Vitamin E as antioxidant in female african catfish (*Clarias gariepinus*) exposed to chronic toxicity of atrazine

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ABSTRACT

The present study was planned aiming to investigate the effects of atrazine herbicide exposure on stress biomarkers acetylcholinesterase activity (AchE) and cortisol, oxidative stress responses and the histopathological changes in liver of female *Clarias gariepinus* and the ameliorative effect of vitamin E supplementation (240mg/kg diet) on the degree of atrazine sublethal toxicity (1.37mg/l) for 6 weeks. Chronic exposure to atrazine revealed a marked inhibition in the activity of AchE. But, significant increase of serum cortisol level was recorded. Moreover, atrazine exposure was associated with a marked induction of oxidative damage in liver tissue as evidenced by increased level of lipid peroxidation (LPO) and reduced glutathione (GSH) content. Atrazine exposure also led to a significant increase in the activities of catalase (CAT) and superoxide dismutase (SOD).Furthermore, histopathological examination of the liver of exposed fish showed dilatation and congestion of blood vessels, fatty degeneration, necrosis and pyknotic nuclei of hepatocytes. However, fish fed diet supplemented with vitamin E exhibited protective effect by minimizing the atrazine-induced toxicity, through measured values more or less similar to the control group fish.

Key words: Atrazine, *Clarias gariepinus*, Cortisol, Oxidative stress, Histopathology.

INTRODUCTION

Atrazine (ATR), is a triazine herbicide (US EPA, 1994) belonging to S-triazine family of herbicides, which are one of the most significant water pollutants in rain, fresh, marine and ground waters (Felding, 1992 and Tasli *et- al.*, 1996). It is used as a selective pre–emergence herbicide for the control of weeds in asparagus, maize, sorghum, sugarcane and pineapple (US EPA, 1994).

Atrazine has been found to affect a variety of physiological processes in aquatic animals. As, animals have been found to accumulate it in a variety of tissues (Du Preez and van Vuren, 1992). In freshwater invertebrates atrazine was known to affect hydromineral balance or gill function in crabs (Silvestre *et al.*, 2002) and hemocyanin function (Prasad *et al.*, 1995). Moreover, in fish atrazine can also cause damage to gill epithelium and kidneys, increasing the renal excretion of sodium, chloride and proteins in rainbow trout (*Oncorhynchus mykiss)* (Fisher–Scherl *et al.*, 1991) and common carp (*Cyprinus carpio)* (Neskovic *et al*., 1993). The brain and muscle tissues of fish contain mostly AchE (Fulton and Key 2001) so, it could be used as a biomarker of exposure to pesticides, as it decreased in fish from the contaminated sites by pesticides (Dorval *et al.,* 2004).

Cortisol, as the main end product of the physiological response to stress, regulates the hydromineral balance and energy metabolism (Wendelaar Bonga, 1997) and has been measured in fish blood and tissues to evaluate the response to several stimuli (Barcellos *et al.*, 2004).

Oxidative stress in aquatic organisms, principally fish, has great importance for environmental and aquatic toxicology. Because oxidative stress is induced by many chemicals, including some pesticides, these contaminants may stimulate reactive oxygen species and alteration in antioxidant systems. Pro-oxidant factor actions in fish can be used to assess pollution of specific areas or worldwide marine pollution (Üner *et al.*, 2006 and Slaninova *et al.*, 2009). Non-enzymatic antioxidants such as α-tocopherol (vitamin E), ascorbate (vitamin C), β-carotene (vitamin A), flavonoids (quercetin, rutin, etc.), selenium and thiol containing compounds such as glutathione (GSH) can also act to overcome the oxidative stress, being a part of total antioxidant system (Sies *et al.*, 1992). Vitamin E is an important biological free radical scavenger in the cell membranes (Horwitt, 1976).

The objective of this work was to study the ameliorative effect of vitamin E supplemented diet on African catfish, *C. gariepinus* exposed to sublethal concentration of atrazine for different periods through the determination of the biochemical and the histopathological changes in liver.
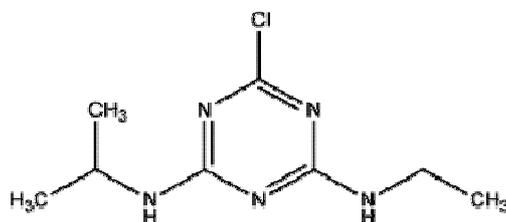
MATERIALS AND METHODS

Fish and rearing conditions:

A total number of 280 female *C. gariepinus* with average body weight 300±50g were collected from Abassa fish farm, El-Sharkya governorate, Egypt. Fish were transferred to the laboratory of Fish Diseases and Management Department, Faculty of Vet. Med., Cairo University in 150 litres in well aerated fiberglass tanks. The fish were examined clinically to assure the absence of any abnormalities or external lesions according to Amlacher (1970). Then, fish were kept in identical glass aquaria (80 x 40 x 40 cm) aerated with air pumps under natural photoperiod and temperature, supplied with dechlorinated tap water and left for 2 weeks for acclimation. Fish were fed commercial ration containing 32% protein (Robinson and Li, 1999).

Experimental chemicals:

a) Atrazine is a white, crystalline solid product of Ciba-Geigy Corp. Greensboro, NC 27419-8300. The molecular weight of atrazine is 215.69 and its chemical formula is: (2 – chloro- 4-ethylamino- 6 - isopropylamino -S- triazine)

b) Vitamin E (DL-α-tocopheryl acetate) was obtained as a soft gelatinous capsules under the patent name of E-viton (Kahira Pharmaceutical and Chemical Industries Co.). It was supplied at a dose level 240 mg/Kg diet according to Gatlin *et al.* (1992).

Experimental design:

Two hundred and forty (240) fish were divided into four groups (60 fish each), distributed in glass aquaria at a rate of 10 fish /aquarium. Each group was exposed for 6 weeks to one of the following treatments:

Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet (32% protein).

Group II: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet (32% protein) supplemented with 240 mg vitamin E/kg diet.

Group III: Fish were exposed to1/10 LC_{50} (1.37mg/l) of atrazine and fed on a commercial diet (32% protein), [according to the previous calculated half lethal concentration of atrazine in Marzouk *et al*. (2012)].

Group IV: Fish were exposed to1/10 LC_{50} (1.37mg/l) of atrazine and fed on a commercial diet (32% protein) supplemented with 240 mg vitamin E/kg diet.

Blood and tissue samples were obtained from fish of the studied groups at 1st, 2nd, 3rd, 4th, 5th and 6th week of chronic exposure, for the investigation of the biochemical parameters and oxidative stress. In addition, tissue specimens were collected for detection of the pathological changes in liver of exposed fish.

Biochemical examination:

Blood samples were collected from the fish caudal vein using plastic syringes in dry sterilized vials. The samples were allowed to clot at room temperature and centrifuged, then serum was separated for determination of AchE using Stanbio kit according to the method described by Kendel and Bottger (1967). serum cortisol was measured by ELISA Reader using Monobind, Inc. kit according to the method described by Foster and Dunn (1974). Samples of liver tissue were homogenized in a Potter–Elvejhem glass/Teflon homogenizer and centrifuged then the supernatant was collected for estimation of LPO using Bio-Diagnostic kit according to the method described by Ohkawa *et al.* (1979). The activity of GSH was determined calorimetrically according to the method described by Beutler *et al*. (1963). The CAT level was estimated by using Bio-Diagnostic kit according to the method described by Aebi (1984). The SOD activity was measured by the calorimetric method described by Nishikimi *et al*. (1972).

Histopathological studies:

For histological examination portions of liver were immediately removed and fixed in 10% neutral buffered formalin and dehydrated through a graded series of ethanol, cleared in xylol and embedded in paraffin, sectioned at 4 μm thickness using a rotary microtome. Sections were prepared and stained with Hematoxylin and Eosin (Bancroft *et al.* 1996 and Roberts 2001).

Statistical Analysis:

Data were statistically analyzed using analysis of variance, one way "ANOVA", and Duncan's multiple range tests to evaluate comparison between means at $P< 0.05$ (SPSS, 2004).

RESULTS AND DISCUSSION

The aquatic environment is continuously being contaminated with chemicals from agriculture activities. Hundreds of pesticides of different chemical structure are extensively used to control a wide variety of agricultural pests and can contaminate aquatic habitats due to leaching and runoff water from treated areas.

AchE activity provides a method for diagnosing poisoning by chemicals such as herbicides. Inhibition of AchE, which is responsible for the degradation of

acetylcholine, will result in an excessive stimulation of cholinergic nerves, resulting in altered swimming behavior, tremors, convulsions and also undesirable effects (Miron *et al.,* 2005). Regarding serum AchE results demonstrated in Table (1), revealed that there was a significant inhibition of AchE in the exposed fish during the chronic exposure in comparison to the control group fish. Also, the inhibition of AchE is correlated with that of exposure concentration, but not with exposure time as reviewed by Roex *et al*. (2003).

Table 1: Serum (AchE) (µ/l) activity of female *C. gariepinus* exposed to different treatments for different periods:

Weeks Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Group I	545.46±8.62 [a]	555.25±2.78[a]	545.10±2.96[a]	557.15±1.95[a]	548.50±6.98[a]	549.60±5.38[a]
Group II	566.05±3.17[a]	562.53±2.91[ab]	561.10±4.44[ab]	550.87±4.84[b]	557.14±3.93[ab]	560.53±4.21[ab]
Group III	463.72±6.72[a]	371.41±4.02[bc]	343.21±3.78[c]	358.20±1.22[bc]	297.89±1.18[d]	270.87±9.09 [d]
Group IV	514.15±3.38[a]	472.21±3.43[b]	382.50±5.09[c]	383.27±5.65[c]	388.14±3.15[c]	362.47±4.65[c]

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same column for each group are significantly different ($P < 0.05$).
Where:
 Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet.
 Group II: Fish were reared in dechlorinated tap water and fed on a commercial diet supplemented with 240 mg vit.E/kg diet.
Group III: Fish were exposed to (1.37mg/l) of atrazine and fed on a commercial diet.
 Group IV: Fish were exposed to1/10 LC_{50} (1.37mg/l) of atrazine and fed on a commercial diet supplemented with 240 mg vit. E/kg diet.

As, when AchE decreased, Ach is not broken and accumulated within synapses, causing overall decline in neural and muscular control (Dutta and Arends, 2003). The obtained results agreed with those of Moraes *et al.* (2009) and Hamed (2010). Also it is more or less in agreement with those of David *et al.*(2007) who recorded an increase in AchE activity of *Cirrhinus mrigala* fish exposed to LC_{50} of malathion (3 micro/l) for 5 and 15 days but on the 25th day of exposure all values approached the normal ones. This may be attributed to recuperation and adaptation phenomena . On the other hand the administration of vitamin E as an antioxidant against the toxicity of atrazine herbicide partially restore the activity of AChE in atrazine–exposed fish. The results were in accordance with Verma *et al.* (2007).

Blood cortisol is the major corticosteroid hormone in fish and may have a significant effect on its dynamics (Wendelar Bonga, 1997). Regarding serum cortisol results demonstrated in Table (2), revealed that there was a significant increase in cortisol level of atrazine exposed fish during the long term of exposure in comparison to the control group. Our investigations agreed with Cericato *et al.* (2008) and Hamed (2010). This may be explained by the activation of hypothalamo–pituitary-inter renal axis with the release of steroid cortisol in blood stream due to stress (Reddy and Leatherland, 1998). Or it could be attributed to the increase in osmotic water- influx, which may cause a cortisol elevation, to restore the hydromineral balance. This osmoregulatory dysfunction might be harmful perse; and owing to sustained high cortisol level which may cause several deleterious physiological changes, affecting the immuno-competence, health status and survival of the fishes (Wendelaar Bonga, 1997). Also, increased cortisol may be important for mobilizing energy for responding to and repairing damage caused by contaminants, but may have negative consequences for disease resistance and growth (Wendelaar Bonga, 1997). On the other hand, the results disagreed with that demonstrated by Nascimento *et al.* (2012)

who stated that no significant alteration in plasma cortisol level of *Prochilodus lineatus* exposed to atrazine. This difference could be attributed to fish species and pesticide variance. While, the administration of vitamin E along with atrazine resulted in statistically significant decrease in serum cortisol level.

Table 2: Serum cortisol (µg/dl) level of female *C. gariepinus* exposed to different treatments for different periods:

Weeks / Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Group I	8.49±0.28[b]	8.71±0.37[ab]	9.14±0.57[ab]	8.99±0.51[ab]	9.14±0.33[ab]	9.91±0.18[a]
Group II	8.34±0.64[a]	8.42±0.27[a]	9.47±0.52[a]	9.02±0.72[a]	8.63±0.67[a]	9.27±0.18[a]
Group III	16.07±0.47[e]	22.10±0.81[d]	31.37±0.46[c]	34.79±0.76[bc]	37.59±0.49[b]	45.61±2.60[a]
Group IV	11.12±0.34[c]	16.76±1.62[b]	21.84±0.76[ab]	27.12±1.21[a]	24.24±0.75[a]	26.16±3.34[a]

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same column for each group are significantly different ($P < 0.05$).
Where:
 Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet.
 Group II: Fish were reared in dechlorinated tap water and fed on a commercial diet supplemented with 240 mg vit.E/kg diet.
Group III: Fish were exposed to (1.37mg/l) of atrazine and fed on a commercial diet.
 Group IV: Fish were exposed to1/10 LC$_{50}$ (1.37mg/l) of atrazine and fed on a commercial diet supplemented with 240 mg vit. E/kg diet.

Many classes of environmental pollutants or their metabolites may exert toxicity related to oxidative stress and can cause oxidative damage in fish (Velisek *et al.*, 2011).

Regarding the LPO levels, Table (3) results revealed that there was a significant increase in LPO level in liver tissue of fish during the chronic exposure in comparison to the control group. The results are in accordance with Jin *et al.* (2010), Velisek *et al.* (2011) and Xing *et al.* (2012). The increase in malondialdehde following atrazine exposure may be attributed to the induction of reactive oxygen species (ROS), which enhance the oxidation of polyunsaturated fatty acids and lead to lipid peroxidation (Liu *et al.*, 2008).

Table 3: (LPO) (nmol/g tissue) level of female *C. gariepinus* exposed to different treatments for different periods:

Weeks / Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Group I	30.34±1.71[a]	31.55±0.55[a]	29.05±1.06[a]	31.09±1.21[a]	28.65±0.63[a]	31.02±0.26[a]
Group II	33.44±2.44[a]	32.89±1.51[a]	29.19±0.61[a]	34.69±1.79[a]	33.24±1.97[a]	31.86±0.76[a]
Group III	73.19±3.87[e]	91.86±1.90[d]	110.82±2.86[c]	151.86±3.58[ab]	131.31±1.80[b]	168.89±0.85[a]
Group IV	46.93±1.86[e]	69.64±5.34[d]	86.40±1.31[c]	116.38±2.42[a]	98.33±1.45[b]	105.48±3.98[b]

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same column for each group are significantly different ($P < 0.05$).

Where:
 Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet.
 Group II: Fish were reared in dechlorinated tap water and fed on a commercial diet supplemented with 240 mg vit.E/kg diet.
Group III: Fish were exposed to (1.37mg/l) of atrazine and fed on a commercial diet.
 Group IV: Fish were exposed to1/10 LC$_{50}$ (1.37mg/l) of atrazine and fed on a commercial diet supplemented with 240 mg vit. E/kg diet.

Take into consideration that thiobarbituric acid reactive substance (TBARS) level may indicate an attempt to adapt and compensate herbicide induced oxidative

stress, due to possible atrazine retention of exposed fish (de Menezes *et al*., 2011). Or may be due to the induction of membrane peroxidation which can cause change in membrane fluidity and permeability, then enhance the rate of protein degeneration, and ultimately cell lysis (Tappel, 1973). But the results disagreed with Moraes *et al.* (2009) who recorded a significant decrease in hepatic (TBARS) levels of the teleost fish, *Leporinus obtusidens* exposed to clomazone and propanil for 90 days. This disagreement may be due to the difference in fish species or the type of the herbicides used. However, administration of vitamin E in combination with atrazine exposure resulted in statistically significant decrease in LPO of liver of the fish when compared with fish exposed to atrazine alone. The results agreed with El-Gharieb *et al.* (2010) and Singh *et al.* (2011).

Concerning the results of reduced glutathione (GSH) demonstrated in Table (4), revealed a marked decrease in liver GSH content of atrazine exposed fish. The results are similar to those of Yi *et al.* (2007) and Jin *et al.* (2010) who recorded a marked decrease in GSH content of *Carassius auratus* and adult female zebra fish, *Danio rerio* exposed to alachlor and atrazine herbicides, respectively. This may be explained by a direct action of atrazine on GSH synthesis or that GSH has been depleted in scavenging free radical resulting in its conversion to the oxidized form GSSG (Bamela and Richard, 1994).

Table 4: (GSH) (mg/g tissue) activity of female *C. gariepinus* exposed to different treatments for different periods:

Weeks / Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Group I	60.99±0.76[ab]	62.90±0.05[a]	59.79±0.44[b]	60.62±1.86[ab]	59.19±0.42[b]	62.14±0.60[ab]
Group II	60.05±0.52[a]	61.23±0.72[a]	60.86±1.39[a]	62.85±0.59[a]	60.37±1.08[a]	62.14±0.59[a]
Group III	53.93±2.11[a]	49.52±0.30[a]	39.32±1.63[b]	33.03±1.61[cd]	38.39±2.94[bc]	29.57±0.41[d]
Group IV	58.56±0.27[a]	55.85±0.90[ab]	52.81±1.88[b]	44.65±1.31[c]	48.48±1.38[c]	45.37±1.44[c]

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same column for each group are significantly different ($P < 0.05$).

Where:
Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet.
Group II: Fish were reared in dechlorinated tap water and fed on a commercial diet supplemented with 240 mg vit.E/kg diet.
Group III: Fish were exposed to (1.37mg/l) of atrazine and fed on a commercial diet.
Group IV: Fish were exposed to1/10 LC_{50} (1.37mg/l) of atrazine and fed on a commercial diet supplemented with 240 mg vit. E/kg diet.

GSH protect the membrane-polyunsaturated fatty acid hydrogen of sulfohydryl group instead of methylene hydrogen of unsaturated lipid (Arima and Shiba, 1992). Moreover, GSH serves as a cofactor for glutathione transferase, which facilitates the removal of certain chemicals and other reactive molecules from the cells. It can also interact directly with certain ROS (e.g., hydroxyl radical) for their detoxification as well as perform other critical activities in the cell (Stara *et al*., 2012). Vitamin E in combination with atrazine exposure showed that liver (GSH) content was significantly improved from that of fish exposed to atrazine individually (p<0.05). The results are in accordance with Singh *et al.* (2011). Vitamin E, a term that compasses a small group of related tocopherols, is a major lipid-soluble antioxidant responsible for protecting the unsaturated fatty acid in membranes against lipid peroxidation (Horwitt, 1986). Also, vitamin E has a capacity to act as free radical scavengers in biological system (Ponti *et al.,* 1978). In addition, it acts as antioxidant

breaking the free radical chain reactions as a result of their ability to transfere a phenolic hydrogen to peroxyl free radical of polyunsaturated fatty acids (Mayes, 1993).

The first line of defense against oxidative stress consists of the antioxidant enzymes CAT and SOD, which convert superoxide anions (O_2^-) into H_2O_2 and then into H_2O and O_2. Concerning the activity of CAT enzyme of atrazine exposed fish, the results demonstrated in Table (5) revealed a significant increase in CAT activity of exposed fish (p<0.05) when compared with that of control group fish. The obtained results agree more or less with the results demonstrated by Jin *et al.* (2010) and Singh *et al.* (2011).

Table 5: (CAT) enzyme (U/g tissue) activity of female *C. gariepinus* exposed to different treatments for different periods:

Weeks / Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Group I	118.37±0.82[ab]	116.74±0.56[b]	112.97±1.36[c]	120.96±1.16[a]	117.27±1.99[ab]	119.86±0.27[ab]
Group II	114.94±1.67[b]	114.69±2.01[b]	121.19±0.59[a]	118.87±1.37[ab]	117.54±2.28[ab]	119.23±0.68[ab]
Group III	128.57±0.38[e]	138.39±1.19[d]	150.99±1.51[c]	178.72±1.27[a]	172.63±1.17[b]	173.43±0.88[b]
Group IV	123.91±2.13[d]	130.49±0.49[c]	140.79±0.79[b]	160.79±2.08[a]	161.22±1.08[a]	158.65±0.48[a]

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same column for each group are significantly different ($P < 0.05$).

Where:
Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet.
Group II: Fish were reared in dechlorinated tap water and fed on a commercial diet supplemented with 240 mg vit.E/kg diet.
Group III: Fish were exposed to (1.37mg/l) of atrazine and fed on a commercial diet.
Group IV: Fish were exposed to1/10 LC$_{50}$ (1.37mg/l) of atrazine and fed on a commercial diet supplemented with 240 mg vit. E/kg diet.

The authors explained that the increase in the activity of CAT enzyme might be contributed to the elimination of ROS from the cell induced by pesticide exposure which converts oxygen free radical (O_2^-) into H_2O_2 and then into H_2O and O_2 (Jin *et al.*, 2010). On the other hand, the results disagreed with those of Moraes *et al.* (2009) and Xing *et al.* (2012). These authors pointed out that the observed decrease in CAT activity could be explained by the flux of superoxide radicals due to the oxidative stress caused by pollutant exposure. Decrease in CAT activity could therefore be caused by excessive production of O_2^- (Bainy *et al.*, 1996). Also, the reduction in CAT activity changes the redox status of the cells; where, ROS are generated in excess or there is not enough oxygen radical scavenging activity, free radical chain reactions are stimulated and interactions with protein, lipids and nucleic acids cause cellular damage and even systemic disease in stressed fish (Sun *et al.*, 2006). However, administration of vitamin E caused a significant decrease in the hepatic CAT activity. The results are in accordance with Singh *et al.* (2011).

It is clearly evident from the data demonstrated in Table (6) that the exposure to atrazine led to a marked increase in the activity of SOD in the liver of exposed fish all over the experimental period (p<0.05) when compared with the control group. The results agree with Jin *et al.* (2010); de Menezes *et al.* (2011) and Velisek *et al.* (2011). The increase in the hepatic SOD level may be attributed to the elimination of ROS from the cell induced by atrazine exposure (Jin *et al.*, 2010). As, SOD plays an important role in the defense against the toxic effects of ROS, which can clean up O_2^-

to protect cells from lesions and maintain the balance between oxidant and antioxidant (Li *et al*., 2003). The results disagreed with Guilherme *et al*. (2012) and Xing *et al*. (2012). The decrease in the hepatic SOD level may be probably due to dismutase $O_2^{\cdot-}$ and to decompose H_2O_2. In some cases, $O_2^{\cdot-}$ by itself or after its transformation to H_2O_2 causes a strong oxidation of the cysteine in the enzyme and decreases the SOD activity (Dimitrova *et al*. 1994).

Table 6: (SOD) enzyme (U/g tissue) activity of female *C. gariepinus* exposed to different treatments for different periods:

Weeks / Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Group I	1318.58±1.19[a]	1313.25±1.80[b]	1316.29±1.61[ab]	1320.36±0.89[a]	1316.44±1.26[ab]	1320.11±1.15[a]
Group II	1314.78±0.94[ab]	1314.08±0.46[ab]	1315.14±1.91[ab]	1313.17±0.75[b]	1316.94±2.32[ab]	1318.18±1.04[a]
Group III	1335.54±1.89[d]	1344.4±2.34[c]	1361.04±2.19[a]	1356.89±1.41[ab]	1359.23±0.64[a]	1351.64±1.90[b]
Group IV	1324.21±1.27[d]	1338.78±0.64[b]	1352.29±1.42[a]	1337.34±2.35[b]	1334.71±1.30[b]	1329.58±1.25[c]

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same column for each group are significantly different ($P < 0.05$).

Where:

Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet.

Group II: Fish were reared in dechlorinated tap water and fed on a commercial diet supplemented with 240 mg vit.E/kg diet.

Group III: Fish were exposed to (1.37mg/l) of atrazine and fed on a commercial diet.

Group IV: Fish were exposed to1/10 LC_{50} (1.37mg/l) of atrazine and fed on a commercial diet supplemented with 240 mg vit. E/kg diet.

Meanwhile, the administration of vitamin E as an antioxidant against the toxicity of atrazine herbicide showed a significant decrease in hepatic SOD activity when compared to fish exposed to atrazine alone. The results are in accordance with Singh *et al.* (2011).

In this study, the normal histological structure of fish liver have been greatly damaged after atrazine exposure. Examination of the liver of *C. gariepinus* fish exposed to atrazine showed necrotic changes, pyknotic nuclei (photomicrographs 2and8), dilatation of central vein (photomicrograph3), infiltration of leucocytic inflammatory cells (photomicrographs 4,6,9 and 10), dilated blood vessel (photomicrographs 6 and 10), vacuolar necrosis (photomicrograph10), degenerative changes (photomicrographs 9and11) and haemorrhage (photomicrographs 5,12 and 13). These results are supported by the findings of Hamed (2010) and Xing *et al.* (2012). The appearance of inflammation in the present study means movement of fluid and leukocytes from blood circulation to tissues (Rubin, 2000). Moreover, the hepatic necrosis and the loss of plasma cell membrane may be a consequence of increased (LPO) and decreased (GSH) levels in the liver of atrazine exposed fish (Velisek *et al.,* 2011 and Xing *et al.,* 2012). The present results disagreed with Velisek *et al.* (2011) who recorded no histopathological alterations in the liver of fish. This may be attributed to the difference in the type of pesticide used (terbutryn) and fish species, *Cyprinus carpio*.

Liver sections from *C. gariepinus* fish exposed to atrazine and fed on vitamin E supplemented diet showed an obvious reduction in damage observed in case of atrazine exposure alone. These ameliorative effect of vitamin E on liver tissues are likely to be related to antioxidant potential. In addition to its other damage preventive properties such as inhibition of nitrosamine, stimulation of immune system and enhancement of cell communication (Van Poppel and Vanden Berg, 1997).

It seems that all manifestation of vitamin E on maintaining cellular structure is secondary to inhibited peroxidation of polyunsaturated fatty acid in biological membrane leading to preservation of plasma membrane integrity and protection against necrosis. Moreover, vitamin E is involved in stabilization of lysosomal membrane, therapy inhibiting the release of hydrolases and subsequent cellular dystrophy (White *et al.*, 1978).

In conclusion, the present study indicated that atrazine at sub-lethal levels under laboratory conditions had the capacity to alter the normal physiological functions of female African catfish, *C. gariepinus*. It also increased oxidative stress and altered antioxidant status in the liver of exposed fish which may compromise their competitive ability and predator avoidance leading to a higher mortality in nature. The incorporation of vitamin E in fish ration of female *C. gariepinus* could minimize the oxidative stress of fish exposed to atrazine toxicity and so enhancing the physiological status of exposed fish and in turn raising their resistance.

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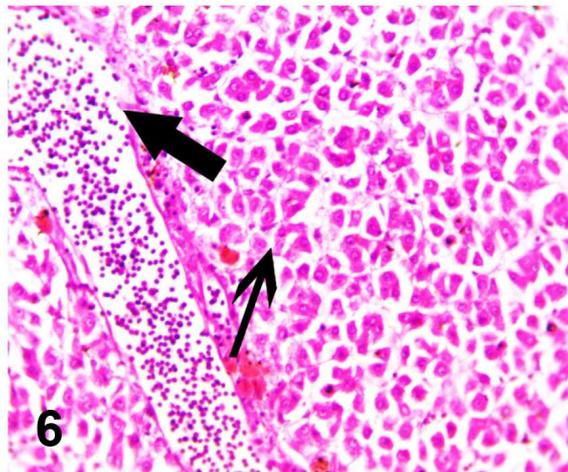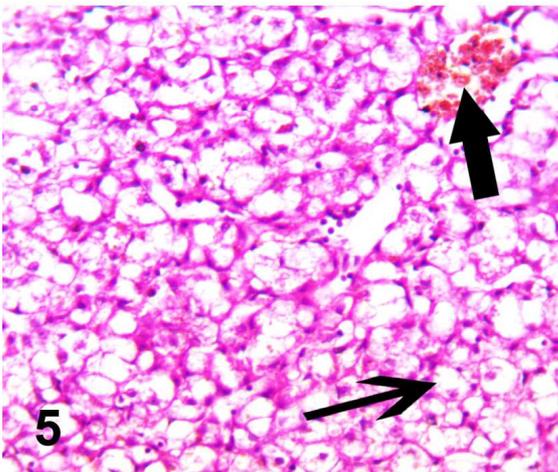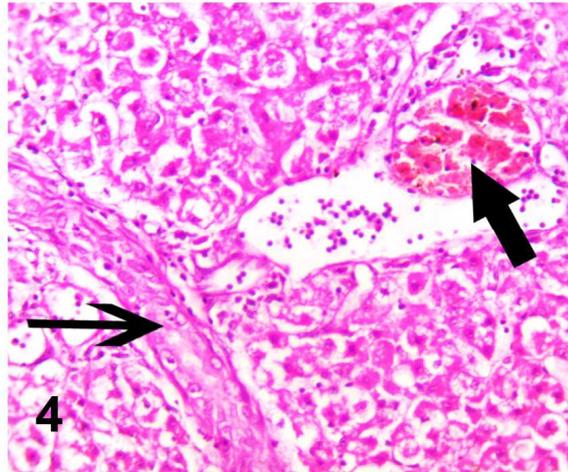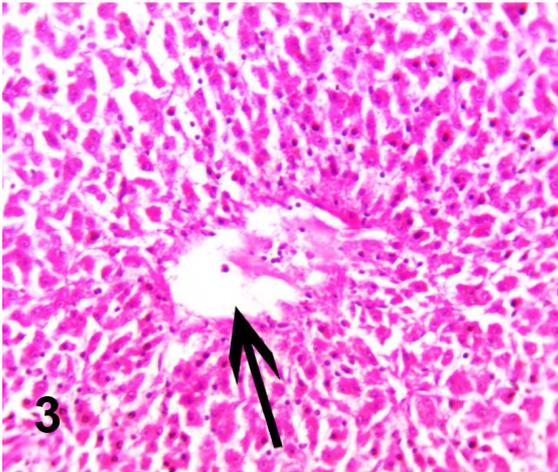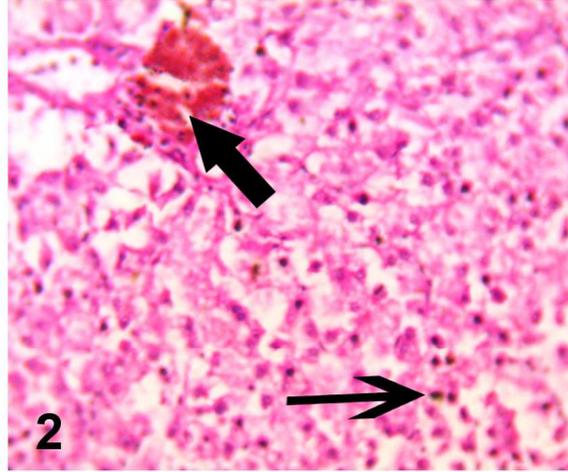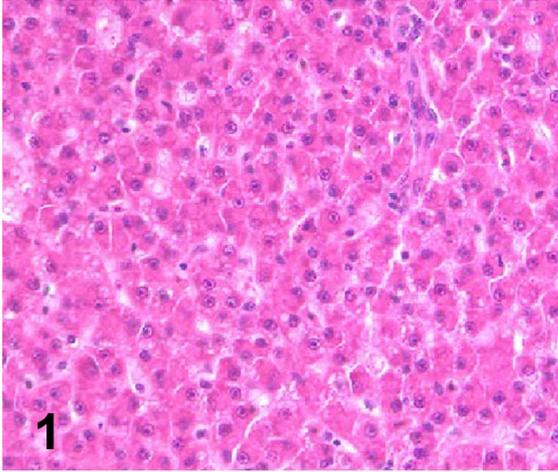
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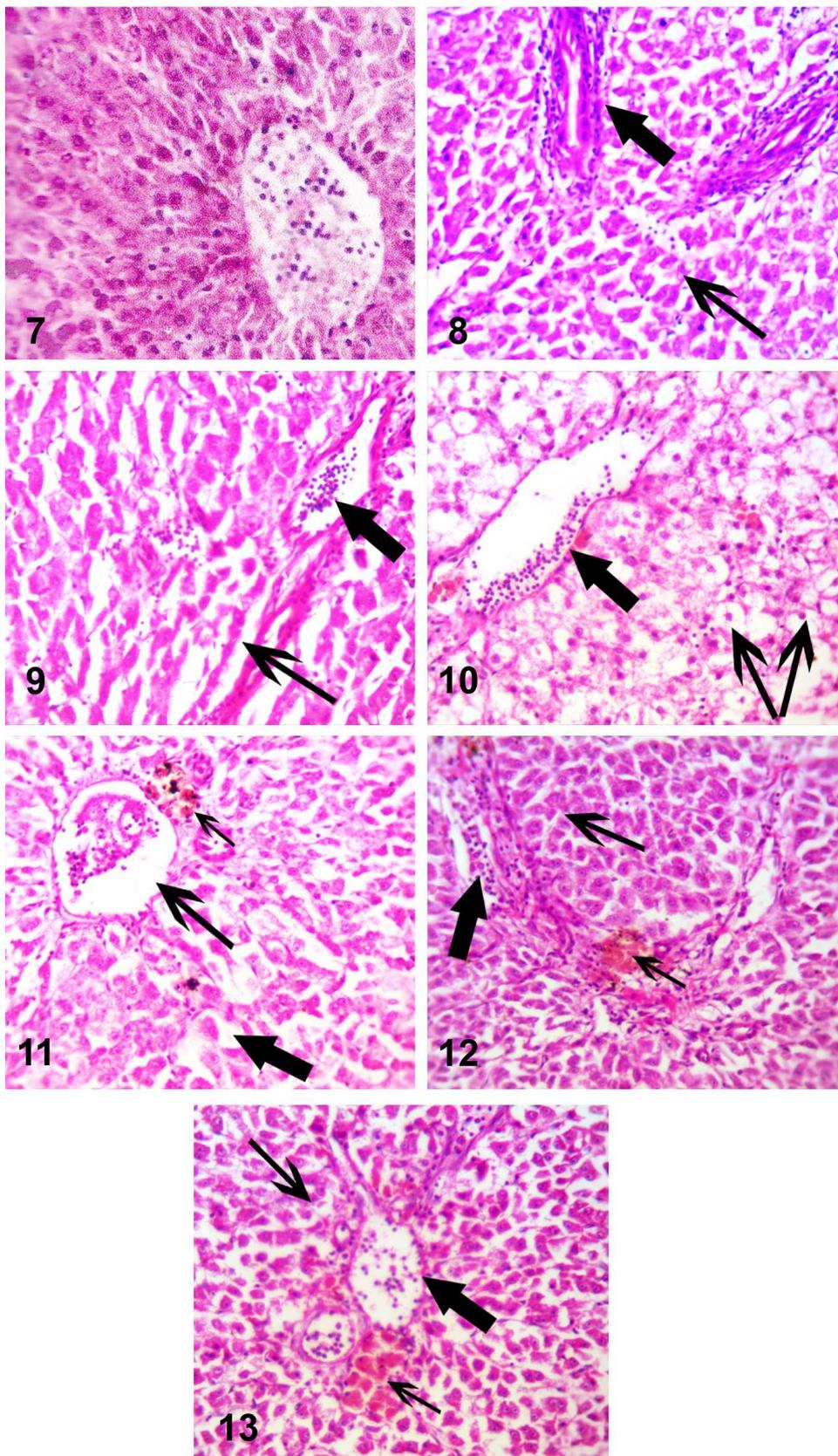
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LEGAND OF PHOTOMICROGRAPHS

Photomicrograph 1: section in liver of control female *Clarias gariepinus* (H&E, X400).

Photomicrograph 2: section in liver of female *Clarias gariepinus* exposed to 1.37mg atrazine /l for 1 week, showing necrotic changes, pyknotic nuclei (thin arrow) which became more dark than that of the control and haemorrhagic signs (thick arrow) (H&E, X 400).

Photomicrograph 3: section in liver of female *C. gariepinus* exposed to 1.37mg atrazine/l for 2 and 3 weeks, showing congested, dilated central vein (thin arrow) (H&E, X 400).

Photomicrograph 4: section in liver of female *C. gariepinus* exposed to 1.37mg atrazine/l for 4 weeks, showing a mass of concentrated bile pigments (thick arrow) surrounded by necrotic liver cells and fibrous tissue invaded with some inflammatory cells (thin arrow) (H&E, X 400).

Photomicrograph 5: section in liver of female *C. gariepinus* exposed to 1.37mg atrazine/l for 5 weeks, showing severe vacuolar necrosis with pyknotic nuclei (thin arrow), haemorrhage is clear through the hepatic cells (thick arrow) (H&E, X 400).

Photomicrograph 6: section in liver of female *C. gariepinus* exposed to 1.37mg atrazine/l for 6 weeks, showing degeneration of some of the hepatic cells (thick arrow), dilated blood vessel (thin arrow) (H&E, X 400).

Photomicrograph7: section in liver of control female *C. gariepinus* fed on diet supplemented with vit E (240 mg/kg diet) (H&E, X 400).

Photomicrograph 8: section in liver of female *C. gariepinus* exposed to1.37mg atrazine/l for 1 week and fed diet supplemented with vit E (240 mg/kg diet), showing necrotic changes (thin arrow), pyknotic nuclei and inflammatory cells within fibre tissue (Thick arrow), dilated sinusoids, infiltration of mononuclear chronic inflammatory cells in a highly thickened wall of blood vessel and between hepatocytes (thick arrow) (H&E, X 400).

Photomicrograph 9: section in liver of female *C. gariepinus* exposed to1.37mg atrazine/l for 2 weeks and fed diet supplemented with vit E (240 mg/kg diet), showing degeneration of some cells (thin arrow), infiltration of chronic inflammatory cells within blood vessel and between hepatocytes (Thick arrow) (H&E, X 400).

Photomicrograph 10: section in liver of female *C. gariepinus* exposed to1.37mg atrazine/l for 3 weeks and fed diet supplemented with vit E (240 mg/kg diet), showing severe vacuolar necrosis (thin arrow) , dilated blood vessel infiltrated with inflammatory cells (thick arrow) (H&E, X 400).

Photomicrograph 11: section in liver of female *C. gariepinus* exposed to1.37mg atrazine/l for 4 weeks and fed diet supplemented with vit E (240 mg/kg diet), showing degeneration of the hepatic cells (thick arrow), dilated central vein filled with inflammatory cells (thin arrow) beside the appearance of iron pigments (haemosiderin) (Short arrow) (H&E, X 400).

Photomicrograph 12: section in liver of female *C. gariepinus* exposed to1.37mg atrazine/l for 5 weeks and fed diet supplemented with vit E (240 mg/kg diet), showing nearly normal hepatic cells (thin arrow), an invasion of inflammatory cells through the hepatic tissue (thick arrow), haemorrhage is quite clear (short arrow) (H&E, X 400).

Photomicrograph 13: section in liver of female *C. gariepinus* exposed to1.37mg atrazine/l for 6 weeks and fed diet supplemented with vit E (240 mg/kg diet), showing nearly normal hepatic tissue (thin arrow), inflammatory cells in the dilated central vein (thick arrow) and haemorrhage is clear (short arrow) (H&E, X 400).

Arabic Summary

فيتامين ه كمضاد للأكسده فى إناث أسماك القرموط الافريقى أثناء التعرض المزمن للأترازين

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خططت هذه الدراسة لمعرفة مدى تأثير مبيد الحشائش الأترازين على نشاط الاسيتيل كولين استريز وردود الأكسدة و التغيرات الهستوباثولوجية فى إناث أسماك القرموط الافريقى وذلك بتعرضها لتركيز 1.37 مللى جرام/لتر، 1/10 التركيز المميت للنصف لمدة 6 أسابيع ، بالأضافة إلى تقييم الدور الوقائى لفيتامين ه المضاف للعليقة (240 مللى جرام/كيلوجرام عليقة) فى تقليل الأضرار المحدثة. وقد أدت نتائج التعرض المزمن للأترازين إلى تثبيط الكولين استريز ، وزيادة ملحوظة فى مستوى الكورتيزول. بينما أوضحت نتائج الضرر التأكسدى فى كبد الأسماك المعرضة للأترازين زيادة معنوية فى مستوى الأكسده الفوقية للدهون يقابلها حدوث انخفاض ملحوظ فى محتوى الجلوتاثيون. كما أدت سمية الأترازين إلى زيادة معنوية فى نشاط كل من انزيمى الكتاليز والسوبرأوكسيد ديزميوتيز. علاوة على ذلك فقد أظهر الفحص الهستوباثولوجى لكبد الأسماك إلى اتساع واحتقان الاوعية الدموية والجيوب الدموية و ظهور بعض بؤر الموت الخلوى بين خلايا الكبد . بينما أكدت الدراسة أن الأسماك المعرضة لنفس التركيز تحت المميت للأترازين والمغذاه على عليقة تحتوى على 240 مللى جرام فيتامين ه /كيلوجرام عليقة, الدور الوقائى لفيتامين ه فى الحد من التأثير الضار لمبيد الأترازين وقيم شبيهه لمثيلاتها من المجموعة الضابطة.