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Negative impacts of Ultraviolet-A radiation on antioxidant and oxidative stress biomarkers of African catfish *Clarias gariepinus*

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Abstract: The present study was carried out to evaluate the ultraviolet-A (UVA) effects on biochemical, oxidative stress and antioxidant changes using aquatic species. The destructive effects of Ultraviolet-A radiation on the African Catfish, *Clarias gariepinus* was revealed in terms of Carbonyl Protein (CP), Lipid peroxidation (LPO), DNA damage, Super oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (Gpx), Glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), Glutathione (GSH) and Total Antioxidant (TAO) in the gills, kidney, liver, muscles and skin. Also, glucose, total lipid, total protein and cortisol of fish serum were detected. These parameters can be used as biomarkers to identify the negative effects of UVA radiation (20, 60 and 180 min/day) for 3 days in different tissues of *Clarias gariepinus*.

There was a significant decrease in the activity of SOD, CAT, Gpx, GSH, G6PDH, LDH, and TAO in all the examined tissues. The pattern of GR activity in UVA exposed groups showed no significant differences with the control group. However, CP, LPO and DNA damage were increased significantly with exposure periods in all the examined tissues. The exposure to different doses of UVA caused hypoglycaemia, hypolipidimia and hypoproteinimia. Cortisol level showed a significant increase after UV exposure when compared with the control group. In conclusion, UVA within different exposure periods has shown negative effects on blood biochemistry, hormonal and antioxidant capacity of *Clarias gariepinus* tissues.

Keywords: UVA, Antioxidant, Oxidative stress, Catfish

Introduction

The increases in ultraviolet radiation level at the ground caused by reduction of ozone in the stratosphere due to the consequence of human activity, which has both positive and negative effects [1]. One of these negative impacts on the aquatic ecosystems UVR decreased the biomass productivity including fish yields[2]. Ultraviolet radiation is a natural stressor to most forms of life. Recent changes in UVR exposure at both the global and local level have, however, renewed concern regarding the potentially damaging effects of this ubiquitous stressor[3]. Exposure to UVR mainly associated with several damage in cell level to the population level[4]. According to wavelength, UVR could divided into three spectral bands UVA, UVB and UVC with wavelengths (315–400 nm), (280–315), and (100–280 nm) respectively. UVA is only slightly affected by ozone levels[5] and is scattered rapidly in water with biologically effective amounts to at least 100 m depth in clear aquatic environments[6].

Many studies on the negative impacts of UVA radiations have been reported, including skin aging, eye damage, physiology, development and immune suppression[7-10]. In many reports it has been suggested that ultraviolet radiation should be associated with the production of reactive oxygen species (ROS) including free radicals of oxygen and other oxygen-derived compounds. Ultraviolet radiation cannot be classified chemically as free radicals, are prooxidants and are capable of generating free radicals during their metabolism[11]. Free radicals have a negative effect on the cells due to the unpaired electron in its ions such as superoxide, hydroxyl and nitric oxide that cause oxidative damage, which leads cell to death[12]. It has been suggested that under oxidative stress conditions, oxygen radicals such as superoxide anions (O^{2-}) and hydroxyl radical (OH) are produced in biological system[13]. These reactive oxygen species can damage DNA cause mutations and chromosomal damage. It also oxidizes cellular thiols and extracts hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids[14]. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of antioxidant enzymes and other redox molecules. Antioxidant defense system, like

Catalase(CAT), Super oxide dismutase (SOD), Glutathione peroxidase (GPx), Glutathione(GSH), Glutathion (GR), Glucose 6-phosphate Dehydrogenase(G6PDH) and Lactate Dehydrogenase (LDH) are present in all aerobic organisms, whose function is to remove ROS, and protect it from oxidative stress[15]. Oxidative stress may produce lipid peroxidation (LPO) when antioxidant defenses are impaired or overcome[16].

Fish, as bio-monitoring species, plays an increasingly important role in the monitoring of aquatic environments, due to its great sensitivity to environmental changes. The aim of this study was to determine oxidative stress biomarkers and antioxidant parameters as a response to ultraviolet radiation exposure. These parameters were evaluated, oxidative: Cp, LPO and DNA damage, enzymatic antioxidant defense (SOD, CAT, GPx, G6PDH and LDH), non-enzymatic (GR, GSH and TAO), in the gills, kidney, liver, muscles and skin of *C. gariepinus* by UVA radiation dose. Moreover, the effect of UVA on *C. gariepinus,* was evaluated through biochemical parameters (glucose, total protein, total lipids and cortisol) generated by UVA in serum of adult, *C. gariepinus*.

Materials and methods

2.1. Specimen collection

Thirty two fish specimens of adult African catfish *Clarias gariepinus* were bought from faculty of Agriculture farm of fish, Assiut University, Egypt. Fish were transported immediately to the fish laboratory in Zoology department, Faculty of Science, Assiut University. A commercial pellet diet (3% of body weight per day) have been fed to fish (232±7 g) and kept together in 160 L rectangular tanks containing tap water (conductivity: 2000 uS/cm); pH 7.4; oxygen 90–95% saturation; temperature 27 °C; photoperiod 12:12 light:dark). After 2 week acclimation, fish were classified into four groups (eight fish per group): control and 3 UVR-treated groups for 3 days/for 20, 60 or 180 min/day. Doses were chosen as described before[8].

2.2. UVA source

The African catfish *Clarias gariepinus* were exposed to UVA (model UVL-56, long wave UV-366 nm, UVP, Inc. San Gabriel, CA, USA using a 6-W self-ballasted long-wave lamp (365 nm) with input voltage 220 V, 60 HZ) as described before [8]. The UVA source was fitted at 20 cm above the aquarium surface (water level was 50 cm).

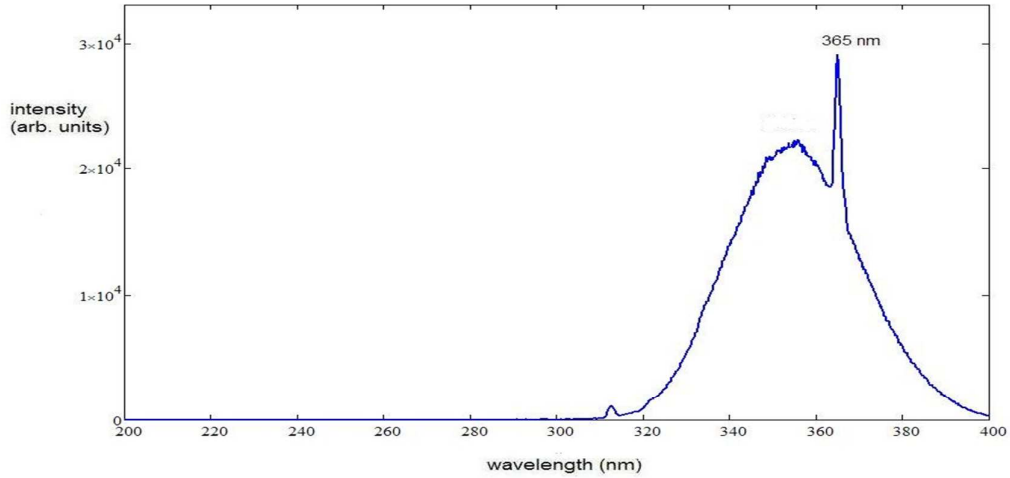

Fig.1 Irradiation spectrum for UV lamp [17]

2.3. Sample preparations

2.3.1 Serum

After 3 days of exposure, blood samples of the control and exposed fish (8 fish/group) were collected and left to coagulate for 15 minutes at 3000 rpm, to separate serum. The fresh serum was subjected to biochemical analysis. Serum glucose (mg/dl), total protein (mg/dl) and total lipids (mg/dl) were estimated using assay kits supplied by Diamond Diagnostic, Egypt.

2.3.2 Hormone assay

Another aliquot of blood samples (2 ml) was collected from the severance of the caudal peduncle and centrifuged (20 min at 5000 rpm), followed by collection of serum, freezing and storing at -70 ºC before hormone analysis. Concentration of cortisol (ng/ml) was determined using competitive chemiluminescent enzyme immunoassay (Immulite 1000, Siemens, Los Angeles, CA). All samples were run in duplicate and assayed at the same time, in a single run with a single lot number of reagents and consumables employed by a single operator, with intra-assay coefficients of variation for all variables less than 5%.

2.3.3 Tissues

Gills, kidney, liver, muscles and skin were carefully excised, surface dried with filter paper, thoroughly washed with 50mM phosphate buffer pH 7.4 and homogenized with 50mM phosphate buffer pH 7.4 containing, 1mM EDTA, 1mM DTT, 0.15M KCl, 0.01% PMSF. Homogenization 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 ◦C. Supernatant was used for antioxidant activities and oxidative stress studies.

2.4 Oxidative stress and Antioxidant biomarkers

2.4.1 Total protein and Lipid peroxidation:

The total protein content was determined as described before [18] using bovine serum albumin (E. Merck-Darmstadt, Germany) as a standard. Lipid peroxidation (LPO) was determined by the procedure of Utley et al.[19]. 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 [20].

2.4.2 DNA fragmentation:

DNA fragmentation was determined according to the procedure of Kurita-Ochiai et al. [21] using a spectrophotometer (Micro Lab 200 Vital Scientific, Dieren, The Netherlands) at 600 nm against a reagent blank. In brief, 0.1 g of tissue was added to 1.0 ml (10% W/V) of buffer (102 mg Tris + 29 mg EDTA + 200 µl triton in 100 ml distilled water). Then the mixture was incubated on ice for 10 min and centrifuged at 8,000 rpm for 10 min. The supernatant was used for the measurement of fragmented DNA while the pellet (P1) was used for the determination of intact DNA. For measuring fragmented DNA, 200 µl of supernatant was added to 200 µl of trichloroacetic acid (TCA), Then centrifuged at 8,000 rpm for 10 min. After centrifugation, 50 µl supernatant was added to 1 ml of diphenylamine

reagent, boiled for 10 min in a water bath and then cooled on ice. The developing blue color was measured at 575 or 600 nm against a blank (diphenylamine solution) to determine the fragmented DNA. For the determination of intact DNA, 1 ml buffer (102 mg Tris + 29 mg EDTA + 200 mg SDS in 100 ml distilled water) was added to the pellet (P1). The mixture was heated in a water bath at 40°C and centrifuged for 10 min at 5,000 rpm. 200 µl of supernatant was added to 200 µl of (TCA) then centrifuged at 5,000 rpm for 10 min. After centrifugation, 50 µl of supernatant was added to 1 ml of diphenylamine reagent, boiled for 10 min in a water bath and cooled on ice. The developing blue color was measured at 575 or 600 nm against a blank (diphenylamine solution). The percentage of fragmented DNA was estimated by the following formula:

Percentage of fragmented DNA = fragmented DNA/ (fragmented + intact DNA) X 100.

2.4.3 Protein carbonyl determination:

Protein carbonyl content was determined by measuring the reactivity of carbonyl groups with 2,4-DNPH, as described by Levine [22]. 0.1 g of tissue was homogenized in 1.5 ml (10% W/V) phosphate buffer (pH 7.4) containing 1.15 % KCl and 3 mmol L-1 EDTA. EDTA was added to prevent further formation of free radicals. Each sample of the homogenate was divided into three aliquots of 0.5 ml. Proteins were precipitated with 10% TCA and centrifuged at 5000 rpm for 10 minutes. One pellet was re-suspended in 1.0 ml of 2 mol L^{-1} HCl (blank), and other two pellets (duplicates) with an equal volume of 0.2 % 2, 4-DNPH in 2 mol L-1 HCl. All samples were incubated at room temperature for one hour.

The samples were precipitated with 10 % TCA and centrifuged as before. The pellets were then washed twice with 1.0 ml of ethanol: ethyl acetate (1:1) to eliminate traces of 2, 4-DNPH. Proteins were finally dissolved in 2.0 ml of 6 mol L^{-1} guanidine HCl, and centrifuged as before. Protein concentration was calculated at 280 nm in the HCl blank using BSA in 6 mol L-1 guanidine HCl as standard. Carbonyl

concentration was determined from the absorbance at 370 nm with the use of a molar absorption coefficient of 22.0 L $mmol^{-1}$ cm^{-1}. The results were expressed as nmol of 2, 4-DNPH bound on mg of protein.

2.4.4. Superoxide dismutase (SOD) activity assay

Cellular total SOD activity was measured following as described by McCord and Fridovich [23] with minor modifications. The activity was measured by monitoring the SOD-induced inhibition of cytochrome *c* reduction by the superoxide radical generated in a xanthine/ xanthine oxidase system. Briefly, the cell pellets were re-suspended in cold 50 mM potassium phosphate buffer (pH 7.5) and sonicated as described above. After protein concentration assay, 30 µg of total protein were added to an assay mixture containing 50 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA, 0.01 mM cytochrome *c*, 0.1 mM Xanthine and 0.003 units of xanthine oxidase in a final volume of 1ml. The rate of increase in absorbance was continuously recorded spectrophotometric ally at 550 nm at 25°C for 7 min.

2.4.5. Catalase activity assay

Cellular catalase activity was measured as it was described by Aebi[12]. Briefly, the cell pellets were re-suspended in cold 100 mM potassium phosphate buffer (pH 7.0) and sonicated with the same procedures as above. After centrifugation, 30 µg of total protein were added to an assay mixture containing 100 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2 in a final volume of 0.5 ml. The decomposition of H2O2 was followed directly by a decrease in absorbance at 240 nm.

2.4.6. Glutathione peroxidase (GPX) activity assay

Cellular total GPX activity was detected using the method delineated by Flohe and Gunler[24]. The assay was based on the GPX catalysis of the glutathione-dependent reduction of hydroperoxides where

reduced glutathione is converted to GSSG (oxidized form). GSSG is reduced to GSH by NADPH in the presence of glutathione reductase. NADPH is continuously oxidized to NADP+ while the GSH concentration was maintained. The reactions are as follows: 30 µg of total protein were added to 50 mM potassium phosphate buffer (pH 7.0) containing 1mM EDTA, 1mM sodium azide, 0.27 unit glutathione reductase and 1 mM GSH. The mixture was incubated for 3 min at room temperature and 20 µl of 3 mM NADPH dissolved in 0.1% NADPH was added. The hydroperoxide-independent NADPH consumption was read at 340 nm for 3 min as a baseline. Then 25 µl of 2.5 mM H2O2 was added through mixing. The decrease in absorbance was detected for another 5 min.

2.4.7. Glutathione (GSH) level assay

The cellular GSH level was measured following the methods described by Cohn and Lyle [25]. The cell pellets were resuspended in 200 µl ice-cold ddH2O and sonicated for 30 seconds. Afterward, 50 µl of 25% metaphosphoric acid was added and vibrated for 2 min and then the mixture was centrifuged at 13000 rpm for 20 minutes. Following the step, 20 µl of supernatant was transferred into cuvette containing 2 ml H_2O. Then 500 µl of 0.1 M sodium phosphate (pH 8.0) and 100 µl of 0.1 % o-phthaladehyde (dissolved in methanol) were added and mixed well. The mixture was kept in dark at room temperature for 20 min. The reaction was read at 340 nm.

2.4.8. Glutathione reductase (GR) activity assay

The GR activity assay was based on that described previously by Styblo and Thomas[26], which involves the GSSG-dependent oxidation of NADPH. Briefly, the cell pellets were re-suspended in cold 100 mM potassium phosphate buffer (pH 7.0). After sonication and centrifugation as described, 30 µg of total protein was added to 100 mM potassium phosphate buffer (pH 7.0) containing 1mM EDTA and 0.1mM oxidized GSH (GSSG). The reaction was initiated by the addition of 0.2 mM NADPH and detected by the decrease in the absorbance at 340 nm by spectrophotometer.

2.4.9. Glucose-6-Phosphate dehydrogenase (G6PDH) and Lactate Dehydrogenase (LDH)

The enzymatic activities of G6PDH (Cat. No. PD 410)and LDH (cat. No.: LD401)were estimated according to the procedure of Anosike and Ejio using a commercially available kit (Randox Lab. Ltd.)

2.4.10. Total Antioxidant (TAO)

The TAO was measured using a colorimetric assay (Cat. No. NX2332) (Randox Laboratories, Crumlin, U.K.). Values are expressed as mmol/L.

2.5. Statistical analyses

Data are expressed as mean ± Std. Err. Statistical significance was evaluated by ANOVA. Differences were considered significant at $P<0.05$ using the statistical software SPSS version 16.

2.6. Ethical statement

Under the accordance of Egyptian laws and University guidelines for the care of experimental animals, all experiments were carried out. All procedures of the current experiment have been approved by the Committee of the Faculty of Science of Assiut University, Egypt.

Results

As indices of antioxidant status, the levels of CP, DNA fragmentation and LPO (assessed by MDA content), SOD, CAT, GPx, G6PDH, LDH, GSH GR and TAO activities were measured for evaluating the presence of oxidative stress in adult *Clarias gariepinus* after exposure to 20, 60 and 180 min/day UVA radiation for 3 days. The biomarkers of oxidative stress and antioxidant analyzed showed significant variations ($p<0.05$) when compared with control. The antioxidant activities were inhibited in a dose dependent manner in UVA treated groups, and significantly decreased in fish dosed for 60 and 180 min/day, respectively. In groups that exposed for 20 min/day, all tissues showed no significant

inhibition detected when compared with control group. The values of CP, MDA and DNA fragmentation showed a significant increase in a dose-dependent manner with an upward trend in exposure groups.

Oxidative stress and antioxidant biomarkers

Lipid peroxidation is indicated by the presence of malondialdehyde (MDA) in tissues. LPO levels showed highly significant increases ($p<0.001$) in liver, kidney and gills when compared with control tissues after exposed to 60 and 180min/day (Fig.2A).

DNA fragmentation percentages of skin and muscles were not significant after they were exposed to 20 min/day of UVA when compared with control. Different tissues that were exposed for 60 and 180 min/day were significantly increased ($p<0.05$) when compared with control (Fig.2B).

Carbonyl protein levels of gills, kidney, skin and muscles weren't significantly different after fish were exposed to 20 min/day of UVA when compared with control. Carbonyl protein levels in all tissues except skin from fish that were exposed to 60 and 180 min/day were significantly increased ($p<0.05$) when compared with control (Fig.2C).

With the exception of kidney, gills and muscles after exposure to 20 min/day, TP concentrations were significantly lower ($p<0.05$) in groups that were exposed to 20, 60 and 180min/day when compared with control tissues (Fig.2D).

Activities of enzymatic antioxidants like SOD, CAT and GPx significantly decreased ($p<0.001$) in groups exposed to 60 and 180 min/day when compared with control (Fig.3A, B & C). However, these parameters were not significantly different from control among fish exposed for 20 min/day, except SOD for liver ($r=0.043$) and kidney ($r=0.041$) when compared with control tissues. Glucose-6-phosphate dehydrogenase and LDH activities significantly decreased ($p<0.001$) in groups that were exposed to 60 and 180 min/day, in different tissues under investigation (Fig.3 D&E). However, both of these parameters were not significantly different from control after exposed to 20 min/day radiation in different tissues except for LDH in muscles.

The non-enzymatic antioxidant GSH was significantly decreased ($p<0.05$) in groups exposed to 60 and 180 min/day when compared with control (Fig.4A). Except for GSH in the skin and kidney, the level in fish exposed to 20 min/day was not significant. No significant variations were recorded in GR between control and groups exposed to different doses of UVA in all tissues (Fig.4B). Total Antioxidant showed the highest activity in control when compared to exposed groups (20, 60 and 180 min/day). All tissues under investigation showed a highly significant difference ($p<0.001$) when compared with control tissues (Fig.4C).

Serum biochemistry

The exposed fish to 60 and 180 min/day of UVA, showed a significant decrease ($p<0.05$) in glucose (hypoglycemia), total lipid (hypolipidimia) and total protein (hypoproteinimia) concentration in serum (Table 1). However, these parameters were not significantly different from control at ($p<0.05$) for the group exposed to 20 min/day.

Also, a significant increase in cortisol level in fish serum was noted in 60 and 180 min/day UVA exposed groups when compared with control. However, the exposed group to 20 min/day wasn't significant at ($p<0.05$) (Table 1).

Discussion

In the present study, increased UVA radiation has increased oxidative stress and impaired antioxidant parameters. UVA exposures of 60 and 180 min/day significantly increased MDA (except skin) and DNA damage in all tissues under investigation except the low dose 20 min/day. Similar results were obtained in zebrafish (*Brachydanio rerio*) by Charron, et al. [27] who found highly damaged DNA and protein from UVBR. The structural and physical characteristics of biological membranes can be modified by LPO products (reviewed in reference 28). For example, in mouse, incorporation of ROS like LOOH decreased the fluidity and increased the permeability of membranes and similar

changes in permeability after lipid peroxidation may occur in other systems like *Clarias gariepinus*[28]. One of the mechanisms in teleost osmoregulation is the selective membrane permeability[9, 10].

During interaction of ROS and lipid peroxidation products with proteins, carbonyl derivatives are formed. Modifications affect protein functions and may lead to protein accumulation and further disruption of normal cell activities[29]. The present study showed a significant increase in carbonyl protein in all tissues except skin. These results were in agreement with previous results[30-32].

The present findings indicated that toxic manifestations induced by ultraviolet radiation may be associated with the enhanced production of reactive oxygen species (ROS), which give an explanation of the multiple types of toxic responses, among which is oxidative damage of tissues and cellular macromolecules. Although it is the least reactive of the ROS, H_2O_2 diffuses throughout mitochondria and crosses cell membranes, enabling it to inflict many types of cellular injury [33].

Oxidative stress in tissues can be initiated by UVA to form ROS through a series of redox reactions[34]. SOD activities significantly decreased after exposure of UVA doses, suggesting a UVA-mediated increase in tissue $^1O_2$ levels. The continued downward trend in SOD activity after exposure of UV-A treated groups support the hypothesis of loss of function as observed in other non-antioxidant enzymes[35, 36]. Pence and Naylor [37] observed a continual significant decrease in epidermal SOD activity in hairless mise. The majority of UVA energy is absorbed by unidentified photosensitizers in murine fibroblasts which are postulated to generate reactive oxygen species (ROS)[38]. The activities of superoxide dismutase and catalase in maize were decreased by increased UV-B radiation[39]. Also, the decrease of SOD or CAT activity in the freshwater fish *Oreochromis niloticus* might be an indicator of damage in the antioxidant mechanisms caused by ROS that formed from metal exposure[40]. In a study on the freshwater cladoceran *Daphnia magna*, Barata et al.[41] concluded that redox cycling toxicants may induce different antioxidant/prooxidant responses depending on their ability to produce ROS and the response of the antioxidant system could differ when organisms are exposed to metals and some other factors.

Catalase activity was significantly decreased in different tissues after exposure to UVA. The lowest CAT activity values were observed in muscles and skin, however the highest was in the gills. Catalase activity showed a positive correlation with SOD (R=0. 894) and GSH (R=0. 876). Similar results were obtained by Chen [13] for apical meristem of *Isatis indigotica* plant seedlings after exposure to UV-B. Otto and Moon [42] reported the highest catalase activities in liver tissue of brown bullheads (*Ameiurus nebulosus*).

In the present study, the observed decrease in GPx activity in UVA exposed tissues was found to parallel the increase in TBARS. GPx activity showed a highly significant negative correlation with LPO (R=-0.509). The observed drop in GPx activity could perhaps be a result of increased degradation of the enzyme for increased phospholipid hydro peroxide glutathione peroxidase (PHGPx) synthesis, an enzyme that catalyzes the repair of peroxidized phospholipids[43]. In addition, a ROS-mediated oxidation of amino acid side-chains could result in a conformational change in GPx leading to loss of function due to a temporary overwhelming of tissues with ROS.

Elevated oxygen consumption in fish could result in higher ROS perhaps contributing to the elevated enzyme activity observed by Peters and Livingstone [44]. Also, it is possible that excess cytosolic GSSG could have been transported to other locations tissues or by the formation of mixed disulfides with proteins thus leading to the observed significant reduction in GSH in tissues under investigation. In addition to GSH directly reducing ROS including singlet oxygen ($^1O_2$), HOx and O_2, GSH also acts as a substrate for GPx in the scavenging of H_2O_2[28].

The present study, has observed a significant decrease in LDH and G6PDH between control and different exposure periods, maybe due to the inactivation of LDH and G6PDH enzymes. A few studies on human lens tissue also referred to such UV-induced inactivation of G6PDH[45, 46]. The G6PDH has a significant positive correlation with LDH activities (R=0. 981) and significant negative correlation between SOD and both LDH (R=-0.405) and G6PDH (R=-0.206). However, GR showed a significant negative correlation with G6PDH (R=-0.226).

The total antioxidant capacity of different tissues, mainly obtained from the diet, decreased with the exposure times. Zamzow [47] noted that the skin is the first defense against ultraviolet radiation in *Clarias gariepinus*. The total antioxidant capacity showed a highly positive correlation with SOD, CAT, GSH and GPx. LDH is generally associated with cellular metabolic activity [10]. Such activity is inhibited under stress, especially after exposure to UVR[9, 10]. Inhibition of the enzyme activity may be due to the formation of an enzyme-inhibition complex, to ion imbalance or to the intracellular action of metal subsequent to initial plasma membrane damage [10].

The present study, showed a significant decrease in total protein concentration in tissues of fish that were exposed to UVA. Sayed, et al. [8] found the same observation in *Clarias gariepinus* after exposure to UVA. Also, the significant decrease in total protein in Rainbow Trout and European Carp (*Cyrinus carpio*) exposed to UVB were noted by Markkula, et al. [48]. The negative impacts on appetite caused by any stress decreases the protein growth of fish by stimulating catabolism[49], or energy allocation to digestion [50]. Atlantic Salmon exposed to UVB showing a build-up of catabolic substrates [51], and decreased feeding in Coho Salmon (*Oncorhyn chuskisutch*) [52] suggest that fish exposed to UVR are more quiescent.

The present study, showed a significant decrease in glucose (hypoglycemia), total lipid (hypolidimia) and total protein (hypoproteinimia) concentrations in serum of fish that were exposed to UVA. Sayed, et al. [8] found a similar observation in *C. gariepinus* after exposure to UVA.

The significant increase in cortisol level in fish serum was noted in this study. Jokinen, et al. [53] observed an increase in cortisol after exposure of Atlantic Salmon, *Salmo salar*, to UV-B. Also, Salo, et al. [54] observed that exposure to UVB radiation causes physiological stress manifested in changes in blood cortisol levels. The cumulative physiological stress may be attributed to repeated acute disturbances of corticosteroids in fish [55]. The inhibition of immune function and disease resistance may be caused by the chronic increases in cortisol levels [56]. We could conclude that high exposure doses of

UVA had a negative impact on the antioxidant system of fish; also, UVA decreased antioxidants and increased oxidative stress due to the formation of free radicals in fish tissues.

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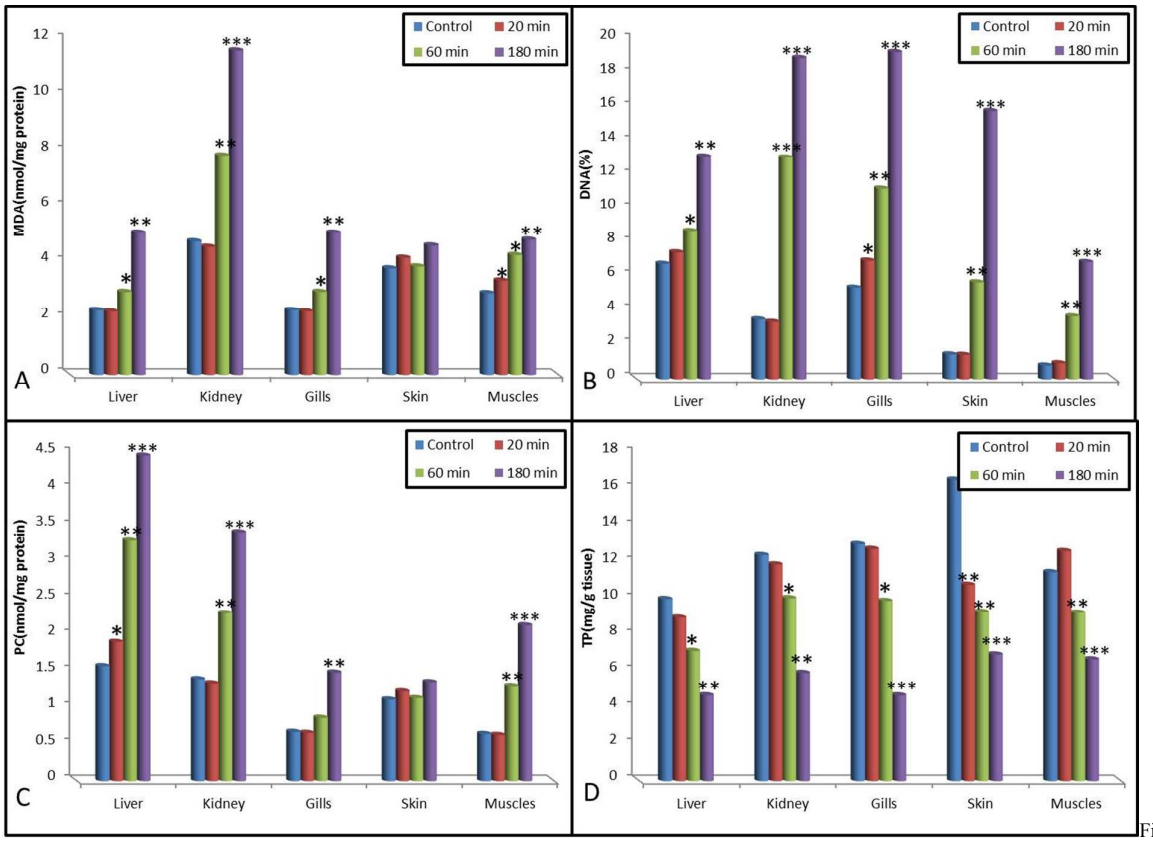

Fig.2.Levels of Oxidative stress biomarkers (A): lipid peroxidation, (B): DNA fragmentation, (C): Carbonyl Protein and (D): total protein in liver, kidney, gills, skin and muscles of C. *gariepinus* exposed to UVA (20, 60 and 180 min/day) for 3 days.
* Significant differences between treatments and control of Oxidative stress biomarkers ($p < 0.05$).
** and *** Significant differences between treatments and control of Oxidative stress biomarkers activities ($p < 0.001$).

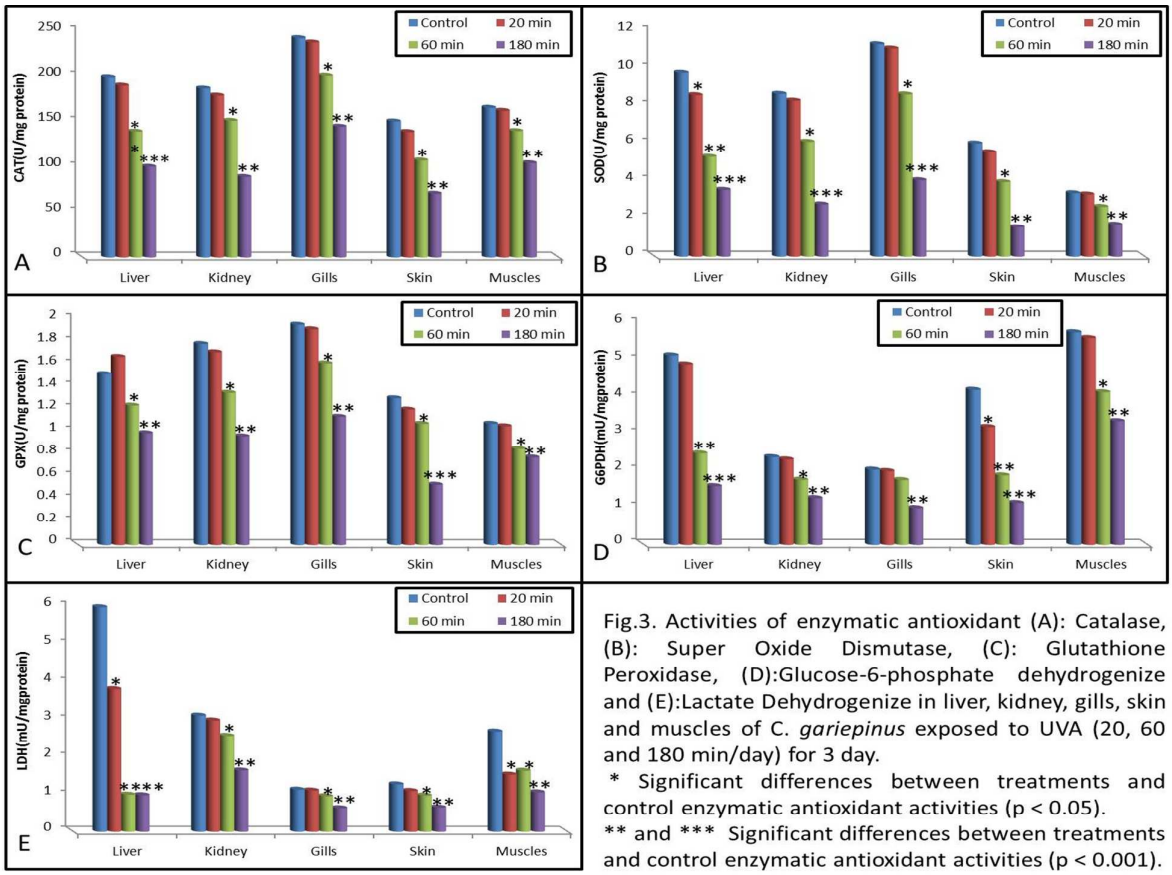

Fig.3. Activities of enzymatic antioxidant (A): Catalase, (B): Super Oxide Dismutase, (C): Glutathione Peroxidase, (D):Glucose-6-phosphate dehydrogenize and (E):Lactate Dehydrogenize in liver, kidney, gills, skin and muscles of C. *gariepinus* exposed to UVA (20, 60 and 180 min/day) for 3 day.

* Significant differences between treatments and control enzymatic antioxidant activities ($p < 0.05$).

** and *** Significant differences between treatments and control enzymatic antioxidant activities ($p < 0.001$).

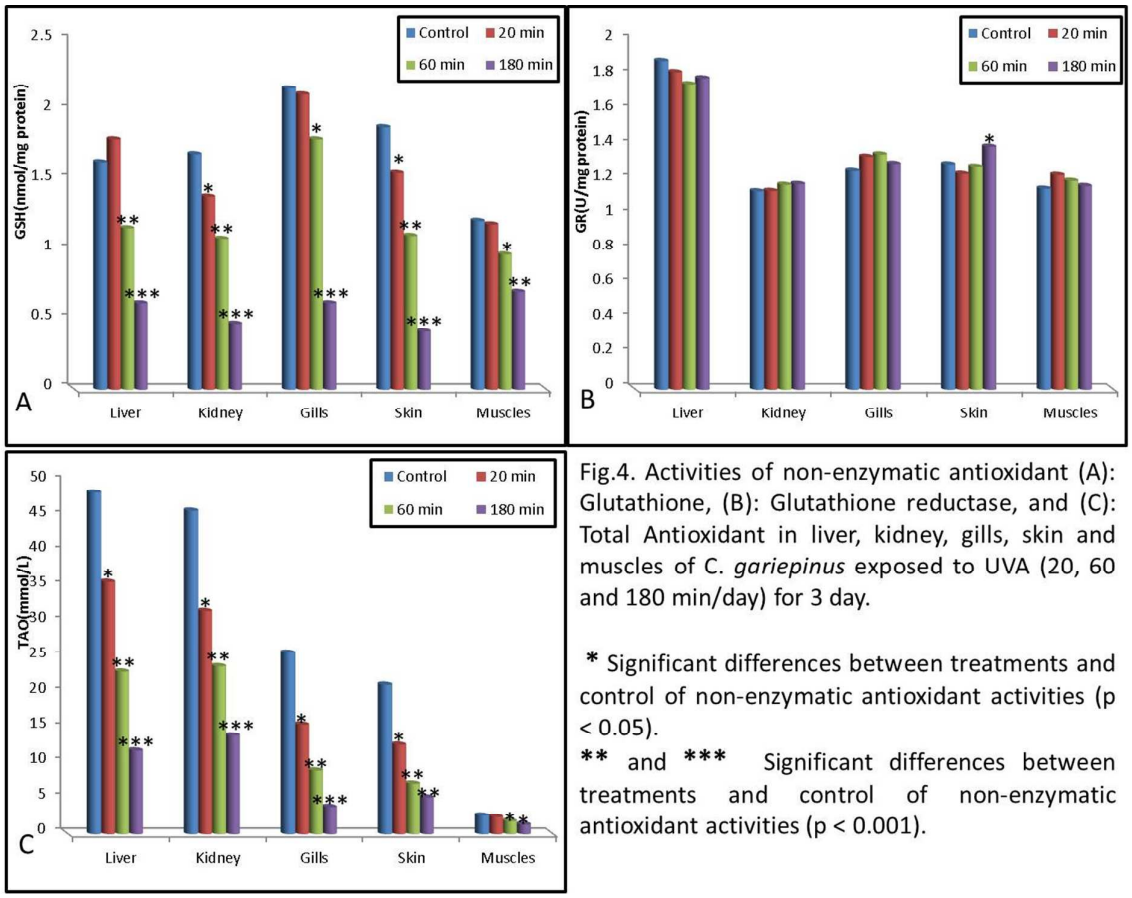

Fig.4. Activities of non-enzymatic antioxidant (A): Glutathione, (B): Glutathione reductase, and (C): Total Antioxidant in liver, kidney, gills, skin and muscles of C. *gariepinus* exposed to UVA (20, 60 and 180 min/day) for 3 day.

* Significant differences between treatments and control of non-enzymatic antioxidant activities ($p < 0.05$).

** and *** Significant differences between treatments and control of non-enzymatic antioxidant activities ($p < 0.001$).

Table 1 Changes in serum content of Total protein, Glucose, Total lipid and cortisol of *Clarias gariepinus* treated with different doses of UV-A radiation. Data are expressed as means± Std. Err.

Treatment	Control	20 min	60 min	180 min
Glucose (mg/dl)	126.15 ± 2.86^{A}	115.42 ± 2.62^{A}	77.22 ± 1.65^{B}	45.24 ± 1.02^{C}
Total Lipid (mg/dl)	105.51 ± 2.25^{A}	95.78 ± 2.47^{A}	53.94 ± 0.67^{B}	18.34 ± 0.23^{C}
Total Protein (mg/dl)	6.11 ± 0.05^{A}	5.55 ± 0.10^{A}	3.65 ± 0.03^{B}	2.48 ± 0.14^{C}
Cortisol (ng/ml)	8.42 ± 0.19^{A}	9.20 ± 0.21^{A}	11.73 ± 0.27^{B}	19.38 ± 1.22^{C}

Different letters show significant differences from the corresponding control group, $P < 0.05$.