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Prophylactic Role of Curcumin and Garlic Acid on Oxyfluorfen Toxicity of *Oreochromis niloticus*: Hematological and Biochemical Responses

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ABSTRACT

The present work aimed to detect the toxicological impacts of oxyfluorfen (herbicide) on hematological and biochemical parameters of *Oreochromis niloticus* and the prophylactic role of garlic acid and curcumin in detoxification. Adult fish were exposed to two sublethal concentrations (0.3 and 0.6ppm) of oxyfluorfen against 5g/ kg of curcumin and 5g/ kg of garlic acid for detoxification role for 2 and 4 weeks. Erythrocyte count (RBCs), hemoglobin content (Hb), hematocrit value (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and leucocytes count (WBCs) as hematological markers were measured. Biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) enzyme activities, serum protein (total protein, albumin and globulin) concentration, urea, creatinine, sodium (Na), potassium (K), glucose, cholesterol (Cho) and triglyceride (Tg). The present investigation showed that oxyfluorfen in different doses led to a significant reduction ($P< 0.05$) in RBCs, Hb, Hct, MCV, MCHC, PLT, WBCS, neutrophils (Neut), monocytes (Mono), serum Cho and Tg. However, compared to the control group, MCHC, lymphocytes (Lymp), AST, ALT, ALP enzyme activities, serum proteins (total protein, albumin and globulin), urea, creatinine, glucose, Na and K showed a significant increase ($P< 0.05$). Garlic acid and curcumin played an optimistic role in the detoxification of oxyfluorfen toxicity. The findings implied that oxyfluorfen had a deleterious impact on fish hematological and biochemical markers. In addition, curcumin and garlic demonstrated an improvement in hematological and biochemical markers regarding the removal of oxyfluorfen toxicity.

INTRODUCTION

The development of industrial, anthropogenic, and agricultural activities is the main factor leading to the increase in contaminants in aquatic ecosystems **(Ibrahim, 2015)**. Thus, its toxicological effects can deteriorate water quality causing a negative impact on human health. Pesticides, which comprise a huge group of harmful compounds frequently employed for pest management are one among many sources of pollutants **(Ibrahim & Banaee, 2014; Ibrahim & Harabawy, 2014)**. Their use is progressively

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declining in most industrialized countries although herbicides make up about 40% of the production of pesticides in the world **(Peixoto *et al*., 2006)**. The contaminants can uptake in fish from water, food, sediments and suspended particulate material, causing deleterious effects on fish health **(Ali *et al*., 2019)**. Oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4 (triXuoromethyl) benzene] is a diphenyl ether herbicide commonly used in agriculture to control broadleaf and grassy weeds with specific recommendations **(Ware & Whitacre, 2004)**. However, run-off surface water may transfer dissolved or soil adsorbed oxyfluorfen from agricultural areas to aquatic environments. The herbicide's effect on algae and plants are well known **(Watanabe *et al*., 2001; Geoffroy *et al*., 2003)**, but data on its impact on biochemical parameters in fish are still scarce **(Hassanein *et al*., 1999; Hassanein, 2002)**. Fish exposed to a variety of chemical agents may experience changes in several hematological and biochemical indicators that are commonly used to assess fish health **(Martnez & Souza, 2002)**. Hematology has always been used to detect physiological changes in response to a variety of stressors. As a result, the most useful application for determining the sublethal effects of contaminants is to use hematological techniques **(Ibrahim, 2015b)**. Fish have long been employed as models for assessing aquatic ecosystems health and in toxicologic disease **(Law, 2003)**. Oxyfluorfen result in stress and immunosuppression, leading to higher mortality and substantial economic losses. Antibiotics and chemotherapy have been used successfully to decrease the negative effects of demanding circumstances on aquatic animal performance **(Chen *et al*., 2020)**. Antibiotic use, on the other hand, has produced a number of detrimental repercussions **(Adel & Dawood, 2021; EU, 2021)**. Antibiotic derivatives have indeed resulted in weakened natural immunity in aquatic animals, as well as the spread of antibiotic-resistant bacterial strains (**Perry *et al*., 2020)**. The human body can be exposed to antibiotics inadvertently and subsequently be at risk (**Leung *et al*., 2020)**. Nutraceuticals are also being proposed as a viable alternative for long-term fish rearing **(Dawood *et al*., 2021; Mehrinakhi *et al*., 2021; Yeganeh *et al*., 2021)**.

The majority of studies show that medicinal plants can be utilized to treat a variety of human diseases in traditional, complementary and alternative ways **(Pratibha & Paul, 2020)**. Garlic is a plant of the Liliaceae family that has been used as a spice, traditional medicine, and functional food to improve physical and mental health for thousands of years **(Saleh *et al*., 2015a; Labrador *et al*., 2016a)**. Potassium, calcium, magnesium, phosphorus, ferrum, manganese, selenium, vanadium, copper and zinc are the minerals found in garlic (**Polyakov *et al*., 2020)**. It is also high in phosphorus, carbs, and calcium, and has a high nutritional value overall **(Saghaei *et al*., 2015; Saleh *et al*., 2015; Labrador *et al*., 2016)**. Garlic also includes a number of essential components, such as silicates, iodine and sulfur salts, which have beneficial effects on the skeletal and circulatory system, cholesterol and the prevention liver diseases **(Labrador *et al*., 2016)**. Garlic is renowned for its antibacterial, anti-carcinogenic, antifungal, and anti-stress characteristics, as well as its role in boosting nutritional indices, immunological and

growth stimulants, antioxidants, and blood pressure regulation **(Kumar and Berwal, 1998; Fazlolahzadeh *et al*., 2011)**. Garlic has been used to control pathogenic bacteria and fungi in animals including fish **(Corzo-Martínez *et al*., 2007)**.

Turmeric (Curcuma longa) is a tropical perennial herb that grows to a height of three to five feet. It is widely cultivated in Asia and other tropical nations. Curcumin is the spice's active component. Turmeric, also known as curcumin, is a zingiberaceae family medicinal plant. Turmeric rhizomes contain of curcumin, a yellowish coloring substance. Curcumin has been demonstrated to have a number of health-related benefits, including hepatoprotective characteristics **(Pal *et al*., 2001)**, anti-inflammatory, immunomodulating, tumor-preventing **(Miquel *et al*., 2002)** and antibacterial activity **(Singh *et al*., 2002)**. Turmeric is used for wound healing, inflammation and acidity **(Jyothi, 2003; Kumar *et al*., 2006)**. Turmeric is potent antioxidant **(El-Bahr *et al*., 2007; Salama and El-Bahr, 2007)**. Turmeric extract supplementation in aquaculture feed is an interesting approach to disease control by strengthening the immune system in a variety of fish, including rohu **Swagatika (2008)**, goldfish **(Harikrishnan and Balasundaram, 2008; Harikrishnan *et al*., 2009)** and marine shrimp such as Pacific white shrimp **(Vanichkul *et al*., 2010)**. To help the fish deal with harsh environmental circumstances, a synthetic curcumin analogue (salicyl curcumin) supplements with the aquaculture feed would be useful. This would increase the survival rate, disease resistance and ultimately the growth rate.

Fish that exposed to different toxins types caused many hematological and biochemical parameters changes, that could be used to estimate fish health by **Ibrahim (2015)**. (**Ibrahim, 2015; Harabawy and Ibrahim, 2014)** employed hematology to detect physiological changes in response to various stressors. As a result, the most frequent methods for determining the sublethal impacts of contaminants are hematological and biochemical parameters techniques **(Harabawy and Ibrahim, 2014; Ibrahim and Banaee, 2014)**. One of the widely distributed freshwater fish is Nile tilapia, *Oreochromis niloticus* **(Ibrahim and Banaee, 2014; Ibrahim, 2015)** that may survive in a polluted environment and be used as a bio-indicator for aquatic environmental contaminants by **Ibrahim (2015)**.

As a result, the goal of this study was to see how two sublethal dosages of oxyfluorfen affect the blood hematology and biochemistry of *Oreochromis niloticus* over two and four weeks. Also, it aimed to determine the garlic and curcumin role in improving oxyfluorfen toxicity.

MATERIALS AND METHODS

1. Chemicals

The diphenyl ether herbicide oxyfluorfen (oxyfluorfen-2-chloro-1-(3ethoxy-4-nitrophenoxy)- 4- fluoromethyl) benzene) (trade name: Goal) a product of Rohm and Haas Company, Italy, was used as commercial material of a concentration of 24%. The used concentrations was prepared by dilution with water on the basis of the LC50 (0.3 mg/l and 0.6 mg/l for *O. niloticus*) according to (**Hassanein *et al.*, 1999)**.

Garlic from local market and curcumin from (El-Gomhouria chemical company, Egypt) was added to the basal diet at concentration (5 and 5 g/kg diet). Garlic and curcumin were blended in maize oil before being combined with a 30 percent protein basal diet (B.D).

2. Sample collection and chemicals

Fifty-four healthy fish *Oreochromis niloticus* (101.5±14.5 g weight, 18.5±2.0 cm length), were caught from the fish fram at New vally (El-kharga). Fish were promptly moved to New Valley University's Science faculty's fish laboratory. Fish were acclimatized for two weeks in aerated glass tanks (100 L capacity) before being utilized in the experiment. The experimental fish were fed pellets twice a day at a rate of 3% of their body weight. Regular aspirate for Feces and residual food were done. The water temperature, pH and dissolved oxygen concentrations (DO) were measured daily (24.3±1.4 C, 7.1±0.2 pH and6.4±1.03 mg/1 DO). Light cycle was 12 h light and 12 h dark.

3. Experimental design

Fishes will be weight, measure and classify randomly into 9 groups (6 fish /tank) according to dose of oxyfluorfen, garlic acid and curcumin and their combinations **(Table, 1)**. The diets (maize and soybeans, 5 g/kg/fish) will be pelleted after addition of curcumin and garlic acid dose for the treated groups and the addition of suitable amounts of molasses and water. The diets will be left to dry at room temperature and store in small bags for fish feeding. Stock solution (1,000 ppm) of oxyfluorfen will be prepared and stored in clean glass bottles and diluted to concentrations of 0.3 and 0.6 mg/l (as 1/10 and 1/5 of LC_{50}).

Oxyfluorfen doses will be prepared and added constantly to the aquarium for 4 weeks. The test water will be replaced daily with the require amount of stock solution to prevent deterioration of water quality and replenish oxyfluorfen levels.

Table 1. The fish groups exposed to oxyfluorfen, curcumin, garlic acid and their combination.

Group / Treatment	C	Oxy_1	Oxy_1 +Cur	Oxy_1 + Ga	Oxy_1 + Cur+ Ga	Oxy_2	Oxy2 + Cur	Oxy2 + Ga	Oxy2 + Cur+ Ga
Oxyfluorfen (mg/L)	0	0.3	0.3	0.3	0.3	0.6	0.6	0.6	0.6
Curcumin (g/kg)	0	0	5	0	5	0	5	0	5
Garlic acid (g/kg)	0	0	0	5	5	0	0	5	5

C: Control, Cur: Curcumin, Ga: Garlic Acid, Oxy1; low oxyfluorfen dose and Oxy2: high oxyfluorfen

4. Hematology

After 2- and 4- weeks exposure, blood samples (6 fish/treatment) of the control and treated fish were collected from the caudal vein of fish in small plastic tubes containing heparin solution (0.2 ml/ml blood) as an anticoagulant. Using an automated technical analyzer, the RBCs, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb) concentration were determined (Mindray Bc-2800).

5. Biochemistry

Fresh serum was prepared after coagulating blood for 15–20 min at 4 °C, then centrifugated for 20 min at 3000 rpm. This serum was used for liver enzymes activity (alanine aminotransferase (ALT, U/I), aspartate aminotransferase (AST, U/I), and Alkaline phosphatase (ALP, U/I)) detection kinetically and Serum urea (mg/l), creatinine (mg/l), glucose (mg/l), cholesterol (mg/l), triglyceride (mg/l), sodium (mg/1) and potassium (mg/1) colorimetry using Spectrum Diagnostics kits. Also, Diamond Diagnostics, Egypt, provided assay kits to evaluate total protein, albumin, and globulin (g/100 ml) concentration. A spectrophotometer was used to measure the samples (Jasco-V530).

6. Statistical analysis

For stated results as the mean ± standard Error, the SPSS 16 computer program (SPSS) was utilized. Analyzing data was carried out for statistical significance between the control and experimental groups with an analysis of variance (one-way ANOVA). P-Values<0.05 were considered statistically significant.

RESULTS

The values of hematological parameters of oxyfluorfen (Oxy), garlic acid (Ga) and curcumin (Cur) treated groups of *O. niloticus* after two and four weeks of exposure are given in **Tables (2 & 3)**. Oxy groups showed a significant decrease ($p<0.05$) in red blood cells (RBCs), hemoglobin concentration (Hb) and Hematocrit percentage (Hct) after both periods of exposure. Curcumin addition to these groups improved RBCs, Hb, and Hct to normal values after both periods of exposure. Such decrease was significantly ($P<0.05$) dose and time dependence, mean corpuscular value (MCV) and mean corpuscular hemoglobin concentration (MCHC) showed a significant decrease ($p<0.05$) with the increase in Oxy doses and time of exposure. However, Ga, Cur+ Ga and Cur addition improves such decrease nearly to the control value ($P>0.05$) after both periods of exposure. The mean corpuscular hemoglobin (MCH) showed a significant increase ($p<0.05$) with the increase in Oxy doses and time of exposure. Cur, Ga addition improves these increase nearly to the control value ($P>0.05$) in both periods of exposure.

Table (3) showed the number of white blood cells (WBCs) and their differential counts, as well as the percentage of lymphocytes (Lymp), monocytes (Mono), neutrophils (Neut), and eosinophils (Eos) in *O. niloticus* after both periods of exposure. WBCs, Neut and Mono showed a significant decrease ($P<0.05$) in Oxy exposed groups. Curcumin and Garlic addition showed an improvement of WBCs toward control values ($P>0.05$) in the low Oxy group only after both periods of exposure. Curcumin and Garlic addition showed an improvement of Neut. to control values ($P>0.05$) in Oxy groups after both periods of exposure. Cur + Ga addition showed an improvement of MONO to control values ($P>0.05$) in the low Oxy group only in both periods of exposure. lymphocytes (Lymp) showed a significant increase ($P<0.05$) in Oxy exposed groups. Ga and Cur+ Ga addition showed an improvement to control values ($P>0.05$) in the low and high Oxy group after 2 weeks of exposure. However, Cur addition showed an improvement for Lymp percentage ($P>0.05$) in the low and high Oxy exposed group after both periods of exposure.

Liver enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALK) activity of *O. niloticus* exposed to oxyfluorfen, garlic acid and curcumin results are presented in **Table (4)**. These enzymes showed a significant elevation ($P<0.05$) in fish that exposed to oxyfluorfen, such significant elevation increase with the increase of oxyfluorfen doses and time of exposure. However, Cur, Cur + Ga addition normalized these enzymes to control level ($P>0.05$) in the low and high Oxy exposed group after both periods of exposure.

Total protein (TP), Albumin and globulin contents of *O. niloticus* that exposed to oxyfluorfen, garlic acid and curcumin for 2 and 4 weeks are present in **Table (4)**. Oxyfluorfen exposed groups showed significant increase ($P<0.05$) in TP, albumin and

globulin. This significant increase was dose and time dependence. However, garlic addition showed a normalization of TP and albumin to control value in the low Oxy group in both periods of exposure. Cur addition showed a normalization of TP and albumin to control value high Oxy exposed group after both periods of exposure. Globulin showed a significant increase ($P<0.05$) in fish that exposed to oxyfluorfen. However, Ga and Cur addition normalized globulin to control values in the low and high Oxy exposed group after 2 weeks of exposure.

The concentration of urea, creatinine, Na and K as kidney function parameters are present in **Table (5)**. These parameters showed a significant increase ($P<0.05$) with the increase of oxyfluorfen doses and time of exposure. However, Cur addition normalized urea and creatinine level to control level ($P>0.05$) in the low Oxy exposed group in both periods of exposure. Also, Cur+ Ga addition normalized urea and creatinine levels only for high oxyfluorfen dose in both periods of exposure. Also, Cur and Cur+ Ga addition normalized Na and K levels only for low oxyfluorfen dose after 2 weeks of exposure.

Glucose, cholesterol (Cho) and Triglyceride (Tg) parameters are present in **Table (5)**. These parameters showed a significant decrease ($P<0.05$) in oxyfluorfen exposed groups. Cur and Cur+ Ga addition normalized Cho and Tg levels only for low oxyfluorfen dose in both periods of exposure. Also, Cur and Cur+ Ga addition normalized Glucose to control vales only for low oxyfluorfen dose after 2 weeks of exposure. However, Ga addition normalized glucose level only for high oxyfluorfen dose after 4 weeks of exposure.

Table 2. The basic data of blood constituent parameters of O. niloticus exposed to Oxyfluorfen(Oxy), garlic acid(Ga) and curcumin(Cur) for 2 and 4 weeks (N = 6).

G	Treat. Period	Hb(g/dl)	RBCS ($X10^6$/iL)	HCT(%)	MCV	MCH(Pg)	MCHC (g/dL)	PLT
Control	2 Weeks	6.72 ± 0.08 ^{A}	1.61 ± 0.03 ^{A}	21.15 ± 0.23 ^{A}	131.37 ± 2.91 ^{DE}	31.76 ± 0.02 ^{E}	41.72 ± 0.93 ^{CD}	374.73± 8.14^{C}
	4 Weeks	6.79± 0.07 ^{A}	1.60± 0.03 ^{A}	21.36± 0.21 ^{A}	133.66± 2.14 ^{C}	31.77± 0.02 ^{E}	42.47± 0.68 ^{BC}	385.75± 4.51 ^{A}
Oxy1	2 Weeks	5.65 ± 0.08 ^{C}	1.45 ± 0.02 ^{B}	15.95 ± 0.24 ^{E}	110.43 ± 1.96 ^{F}	35.42 ± 0.03 ^{C}	39.12 ±0.67 ^{E}	346.75± 6.79^{D}
	4 Weeks	5.15± 0.05 ^{E}	1.30± 0.01 ^{C}	14.45± 0.15 ^{E}	111.62± 1.37 ^{E}	35.64± 0.02 ^{C}	39.78± 0.47 ^{DE}	212.00± 5.32 ^{E}
Oxy1+Cur	2 Weeks	6.83 ± 0.07 ^{A}	1.58± 0.01 ^{A}	21.48± 0.20 ^{A}	136.20± 0.87 ^{CD}	31.78± 0.01 ^{E}	43.29± 0.29 ^{C}	440.73± 5.84^{A}
	4 Weeks	5.43± 0.05 ^{C}	1.44± 0.02 ^{B}	17.30± 0.14 ^{B}	120.24± 2.20 ^{D}	31.41± 0.02 ^{FG}	37.76± 0.71 ^{E}	328.27± 5.99 ^{BC}
Oxy1+Ga	2 Weeks	5.37± 0.02 ^{D}	1.37± 0.01 ^{C}	17.10± 0.05 ^{CD}	125.16± 1.01 ^{E}	31.38± 0.01 ^{G}	39.28± 0.32 ^{E}	413.00± 3.42^{B}
	4 Weeks	5.50± 0.03 ^{C}	1.33± 0.01 ^{C}	7.50± 0.09 ^{B}١	131.96± 1.65 ^{C}	31.43± 0.01 ^{F}	41.47± 0.53 ^{CD}	322.73± 4.94 ^{BC}
Oxy1+Cur+ Ga	2 Weeks	6.13± 0.02 ^{B}	1.24± 0.00 ^{D}	17.38± 0.06 ^{C}	140.56± 1.04 ^{BC}	35.25± 0.01 ^{D}	49.55± 0.36 ^{A}	252.73± 6.58 ^{F}
	4 Weeks	5.81± 0.02 ^{B}	1.13± 0.01 ^{D}	16.43± 0.06 ^{D}	145.85± 1.17 ^{B}	35.36± 0.01 ^{D}	51.58± 0.41 ^{A}	316.27± 1.74 ^{C}
Oxy2	2 Weeks	4.18± 0.05 ^{F}	1.06± 0.01 ^{E}	11.54± 0.15 ^{G}	109.20± 2.07 ^{F}	36.22± 0.04 ^{A}	39.55± 0.72 ^{DE}	315.25± 7.76 ^{E}
	4 Weeks	3.06± 0.02 ^{G}	0.69± 0.03 ^{G}	8.19± 0.07 ^{G}	119.57± 4.90 ^{D}	37.40± 0.04 ^{A}	44.73± 1.86 ^{B}	170.60± 4.77 ^{F}
Oxy2+Cur	2 Weeks	5.23± 0.04 ^{D}	1.07± 0.02 ^{E}	16.68± 0.11 ^{D}	156.39± 2.92 ^{A}	31.33± 0.01 ^{G}	49.00± 0.92 ^{A}	343.75± 5.27^{D}
	4 Weeks	5.10± 0.01 ^{E}	1.00± 0.01 ^{E}	16.29± 0.03 ^{D}	163.68± 0.56 ^{A}	31.29± 0.00 ^{H}	51.21± 0.17 ^{A}	394.50±5.12 ^{A}
Oxy2+Ga	2 Weeks	5.66± 0.08 ^{C}	1.23± 0.02 ^{D}	17.98± 0.23 ^{B}	145.82± 1.36 ^{B}	31.48± 0.02 ^{F}	45.90± 0.44 ^{B}	341.00± 3.65^{D}
	4 Weeks	5.30± 0.03 ^{D}	1.05± 0.02 ^{E}	16.90± 0.09 ^{C}	161.68± 3.34 ^{A}	31.36± 0.01 ^{G}	50.71± 1.06 ^{A}	332.73± 4.76 ^{B}
Oxy2+Cur+ Ga	2 Weeks	4.47± 0.05 ^{E}	0.88± 0.02 ^{F}	12.41± 0.15 ^{F}	140.91± 4.25 ^{BC}	36.02± 0.03 ^{B}	50.76± 1.52 ^{A}	332.73± 2.37^{D}
	4 Weeks	4.42± 0.05 ^{F}	0.83± 0.01 ^{F}	12.26± 0.14 ^{F}	147.12± 0.09 ^{B}	36.05± 0.03 ^{B}	53.04± 0.03 ^{A}	253.25± 2.89 ^{D}

The data are presented as Means±S.E.

Oxy1and 2: low and high Oxyfluorfen dose (0.3 and 0.6 ppm), Cur: curcumin (5 g/kg) and Ga: garlic acid (5 g/kg).

Different letters indicate significant difference at $p<0.05$.d

blood cells (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC).

Table 3. The basic data of blood constituent parameters of *Oreochromis niloticus* exposed to Oxyfluorfen(Oxy), garlic**.** acid(Ga) and curcumin(Cur) for 2 and 4 weeks (N = 6).

G	Treat Period	WBCS(x10^3)	Neutro(%)	Lymph(%)	Mono(%)
Control	2 Weeks	25.23 ± 0.78 ^{A}	56.39 ± 0.73 ^{AB}	39.28 ± 1.27 ^{C}	4.50 ± 0.03 ^{A}
	4 Weeks	24.05± 0.47 ^{A}	54.85± 0.20 ^{BC}	42.37±0.20 ^{ABC}	4.40± 0.11 ^{A}
Oxy1	2 Weeks	15.50 ± 0.31 ^{BC}	54.00 ± 0.32 ^{D}	41.63 ± 0.49 ^{A}	3.75 ± 0.02 ^{B}
	4 Weeks	11.79± 0.15 ^{C}	54.70± 0.16 ^{C}	43.10± 0.29 ^{AB}	3.48± 0.04 ^{C}
Oxy1+Cur	2 Weeks	14.96 ± 0.23 ^{CD}	55.80 ± 0.26 ^{B}	41.40 ± 0.35 ^{AB}	2.80 ± 0.11 ^{D}
	4 Weeks	13.03± 0.07 ^{B}	55.97± 0.42 ^{B}	40.83± 1.05 ^{CD}	2.50± 0.03 ^{E}
Oxy1+Ga	2 Weeks	16.27 ± 0.26 ^{B}	55.28 ± 0.23 ^{BC}	41.00 ± 0.74 ^{AB}	2.27 ± 0.04 ^{E}
	4 Weeks	12.57± 0.07 ^{B}	56.00± 0.14 ^{B}	40.70± 0.32 ^{D}	2.93± 0.04 ^{D}
Oxy1+Cur+Ga	2 Weeks	12.27 ± 0.03 ^{E}	57.70 ± 0.27 ^{A}	36.73 ± 0.30 ^{D}	4.60 ± 0.03 ^{A}
	4 Weeks	11.69± 0.06 ^{C}	53.37± 0.70 ^{D}	43.42± 0.45 ^{A}	4.23± 0.08 ^{A}
Oxy2	2 Weeks	10.20 ± 0.08 ^{G}	53.85±0.12 ^{D}	41.85 ± 0.96 ^{A}	3.30±0.24 ^{C}
	4 Weeks	8.28± 0.06 ^{F}	54.25± 0.47 ^{CD}	41.12± 0.74 ^{CD}	2.58± 0.15 ^{E}
Oxy2+Cur	2 Weeks	11.24 ± 0.10 ^{F}	57.33± 0.92 ^{A}	34.20± 0.80 ^{E}	2.53± 0.10 ^{DE}
	4 Weeks	9.55± 0.08 ^{E}	54.93± 0.37 ^{BC}	38.67± 0.21 ^{E}	4.38± 0.10 ^{A}
Oxy2+Ga	2 Weeks	14.13± 0.29 ^{D}	55.22± 0.24 ^{BC}	42.50± 0.47 ^{A}	2.73± 0.11 ^{D}
	4 Weeks	10.73± 0.16 ^{D}	57.27± 0.28 ^{A}	38.65± 0.38 ^{E}	3.97± 0.13 ^{B}
Oxy2+Cur+Ga	2 Weeks	10.87± 0.07 ^{FG}	55.10± 0.30 ^{BC}	42.53± 0.72 ^{A}	3.77± 0.05 ^{B}
	4 Weeks	9.63± 0.20 ^{E}	55.85± 0.15 ^{B}	41.77±0.13 ^{BCD}	2.40± 0.05 ^{E}

The data are presented as Means± Standard Error.

Oxy1and 2: low and high Oxyfluorfen dose (0.3 and 0.6 ppm), Cur: curcumin (5 g/kg) and Ga: garlic acid (5 g/kg). Different letters indicate significant difference at $p<0.05$.

White blood cells (WBC), Neutrophils (NEUT). Lymphocytes (LYM), and Monocytes (Mono).

Table 4. The basic data of blood constituent parameters of *Oreochromis niloticus* exposed to Oxyfluorfen(Oxy), garlic acid(Ga) and curcumin(Cur) for 2 and 4 weeks(N = 6).

G	Treat Period	AST(U/l)	ALT(U/l)	ALK(U/l)	T.PRO(g/dl)	Albumin (g/dl)	glob(g/dl)	albglo(g/dl)
Control	2 Weeks	57.07± 0.87 ^{G}	11.67± 0.25 ^{G}	41.20± 0.96 ^{G}	2.87± 0.04 ^{E}	0.77± 0.01 ^{E}	2.09± 0.04 ^{E}	0.37± 0.01 ^{C}
	4 Weeks	56.50± 1.02 ^{G}	11.45± 0.25 ^{G}	42.95± 1.54 ^{G}	2.85± 0.05 ^{E}	0.78± 0.02 ^{F}	2.07± 0.07 ^{D}	0.38± 0.02 ^{C}
Oxy1	2 Weeks	87.00± 1.14 ^{D}	45.35± 0.95 ^{D}	61.98 ± 1.20 ^{B}	3.18 ± 0.04 ^{D}	1.08± 0.04 ^{C}	2.10± 0.00 ^{E}	0.51± 0.02 ^{A}
	4 Weeks	156.75± 3.22 ^{C}	64.25± 1.28 ^{D}	82.50± 0.81 ^{B}	4.28± 0.07 ^{C}	1.40± 0.03 ^{C}	2.88± 0.09 ^{C}	0.49± 0.03 ^{B}
Oxy1+Cur	2 Weeks	58.07± 0.86 ^{G}	22.67± 0.80 ^{F}	43.00± 0.84 ^{FG}	3.80± 0.09 ^{C}	0.90± 0.03 ^{D}	2.90± 0.06 ^{C}	0.31± 0.00 ^{D}
	4 Weeks	73.33± 0.48 ^{F}	34.00± 0.32 ^{F}	45.27± 1.14 ^{G}	3.83± 0.02 ^{D}	0.99± 0.03 ^{E}	2.84±0.05 ^{C}	0.35±0.02 ^{CD}
Oxy1+ Ga	2 Weeks	81.67± 0.97 ^{E}	57.00± 0.84 ^{C}	54.33± 0.48 ^{D}	3.04± 0.02 ^{DE}	0.81± 0.00 ^{E}	2.24± 0.02 ^{E}	0.36± 0.01 ^{C}
	4 Weeks	113.33± 2.42 ^{D}	70.00± 1.76 ^{C}	67.20± 3.45 ^{D}	3.83± 0.02 ^{D}	0.90± 0.00 ^{E}	2.93± 0.02 ^{C}	0.31± 0.00 ^{DE}
Oxy1+ Cur+Ga	2 Weeks	64.33± 1.28 ^{F}	25.33± 0.80 ^{F}	45.73± 1.68 ^{F}	3.87± 0.10 ^{C}	0.90± 0.00 ^{D}	2.96± 0.10 ^{C}	0.30± 0.01 ^{D}
	4 Weeks	73.33± 1.11 ^{F}	46.00± 0.55 ^{E}	53.33± 0.18 ^{F}	4.40± 0.05 ^{C}	1.00± 0.01 ^{E}	3.40± 0.06 ^{B}	0.29± 0.01 ^{E}
Oxy2	2 Weeks	137.75± 2.40 ^{A}	82.65± 2.41 ^{A}	72.18± 0.89 ^{A}	4.73± 0.06 ^{B}	1.57± 0.04 ^{A}	3.16± 0.03 ^{B}	0.50± 0.01 ^{AB}
	4 Weeks	220.75± 3.04 ^{A}	117.50±1.36 ^{A}	92.53± 0.50 ^{A}	5.10± 0.05 ^{B}	1.88± 0.02 ^{A}	3.23± 0.07 ^{B}	0.58± 0.02 ^{A}
Oxy2+ Cur	2 Weeks	80.00± 2.30 ^{E}	31.25± 0.86 ^{E}	57.44± 1.26 ^{C}	3.68± 0.09 ^{C}	0.95± 0.05 ^{D}	2.73± 0.08 ^{D}	0.35± 0.02 ^{C}
	4 Weeks	101.25± 1.56 ^{E}	48.75± 0.66 ^{E}	58.83± 2.78 ^{E}	3.95± 0.09 ^{D}	1.26± 0.04 ^{D}	2.70± 0.08 ^{C}	0.47± 0.02 ^{B}
Oxy2+Ga	2 Weeks	116.67± 1.59 ^{B}	73.33± 0.48 ^{B}	63.50± 0.50 ^{B}	4.60± 0.06 ^{B}	1.47± 0.02 ^{B}	3.13± 0.05 ^{B}	0.47± 0.00 ^{B}
	4 Weeks	181.67± 0.97 ^{B}	88.67± 0.97 ^{B}	73.77± 0.24 ^{C}	5.13± 0.12 ^{B}	1.73± 0.04 ^{B}	3.40± 0.08 ^{B}	0.51± 0.00 ^{B}
Oxy2+ Cur+Ga	2 Weeks	92.00± 0.32 ^{C}	45.00± 0.55 ^{D}	50.52± 0.32 ^{E}	5.43± 0.08 ^{A}	1.05± 0.02 ^{C}	4.38± 0.07 ^{A}	0.24± 0.00 ^{E}
	4 Weeks	98.00± 1.58 ^{E}	63.00± 0.55 ^{D}	62.67± 0.48 ^{DE}	5.83± 0.10 ^{A}	1.37± 0.07 ^{C}	4.47± 0.16 ^{A}	0.31± 0.03 ^{DE}

The data are presented as Means±S.E.

Oxy1and 2: low and high Oxyfluorfen dose (0.3 and 0.6 ppm), Cur: curcumin (5 g/kg) and Ga: garlic acid (5 g/kg).

Different letters indicate significant difference at $p<0.05$.

Aspartate Aminotransferase (AST), alanine Aminotransferase (ALT), , alkaline phosphates (ALK), Total Protein (TP), Albumin and Globulin.

Table 5. The basic data of blood constituent parameters of *Oreochromis niloticus* exposed to Oxyfluorfen(Oxy), garlic acid(Ga) and curcumin(Cur) for 2 and 4 weeks (N = 6).

G	Treat Period	Urea (mg/dl)	Creat (mg/dl)	Na (mEq/L)	K (mEq/L)	Chol (mg/dl)	Trig (mg/dl)	Glucose (mg/dl)
Control	2 Weeks	5.60± 0.06 ^{F}	0.24± 0.02 ^{F}	133.23± 0.86 ^{D}	4.50± 0.03 ^{E}	228.00± 1.38 ^{B}	170.00± 0.63 ^{A}	78.43± 1.15 ^{E}
	4 Weeks	5.68± 0.11 ^{G}	0.26± 0.02 ^{H}	135.00± 0.84 ^{D}	4.55± 0.02 ^{G}	228.00± 2.92 ^{A}	169.98± 2.57 ^{A}	79.45± 1.10 ^{C}
Oxy1	2 Weeks	15.58±0.38 ^{B}	0.73±0.04 ^{BC}	153.63± 0.50 ^{B}	7.95± 0.05 ^{C}	184.00± 1.22 ^{E}	119.85± 1.25 ^{D}	39.70± 2.16 ^{G}
	4 Weeks	27.90±0.73 ^{B}	1.00± 0.03 ^{C}	79.95± 1.31 ^{F}	13.08± 0.17 ^{D}	149.25± 1.07 ^{E}	103.50± 0.81 ^{E}	27.10± 0.66 ^{E}
Oxy1+Cur	2 Weeks	5.87± 0.10 ^{F}	0.35± 0.02 ^{E}	122.13± 0.96 ^{E}	4.43± 0.32 ^{E}	237.40± 2.80 ^{A}	169.67± 2.15 ^{A}	58.63± 0.79 ^{F}
	4 Weeks	12.30±0.35 ^{E}	0.62± 0.03 ^{F}	148.23± 4.08 ^{C}	7.80± 0.19 ^{F}	216.00± 1.67 ^{B}	151.00± 1.45 ^{C}	97.57± 3.99 ^{A}
Oxy1+Ga	2 Weeks	10.26±0.27 ^{C}	0.47± 0.02 ^{D}	157.37± 4.46 ^{B}	6.27± 0.11 ^{D}	227.67± 2.87 ^{B}	169.67± 1.49 ^{A}	150.77± 0.70 ^{A}
	4 Weeks	15.27±0.31 ^{D}	0.78± 0.01 ^{E}	164.97± 3.52 ^{B}	18.73± 0.67 ^{B}	216.67± 1.62 ^{B}	160.33± 1.59 ^{B}	57.97± 0.81 ^{D}
Oxy1+Cur+Ga	2 Weeks	5.07± 0.05 ^{F}	0.26± 0.01 ^{F}	122.90± 2.27 ^{E}	4.23± 0.10 ^{E}	220.00± 0.63 ^{C}	161.67± 0.48 ^{B}	89.30± 1.29 ^{D}
	4 Weeks	7.90± 0.08 ^{F}	0.33± 0.01 ^{H}	170.83± 1.47 ^{B}	11.77± 0.75 ^{DE}	209.67± 0.48 ^{C}	155.33± 0.80 ^{C}	91.30± 0.36 ^{B}
Oxy2	2 Weeks	24.13±0.59 ^{A}	1.11± 0.02 ^{A}	151.35± 4.26 ^{B}	10.05± 0.51^{B}	146.75± 3.44 ^{F}	94.50± 1.57 ^{F}	97.90± 2.53 ^{C}
	4 Weeks	31.93±0.39 ^{A}	1.60± 0.05 ^{A}	59.00± 1.23 ^{G}	9.01± 0.42 ^{F}	109.65± 0.61 ^{H}	66.50± 1.50 ^{G}	82.38± 3.47 ^{C}
Oxy2+Cur	2 Weeks	9.08± 0.12 ^{D}	0.74± 0.02 ^{B}	140.30± 1.04 ^{C}	6.80± 0.25 ^{D}	150.50± 1.16 ^{F}	91.35± 1.79 ^{F}	58.13± 1.34 ^{F}
	4 Weeks	14.55±0.22 ^{D}	1.20± 0.01 ^{B}	177.50± 1.78 ^{A}	14.68± 0.27 ^{C}	140.00± 0.71 ^{F}	80.75± 1.62 ^{F}	55.20± 1.31 ^{D}
Oxy2+Ga	2 Weeks	15.77±0.51 ^{B}	0.67± 0.02 ^{C}	136.63±1.30 ^{CD}	13.50± 0.36 ^{A}	149.07± 0.92 ^{F}	98.33± 0.48 ^{E}	74.53± 3.48 ^{E}
	4 Weeks	23.33±0.30 ^{C}	0.92± 0.01 ^{D}	123.90± 2.23 ^{E}	10.80± 0.22 ^{E}	129.00± 1.14 ^{G}	79.67± 0.48 ^{F}	24.20± 1.31 ^{EF}
Oxy2+Cur+Ga	2 Weeks	6.77± 0.12 ^{E}	0.36± 0.01 ^{E}	172.33± 0.49 ^{A}	4.97± 0.07 ^{E}	213.23± 0.93 ^{D}	149.67± 0.66 ^{C}	112.73± 2.52 ^{B}
	4 Weeks	12.10±0.06 ^{E}	0.42± 0.00 ^{G}	164.87± 1.96 ^{B}	20.77± 0.76 ^{A}	196.33± 0.97 ^{D}	124.67± 2.06 ^{D}	19.23± 1.20 ^{F}

The data are presented as Means±S.E.

Oxy1and 2: low and high Oxyfluorfen dose (0.3 and 0.6 ppm), Cur: curcumin (5 g/kg) and Ga: garlic acid (5 g/kg).

Different letters indicate significant difference at $p<0.05$

urea, creatinine, sodium (Na), potassium (K), cholesterol (Cho), triglyceride (Tg), and glucose.

DISCUSSION

Oxyfluorfen is a diphenyl-ether herbicide which is used for broad spectrum pre- and post-emergent control of annual broadleaf and grassy weeds in a variety of tree fruit, nut, vine, and field crops **(Pirasath *et al.*, 2021)**. It is structurally related to lactofen and acifluorfen which inhibits protoporphyrinogen oxidase, involved in heame biosynthesis pathway by **Poletika (2001)**. The toxicity occurs through acute oral, dermal and inhalation methods. The moderate toxicity can occur by ingestion and slightly toxicity can occur by dermal absorption by **Cheng (1989)**.

Hematology is a valuable tool for monitoring health, diagnosing illness, and tracking disease progression and treatment response. Hematological characteristics could be utilized as a reliable indicator to observe physiological changes following exposure to any harmful compounds, as blood is a pathophysiological in dicator organ that reflects total body status **(Harabawy and Ibrahim, 2014)**. Many hematological indicators, such

as RBCs, Hb, Hct, and WBCs, might be utilized to assess the blood of exposed fish **(Shah and Altindag, 2004; Ibrahim and Banaee, 2014; Ibrahim and Harabawy, 2014)**. This evidence clearly demonstrates how fish react to contaminants like oxyfluorfen. The acquired data revealed that oxyfluorfen exposure resulted in a considerable drop in RBCs, Hb, and Hct in *O. niloticus*, indicating that the fish suffered from anemia as a result of the reduced parameters following exposure to toxicants **(Kori-Siakpere *et al*., 2005)**. The significant decrease in RBCs, hemoglobin and hematocrit in fish may be attributed to the lowering of the oxygen content of the water. It was reported that the oxygen tension in the water is decreased in presence of herbicide **Ojala (1966)**. Similar results were obtained by **(Hussein *et al*., 1996; Mekkawy *et al*., 1996; Shalaby *et al*., 2007).** After being exposed to sublethal quantities of atrazine, observed a significant drop in RBCs, Hb content, and Hct % in rainbow trout. Deficiency can be attributed to one or more of the following factors: (i) heam dilution of blood as a result of damage and subsequent bleeding in the gills, as well as the removal of RBCs as a result of blood extravasations **(Abo-Hegab *et al*., 1993)** and (ii) disequilibrium of the osmotic pressure inside and outside the blood cell due to gain of water in the extracellular fluid with a subsequent increase in size by **Heath (1987)**.

The hematological parameters, MCV, MCH and MCHC are important indicators to detect the type of anemia in different animals **(Vaseem *et al*., 2012)**. MCV and MCHC of the present study showed a significant decrease in oxyfluorfen exposed groups. This shows that exposed fish are suffering from hypochromic macrocytic anemia. Chemical-induced changes in MCV, MCH, and MCHC were attributed to direct or feedback responses to structural damage to RBC membranes, which resulted in hemolysis and impaired hemoglobin synthesis, stress-related RBC discharge from the spleen, and hypoxia **(Marie *et al*., 1998; Shah, 2006)**.

According to **(Palanisamy Arunkumar, 2016; Jamal and Al-Faragi, 2017)** curcumin and garlic play important role in improving the hematological and biochemical health of fish. The present results showed that Cur and Ga improved the hematological parameters in both periods of exposure. However, Cur and Ga were more powerful to improve those hematological changes especially after exposure to the high dose of oxyfluorfen. Many studies have verified Cur role in hematological indices improvement in fish **(Palanisamy-Arunkumar, 2016; Jamal and Al-Faragi, 2017)**. The findings were similar to those of **(Yonar *et al*., 2019)**, who found that rainbow trout (*Oncorhynchus mykiss*) administered dietary curcumin supplementation had higher Hct, Hb, and RBC levels. (**Fawole *et al*., 2020; Anene *et al*., 2021)** found an increase in MCV in *Claris gariepinus* fed a diet supplemented with pawpaw seed and onion peel powder. The results of (**Zare *et al*., 2021)** study showed that garlic powder, at 10 g/kg, raise RBC levels in Eurasian Perch *Perca fluviatilis* juveniles. Garlic powder has demonstrated similar outcomes in rainbow trout at 0.5, 1, 5, and 10 g kg1 **(Nya and Austin, 2009)** and rohu at 10 g/kg at similar doses **(Sahu *et al*., 2007)**.

Variations in white blood cell indices (WBC, lymphocytes, neutrophils, and monocytes) as a non-specific immune cell ,are used as a stress indicator in fish **(Abarghouei *et al*., 2016)**. WBC is the regulators fish immunity **(Gaber *et al*., 2013)** and body defense against both foreign bodies and infectious disease. In the present work, WBCs, Neut and Mono showed a significant decrease in oxyfluorfen exposed groups. On Lymp., on the other hand, increased considerably after exposure to oxyfluorfen compared to the control group. WBCs may decrease in response to stress factors due to oxyfluorfen toxicity, whereas increasing values indicate a reaction to stress or infection **(Adams *et al*., 2006)**.The lymphocyte is the most dominant differential leukocyte and responsible for many functions of the immune system in fish.

The present study indicates that the WBC count was significantly decreased in the groups that were exposed to oxyflurfen. Similar results were obtained by **(Köprücü *et al*., 2006)** and **Far *et al*. (2012),** in fishes that exposed to diazinon. During exposure to sub-lethal concentrations of diazinon, the leucocyte count of common carp decreased significantly (**Banaee *et al*., 2007)**. These changes could be directly harmful to the kidneys and spleen (hematopoietic tissue). Probable cause of neutrophilia can be induced by the phagocytic cells in host defense **(Lusková *et al*., 2002; Svobodová *et al*., 2003; Far *et al*., 2012)**. Increase of lymphocytes was observed in the present study. Similar result was observed by **(Parrish, 1985; Gill and Bruland, 1990),** who reported that the stimulation of the immune system causes an increase in lymphocytes by injury or tissue damage. An increase in lymphocytes number may be a compensatory response of lymphoid tissues to the destruction of circulating lymphocytes **(Shah and Sciences, 2005)**.

Aly *et al*. (2008) study showed a clear effect on the physiological traits of common carp. When fed on diets fortified with different levels of powder, garlic fortified diets had a significant effect in increasing the number of red blood cells, while the number of white blood cells increased. In fish fed diets containing 5% garlic powder, compared to other experimental treatments. **Marentek *et al*. (2013)** study showed that when feeding tilapia (10.4 grams) on fortified diets of 2% garlic powder for 10 weeks, growth performance improved and macrophage cell activity increased, as well as an increase in types of white blood cells compared to fish control group. The results of **Zare *et al*. (2021)** showed that garlic powder, at 10 g/kg, raised WBC levels in Eurasian Perch *Perca fluviatilis* juveniles. The study of **Martins *et al*. (2002)** confirmed that the addition of garlic powder to the dietary diet of fish leads to clear changes in blood standards and varied depending on fish species and experimental conditions.

The results of **Mooraki *et al*. (2019)** study to assess the effect of turmeric powder given as a dietary supplement on *Andinoacara rivulatus* revealed that fish fed a meal supplemented with 0.3% turmeric powder had a significantly higher quantity of red and white blood cells than the control group.

Arunkumar *et al.* (2016) recorded 15 days after feeding common carp on turmeric-fortified diets with an increase in the numbers of red and white blood cells, while the lowest numbers of cell types were recorded for the same fish after 45 feeding in the fortified feed at a concentration of 0.3 ppm. The results of (**Ashry *et al.*, 2021)** found that dietary curcumin enhanced WBC count in gilthead seabream, indicating a powerful immunological response. Curcumin supplementation raised the number of white blood cells in common carp **(Yonar, 2018)** and rainbow trout **(Yonar *et al.*, 2019)**.

Pollution in the aquatic environment has an impact at the cellular or molecular level, resulting in major changes in biochemical parameters of organisms **(Ibrahim and Harabawy, 2014; Ibrahim and Harabawy, 2015)**. According to toxicological reports, the discovery of fish liver biomarkers such as biochemical characteristics offers information about how poisons affect fish health **(Authman *et al.*, 2013)**. Liver enzymes like AST, ALT, and ALP are sensitive to toxins and they are used to assess hepatocellular damage and a variety of hepatic disorders **(Ibrahim and Mahmoud, 2005)**. Presence of these enzymes in blood plasma may be due to tissue injury or organ disfunction **(Ibrahim and Banaee, 2014; Ibrahim and Harabawy, 2014)**. AST, ALT and ALP enzymes activity of the present results showed a significant increase in after oxyfluorfen exposure. **(Mahmoud *et al.*, 2012; Authman *et al.*, 2013)** have stated that the increase of liver enzyme activities has been proven to reflect liver damage, while increase in the ALP level may be indicator for renal and liver damage; and the alterations in enzymes activities in the serum directly indicates major pathologic changes in permeability of cell membrane or hepatic cell rupture. Also, the increase in the activity of ALP and AST in blood might be attributed to the necrosis of the liver and kidney as reported by **(Mona *et al.*, 2013)**.

This is in accordance with the finding of **(Ibrahim and Banaee, 2014)** in *Oncorhynchus mykiss* exposed to oxyflurfen. They also attributed that increase in AST, ALT and ALP to histopathological changes and tissue injury or organ disfunction. Curcumin and garlic addition to oxyflurfen groups recover liver enzymes (ALT, AST, and ALP) to control values. Similar results were obtained by **El-Barbary (2016)**. **(Panahi *et al.*, 2019; Rahmani *et al.*, 2016)** found statistically significant reductions in ALT and AST levels after supplementing their subjects with 3 × 500 mg/day (100 mg curcuminoids per capsule) and 500 mg/day of an amorphous dispersion preparation containing 70 mg curcuminoids for 8 weeks, respectively. Regardless of the supplement administered or the length of supplementation, non-statistically significant reductions in ALT levels were found **(Shalaby *et al.*, 2006; Metwally, 2009; Hariri *et al.*, 2020; Saberi-Karimian *et al.*, 2020; Kelardeh *et al.*, 2020**; **Yousefi *et al.*, 2020)**.

The increase of oxyfluorfen doses exposure caused a significant increase in urea and creatinine levels as kidney functions indicators. These results were confirmed by **(Hussein *et al.*, 1996; Shalaby *et al.*, 2007)** who reported that urea and creatinine

concentrations increased significantly after atrazine exposure of *Chrysichthyes auratus* and *Oreochromis niloticus*, suggesting nephrotoxicity. The significant increase of urea nitrogen in exposed *Chrysichthyes auratus* to atrazine may be due to necrosis of endothelial cells and renal hemopoietic tissue **(Hussein *et al*., 1996)**. This opinion supported by **(Fischer-Scherl *et al*., 1991)**. **(Gunkel and Streit, 1980)** indicated that atrazine was accumulated via the gills and blood during its exposure phase. This accumulation of atrazine in the gills caused their dysfunction and resulted in kidney stress which led to increase of urea in the blood. The nutritional supplement of (Cur, Ga, Cur + Ga) can significantly decrease urea and creatinine levels in serum after intense exercise **(Verma, 1997; El-Sayed and Khalil, 2009; Mathuria and Verma, 2007).**

Na+ and K+ are biomarkers for kidney function. The present study revealed an elevation of serum Na+, K+, with increase of Oxyfluorfen doses exposure . This elevation in the previous parameters may be attributed to kidney dysfunction. Kidney dysfunction may be explaining the increase in serum Na+ and K+ (**Abu *et al*., 2009; Hadi *et al*., 2009; Zaki *et al*., 2010**). **Abu *et al*. (2009)** reported that addition of Cur and Ga to the treated oxyfluorfen groups improved Na+ and K+ to normal values.

Blood proteins are used as biomarkers of negative effects on animals and considered as important tool for assessment of fish health status **(Kovyrshina and Rudneva, 2012)**. Blood contains different types of proteins that transport various metabolites and exogenous chemicals, provide fish protection against infections and help in other body functions **(Kovyrshina and Rudneva, 2012)**. Albumin and globulin are two protein components of blood used to assess liver health status **(Sunmonu and Oloyede, 2007)**. The liver is the factory of albumin which regulate the colloidal osmotic pressure of blood and transport the exogenous chemicals such as drugs and toxicants **(Kovyrshina and Rudneva, 2012)**. However, globulins play important role in immune system **(Sunmonu and Oloyede, 2007)**. The present results showed a significant increase in total protein, albumin and globulin concentrations in oxyfluorfen exposed groups. Increase in the serum protein, albumin and globulin levels is thought to be associated with a stronger innate immune response in fish **(Wiegertjes *et al*., 1996)**. The addition of curcumin and garlic to oxyflurfen-treated groups improves total protein, albumin, and globulin recovery to control levels **(Pal *et al*., 2005).** They demonstrated that combining curcumin with a hepatotoxic medication reduced elevated transaminases to normal levels. **Metwally (2009)** demonstrated that garlic oil treatment improved liver and other organ function in the production of plasma protein.

Carbohydrates (glucose) and lipids (cholesterol and triglyceride) the main sources of animals energy and indicators of stress resulting from toxins **(Authman *et al*., 2013)**. Lipids have a fast metabolic transformation **(Ibrahim and Harabawy, 2015)**. The present results showed a significant decrease in serum glucose level (hypoglycemia). Triglyceride and cholesterol of *Oreochromis niloticus* that exposed to oxyfluorfen doses.

Hussein *et al*. (1996) noted that the decrease glucose availability in exposed fish. Glucose is essential for triglycerides synthesis because it forms alpha glycerophosphate which is the specific precursor of glycerol with which fatty acids are esterified for triglycerides formation **(Bergman, 1983)**. In addition glucose furnishes NADPH which is required as a reducing agent in the synthesis of long chain fatty acids **(Hussein *et al*., 1996)**. Increases in blood glucose concentration (termed hyperglycemia) occur as a result of seasonal osmoregulatory changes, presence of stressors, and or shifts in diet compositions, according to a review on carbohydrates metabolism in fishes **(Polakof *et al*., 2012)**, whereas decreases in blood glucose concentration (termed hypoglycemia) occur as a result of food deprivation and or environmental perturbations (crowding stress and or hypoxia). The obtained results demonstrated that curcumin garlic extract and olive oil supplementation have potential effects in preventing hyperlipidemia, diabetes and on cardiovascular protection. **Chen *et al*. (2015)** suggested that selenium in curcumin and garlic might play an insulin-like role to normalize glucose metabolism and improve glucose uptake and metabolism in the liver. Selenium could restore glucagon-like peptide 1 receptor expression and suppress insulin receptor in the liver, which may reduce the hypoglycaemic effect of toxins **(Barakat *et al*., 2012)**. **Sallam (2012)** reported that selenium supplementation recovers cholesterol and triglycerides levels to the normal level of control in Nile tilapia.

CONCLUSION

the results obtained of *O. niloticus* that exposed to two doses of oxyfluorfen induced hematological and biochemical. Hence, our results give a good indication about the response of *Oreochromis niloticus* to sublethal doses of oxyfluorfen However, Curcumin and garlic addition to the treated fish showed a good improvement in hematological and biochemical parameters.

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