender, K. Dirican, S. Kalender, A. Ogutcu, F. Buyukkomurcu, (2007) Acute, subacute and subchronic administration of methyl parathion- induced testicular damage in male rats and protective role of vitamins C and E, ***Pesticide Biochemistry and Physiology,*** **87:**115-122.

[35]. E.L. Rodrigues, E. Fanta, (1998) Liver histopathology of the fish Brachydanio rerio after acute exposure to sublethal levels of the organophosphate dimethoat 500, ***Revista Brasileira de Zoologia,*** **15:** 441-450.

[36]. E. Fanta, F.S. Rios, S. Romao, A.C.C. Vianna, S. Freiberger, (2003) Histopathology of the fish Corydoras paleatus contaminated with sublethal levels of organophosphorus in water and food, ***Ecotoxicology and Environmental Safety,*** **54:** 119-130.