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Full Length Research Paper

Protective effects of tomato paste and Vitamin E on Atrazine-Induced Hematological and biochemical characteristics of *Clarias gariepinus* (Burchell,1822)

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The pesticides have different adverse impacts on different life stages of fish species with attempts to use dietary antioxidants to counteract their effects. So, the present study investigated the potential protective effects of vitamin E (50 mg/kg BW), tomato paste (in terms of 9 mg lycopene/kg BW) and their combination versus atrazine-induced changes in *Clarias gariepinus*. This species were exposed to sublethal doses of atrazine of 1.7 and 3.4 mg/L for 15 and 30 days. Atrazine significantly (P<0.05) induced free radicals in serum constituents. It caused a significant (P<0.05) increase in serum glucose, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and lipid peroxidation in liver. Addition of vitamin E and/or tomato paste to the diet of atrazine-treated fish improved the serum glucose, MCV, MCH and LPO levels in comparison to control fish. On the other hand, atrazine significantly (P<0.05) led to decline in serum total protein, total lipid, blood hemoglobin, total erythrocytes count, packed cell volume, and mean corpuscular hemoglobin concentration.These atrazine-induced parameters were improved with the dietary supplemented tomato paste and/or vitamin E.

Keywords: *Clarias*, Atrazine, Tomato-past, vitamin-E haemotology, biochemistry.

INTRODUCTION

The aquatic ecosystems have known to receive a wide spectrum of pollutants, which may be introduced to it directly or indirectly. Pesticides are one of these pollutants representing a serious environmental problem (Fischer-Scherl et al., 1991; Joseph and Raj, 2011; Khidr et al., 2001; Mekkawy et al., 1996; Okayi et al., 2013a; Okayi et al., 2013b). The major source of contamination by these pesticides is the deposits resulting from their application to control agriculture pests and harmful aquatic herbs (Egaas et al., 1993; Fischer-Scherl et al., 1991; Mekkawy et al., 1996; Yadav et al., 2010). The pesticides have different diverse impacts on the aquatic animals especially fishes which are of economic importance and high value from the point of biological conservation. Different worldwide efforts and scientific studies have been carried out to evaluate and hence prevent the adverse impacts of these chemicals to the extent of their banning especially in the advanced countries (EUC, 2007).

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In Egypt, waste water and agriculture drains containing pesticides and heavy metals residue are discharged directly into the Nile and its tributaries and the northern lakes (Mekkawy et al., 1996). Pesticides enter these water bodies from industrial planes, aerial spraying of field and water drainage system (El-Elaimy et al., 1990). The increasing use of pesticides in insect and herbal control as well as in public and private gardens has resulted in a great danger to aquatic organisms and man (El-Missiry and Othman, 1993).

Atrazine (AT) is one of the herbicides used in Egypt. It belongs to the s-triazine family of herbicides, which are one of the most significant water pollutants in rain, fresh, marine and ground waters (Egaas et al., 1993; Felding, 1992; Fischer-Scherl et al., 1991; Tasli et al., 1996). Atrazine has been detected in various concentrations in rainwater, surface water, ground water and drinking water reservoirs (Braun et al., 1987; Haberer and Normann, 1988; Oehmichen and Haberer, 1986). Fish species are one of the aquatic organism which suffer severely from the herbicides especially atrazine. Different studies have been carried out to evaluate its adverse impacts on the biological system of fishes and to determine the corresponding biological, molecular and genetic biomarkers (e.g. (Khidr et al., 2001; Nwani et al., 2010; Okayi et al., 2013a; Ovie et al., 2007; Wassif et al., 2000; Yadav et al., 2010). Also, further studies are published to counteract the adverse impacts of pollutants especially in aquaculture through the enhancement of the natural antioxidant system and addition of different exogenous antioxidants in diets (Mekkawy et al., 2011; 2012).

Carotenoids are a family of fat-soluble pigments found in tomatoes and its products, some other fruits and vegetables. Mmany studies have investigated the potentials of these carotenoids in the oxidative stress. Tomato carotenoids include lycopene and other similar carotenoids (Cohen, 2002; Mekkawy et al., 2011; 2012; Tapiero et al., 2004; Visioli et al., 2003). Recently, tomato paste and its individual carotenoids have received particular attention in counteracting pollutant-induced oxidative stress especially in human and rats because of their highly efficient scavenging capacity for singlet-oxygen and free radicals (Heber and Lu, 2002; Mekkawy et al., 2011; 2012; Stahl and Sies, 2003; Wertz et al., 2004). The potentials of tomato and their products and catotenoids especially lycopene in treatment of different types of human cancers were evident especially in prostate cancers (Boileau et al., 2003; Mekkawy et al., 2011). Lycopene can be considered as a biomarker for the additive or synergistic anticancer effects of tomato phytonutrients. Boileau et al. (2003), supporting this observation, concluded that consumption of tomato powder but not lycopene inhibited prostate carcinogenesis. Mekkawy et al. (2011; 2012) referred to its validity in improving liver function and other biochemical and liver histological characteristics of cadmium-exposed Oreochromis niloticus.

Vitamin E is an indispensable nutrient required to maintain flesh quality, immunity, normal resistance of red blood corpuscles to haemolysis, permeability of capillaries and heart muscle (Halver, 2002). It functions as a lipid soluble antioxidant and protects biological membranes, lipoproteins and lipid stores against oxidation. Its main function is to protect unsaturated fatty acids against free radical-mediated oxidation (Hamre et al., 1998). Different studies have been recorded its antioxidant roles in preventing and improving pollutant adverse effects including those induced by atrazine on the biological systems of aquatic animals.

According to the aforementioned findings and low price of tomatoes in a definite season in Egypt, the present work was suggested and aimed to study the protective roles of tomato paste and/or vitamin E on atrazine-induced changes in some haematological and biochemical parameters of the Nile catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Sample collection and treatment manipulation

One hundred and twenty healthy fish of The Nile catfish, Clarias gariepinus were caught from the River Nile at Assiut, Egypt. Fishes immediately were transported to the fish laboratory in the Department of Zoology, Faculty of Science, Assiut University. The experimental fishes were reared in aerated glass tanks (100 L capacity) and acclimatized for two weeks before being used in the experimental study. The experimental fish fed pellets at a rate of 4.5 % of wet weight twice daily. Feces and residual food were aspirated regularly. The water temperature, pH and dissolved oxygen (DO) concentrations were measured daily (18.44±1.43˚C, 6.94±0.11 pH, 6.71±2.52 mg L^{-1} DO) with photoperiod of 12 light/12 dark.

Preparation of tomato paste to adjust the lycopene dose

Preparation of tomato paste to adjust the lycopene dose follow Mekkawy et al. (2011). Tomatoes used for the experiment were obtained from the local market. Fresh peeled, deseeded tomatoes were pulped well to a smooth consistency in a Warring blender. The lycopene content in tomato paste was estimated spectrophotometrically according to the methods of Ranganna (1976) and Choudhari and Anantharayan (2007). The lycopene concentration in the tomato paste was 30.28 mg kg-1. In addition to lycopene, tomato paste composition include water, proteins, carbohydrates, fibers, calcium, potassium, zinc, copper, manganese, iron, vitamin C, vitamin E, β-caroteinoids and other

Table 1. The fish groups exposed to atrazine (AT1, AT2) doses, tomato paste (TP), vitamin E (VE) and their combinations.

Treatments	C	VE	TP	TP+ VE	AT1	AT1+ VE	AT1+ TP	AT1+T P+VE	AT2	AT2+ VE	AT2+ TP	AT2+ TP+ VE
Atrazine (mg/L)	0	0	0	0	1.7	1.7	1.7	1.7	3.4	3.4	3.4	3.4
Vitamin E (mg/kg)	0	50	0	50	0	50	0	50	0	50	0	50
Tomato paste (mg/kg)	0	0	9	9	0	0	9	9	0	0	9	9

C= control, VE= vitamin E, TP= tomato paste and AT1, AT2= atrazine doses

phytonutrients (Okajima et al., 1998).

Experimental design

Fishes were weighed, measured and classified randomly into 12 groups (10 fishes/tank) according to doses of atrazine (AT), tomato paste (TP), vitamin E (VE) and their combinations (Table 1). The diets were pelleted after addition of tomato paste and vitamin E doses and addition of suitable amounts of molasses and water. The diets were dried at room temperature and stored in small bags for fish feeding.

Stock solution of the herbicide, atrazine were prepared and stored in clean glass bottles and diluted to two sublethal concentrations of 1.7 and 3.4 mg L^{-1} (96h- LC50 equal 7.6 mg L^{-1} for catfish (WHO/FAO, 1996). Atrazine doses were prepared and added constantly to the aquarium for four weeks. The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish atrazine levels.

Blood analyses

After 15 and 30 day periods, blood samples of the control and treated fish (3 fish/treatment) were collected from caudal vein of the fish in a small plastic tubes containing heparin solution (0.2 mL mL-1 blood) as anticoagulant. These blood samples were used for determining erythrocyte count according to Dacie and Lewis (1984) using hemocytometer. Haemoglobin (Hb) was estimated where it was converted into red cyanomethemoglobin under the influence of potassium ferricyanide and potassium cyanide according to Vankampen (1961). Haemoglobin level was determined by using suitable Kits (Diamond Diagnostics, Egypt) according to Stoskopf (1993). Haematocrit value (Hct) was calculated according to the formula mentioned by Stoskopf (1993). Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were calculated according to Cloes (1980).

Some other blood samples were collected and left to coagulate for 15-20 minutes at 4ºC prior to centrifugation for 20 minutes at 3000 rpm to separate serum. The fresh serum was subjected to biochemical analysis. Serum glucose (mg L-1) was determined, using assay kit supplied by (Spectrum Diagnostics, Egypt). Total protein (g 100mL-1) content and total lipids (g L-1) content were determined colorimetrically using assay kit supplied by Diamond Diagnostics, Egypt. The samples were measured by spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd).

Lipid peroxidation measurement

Lipid peroxidation was measured according to the method of Ohkawa *et al*. (1979). 10(w/v) tissue homogenate from liver was used (this homogenate contained 1% v/v dimethyl sulfoxide to prevent further oxidation). 0.2 ml aliquots of tissue homogenate were added to 0.2 ml sodium dodecyl sulfate solution (8.1% w/v) and 1.5 ml acetic acid solution (20% v/v). The mixture was made up to 4.0 ml with distilled water and heated to 95 ºC for 1 h. The samples were cooled, centrifuged at 2000 rpm for 10 min. and measured at 532 nm using spectrophotometer (Ultrospec 3100 *pro*, Biochrom Ltd). The results were expressed as nmol malondialdehyde formation per g tissue.

Statistical analysis

The basic statistics, means, standard errors and ranges of the measured parameters were estimated. The patterns of variation due to atrazine, tomato paste and vitamin E doses and their combinations were studied by three and four-way analysis of variance using the SPSS transformed data. So, the homogeneity of variance was

Table 2. Values of blood constituent parameters of *Clarias gariepinus* exposed to atrazine, vitamin E, tomato paste and their combinations for 15 days.

Parameters \ Treatments	C	VE	TP	TP+VE	AT1	AT1+VE
RBCs(million/mm^3)	3.18±0.02^{b} (3.15-3.21)	3.41±0.24^{b} (3.00-3.82)	3.65±0.03^{b} (3.61-3.70)	2.23±0.05^{a} (2.13-2.32)	1.90±0.42^{a} (1.17-2.63)	2.27±0.23^{a} (1.86-2.67)
Hb(g/100ml blood)	8.32±0.47^{ab} (7.50-9.15)	10.61±0.20^{c} (10.26-10.96)	12.49±0.86^{d} (11.00-13.97)	10.79±0.18^{c} (10.52-11.15)	7.32±0.07^{ab} (7.20-7.43)	8.80±0.12^{b} (8.60-9.00)
Hct(%)	24.10±1.33^{ab} (21.80-26.39)	30.51±0.57^{c} (29.53-31.49)	35.77±2.40^{d} (31.60-39.92)	31.00±0.51^{c} (30.26-31.99)	21.29±0.18^{ab} (20.96-21.60)	25.44±0.32^{b} (24.88-26.00)
MCV(μ^3)	75.80±4.41^{a} (68.55-83.78)	90.44±7.10^{a} (77.30-101.69)	97.98±6.94^{a} (85.41-109.36)	139.52±5.77^{a} (130.41-150.20)	125.15±30.09^{a} (79.70-182.02)	115.83±12.58^{a} (93.18-136.77)
MCH($\mu\mu^3$)	26.17±1.58^{a} (23.58-29.02)	31.46±2.48^{a} (26.86-35.37)	34.21±2.47^{a} (29.73-38.27)	48.54±2.03^{a} (45.34-52.30)	43.02±0.34^{a} (27.38-62.56)	39.72±4.36^{a} (32.21-47.31)
MCHC(%)	34.52±0.07^{bc} (34.40-34.63)	34.78±0.02^{de} (34.75-34.81)	34.91±0.06^{e} (34.81-35.00)	34.79±0.01^{de} (34.77-34.82)	34.37±0.01^{abc} (34.35-34.39)	34.59±0.01^{cd} (34.57-34.62)
Gl(mg/dl)	25.08±1.75^{a} (22.05-30.11)	26.90±0.53^{ab} (25.98-27.82)	27.31±0.61^{ab} (26.25-30.36)	47.50±2.26^{cd} (43.25-50.96)	41.61±3.64^{c} (35.43-48.03)	38.58±2.88^{abc} (33.59-43.57)
TP(g/dl)	5.43±0.05^{de} (5.34-5.51)	6.41±0.01^{ef} (6.39-6.43)	5.13±0.16^{cd} (4.85-5.40)	6.62±0.25^{f} (6.18-7.06)	4.57±0.45^{bcd} (3.82-5.36)	4.27±0.11^{bcd} (4.07-4.46)
TL(g/l)	8.32±0.13^{d} (8.10-8.54)	8.37±0.27^{d} (7.89-8.84)	7.10±0.66^{cd} (5.95-8.24)	8.05±0.25^{d} (7.61-8.49)	7.08±0.07^{cd} (6.98-7.21)	8.19±0.12^{d} (7.98-8.40)
LPO(nmol/mg tissue)	1.39±0.59^{ab} (0.37-2.41)	1.13±0.11^{a} (0.94-1.32)	1.49±0.19^{ab} (1.15-1.82)	1.5±0.09^{ab} (1.32-1.61)	4.20±0.33^{d} (3.63-4.77)	2.61±0.18^{bc} (2.24-2.79)

The data are presented as Means ± S.E. (Min-Max).
Different letters indicate significance at $p<0.05$
Red blood cells (RBC), Haemoglobin (Hb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Glucose (Gl), Total protein (TP), Total lipids (TL) and Lipid peroxidation (LPO).

Table 2. Cont.

Parameters \ Treatments	AT1+TP	AT1+TP+VE	AT2	AT2+VE	AT2+TP	AT2+TP+VE
RBCs(million/mm^3)	2.17±0.21^{a} (1.80-2.54)	1.97±0.25^{a} (1.53-2.40)	1.49±0.21^{a} (1.13-1.85)	1.98±0.12^{a} (1.76-2.19)	1.71±0.03^{a} (1.66-1.75)	1.73±0.01^{a} (1.70-1.75)
Hb(g/100ml blood)	7.68±0.22^{ab} (7.30-8.05)	7.00±0.18^{a} (6.69-7.30)	6.84±0.21^{ab} (6.47-7.20)	7.30±0.40^{ab} (6.58-7.98)	7.49±0.30^{ab} (7.02-8.00)	6.90±0.46^{a} (6.10-7.70)
Hct(%)	22.29±0.61^{ab} (21.24-23.34)	20.39±0.49^{a} (19.53-21.24)	19.94±0.59^{ab} (18.92-20.96)	21.18±1.13^{ab} (19.22-23.15)	21.77±0.79^{ab} (20.46-23.20)	20.12±1.29^{a} (17.88-22.36)
MCV(μ^3)	104.56±9.83^{a} (91.89-123.91)	106.57±11.40^{a} (88.50-127.66)	139.83±21.46^{a} (102.25-176.57)	107.30±1.04^{a} (105.68-109.23)	127.68±5.60^{a} (116.89-135.67)	116.42±6.52^{a} (105.18-127.77)
MCH($\mu\mu^3$)	36.00±3.38^{a} (31.69-42.67)	36.56±3.88^{a} (30.42-43.73)	47.94±7.38^{a} (34.97-60.53)	36.87±0.30^{a} (36.44-37.39)	43.92±1.98^{a} (40.11-46.78)	39.92±2.34^{a} (35.88-44.00)
MCHC(%)	34.43±0.03^{abc} (34.37-34.49)	34.31±0.03^{ab} (34.25-34.37)	34.30±0.04^{abc} (34.20-34.35)	34.36±0.07^{ab} (34.23-34.48)	34.40±0.05^{abc} (34.32-34.48)	34.30±0.09^{a} (34.12-34.44)
Gl(mg/dl)	46.98±5.46^{cd} (38.58-57.22)	64.29±2.26^{e} (60.52-68.32)	58.85±1.93^{c} (55.40-62.08)	41.21±0.61^{c} (40.16-42.26)	39.77±0.53^{bc} (38.85-40.68)	65.11±6.53^{e} (55.38-77.53)
TP(g/dl)	4.53±0.16^{cd} (4.26-4.82)	4.35±0.07^{bcd} (4.23-4.46)	4.11±0.54^{cd} (3.17-5.04)	3.00±0.33^{a} (2.42-3.58)	3.93±0.33^{abc} (3.35-4.50)	3.77±0.15^{ab} (3.52-4.01)
TL(g/l)	4.59±0.06^{a} (4.47-4.68)	5.51±0.25^{ab} (5.07-5.95)	6.06±0.06^{cd} (5.95-6.16)	5.27±0.36^{ab} (4.65-5.88)	5.85±0.29^{b} (5.35-6.34)	4.55±0.15^{a} (4.30-4.79)
LPO(nmol/mg tissue)	6.47±0.21^{e} (6.12-6.83)	2.64±0.58^{bc} (1.99-3.80)	3.23±0.18^{cd} (2.92-3.55)	1.21±0.04^{a} (1.15-1.30)	5.70±0.10^{e} (5.53-5.85)	2.1±0.23^{abc} (1.70-2.50)

The data are presented as Means ± S.E. (Min-Max).
Different letters indicate significance at $p<0.05$
Red blood cells (RBCs), Haemoglobin (Hb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Glucose (Gl), Total protein (TP), Total lipids (TL) and Lipid peroxidation (LPO).

Table 3. Values of blood constituent parameters of *Clarias gariepinus* exposed to atrazine, vitamin E, tomato paste and their combinations for 30 days.

Parameters \ Treatments	C	VE	TP	TP+VE	AT1	AT1+VE
RBCs(million/mm^3)	3.40±0.16^{d} (3.12-3.67)	3.31±0.03^{d} (3.25-3.36)	3.77±0.15^{d} (3.52-4.01)	2.23±0.10^{abc} (2.06-2.39)	1.92±0.11^{abc} (1.75-2.12)	2.55±0.12^{c} (2.35-2.75)
Hb(g/100ml blood)	9.11±0.34^{d} (8.53-9.70)	9.32±0.31^{d} (8.79-9.85)	12.15±0.68^{e} (10.96-13.33)	8.42±0.38^{bcd} (7.76-9.08)	6.66±0.21^{a} (6.29-7.02)	7.41±0.22^{abc} (7.02-7.79)
Hct(%)	26.31±0.95^{d} (24.68-27.96)	26.90±0.86^{d} (25.41-30.38)	34.81±1.91^{e} (31.49-38.12)	24.38±1.07^{bcd} (22.53-26.22)	19.44±0.59^{a} (18.41-20.46)	21.54±0.62^{abc} (20.46-22.61)
MCV(μ^3)	77.53±0.85^{a} (76.19-79.12)	81.30±1.81^{a} (78.19-84.46)	92.29±1.62^{ab} (89.45-95.07)	109.46±0.13^{ab} (109.31-109.72)	101.35±2.57^{ab} (96.49-105.21)	84.92±5.34^{a} (74.39-91.69)
MCH(μμ^3)	26.84±0.27^{a} (26.43-27.34)	30.18±0.66^{a} (27.05-29.32)	32.20±0.61^{ab} (31.15-33.24)	37.81±0.10^{ab} (37.67-37.99)	34.70±0.84^{ab} (33.11-35.94)	29.20±1.86^{a} (25.53-31.53)
MCHC(%)	34.63±0.04^{d} (34.56-34.69)	34.65±0.03^{d} (34.59-34.71)	34.89±0.04^{e} (34.81-34.96)	34.54±0.05^{cd} (34.45-34.62)	34.24±0.05^{a} (34.16-34.32)	34.39±0.04^{abc} (34.32-34.45)
Gl(mg/dl)	26.59±1.05^{a} (24.77-30.40)	30.43±1.17^{a} (30.40-32.46)	27.95±0.96^{a} (26.30-29.61)	26.15±0.44^{a} (25.38-26.89)	58.76±5.67^{c} (48.94-68.58)	37.95±1.34^{ab} (35.45-40.02)
TP(g/dl)	6.06±0.13^{e} (5.82-6.26)	7.62±0.43^{f} (6.88-8.36)	7.26±0.40^{f} (6.56-7.96)	5.08±0.07^{cde} (5.00-5.21)	4.25±0.15^{bc} (4.02-4.50)	5.30±0.20^{cde} (4.95-5.65)
TL(g/l)	8.53±0.25^{d} (8.12-8.97)	9.27±0.44^{d} (8.50-10.04)	8.65±0.04^{d} (8.58-8.72)	9.23±0.25^{d} (8.78-9.63)	6.07±0.12^{abc} (5.85-6.30)	5.03±0.04^{ab} (4.96-5.09)
LPO(nmol/mg tissue)	2.77±0.06^{a} (2.66-2.88)	6.83±0.05^{bcd} (6.75-6.92)	6.30±0.44^{bc} (5.40-6.79)	5.04±0.59^{b} (4.01-6.07)	6.73±0.35^{bcd} (6.12-7.34)	7.71±0.49^{bc} (7.00-8.64)

The data are presented as Means ± S.E. (Min-Max).
Different letters indicate significance at $p<0.05$
Red blood cells (RBCs), Haemoglobin (Hb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Glucose (Gl), Total protein (TP), Total lipids (TL) and Lipid peroxidation (LPO).

Table 3. Cont.

Parameters \ Treatments	AT1+TP	AT1+TP+VE	AT2	AT2+VE	AT2+TP	AT2+TP+VE
RBCs(million/mm^3)	2.49±0.02^{bc} (2.45-2.53)	1.90±0.36^{abc} (1.27-2.53)	1.44±0.01^{a} (1.42-1.45)	2.00±0.12^{abc} (1.80-2.20)	2.42±0.16^{bc} (2.15-2.70)	1.70±0.29^{ab} (1.19-2.20)
Hb(g/100ml blood)	8.91±0.20^{d} (8.56-9.26)	8.10±0.13^{bcd} (7.86-8.31)	6.65±0.10^{a} (6.47-6.82)	7.16±0.08^{ab} (7.02-7.30)	8.63±0.23^{cd} (8.24-9.05)	8.11±0.31^{bcd} (7.56-8.64)
Hct(%)	25.75±0.57^{d} (24.77-26.73)	23.49±0.37^{bcd} (22.81-24.07)	19.41±0.30^{a} (18.92-19.90)	20.85±0.23^{ab} (20.46-21.24)	24.96±0.66^{cd} (23.87-26.15)	23.52±0.87^{bcd} (21.97-24.99)
MCV(μ^3)	103.38±1.31^{ab} (101.09-105.64)	133.17±25.15^{ab} (93.25-179.59)	135.13±2.37^{ab} (130.46-138.17)	104.81±4.94^{ab} (96.55-113.64)	103.90±6.20^{ab} (96.81-116.26)	158.83±31.25^{b} (107.24-210.02)
MCH(μμ^3)	35.77±0.48^{ab} (34.94-36.60)	45.93±8.65^{ab} (32.17-61.89)	46.27±0.84^{ab} (44.62-47.36)	35.99±1.68^{ab} (33.18-39.00)	35.92±2.15^{ab} (33.52-40.19)	51.36±10.84^{b} (37.00-72.61)
MCHC(%)	34.60±0.03^{d} (34.56-34.65)	34.50±0.02^{bcd} (34.46-34.53)	34.24±0.02^{a} (34.20-34.30)	34.34±0.01^{ab} (34.32-34.37)	34.57±0.03^{d} (34.52-34.62)	34.49±0.05^{bcd} (34.41-34.57)
Gl(mg/dl)	30.25±0.79^{a} (26.89-29.61)	38.82±0.79^{ab} (37.46-40.18)	80.63±5.72^{e} (69.42-88.22)	34.75±2.27^{a} (30.82-38.67)	29.46±2.36^{a} (25.38-33.54)	47.96±1.41^{b} (45.36-50.21)
TP(g/dl)	5.65±0.19^{de} (5.31-5.98)	4.58±0.23^{bcd} (4.21-5.00)	2.80±0.18^{a} (2.48-3.11)	4.86±0.09^{cd} (4.68-4.97)	3.68±0.01^{ab} (3.66-3.69)	3.69±0.31^{ab} (3.13-4.20)
TL(g/l)	8.42±0.42^{d} (7.69-9.15)	7.20±0.19^{c} (6.84-7.50)	5.32±0.33^{ab} (4.74-5.89)	4.78±0.44^{a} (4.06-5.59)	5.13±0.05^{ab} (5.04-5.21)	6.26±0.41^{bc} (5.55-6.96)
LPO(nmol/mg tissue)	8.05±0.29^{cd} (7.55-8.56)	12.58±0.81^{f} (11.17-13.99)	8.43±0.22^{d} (8.05-8.81)	15.37±0.17^{g} (15.07-15.66)	10.63±0.42^{e} (9.82-11.25)	8.27±0.02^{d} (8.23-8.31)

The data are presented as Means ± S.E. (Min-Max).
Different letters indicate significance at $p<0.05$
Red blood cells (RBCs), Haemoglobin (Hb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Glucose (Gl), Total protein (TP), Total lipids (TL) and Lipid peroxidation (LPO).

package (SPSS, 1998) at the 0.05 significance level. Null hypothesis for raw, log-transformed and SQRT- Levene's test of equality of error variance of the dependent variables was applied, with rejection of the assumed for raw data. The pattern of variations was also recorded by one-way analysis of variance, revealing significant difference due to atrazine, tomato paste and vitamin E ($P < 0.0001$); The Tukey-HSD test was considered for multiple comparisons. Moreover, the Dunnett-T test was applied, measuring the control against other treatments.

RESULTS

Behavioral changes

After exposure to atrazine some of the fishes exhibited hyperactivity characterized by rapid and erratic swimming or darting, partial loss of equilibrium, rapid pectoral fins and opercular movements, reduction in the feeding activity, fins haemorrhage and loss of some skin parts especially in those exposed to atrazine without vitamin E and/or lycopene. Quick ventilation movements were recorded especially in aquaria with high concentration of atrazine. The mortality rate of 2.3 was recorded at the first period in aquaria with only atrazine. The dead fishes exhibited changes and abnormality in eyes, gills, gall bladder, spleen and liver color. On the other hand, fish groups exposed to the same doses of atrazine in combination with tomato paste and/or vitamin E did not show such behavioral abnormalities. Moreover, Atrazine-free fishes treated with vitamin E and/or tomato paste were noticed in better conditions.

Blood constituents

The basic data are given in Tables 2 and 3. Correlation matrix of blood constituents parameters in the first and second periods of exposure are given in Tables 4 and 5. Many significant correlations between most of such parameters were recorded. For example, RBCs were found to be correlated with all parameters.

A- Haematological parameters

The normal values of red-blood-cells (RBCs) count, haemoglobin (Hb) content and haematocrit (Hct) value of *Clarias gariepinus* in the two periods are given in Tables 2and3. The atrazine main effect, was highly significant ($P<0.0001$) in both periods for the three previous parameters. The tomato paste main effect was significant in both periods except for RBCs. Vitamin E main effect was significant only at the second period for RBCs, Hb and Hct. TP-VE interaction effect was highly significant in both periods. AT-TP-VE interaction was only significant in the second period. The time of exposure main effect was not significant and positive correlations were recorded between RBCs, Hb and Hct in the two periods (r RBCs= 0.948, r Hb and r Hct= 0.763).

The normal value of mean corpuscular value, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of *Clarias gariepinus* in the two periods are given in Tables 2 and 3. The atrazine main effect was highly significant ($P<0.0001$) in the two periods for MCHC while MCV and MCH showed significant effects only in the second period. The tomato paste main effect was significant for MCH, MCV and MCHC in the second period only. Vitamin E had no significant effect at the two periods except for MCHC. TP-VE interaction effect was significant for MCH, MCV and MCHC in the second period while MCHC showed significant effect in the first period only. AT-TP-VE interaction didn't show significant effect for the three parameters. The time of exposure main effect was not significant and positive correlations were recorded between MCV, MCH and MCHC in the two periods (r MCV= 0.544, r MCH= 0.517 and r MCHC= 0.689). Supplementation of tomato paste and/or vitamin E improved the haematological parameters of AT- exposed fishes (Tables 2 and 3).

B- Biochemical parameters

Glucose level

The normal glucose level of *Clarias gariepinus* in the two periods is given in Tables 2and3. Atrazine main effect was significantly increased in both periods ($P< 0.0001$). The main effect of tomato paste and/or vitamin E in the two periods was highly significant ($P<0.0001$). AT-TP-VE interaction showed significant effect in the two periods. The time of exposure main effect was significant ($P<0.0001$) and a positive correlation was obtained between glucose levels in the two periods ($r =0.526$). Dietary supplementation with vitamin E and/or tomato paste improved AT-induced hyperglycemia in comparison with control.

Total protein level

The total protein level of *Clarias gariepinus* in the two periods is given in Tables 2and3. Atrazine reflected high significant decrease in total protein level at both periods ($P<0.0001$). The main effect of tomato paste or vitamin E in the two periods was not significant. Highly significant interaction effect between AT- TP- VE was recorded in the second period only. The time of exposure main effect was highly significant ($P<0.0001$) and a positive correlation was obtained between the total protein levels total protein in AT-exposed fish.

Table 4. Correlation matrix of blood constituent parameters of *Clarias gariepinus* subjected to different treatments of atrazine, tomato paste, vitamin E and their combinations for 15 days.

Parameters	RBCs	HB	HCT	MCV	MCH	MCHC	Glucose	TP	TL	LPO
RBCs	1.000									
HB	0.721**	1.000								
HCT	0.721**	1.000**	1.000							
MCV	-0.730**	-0.113	-0.113	1.000						
MCH	-0.711**	-0.086	-0.086	1.000**	1.000					
MCHC	0.725**	0.980**	0.980**	-0.127	-0.100	1.000				
Glucose	-0.629**	-0.577**	-0.577**	0.256	0.241	-0.630**	1.000			
TP	0.573**	0.656**	0.656**	-0.165	-0.156	0.685**	-0.357*	1.000		
TL	0.524**	0.591**	0.591**	-0.121	-0.104	0.635**	-0.620**	0.686**	1.000	
LPO	-0.435**	-0.421*	-0.421*	0.215	0.205	-0.391*	0.206	-0.277	-0.430**	1.000

* Significant at P<0.05
** Significant at P<0.01

Table 5. Correlation matrix of blood constituent parameters of *Clarias gariepinus* subjected to different treatments of atrazine, tomato paste, vitamin E and their combinations for 30 days.

Parameters	RBCs	HB	HCT	MCV	MCH	MCHC	Glucose	TP	TL	LPO
RBCs	1.000									
HB	0.789**	1.000								
HCT	0.789**	1.000**	1.000							
MCV	-0.758**	-0.254	-0.254	1.000						
MCH	-0.746**	-0.235	-0.235	1.000**	1.000					
MCHC	0.780**	0.972**	0.972**	-0.257	-0.238	1.000				
Glucose	-0.608**	-0.586**	-0.586**	0.399*	0.386*	-0.681**	1.000			
TP	0.827**	0.707**	0.707**	-0.538**	-0.527**	0.701**	-0.646**	1.000		
TL	0.566**	0.627**	0.627**	-0.240	-0.230	0.674**	-0.495**	0.681**	1.000	
LPO	-0.485**	-0.320	-0.320	0.362*	0.358*	-0.320	0.125	-0.359*	0.575**	1.000

* Significant at P<0.05
** Significant at P<0.01

in the two periods (r =0.608). In the present study, diet supplementation with tomato paste and/or vitamin E for 15 and 30 days showed a marked elevation in the level of

Total lipids level

The total lipids level of *Clarias gariepinus* in the two periods is given in Tables 2and3. Atrazine reflected high significant decrease in total lipids level (P<0.0001) in the two periods of exposure. The main effect of tomato paste in the two periods was significant (P<0.0001). Vitamin E and its interaction with tomato paste didn't show significant effect in the two periods. Highly significant interaction effect between AT-TP and AT-VE was recorded in the two periods. The time of exposure main effect was highly significant (P<0.0001) and a positive correlation was obtained between the total lipids levels in the two periods (r =0.387). Dietary supplementation of tomato paste and vitamin E corrected markedly the level of total lipids in comparison of that of control and AT-treated group (P<0.0001) especially in the second period.

Lipid peroxidation measurement

The liver lipid peroxidation (LPO) values are given in Tables 2 and 3. The main effects of AT, TP, VE and their interactions were highly significant (P<0.0001) in the two periods except for AT-VE interaction in the second period. The time of exposure main effect was highly significant (P<0.0001) and a positive correlation was recorded between LPO in the two periods (r =0.17).The values of lipid peroxidation were significantly (P< 0.0001) decreased in liver of AT-exposed fishes fed diets supplemented with vitamin E and/or tomato paste especially in the first period.

DISCUSSION

In the present study, Clarias gariepinus exhibited some

behavioral changes due to exposure to sublethal concentrations of atrazine. Similar behavioral changes were recorded by El-Banhawy et al. (1986); Abdel-Rahman (1997); Wassif et al. (2000); Hussein and Mekkawy (2001); Saha and Kaviraj (2003); Viran et al. (2003); Calta and Ural (2004); Köprücü et al. (2006), Sobha et al. (2007) and Mekkawy et al. (2011) in fishes exposed to pesticides and heavy metals. Such atrazine-induced behavioral changes were counteracted by dietary supplementation with tomato paste and/or vitamin E; a result confirmed by Mekkawy et al. (2011) for cadmium induced behavioral changes of *O. niloticus*. Moreover, the control fishes of *C. gariepinus* and *O. niloticus* treated with vitamin E and/or tomato paste were noticed in better conditions.

The atrazine-induced decrease in RBCs, Hb and Hct of *Clarias gariepinus* recorded in the present study is in agreement with those of Mekkawy et al. (1996) working on O. niloticus and Chrysichthys auratus exposed to atrazine, Koprücü et al. (2006) working on Silurus glanis exposed to diazinon and Siang et al. (2007) working on Monopterus albus exposed to endosulfan. The reduction of these parameters at sublethal levels of these pesticides might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemsynthesis that is affected by these pollutants (Khadre, 1988; Wintrobe, 1978). Also, this reduction may be indirectly attributed to pesticide-induced decrease in growth and other food utilization parameters which results in severe anemia (James and Sampath, 1999). Heath (1987) and Abo-Hegab et al. (1993) interpreted stress-induced decrease in the haemoglobin and haematocrit values in terms of heam dilution of blood and elimination of RBCs as well as disequilibrium of the osmotic pressure inside and outside the blood cell.

The atrazine-induced changes towards increase or decrease in MCV, MCH and MCHC values were reported in the present work. Similar findings were recorded by (Mekkawy et al., 1996) working on Oreochromis niloticus and *Chrysichthyes auratus* and (Köprücü et al., 2006) working on Silurus glanis after exposure to the pesticides, atrazine and diazinon respectively.

Adeyemo (2007) found significant increase in MCV, MCH and MCHC values of Clarias gariepinus exposed to lead while Siang et al. (2007) working on Monopterus albus exposed to endosulfan found decrease in MCH and MCHC and increase in MCV values.

These chemicals-induced alterations in MCV, MCH and MCHC were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in hemoglobin synthesis, stress-related release of RBCs from the spleen and hypoxia (Marie et al., 1998; Shah, 2006). Except for those of Mekkawy et al.(2012) on O. niloticus, no literature was in hand with regards to lycopene and tomato paste benefits for fish stressed haematological parameters. However, many studies were in concern with the beneficial supplementation of vitamin E to counteract the pesticide sublethal effects on fish haematology (Andrade et al., 2007; Montero et al., 2001) confirming the present results.In the present study, atrazine-induced hyperglycemia was revealed in *Clarias gariepinus*. Similar findings were observed by Mekkawy et al. (1996); Elowa and El-Elaimy (2001); Fayed et al. (2001); Das and Mukherjee (2003) and Fouda (2004) after exposure to different doses of pesticides. The source of such hyperglycemia seems to be due to the liver glycogenolysis, resulting from the increased plasma catecholamines and corticosteroid hormones (Mazeaud et al., 1977; Pickering, 1981) as well as amino acids through the activation of gluconeogenesis process (Abo-Hegab and Hanke, 1984). Tomato paste and/or vitamin E reduced atrazine-induced hyperglycemia revealed in Clarias gariepinus. Similar results were recorded by Mekkawy et al. (2011) for O. niloticus stressed by cadmium. Martins et al. (2007) reported a reduced blood glucose level after stress in Atlantic halibut (*Hippoglossus hippoglossus* L.) fed vitamin E supplemented diets.

In the present study, decrease in serum total protein level (hypoproteinemia) was recorded in atrazine-exposed C. gariepinus. Similar findings were recorded by Assem et al. (1992), Mekkawy et al. (1996; 2011), Das and Mukherjee (2003), Fouda (2004), Gad (2005) and Shalaby (2007) after exposure to different doses of pesticides and heavy metals. Such hypoproteinemia may be due to direct effect of the utilization of body protein as an energy supply to meet the increasing physiological demands to overcome the stress in the polluted medium (Abd El-Salam et al., 1994; El-Sayed et al., 1996; Fontana et al., 1998) hypoproteinemia may also be attributed to several pathological processes including plasma dissolution, renal damage and elimination in the urine, decreased liver protein synthesis, alteration in hepatic blood flow and / or hemorrhage into the peritoneal cavity and intestine (Keith and Weber, 1979). The present results and those of Mekkawy et al. (2011) confirm the validity of tomato paste and/or vitamin E in counteracting stress-induced hypoproteinemia. Elkomy and Hassan (2005) observed an increase in the stressed total protein level in male rat fed Tomato-juice as supplemented diets. Kalender et al. (2005) and Ogur et al. (2005) recorded an increase in total protein level after Diazinon-induced and Nitrate-induced stress in male rats fed vitamin E as supplemented diets.

In the present study, decrease in serum total lipids level was recorded in atrazine-treated *C. gariepinus*. Similar findings were observed by Mekkawy et al. (1996; 2011); Fayed et al. (2001) and Fouda (2004) after exposure to different doses of pesticides and heavy metals. Such induced decrease in serum total lipids level may be due to the increase in secretion of catecholamines and corticosteroids with enhanced metabolic rate and in turn reduced metabolic reserves (Fayed et al., 2001). Tomato paste and/or E were found

to be valid in counteracting stress-induced changes in total lipid level in the present work and that of Mekkawy et al. (2011). Yilmaz et al. (1997) found that vitamin E as a diet supplementation improved total lipids level in muscle and liver tissues of rats.

The present results have clearly demonstrated the ability of atrazine sublethal doses to induce oxidative stress in fish liver as evidenced by increased thiobarbituric acid reactive substance after atrazine-exposure. The rise in lipid peroxidation level in liver means a modification in the physical characteristics of cell membrane (Ursini et al., 1991) since lipid peroxidation leads to hydrolysis of phospholipids into hydroperoxy fatty acids (Salgo et al., 1993). The atrazine-induced oxidative stress in tissues by increasing lipid peroxidation and by altering the antioxidant status was postulated by many authors (Geret et al., 2002; Mekkawy et al., 2011; 2012; Nwani et al., 2010; Romeo et al., 2000; Romeo and Gnassia-Baralli, 1997; Sarkar et al., 1997).

In the present study, the level of lipid peroxidation was decreased in liver of atrazine-exposed fishes fed diets supplemented with vitamin E and/or tomato paste especially in the first period. Similar results were recorded by Mekkawy *et al.*(2011) in concern with cadmium-exposed *O. niloticus*. The benefits of vitamin E in elimination of stress-induced lipid peroxidation were recorded for different fish species including *Clarias gariepinus* (Baker and Davies, 1996; 1997), *Salmo salar* (Scaife et al., 2000), *Salmo gairdneri* (Chaiyapechara et al., 2003; Frigg et al., 1990; Hung and Slinger, 1982), hybrid tilapia, *Oreochromis niloticus x O. aureus* (Huang et al., 2003; 2004) and red hybrid tilapia, *Oreochromis* sp. (Wang et al., 2006). In rats, tomato and its products including lycopene were found by many authors to improve stress-induced lipid peroxidation (Atessahin et al., 2006; Bhuvaneswaria et al., 2001; El-Demerdash et al., 2004; Elkomy and Hassan, 2005; Moreira et al., 2005; Velmurugan et al., 2001; 2002; Yilmaz et al., 2006).

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