

The Radioprotective nature of MRN-100 on Nile Tilapia (*Oreochromis niloticus*) under γ -radiation-Induction

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ABSTRACT

Iron is an element that exists in various forms and compositions based on the overall electron charge on Fe-. Relevantly, one of the existent compounds derived from the ferrates group are the hydro ferrates and inclusively encompasses the MRN-100 ionic based compound. MRN-100 is a potent and chemically stable antioxidant compound derived from bivalent and trivalent ferrates (Fe²⁺/Fe³⁺). Potently, the experiment done was factually based on the concept of ionization radiation and the effects rendered on tissue. An analysis and examination of the defensive influence of MRN-100 counter to γ -radiation-induced lethality and reparation to hematopoietic tissues in fish species-Nile tilapia of interest. The modules of the experiment included specimen samples of 216 Nile tilapia (*Oreochromis niloticus*) equally divided

between groups. Group 1 was configured as a control, Group 2 induced to γ -radiation (single dose of 15 Gy), Group 3 and 4 were ethologically pre-treated with doses of 1 ml of MRN-100 per litre of water and 3 ml/L in water for a period of 1 week, and then exposed to radiation, while continuing to receive MRN-100 for 1 week and 4 weeks respectively. The current study discussed parametric analysis of biochemical components in blood serum as well as lipid peroxidation in muscular tissue versus γ -radiation-Induction and MRN-100 effect on radioprotective ability. Conclusion is made that MRN-100 is viably a radioprotective agent in ichthyees/Pisces and validly can be used as a counteract for ionization radiation.

Keywords: *Hydroferrate fluid, MRN-100, Oreochromis niloticus, γ -radiation*

BACKGROUND AND SIGNIFICANCE

Ionizing radiation is defined as the energy resultant from radiation that ideally carries supra-energy enough to free electrons from atoms and/or molecules; the process of freeing electrons results in ionization of compounds, atoms and molecules depending on magnitude and overall charge. There are various ionization radiation sources but of primal interest are ultraviolet, Gamma rays (γ -radiation) and X-rays. There is however potent evidence that ionizing radiation can cause a series of significant damage to macromolecules in cell tissues and holistically degrade and demise of hematopoietic system.

On factual basis of case studies, a number of prospects have been delved into the articulation and enumeration of the ionizing radiation. Moreover, many authors have directed their research plans toward discovering effective treatment for radiation negative effect which able to prohibit or at least

diminish the negative deleterious effect concerning the free radicals that produced by ionizing radiation, the radiation composed of particles, gamma rays or X-rays with enough energy to cause ionization in the medium through which it passes. Ideally, there has been candid research investigation on inferential experimentations deducting relevant and effective radio prospective treatments, the value of such treatment is to contain, manage or expedite the eradication of effects caused by ionizing radiation. The revelations from case studies point out that several agents and compounds exhibit effective radio protective characteristics. Notably, they include the artificially synthetic factors ethiofos and amifostine [4], with some various dietary supplements. Understandably, these primal products provide some source of revitalization in terms of curbing and alleviating the negative impacts resulted from radiation. However, synthetic artificial compounds have been accosted with susceptibility to create poisoning impact in vitro and vivo.

Investigation and insightful inferential analysis was equally done on

some dietary agents that have radioprotective role like genistein [6], arabinoxylan rice bran (MGN-3/Biobran) [5], Panax ginseng [8], bael leaf (*Aegle marmelos*) [7], and eckol (from marine alga) [9]. Further findings indicate that viable antioxidants (vitamin E and vitamin C) can be potent efficient radioprotectors successful in producing a desired or intended result [10].

MRN-100 (hydroferrate fluid), a potent and chemically stable iron-based antioxidant compound originated from trivalent and bivalent ferrates ($\text{Fe}^{2+}/\text{Fe}^{3+}$) exhibited strong antioxidant properties by prohibiting radicalization. On the other hand, fish are used since deductive analysis shows that they offer remarkable modeling avenues for studying radiation impact on hematopoietic tissues. Increased productivity of ROS is primarily the influence parameter of ionization radiation effect in living cells and therefore this case research was angled to test the impact of radioprotective (MRN-100) versus γ -radiation with induction of lethality and haematopoietic tissue hurt [13].

Consciously, due to the properties of preventive radicalization capabilities exhibited by MRN-100, it is essential to study its exhibition as radioprotector versus γ -radiation in ichthyoes (fish), *Oreochromis niloticus* [13].

MATERIALS AND METHODS

1. MRN-100

MRN-100 was prepared in distilled water (DW) with the concentration of Fe^{2+} and Fe^{3+} ions at about 2×10^{-12} mol/l. MRN-100 is obtained from phytosin, a plant extract that contains iron and neutral lipid compounds and can be found in rice, wheat, or radish seeds. First, dispersion of 1 unit of Phytosin is made in the prepared solution of 100ml distilled water and ferric chloride. Ferric chloride is chemically intonated as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. Removal of lipid compound is done via liquid-liquid extraction with the aid of a separation funnel. Residual liquid is filtered and filtrate conjured is respectively evaporated and condensed-water bath. Fractional determination is done to identify the ferrate ions present for generation of MRN-100; delivered by ACM Co Ltd, Japan.

2. Schedule of Radiation

A particular radiation schedule was followed; γ -radiation at a primed value of 4-MCi ^{137}Cs at Department of Radiotherapy, Mansoura University Hospital, Egypt. Dose of 15 Gy given once at a distance of 27cm and subsidiary rate of exposure rate- 200 round/min. throughout the course of radiation, each set was preserved in glass vessels “small and oblong-shaped” containing 2 litres of tap water (aged). after radiation, fish were transferred immediately into 200-L large tanks, and ten percent of the water have been changed once a day till the experiment end. Freshly prepared solution of MRN-100 has been used daily. Fish Survival was observed and checked two times per day at night and morning for 4 weeks after radiation [3].

3. *Oreochromis niloticus*

The ichthyoes ($\sim 50 \pm 15$ g body weight, 6-8 weeks old, length $\sim 16.5 \pm 10$ cm) have been purchased from tilapia fish farm (Kafr El-Sheikh; Egypt) and accommodated for 7 days prior to experiment. The fish were set in tanks (fifty four fish for each tank). Each one contained 2 hundred liters of tap water (aged and dechlorinated). The fish

number for each litre hired in the present study was within the vicinity of another published study [2]. Throughout the experimental period, the fish were outdoors kept. In all aquaria, the water was aerated constantly in a continuous manner. The temperatures were kept at $\sim 22 \pm 2^\circ\text{C}$. Standard laboratory floating pellets were the fish diet twice a day (at eight AM and one PM). The different parameters of the water stayed fixed via the whole experiment; dissolved oxygen (6-8 mg/l), CO_2 (10 mg/l), pH (7.3-8.6), NH_3 (0.02 mg/l), hardness (180 mg/l), alkalinity (150 mg/l), NO_2 (0.01 mg/l) and NO_3 (0.4 mg/l).

4. Experimental Protocol

A total of two hundred sixteen, Nile tilapia were indiscriminately classified to 4 sets (G1-G4) of fifty four fish for each set. Forty seven fish of these were hired for recording daily survival post-exposure to radiation from each group. One week after, five to seven fishes from each group have been utilized for required studies. On the last day of the present study “at four weeks” 5-7 fish from each set were used for follow-up studies. G1 acts as control (neither radiation nor MRN-100 administration);

G2 was exposed to whole-body γ -radiation only; G3 and G4 were treated with doses of one ml and three ml of MRN-100 per litre of water, respectively, for 1 week, and hence subjected to radiation, while continuing to receive MRN-100 (Table. 1). The effect of MRN-100 and radiation on the

survival rate of fish was noticed by recording the dead fish among the 4 groups daily. Biochemical analysis was carried out on the survived fish at one and four weeks post-exposure to γ -radiation. MRN-100 Delivery: Fish at groups 3 and 4 will receive MRN-100 mixed with water (freshly prepared).

Experimental Design					
Groups	Number of fish/group	Feed MRN-100 prior to γ -radiation	γ -radiation Exposure	Week 1 post γ -radiation Exposure	Week 4 post γ -radiation Exposure
G-1	60	No MRN-100	No γ -radiation	1-Record death of fish 2- 10 live fish use for the required studies.	1- Record final survival 2-kill remainder alive for the required studies
G-2	60	No MRN-100	γ -radiation only	1-Record death of fish 2- 10 live fish use for the required studies.	1- Record final survival 2-kill remainder alive for the required studies.
G-3	60	Add 1 ml MRN-100	γ -radiation + MRN-100	1-Record death of fish 2- 10 live fish use for the required studies.	1- Record final survival 2-kill remainder alive for the required studies.
G-4	60	Add 3 ml MRN-100	γ -radiation + MRN-100	1-Record death of fish 2- 10 live fish use for the required studies.	1- Record final survival 2-kill remainder alive for the required studies.

Table 1: Experimental Design for γ -radiation 1.5 k Rad and its Counteract Using MRN-100 in Tilapia

5. Sample collection

5.1. Serum Analysis

7 fishes per group were collected at one and four weeks' post-irradiation and patronized with a mix of clove oil and alcohol for 3 minutes. Fishes were put on a clean towel, then 2 ml blood was

withdrawn using a sterilized syringe from the caudal vein. Two ml blood for each fish was left for coagulation, followed by centrifugation for collection the sera. Serum measurements, including the ratio of Aspartate amino transferase (AST or SGOT), Alkaline phosphatase

(ALP), Albumin, Total bilirubin, Total protein, Creatinine, Urea, Glucose, Calcium, Magnesium, Total lipids, Cholesterol, Glucose-6-phosphatase, Cholinesterase and lactate dehydrogenase (LDH) in accordance with the protocol provided by the kit's manufacturer of SGMitalia Company U.S.A.

5.2. Lipid Peroxidation Analysis

The method of **Ohkawa et al. (1979)** was followed for measured lipid peroxidation. Ten (w/v) tissue homogenate from muscular tissue has been used (this homogenate has 1% v/v dimethyl sulfoxide for preventing further oxidation). 0.2 ml aliquots of tissue homogenate were added to 0.2 ml sodium dodecyl sulfate soln (8.1% w/v) and 1.5 ml acetic acid solution (20%

v/v). The mix was made up to 4.0 ml with distilled water and heated to 95 °C for one hour. Then the samples were cooled, centrifuged at 2000 rpm for ten min, and measured at 532 nm using spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd). The results were expressed as nmol malondialdehyde formation per gram tissue.

6. Statistical Analysis

The results were reported as mean \pm SEM*. The statistical analysis was conducted using Shapiro-Wilk test for assessment of the normality of data, one way ANOVA* with LSD* Post Hoc test, T student test and the Pearson correlation coefficient. Values were considered significant at $P < 0.05$. The software SPSS, version 18 was used.

RESULTS

There was quantitative analysis and inferential study of two statuses: Presence of MRN-100 and absence of MRN-100. Deductive significance was based on the parametric aspects of: Albumin, total protein, bilirubin, Urea, Lipid, Glucose and other biochemical

aspects as well as lipid peroxidation quantitative detection in muscular tissue of *O. niloticus*. The results were reported as mean \pm SEM*. The statistical analysis was conducted by using Shapiro-Wilk test for assessment of the normality of data, one way ANOVA* with LSD* Post Hoc test, T student test and the

Pearson correlation coefficient. Values were considered significant at $P < 0.05$. The software SPSS, version 18 was used. * SEM (Standard Error of Mean); ANOVA (analysis of variance); LSD (Least Significant Difference). The values' significance was based at $P < 0.05$.

Findings projected that there was relevantly stable disparities after 1 week of radiation exposure. G3 and G4 showed primal differences between first and fourth week.

From the comparative deduction analysis done, it is noted that albumin is higher in week 4 than in week 1; this is as per the data with the control group having 5.08 (week 4) and 4.96 (week 1). However, a record drop is registered in G2 (γ -radiation only) from 3.27-2.94 as well as in G3 (1ml MRN 100+ γ -radiation) and G4 (3ml MRN100+ γ -radiation) respectively but not as much as in G2). The protein amount recorded in week 4 is relatively higher than in week 1 as per analyzed data in week 4 and week 1. Groups 3 and 4 showed increase in protein level thereby indicating that there was no influence from radiation effects hence normalcy

while G2 showed diminish in protein level.

Control magnesium levels in the first period of the current experiment showed significant difference ($P < 0.05$) with gamma radiation group. However, in the second one, there is significant effect between control vs γ -radiation $P < 0.01$, 1 ml MRN-100 ($P < 0.05$) as well as 3 ml MRN-100 ($P < 0.05$). Moreover, lactate dehydrogenase (LDH) deduced significance effect ($P < 0.01$) between Control against γ -radiation ($P < 0.05$), γ -radiation group versus 3 ml of MRN-100 group as well as between 1 ml MRN-100 group versus 3 ml MRN-100 (G4) ($P < 0.05$).

Analysis was also made on the levels of Creatinine, Urea and Glucose with regards to exposure time and experimental parameters of each group (G1-G4). The results deduced that levels of Creatinine, Urea and Glucose dropped in G1, G3 & G4 but elevated in G2. This indicated that there was ionizing radiation effect in G2 while G3 and G4 were protected by the protective MRN 100 addition against γ -radiation. There was similar analysis made in the other period.

The total lipid level in the serum *O. niloticus* of Control group (G1) denoting normalcy in two periods, G2 showing drop in levels for all parameters while G3 and G4 exhibited protective effects of MRN-100 to record close state to normalcy.

The normal lipid peroxidation level of *O. niloticus* for both intervals is given in Tables 2 and 3. The fundamental impact of γ -radiation was increased significantly in both intervals. The essential impact of MRN-100 in the two periods was highly significant. Dietary supplementation with MRN-100 improved its value in comparison with control. lipid peroxidation in muscular tissue showed significant effect for the 4th week period between G1 vs radiated fishes while no

significant difference found in all groups (G1-G4) in the first period. Clarification of the values is showed in tables 2 and 3. The levels of AST and ALP of *O. niloticus* in the first period (Table 2) reflected no mentioned significant difference between groups (G1-G4). However, elevation in their values was found in gamma radiation exposed group and drop occurred in G3 and G4. Comparatively, in the second part (Table 3), AST and ALP levels denoting significant increase of AST between control and G2 ($P < 0.05$) and the same for ALP between control and gamma radiation group ($P < 0.01$), as well as gamma radiation group versus 3 ml of MRN-100 ($P < 0.01$),

Table 1. The Data of biochemical components in *O. niloticus* vs. γ -radiation-Induction and MRN-100 effect on radioprotective ability (first week post radiation).

GROUP	<i>EXPERIMENTAL GROUP (for 1 week)</i>			
	Control	G2 (γ -radiation)	1 ml of MRN-100	3 ml of MRN-100
Albumin (g/dl)	4.96 \pm 0.72 3.8 - 6.29	3.27 \pm 0.42 2.8 - 4.1	3.57 \pm 0.84 2.4 - 5.2	3.95 \pm 0.1 3.8 - 4.14
Total protein (g/dl)	4.59 \pm 0.56 3.92 - 5.7	4.71 \pm 0.46 3.4 - 4.92	4.18 \pm 0.31 3.82 - 4.8	4.68 \pm 0.53 3.99 - 5.8
Total bilirubin (μ mol/L)	0.36 \pm 0.04 0.32 - 0.44	0.44 \pm 0.03 d 0.38 - 0.48	0.37 \pm 0.01 0.36 - 0.39	0.34 \pm 0.01 b 0.32 - 0.36
AST (U/L)	100.2 \pm 1.22 98.2 - 102.2	110.73 \pm 5.99 102 - 122.2	102.73 \pm 1.71 100.2 - 106	102.27 \pm 1.19 100 - 104
ALP (U/L)	20.43 \pm 1 19.1 - 22.4	27.33 \pm 3 21.8 - 32.1	25.66 \pm 2.89 21.4 - 31.18	21.49 \pm 0.79 20.18 - 22.9
Creatinine (g/dl)	0.15 \pm 0.01 b 0.14 - 0.17	0.25 \pm 0.05 a 0.19 - 0.35	0.19 \pm 0.02 0.16 - 0.22	0.18 \pm 0.01 0.17 - 0.19
Urea (mmol/l)	0.6 \pm 0.06 0.52 - 0.72	0.73 \pm 0.08 0.63 - 0.89	0.63 \pm 0.02 0.59 - 0.66	0.61 \pm 0.02 0.58 - 0.64
Glucose (mg/dl)	66 \pm 9.69 47.2 - 79.5	77.27 \pm 7.24 62.8 - 84.8	70.7 \pm 3 65.4 - 75.8	69.47 \pm 5.65 58.2 - 75.8
Calcium (mmol/l)	4.36 \pm 0.32 3.9 - 4.97	3.77 \pm 0.38 3.2 - 4.5	4.17 \pm 0.43 3.4 - 4.9	4.27 \pm 0.3 3.8 - 4.82
Magnesium (mmol/l)	1.29 \pm 0.06 b 1.19 - 1.4	1.03 \pm 0.12 a 0.8 - 1.2	1.16 \pm 0.02 1.12 - 1.2	1.17 \pm 0.03 1.14 - 1.22
Total lipids (g/l)	18.22 \pm 6.46 11.39 - 31.12	24.49 \pm 7.63 12.4 - 38.6	16.26 \pm 3.34 11.8 - 22.8	11.74 \pm 0.31 11.14 - 12.2
Cholesterol (mg/dl)	208.17 \pm 18.64 184.8 - 245	224.13 \pm 14.8 199.2 - 250.4	204.93 \pm 8.95 192.8 - 222.4	190.33 \pm 2.79 186.6 - 195.8
G6PHDH (g/dl)	56.49 \pm 6.46 48.1 - 69.18	62.1 \pm 8.48 52.4 - 78.99	58.33 \pm 5.65 50.8 - 69.4	54.1 \pm 5.72 44.9 - 64.6
LDH (U/l)	1210 \pm 9.19 b 1192 - 1220	1354 \pm 37.78 ad 1281 - 1400	1325 \pm 23.36 d 1340 - 1420	1254 \pm 32.89 bc 1218 - 1320
Cholinesterase (U/l)	46093 \pm 6303 35420 - 57240	40840 \pm 8355 31400 - 57500	44155 \pm 6746 34210 - 57023	47838 \pm 5300 40200 - 58023
Lipid peroxidation (nm/mg tissue)	11.85 \pm 0.36 11.24 - 12.48	15.07 \pm 1.7 12.4 - 18.22	13.17 \pm 0.86 11.86 - 14.8	12.97 \pm 0.83 11.9 - 14.6

The data expressed as Mean \pm SEM with (Min - Max). Different letters signalize significance at $P < 0.05$; ^a significant vs control (1weeks), ^b significant vs γ -radiation only (1 weeks), ^c significant vs 1 ml of MRN-100 (1 weeks), ^d significant vs 3 ml of MRN-100 (1 weeks).

Total bilirubin: γ -radiation with MRN-100 (3 ml) $P < 0.05$; **Creatinine:** Control with γ -radiation $P < 0.05$; **Magnesium:** control with γ -radiation $P < 0.05$; **LDH:** Control with γ -radiation $P < 0.05$; γ -radiation with MRN-100 (3 ml) $P < 0.01$; MRN-100 (1 ml) with MRN-100 (3 ml) $P < 0.05$.

Table 2. The data of biochemical components in *O. niloticus* vs. γ -radiation-Induction and MRN-100 effect on radioprotective ability (4th week post radiation).

GROUP	<i>EXPERIMENTAL GROUP (for 4 weeks)</i>			
	Control	G2 (γ -radiation)	1 ml of MRN-100	3 ml of MRN-100
Albumin (g/dl)	5.08±0.4 bc 4.31 – 5.66	2.94±0.38 a 2.52 – 3.69	3.51±0.76 a 2.16 – 4.68	3.95±0.09 3.42 – 3.73
Total protein (g/dl)	5.6±0.6 4.47 – 6.5	4.04±0.21 4.45 – 5.13	4.52±0.51 3.81 – 5.51	5 ±0.35 4.28 – 5.38
Total bilirubin (μ mol/L)	0.37±0.01 b 0.35 – 0.4	0.49±0.03 acd 0.43 – 0.54	0.41±0.01b 0.4 – 0.44	0.38±0.01b 0.36 – 0.4
AST (U/L)	104.53±6.38 b 92.16 – 113.4	124.02±6.71 a 114.24 – 136.86	115.06±1.92 112.22 – 118.72	114.54±1.33 112 – 116.48
ALP (U/L)	18.39±0.9 b 17.19 – 20.16	28.17±1.82 ad 24.72 – 30.91	23.09±2.6 19.26 – 28.06	19.34±0.71 b 18.16 – 20.61
Creatinine (g/dl)	0.16±0.02 b 0.13 – 0.19	0.28±0.06 a 0.21 – 0.39	0.21±0.02 0.18 – 0.25	0.2±0.01 0.19 – 0.21
Urea (mmol/l)	0.63±0.09 0.5 – 0.79	0.81±0.09 0.71 – 1.0	0.71±0.02 0.66 – 0.74	0.69±0.02 0.65 – 0.72
Glucose (mg/dl)	70.26±14.02 42.48 – 87.45	86.54±8.1 70.34 – 94.98	79.18±3.36 73.25 – 84.9	77.8±6.33 65.18 – 84.9
Calcium (mmol/l)	4.59±0.61 3.51 – 5.64	3.39±0.35 2.88 – 4.05	3.75±0.39 3.06 – 4.41	3.84±0.27 3.42 – 4.34
Magnesium (mmol/l)	1.35±0.11 bcd 1.15 – 1.54	0.93±0.11 a 0.72 – 1.08	1.05±0.02 a 1.01 – 1.08	1.05±0.02 a 1.03 – 1.1
Total lipids (g/l)	19.36±7.46 10.93 – 34.23	27.43±8.55 13.89 – 43.23	18.21±3.74 13.22 – 25.54	13.15±0.35 12.48 – 13.66
Cholesterol (mg/dl)	214.86±5.79 b 203.28 – 220.79	251.03±16.57 ad 223.1 – 280.45	229.53±10.03 215.94 – 249.09	213.17±3.13 b 208.99 – 219.3
G6PHDH (g/dl)	59.2±8.73 46.96 – 76.1	69.55±9.5 58.69 – 88.47	65.33±6.33 56.9 – 77.73	60.59±6.41 50.29 – 72.35
LDH (U/l)	1089±8.27 bd 1073 – 1098	1388±126 a 1153 – 1588	1245±21.03 1206 – 1278	1129±29.6 a 1097 – 1188
Cholinesterase (U/l)	53578±5937 45024 – 64986	46936±9811 30789 – 64664	45741±9357 35168 – 64400	51625±7060 39670 – 64109
Lipid peroxidation (nm/mg tissue)	12.34±0.63 b 11.23 – 13.42	16.88±1.9 a 13.89 – 20.41	14.75±0.97 13.28 – 16.58	14.52±0.93 13.33 – 16.35

Data expressed as Mean \pm SEM with (Min – Max). Different letters indicate significance at $P < 0.05$; a significant vs control (4 weeks), b significant vs γ -radiation only (4 weeks), c significant vs 1 ml of MRN-100 (4 weeks), d significant vs 3 ml of MRN-100 (4 weeks).

Albumin: Control with γ -radiation $P < 0.05$; control with 1 ml MRN-100 $P < 0.05$; **Bilirubin:** Control with γ -radiation $P < 0.01$; γ -radiation with 1 ml MRN-100 $P < 0.01$; γ -radiation with 3 ml MRN-100 $P < 0.05$; **AST:** Control with γ -radiation $P < 0.05$; **ALP:** Control with γ -radiation $P < 0.01$; control with 1 ml MRN-100 $P < 0.01$; **Creatinine:** Control with γ -radiation $P < 0.05$; **Magnesium:** γ -radiation with with 3 ml MRN-100 $P < 0.01$; control with 1 ml MRN-100 $P < 0.05$; control with 3 ml MRN-100 $P < 0.05$; **Cholesterol:** Control with γ -radiation $P < 0.05$; γ -radiation with 3 ml MRN-100 $P < 0.05$; **LDH:** Control with γ -radiation $P < 0.05$; control with 3 ml MRN-100 $P < 0.05$; **Lipid peroxidation:** Control with γ -radiation $P < 0.05$.

DISCUSSION

Liver is the largest vital organ in our body. Any disturbance in function of the liver which can cause illness is termed as liver disease. It is also called hepatic disease. It is a major leading cause of morbidity and mortality worldwide. Liver function tests (used for the evaluation of liver function) include measurement of serum aspartate and alanine transaminases, serum bilirubin, serum albumin, serum protein and alkaline phosphatase. The current outcome has obviously showed the capability of Gamma radiation stressor for inducing oxidative stress in fish liver.

The activity of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) enzymes were demonstrated to rise in response to gamma radiation. The same was found by many authors on nonylphenols [11] and heavy metals [1]. The response was dose-dependent. [12] Working on the same species reported that AST and ALT elevated with elevation in NP doses till 0.5 and 0.75 mg/L then declined with 1 mg/L. Increase in the levels of AST and ALT has been demonstrated to

reflect liver injury [11]. Elevation of alkaline phosphatase activity in serum may result from pathological or physiological enzyme production and release from non-liver tissue sources.

Albumin level is higher in the control group (G1) in week four as the rest of the other parameters studied due to the fact that there is no ionizing radiation effect rendered on the fish samples in G1. However, G2 shows decline in all parameters due to the gamma radiation induced in all the fish samples in G2. As a result there is hematopoietic tissue damage as well as cell damage; these cause organs such as liver, kidney and gills to be distorted and hence function abnormally. G3 and G4 exhibit states of normalcy close to that in G1. Concentration of albumin probably dropped for causes related to failure of liver synthesis [14].

bilirubin in the blood of high level is a bad indicator. A small amount of older red blood cells are replaced by new blood cells every day. Bilirubin is left after these older blood cells are removed. The liver helps break down bilirubin so that it can be removed from the body in

the stool. A high level of bilirubin in the blood can lead to jaundice. Serum bilirubin may be raised because of increased erythrocyte breakdown rather than because of failure of hepatic clearance [15].

Creatinine is a waste molecule which is produced from the metabolism of the muscle. Creatinine is a chemical waste product of creatine. Creatine is a chemical made by the body and is used to supply energy mainly to muscles. Creatinine is created from creatine which is a molecule of main significance required for production of energy in muscles. Approximately 2 percent of the creatine in our body is transformed to creatinine each day. Creatinine is carried to the kidneys throughout the bloodstream. The kidneys filtrate most of the creatinine and get rid of it in the urine. The production of creatinine remains normally basically without daily, for the reason that the mass of muscle in the body is constant. The kidneys keep the creatinine of the blood within ordinary range. As the kidneys become impaired for any reason, the level of the creatinine in the blood will

elevate for poor creatinine clearance by means of the kidneys. Elevated abnormal levels of creatinine warning of failure of the kidneys of possible malfunction. For this reason, standard blood tests routinely examine the quantity of creatinine in the blood. Hadi et al. (2009) reported that the increase of creatinine level might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrates metabolism [16].

Cholinesterase is a family of enzymes that catalyse the hydrolysis of the neurotransmitter acetyl choline into choline and acetic acid. The two types of cholinesterase found in the human blood are acetyl cholinesterase “true” cholinesterase in red cells and butyryl cholinesterase “non-specific, pseudo cholinesterase” in serum. Cholinesterase is synthesized mainly in hepatocytes and released into the blood. Serum activity is reduced in liver dysfunction due to reduced synthesis. The predominant hepatic source of serum cholinesterase, the marked decrease in its synthesis with hepatocyte dysfunction and restoration of synthesis with hepatocyte recovery suggests that serum cholinesterase

activity might be a more specific indicator of liver dysfunction than the traditional liver function tests [17].

In the current research study, hypoproteinemia, decrease in total protein level in the serum was listed in gamma radiated groups. The same was recorded by [18]; [19]; [20]; [21]; [22]; [23]; [24] following exposure to various doses of heavy metals and pesticides. Such hypoproteinemia may be as a result of the direct impact and effect of the exploitation of protein in the body as an energy supply to meet the increase in physiological demands to overcome the stress present in the polluted media [28]. Also, there are several pathological processes that may be the reason for hypoproteinemia like renal dysfunction and elimination in the urine, plasma dissolution, liver protein synthesis decrease, hepatic blood flow alteration and hemorrhage into the intestine and peritoneal cavity [29]. [30] noticed an elevation in the level of stressed total protein in male rat administrated juice of tomato supplemented diets. [31] observed an enhancement in total level of protein following Diazinon induction and also Nitrate induction stress in male

rats administrated vitamin E as supplemented diets.

There is a significant decrease in serum protein observed in gamma radiated fishes and this may be due to the stress which may reduce protein content in tissues. This is supported by [32] that proteins are mainly involved in the architecture of the cell. During chronic period of stress, they are also a source of energy. During stress condition, fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amount of carbohydrate, the next alternative source of energy is protein to meet the increased energy demand. The depletion of protein may have been due to their degradation and possible utilization of degraded products for metabolic purposes. [33] also observed that protein content drop as noticed in most of fish tissues may be as a result of orientating the free amino acids in particular for the protein synthesis, or for the osmo and ionic regulation maintenance.

Cholesterol was found to elevate considerably in γ -radiated fishes and declined in G3 and G4 which may be due to utilization of stored and

circulatory cholesterol and other lipid fractions in the treated fish to counteract toxic effects produced. This result conforms closely with [34] who observed increase level of cholesterol in *Channa punctatus* exposed to phorate. [35] And [36] also observed the same trend in *Notopterus notopterus* during stress. Elevation of Concentration of blood cholesterol is considered an important index of liver function impairment due to lipid homeostasis which is one of the main function of the liver. Several research studies are in concern with such measured parameters to estimate the stress degree in fishes and the nature [37]; [38] and [39] .

Ions are essential for any living organism as they are involved in most of biological processes like muscle contraction, respiration, nerve impulses transmission, absorption, acid-base balance, osmoregulation and excretion in fish. A lot of research studies paid an interest toward the harmful effects of several types of stress on biological processes considered in fishes. In present work, some ions decreased significantly

(Mg^{2+} & Ca^{2+}) under stress on *O. niloticus*. Hypomagnesemia,

an electrolyte disturbance in which there is a low level of magnesium in the blood and Hypocalcemia, low level of calcium in the Blood were identified and detected in *O. niloticus* exposed to gamma radiation for both periods. Kidney dysfunction (a common cause), which results in more calcium excreted in urine and makes the kidneys less able to activate vitamin D. Causes also increased urinary loss, and poor absorption from the intestines [2].

The blood glucose, G6PDH, LDH are considered as important bioindicators of tension occurred in the well known fish, *C. gariepinus* [12]. In the current study, gamma radiation induced hyperglycemia (glucose level elevation) in *O. niloticus*. The same observations were recorded by [19] and [20] following to exposure to several various doses of pesticides. The origin of such hyperglycemia is likely to be as a result of liver glycogenolysis, resulting from the elevation in plasma catecholamines and corticosteroid hormones as well as amino acids via gluconeogenesis process activation. Basically, the same results were noticed by [20] for *O. niloticus* tested by cadmium. Also, [40] reported a reduction

in the level of blood glucose following to stress in *Hippoglossus hippoglossus* fed supplemented diets with vitamin E. Glucose increase is a general response of fish to acute and sub-lethal pollutant effects. Elevation in the levels of serum glucose in stressed fish was illustrated by [41]; [42] and [43]. This can be regarding to variety of agents. The diminution in the specific activity for some enzymes as lactate dehydrogenase, phosphofructokinase and citrate kinase that decrease the capacity of glycolysis is one of these agents.

Seriously, LDH seems to be associated with cellular metabolic activity. As a result, its suppression that may be as a result of plasma membrane damage or ion imbalance [44] and also may be due to the formation of complex called enzyme inhibitor complex [45]. [46] mentioned that LDH elevated in accordance to NP increase to higher value (159.09 ± 2.84) at NP of 0.5 mg/l then depleted to 52.18 ± 0.95 at NP of 1 mg/l. [47] stated that the increase in the activities of G6PDH safeguard versus increased values of reactive oxygen species in cells subjected to stress via the increased production of NADH.

In the present study, increase in the level of total lipids in sera was on record in gamma radiated tested *O. niloticus*. The same research findings were noticed by [19]; [48] and [49] after exposure to various doses of heavy metals and pesticides. That increase in the level of total lipids may resulted from the decrease in secretion of catecholamines and corticosteroids with increased metabolic rate and in turn increased metabolic reserves [50]. Hydroferrates were found to be valid in counteracting stress-induced changes in total lipid level in the present work.

The rise in lipid peroxidation level in muscular tissue means a modification in the physical characteristics of cell membrane [51] since lipid peroxidation leads to hydrolysis of phospholipids into hydroperoxy fatty acids [52]. The gamma radiation induced oxidative stress in tissues by increasing lipid peroxidation and by altering the antioxidant status was postulated by many authors [53] and [54]. In the present study, the level of lipid peroxidation was dropped in muscular tissue of gamma radiated exposed fishes fed diets supplemented with MRN-100.

lipid peroxidation is analyzed with core relationship between the antioxidant proxy ability of MRN-100 in sets (G3-G4) and absence in G2 and G1. It is noted that there is a proliferation in the amount of lipid produced due to peroxidation in the muscles that results from the influx production of Glucose and sugar that are subsequently stored as lipids/fats. Group 2 recorded the highest peroxidation quotient based on the gamma radiation full influence without MRN-100 Protection. G3 and G4 show restricted lipid peroxidation that can be accounted for due to MRN-100 antioxidant proxy ability. Antioxidants such as vitamin C and vitamin E decreases in tissues as a result of increase in lipid peroxidation. In a similar way, lipid peroxidation elevated and drop in vitamin C and E in tissues under hypoxic stress occurred.

Considerable interest toward oxygen has been paid, as low concentrations of ambient oxygen are well-known to have an effect on food consumption, growth, and the physiological state of fish [27]. All aerobic individuals rely on presence of oxygen in the surrounding environment that use it for production

and generation of energy through oxidative phosphorylation [27]. The generation of different oxygen metabolism by-products, known as reactive oxygen species (ROS) “is the other side of the coin”. They involve hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), hydroxyl radical (-OH) and others. If defenses for the antioxidant are efficient in detoxify ROS, then no deleterious resulted outcome will occurred in tissues [26]. However, if there is severity for ROS attack, afterward antioxidant defense systems may be overwhelmed, resulting in antioxidant enzymes inhibition and oxidative damage to protein, lipid, DNA as well as other important key molecules. Reactive oxygen species “ROS” are produced via ordinary physiologic processes that are extremely important to normal cellular function. Some vitamins possess an antioxidant activity which keep the cells from the harm resulted from the free radicals and by preventing the formation of free radical that play an essential role in the antioxidant defense. Moreover, Stress has the ability to generate the free radicals, like HO and O₂⁻. [26] reported

that these free radicals can destroy cell membranes through encouraging lipid peroxidation of polyunsaturated fatty acids present in cell membrane.

Accountably, there are three fundamental alterations that prelude the induction by ionizing radiation: (1) lipid peroxidation (2) protein modification (3) tissue damage resulting to cell death. It is therefore inferential as to why data in week 1 differs substantially to that in week 4 (Table 2 and 3). As such, there is valid evidence from the depreciation and appreciations in the total protein, total lipids, albumin and other mentioned experimental parameters due to modification caused by radiation. On the other hand, the parameters value ratios as seen in the results where (albumin, protein, glucose etc.) total count in week 1 accounts drop in comparing to week 4. However, parametric disparity levels exists in the groups whereby G1 (control group) showed normalcy during the tenure of exposure; G2 (exposed to γ -radiation) showed the greatest disparity of degradation and damage since it lacks any agent to protect or counteract the effects of exposure. G3 and G4 showed limited differences after and during

exposure due the reversion of MRN-100 that acts as a radioprotective agent. Therefore, albumin, protein, glucose lipid and other parametric levels gradually increase from week 2 to week 4. This can be validated by the radio protective element of MRN-100 on gamma radiation. Their level is higher in the control group (G1) in week four as the rest of the other parameters studied due to the fact that there is no ionizing radiation effect rendered on the fish samples in G1. However, G2 shows decline in all parameters due to the gamma radiation induced in all the fish samples in G2. As a result there is hematopoietic tissue damage as well as cell damage; these cause organs such as liver, kidney and gills to be distorted and hence function abnormally. G3 and G4 exhibit states of normalcy close to that in G1. Therefore, albumin, protein, glucose lipid and other parametric levels gradually increase from week 2 to week 4. This can be validated by the radio protective element of MRN-100 on gamma radiation.

Factually, it can be advanced that MRN-100 act as “a radioprotective agent” in two different ways: (1)

Hematopoietic tissue protector (2) viable antioxidant proxy. Further relevance is based on the examination that shows that fish induced with MRN-100 prior to irradiation exhibited limited lesion and tissue damage as validated by the results [13]. Effectively, deduction is made from the results in first week after radiation exposure and week 4.

Based on the inferential analysis of the experiment conducted, it can be validated that MRN-100 is viably a radioprotective agent that can be used to reduce and control the adverse effects of ionization radiation. There are broad arrays of effects that are in-vitro based in terms of exposure to ionizing radiation with regards to living things. The scientific name given to the series of adverse effects resultant from exposure to ionizing radiation is called acute radiation syndrome. The principle of action of the ionizing radiation is that it promotes the oxidative induction of stress that proliferates the production of ROS. Subsequently, there is an imbalance created between pro-oxidant and antioxidant aspects of the body [25]. ROS is deduced to play a primal role in tissue attenuation and damage; it does so

on the basis of inducing lipid peroxidation and hence that's why there is reported biochemical imbalances in subjects exposed to ionizing radiation (As can be related to the findings in the current experiment).

In conclusion, it is in order to validate the hypothesis construed in this experiment that purported that MRN-100 offered potent radioprotective characteristics on induced groups under exposure to γ -radiation. It is therefore essential to accept the hypothesis based on the results from the experiment conducted. The prospects that are ideally presented by the MRN-100 agent are formidable in alleviating adverse effects caused by ionizing radiation. It is therefore ideal to further bolster research on MRN-100 and thereby generate the best outcome possible; this is in light of suggested MRN-100 safety and its use in animals and human beings. On the basis of this research study, it is denoted that the MRN-100 agent is a secure reliable and toxic-free product that if used correctly can provide the best radioprotective effects. We can therefore conclude that MRN-100 viably serves as a radioprotective proxy via creating

candid protection versus mortality due to influencing radiation.

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REFERENCES

- [1] Oner M, Atli G, Canli M (2008). Changes in serum biochemical parameters of freash water fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. *Environ. Toxicol. Chem.*, 27(2): 360-336.
- [2] Tram, N.D.Q., Ngoan, L.T., Hung, L.T. & Lindberg, J.E. (2011) A comparative study on the apparent digestibility of selected feedstuffs in hybrid catfish (*Clarius macrocephalus* × *Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*). *Aquacult. Nutr.*, 17, 636–643.
- [3] Jagetia, G., Venkatesh, P., & Baliga, M. (2004). Evaluation of the radioprotective effect of bael leaf (*Aegle marmelos*) extract in mice. *International Journal Of Radiation Biology*, 80(4), 281-290. <http://dx.doi.org/10.1080/09553000410001679776>.
- [4] Anne, P.R. Phase II trial of subcutaneous amifostine in patients undergoing radiation therapy for head and neck cancer. *Semin Oncol.* 2002;29:80–83.
- [5] Ghoneum, M., Abedi, S. Enhancement of natural killer cell activity of aged mice by modified arabinoxylan rice bran (MGN-3/Biobran). *J Pharm Pharmacol.* 2004;56:1581–1588.
- [6] 16. Kruk I, Aboul-Enein HY, Michalska T, Lichszeld K, Kladna A. Scavenging of reactive oxygen species by the plant phenols genistein and oleuropein. *Luminescence.* 2005;20:81–89. doi: 10.1002/bio.808.[PubMed].
- [7] Maity P., Hansda D., Bandyopadhyay U. & Mishra D.K., (2009) “Biological activities of crude extracts of chemical constituents of Bael, *Aegle marmelos* (L.) Corr.” *Indian Journal of Experimental Biology*, Vol 47, p.p. 849-861.
- [8] E. Ernst, “Panax ginseng: an overview of the clinical evidence,” *Journal of Ginseng Research*, 2010; vol. 34, no. 4, pp. 259–263.
- [9] Sugiura, Y.; Matsuda, K.; Yamada, Y.; Nishikawa, M.; Shioya, K.; Katsuzaki, H.; Imai, K.; Amano, H. Isolation of a new anti-allergic phlorotannin, phlorofucofuroeckol-B, from an edible brown alga, *Eisenia arborea*. *Biosci. Biotechnol. Biochem.* 2006, 70, 2807–2811.
- [10] Zhang, S.; Hunter, D.J.; Forman, M.R.; Rosner, B.A.; Speizer, F.E.; Colditz, G.A.; Manson, J.E.; Hankinson, S.E.; Willett, W.C. Dietary carotenoids and vitamins A, C, and E and risk of

breast cancer. *J. Natl. Cancer Inst.* 1999, 91, 547–556.

[11] Bhattacharya H, Xiao Q, Lun L (2008). Toxicity studies of nonylphenol on rosy barb (*Puntius conchonius*): A biochemical and histopathological evaluation. *Tissue Cell*, 40: 243-249.

[12] Satyanarayanan SK, Chokkalingam K, Mathan R, Tamara G (2011). Toxicity studies of nonylphenol and octylphenol: hormonal, hematological and biochemical effects in *Clarias gariepinus*, *J. Appl. Toxicol.*, DOI 10.1002/jat.1629.

[13] Ghoneum, M., Matsuura, M., &Gollapudi, S. (2009). An iron-based beverage, HydroFerrate fluid (MRN-100), alleviates oxidative stress in murine lymphocytes in vitro. *Nutrition Journal*, 8(1).
<http://dx.doi.org/10.1186/1475-2891-8-18>

[14] McPherson RA. Specific proteins. In: McPherson RA, Pincus MR, eds. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia, PA: Elsevier Saunders; 2011:chap 19.

[15] Pratt DS. Liver chemistry and function tests. In: Feldman M, Friedman LS, Brandt LJ, eds. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management*. 10th ed. Philadelphia, PA: Elsevier Saunders; 2016:chap 73.

[16] Landry DW, Bazari H. Approach to the patient with renal disease. In: Goldman L, Schafer AI, eds. *Goldman's*

Cecil Medicine. 25th ed. Philadelphia, PA: Elsevier Saunders; 2016:chap 114.

[17] Nelson LS, Ford MD. Acute poisoning. In: Goldman L, Schafer AI, eds. *Goldman's Cecil Medicine*. 25th ed. Philadelphia, PA: Elsevier Saunders; 2016:chap 110.

[18] Assem H, Abo-Hegab S, Belal I (1992). Comparison of haematological effects of some toxicants on *Clarias gariepinus*. *Journal of Egyptian- German Society of Zoology*. 9, 33-50.

[19] Mekkawy IAA, Hussein SY, Abd El-Nasser M, Ahmed SM (1996). Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt. *J. Egyptian-German Society of Zoology*. 19, 283-319.

[20] Mekkawy IAA, Mahmoud UM, Wassif ET, Naguib M (2011). Effects of Cadmium on Some Hematological and Biochemical Characteristics of *Oreochromis niloticus* (Linnaeus, 1758) Dietary Supplemented with Tomato paste and vitamin E. *J. Fish Physiol. Biochem*.

[21] Das BK, Mukherjee S (2003). Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. *Comparative Biochemistry and Physiology*. 134, 109-121.

[22] Fouda FM (2004). Hematological and biochemical studies on the effects of a biological and chemical pesticides on

the Nile catfish, *Clarias gariepinus*. J. Egyptian-German Soc. Zool. 43, 77-97.

[23] Gad NS (2005). Impact of environmental pollution in the southern region of Lake Manzalah Egypt on some biochemical parameters of *Tilapia Zilii*. J. Egyptian-German Soc. Zool. 48, 279-298.

[24] Shalaby AME (2007). Effect of EDTA on reduction of cadmium toxicity on growth, some haematological and biochemical profiles of Nile tilapia (*Oreochromis niloticus*). J. Fisheries Aquatic Sci. 2, 100-109.

[25] Ping, X., Junqing, J., Junfeng, J., & Enjin, J. (2012). Radioprotective effects of troxerutin against gamma irradiation in mice liver. *International Journal Of Radiation Biology*, 88(8), 607-612. <http://dx.doi.org/10.3109/09553002.2012.692494>

[26] Yin, H.; Xu, L. & Porter, N.A. (2011) Free radical lipid peroxidation: mechanisms and analysis. *Chemical Reviews*. 111: 5944-5972. ISSN: 0009-2665

[27] K. Pichavant* 1 , J. Person-Le-Ruyet1 , N. Le Bayon1 , A. Severe1 , A. Le Roux1 and G. Boeuf1, 2. Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. *Journal of Fish Biology* October 2001; 59 (4) : 875 – 883.

[28] Fontana L, Moreira E, Torres M, Fernandez I, Rios A, Sanchez DF, Gil A (1998). Dietary nucleotides correct plasma and liver microsomal fatty acids

alterations in rats with liver cirrhosis induced by oral intake of thioacetamide. *J. Hepatol.* 28, 662-669.

[29] Keith FP, Weber JL (1979). The effect of carbon tetrachloride on the total plasma protein concentration of rainbow trout, *Salmo gairdneri*. *Comparative Biochemistry and Physiology*. 63, 37-42.

[30] Elkomy MM, Hassan HA (2005). The role of tomato-juice as a protective agent against thioacetamide hepatotoxicity in male rats. *Comparative Physiology*. 46, 217-234.

[31] Kalender S, Ogutcu A, Uzunhisarcikli M, Acikgoz F, Durak D, Ulusoy Y, Kalender Y (2005). Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. *Toxicology*. 211, 197-206.

[32] Ogur R, Coskun O, Korkmaz A, Oter S, Yaren H, Hasde M (2005). High nitrate intake impairs liver functions and morphology in rats; protective effects of α -tocopherol. *Environmental Toxicology and Pharmacology*. 20, 161-166.

[33] K. Shobha, A. Poornima, P. Harini, K. Veeraiah, A study on biochemical changes in the fresh water fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant Cadmium chloride. *Kathmandu University Journal of Science, Engineering and Technology*. 1(4): 1-11. 2007

[34] V. Tiwari, A. Singh, Biochemical stress response in freshwater fish, *Channa punctatus* induced by aqueous extracts of *Euphorbia truncalli* plant. *Chemosphere* 64: 36-42. 2006.

- [35] A.S. Rani, R. Sudharsan, T.N. Reddy, P.U.M. Reddy, T.N. Raju, Effects of arsenite on certain aspects of protein metabolism in freshwater teleost, *Tilapia mossambica* (Peters). *Journal of Environmental Biology*. 22(2): 101-104. 2001.
- [36] 48. D.S. Shankar, R.S. Kulkarni, Tissue cholesterol and Serum cortisol level during different reproductive phases of the female freshwater fish, *Notopterus notopterus*. *Journal of Environmental Biology*. 28(1):137-139. 2007.
- [37] Poléo A, Hytterød S (2003). The effect of aluminium in Atlantic salmon (*Salmo salar*) with special emphasis on alkaline water. *J. Inorg. Biochem.*, 97: 89-96.
- [38] Rosety-Rodriguez M, Ordonez F, Rosety I, Rosety J, Rosery M (2005). Erythrocyte antioxidant enzymes of gilthead as early-warning bioindicators of oxidative stress induced by malathion. *Haema*, 8: 237- 240.
- [39] Sayed AH, Ibrahim ATh, Mekkawy IAA, Mahmoud UM (2007). Acute effects of Ultraviolet-A radiation on African Catfish *Clarias gariepinus* (Burchell, 1822). *J. Photoch. Photobiol. B*, 89: 170-174.
- [40] Martins DA, Afonso LOB, Hosoya S, Lewis-McCrea LMP, VLM, Lall SP (2007). Effects of moderately oxidized dietary lipid and the role of vitamin E on the stress response in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*. 272, 573-580.
- [41] Kalpana, K., Devipriya, N., Srinivasan, M., Vishwanathan, P., KuppsamyThayalan, & Menon, V. (2011). Evaluating the radioprotective effect of hesperidin in the liver of Swiss albino mice. *European Journal Of Pharmacology*, 658(2-3), 206-212. <http://dx.doi.org/10.1016/j.ejphar.2011.02.031>
- [42] Melzer, P. (2012). Radiation Dose-Rate and DNA Damage. *Environmental Health Perspectives*, 120(11), a417-a417. <http://dx.doi.org/10.1289/ehp.1205595>
- [43] Zhang, C., Ni, J., Li, B., Gao, F., Liu, H., & Liu, W. et al. (2013). CpG-Oligodeoxynucleotide Treatment Protects against Ionizing Radiation-Induced Intestine Injury. *Plos ONE*, 8(6), e66586. <http://dx.doi.org/10.1371/journal.pone.0066586>
- [44] Sastry KV, Gupta PK (1980). Alterations in the activities of a few dehydrogenases in the digestive system of 2 teleost fishes exposed to lead nitrate. *Ecotoxicol. Environ. Safe*, 4: 232-239
- [45] Rajanna B, Chetty CS, McBride V, Rajanna S (1999). Effects of lead on K+-para-nitrophenyl phosphatase activity and protection by thiol reagents. *Biochem. Int.*, 20: 1011-1018.
- [46] Satyanarayanan SK, Chokkalingam K, Mathan R, Tamara G (2011). Toxicity studies of nonylphenol and octylphenol: hormonal, hematological and biochemical effects in *Clarias gariepinus*. *J. Appl. Toxicol.*, DOI 10.1002/jat.1629.

- [47] Leopold JA, Zhang YY, Scribner AW, Stanton RC, Loscalzo J (2003). Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. *Arterioscler. Thromb. Vasc. Biol.*, 23: 411-417.
- [48] Fayed HM, Zaghoul KH, Abd El-Monem S, Mohamed HA (2001). Biological responses of the Nile Tilapia, *Oreochromis niloticus* and the Nile catfish, *Clarias gariepinus* induced by agricultural and industrial pollutants. *J. Union of Arab Biol.* 16, 543-568.
- [49] Fouda FM (2004). Hematological and biochemical studies on the effects of a biological and chemical pesticides on the Nile catfish, *Clarias gariepinus*. *J. Egyptian-German Soc. Zool.* 43, 77-97.
- [50] Fayed HM, Zaghoul KH, Abd El-Monem S, Mohamed HA (2001). Biological responses of the Nile Tilapia, *Oreochromis niloticus* and the Nile catfish, *Clarias gariepinus* induced by agricultural and industrial pollutants. *J. Union of Arab Biol.* 16, 543-568.
- [51] Ursini F, Maiorino M, Sevanian A (1991). Membrane hydroperoxides in oxidative stress. In: H. Sies, (Ed.), *Oxidants and Antioxidants*. NHarcort Brace Jovanovich, London, pp. 319-336.
- [52] Salgo MG, Corongiu FP, Sevnian A (1993). Enhanced interfacial catalysis and hydrolytic specificity of phospholipase A2 toward peroxidized phosphatidylcholine vesicles. *Archives of Biochemistry and Biophysics.* 304, 123-132.
- [53] Romeo M, Gnassia-Baralli M (1997). Effect of heavy metals on lipid peroxidation in the Mediterranean clam, *Ruditapes decussates*. *Comparative Biochemistry and Physiology.* 118, 33-37.
- [54] Sarkar S, Yadav P, Bhatnagar D (1997). Cadmium-induced lipid Peroxidation and the antioxidant system in rat erythrocytes: the role of antioxidants. *J. Trace Elements in Med. Biol.* 11, 8-13.
- [55] Baker RTM, Davies SJ (1996). Changes in tissue α -tocopherol status and degree of lipid peroxidation with varying α -tocopheryl acetate inclusion in diets for African catfish. *Aquacult. Nutr.* 2, 71-79.
- [56] Scaife JR, Onibi GE, Murray I, Fletcher TC, Houlihan DF (2000). Influence of α -tocopherol acetate on the short- and longterm storage properties of fillets from Atlantic salmon *Salmo salar* fed a high lipid diet. *Aquaculture Nutrition.* 6, 65-71.
- [57] Chaiyapechara S, Casten MT, Hardy RW, Dong FM (2003). Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E. *Aquaculture.* 219, 715-738.
- [58] Huang SL, Weng YM, Huang CH (2004). Lipid peroxidation in sarcoplasmic reticulum and muscle of tilapia is inhibited by dietary vitamin E supplementation. *Journal of Food Biochemistry.* 28, 101-111.

[59] Wang Y, Yuen KH, Ng WK (2006). Deposition of tocotrienols and tocopherols in the tissues of red hybrid tilapia, *Oreochromis sp.*, fed α -tocotrienol-rich fraction extracted from crude palm oil and its effect on lipid peroxidation. *Aquaculture*. 253, 583-591.