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Hematotoxic and Genotoxic Potential of Ultraviolet-A Radiation on the African Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract: Ultraviolet-A radiation (320-400 nm) is scattered rapidly in water with biologically useful amounts to at least 100 m deep in clear aquatic environments. The present study aimed to elucidate the hematotoxic and genotoxic potential of UVA on the African catfish, *Clarias gariepinus* by investigate the impact of different UVA doses (3 h for 3 days and 5 h for 3 days exposure) on the hematological parameters, biochemical variables, micronuclei and binuclei formation. In the present result, a significant ($p<0.05$) decrease in Red Blood Cell counts (RBCs), Hemoglobin concentration (Hb) and Hematocrit value (Ht) was recorded in the groups of fish exposed to UVA comparing to the control groups. Such decrease was significantly ($p<0.05$) increased with the increasing of exposure time. The exposure to different doses of UVA induced marked red cell shrinkage (increased Mean Cell Hemoglobin Concentration (MCHC)) and showed an elevation in Mean Cell Volume (MCV) and Mean Cell Hemoglobin (MCH) in the blood of the exposed fish comparing to the control ones. Concerning to the total White Blood Cell count (WBCs), a significant ($p<0.05$) reduction was recorded in the blood of exposed fish comparing to the control. The biochemical parameters (blood glucose, total plasma protein, blood cholesterol, plasma creatinine, Aspartic Amino Transferase (AST) and Alanine Amino Transferase (ALT) exhibited a significant increase in the blood of fish exposed to different doses of UVA. The groups exposed to UVA subjected to the Nicronuclei (MN) and Binuclei (BN) tests showed a statistically significant increase ($p<0.05$) of MN and BN frequencies with the increasing of exposure time. In conclusion, the results confirmed the sublethal effect of UVA on *C. gariepinus* by using a set of hematological and biochemical parameters. The recorded changes in the hematological and biochemical parameters and the formation of MN and BN in the blood of the exposed fishes revealed the hematotoxic and genotoxic effect of UVA. The selected biochemical parameters could be effectively used as potential biomarkers of UVA toxicity to the freshwater fish in the field of environmental biomonitoring and they could be ranked as possible biomarkers of pollution. It is concluded that the fishes can effectively used as monitors of water quality with respect to radiation.

Key words: UV, hematological parameter, African catfish, micronuclei, genotoxic potential, hematotoxic stress

INTRODUCTION

Studies of environmental stressors have traditionally included human-produced contaminants such as pesticides, metals and industrial chemicals but have come to include anthropogenic changes to natural features of environments like temperature, salinity and ultraviolet radiation (Mahmoud *et al*., 2009; Mekkawy *et al*., 2009). The reduction of ozone in the stratosphere as a consequence of human activity led to an increase in the level of Ultraviolet Radiation (UVR) at the ground. Ultraviolet radiation is part of the spectrum of electromagnetic radiation emitted by the sun. It is arbitrarily divided into 3 categories of different wavelength: Ultraviolet A (UVA) 400-320 nm, Ultraviolet B (UVB) 320-290 nm and Ultraviolet C (UVC) 290-200 nm and has long been known to cause adverse effects to aquatic organisms (Dong *et al*., 2007). Plenty of evidence has been gathered concerning the harmful effects of exposure of fish even to current levels of UV radiation (Sjobeck *et al*., 1984) including a destruction of the fish immune system (Cox *et al*., 1995; WHO, 1994) and alteration of the biochemical, hematological and histopathological characteristics of fishes. The role of UVA radiation as a noxious environmental agent has been much less studied despite the fact that it comprises

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the main component of solar ultraviolet radiation and has greater penetration in water than UVB radiation (Mekkawy *et al*., 2009; Sayed *et al*., 2007). Ultraviolet-A radiation (320-400 nm) is scattered rapidly in water with biologically useful amounts to at least 100 m deep in clear aquatic environments (Sayed *et al*., 2007).

Hematological parameters are closely related to the response of the animal to the environment (Fernandes and Mazon, 2003) and used as reliable indicators of fish health status to detect physiological changes following different stress conditions (Blaxhall and Daisley, 1973). Hematological indices is important for toxicological research, environmental monitoring and as indicators of disease and stress (Blaxhall and Daisley, 1973). Many studies have demonstrated changes in blood variables as a result of environmental conditions and presence of contaminants (Houston and Bedard, 1994; Zbanyszek and Smith, 1984). In addition to the hematological parameters, biochemical variables are of fundamental importance in the physiopathological evaluation of animals (Osman *et al*., 2010b). Biochemical parameters were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances.

The Micronucleus (MN) test is the most widely applied method since it detects the genotoxicity of a wide range compounds especially with fish as bioindicators (Cavas and Gazukara, 2005; Cavas *et al*., 2005; Heddle *et al*., 1991; Russo *et al*., 2004). Some of the advantages of the micronucleus test are its simplicity, reliability and sensitivity (Ayllon and Garcia-Vazquez, 2000). Nuclear Lesions (NL) including Binuclei (BN) is genotoxic analogues of micronuclei that may also be the result of the action of a genotoxic agent (Ayllon and Garcia-Vazquez, 2000; Osman *et al*., 2010a). These lesions have been considered to be of genotoxic origin by some authors (Metcalfe, 1988; Pacheco and Santos, 1997, 1998, 1999) and used by others (Metcalfe, 1988; Pacheco and Santos, 1997) as a signal of cytogenetic damage in fish species (Osman *et al*., 2010a).

In aquatic ecosystems, fish are regarded as bioindicators of overall system health. Fish can be affected directly or indirectly. The direct effects are initiated at the lower level of biological organization (molecular level). Indirect affects are where the effect is on the food chain and the behavior of the organism. The African catfish *Clarias gariepinus* is among the most widespread freshwater fishes in Africa (Osman *et al*., 2007, 2008a, b). It inhabits tropical swamps, lakes and rivers (Nguyen and Janssen, 2002). The economic importance of this species has increased tremendously in recent years as a result of its extensive use in aquaculture (Nguyen and Janssen, 2002). Besides being an excellent candidate for aquaculture, *C. gariepinus* has also been used in fundamental research and for ecotoxicological studies (Liena *et al*., 1997; Nguyen *et al*., 1999; Olaifa *et al*., 2003; Osman *et al*., 2008a, b). Accordingly, the present study aimed to elucidate the hematotoxic and genotoxic potential of UVA on an economically important African catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Specimen collection: Specimens of adult *C. gariepinus* were collected from the river Nile at Assiut. The fish (250-270 g) were fed on a commercial pellet diet (3% of body weight per day) and kept together in 30 L rectangular tanks containing tap water (conductivity 2000, pH 7.5; oxygen 90-95% saturation; temperature 25°C; photoperiod 12:12 light:dark). After 2 weeks acclimatization, fishes were classified into three groups: control, UVR-treated group (3 h for 3 days), UVR-treated group (5 h for 3 days) (Hakkinen and Oikari, 2004).

UV-A source: The African catfish were exposed to UVA (ULTRA-VIOLET Products, Inc. San Cabrial, CA, model UVL-56) using a 6-W self-ballasted long-wave lamp (366 nm) with input voltage 220 V, 60 HZ. The UVA source was fitted at 20 cm the aquarium bottom (water level was 15 cm) (Hakkinen and Oikari, 2004).

Hematological and biochemical analyses: Blood samples were taken from the caudal vein into heparinized tubes. The whole blood was used for the estimation of Hemoglobin concentration (Hb), Hematocrit value (Ht), Red Blood Cells count (RBCs) and White Blood Cells count (WBCs) immediately.

The reminders of blood samples were centrifuged at 5000 rpm for 20 min to separate the plasma for biochemical analysis. The RBCs, WBCs, Hematocrit (Ht) and Hemoglobin (Hb) were determined by using automated technical analyzer (Mindray Bc-2800). Mean Cell Hemoglobin Concentration (MCHC), Mean Cell Hemoglobin (MCH) and Mean Cell Volume (MCV) were calculated using the formulae mentioned by Dacie and Lewis (1991):

$$MCHC\ (g\ dL^{-1}) = Hb/Ht \times 100$$
$$MCH\ (pg) = Hb/RBCs \times 10$$
$$MCV\ (\mu m^3) = Ht/RBCs \times 10$$

Plasma samples were analyzed for Creatinine (Cr), Aspartic Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), glucose, cholesterol and total protein by kits of SGMitalia Company U.S.A.

Micronuclei (MN) and Binuclei (BN) tests: Blood cells were used for analysis of Micronuclei (MN) and Binuclei (BN) formation as described by Cavas *et al.* (2005) and Osman *et al.* (2010a). Blood samples were smeared on clean microscope slides. After fixation in pure ethanol for 20 min, slides were air-dried and then stained with 5% Giemsa solution for 30 min. Ten slides per treatment were prepared. From each animal, 1000 cells were scored under 1000 x magnification to determine the frequencies of micronucleated and binucleated cells. Coded and randomised slides were scored using a blind review by a single observer.

Scoring criteria for micronuclei: Only the cells clearly isolated from the surrounding cells were scored. According to Cavas *et al.* (2005), the criteria for the identification of micronuclei were as follows:

- MN must be smaller than one-third of the main nuclei
- MN must be clearly separated from the main nuclei
- MN must be on the same plane of focus and have the same colour

Cells with more than four MN were discarded to exclude apoptotic phenomena.

Statistical analyses: All values from chemical analyses are presented as mean±SD. Data obtained from the experiment were subjected to one way Analysis of Variance (ANOVA) test using the Statistical Package for the Social Sciences (SPSS). Means were tested using Least Significant Difference (LSD) test to compare between the hematological and blood biochemistry values between control and treated groups. In all cases, $p<0.05$ was the accepted significance level. For the Micronuclei and Binuclei, the mean±SD were considered. The frequencies of micronuclei and binuclei were expressed per 1000 cells. The patterns of variation (in MN and BN frequencies) due to the exposure time and treatments and their interaction were studied by a one way analysis of variance ANOVA considering non-parametric Bonferroni. Significance was accepted at $p<0.001$.

Ethical statement: All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science of Al-Azhar University, Egypt.

RESULTS AND DISCUSSION

Hematological and biochemical parameters: As a result of the analyses, the differences between the selected

Table 1: Changes in the hematological parameters levels (Mean±SD) in the African catfish *Clarias gariepinus* exposed to UVA 3h, UVA 5h for 3days. Significant differences with the control groups are accepted at $p<0.05$

Parameters	Control	UVA 3h	UVA 5h
RBCs x 10^{12} L^{-1}	5.80±0.16	3.80±0.16*	3.13±0.09*
Hb (g dL^{-1})	13.50±0.41	11.53±0.41*	10.80±0.43*
Ht (%)	41.39±0.55	33.68±0.48*	31.04±0.77*
MCV (μm^3)	87.73±2.02	121.73±0.90*	131.70±0.92*
MCH (pg)	35.00±0.82	34.33±1.25	36.07±1.72
MCHC (g dL^{-1})	30.27±0.52	30.80±0.59	30.80±0.59
WBCs x 10^9 L^{-1}	9.77±0.12	7.27±0.68*	5.07±0.12*
Monocytes (%)	2.33±0.47	2.67±0.47	3.33±0.94*
Eosinocytes (%)	1.67±0.47	2.67±0.47*	3.33±0.47*
Basocytes (%)	0.67±0.47	1.67±0.47*	1.67±0.47*

(RBCs) Red Blood Cells, (Hb) Hemoglobin concentration, (Ht) Hematocrit value, (MCV) Mean Cell Volume, (MCH) Mean Cell Hemoglobin, (MCHC) Mean Cell Hemoglobin Concentration and (WBCs) white blood ells; *Significant comparing to the control at 0.05 levels

Table 2: Changes in the biochemical blood parameters levels (Mean±SD) in the African catfish *Clarias gariepinus* exposed to UVA 3h, UVA 5h for 3 days each. Significant differences with the control groups are accepted at $p<0.05$

Parameters	Control	UVA 3h	UVA 5h
AST (μ L^{-1})	36.00±0.82	54.00±1.63*	63.33±0.94*
ALT (μ L^{-1})	15.67±1.25	49.33±1.25*	53.33±1.25*
ALP (μ L^{-1})	51.67±0.94	49.33±1.25	46.33±1.25*
Creatinine (mg dL^{-1})	0.43±0.12	0.53±0.17	0.87±0.05*
Total protein (mg dL^{-1})	6.33±0.25	7.13±0.13	8.47±0.09*
Total cholesterol (mg dL^{-1})	212.00±0.82	212.00±0.82	217.33±1.25*
Glucose (mg dL^{-1})	116.33±3.68	118.00±0.82	125.00±1.41*

(AST) Aspartic Amino Transferase, (ALT) Alanine Amino Transferase and (ALP) Alkaline Phosphatase; *Significant comparing to the control at 0.05 levels

hematological and biochemical variables of the African catfish (*Clarias gariepinus*) exposed to UVA (3 h), UVA (5 h) for 3 days each were found to be statistically important (Table 1 and 2). The hematological analysis revealed a highly significant ($p<0.05$) reduction in Red Blood Cells (RBCs) count from 13.5 g dL^{-1} in the control catfish (*C. gariepinus*) to 3.8 and 3.1 10^{12} L^{-1} in the fish exposed to UVA for 3 and 5 h, respectively (Fig. 1a). Also a significant decrease was recorded in Hemoglobin concentration (Hb) from 13.5 g dL^{-1} in the control catfish to 11.8 and 10.8 g dL^{-1} in the fish exposed to UVA for 3 and 5 h, respectively (Fig. 1b). Moreover, Hematocrit value (Ht) was significantly ($p<0.05$) reduced from 41.4% in the control catfish to 33.7 and 31.04% in the exposed fish (Fig. 1b). The calculations of the three absolute values of the erythrocyte indices, MCV, MCH and MCHC exhibited significant ($p<0.05$) differences in their values by exposure to UVA when compared to the control groups (Fig. 1c). A significant ($p<0.05$) increase in MCV value (121.7 and 131.7 μm^3 in the blood of fish exposed to UVA (3 h for 3 days) and UVA (3 h for 3 days), respectively) were recorded comparing to the control (87.7 μm^3). The value of MCH was insignificantly increased in the groups exposed to UVA comparing to the control one. Mean Cell Hemoglobin Concentration (MCHC) exhibited

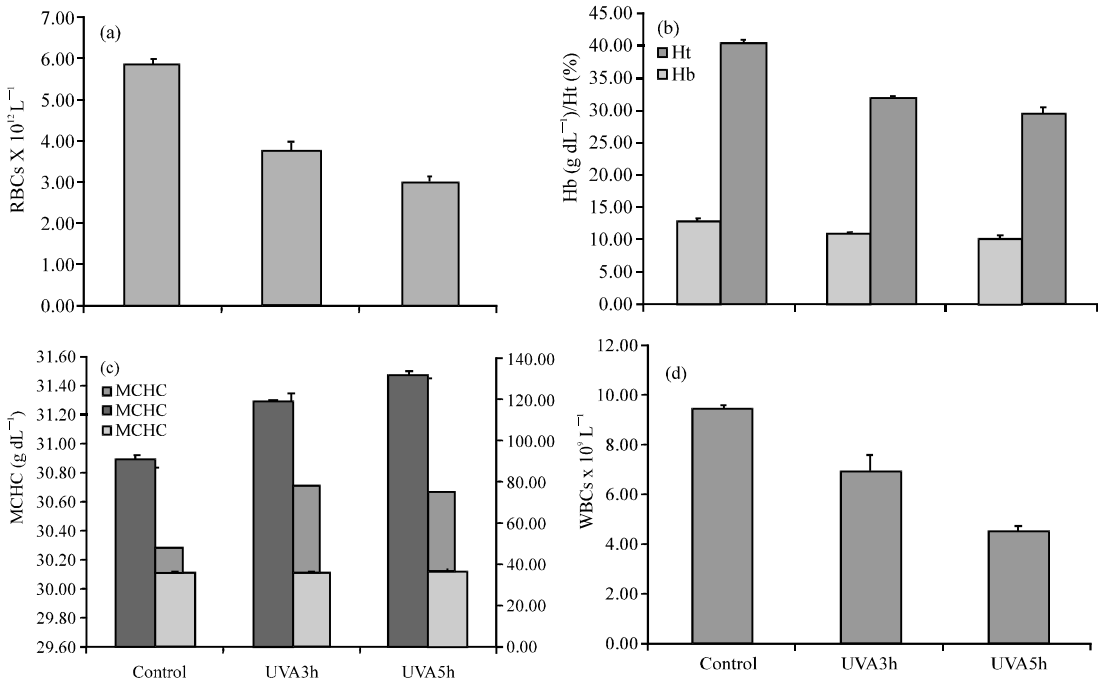

Fig. 1: Hematological parameters: a) Red Blood Cells count (RBCs), b) Hemoglobin concentration (Hb) and Hematocrit value (Ht), c) Mean Cell Hemoglobin Concentration (MCHC), Mean Cell Hemoglobin (MCH) and Mean Cell Volume (MCV) and d) White Blood Cell count (WBCs) in *Clarias gariepinus* after 3 days of exposure to UVA for 3 and for 5 h. Data are presented as the mean±SD. Significant differences with the control groups are accepted at $p<0.05$

an insignificant ($p<0.05$) increase in the exposed groups comparing to the control (Fig. 1c). Total White Blood Cells (WBCs) count was significantly ($p<0.05$) decreased in the exposed groups comparing to the control one. Such reduction was significantly increased with the increasing of exposure time (Fig. 1d). The number of Monocytes, Basocytes and Eosinocytes was increased significantly ($p<0.05$) in the blood of the exposed fishes (Table 1) comparing to the control. The glucose concentration of blood of the African catfish was increased from 116.3 in the control to 118 mg dL^{-1} in the group exposed to UVA (5 h for 3 days) and then 125 in the group exposed to UVA (5 h for 3 days) (Fig. 2a).

Total plasma protein was increased from 6.3 in the control to 7.1 in the group exposed to UVA (3 h for 3 days) and 8.5 in the blood of fish exposed to UVA (5 h for 3 days) (Fig. 2b). Total cholesterol was seemed to be constant between the control and the group exposed to UVA (3 h for 3 days) recording 212 and slightly increased (217.3) in the group exposed to UVA (5 h for 3 days). The changes in Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) and creatinine are shown in Fig. 2a. Data presented in such figure indicated that treatment of *C. gariepinus* with UVA induced a significant ($p<0.05$) increase in AST and ALT with the increasing of exposure time (Fig. 2d).

Table 3: Percentage frequency of Micronuclei (MN) and Binuclei (BN) in erythrocytes of the African catfish *Clarias gariepinus* after exposure to UVA at different exposure time (3h and 5h for three days each)

Groups	MN/1000 cells±SD	BN/1000 cells ±SD
Control	9.3±0.6	22.7±2.1
UVA 3 h	24.7±9.5*	47.7±8.1*
UVA 5 h	45.0±5.0*	88.3±7.6*

*Significant comparing to the control at 0.001 levels, n = 1000

ALP was insignificantly decreased with the increasing of exposure time (Fig. 2e). The concentration of the creatinine was significantly ($p<0.05$) higher in the groups exposed to UVA comparing to the control (Fig. 2f).

Micronuclei and Binuclei formation: As a result of UVA exposure micronuclei and binuclei (Fig. 3) were detected in the erythrocytes of the exposed fishes. The results of the mean and SD of the micronuclei and binuclei frequencies are shown in Table 3. There were statistically significant differences ($p<0.001$) in the MN and BN frequencies between the control group and those exposed

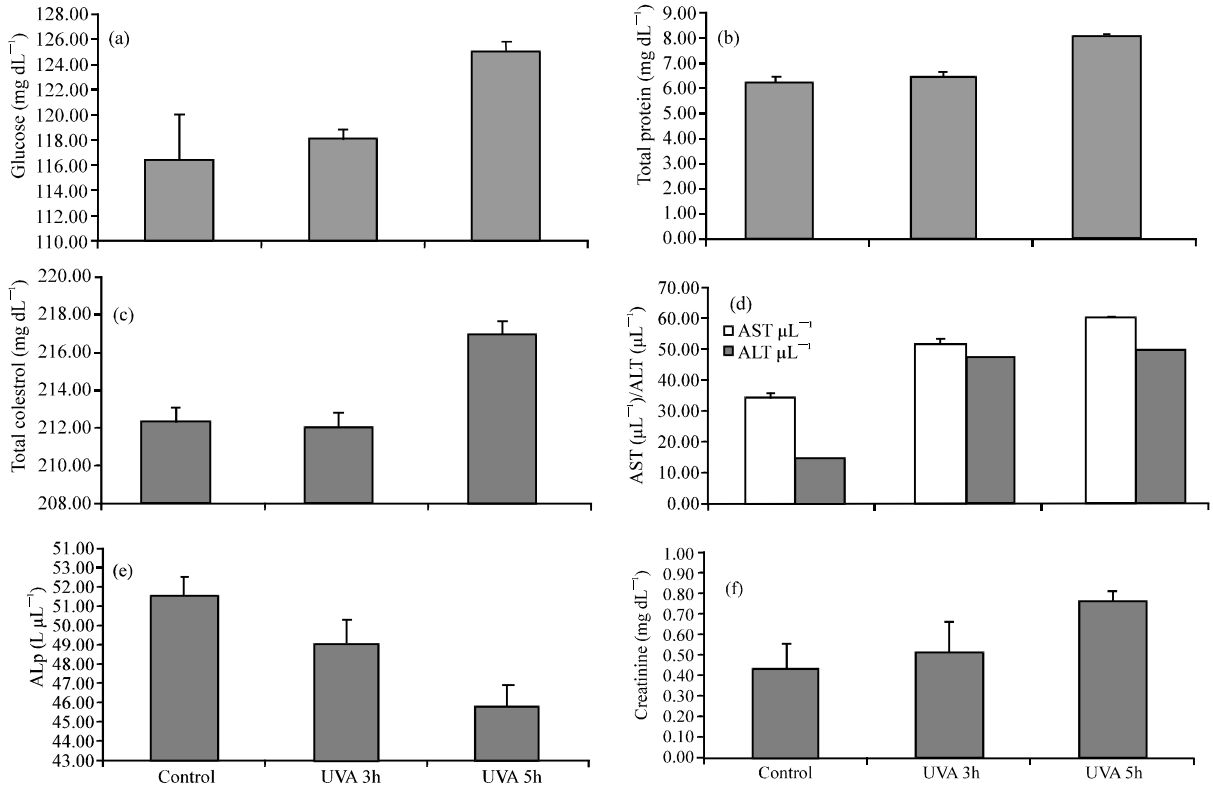

Fig. 2: Biochemical blood parameters: a) glucose, b) total protein, c) total cholesterol, d) aspartic amino transferase (ASL) and Alanine amino transferase (ALT), e) Alkaline Phosphatase (ALP), f) creatinine in *Clarias gariepinus* after 3 days of exposure to UVA for 3 and for 5 h. Data are presented as the mean±SD. Significant differences with the control groups are accepted at $p<0.05$

to different dose of UVA (3 and 5 h for 3 days each) (Table 3, Fig. 4). The frequency of MN in the control group was equal to 9.3 MN/1000 cells. In the exposed fish, the levels of micronuclei frequencies were 24.7 MN/1000 cells and 45 MN/1000 cells in the groupsexposed to UVA for 3h and UVA for 5 h, respectively.

This means the frequencies of micronuclei was significantly ($p<0.001$) increased with the increasing of exposure time. The frequencies of the BN in the control group were 22.7 BN/1000 cells. Significant differences were observed between such control group and the groups exposed to UVA.

The frequencies of the BN in the exposed groups increased significantly ($p<0.001$) with the increasing of exposure time (Table 3, Fig. 4). A correlation was observed between the frequencies of MN and BN in the exposed groups (R^2 0.985, $p<0.001$). The results obtained in this research with the selected UVA doses show that these radiations induced changes in the hematological and blood biochemical values and formation of micronuclei and binuclei which reflect alteration of physiological and cytological state. Changes in the hematological and blood biochemical variables were previously recorded by Osman *et al.* (2010b) after UVA exposure.

Blood parameters can be useful for the measurement of physiological disturbances in stressed fish and thus provide information about the level of damage in the fish (Osman *et al.*, 2010b). The study of blood characteristics may corroborate important subsidies of diagnoses and prognoses of morbid conditions in fish populations and therefore, contribute to better comprehending comparative physiology, phylogenetic relations, feeding conditions and other ecological parameters (Osman *et al.*, 2010b).

Red Blood Cells count (RBCs), Hemoglobin concentration (Hb) and Hematocrit value (Ht) revealed a highly significant reduction in the erythrocytes of catfish exposed to different dose of UVA comparing to the control groups. Similar results were described for *Clarias gariepinus* (Osman *et al.*, 2010b). The reduction in RBCs count, Hb value and Ht in the exposed catfish might have

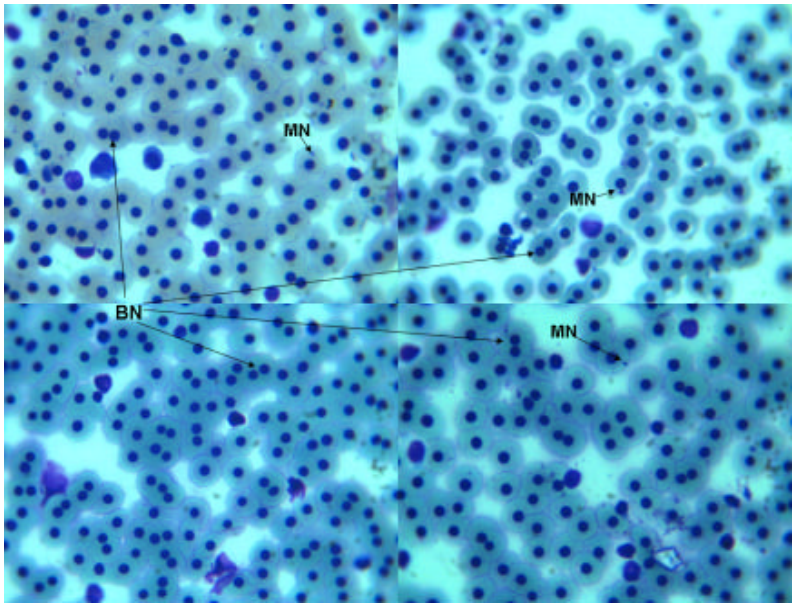

Fig. 3: Micronuclei (MN) and Binuclei (BN) in the erythrocytes of the African catfish *Clarias gariepinus* after 3 days of exposure to UVA for 3 and 5 h

resulted from sever anaemic state. Such decrease in RBCs number, Hb and Ht may be due to hemolysis as a consequence of toxicity or stress (O'Connor and Fromm, 1975). Some researchers suggested that in toxicity experiment the decrease in RBCs, Hb and Ht level could be related to the conditions of confinement or stress induced by the lack of food (Affonso *et al*., 2002). The exposure to UVA for 3 days may lead to suppression in the activity of some hematopoietic tissues which intern led to a reduction in erythropoisis and impeded the formation of RBCs (Osman *et al*., 2010b). The perturbation in these blood indices may be attributed to a defense reaction against toxicity through the stimulation of erythropoiesis. The significant ($p<0.05$) decrease in the Hb concentration may also be due to either an increase in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis. Hematocrit values were previously used as a tool in aquaculture and fishery management for checking anaemic condition (Blaxhall and Daisley, 1973).

The mean haematocrit values in the exposed catfish during the research were ranged from 31-33%. The related decrease in hematological indices proved the toxic effect of UVA that affect both metabolic and hemopoietic activities of *Clarias gariepinus*. The findings show that the exposure to UVA for 3 days induced an elevation in MCV and MCH in the blood of the exposed fish comparing to the control. Such elevation was increased with the increasing of exposure time. Mean cell

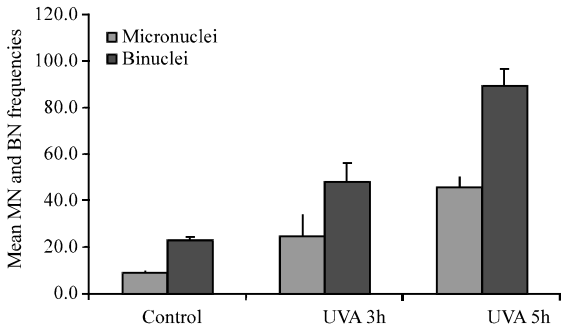

Fig. 4: The frequencies of Micronuclei (MN) and Binuclei (BN) in erythrocytes of the African catfish *Clarias gariepinus* after 3 days of exposure to UVA for 3 and for 5 h

hemoglobin concentration measure was used to assess the amount of red cell swelling (decreased MCHC) or shrinkage (increased MCHC) present (Milligan and Wood, 1982). The present study revealed that exposure to UVA induced marked red cell shrinkage (increased MCHC) and showed insignificant increase of MCH. Concerning to the total WBC, a significant reduction was recorded comparing to the control. The number of Monocytes, Basocytes and Eosinocytes was increased significantly ($p<0.05$) in the blood of the treated fishes. This means, UVA leads to a redistribution of white blood cell, diminishing the total number of lymphocytes in the blood circulation of the exposed fishes comparing to the

control. Glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in plasma. Glucose levels are maintained principally through the conversion of liver glycogen. Blood glucose levels have long been used as indicators of stress in fish. Blood glucose levels were significantly ($p<0.05$) higher in fish exposed to UVA as compared to the control groups.

The level of glucose increased with the increasing of exposure time. Increases in blood glucose levels may be due to increased glucose production or release. This might be due to the vulnerable stress induced by UVA resulted in hyperglycemia. The same results have been recorded in the blood of other fishes exposed to heavy metals and other pollutants (Poleo and Hytterod, 2003; Rosety-Rodriguez *et al*., 2005). In the present research, mean values of total plasma protein were increased significantly ($p<0.05$) in the blood of fish exposed to UVA comparing to the control. The same result was recorded for the protein content of rainbow trout, *Salmo gaivdneri,* following aluminum toxicity (Gross and Wood, 1988). Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Hadi *et al*., 2009; Shalaby *et al*., 2006). Thus, the influence of toxicants on the total protein concentration of fish has been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy (Hadi *et al*., 2009).

Cholesterol is the most important sterol occurring plasma and red blood cells. The cholesterol occurs as white (or) faintly yellow almost odorless granules. In the present investigation, the blood cholesterol level was significantly ($p<0.05$) increased in UVA exposed fish. Triglycerides and cholesterol are known to participate in the rise of total lipid (Osman *et al*., 2010b). The rise of these energy reserves in response to pollution could be due to the fact that excess energy reserves (as glucose, triglycerides and cholesterol) are required by organisms to mediate the effects of stress (Lee *et al*., 1983). Such increase in the levels of cholesterol develops weakness in the body and swimming ability of the fish was observed in the study.

Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate (Osman *et al*., 2010b). In this research, exposure to UVA resulted in a significant ($p<0.05$) increase in the activities of plasma creatinine, AST and ALT as compared with control. AST and ALT belong to the plasma non functional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs (Hadi *et al*., 2009). It is also considered to be important in assessing the state of the liver and some other organs (Verma *et al*., 1981). Their presence in blood plasma may give information on tissue injury or organ dysfunction (Osman *et al*., 2010b). Monitoring of liver enzymes leakage into the blood has proved to be a very useful tool in liver toxic studies (Salah El-Deen and Rogers, 1993).

This rise in creatinine might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism (Hadi *et al*., 2009). ALP enzyme is a sensitive biomarker to metallic salts, since it is a membrane bound enzyme related to the transport of various metabolites (Hadi *et al*., 2009; Lakshmi *et al*., 1991). Osman *et al*. (2010b) reported that the increase in the activity of ALP in blood might be due to the necrosis of liver, kidney and lung.

Micronuclei and binuclei have been assessed in fish as a biological indicator of pollution in wild areas and also for genotoxicity evaluation of physical and chemical agents after direct or indirect exposure *in vivo* (Bahari *et al*., 1994; Nepomuceno *et al*., 1997; Sanchez-Galan *et al*., 1999). They are well established indicators of cytotoxicity and an association between the frequency of such lesions and the exposure to toxic agents have been recorded (Hose *et al*., 1987; Metcalfe, 1988; Pacheco and Santos, 2002; Sanchez-Galan *et al*., 1999). However, to date there is no available literature concerning with the detection of MN and BN after UV exposure on fishes. In the present research, we have described the genotoxic potential of UVA on erythrocytes of the Africa catfish *Clarias gariepinus* for the first time by two complementary tests (MN and BN). The genotoxic effect of some toxic physical and chemical substances has previously been demonstrated in whole blood cells from fishes (Ateeq *et al*., 2005; Ayllon and Garcia-Vazquez, 2000, 2001; Bolognesi *et al*., 2006; Bombail *et al*., 2001; Cavas and Gazukara, 2005; Russo *et al*., 2004), making it possible to compare the results with UVA and evaluate its genotoxic potential. In the present results the African catfish exposed to different dose of UVA subjected to the MN and BN test showed statistically significant ($p<0.001$) differences in MN and BN frequencies with respect to the control ones.

Also, a significant increase ($p<0.001$) of micronuclei and nuclear lesions frequencies were recorded with the increasing of exposure time. A correlation was observed between the frequencies of MN and BN in the groups exposed to UVA ($R^2 = 0.985$ $p<0.001$), suggesting the importance for recording this BN in order to improve the information obtained with MN test. Therefore, the results suggest that the BN found here should be considered indicators of genotoxicity in addition to the MN and should be included in routine tests when fish are employed for toxicological experiments.

CONCLUSION

In this study, the results confirmed the hematotoxic potential of UVA on *C. gariepinus* by using a set of hematological and biochemical parameters. The recorded decrease in the level of hemoglobin, hematocrit and RBC count revealed the hematotoxic effect of UVA. The biochemical parameters (glucose, total protein and cholesterol, AST, ALT) could be effectively used as potential biomarkers of UVA toxicity to the freshwater fish in the field of environmental biomonitoring and they could be ranked as possible biomarkers of pollution.

The genotoxic potential of UVA on erythrocytes of the Africa catfish *Clarias gariepinus* was confirmed here for the first time by two complementary tests (MN and BN). The results suggest that the BN found here should be considered indicators of genotoxicity, in addition to the MN and should be included in routine tests when fish are employed for toxicological experiments. It is concluded that the fishes can effectively used as monitors of water quality with respect to radiation.

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