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Effect of sub-acute oscillating magnetic field exposure of 1.65mT on biochemical and haematological parameters of rats fed with fertilized vegetables

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***Abstract*—**The biochemical and hematological effects of rats fed with vegetables planted on fertilized soil after exposure to sub-acute oscillating magnetic field was investigated. Five different types of soil were sampled along with three types of commonly eaten vegetable plants nursed on two types of fertilizers. Each of the vegetable plants was planted in five local pots, each containing a sampled soil. Other sets of local pots matching this description were fertilized differently with *Telfaria occidentalis* (Fluted pumpkin), *Amaranthus hybridus* (Green amaranth) and *Basella alba* (Malabar spinach) respectively. The control group consists of 3 pots of vegetable plants without fertilizer while the experimental group consists of 6 pots of plants planted with fertilizer. After the vegetables have been matured, they were harvested, dried and grinded into powdered form. The elemental levels in both the soil and vegetables were measured using the Atomic Absorption Spectrophotometer (AAS). Three groups of five rats each were formed from fifteen selected rats. Each group was fed differently with the vegetables harvested from experimental groups, and later exposed to a sub-acute oscillating magnetic field for about 30 days. The animals were sacrificed and the liver was excised for biochemical and haematological analysis. The results show that exposure of rats to magnetic field caused oxidative stress. The accumulated level of heavy metals in the vegetable did not cause significant stress probably due to the presence of bioactive phytochemicals. Data obtained suggest that some haematological parameters of human could be altered if exposed to either sub-acute oscillating magnetic field or consumption of fertilized vegetables.

***Keywords*—**biochemical, haematological, vegetables, fertilizer, oscillating, magnetic field.

I.INTRODUCTION

Vegetables are edible portion of a plant that form a crucial component of the human diet since they contain several food components ranging from carbohydrates, proteins, vitamins, minerals and fibres [1]. Due to their importance in human diet, the need to increase the production of vegetables in Nigeria today using fertilizers becomes very essential [2].

Other than liming materials, any materials of natural or synthetic origin applied to the soil which supply essential nutrients to enhance the growth of plants, thereby increasing the yield are regarded as fertilizers. Different types of fertilizers are available in market today, this include NPK (Nitrogen, Phosphorus and Potassium), Urea fertilizer (46% of $N\text{-}NH_2$), Magnesium fertilizer (11% Mg, 14% S and 42% SO_4) and Calcium Nitrate fertilizer (14.4% $N\text{-}NO_3$, 1.1% $N\text{-}NH_4$ and 19% Ca) [3].
However, NPK, Urea, and Calcium Nitrate fertilizers have been described to be the best fertilizer for the growth of vegetable plants. The application of fertilizer to the soil also increases the metal content in the soil due to their constituents. Climate, soil concentrations of heavy metals, soil type, fertilizer applied, and plant maturity level at harvest time are some of the variables that have an impact on the bioaccumulation of metals in vegetables. This may alter the physicochemical characteristics of the soil, such as pH, organic matter, and the bioavailability of metals in the soil, and also influencing the uptake of heavy metals by the soil [4,5,6]. Cultivation areas close to highways are also subjected to air pollution from metal-containing aerosols. These aerosols can either be directly exposed to the leaves and fruits, where they are then absorbed, or they can be exposed to the soil, where they are then absorbed indirectly by the vegetables [4,7,8,9].

II. RELATED WORK

Rats fed on fish contaminated with heavy metals and their haematological and biochemical characteristics after being exposed to a 47mT oscillating magnetic field has been previously studied [10]. The haematological status of the rats exposed to oscillating magnetic field after being fed fish feed contaminated with metal showed no adverse effects [10]. In addition, the activity of alanine aminotransferase (ALT) in liver has also been shown to be unaffected following exposure to the magnetic field and heavy metals [10]. The result obtained from this work has been shown to depict certain combined deleterious effects of both magnetic fields and heavy metals exposure and in particular on the cell's antioxidant defense system in animals [10].

However, the aim of the present study is to investigate the metals level in the soil samples and the level of metals the vegetable plant has absorbed from the soil fertilized with NPK and Urea. It is also desired to investigate the changes in both biochemical and haematological parameters on the rats fed with the harvested fertilized vegetables exposed to sub-acute oscillating magnetic field of 1.65mT.

III. MATERIALS AND METHODS

Laboratory animals

Fifteen albino male wistar rats were purchased from a commercial seller in Owo market, Ondo State, Nigeria.

Vegetable plants materials

Three types of vegetables *Telfaira occidentalis*, *Amaranthus hybridus* and *Basella alba* seeds were obtained from a vegetable garden in Adekunle Ajasin University, Akungba-Akoko, (AAUA) Ondo State.

Soil Samples

Five types of soil were obtained from the AAUA School garden; loamy, clay, humus, brown sandy soil and white sandy soil. These soils were packed inside a polythene bag and labeled. The soil were first weighed before drying and weighed again after drying. This was followed by detection using a flame photometer for analysis of sodium (Na) and potassium (K) in the soil.

Methods

Atomic absorption spectrophotometer (AAS) was used to determine the amount of zinc (Zn), iron (Fe), calcium (Ca), magnesium (Mg), copper (Cu) and lead (Pb) that were present in the soil. A glass electrode pH meter was used to measure the pH at a soil to water ratio of 1:1, and the soil's electrical conductivity was also examined using the Horiba (LAQUAtwin) conductivity meter. Hydrometers were used to determine particle size analysis using a modified Day method [11]. Using Jackson's reported chromic acid wet oxidation method, the soil's organic carbon content was measured [12]. According to Jackson, the micro-kjeldal technique was used to determine the nitrogen (N) concentration [12]. Using Bray No. 1 P solution, phosphorus (P) was extracted [13] and the P in the extract was measured calorimetrically using Murphy and Riley's molybdenum blue color technique [14]. IN neutral ammonium acetate solution was used to extract the exchangeable bases. Using the EDTA titration method, the volumetric amounts of Ca and Mg were determined [15]. By using flame photometry, the concentrations of K and Na were determined, and the magnesium concentration was determined by the difference. This was established using McLean's KCI extraction and titration techniques [16]. We calculated the effective cation exchange capacity as the sum of exchangeable bases (Ca, Mg, K, and Na) and exchangeable acidity. Utilizing Soon and Abboud's methodology, the Pb was calculated [17].

Treatment of planted vegetables

Each of the vegetables was planted in different pots using five different types of soil in triplicate giving rise to fifteen samples. The first vegetable pot triplicate had NPK fertilizer added while the second had urea fertilizer added and neither NPK nor urea fertilizers was added to the third (control) of the triplicate set. After 3 months of plantation, the matured vegetables were harvested. These matured vegetables were dried in an oven for 2 days, after which it was grinded into powder form and kept in a 100 ml standard flask for AAS analysis.

Preparation of plant materials for AAS

The plant materials were digested using a combination of $HClO_4$, HNO_3, and H_2SO_4 acids [18]. The mineral ions (K, Na, Ca, Mg, Fe, Mn, Zn and Pb) were determined by the use of atomic absorption spectrophotometer. The amount of P content was determined following AOAC [19] in which the micro-kjeldal method of Jackson [12] was employed for N determination while the perchloric acid digestion (wet oxidation) method was used. The Azmat and Haider method was used to calculate the protein contents [20].

Consumption of fertilized vegetables/treatment of rats

The remaining vegetables were used to feed albino rats. The rats were housed in a well -ventilated cage for acclimatization for a week and were split up into three groups, each with five rats. After acclimatization, the first group of rats were taken and housed in an oscillating magnetic field cage and were fed with vegetables planted with fertilizer, while the second group were also taken and housed in an oscillating magnetic field cage, fed with standard rat chow. The third group of rats (control) was left to stand alone in their cage. The voltage and current of the oscillating magnetic cage were recorded daily. After a month, the experimental rats were sacrificed by cervical dislocation while samples of blood were collected into plain sample bottles for haematological analysis. The liver was also excised during this process for biochemical analysis.

Biochemical Analysis

Malondialdehyde (MDA) level was calculated using the technique described by Guttridge and Wilkings [21]. The Misra and Fridovich method was used to measure the superoxide dismutase (SOD) activity [22]. The Cohen *et al*. method was used to measure the catalase (CAT) activity [23].

Haematological Analysis
Haematological analyses were carried out on the plasma using an automated haematological analyzer.

Description of solenoid
A controlled oscillating magnetic field used for this research was created by constructing a solenoid using a coil, long cylinder, bar magnet and a d.c power supply. A long wire was wound around a made cylindrical cage to create a solenoid, which is a helical coil. The magnetic field generated by a solenoid is the sum of the magnetic fields created by the current in each turn. The magnetic field created by a solenoid depends on the position inside a solenoid and the current through a solenoid. The typical solenoid is several times longer than its width. The wire is tightly twisted in the shape of a small-pitch helix around the exterior of the long cylinder. A uniform magnetic field is created inside the cylinder especially far from the end of the solenoid. The larger the ratios of the length to the diameter, the more uniform the field near the middle.

Fig. 1: Oscillating magnetic field cage

The magnetic field's approximate value is provided by

B=μnI…………………………………………………….(i)
B is the magnetic field,**μ** is the permeability of free space, **n** is the number of turns of wire per unit length.
n=N/L ……………………………………………………(ii)
N is the number of turns of the coil and **L** is the length of the solenoid.
Number of turns of the coil in the solenoid, **n**= 450
Length of the solenoid, **L**= 1.9 m
450 x 1.9 = 855 m
N= 855 m
Permeability of free space, **μ** = 1.26×10^{-6} H/M
Current flowing through the coil, **I**= 2.9 A
B= Magnetic field inside the solenoid
Therefore, the field intensity was calculated as shown after combining eqn (i) and (ii)
B= μNI/L = $1.26 \times 10^{-6} \times 855 \times 2.9/1.9$
B= 1.65×10^{-3} T or 1.65 mT
Note: 1 mT = 0.001T; 1.65/1000 = 1.65 mT (mT= Millitesla; T= Tesla)

Data Analysis
Genstat statistical package version 6.1 [24] was used to analyze the data. Duncan's Multiple Range Test was used to test for the Significant level of the group means at $p<0.05$.

IV. RESULTS AND DISCUSSION

Table 1reveals the amount of metals present in the selected soil samples while Table 2 shows the pH and electrical conductivity of the sampled soils. Loamy, humus and clay soils are richer in Mg, Na, K whereas brown sandy and white sandy soils are richer in Ca, Fe, Zn, Pb and Cu. The pH of the soil samples range from 7.91 to 9.10 which indicate that the soils are basic and not acidic. Brown sandy soil shows high electrical conductivity followed by humus soil while the clay soil shows the least electrical conductivity.

Table 1: The amount of metals presents in the selected soil samples

Soil	Mg (ppm)	Na (ppm)	K (ppm)	Ca (ppm)	Fe (ppm)	Zn (ppm)	Pb (ppm)	Cu (ppm)
Brown sandy	22.57	3.2	7.0	30	1.20	0.21	0.04	0.03
White sandy	16.38	2.0	14	33	0.95	0.22	0.04	0.03
Clay	32.65	2.3	24	26	0.27	0.12	0.01	0.01
Humus	32.75	3.2	48	24	0.25	0.06	0.01	0.02
Loamy	40.01	2.4	22	24.5	0.58	0.11	0.02	0.01

Table 2: The pH and electrical conductivity of the soil samples

Soil	pH	Electrical conductivity ($^{o}/_{oo}$)
Brown sandy	8.00	122.3
White sandy	8.44	96.3
Clay	7.91	66.3
Humus	9.10	115.5
Loamy	8.10	74.4

Table 3- Table 7 showed the amount of metals in vegetables on loamy soil, clay soil, humus soil, brown sandy soil and white sandy soil respectively. The results show that the sampled plants accumulated more of the metals when planted on fertilized soil than when planted on natural soil.

Table 3: The amount of metals in vegetables on loamy soil

S/N	PLANT	SAMPLE	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	P (ppm)	N (ppm)	Fe (ppm)
1	*Basella alba*	Natural	18.26	22.46	19.56	3.55	17.57	12.68	3.18
2	*Telfaira occidentalis*	Natural	17.17	20.77	22.16	4.35	22.10	12.70	2.98
3	*Amaranthushybridus*	Natural	16.22	18.22	24.73	3.25	16.43	10.22	2.26
4	*Basella alba*	NPK	25.62	32.11	31.04	8.40	31.10	14.68	3.23
5	*Telfaira occidentalis*	NPK	20.23	34.57	39.26	4.46	23.57	14.12	3.49
6	*Amaranthushybridus*	NPK	19.23	30.29	38.40	5.20	20.21	16.23	3.83
7	*Basella alba*	Urea	26.11	63.25	43.77	9.10	57.50	18.24	4.88
8	*Telfaira occidentalis*	Urea	40.12	43.44	49.88	6.44	32.44	20.22	4.81
9	*Amaranthushybridus*	Urea	44.10	30.76	52.32	5.67	62.40	22.80	4.45

Table 4: The amount of metals in vegetables on clay soil

S/N	PLANT	SAMPLE	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	P (ppm)	N (ppm)	Fe (ppm)
1	*Basella alba*	Natural	20.71	26.43	31.18	5.56	39.02	15.15	2.62
2	*Telfaira occidentalis*	Natural	21.48	25.33	16.21	5.88	29.42	14.66	4.02
3	*Amaranthushybridus*	Natural	20.22	26.52	28.52	4.64	31.40	13.45	3.65
4	*Basella alba*	NPK	23.85	29.32	32.70	10.44	41.15	16.19	6.82
5	*Telfaira occidentalis*	NPK	21.93	31.41	30.69	8.60	45.26	14.90	4.70
6	*Amaranthushybridus*	NPK	29.70	30.33	35.18	6.42	39.42	18.11	6.17
7	*Basella alba*	Urea	25.72	30.18	48.17	11.45	55.71	17.12	8.72
8	*Telfaira occidentalis*	Urea	25.19	37.42	51.27	13.25	60.46	38.13	8.84
9	*Amaranthushybridus*	Urea	37.10	56.31	40.19	10.45	41.09	30.15	6.45

Table 5: The amount of metals in vegetables on humus soil

S/N	PLANT	SAMPLE	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	P (ppm)	N (ppm)	Fe (ppm)
1	*Basella alba*	Natural	28.16	30.49	16.20	4.78	35.67	14.33	3.23
2	*Telfaira occidentalis*	Natural	19.42	28.40	19.56	3.89	22.10	16.18	3.21
3	*Amaranthushybridus*	Natural	16.24	27.40	26.16	3.42	24.29	16.44	2.11
4	*Basella alba*	NPK	30.33	39.20	30.55	7.35	36.30	16.57	4.50
5	*Telfaira occidentalis*	NPK	21.17	32.15	37.12	4.22	26.45	18.48	4.46
6	*Amaranthushybridus*	NPK	23.16	36.28	30.33	4.24	33.45	16.63	3.32
7	*Basella alba*	Urea	37.26	50.11	40.44	8.93	38.65	20.67	4.94
8	*Telfaira occidentalis*	Urea	29.08	46.13	41.88	14.25	35.31	35.31	5.10
9	*Amaranthushybridus*	Urea	26.34	50.77	46.10	5.60	34.25	21.22	5.13

Table 6: The amount of metals in vegetables on brown sandy soil

S/N	PLANT	SAMPLE	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	P (ppm)	N (ppm)	Fe (ppm)
1	*Basella alba*	Natural	18.33	43.29	38.57	3.74	39.40	13.48	3.17
2	*Telfaira occidentalis*	Natural	19.56	38.47	33.40	9.47	25.67	12.45	3.95
3	*Amaranthushybridus*	Natural	21.40	38.48	40.33	6.75	23.46	14.33	3.13
4	*Basella alba*	NPK	24.20	44.20	48.20	13.45	42.50	16.57	10.70
5	*Telfaira occidentalis*	NPK	22.46	40.36	50.50	9.49	38.25	15.20	5.10
6	*Amaranthushybridus*	NPK	23.38	42.30	49.86	12.49	44.01	18.40	4.19
7	*Basella alba*	Urea	30.12	66.45	51.29	8.55	40.41	20.38	18.10
8	*Telfaira occidentalis*	Urea	28.30	56.38	50.55	10.20	28.20	27.20	12.80
9	*Amaranthushybridus*	Urea	26.29	51.30	55.44	12.80	39.41	20.73	20.27

Table 7: The amount of metals in vegetables on white sandy soil

S/N	PLANT	SAMPLE	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	P (ppm)	N (ppm)	Fe (ppm)
1	*Basella alba*	Natural	25.17	29.30	30.16	15.02	23.08	15.16	3.14
2	*Telfaira occidentalis*	Natural	25.78	30.14	20.88	14.15	22.45	15.70	5.17
3	*Amaranthushybridus*	Natural	26.94	32.67	25.16	8.55	16.96	14.27	2.39

4	*Basella alba*	NPK	25.71	30.34	33.94	15.21	27.52	17.50	4.32
5	*Telfaira occidentalis*	NPK	33.33	30.14	40.72	21.75	39.47	16.73	8.16
6	*Amaranthushybridus*	NPK	38.10	42.50	48.23	14.65	41.49	17.16	3.12
7	*Basella alba*	Urea	38.33	40.33	62.07	21.70	27.56	30.72	6.18
8	*Telfaira occidentalis*	Urea	26.48	41.20	43.98	22.45	42.30	28.40	19.20
9	*Amaranthushybridus*	Urea	60.43	42.50	57.81	20.65	45.66	33.23	10.62

Biochemical effects of rats fed with metals contaminated vegetables

The results reveal a notable rise in MDA levels in both serum and liver (Fig. 2) coupled with a notable fall in SOD activity in liver and CAT activity in serum (Fig. 3 & Fig. 4) when rats fed with fertilized vegetables were exposed to sub-acute oscillating magnetic field (OS). Rats fed with only fertilized vegetables (VG) showed significant increase in MDA level in serum while a significant reduction was observed in their SOD and CAT activities in serum coupled with significant reduction in SOD activity in the liver when compared to control (see Fig. 3 & Fig. 4).

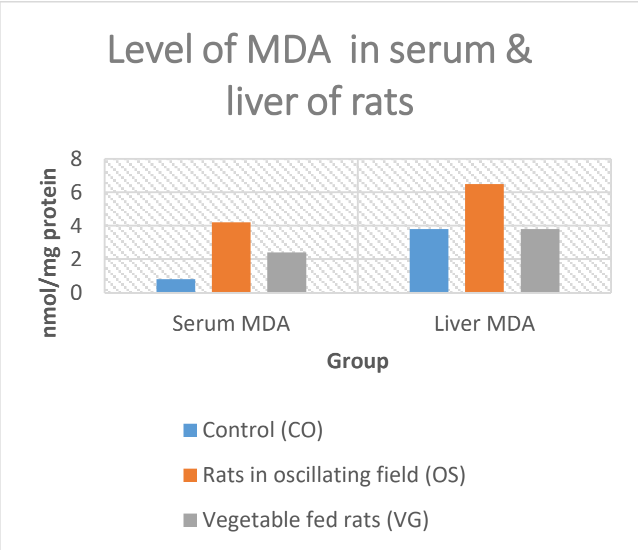

Fig. 2: Level of MDA in serum and liver of rats

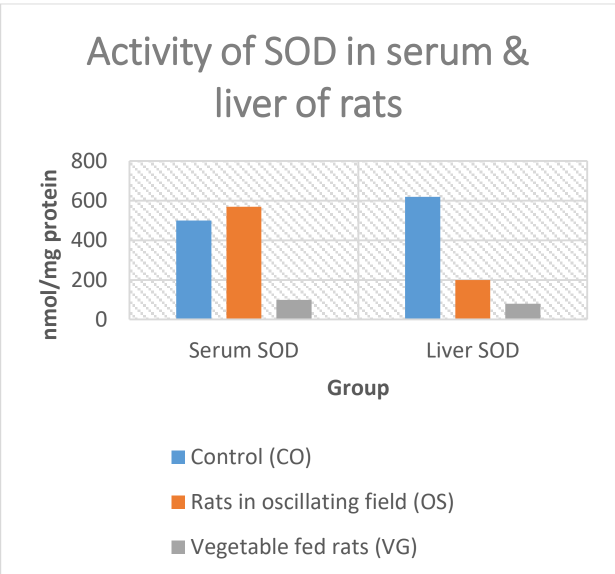

Fig. 3: Activity of SOD in serum and liver of rats

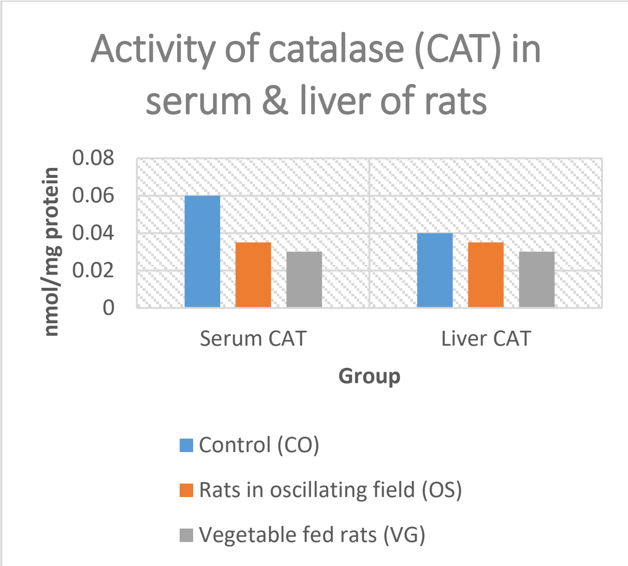

Fig. 4: Activity of catalase in serum and liver of rats

The result of the alanine aminotransferase (ALT) activities within both liver and serum of rats show a significant rise in liver ALT in rats fed with fertilized vegetables and exposed to sub-acute oscillating magnetic field (OS) whereas, a significant decrease in liver ALT in rats fed with only fertilized vegetables (VG) was observed when compared to control (Fig. 5).

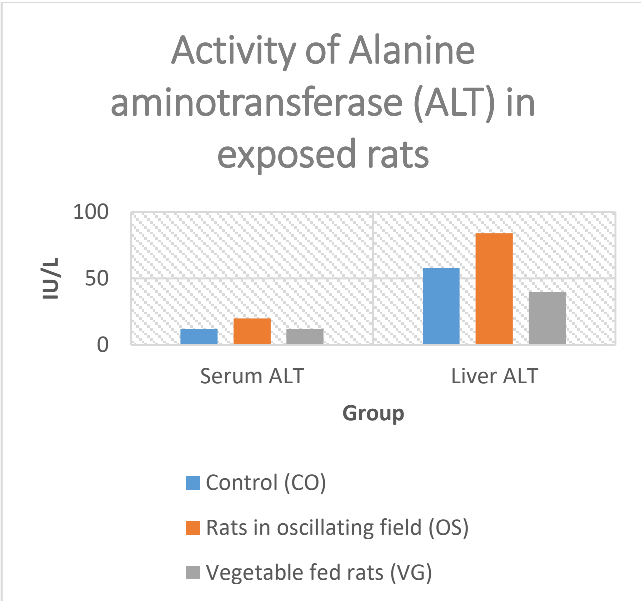

Fig. 5: ALTactivity in serum and liver of rats

Haematological effects of rats fed with metals contaminated vegetables

The results show significant decrease in WBC, neutrophils and haemoglobin values but increase in lymphocytes and MCH values in the group of rats fed with only fertilized vegetables (VG) when compared to control. Significant decrease in lymphocyte and MCV values coupled with significant increase in haemoglobin level were also observed in rats fed with fertilized vegetables and exposed to sub-acute oscillating magnetic field (OS) when compared to control (Fig. 6 – Fig. 10).

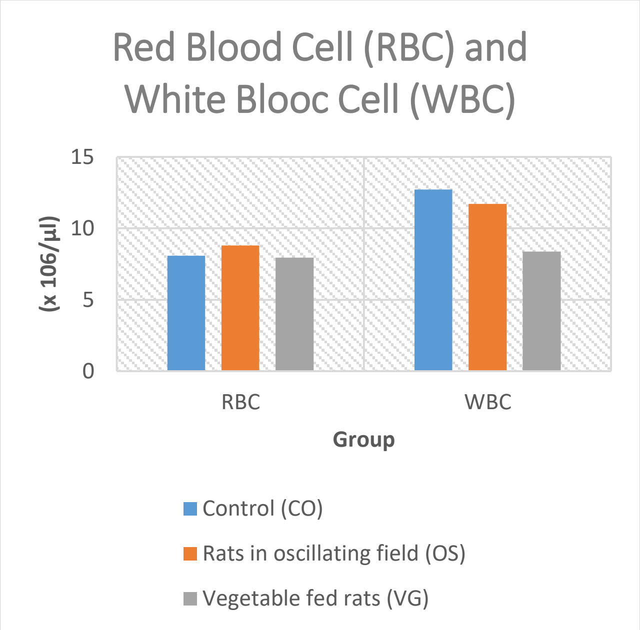

Fig. 6: RBC and WBC of rats exposed to *OS and *VG
*OS= Oscillating magnetic field *VG= Vegetable fertilized

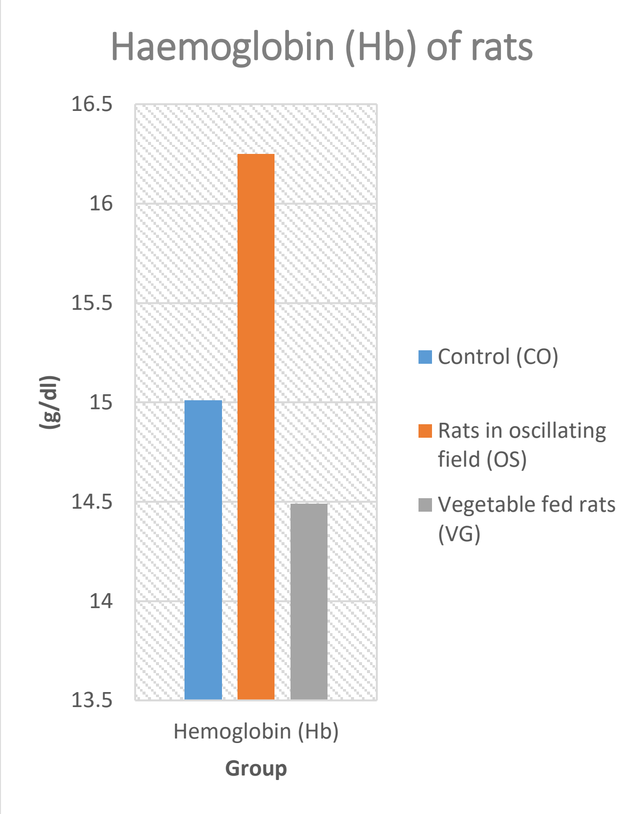

Fig. 8: Haemoglobin of rats exposed to OS and VG

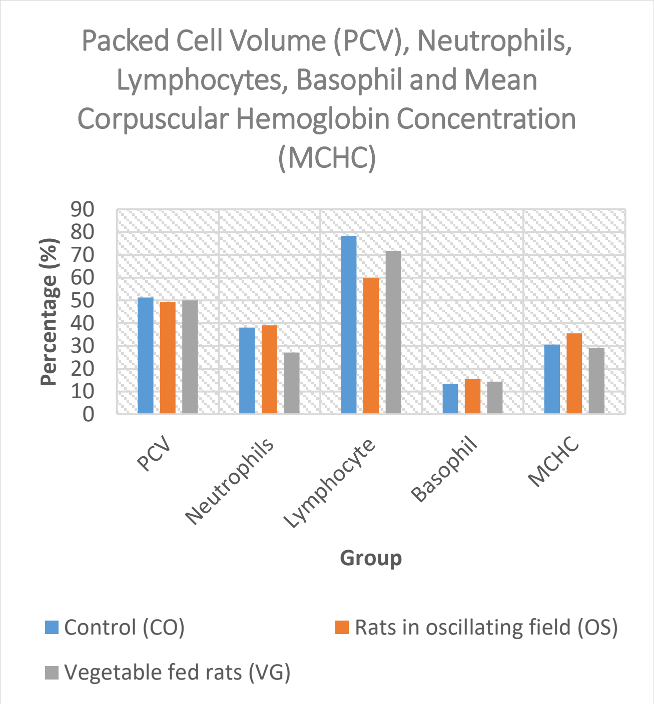

Fig. 7: PCV, Neutrophils, Lymphocytes, Basophil and MCHC of exposed rats to OS and VG

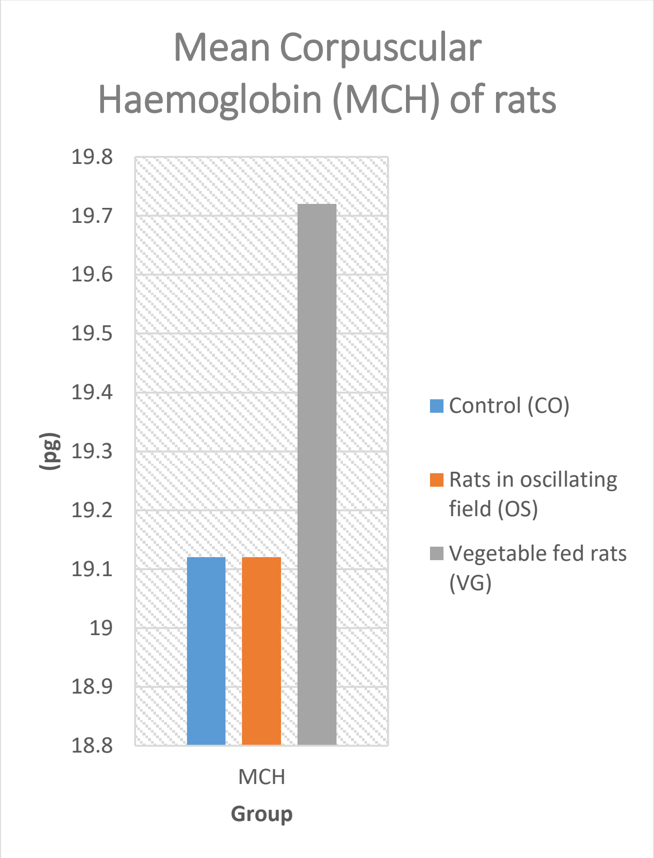

Fig. 9: Mean Corpuscular Haemoglobin of rats exposed to OS and VG

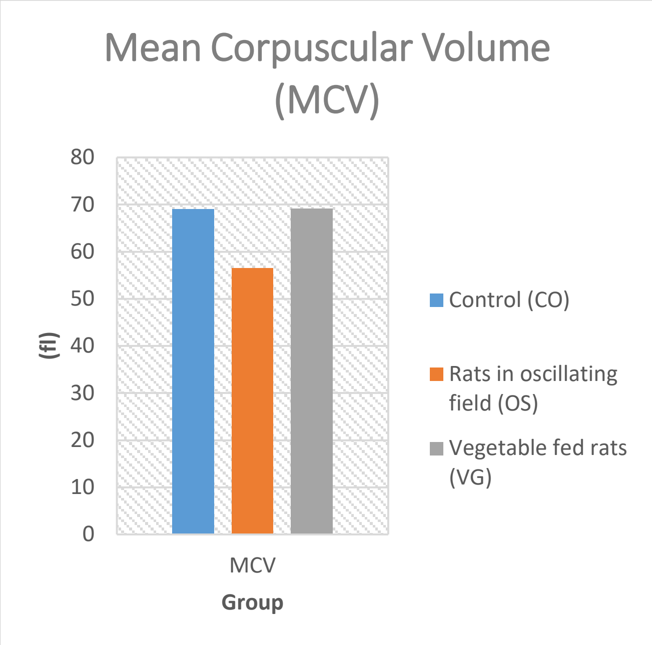

Fig. 10: Mean Corpuscular Volume of rats exposed to OS and VG

Several researches from earlier years have shown potential biological impacts of magnetic fields on human health [25,26]. Recently, effect of oscillating magnetic field has been revealed to have no negative effects on the haematological status of the rats when they are exposed via feeding with metal-contaminated fish feeds [10]. Epidemiological examinations have neglected to track down a connection between the constant presence of magnetic field at various forces and the presence of a specific pathology in live subjects [27,28,29,30]. Conversely, a few recent studies have revealed an increase in childhood leukemia and other associated diseases in children from populations exposed to electromagnetic fields [31,32]. Additionally, a number of reports show that magnetic field is engaged with malignant growth enlistment as co-cancer-causing factor ready to enhance the impacts of other mutagenic substances [33,34]. Studies in biochemistry have been done to assess how magnetic fields affect the metabolism of cell cultures, animals, and people [35]. The metabolism of proteins, lipids, and carbohydrates has been shown to be significantly disrupted in previous studies, as evidenced by altered blood glucose levels and increased glycolysis and glycogenolysis [36].

In rodents, magnetic fields have been shown to have substantial lipolytic and glycogenolytic effects [37,38], including a conspicuous increment in blood cortisol, glucagon and thyroxin levels. Animals have undergone examinations that are more intrusive. High *et al*. [39] discovered that exposure of rodents to sub-acute oscillating magnetic field (SMF) had no appreciable effects on spatial memory, death rates, heart rate fluctuations, body weight, or food and water intake. Static magnetic field exposure in mice reduced their appetite and drinking, and significantly raised their blood urea nitrogen, glucose, and creatinine levels [35].

In rats, the magnetic field had a disproportionately dominating influence on their hematological, digesting, and thermoregulation [40,41,42,43,44]. Given the lack of consensus about the biological effects of static magnetic fields, the goal of this study is to look into how exposure to SMF affected the blood cell count and plasma biochemical markers in adult male rats. Rats exposed to a magnetic field developed membrane lipid peroxidation, as evidenced by the rise in serum and liver MDA levels (Fig. 2). The cell membrane may have been compromised as a result of the magnetic field exposure because the polyunsaturated connection in the phospholipid bilayer was broken.

Numerous pathological diseases have been linked to lipid peroxidations [45,46]. The group fed with vegetable showed lower level of lipid peroxidation. This could be attributed to the phytochemical constituents (saponins, alkaloids and phenols) of the vegetables which have been reported to be potent antioxidant that could protect the membrane lipids from peroxidation.

In this study, the levels of the antioxidant enzymes SOD and CAT were examined. Rats exposed to a magnetic field resulted into a decrease in SOD and CAT levels (Fig. 3 and Fig. 4). The decrease may be due to increased oxidative stress by being exposed to a magnetic field, which lowers the amount of these enzymes. The decline could potentially be brought on by the magnetic field inactivating or inhibiting certain enzymes. Rats that consumed vegetables also had lower levels of SOD and CAT, which was likely due to the high concentration of heavy metals in the vegetables. It has been found that rats exposed to magnetic fields and heavy metals had reduced antioxidant enzyme activity, which could eventually lead to oxidative stress [10].

An increase in the ALT level in the serum and liver is an indication of tissue injury. The peroxidation of membrane lipid by magnetic field will compromise the cell membrane causing leakage of ALT from the liver to the serum (Fig. 5). Rats fed vegetables did not change significantly from controls, but they did differ significantly from the group that was only exposed to magnetic fields. This may be as a result of bioactive antioxidant compounds in the vegetable. The findings of this study demonstrated that rats fed fertilized vegetables and subjected to an oscillating magnetic field had improved haematological parameters. Also, though an immune response was elicited by the treatments, it was not severe. In toxicological terms, the exposure of rats to oscillating magnetic field did not adversely affect haematological status of the rats, with respect to RBC, WBC and Hb indices; it may have even improved it. The increase in RBC, PCV, total WBC, and lymphocytes counts after exposure to oscillating magnetic field may signify the positive effects of fertilized vegetables on the haemopoietic system of experimental rats and might be capable of improving the haematological abnormalities associated with some diseases. The liver activity is not compromised in the test rats as indicated by the ALT; showing that the treatment did not hamper the liver function.

V. CONCLUSION AND FUTURE SCOPE

Exposure of rats to magnetic field caused oxidative stress. The accumulated amounts of heavy metals in the vegetable did not cause significant stress as inferred from the result of this study probably due to the presence of bioactive phytochemicals such as saponin which counteract the destructive effect of the metal as well as that of oscillating magnetic field. Result obtained from this study suggests that haematological parameters of human could be altered if exposed to either sub-acute oscillating magnetic field or consumption of fertilized vegetables.

Further analysis is required to elucidate the mechanism behind haematological and the biochemical changes induced in serum and liver of rats when exposed to sub-acute oscillating magnetic field.

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