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Protective Effects of Curcumin and/or Ginger Supplementation against *Oreochromis niloticus* Oxidative Stress Induced by Ultraviolet-A radiation

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ABSTRACT

Ginger and curcumin showed a variety of important properties in fish, including antioxidant, anti-inflammatory, and immunostimulatory actions. The protective effects of curcumin and/or ginger supplementation have been evaluated against oxidative stress caused by ultraviolet (UVA) radiation in terms of hemo-biochemical parameters, lipid peroxidation, DNA fragmentation, and antioxidant enzymes. 160 healthy *Oreochromis niloticus* fish (n= 20/group) were subjected to 8 groups (1) control, (2) fed with Curcumin (10 mg/kg body weight), (3) fed with Ginger (10 mg/kg body weight), (4) were fed with Curcumin + Ginger, (5) exposed to ultraviolet (UVA) 60 min/ day for three days, (6) exposed to UVA+ Curcumin, (7) exposed to UVA + Ginger, (8) exposed to UVA + Curcumin + Ginger for three days. The findings demonstrated that after exposure to UVA, hematological indices including hemoglobin (Hb), red blood cells (RBCs), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells, and lymphocytes significantly decreased, whereas mean corpuscular volume (MCV), neutrophils, and eosinophils significantly increased. For biochemical markers including total protein, cholesterol, triglycerides, urea, creatinine, ALT, and AST were significantly decreased, whereas glucose significantly increased. Curcumin and ginger supplements were able to reverse abnormalities in the hemo-biochemical parameters Additionally, after exposure to UVA, the antioxidant enzymes such as Superoxide dismutase, Catalase, and Total Antioxidant Capacity significantly decreased in the liver, kidney, and gill. Whereas lipid peroxidation and DNA fragmentation (%) levels were significantly increased in the same organs. Curcumin and ginger supplements reduce UVA-induced oxidative stress and also restore antioxidant enzymes.

INTRODUCTION

Emitting the sun with ultraviolet radiation (UVR) induces several regions according to its wavelength, the most active radiation whose length is located between 100 and 280 nm and is known as the ultraviolet C (UVC), this type of radiation doesn't arrive on earth. The region whose length is located between 315 nm and 400 nm which is termed

the ultraviolet A (UVA) is the lowest active. The most active radiation which arrives on the surface of the earth and exists between the two regions is called ultraviolet B (UVB) **(Araújo *et al*., 2021)**. The ability of UVR to penetration of fresh and marine water reach 60 m which has been reported to cause deleterious effects on organisms present in these aquatic systems extending from bacteria to many types of vertebrates **(Llabrés *et al*., 2013; Häder *et al*., 2015; Alves and Agusti, 2020; Peng *et al*., 2020)**, this effect may extended to cause a change in population level **(Häder *et al*., 2007)**. Exposure of fish to UVR causes several harmful effects such as a decrease in growth, behavior alterations, and changes in the skin and eyes **(Hunter *et al*., 1981; Sandrini *et al*., 2009)**. The skin hazards and their development in farmed fishes are related to elevation mortality rates **(Bullock, 1988)**. Other studies proved the hazards caused by exposure to UVA represented in hematology and blood biochemical effects and other genotoxic changes which show the cytological and physiological modulation **(Osman *et al*., 2010; Ibrahim *et al*., 2019a; Osman *et al*., 2019)**.

The cytological changes occurred after UVA exposure leads to breaking DNA strands which in turn damage the cellular DNA **(Mekkawy *et al*., 2010; Petersen and Nelson, 2010)**. Exposure to UVA cause fish immunity destruction **(Sayed and Mitani, 2017)**. This suppression in fish immunity decreases the fish resistance to bacteria, parasites and fungi which make them more vulnerable to infections **(Fabacher and Little, 1995; Ghanizadeh Kazerouni *et al*., 2017)**. The negative impacts of UVR on fish immunity and different photoprotective ways used by some fishes to deliver the bad effects after exposed to UVR was determined (Lawrence et al., 2020). General studies included the UVR effects on fish as the producing of reactive oxygen species (ROS) which cause cellular oxidative stress **(Zagarese and Williamson, 2001; Häder *et al*., 2007; Häder *et al*., 2015)**.

Pay attention to using natural products and adding them to food for decreasing the harmful hazards of feed contaminants and to keep the fish healthy increased **(Galal *et al*., 2018; El-Houseiny *et al*., 2019; Ibrahim *et al*., 2019b)**. Curcumin (CUR) or diferuloymethane, is a yellow extraction coming from the *Curcuma longa L* rhizome plant **(Mirzaei *et al*., 2017)**. Using curcumin as a phytogenic feed additive may be a benefit to promoting growth and health, also it does not cause any harmful effects on fish, or environment, or humans **(Mahmoud *et al*., 2017)**. The rhizome of this plant gives powder known as curcumin which was used in china in ancient times as a medicine for different diseases **(Kumar *et al*., 2020)**. Curcumin plays a role in protecting the fish liver tissues **(Manal, 2018)** and can raise the ability of digestion and absorption in the intestine which increases the growth rate **(Jiang *et al*., 2016; Sruthi *et al*., 2018)** due to its good anti-inflammatory and antioxidant characters **(Bellio *et al*., 2014; Cao *et al*., 2015)**. Curcumin is known for its effects on the immune system and can increase the ability of fish to the resistance of parasites and bacteria **(Elgendy *et al*., 2016; Baldissera *et al*., 2018)**. Many studies showed the ability of curcumin to treat the harmful impacts resulting

from pollutant exposure, for example adding curcumin help in recovering the damage in liver tissues in Jian carp after exposure to tetrachloride **(Cao *et al*., 2015)** and ameliorating the liver toxicity that occurred in Nile tilapia after exposure to aflatoxin B1**(Mahfouz, 2015)**.

Ginger root (*Zingiber officinale Roscoe*) is famous as a condiment and as a useful medicinal plant which extensively used in traditional medicine due to containing different compounds like carotenoids, flavonoids, steroids, polyphenols, and alkaloids **(Shirin and Jamuna, 2010)**. Also Ginger has other bioactive important compounds such as gingerdiol, gingerdione, and gingerol **(Shahrajabian *et al*., 2019)** which have several excellent characteristics as anti-inflammatory, antimicrobial, and natural antioxidants **(Nya and Austin, 2009; Reverter *et al*., 2017; Mao *et al*., 2019)**. Many Collective studies showed the useful effect of ginger on different fish growth, hematology, and immunity **(Dügenci *et al*., 2003; Nya and Austin, 2009; Sukumaran *et al*., 2016; Oh *et al*., 2022)**. Also, it has a good effect on preventing hyperglycemia and digestive disorders **(Mao *et al*., 2019; Shang *et al*., 2019; Chowdhury *et al*., 2021)**. The good effect of ginger on these parameters is due to the change in gene expression linked with them **(Sukumaran *et al*., 2016)**. Some studies recorded that ginger decrease stress results from high density **(Fazelan *et al*., 2020)**. Many phyto- additives have various effective properties, however, their effect increase after combination as shown in several studies **(Chandran *et al*., 2016; Jahanjoo *et al*., 2018).**

Nile tilapia (*Oreochromis niloticus*) is a fresh water fish have a great concern in aquaculture sharing in increasing global protein production **(FAO, 2018)**. Tilapia is usually introduced as fish fillets meal with (23%) protein and (66%) unsaturated fatty acids **(Monteiro *et al*., 2017; USDA, 2018)**. *Oreochromis niloticus* is considered as a biomarker to reflect the toxic effects of toxicants such as UVA radiation expose that cause many physiological, genotoxic and cytological alterations **(Ibrahim *et al*., 2019a)**.
To our knowledge, the impact of curcumin and/or ginger supplementation on UVA-exposed aquatic animals has rare. Our current research was planned to study the hazards resulting from *O. niloticus* exposure to UVA on hemo-biochemical parameters, lipid peroxidation, DNA fragmentation, and antioxidant enzymes. Based on the several characteristics of curcumin and ginger, we also examine the benefits of their supplementation to diet to improve the same biomarkers.

MATERIALS AND METHODS

Specimen's collection

We started our experiment by collecting one hundred Sixty healthy *O. niloticus* fish from the fish market (125.5±13.0 g weight, 20.5±3.0 cm length). After reaching the fish laboratory in the Faculty of Science, South Valley University, the healthy experimental fishes were kept in aerated glass tanks (100 L capacity) and leave it for acclimation for 2 weeks before using fish in the experimental study. The experimental fish fed pellets at a

rate of 3% of the fish's body weight twice daily. Regular aspirate for feces and residual food was done. The water temperature, pH, and dissolved oxygen concentrations (DO) were measured daily (22.2 ± 1.5 C, 6.9 ± 0.2 pH, and 6.5 ± 1.03 mg L-1 DO). The light cycle was 12 h light and 12 h dark.

UVA source

O. niloticus fish were exposed to UVA radiation (model UVL-56, 6 W self-ballasted long wave UV-365 nm, UVP, Inc. San Gabriel, CA, USA with input voltage 220 V, 60 Hz) as used in a previous study **(Ibrahim, 2015)**. The UVA source was fitted 20 cm above the level of the aquarium water surface (the water level was 50 cm).

Extract preparation

Curcumin (*Curcuma longa*) and ginger (*Zingiber officinale*), rhizomes were freshly collected and then dried. After drying, curcumin and ginger were steeped in de-ionized water 100 g/l **(Mohankumar and McFarlane, 2011; Manuhara *et al*., 2018)**. Then stirring of rhizomes occurred at 50°C for 4h and filtered to obtain a yellow extract this extract was used fresh. The doses of curcumin and ginger were (10 mg/kg) body weight according to **(Mohankumar and McFarlane, 2011; Manuhara *et al*., 2018).**

Experimental setup

After the acclimation period, fish were distributed into 8 groups (6 fish each); control and seven treated groups as shown in table 1. The UVA dose was 60 min/ day for three days based on a study by **Ibrahim (2015)**. Curcumin and/or ginger aqueous extraction dose were 5% from the basal diet according to **Mohankumar and McFarlane (2011) and Manuhara *et al*. (2018)**. Water was completely renewed daily.

Blood sampling

Once the experiment period finished, two blood samples were obtained from the fish caudal vein. The first one was obtained fresh and put in a glass tube with an anticoagulant or heparin solution (0.2 ml/ml blood). This sample was useful for the analysis of the hematological variables. The second one was obtained and allowed to be coagulated for 15–20 min at 4ºC before centrifugation for 20 min at 3,000 rpm to separate serum. The obtained serum was used in the analysis of biochemical variables.

Hematological parameters

Whole blood was used for the estimation. By a hemocytometer, the erythrocyte and leucocyte counts were determined as in (**Natt and Herrick, 1952)**. The hemoglobin concentration (Hb) was estimated by using Drabkin's reagent read at 540 nm **(Drabkin, 1946)**, and the hematocrit (Hct) was estimated by a microhaematocrit centrifugation technique as in Vonti, 2008. The erythrocyte indi-ces (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration

(MCHC) were calculated by formulas with the obtained data of the Hct, RBC, and Hb **(Jain, 1993; Sağlam and Yonar, 2009)**.

Biochemical parameters

Colorimetric estimations of biochemical indices were conducted by a spectrophotometer (Jasco-V530). Aspartate aminotransferase (AST, U/I), alanine aminotransferase (ALT, U/I) activities, urea (mg/l), creatinine (mg/l), glucose (mg/l), cholesterol (mg/l) and triglyceride (mg/l) were estimated using specialized kits (Spec-trum Diagnostics, Egypt). Total protein (g/100 ml) content was estimated using specialized kits (Diamond Diagnostics, Egypt).

Tissues preparation

One gram of liver, kidney, and gills was carefully taken, then using filter paper to dry the specimen surface, then completely washed with 50mM phosphate buffer with pH 7.4 and homogenized with 50mM phosphate buffer with pH 7.4 containing, 1mM EDTA, 1mM DTT, 0.15M KCl, 0.01% PMSF. Homogenization of the chosen organs was carried out at 4 ◦C using 12–15 strokes of a motor-driven Teflon Potter homogenizer and centrifuge at 10,000 rpm for 20 min at 4 ºC. The supernatant was used for antioxidant activities and oxidative stress studies.

Lipid peroxidation & DNA fragmentation (%) measurements

Lipid peroxidation (LPO) in the selected tissues was estimated by the method of **(Utley *et al*., 1967)**. The absorbance of each aliquot was measured at 535 nm. The rate of lipid peroxidation was expressed as nmol of thiobarbituric acid reactive substance (TBARS) formed per hour per milligram of protein using a molar extinction coefficient of 1.56 M-1 cm-1 **(Buege and Aust, 1978)**. DNA fragmentation was estimated by the method of **(Kurita-Ochiai *et al*., 1999)** using a spectrophotometer (Jasco-V530) at 575 or 600 nm against a reagent blank. The percentage of fragmented DNA was estimated by the following formula: % of fragmented DNA = fragmented DNA / (fragmented + intact DNA) X 100.

Antioxidant enzyme measurements

Catalase activity was determined using Biodiagnostic Kit No.CA 25 17, which is based on the spectrophotometric method described by Aebi (1984) and values are expressed as Ug-1. Superoxide dismutase activity was determined using a colorimetric Sigma-Aldrich Kit No. 19160; the absorbance was measured at 450 nm and values are expressed as inhibition rate %. The TAO was measured using a colorimetric assay kit (Randox Laboratories, Crumlin, U.K.) and values are expressed as mmol L-1.

Statistical Analysis

The basic statistics (means and standard errors) of the measured parameters were estimated. The statistical significance was tested by using one-way ANOVA. Differences were considered significant at $P<0.05$ using the statistical software SPSS version 16.

Ethical statement

Our experiments were carried out according to Egyptian laws and University guidelines for the care of experimental animals. Experiments and fish handling of the current investigation have been approved by the Research Ethical Committee of the Faculty of Science, South Valley University, Egypt (007/12/22).

RESULTS

Haematological parameters

The present study explored the effect of the aqueous extraction of Curcumin and/ or Ginger on the harmful impacts of Ultraviolet -A radiation. No mortality was recorded during the exposure period of our study in control or exposed groups. The effects of UVA, Curcumin, Ginger, and their combination on the hematological parameters of *O. niloticus* after 1h/3 days are given in Table 2. Red blood cells (RBC), Hemoglobin (Hb), and Hematocrit (Hct) showed a significant drop ($p<0.05$) in the UVA group when compared with the respective control. Curcumin plus ginger addition to UVA exposed groups caused normalized RBC, Hb, and Hct ($p>0.05$) values. Curcumin addition to UVA exposed group caused normalized Hb and Hct ($p>0.05$) values.

MCV showed a significant increase ($p<0.05$) while MCHC and MCH showed a significant decrease ($p<0.05$) in the UVA group when compared with the control group. Curcumin plus ginger addition to UVA exposed groups caused normalized MCV, MCH, and MCHC ($p>0.05$) values. Curcumin addition to UVA-exposed groups caused normalized MCV and MCHC ($p>0.05$) values.

White blood cells (WBC) and Lymphocytes (Lym) showed a significant decrease ($p<0.05$). However, Neutrophils (Neutro) and Eosinophils (Eos) showed a significant increase ($p<0.05$) while monophils (Mono) showed no significant changes ($p<0.05$) in UVA exposed group when compared with the control one. The addition of curcumin plus ginger normalized WBC's, lymphocytes, neutrophils, and eosinophils ($p>0.05$) in the group that was exposed to UVA. Curcumin addition improves lymphocytes, neutrophils, and eosinophils percentage ($p>0.05$) in the group that was exposed to UVA.Only eosinophil percentage was improved by Ginger addition in the group that was exposed to UVA (Table 2).

Biochemical parameters

The effects of UVA, Curcumin, Ginger, and their combination on the biochemical parameters of *O. niloticus* after 1h/3 days are given in Table 3. Serum total protein (Tp)

showed a significant increase ($p<0.05$) in UVA exposed group when compared with the control. However, the addition of curcumin and/or ginger normalized Tp content ($p<0.05$) in UVA exposed group. Also, Cholesterol (Chl) and Triglyceride (Tg) showed a significant increase ($p<0.05$) in UVA exposed group. Curcumin plus ginger additions normalized Chl and Tg values ($p>0.05$). Also, Chl values were normalized ($p<0.05$) in the curcumin group that was exposed to UVA. Glucose levels showed a significant decrease ($p<0.05$) in the group that was exposed to UVA. Curcumin and Curcumin plus ginger normalized serum glucose level ($p>0.05$) in UVA exposed group.

Urea and Creatinine content as kidney functions showed a significant increase ($p<0.05$) in the UVA group. However, Curcumin and/ or ginger additions normalized their values ($p>0.05$) in UVA-exposed groups. Also, ALT and AST as liver function showed a significant increase ($p<0.05$) in the UVA groups. However, Curcumin addition normalized their values ($p>0.05$) in UVA-exposed groups. Also, AST values were normalized ($p>0.05$) in the ginger group that was exposed to UVA while ALT values were normalized ($p>0.05$) by Curcumin plus ginger addition in UVA exposed group (Table 3).

Lipid peroxidation & DNA fragmentation (%) measurements

Lipid peroxidation (LPO) results (Malonaldehyde level) are presented in Table (4). The LPO level was significantly increased in the liver, kidney, and gills samples of the UVA groups. However, Curcumin additions normalized LPO values ($p>0.05$) in the liver, kidney, and gills of UVA-exposed groups. Also, Ginger addition normalized LPO values ($p>0.05$) in the kidney and gills of UVA-exposed groups.DNA fragmentation (%) levels are presented in Table 4. The DNA fragmentation (%) level was significantly increased ($p<0.05$) in the liver, kidney, and gills samples of the UVA groups. Only, Curcumin or Ginger additions normalized DNA fragmentation (%) level ($p>0.05$) in the gills of UVA-exposed groups

Antioxidant enzymes

Superoxide dismutase (SOD), Catalase (CAT), and Total Antioxidant Capacity (TAOC) levels are presented in Table (5). The SOD level was significantly decreased in the liver, kidney, and gills samples of the UVA-exposed group. However, Curcumin plus Ginger additions normalized SOD level ($p>0.05$) in the liver, kidney, and gills of UVA-exposed groups. The CAT level was significantly decreased ($p<0.05$) in the liver, kidney, and gills samples of the UVA-exposed group. However, Curcumin plus Ginger additions normalized CAT level ($p>0.05$) in the liver, kidney, and gills of UVA-exposed groups. The TAOC level was significantly decreased ($p<0.05$) in the liver, kidney, and gills samples of the UVA-exposed group. However, Curcumin or Ginger additions normalized the TAOC level ($p>0.05$) in the liver while Curcumin plus Ginger additions normalized the TAOC level ($p>0.05$) in the gills of UVA exposed groups.

Table 1. The fish groups exposed to 1h UVA for 3 days, Curcumin (10 mg/kg) , Ginger (10 mg/kg) and their combinations.

Group / Treatment	Control	Cur	Gin	Cur + Gin	UVA	UVA + Cur	UVA + Gin	UVA + Cur + Gin
UVA	0	0	0	0	60 min	60 min	60 min	60 min
Curcumin (10mg/kg)	0	5%	0	5%	0	5%	0	5%
Ginger (10mg/kg)	0	0	5%	5%	0	0	5%	5%

Table 2. The basic data of blood constituent parameters (N =6) of *Oreochromis niloticus* exposed to Ultraviolet radiation (1 hour), Curcumin (10 mg/kg), Ginger (10 mg/kg) and their combinations for 3 days.

Treatments / parameters	Control	Cur	Gin	Cur + Gin	UVA	UVA+Cur	UVA+Gin	UVA+Cur +Gin
RBC (Million/mm3)	2.00±0.02^{a}	2.09±0.03^{ab}	2.06±0.03^{a}	2.19±0.06^{b}	1.12±0.03^{e}	1.88±0.03^{c}	1.61±0.04^{d}	2.11±0.06^{ab}
Hb (g/dl)	3.69±0.04^{ab}	3.75±0.04^{b}	3.77±0.03^{b}	3.67±0.08^{ab}	1.96±0.09^{d}	3.50±0.07^{a}	3.15±0.05^{c}	3.59±0.09^{ab}
HCT (%)	40.53±0.52^{ab}	40.72±0.25^{b}	40.85±0.33^{b}	41.08±0.34^{b}	25.05±0.94^{d}	39.22±0.36^{ac}	37.98±0.40^{c}	40.98±0.28^{b}
MCV (µm³)	149.95±2.75^{ab}	144.71±1.51^{ab}	147.14±2.30^{ab}	139.31±4.30^{b}	175.52±5.00^{c}	154.23±2.57^{a}	165.71±3.60^{c}	144.64±4.38^{ab}
MCH (Pg)	18.42±0.25^{a}	18.04±0.36^{bc}	18.33±0.21^{abc}	16.82±0.57^{c}	17.53±0.43^{bc}	17.14±0.67^{bc}	19.69±0.67^{b}	18.59±0.47^{ab}
MCHC (%)	9.10±0.17^{a}	9.22±0.11^{a}	9.22±0.10^{a}	8.94±0.20^{a}	7.83±0.10^{c}	8.92±0.16^{a}	8.31±0.18^{b}	8.77±0.20^{a}
WBC (Thousands/mm3)	49.73±0.49^{a}	51.07±0.49^{a}	50.22±0.59^{a}	49.80±0.39^{a}	30.62±0.39^{d}	47.07±0.37^{b}	42.84±0.79^{c}	50.60±0.44^{a}
Lym (%)	56.83±0.60^{a}	57.17±1.08^{a}	55.67±1.09^{a}	57.00±0.82^{a}	37.33±0.71^{c}	56.00±0.63^{a}	49.33±0.49^{b}	56.17±0.79^{a}
Neutro (%)	28.00±0.58^{a}	27.33±0.84^{a}	27.50±0.67^{a}	29.00±0.37^{a}	45.50±0.62^{c}	31.00±1.06^{a}	28.67±0.49^{b}	29.00±0.37^{a}
Eos (%)	5.00±0.37^{ab}	5.00±0.37^{a}	5.17±0.31^{a}	5.33±0.33^{a}	6.67±0.49^{c}	5.17±0.31^{b}	5.83±0.54^{a}	6.17±0.65^{a}
Mono (%)	6.67±0.21^{ab}	6.33±0.21^{ab}	6.50±0.34^{ab}	6.50±0.22^{ab}	7.50±0.43^{b}	6.00±0.58^{a}	6.83±0.48^{ab}	6.00±0.37^{ab}

The data are presented as Means ± S.E.Different letters indicate significant difference at $p<0.05$.

Red blood cells (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), White blood cells (WBC), Lymphocytes (Lym), Neutrophils (Neutro), Eosinophils (Eos) and Monocytes (Mono).

Table 3. The basic data of serum biochemistry parameters (N =6) of *Oreochromis niloticus* exposed to Ultraviolet radiation (1 hour), Curcumin (10 mg/kg), Ginger (10 mg/kg) and their combinations for 3 days.

Parameters \ Treatments	Control	Cur	Gin	Cur+Gin	UVA	UVA+Cur	UVA+Gin	UVA+Cur+Gin
Total protein (g/dl)	3.53±0.07^{ab}	3.61±0.06^{a}	3.70±0.04^{a}	3.64±0.05^{a}	6.08±0.13^{c}	3.73±0.11^{a}	4.03±0.16^{b}	3.58±0.06^{a}
Cholesterol (mg/dl)	203.80±1.94^{a}	200.50±1.77^{a}	200.17±1.68^{a}	200.18±3.94^{a}	310.10±5.02^{c}	206.83±2.02^{ab}	212.17±2.89^{b}	208.00±2.70^{ab}
Triglyceride(mg/dl)	198.02±5.11^{a}	193.67±2.89^{ab}	188.17±2.65^{b}	191.42±2.17^{ab}	289.50±1.77^{d}	211.50±2.23^{c}	212.17±2.77^{c}	200.67±4.14^{a}
Glucose (mg/dl)	94.70±1.86^{a}	97.50±0.67^{c}	95.33±1.26^{bc}	92.67±1.12^{ab}	69.07±1.21^{d}	90.83±1.47^{ab}	90.67±0.80^{b}	92.00±2.39^{ab}
Urea (mg/dl)	35.00±1.59^{a}	32.33±1.15^{a}	32.83±0.79^{a}	32.20±0.83^{a}	81.15±1.79^{b}	34.32±1.02^{a}	34.83±0.95^{a}	31.50±1.38^{a}
Creatinine (mg/dl)	0.38±0.01^{a}	0.33±0.01^{a}	0.34±0.01^{a}	0.34±0.02^{a}	1.33±0.03^{b}	0.37±0.02^{a}	0.39±0.02^{a}	0.33±0.01^{a}
ALT (µ/l)	12.83±0.53^{a}	11.87±0.39^{a}	11.92±0.49^{a}	13.20±0.49^{a}	50.79±2.37^{c}	14.10±0.32^{ab}	16.50±0.80^{b}	14.75±0.67^{ab}
AST (µ/l)	24.41±0.55^{a}	23.81±0.49^{a}	22.75±0.63^{ab}	22.84±0.60^{ab}	50.43±0.63^{C}	23.45±0.89^{ab}	24.47±0.52^{ab}	21.75±0.44^{b}

The data are presented as Means ± S.E.Different letters indicate significant difference at $p<0.05$.

Table 4. Changes in the level of Lipid peroxidation (nmol/mg protein) and DNA fragmentation (%) in liver, kidney, and gills of *Oreochromis niloticus* (n=6) treated with different doses of Ultraviolet radiation (1 hour), Curcumin (10 mg/kg), Ginger (10 mg/kg) and their combinations for 3 days.

Tissues \ Treatments		Control	Cur	Gin	Cur+Gin	UVA	UVA+Cur	UVA+Gin	UVA+Cur+Gin
LPO (nmol/mg protein)	**Liver**	2.03±0.03^{a}	1.76±0.06^{abc}	1.57±0.09^{bc}	1.39±0.08^{c}	16.87±0.34^{d}	1.99±0.09^{ab}	1.73±0.12^{abc}	1.54±0.07^{bc}
	Kidney	1.80±0.03^{a}	1.39±0.06^{b}	1.48±0.06^{b}	1.07±0.04^{c}	11.82±0.22^{d}	1.57±0.09^{ab}	1.64±0.09^{ab}	1.34±0.05^{bc}
	Gills	0.87±0.01^{a}	0.76±0.02^{a}	0.75±0.02^{a}	0.75±0.04^{a}	4.44±0.10c	0.86±0.03^{a}	0.82±0.03^{a}	0.72±0.04^{b}
DNA (%)	**Liver**	6.02±0.09^{a}	4.62±0.27^{b}	4.22±0.22^{bc}	3.56±0.18^{c}	26.60±0.53^{d}	5.08±0.24^{b}	4.69±0.25^{b}	4.69±0.28^{b}
	Kidney	5.40±0.12^{a}	3.92±0.36^{bc}	4.13±0.18^{bc}	3.72±0.12^{c}	20.12±0.28^{d}	4.32±0.37^{bc}	4.61±0.27^{b}	4.55±0.17^{b}
	Gills	2.25±0.04^{ac}	2.24±0.08^{ac}	1.96±0.02^{ab}	2.01±0.05^{ab}	8.76±0.19^{d}	2.48±0.14^{c}	2.18±0.07^{ac}	1.79±0.05^{b}

The data are presented as Means ± S.E.Different letters indicate significant difference at $p<0.05$.

Table 5. Changes in the level of Super oxide dismutase (SOD), Catalase (Cat) and Total Antioxidant Capacity (TAOC) in liver, kidney, and gills of *Oreochromis niloticus* (n=6) treated with different doses of Ultraviolet (UVA) radiation, Curcumin (Cur), Ginger (Gin) and their combinations for 1h UVA for 3 days**.**

Enzymes	Treatments / Tissues	Control	Cur	Gin	Cur+Gin	UVA	UVA+Cur	UVA+Gin	UVA+Cur +Gin
SOD	Liver	2.11±0.03^{a}	2.09±0.05^{a}	2.15±0.03^{a}	2.13±0.02^{a}	1.38±0.03^{c}	1.78±0.06^{b}	1.72±0.07^{b}	2.02±0.04^{a}
	Kidney	3.01±0.05^{a}	3.00±0.03^{a}	3.13±0.00^{a}	3.10±0.03^{a}	2.13±0.05^{c}	2.62±0.07^{b}	2.68±0.11^{b}	3.09±0.05^{a}
	Gills	3.94±0.06^{a}	4.07±0.04^{a}	4.04±0.05^{a}	4.08±0.05^{a}	3.00±0.04^{c}	3.56±0.10^{b}	3.46±0.19^{b}	4.09±0.07^{a}
CAT	Liver	294.48±4.80^{a}	294.70±4.04^{a}	296.26±3.68^{a}	301.91±3.60^{a}	210.45±4.22^{c}	257.44±8.12^{b}	253.97±9.94^{b}	300.44±2.72^{a}
	Kidney	364.88±5.28^{a}	379.65±2.99^{ab}	384.02±1.43^{b}	383.09±1.65^{b}	295.88±5.40^{d}	331.57±8.99^{c}	328.82±9.93^{c}	377.94±2.43^{a}
	Gills	474.93±6.87^{a}	482.16±2.23^{a}	469.65±4.54^{a}	479.37±2.97^{a}	393.71±6.42^{c}	420.98±10.21^{b}	402.48±14.67^{bc}	472.69±4.72^{a}
TAOC (nmol/mg protein)	Liver	40.19±0.58^{ab}	42.44±1.31^{bc}	44.36±1.14^{cd}	47.17±1.00^{d}	19.12±0.38^{e}	37.17±1.65^{a}	37.88±1.67^{a}	44.17±1.06^{cd}
	Kidney	38.04±0.84^{a}	38.05±1.56^{a}	39.40±1.09^{ab}	41.07±0.55^{ab}	19.75±0.28^{d}	33.29±1.55^{c}	33.49±0.58^{c}	41.48±0.65^{b}
	Gills	21.36±0.31^{a}	21.22±0.27^{a}	20.54±0.42^{a}	21.45±0.53^{a}	7.52±0.11^{c}	18.22±0.56^{b}	18.04±0.46^{b}	20.29±0.38^{a}

The data are presented as Means ± S.E.Different letters indicate significant difference at $p<0.05$.

DISCUSSION

UVB and UVA are the two main categories of ultraviolet rays that reach the surface of the earth. Additionally, UVA radiation age the skin prematurely by penetrating deeper into the epidermis and contributing more to the development of wrinkles (photoaging) **(D'Orazio *et al*., 2013)**. Blood parameters are a critical component of how the body works and serve as the entire body's physiological mirror **(Keller, 2019)**. They serve as trustworthy indicators for identifying the physiological alterations induced by toxicant exposure **(Seibel *et al*., 2021)**. Hematological and biochemical alterations were previously reported by some investigators in several animals after exposure to UVA or UVB **(Ibrahim, 2015; Ibrahim *et al*., 2019a; Osman *et al*., 2019)**.

Here, RBC, Hb, and Hct levels were significantly decreased ($P < 0.05$) in UVA exposed group. When *Clarias gariepinus* and *Oreochromis niloticus* were exposed to UVA in previous studies **(Ibrahim *et al*., 2019a; Osman *et al*., 2019)**, similar outcomes were obtained. The alterations in hematological parameters after UVA exposure observed in this study have also been reported in *O. niloticus* exposed to three doses of ultraviolet A radiation **(Ibrahim *et al*., 2019a)** and in *C. gariepinus* exposed to UVA, 4-nonylphenol, arsenic and sodium dodecyl sulphate (SDS) (**Sayed and Authman, 2018; Osman *et al*., 2019; Mekkawy *et al*., 2020; Sayed *et al*., 2022)**. One explanation for the decline in these measures following UVA-induced stress is hemolysis because UVA can inactivate hematopoietic tissues, which then results in a decrease in erythropoiesis **(Narayanan, 2009; Sayed, 2016)**. Hb levels have decreased significantly ($P < 0.05$), which could be the result of either a rise in Hb degradation or a fall in Hb production rates.

Red cell indices are another name for MCV, MCH, and MCHC, which are components of a complete blood count. It indicates that they display the erythrocyte's size and hemoglobin concentration. The erythrocyte size is determined by MCV, where a high value denotes a macrocytic condition and a low value denotes a microcytic condition. Since MCV significantly increased ($P < 0.05$) when compared to the control fish in the current example, UVA radiation exposure caused macrocytic anemia in the fish. This crucial state develops when a cell grows constantly to higher sizes yet is unable to produce DNA quickly enough to divide when it should **(Benz *et al*., 2009)**. **Osman *et al*. (2010)** found a similar outcome after exposing African catfish *C. gariepinus* to UVA for three days and three hours each.

The average hemoglobin content of erythrocytes is described differently by MCH and MCHC. While MCHC reflects the amount of hemoglobin per unit volume of red cells, MCH expresses the amount of hemoglobin per red cell **(Wintrobe, 1981)**. Hypochromic anemia is indicated by low MCHC levels. When compared to the control group, MCH, and MCHC in the UVA-exposed group showed a significant decline ($P < 0.05$). When exposed to the effluents of the paint, dye, and petroleum industries, fish L. rohita showed a similar trend of increasing MCV and decreasing MCHC levels **(Zutshi *et***

***al*., 2010)**. When exposed to the heavy metal Cd, *O. mossambicus* showed a considerable increase in MCV and a decrease in MCH and MCHC values, according to (**Shalaby, 2001)**. The alterations in the previous blood indices after UVA exposure observed in this study have also been reported in *O. niloticus* exposed to silver nanoparticles **(Ibrahim, 2020)** and *C. gariepinus* after exposure to arsenic and silver nanoparticles **(Mekkawy *et al*., 2019; Mekkawy *et al*., 2020)**. However, adding curcumin and ginger restored RBC, Hb, Hct, MCV, MCH, and MCHC levels in the UVA-exposed group. According to earlier investigations, dietary curcumin supplementation restored the abnormal hematological markers **(Abd El-Hakim *et al*., 2020; Giri *et al*., 2021)**. Curcumin has been shown to have a comparable beneficial effect on hematological parameters in rainbow trout (Yonar *et al*., 2019). Previous investigations on *Lates calcarifer* **(Talpur *et al*., 2013)**, *Oncorhynchus mykiss* **(Nya and Austin, 2009)**, and *Huso huso* **(Gholipour Kanani *et al*., 2014)** have likewise documented the positive effects of ginger supplementation on RBC, Hb, and HCT.

Leukocyte activity and count can provide information about a fish's health and play a significant part in nonspecific or innate immunity **(Secombes, 1996)**. When comparing the UVA- exposed group to the control group, white blood cells (WBC) and lymphocytes exhibited a significant decline ($P < 0.05$), whereas neutrophils and eosinophils showed a significant increase ($P < 0.05$). Both *O. niloticus* **(Ibrahim *et al*., 2019a)** and *C. gariepinus* **(Osman *et al*., 2019)** exposed to ultraviolet A radiation showed similar reduced White blood cells. Other fish species, including common carp, rainbow trout, and Atlantic salmon **(Markkula *et al*., 2006; Markkula *et al*., 2009; Jokinen *et al*., 2011)**, have varying WBC counts. In order to properly establish the process of UVA-mediated immunosuppression, the mechanisms behind these phenomena should be further investigated in fish. Leukocytes are one of the immune system's basic responses, according to **Castro-Pérez (2004).** Perhaps the kidney and spleen are directly harmed by these changes in a toxic way (hematopoietic tissue). In the group exposed to UVA, the addition of curcumin and ginger restored normal WBC, lymphocyte, neutrophil, and eosinophil counts. According to **Jagetia and Aggarwal (2007)**, curcumin enhances innate immunity by activating neutrophils and macrophages. Additionally, according to **Antony *et al*. (1999)**, curcumin controls immunological responses by raising WBC numbers. In *Lates calcarifer* (**Talpur *et al*., 2013)**, *Oncorhynchus mykiss* **(Nya and Austin, 2009)**, and *Huso huso* **(Gholipour Kanani *et al*., 2014)**, a considerable increase in the leukocyte counts following ginger administration has also been documented.

Before environmental contamination affects fish health, it is possible to detect its effects using serum biochemical variables (also known as biomarkers) **(Hamed and Osman, 2017)**. The most significant and numerous macromolecules in living things, proteins are crucial for cellular metabolism **(Alberts *et al*., 2002)**. Blood protein concentration is a useful method for evaluating the physiological, biochemical, and

ecological conditions of fish. It is crucial for the movement of several metabolites and exogenous substances and safeguards the organism from pathogens, parasites, and xenobiotics **(Kovyrshina and Rudneva, 2012)**. However, the group that had been exposed to UVA showed a considerable rise in total protein. After fish exposure to UVA, a similar finding was confirmed by **Ibrahim *et al*. (2019a)** and **Osman *et al*. (2010)**.

Animals rely on glucose for energy, and it has long been used to detect stress in fish brought on by chemicals and environmental variables **(Ibrahim *et al*., 2013)**. Here, the group that was exposed to UVA showed a considerable drop in glucose levels. The results of **Ibrahim (2015)** and **Sayed *et al*. (2007)** on *C. gariepinus* after exposure to UVA provide credence to the current finding. The addition of ginger and curcumin, however, restored normal total protein and glucose levels in the UVA-exposed group. Additionally, prior research has demonstrated that feeding Nile tilapia fish curcumin at levels of 5, 10, or 20 g/kg improved hematological parameters, serum total protein, liver heat shock protein, glycogenesis, and bactericidal activity **(Priyadarsini, 2014; El-Barbary, 2016; Cui *et al*., 2017)**. Other researchers have also shown the beneficial effects of ginger supplementation on the blood total protein, albumin, and globulin in *Lates calcarifer, Danio rerio, Huso huso*, and *Oncorhynchus mykiss* **(Nya and Austin, 2009; Talpur *et al*., 2013; Gholipour Kanani *et al*., 2014; Ahmadifar *et al*., 2019)**.

Lipids, the transient body material, are the source of chemical energy stored in the body of the organism. Lipids thus constitute temporary body material **(Ramadan *et al*., 2011)**. The most important sterol found in plasma and red blood cells is cholesterol **(Ibrahim *et al*., 2019a)**. The data collected showed that following exposure to UVA, cholesterol and triglycerides significantly increased ($P < 0.05$). **Osman *et al*. (2010)** and **Ibrahim (2015)** support our finding; they found similar high cholesterol results in C. gariepinus following UVA radiation exposure. Living organisms need to build up their energy stores in reaction to stress in order to lessen its effects **(Rabasa and Dickson, 2016)**. Weakness in the body was generated as a result of this rise in cholesterol levels, as seen in our study. The disruption of lipid metabolism brought on by UVA radiation exposure may be to blame for this condition **(Ibrahim, 2015)**. However, the cholesterol and triglyceride values in the UVA-exposed group were normalized by curcumin and ginger addition. Curcumin therapy for hypercholesterolemia caused by a high-fat diet Rats had reduced levels of triacylglycerol and serum total cholesterol Hussein et al. (2014). As for the improvement in ginger, a study by Shokr and Mohamed (2019) showed that different doses of ginger caused decreased in cholesterol and triglycerides in the Nile tilapia.

Kidney functions such as urea and creatinine showed a significant increase ($P < 0.05$) in the UVA groups. In addition, **Osman *et al*. (2010)** found that African catfish *C. gariepinus* exposed to UVA radiation for three days and three hours each had a similar pattern of rise in urea and creatinine, and **Kaur *et al*. (2012)** found that *O. niloticus* showed a similar pattern after UVA exposure, suggesting nephrotoxicity. Additionally,

the elevated creatinine found in this study may be a result of the renal damage caused by UVA radiation. **Abcar *et al*. (2004)**, who stated that a rise in the blood creatinine level is a sign of compromised renal function, endorsed this point of view. In those exposed to UVA, however, the addition of curcumin and/or ginger corrected the levels of urea and creatinine in our study. The ameliorating effects of curcumin supplementation on renal markers as creatinine value were studied by **Abdelkhalek *et al*. (2021)** in Nile tilapia monosex exposed to fipronil. According to **Olaniyi *et al*. (2020)**, African catfish (*C. gariepinus)* can increase the level of creatinine by consuming 15% ginger in their diet.

Typically, liver function tests are seen as trustworthy indications of hepatic metabolism. One of the most helpful strategies in toxicological investigations is the monitoring of liver enzymes that enter the blood **(Osman *et al*., 2010; Harabawy and Ibrahim, 2014; Ibrahim, 2015)**. By monitoring biochemical parameters and selecting the appropriate biomarkers, it is possible to learn more about tissue damage or organ malfunction and gain insight into the effects of toxins on fish health **(Osman *et al*., 2010; Barhoumi *et al*., 2012; Ibrahim *et al*., 2013)**.

The nonfunctional plasma enzymes aspartic aminotransferase (AST) and alanine aminotransferase (ALT) are typically found within the cells of the liver **(Huang *et al*., 2006)**. They serve as the primary enzymes in the assessment of hepatocellular damage and many hepatic disorders and are sensitive responders to toxins **(Ibrahim and Mahmoud, 2005)**. In the current study, ALT and AST levels following exposure to UVA radiation were found to significantly increase. After three hours for three days of UVA exposure, **Osman *et al*. (2010)** found a similar outcome in the African catfish *Clarias gariepinus*.

Other researchers also observed comparable results for AST and ALT in fish serum, including *O. niloticus* exposed to silver nanoparticles **(Ibrahim, 2020)**, C. gariepinus exposed to silver nanoparticles, arsenic **(Mekkawy *et al*., 2020; Naguib *et al*., 2020)**, *C. carpio* subjected to Chromium, lead **(Parvathi *et al*., 2011; Giri *et al*., 2021)**. According to **Ibrahim *et al*. (2013)**, an increase in liver enzyme activity has been shown to reflect liver damage indicating significant pathological alterations in cell membrane permeability or hepatic cell rupture.

In our investigation, curcumin supplementation effectively restored the changes in liver function markers caused by UVA. Following chromium exposure, *O. niloticus* showed comparable curcumin-induced improvement in liver function markers **(Mohamed *et al*., 2020)**. After CCl4-induced liver damage, dietary curcumin prevents the rise in blood AST and ALT activity in Jian carp **(Cao *et al*., 2015)**. **Olaniyi *et al*. (2020)** concluded that 15% ginger can be included in the diet of African catfish (*C. gariepinus)* to enhance the levels of AST and ALT activity.

The definition of oxidative stress is an imbalance between the generation of free radicals and reactive metabolites and their removal by antioxidant-based defense systems **(Yonar *et al*., 2011; Piner and Üner, 2013)**. Reactive oxygen species are produced when

there are not enough antioxidants, which can lead to oxidative damage **(Yonar, 2013)**. Lipid peroxidation is regarded as an important sign of oxidative damage to cellular constituents **(Ferreira *et al.*, 2005)**. One of the markers of lipid peroxidation can be MDA, which is the primary end product of lipid peroxidation **(Yonar *et al.*, 2014)**.

In the present study, UVA radiation has elevated oxidative stress and compromised antioxidant markers. In all tissues under study, UVA radiation significantly elevated DNA fragmentation and MDA. Similar findings were made by **Ibrahim (2015)** in *C. gariepinus* and **Charron *et al.* (2000)** in zebrafish (*Brachydanio rerio*) who discovered UVBR-induced significantly damaged DNA and protein. LPO products can alter the structural and physical properties of biological membranes **(Charron *et al.*, 2000)**. As opposed to the UVA group, CUR and/or ginger supplementation dramatically reduced the MDA level in the present study. After pb-induced increase in MDA level, dietary curcumin prevents this rise in *C. carpio* **(Giri *et al.*, 2021)**. Effects of dietary ginger also appeared in improving the MDA level of C. carpio reared under high stocking density **(Fazelan *et al.*, 2020)**.

To counteract the detrimental effects of UVR-mediated ROS and safeguard cells from oxidative stress, organisms contain both enzymatic (such as SOD, CAT, and TAC) and non-enzymatic antioxidant defense systems **(Dahms and Lee, 2010; Birben *et al.*, 2012; Rastogi *et al.*, 2014)**. According to earlier studies, fish exposed to UVR can have antioxidant enzyme activities that are unchanged, increase, or decrease, indicating that various fish species employ various methods to minimize oxidative damage **(Charron *et al.*, 2000; Lesser *et al.*, 2001; Boily *et al.*, 2011; Singh *et al.*, 2013)**.

After exposure to UVA radiation, antioxidant enzyme activities (SOD, CAT, and TAC) were typically reduced in all tissues examined in this investigation. **Ibrahim (2015)** discovered that UVA radiation significantly reduced the activity of SOD, CAT, Gpx, GSH, G6PDH, LDH, and TAO in *C. gariepinus*'s gills, kidney, liver, muscles, and skin. Zebrafish exposure to absolute UVB levels demonstrated a decrease in muscle/skin SOD activity, which is in line with our findings **(Charron *et al.*, 2000)**. **Ibrahim (2015)** noticed a reduction in SOD activity in the African catfish's gills, kidney, liver, muscle, and skin after three days of UVA exposure (3 h/ day). However, it was discovered that curcumin might mitigate the oxidative stress and lipid peroxidative damage brought on by melamine in *O. niloticus* (**Abd El-Hakim *et al.*, 2020**). When *C. gariepinus* exposed to cadmium consumes curcumin and black pepper powder, antioxidant activity is restored (**El-Houseiny *et al.*, 2019**).

SOD, GPx, GST, GSH, and G-Rd antioxidant activity in the livers of grass carp infected with Aeromonas hydrophila are increased by dietary curcumin **(Ming *et al.*, 2020)**. This suggests that curcumin encourages antioxidant activity, which may be the route to resistance to oxidative damage brought on by Pb. SOD and CAT activities in C. carpio groups that were fed ginger were significantly higher **(Mohammadi *et al.*, 2020)**.

Ginger is a powerful antioxidant **(Si *et al*., 2018)**, and studies by **Fazelan *et al*. (2020)**, **Sukumaran *et al.* (2016)**, and **Ahmadifar *et al*. (2019)** have shown that ginger has positive effects on fish's antioxidant system. These positive effects are attributed to ginger's presence of bioactive compounds, particularly phenols, tocopherols, and flavonoids **(Dalsasso *et al*., 2022; Kurniasari *et al*., 2022)**.

According to **Chowdhury *et al*. (2021)**, the combination of dietary curcumin and ginger significantly improved hepatic SOD activity in *Labeo rohita* fingerlings. These effects were likely mediated by the bioactive compounds like phenolic, flavonoid, and trace metals (Zn, Mn, and Se) of these herbs **(Ravipati *et al*., 2012)**. In fact, the presence of tocopherols, including a-tocopherol and d-tocopherol, is thought to be the cause of the antioxidant action implicated by ginger **(Sukumaran *et al*., 2016)**.

Oxidative stress can result from an imbalance between the antioxidant defense system and intracellular ROS **(Ibrahim *et al*., 2019b)**. Reduced total antioxidant capacity raises the possibility of oxidative damage **(Ibrahim *et al*., 2021)**. Our findings showed that fish exposed to UVR had lower levels of total antioxidant capacity. African catfish *C. gariepinus* **(Sayed *et al*., 2021)** and *C. carpio* **(Banaei *et al*., 2022)** exposed to MPs both showed a significant decrease in total antioxidants. On toxicity of chlorpyrifos, common carp (*C. carpio*) showed the similar decrease in total antioxidants **(Banaee *et al*., 2022)**. In contrast to the UVA group, curcumin and/or ginger administration significantly elevated total antioxidant capacity levels in the liver and gills. Curcumin when taken as a dietary supplement, it can increase antioxidant capacity by up to 80%. **(Jayaprakasha *et al*., 2006)**. It has antioxidant capabilities because it can interact with free radicals and give them a hydrogen atom **(Wei *et al*., 2006)**. To determine their absorption, metabolism, and biological action in connection to their effects on the fish antioxidant system, as well as to identify the compounds found in ginger and curcumin that are involved in the antioxidant activity in fish, additional research is required.

CONCLUSION

In general, the results showed that O. niloticus exposed to UVA radiation caused hematological and biochemical disturbance with disruption in lipid peroxidation, DNA fragmentation, and oxidative stress in fish tissue. Consequently, our findings provide a clear indicator of *O. niloticus* reaction to UVA exposure. However, most of the previous UVA-induced stress in the treated fish improved significantly with the addition of Curcumin and Ginger. Additionally, Curcumin and Ginger demonstrated complete recovery in UVA-treated groups, demonstrating Curcumin and Ginger's superior efficacy to curcumin or ginger alone in controlling UVA-induced stress. Further research should be done at the receptor and bioinformatics levels to determine how curcumin or ginger shields fish from UVA modification.

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