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Haematological and Biochemical Parameters of Rats Fed with Heavy Metaled Fish after Exposure to a 47mT Oscillating Magnetic Field

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Abstract This study investigated the effect of oscillating magnetic field (OSMF) of 47mT on the haematological and biochemical parameters of rats fed with heavy metaled fishes obtained from Alape River and the Atlantic Ocean of Ilaje local government of Ondo state. Thirty rats and two species of fishes, Tilapia and Catfish, were the animals used for this study. The rats were divided into three groups of ten animals each. The first was the control group and were not fed with the fishes neither were they exposed to OSMF, and the other two were the experimental groups. The rats in the second group were fed with a mixture of the powdered form of the Tilapia and Sea catfish for twenty days, there after exposed to oscillating magnetic field for thirty days. The rats in the third group were fed with the powdered form of the fishes but were not exposed to OSMF. The rats in the three groups were sacrificed after the chosen period of exposure and their blood and liver samples were analyzed for hematological and biochemical indices respectively. The results showed that there was no significant difference in hematologic parameters in the groups. However, there was significant increase ($p<0.05$) in malondialdehyde levels as well as reduced activities of superoxide dismutase and catalase in liver and serum of rats in experimental groups, when compared to the control group, but alanine aminotransferase activities were unaffected. This suggests that there are additive, injurious effects in the combined exposure of the animals to heavy metals and oscillating magnetic field by hampering their ability to detoxify free radicals.

Keywords: *oscillating magnetic field, haematological parameters, biochemical parameters, catalase, superoxide dismutase, malondialdehyde*

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1. Introduction

Fish is the main source of protein to the people of Ilaje local government area of Ondo State and its environs. The types of fish normally harvested and consumed are Tilapia, Catfish, Crocker, Sea catfish from both fresh water and the Atlantic Ocean. Unreported Hospitals records have in recent time showed a constant complain of typical diseases by inhabitants of these areas. Also, related diagnoses have revealed that these diseases are caused by metallic substances within the human body.

Over the years, the natural food in takes by the fishes in riverine area of Ilaje Local government have greatly been distorted due to various discharge of domestic, industrial, agricultural, sewage wastes due to oil spillage from oil exploration and amongst others. These products are transported from point of discharge to distance locations by the streams into both fresh water and Atlantic Ocean. Thus the major sources of food for these fishes are mostly this waste and other aquatic animals which have been exposed to this same wastes. These products accumulate in the fishes through digestion and absorption into the tissues. A number of reports have shown that heavy metals such as Cadmium (Cd), Chromium (Cr), Cobalt (Co), Nickel (Ni), Zinc (Zn), Copper (Cu) and Arsenic (As) are all found in fishes collected from fishermen around Ogun river estuary, Ubeji river in Warri and Eleyele river in Ibadan [1,2,3]. The locations of these rivers are all around one industry or the other as that of our study area. Then the fishes obtained from our considered study area are assumed to contain some metals. This calls for a critical monitoring of the level of these metals, if there is any, in foods knowing fully that contamination of the general environment through waste products is on the increase [4]. It has been observed that concentration of heavy metals in human being is associated with a number of diseases, such as cardiovascular, renal, neurological, and bone diseases [5,6,7].

Several studies have revealed possible bio-effects of magnetic fields on human health [8]. It has been equally reported that some adverse health effects are observed when some rats were exposed to static magnetic field (SMF) at relatively higher frequencies and long duration of exposure [9]. Up to now, there are no reported cases of adverse health effect when rats are exposed to oscillating magnetic field (OSMF) at low or high frequency.

From the review above it is observed that both heavy metals and oscillating magnetic can cause some health challenges in human being unnoticed. Thus, this study seeks to determine the type of heavy metals present in the fishes from our area of study, and there after investigate the effect of both the heavy metals and exposure to OSMF combined within the body of rats by examining the level of changes in the hematological parameters of the blood and biochemical parameters of the serum and the liver of the rats.

2. Materials and Methods

2.1. Materials

2.1.1. Animals

The animals used for this study were Tilapia (Oriochromis Niloticus) and Sea catfish (Arius species) and thirty white rats. These sets of fishes were gotten from Alape River and Atlantic Ocean. They were dried in an oven in the chemistry laboratory of Adekunle Ajasin University for four days, and thereafter grinded into a powdered form. The powdered sample was loaded into the chamber of the spectrometer with a voltage of 30kv (maximum) and a current 1 mA (maximum) applied to produce a value of X-rays that excite the sample for a preset time (10mins in this case). The X-rays interact in the detector which releases a pulse of charge proportional to the energy deposited. The signal processing electronics sum the results of many interactions. The analysis software detects which peak is present and computes the concentration of each element in the sample. This experimental procedure was carried out at the Centre for Energy Research and Development, Obafemi Awolowo University, Ile –Ife in X-Ray Fluorescence (XRF) Laboratory.

2.1.2. Oscillating Magnetic Field, (OSMF)

The Oscillating magnetic field used was obtained from a solenoid built with a 0.85mm copper wire. Four hundred and fifty (450) turns of this wire was wound round a cylindrical wooden box of diameter 61cm. The solenoid was connected to an AC main 55 Hz frequency through a step down transformer of 12v. The current value used for this set up was 2.6A. With this a magnetic field of 47 mT was obtained.

2.2. Methods

2.2.1. Experimental Design

Thirty albino rats were obtained from Owo town in Ondo state. The rats were divided into three groups, each containing ten rats. Two of these groups were taken as the experimental groups and the third group is the control group. The experimental groups were fed with the mixture of powdered form of both the Tilapia and the sea catfish. Group one of the experimental groups was exposed to Oscillating magnetic fields for four weeks, while the second experimental group was not exposed to OSMF. The control group was fed with normal standard mice pellet diet and housed in clear plastic substance. The three groups had access to adequate water, and were kept under standard conditions with 12hrs light/dark cycle.

The control and experimental rats sacrificed after forty experimental days. Their blood samples were taking to the state specialist hospital, Ikare Akoko for Haematological analysis while the liver and serum were taken to Biochemistry Department of Adekunle Ajasin University, Akungba-Akoko, where a detailed biochemical analysis was done.

2.2.2. Blood Collection

The blood samples were obtained via jugular puncturing and collected in heparinized tubes The percentage packed cell volume (PCV) was determined according to the hematocrit method while the blood hemoglobin concentrations (BHC) in all samples were estimated according to the cyamomethaemoglobin method. The total white Blood cells (WBC), white blood cell differentials, red blood cell (RBC) were estimated using the improved Neubauer counting chamber [10,11]. The mean corpuscular Haemoglobin volume (MCV), mean corpuscular Haemoglobin (MCH) and mean corpuscular Haemoglobin concentrations (MCHC) were calculated by dividing the haemoglobin by the haematocrit. All these were carried out at the Ondo state Specialist Hospital in Ikare.

2.2.3. Statistical Analysis

The software packages SPSS 11.0, (Inc., Chicago, Illinois, USA), and GraphPad Prism 6.0(San Diego, CA, USA) were used to analyze the results obtained from this study. Differences were considered statistically significant at $p<0.05$.

3. Results

The results generated for this study are presented in this section.

The identified metals by the XRF and their percentage weight in the fishes, (Tilapia and the Sea Catfish) are presented in Table 1 and Table 2 respectively.

Table 1. The Metals Present and Their Percentage by Weight in Sea Catfish, (*Arius*)

Elements	*Conc Value*	*Conc Error*	*Unit*
K	3.9984	± 0.1035	wt.%
Ca	43.3510	± 0.3407	wt.%
Mn	2829	± 275	Ppm
Fe	1.5126	± 0.0551	wt.%
Ni	5779	± 276	Ppm
Zn	3.7155	± 0.0628	wt.%
Se	1640	± 125	Ppm
Mo	1119	± 154	Ppm
Rb	978	± 101	Ppm
Sr	1084	± 0.0363	wt.%
Ru	1533	± 203	Ppm
Br	717	± 83	Ppm

Table 2. The Metals Present and Their Percentage by Weight in Tilapia Fish (*Oreochromis niloticus*)

Elements	Conc Value	Conc Error	Unit
K	4.2027	± 0.1068	wt.%
Ca	46.4744	± 0.3553	wt.%
Mn	3616	± 313	Ppm
Fe	1.7440	± 0.0598	wt.%
Ni	4303	± 241	Ppm
Zn	7776	± 278	Ppm
As	113	± 30	Ppm
Se	765	± 76	Ppm
Mo	1026	± 131	Ppm
Rb	478	± 63	Ppm
Sr	6834	± 257	Ppm
Ru	1019	± 158	Ppm
Br	819	± 79	Ppm

The figures captured as a and b were obtained after the liver and the serum had been extracted, dried and powdered. The pulverized organs were thereafter taken to the Centre for Energy Research and Development, Obafemi Awolowo University, Ile –Ife in X-Ray Fluorescence (XRF) Laboratory.

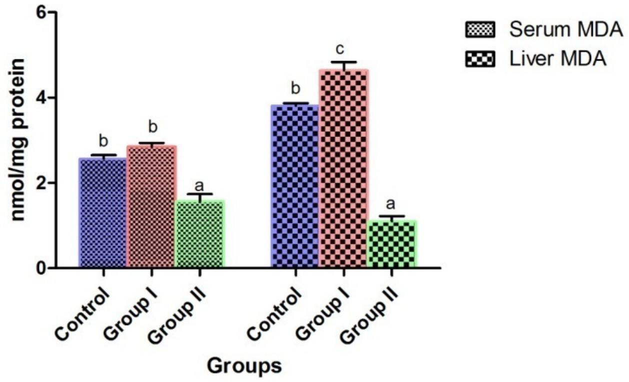

Figure 1. Malondialdehyde (MDA) Levels of Serum and Liver of Rats Fed with Heavy Metaled Fish and/or Exposed to OSMF

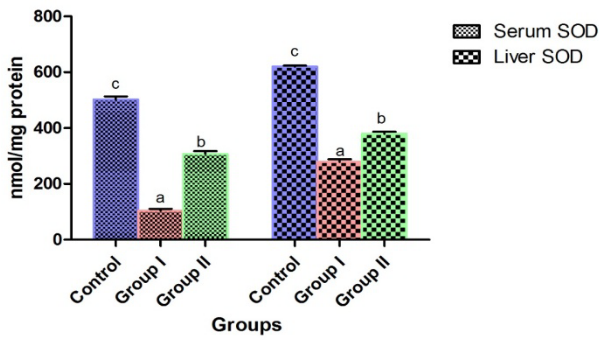

Figure 2. Superoxide Dismutase (SOD) Activity in Serum and Liver of Rats Fed with Heavy Metaled Fish and/or Exposed to OSMF

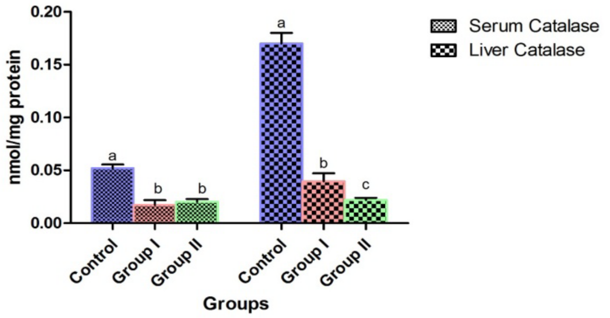

Figure 3. Catalase Activity in Serum and Liver of Rats Fed with Heavy Metaled Fish and/or Exposed to OSMF

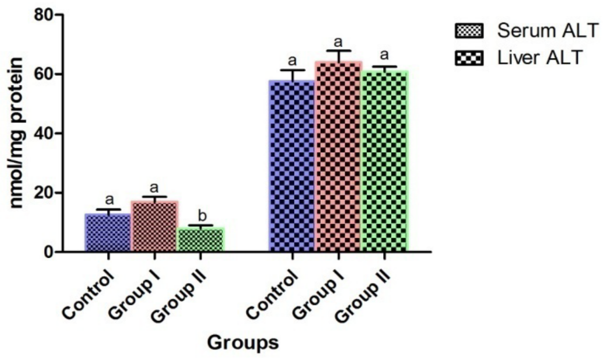

Figure 4. Alanine aminotransferase (ALT) Activity in Serum and Liver of Rats Fed with Heavy Metaled Fish and/or Exposed to OSMF

Table 3. Hematological Parameters of Rats Fed with Heavy Metaled Fish and/or Exposed to OSMF

Group/Test	Control group	Experimental group 1: Rats fed with the mixture of powdered Tilapia Fish and Sea Catfish not exposure to OSMF	Experimental group 2: Rats fed with the mixture of powdered Tilapia and Sea Catfish Sea Fishes exposed to OSMF
RBC (10^6/µl)	5.66±2.43 ^{a}	7.72±0.86 ^{a}	7.10±1.69 ^{a}
PCV (%)	36.40±14.90 ^{a}	47.82±5.61^{ab}	39.88±9.37 ^{a}
Hb (g/dl)	9.63±5.38 ^{a}	13.96±1.08 ^{ab}	13.53±2.72 ^{ab}
WBC (10^6/µl)	7.79±4.92 ^{a}	10.89±4.34 ^{ab}	8.84±2.87 ^{a}
Neutrophils (%)	23.56±14.55 ^{a}	14.53±5.25^a	13.00±7.05 ^{a}
Lymphocyte (%)	63.43±14.87 ^{a}	69.30±12.15 ^{a}	57.73±2.05 ^{a}
Basophil (%)	13.00 ± 0.43 ^{a}	16.13 ± 6.96 ^{ab}	10.3 ± 5.27 ^{a}
MCH (pg)	16.13±2.99 ^{a}	19.26±0.83 ^{ab}	16.13±2.99 ^{a}
MCHC (%)	25.00±5.64^a	29.37±1.20^{ab}	34.20±1.40^b
MCV (fl)	65±4 ^{a}	61.66 ±1.53 ^{a}	56±0.50 ^{b}

Values are expressed as mean ± standard deviation (SD).
Values with different superscript are significantly different ($p<0.05$) as compared with the control group.

Table 4. Comparison of X-ray Fluorescence Spectroscopic (XRF) Analysis for Sea catfish and Tilapia with WHO Standard

Element	Tilapia Fish (ppm)	Sea Catfish (ppm)	Combined content of metals in the Liver and Serum of the rats in the experimental groups(ppm).		WHO Standard Limit (ppm)
			Rats exposed to OSMF [a]	Rats not exposed to OSMF [b]	
K	420	399	205	202	150
Ca	4647	4335	410	530	200
Fe	174	151	73	69	40
Mn	3616	2829	613	596	30
Zn	7776	3771	1006	1675	100
Ni	4303	5779	225	279	15

4. Discussion

This study is designed to identify the heavy metals present in our study fishes and possible hematological and Biochemical changes experienced by rats fed with these fishes after exposure to oscillating magnetic field.Tables one and two contain the identified heavy metals with their respective weight in the fishes used for this study and as measured by the XRF.

These tables show that the fishes contain the same number of heavy metals but in varying degree. These metals are Potassium (K), Calcium (Ca), Magnesium (M), Iron (Fe), Nickel (Ni), Zinc (Zn), Selenium (Se), Molybdenum (Mo), Rubidium (Rb), Selenium (Sr), Ruthenium (Ru). Heavy metals such as Cadmium, Chromium(Cr), Cobalt, Nickel, Zinc, Copper and Arsenic were found in fishes collected from fishermen around Ogun river estuary, Ubeji river in Warri, and Eleyele river in Ibadan according to earlier studies [1,2,3]. In our study, the same set of metals have been identified in the fishes collected from Alape and the ocean except Cd, Cr and Co, contrasting slightly with those obtained by previous reports [2,3]. This difference may largely be to the difference in various discharges into the rivers at the area of studies. The difference may equally be that the bioaccumulation of the heavy metals was species-related.

Table four shows the percentage weight of the metals, (K, Ca, Fe, Mn, Zn and Ni) in the fishes used for this study, the percentage weight of Ca, Mn and Nickel in the Liver and the serum of the rats fed by our study fishes as compared with the WHO recommended values of the metals needed for functioning of the body system. The values for both the Catfish and the Tilapia on this table are about 100% higher than the WHO recommended % value. This may likely have some health implications on the people in the area of our study, as a result of bioaccumulation of these metals by the fishes.

Evaluation of Haematological parameters was used in this study to determine the possible changes in the blood parameters due to the combined effect of the consumed metals and the OSMF. The changes in the mean Red blood cell count (RBC), Packed cell volume (PCV), Haemoglobin concentration (HGB), as well as the MCHC, MCH and MCV, total white blood cell count (WBC) and White blood differentials are presented in Table 3. The values computed for the lymphocyte count and differentials of the rats showed that there was no significant difference across the experimental groups when compared with the control group. This could be as a result of the relatively short period of time the animals were exposed to these fishes and the oscillating radiation. Lymphocytes are the main effector of cells of the immune system, and exposure to heavy metals such as cadmium, mercury and lead have been demonstrated to interfere with immune cell function by competing with Iron in certain iron transfer proteins such as ferritin [12]. The absence of metals such as lead and cadmium in our fish samples may have contributed to the relatively normal levels of WBC counts observed in both experimental groups.

There was increase in Hb, RBC and PCV in the experimental groups (1 and 2) when compared to the control group, which were nonsignificant at ($p<0.05$). There was also no significant difference in MCH levels across the three groups. The increases observed in the RBC count and PCV indicate the balance between production and destruction of red blood cell but tilting towards production.

Hemoglobin tests measure the amount of Hb in grams per 1dl of whole blood and provides an estimate of oxygen carrying capacity of the RBC's. The observed mean corpuscular hemoglobin concentration, MCHC, of the experimental rats were higher compared to control group, indicating that there was more Hb per unit of RBCs. The reason for the increase in the level of Hb, MCHC and PCV in rats fed contaminated fish feed could be attributed to the presence of iron in both fish samples. Iron (II) ions are an essential in the biosynthesis of heme, such that it is the central coordinating ion in the porphyrin ring of the hemoglobin molecule [13]. Also the absence of certain heavy metals in the fish samples such as lead which competes with iron during heme synthesis also contributed to the observed increase in hemoglobin and PCV levels [14]. Another reason is that it could be due to an increase in the protection of RBCs from oxidative destruction. The elevation in the levels of haemoglobin culminated to protein utilized by red blood cell for the distribution of oxygen to other tissue and cells of the body is an indication of induced synthesis of the porphyrins which is required by the detoxifying enzymes [15]. The mean corpuscular volume (MCV) reflects the size of red blood cells by expressing the volume occupied by a single red blood cell [16]. MCV values of the treated rats were largely unaffected. This implies that neither the fish samples nor the oscillating magnetic field had effect on the MCV values.

Blood lymphocytes, neutrophils, eosinophils and basophils are important cells of the immune system in mammals. They are involved in engulfing foreign pathogens, secreting cytotoxic substances and producing immunomodulatory cytokines which improves the overall immune response [17]. Neutrophils are usually the first line of defense in the innate immune response, and they are the most abundant of the leucocytes in circulation [18]. The B and T lymphocytes, play their roles in cellular immunity by producing antigen-specific antibodies and secreting toxic chemical substances to destroy invading foreign cells, respectively. Blood basophils, on activation by a subclass of the immunoglobulins, IgE, release pharmacologically active mediators, which culminate in pathogen destruction [18].

The neutrophils, lymphocyte and basophil count in the rats in experimental groups are lower than the that of the control group. The low value is more pronounced in the experimental group exposed to OSMF. This could mean that the exposure to magnetic radiation and the fish sample did not elicit an immune response from the animals in this study. Heavy metals such as cadmium, mercury, have immunosuppressive effects on cells of the immune system [12]. In similar fashion, it has been reported that cadmium, palladium, platinum, and vanadium interact with these cells, causing them to overproduce free radicals, causing cellular toxicity as a result [19]. However, these were not present in the fish sample which were fed to the animals.

The increased level of serum and liver malondialdehyde levels showed that exposure of rats to magnetic field resulted in membrane lipid peroxidation (Figure 1). The exposure to magnetic field could have caused a disruption of the polyunsaturated bond in the phospholipid bilayer of the cell membrane thereby compromising the membrane. Lipid peroxidation have been implicated in several pathological diseases [20]. Also, magnetic field contributes to cellular injury by elevating nitric oxide and malondialdehyde levels [21]. The group fed with fish under magnetic field showed slight increase when compared to the control but lower level of lipid peroxidation when compared to the group exposed to magnetic field. Although fish consist of unsaturated fatty acids making it susceptible to high level of oxidation leading to rancidity, but contain of a lot of both enzymatic and nonenzymatic antioxidants to protect their membrane lipids from oxidation. The lower level of lipid peroxidation observed in the group exposed to magnetic field but fed with fish could be attributed to the presence of antioxidant such as catalase, peroxidase and glutathione and superoxide dismutase), carotenoids, peptides, amino acids and phenolic compounds (tocopherols, ubiquinones).

The activities of the antioxidant enzymes (Catalase and SOD) on the liver and serum of the animals were investigated in this study. It was observed that there was reduction in levels of SOD and catalase in the liver and the serum of the experimental rats as compared with the control group. This observation is presented in the bar chat diagrams in Figure 2 and Figure 3. Fish ingestion by the rats caused a reduction in the level of SOD and catalase and this could be attributed to the high level of heavy metals in the fish, which could alter the mechanisms of antioxidant enzymes by forming covalent interactions with sulfydryl groups of these enzymes, inhibiting their activities, and inactivating them in the process [12] thus giving rise to oxidative stress in the long term. The decrease may also be due to enzyme inhibition or inactivation by exposure to the magnetic field. Magnetic fields have been reported to decrease the activity of superoxide dismutase (SOD), glutathione peroxidase [22] and catalase by possible interactions with the electron transport(redox) mechanisms of these enzymes [21]. Thus, both heavy metals and the oscillating magnetic field, either singly or in combination had adverse effects on the antioxidant defence systems of the animals in this study.

Alanine aminotransferase is one of the marker enzymes measured to access proper functional state of the tissues such as liver and kidney. The liver in particular is the main target organ for xenobiotics, as it plays the most vital role in their biotransformation, detoxification and elimination from the body [12]. There was no significant difference in liver ALT activity in both control and the groups exposed to heavy metaled fish and/or oscillating magnetic field as displayed in Figure 4. These results are in accordance with those of previous investigators [23,24] who reported that sub-acute exposure of rats to static magnetic field had no effect on ALT activities. However, an earlier study showed that at sublethal concentrations of Cd, Zn, Pb, Fe and Mn, cause reduction in liver enzyme activities by binding to thiol groups of these proteins [25]. The reason for disparity in these results could be that the concentration of metals in the fish sample were not high enough to cause substantial liver damage. In relation to that, the liver is capable of protecting itself from small doses of heavy metals by expressing certain metal binding proteins called the metallothioneins [26]. Another reason is that, the cadmium was not among the metals detected in the fish sample.

5. Conclusion

The exposure of rats fed with metal-contaminated fish feeds and exposed to oscillating magnetic field had no adverse effects on the haematological status of the animals. Also, the liver ALT activity was unaffected as a result of exposure to the heavy metals and the magnetic field. However, exposure of rats to magnetic field and heavy metals caused a reduction in activity of antioxidant enzymes, which could give rise to oxidative stress on the long run. The results also depicts certain additive, injurious effects of both heavy metals and magnetic fields exposure, particularly on cellular antioxidant defence system of the animals.

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