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## Effect of *Moringa oleifera* leaves extract against electromagnetic field impairments on hemoglobin and testes of rat

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### Abstract:

The present study has revealed the effect of *Moringa oleifera* leaves extract against electromagnetic field induced impairments. Forty-two adult white Albino male rats, weighting 150-180 g were involved in this study and divided into six equal groups. The first group was the control while the second group was daily supplemented with 200 mg/kg *Moringa oleifera* leaves extract orally for six days. The third group was exposed to an electromagnetic field of 1.5 mT, 50 Hz for 9 hours. The other three groups were exposed to an electromagnetic field of 1.5 mT, 50 Hz for 9 hours, and received the *Moringa oleifera* leaves extract in different supplemented ways. Blood samples were collected for the absorption spectrum of hemoglobin molecules analysis and serum total testosterone was studied. Samples of testes were taken for histopathological observations. The results showed that exposure of the animals to the electromagnetic field resulted in changing the absorption spectrum of hemoglobin, a highly significant reduction in testosterone level, and degenerative changes in testes. All these induced changes were decreased in groups treated by *Moringa oleifera* leaves extract in all ways. So through this study, one can conclude that damages induced by exposure to the electromagnetic field can be decreased by *Moringa oleifera* leaves extract.

**Keywords:** *Moringa oleifera*, electromagnetic field, hemoglobin molecules, testosterone, testes of rat

### 1. Introduction:

Technology allowed the expansion of many different apparatuses that emitting electromagnetic fields (EMF) such as mobile phones, satellite signals, television sets, microwaves, and computers. So we are exposed to EMF daily (Abd El-Hady and El-Tahawy, 2015). EMF might adversely effect the electrical properties of membrane proteins, so there are reports that EMF could be dangerous (Yildiz-

Gulay *et al.* 2017). Hassan and Abdelkawi, (2010) stated animals exposed to static magnetic fields have showed changes in the absorption spectrum of hemoglobin (Hb) molecules. Azab *et al.* (2018) reported that exposure to EMF resulted in a decrement in serum levels of testosterone, sperm motility, count, and induced sperm abnormalities. The testis in which the male reproductive gonad is very sensitive to many factors such as inflammation,

hyperthermia, and radiation (Bahaodini *et al.* 2015). Elbaz and Ghonimi, (2015) studied the effects of exposure to a 0.1 mT magnetic field on mature male albino rats. Their results showed pathological injuries in testis. Adah *et al.* (2018) concluded that EMF induced changes in the male reproductive system. These changes include spermatozoa decreased motility, morphometric abnormalities, increased peroxidation due to oxidative stress and histopathological changes in the testes.

*Moringa oleifera* is the most cultivated in tropical and subtropical countries. The leaves of these plants are highly valued nutritious, they rich with vitamins A, B, C and E,  $\beta$ -carotene, nicotinic acid, folic acid, protein, various phenolic compounds, amino acids, and minerals (Khalafalla *et al.* 2010). Bin-Meferij and El-kott, (2015) showed that polyphenolic-rich *Moringa oleifera* leaves extract (MOLE) able to protecting rat testis against EMF induced impairments. Also, Syarifuddin *et al.* (2017) reported that MOLE supplementation increased testosterone and motility of sperm. The aim of this study is to investigate the effect of MOLE against damage induced by EMF in rats.

## **2. Materials and Methods:**

### **Experimental animals:**

In this study (42) white Albino male rats, weighing 150-180 g were involved. The rats were divided into six equal groups; each group containing 7 rats.

**1- Group (A):** was the control group received an only standard diet.

**2- Group (M):** was supplemented daily and for six days with 200 mg/kg MOLE orally.

**3- Group (B):** was exposed to EMF of 1.5mT, 50 Hz for 9 hours.

**4- Group (N):** protection model, was supplemented daily, and for six days with 200 mg/kg of MOLE then exposed to EMF of 1.5mT for 9 hours.

**5- Group (T):** treatment model, was exposed to EMF of 1.5mT, 50 Hz for 9 hours then supplemented daily and for six days with 200 mg/kg of MOLE.

**6- Group (C):** contain both protection and treatment models, was supplemented daily, and for three days with 200 mg/kg of MOLE then exposed to EMF of 1.5mT, 50 Hz for 9 hours then supplemented daily and for three days with 200 mg/kg MOLE.

### **Electromagnetic field exposure device:**

The exposure device consists of a coil placed on a wooden rack which with 320 turns of 2mm copper wire wounded around a copper cylinder of 2 mm thick, 50 cm in diameter and 60 cm in length. The coil ends were connected to a variac that was fed from the mains (220 Vpp and 50 Hz) to produce the electromagnetic field as shown in fig. (1). The magnetic field of 1.5mT (in the area where the animals housed) was adjusted by Gauss/Tesla Meter, Model CYHT208 No: BH13049 - Chenyang.



Fig. (1): The exposure device

**Ethanollic *Moringa oleifera* leaves extract:**

*Moringa oleifera* leaves ethanolic extract was prepared according to the technique mentioned by **Okechukwu et al. (2013)**. Firstly, the leaves were dried at 29-35°C for three weeks then grinded. The grinded leaves were extracted (using a soxhlet extractor unit) by absolute ethanol and left for 48 hours. The extraction was evaporated to dryness using a rotary evaporator at 40-45°C. The extract was diluted using a polysaccharide to 1000 ml as a carrier and kept in the fridge. Finally, the diluted extraction was lightened with diluted by distilled water to equilibrate 1 kg of leaves powder/Liter.

**Absorption spectrum analysis:**

The rats under studying were anaesthetized with diethyl ether. Blood samples were taken from them by draining the blood from their eyes into tubes containing heparin, using capillary tubes. The tubes sealed and gently checked ready for performing measurements. Hemoglobin was extracted by the method of **Trivelli et al. (1971)** with modification. After hemoglobin extraction, its concentration was adjusted by appropriate dilution with deionized water at room temperature  $25 \pm 1^\circ\text{C}$  on the base of the heme absorption band at 576 nm where the absorbance of Hb at 576 nm equals 0.5. The absorption spectrum of hemoglobin was measured in the wavelength range between 200-800 nm at room temperature ( $25 \pm 1^\circ\text{C}$ ) using an automatic recording double beam UV –Vis spectrophotometer type Perkin Elemer precisely (Lambada 45) - Germany.

**Total testosterone measurement:**

Blood samples were taken from Albino male rats by draining the blood from their eyes into tubes without anticoagulants; using capillary tubes and allowed to clot. The clotted blood samples were centrifuged at 2000 rpm for 15 min to obtain the serum. The serum was stored at  $-25^\circ\text{C}$  until analysis.

**Histological procedure:**

The rats were anaesthetized with diethyl ether and tests of both control and experimental rats were removed and prepared according to the method described by **Drury et al. (1973)**. The method involves the fixation of the specimens in Boun's fluid and formalin 10% for 48 hours then dehydrated in a series of descending grades ethyl alcohol 70, 80, 90, and 100%, after that cleared by using cedar wood oil as a clearing agent. The cleared specimens were embedded in paraffin wax at ( $56-58^\circ\text{C}$ ), then sectioned at  $5\mu$  thickness by using a microtome. The sections were then stained with haematoxylin and eosin for microscopic examination.

**Statistical Analysis:**

Data were statistically analyzed by ANOVA test (SPSS version17).

**3. Results:****Absorption spectrum of hemoglobin:**

Figures (2&3and 4) shows the absorption spectrum of Hb extracted for control and different experimental groups. Table (1) indicates the absorbance values for all groups and the ratio  $A_{576}/A_{541}$ . The absorption spectrum of Hb- control group shows the different bands in wave length range of 230-680 nm. The bands were named as follow: 270nm referred to a globin band, peak at 345 nm is a globin heme interaction band and referred to the non-covalent bond between globin's histidine and heme iron, peak at 419 nm is a soret band, peak at 540 nm is the Nitrogen-iron band and peak at 576 nm is the heme-heme interaction band. The difference between absorbance values for all bands between group (A) and (M) is very small. Great differences were detected in all Hb-absorption spectrum bands for EMF exposed group (B) and there is an increase in  $A_{576}/A_{541}$  ratio (its value is accompanied with the appearance of a new band at wavelength 630nm). The difference decreased with using MOLE in all used ways.

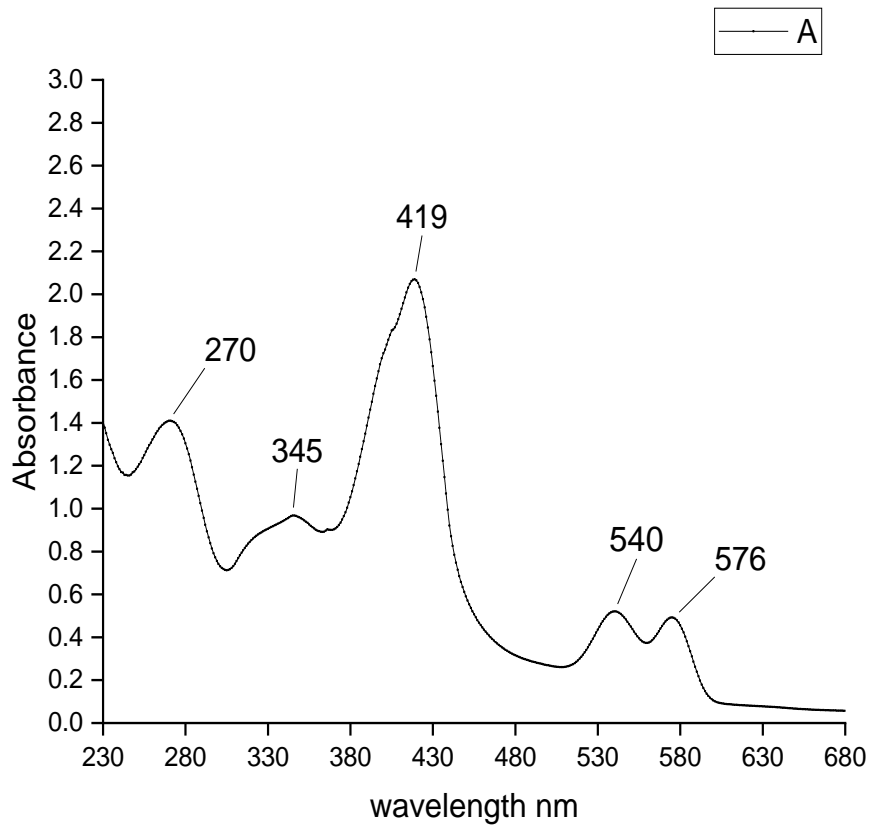


Fig. (2) Absorption spectrum of normal hemoglobin for control rat group (A) showing a characteristic bands

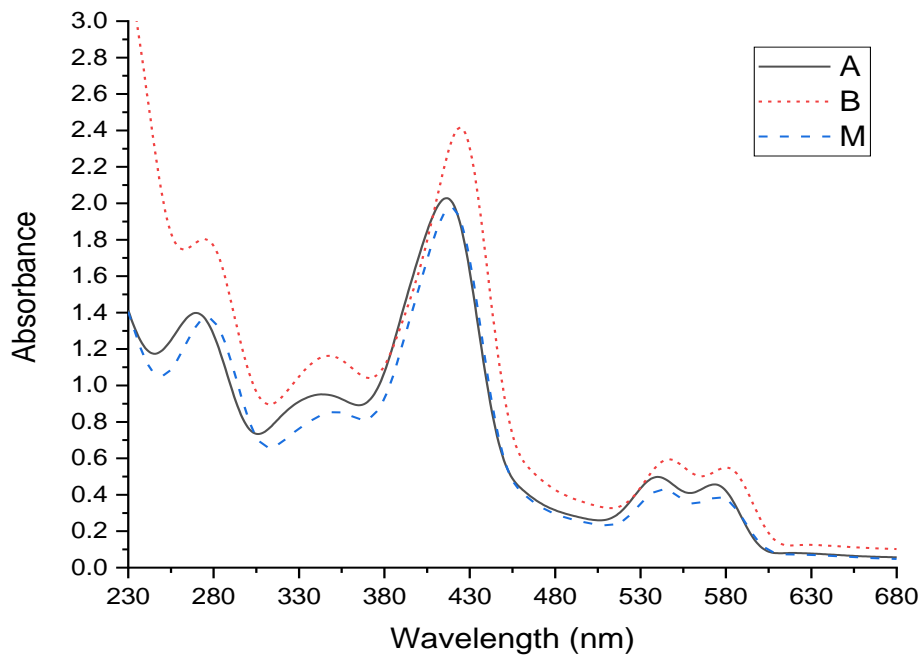


Fig.(3) Absorption spectrum of hemoglobin of rats which exposed to EMF of 1.5mT for 9 hours group (B) in comparison with group (M) and control one (A).

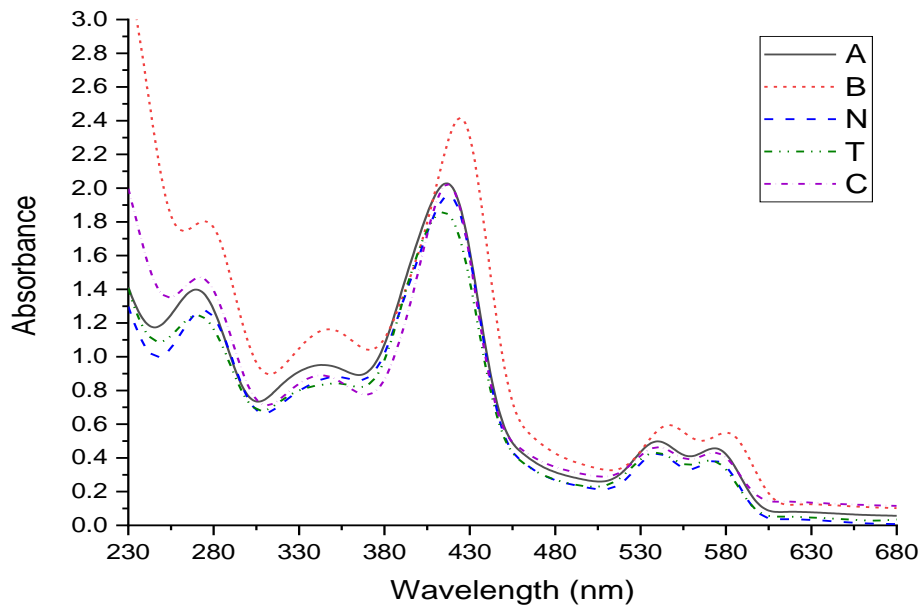


Fig. (4) Absorption spectrum of hemoglobin of rats which exposed to EMF of 1.5mT for 9 hours and treated with *Moringa* in different ways (N&T and C) in comparison with a group(B) and the control one (A).

Table (1): Absorption values of hemoglobin for experimental groups compared to the control one.

Group	Globin Band 270	Globin-heme interaction 345	Soret band 419	Nitrogen-iron band 540	Heme-heme interaction 576	Ratio $A_{576}/A_{540}$
A	1.4097	.968	2.069	.534	.498	0.933
B	1.752	1.158	2.357	.546	.524	0.961
M	1.330	.854	2.018	.507	.475	0.937
N	1.265	.889	2.008	.515	.484	0.941
T	1.253	.851	1.866	.523	.472	0.902
C	1.480	.898	2.090	.503	.481	0.956

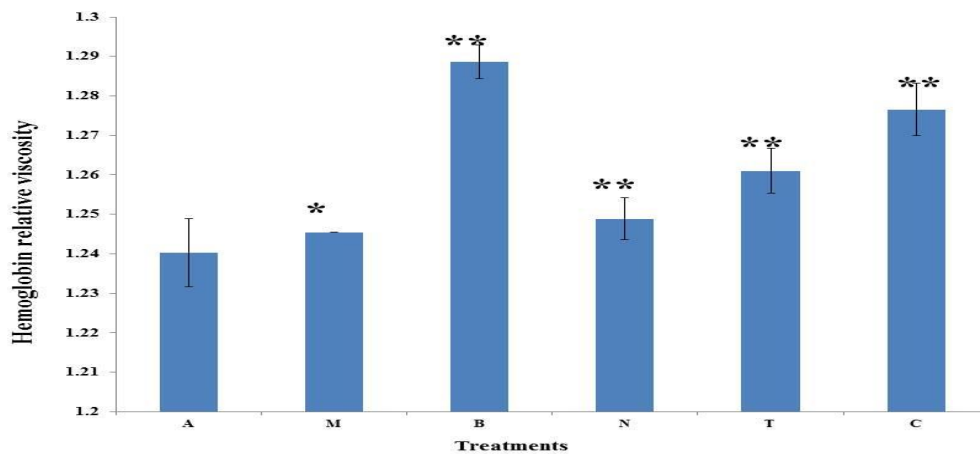
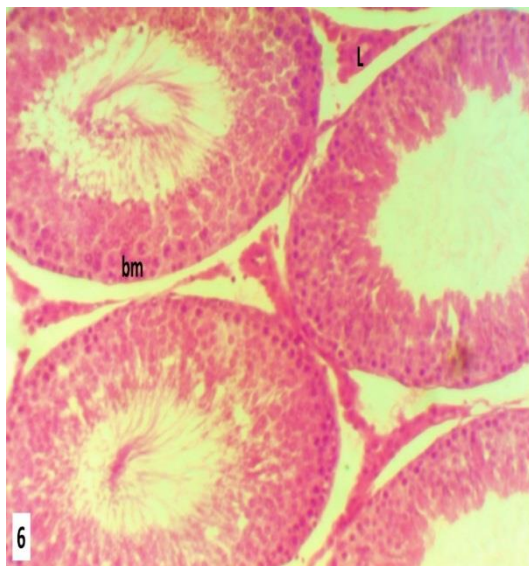
**Total testosterone measurement:**

Fig.(5):Histogram of serum testosterone  $\pm$ SD for control and different experimental groups.\*:high significant increase ( $P \leq 0.001$ ) \*\*:High significant decrease ( $P \leq 0.001$ )

Fig. (5): Summarizes the effects of 1.5mT EMF exposure on male rats testosterone and treatment with MOLE before (N), after and before, and after exposure to EMF. The results showed a high significant increase in the serum levels of testosterone ( $P \leq 0.001$ ) for (M) group. On the other hand, high significant decrease on the serum levels of

testosterone was observed ( $P \leq 0.001$ ) in EMF exposed group (B). The other three groups which take MOLE showed an increment in serum levels of testosterone in comparison with the exposed group, however, it still showed high significant decreases when compared with the control one.

**Histopathological observations:**

Figs. (6 and7) micrographs of transverse section of testis of groups (A) and (M) respectively showing normal seminiferous tubule. The seminiferous tubule has well-developed basement membrane (bm), numerous sertoli cell (sc) and sperms (s) in the lumens. Normal interstitial tissue in between the tubules which containing Leydig cells (L) (H&E,6 X 200, 2 X 400).

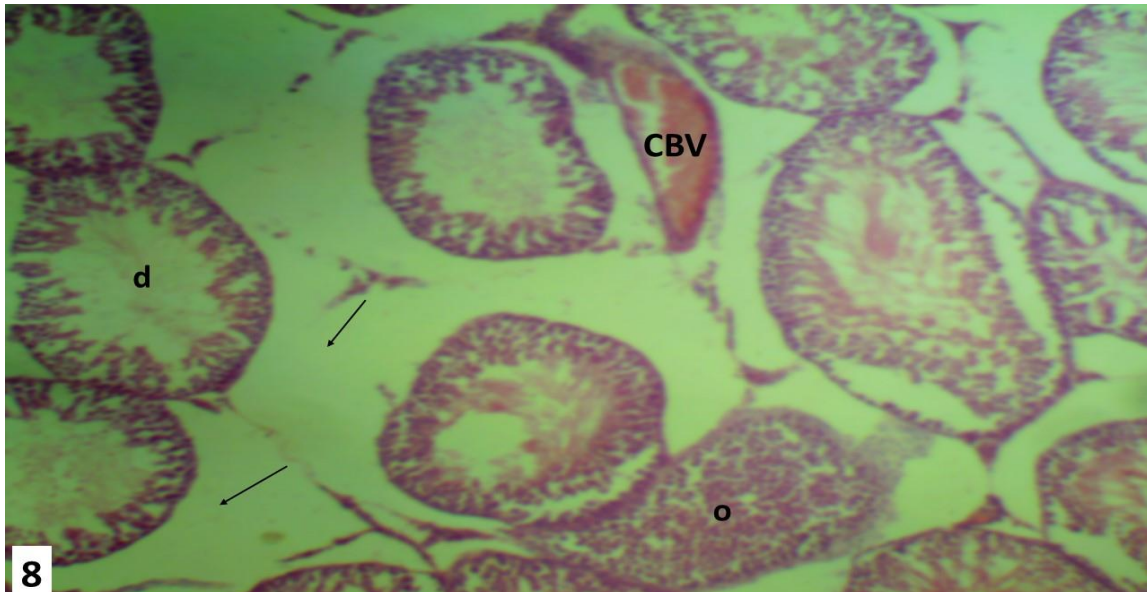
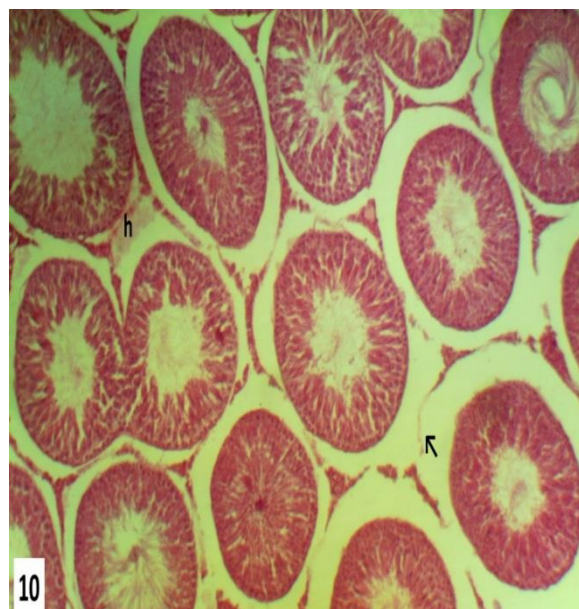
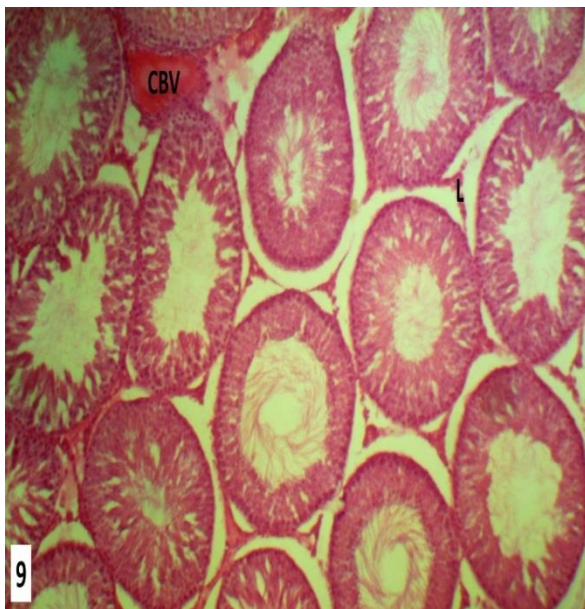
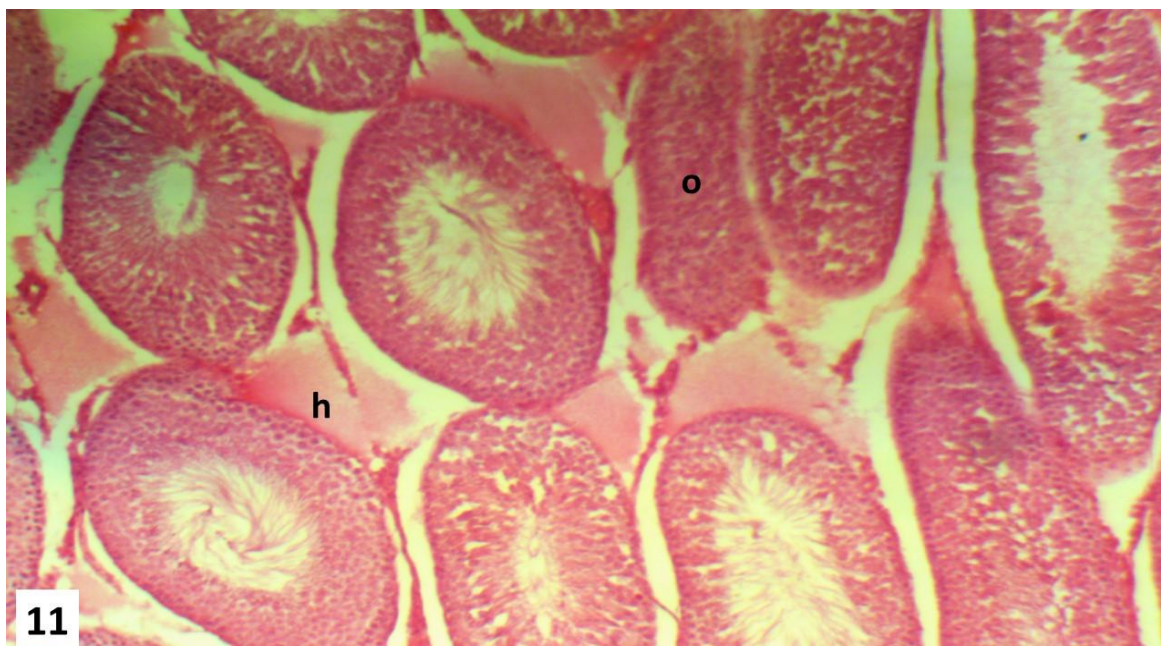


Fig.(8) Micrograph of transverse section of testis of group (B) showing disorganization (o) degeneration (d), decreased diameters and atrophy in some seminiferous tubules ,decreased interstitial tissue, widening of interstitial spaces (arrows) , highly reduced sperms in the lumen of the seminiferous tubules and reduction in Leydig cells **H&E, X 100**).





Figs. (9,10 and 11) Micrographs of a transverse section of testis of groups N, T and C respectively showing enhancement in the seminiferous tubules with the reappearance of Leydig cells (L) and sperms. However, some changes still found such as congested blood vessel (CBV), widening of interstitial spaces (arrows), disorganization (o), hemorrhage and atrophy in some seminiferous tubules (H&E, X 100).

Examination of hematoxylin and eosin-stained sections of control testes (Group A) and group (M) showed normal histological structure of testis tissue which showed normal seminiferous tubules, Leydig cells which were randomly distributed in between the seminiferous tubules, basement membranes and normal mature sperms which collected in the middle of the seminiferous tubules (Figs. 6 and 7). Sertoli cells which are large epithelial cells and have a large nucleolus showed also normal appearance. On the other hand, the sections of testis which exposed to EMF showed a degenerative changes represented in disorganization, degeneration and atrophy in some seminiferous tubules tissue, highly reduced number of Leydig cells, congested blood vessel (CBV) and dilation in interstitial spaces. Reduction in spermatogenic layers and cells and reduction in the number of mature sperms were also noticed (Fig.8). The examination of the testicular tissues of rats

treated with *Moringa oleifera* leaves extract in all treatment designs showed somewhat normal appearance. However, some changes still found such as congested blood vessels, widening of interstitial spaces, disorganization, hemorrhage, and atrophy in some seminiferous tubules (Figs. 10-12).

#### 4. Discussion:

The hemoglobin molecule is affected by any alteration in its environment. The absorption spectrum of the Hb molecule can give some evidence about variations in its conformation (El-Bediwi *et al.* 2013). The results of this study showed that exposure of Hb molecules to EMF was induced great changes in its absorption spectrum and these results in agreement with the study of (Hassan and Abdelkawi, 2010) who indicated that exposure of the animals to magnetic fields caused changes in the absorption spectrum of Hb molecules. The results presented in this study clearly demonstrated that



exposure of adult male rats to 1.5 mT,50Hz EMF for a period of 9 consecutive hours had a significant reduction effect on serum testosterone. This result is in agreement with **Yildiz-Gulay et al. (2017)** who found that exposure of rabbits to EMF results a decrement in testosterone concentrations. The present results of histological observations showed degenerative changes in the testis exposed to EMF. These findings are consistent with that of **Elbaz and Ghonimi (2015)** who reported that exposure of mature male rats to 50 Hz, 0.1mT Magnetic Field results in severe pathological lesions in the testis. In the present study, MOLE was showed a protective role against induced effects and this is due to *Moringa oleifera* micronutrients contain antitumor, anti-epileptic, anti-diuretic, anti-inflammatory and venomous bite characters. *Moringa oleifera* extract (MOE) contains specific plant pigments with demonstrated powerful anti-oxidative ability such as vitamins C, E, A, caffeoylquinic acids, carotenoids-lutein, alpha-carotene, and beta carotene, kaempferol, quercetin, rutin (**Akunna et al., (2012) and Maida et al. (2005)**). Our results are in agreement with **Bin-Meferij and El-kott, (2015)** who indicated that chronic exposure to EMF marked testicular damage which can be decreased by MOLE.

### **Conclusion:**

This study indicates that exposure to the electromagnetic field may cause conformational changes in hemoglobin molecules and significantly decrease serum testosterone. The results showed also degenerative changes in the testis of male albino rats. However, all these changes were decreased by oral sublimation of *Moringa oleifera* leaves extract. Therefore it can be concluded that MOLE had a protective role against the EMF induced effects. So one who forced to exposed to EMF can be recommended to take *Moringa oleifera* leaves extract in his diet by suitable dose defined by the doctor.

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