

Protective Effect of Spirulina Versus Vitamin E on Electromagnetic Waves Induced Thyroid Gland Damage in the Adult Albino Rat: A Histological and Immunohistochemical Study

Original
Article

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ABSTRACT

Introduction: The widespread use of several electrical and telecommunication applications that produce electromagnetic fields is increasing rapidly. One of the most important organs which affected by numerous types of electromagnetic waves is the thyroid gland.

Aim of the Study: This study targets to explore the effects of electromagnetic fields exposure on the thyroid and the protective influence of Spirulina platensis and vit E .

Material and Methods: Fifty healthy rats of adult male albino type were separated into five equal groups. Control group, EMW-exposed at which, rats were subjected to the effect of electric magnetic field individually 4 hours daily for a period of one month. EMW exposed+ Spirulina treated group at which rats received Spirulina at a dosage of 300 mg/kg b.wt. just previous to EMW exposure daily for one month. EMW exposed + Vit E treated group at which , rats were subjected to EMF like that of group II and given Vit E orally at a dosage of 200 mg/kg. b.wt and a recovery group at which the rats kept for one month after one month of exposure to low frequency electro magnetic field then the thyroid tissues were examined for histopathological and immunohistochemical changes.

Results: The group subjected to EMW showed thyroid follicles with degeneration and disorganization. Also numerous collagen fibers were present within the CT septa in the masson stained thyroid sections .The amount of PAS positive central colloid showed obvious decrease, with Strong Ki-67 immunoreactivity and TGF β 1 immuno expression. Spirulina platensis and vit E reduces the effect of EMW on thyroid gland , but vit E revealed obvious increase in the PAS positive central colloid amount, a significant decrease in collagen fibers, Ki-67 immunoreactivity and TGF β 1 immuno expression compared with that in spirulina group.

Conclusion: The use of spirulina platensis during the period of EMW exposure considered to has a protecting influence. Meanwhile the utilization of vit E has a more protection than spirulina

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INTRODUCTION

The widespread use of various electrical and telecommunication applications that produce electromagnetic fields (EMFs) is increasing rapidly in the past few decades^[1]. These (EMFs) affect human health as they are transformed partially into radiation waves^[2].

So, the electromagnetic waves (EMW) exposure becomes the most important research topic, as it leads to essential alterations and harmful biological properties^[3]. The electromagnetic field affects by its thermal properties through absorption of energy produced by electromagnetic field and by non-thermal effect through the long-term contact^[4].

Mobile radiation is said to disturb the thyroid gland metabolism owing to its non-thermal properties. The body tissues near to the mobile such as the thyroid and the brain

acquire more energy than the tissues away. These EMW initiate from the antenna, which is a part of the body of a mobile phone. These waves are highest at the antenna and its energy decrease as they travel away from the mobile^[5].

Electromagnetic waves can increase the generation of oxidative stress and cause a discrepancy between the reactive oxygen species (ROS) generation and the antioxidant protecting system, causing cellular destruction^[6].

One of the most important organs which affected by the electromagnetic waves is the thyroid gland^[7].

Spirulina platensis is a blue-green microalgae present in the regions where warm lakes are found like in the tropical and subtropical areas. It is recognized by its great dietary value because of its great content of protein, numerous vitamins, amino acids and minerals^[8].

Spirulina has antioxidant properties which attributed mainly to the polyunsaturated fatty acids, phycocyanin and phenolic contents^[9] also it has radioprotective properties^[10].

The phycocyanin components of the Spirulina ameliorates oxidative stress and apoptosis, by inhibiting the generation of ROS by the mitochondria and later alterations in redox state of the cells^[11].

Vitamin E is considered as one of the most important antioxidants; it plays an important role in decreasing oxidative stress^[12]. Vitamin E is a component of cell membranes because it is one of the fat soluble vitamins, so protected them from the oxidative damage^[13].

Also, it is a powerful antioxidant that prevents the cell damage by destroying the reactive oxygen species^[14].

In the current study, the outcome of using Spirulina platensis versus vitamin E on EMWs effect on the thyroid gland have been investigated.

MATERIALS AND METHODS

1- Animals and experimental design

This study was done on fifty adult albino male rats (2 months-old) their weight were 180-200 gram. We got the rats from laboratory animals unit, present in Faculty of Veterinary Medicine, Zagazig University, Egypt. Beneath environmental laboratory circumstance at temperature $20 \pm 2^\circ\text{C}$, The rats were kept in plastic cages to evade any metallic contact. exposure to an organized illumination period (light/: dark 14 hour/: 10 hour), water and typical diet were allowed. The procedure protocol was accepted by the Committee of Ethical research of Benha University. After 1 week of accommodation, the rats were divided into 5 equal groups each ten rats.

Group I (Control group): Ten rats were divided into 3 subgroups;

Group Ia: Three rats were put in the experimental situation like the exposed group but without exposure to radiation waves (the device of exposure was off) and was as a sham-exposed group.

Group Ib: Four rats ,two of them were given corn oil (5mg/kg body weight/day) orally by gavage needle and the other two rats were given distilled water orally by agavage needle and was as sham-exposed group.

Group Ic : Three rats were given only standard diet and tap water.

Group II (EMW-exposed): The rats were subjected individually 4 hours/day to the effect of electromagnetic waves for a period of one month.

Group III: (EMW exposed+ Spirulina treated group): The rats received Spirulina platensis daily just before EMW exposure throughout the experimental period which is one month. The Spirulina platensis powder was suspended in the distilled water at a dose of 300 mg/kg, body weight given orally by a gavage needle^[15].

Group IV (EMW exposed + Vit E treated group): The rats were given Vit E orally by a gavage needle at a dose of 200 mg/kg, body weight liquefied in the corn oil just before to EMW exposure daily throughout the experimental period which is one month^[16].

Group V: The rats kept as a recovery group for one month after one month of exposure to low frequency electro magnetic waves as group II (total period of 2 months).

2- Reagents

Spirulina is a blue-green tablet. Spirulina tablets were manufactured by Puritan's Pride Company, Inc. (USA). They were obtained from Delta Trade Company, Alexandria. Each tablet comprises 500 mg of the active component.

Vitamin E is used as DL-alpha-tocopherol acetate (soft gelatin capsules; 400 mg each). It was purchased from pharco Company and given orally at a dose of 200 mg/kg, body weight liquified in the corn oil.

3- Experimental design and Magnetic field producer system

A generator of magnetic field was used to generate uniform Magnetic field. which was made by Department of Physics in Faculty of Science, Benha University. It is made of copper wire which is electrically insulated 2 mm thickness coiled in a homogenous way around a cylinder of copper , its thickness is 1.5 mm, its diameter is 30 cm and its length is 20 cm forming a solenoid that contains 250turns and to eliminate the effects of electrical field, the cylinder was earthed.

During the period of exposure, a water pump was used to control the temperature of the generator. A variac fed (220 v, 50 Hz) was connected to the coils' ends from the mains with the power of the field was about 20 G (gauss). The cage containing the rats was put in the center of the coil to obtain a higher and homogenous magnetic field.

4-Hormonal assessment

The samples of the blood were collected at the end of the experiment according to the time mentioned in each group from all the animals tail veins in glass tubes (without anticoagulant) to coagulate and centrifuged for ten minutes at 3000 rpm to acquire the serum. The sera that collected were separated and saved at temperature -20°C until the hormonal analysis. By using radioimmunoassay (RIA) the total T3 and T4 levels were measured using commercial kits (Coat-A-Coat) and the Serum TSH were measured by using a particular rat TSH kits (provided by Diagnostic Products Corporation, Los Angeles, USA).

5- Histological and immunohistochemical examinations

At the end of experiment according to timing mentioned in each group, the rats have been anesthetized using an intraperitoneal sodium pentobarbital injection

(Nembutal, 30 mg/kg body weight) for sacrificing. The thyroid tissues have been processed for a light microscopic study. They were fixed in 10 % formaline for 1 day and were handled to make paraffin sections at 5 μ m thickness.

To evaluate the degree of inflammation after radiation exposure, the sections were stained with hematoxylin and eosin (H&E) in each group and stained with periodic acid-schiff (PAS) for glycoproteins and stained with Masson's trichrome stain to detect the collagen fibers in the tissues, respectively these sections were microscopically examined for any histopathological alterations^[17].

Immunohistochemical analysis

TGF β 1

After deparaffinization, the sections were treated successively with 1% H₂O₂ for a period of 10 min and washed thoroughly with PBS. For minimizing nonspecific IgG binding, 2% normal goat serum in PBS is used to block the sections at room temperature for 60 min, then kept with rabbit anti-TGF- β 1 (diluted 1:50; Santa Cruz Biotechnology, USA). After washing with PBS, the samples were incubated with biotin-conjugated secondary IgG (diluted 1:200; Vector Laboratories, Burlingame, CA, USA), at room temperature for 60 min, it is diluted in 2% normal blocking serum and then it kept for 60 min at room temperature with avidin-biotin-peroxidase complex. After that the samples were washed away with PBS and kept with diaminobenzidine tetrahydrochloride for 3 minutes to improve the color, 0.05% hydrogen peroxidase was used. The samples were then stained with Mayer's hematoxylin stain. By using light microscopy the sections were visualized and the digital images were taken and then analyzed^[18].

Ki-67

Fixation of the tissues in 10% formalin then dried and cleared and then inserted in paraffin. 5 μ m Sections were deparaffinized and then hydrated in an ascending sequences of alcohols. The tissue sections were kept with 3% H₂O₂ solution for a period of 20 minutes at a temperature 27°C. then kept at temperature 27°C with standard goat serum working solution for 30 minutes. Then, the sections were kept overnight with mouse monoclonal Ki-67 antibody at temperature 4°C (dilution, 1:500; cat. no. WH0004288M1) from Sigma (St. Louis, MO, USA). Then incubated at 27°C for 20 min with Goat anti-mouse IgG secondary polyclonal antibodies (dilution, 1:1,000; cat. no. ab6789; Abcam, Cambridge, MA, USA) Lastly, the sections were kept at 27°C with horse-radish peroxidase-labeled streptavidin working solution for a period 20 min. The sections were washed 3 times with PBS, 5 minutes each time, with adding of DAB solution. The sections were counterstained with hematoxylin to stain nuclei, dehydrated, cleared in xylene and mounted with DPX^[19].

The sections were examined by using a camera which attached to a Leica DM LS2 microscope at Human Anatomy

and Embryology Department, Faculty of Medicine, Benha University.

6- Morphometrical study

Serial sections stained with Ki-67 were morphometrically analyzed for revealing the area percent of brownish coloration & also Masson's trichrome stained sections for revealing the area percent of collagen fibers in the septa, around the follicles and the blood vessels by using 500 image analyzer computer system of Leica Qwin. The standered area measuring frame of a standard area is equal to 7286, 78 μ m². This was performed in 5 non overlying fields of 5 different sections from 5 different rats in each group at \times 400.

7-Statistical analysis

Data of all groups were expressed as (mean \pm standard deviation). The data achieved from the biochemical records and image analyzer were subjected to (SPSS program; version 20.0 for windows, SPSS Inc.,Chicago,IL). Statistical analysis using (ANOVA). The results were considered to be significant when the (*P*) value was \leq 0.05.

RESULTS

Histological results

H & E stain

Group I (Control group): Tissue analysis of control subclasses; Ia, Ib and Ic revealed nearly the same configuration. We used figures of the control subgroup Ic to differentiate with other groups. Tissue sections of the thyroid gland of the control group (GI) revealed regular, tightly packed follicles with narrow interfollicular septa in between, which contains connective tissues with blood capillaries and clusters of interfollicular cells (Figure 1A). The thyroid follicles are variable in size and shapes, filled with a homogeneous acidophilic colloid with peripherally fine vacuolated regions. The follicles are lined by a single layer of low cuboidal cells having rounded nuclei (Figure 1A). Also, interfollicular cells (C-cells) were detected between the follicles and had large pale nuclei.

Group II (EMW Exposed group): Thyroid in EMW exposed group exhibited degeneration and disorganization of the follicles. Some follicles indicated interrupted follicular wall. Congested blood vessels were perceived in connective tissue septa (Figure 2A).

Also, some follicular cells had dark nuclei and interfollicular cellular infiltration. Desquamated epithelial follicular cells in the lumen were also seen.

The thyroid follicles appeared with empty lumina, lined mostly with high cuboidal cells containing hyperchromatic nuclei. Some of the follicular cells exhibited apparent cytoplasmic vacuolation. Also, both follicular cells and the interfollicular cells revealed moderate hyperplasia with desquamation of follicular cells in the lumen (Figure 3A).

Group III (EMW exposed+ Spirulina treated group): Some thyroid follicles in group III that treated with Spirulina before EMW exposure retained the appearance of controlled follicles. Follicular cells appeared cuboidal in shape with rounded nuclei. Some follicles were distended with colloid with peripheral vacuolation (Figure 4A). Some follicles had irregular outline and other follicles were disrupted with vacuolated colloid (Figure 4A). Slight improvement in the architecture of the thyroid gland tissues was observed. Variable-sized thyroid follicles, with partially filled colloid, were detected.

Group IV (EMW exposed + Vit E treated group): The vacuolated follicular cells have become less than that appeared in the EMW & Spirulina treated group. Typical rounded thyroid follicles were more or less restored (Figure 5A). Also, the thyroglobulin has become more homogeneous and filled the follicular lumina of the thyroid tissue (Figure 5A)

Group V (recovery group): Some of the thyroid follicles were obliterated due to the noticeable highly cellular hyperplasia of the follicular cells. vacuolation of the cytoplasm of follicular cells with vacuoles in interfollicular tissue were seen (Figure 6A). The lumina of the follicles were excessively filled with vacuolated thyroglobulin or empty. Fibrosis of interfollicular tissue was observed (Figure 6A).

Masson trichrome stain

Little amount of collagen fiber was detected in connective tissue septa of Masson stained thyroid sections of control group (Figure 1B)

Many collagen fibers were noticed in the connective tissue septa of Masson stained thyroid sections of (EMW exposed group) with congested blood vessels were detected (Figure 2B).

Moderate amount of collagen fibers could be seen in the interfollicular spaces of Masson stained thyroid sections of (EMW exposed+ Spirulina treated group) (Figure 3B).

Minimal collagen fibers were seen in interfollicular connective tissue septa of Masson stained thyroid sections of (EMW exposed + Vit E treated group) (Figure 4B).

Many collagen fibers were detected in the connective tissue septa of Masson stained thyroid sections of recovery group with congested blood vessels were also detected (Figure 5B).

Periodic acid Schiff (PAS)

Periodic acid Schiff (PAS) reaction of the thyroid tissue sections from control group exhibited the magenta coloured colloid and the basement membrane surrounding the follicles. The colloid of the peripheral follicles revealed few marginal vacuoles (Figure 1C).

PAS reaction of the thyroid tissue sections of EMW exposed group (group II) showed marked decrease in the amount of PAS positive central colloid, some follicles are completely empty (Figure 2C).

PAS reaction of the thyroid tissue sections of (EMW exposed+ Spirulina treated group) revealed a moderate magenta coloured reaction. Marginal vacuoles were observed in the colloid especially in the peripheral follicles (Figure 3C).

PAS reaction of the thyroid glands of (EMW exposed + Vit E treated group) exhibited a similar reaction to that of (group I) but with more marginal vacuoles in the colloid (Figure 4C).

PAS reaction of the thyroid glands of recovery group (group V) revealed decrease in the amount of magenta coloured colloid with more marginal vacuolation. In addition, completely empty follicles from colloid were observed (Figure 5C).

Immunohistochemical results

Ki-67

The immunohistochemical results revealed the negative expression of Ki-67 in the nuclei of the follicular epithelial cells in the thyroid tissue of the control group (G1) (Figure 1D).

A strong Ki-67 immunoreactivity was shown in thyroid gland sections of the EMW exposed group (G2) (Figure 2D).

Nuclei of the epithelial cells lining the thyroid follicles of Group III (EMW exposed+ Spirulina treated group) revealed weak Ki-67 expression (Figure 3D) in comparison with both the control group and EMW exposed group.

Also nuclei of the epithelial cells lining the thyroid follicles of Group IV (EMW exposed + Vit E treated group) revealed weak Ki-67 expression (Figure 4D).

While in the recovery group (group V), there is moderate Ki-67 expression (Figure 5D).

TGF β 1

Examination of TGF β 1 immuno expression of the thyroid gland of the control group revealed negative reaction (Figure 1E).

TGF β 1 immuno expression of the thyroid glands of EMW exposed group revealed a dark brown color in the cytoplasm and nuclei of most of the follicular epithelium and colloid (Figure 2E).

Examination of TGF β 1 immunoexpression of the thyroid glands of EMW exposed+ Spirulina treated group revealed weak positive reaction (moderate brown color) in the cytoplasm of some of follicular cells (Figure 3E).

Immunohistochemical examination of TGF β 1 immunoexpression of the the tissue of (EMW exposed - Vit E treated group) revealed also a faint brown color in the cytoplasm of some of the follicular cells nearly similar to control (Figure 4E).

TGF β 1 immunorexpression of the thyroid gland of recovery group revealed a dark brown color in the cytoplasm and nuclei of some follicular cells (Figure 5E).

Hormonal results

Results of the thyroid hormones level (T3, T4, and TSH) of all groups are represented in (Table 1, Histogram 1). After EMW exposure, level of T3 and T4 in circulation was significantly decreased, while the level of TSH increased significantly compared to the control group and groups III & IV. Whereas the treatment of EMW exposed group with Spirulina exhibited an improvement in the level of the hormones. But the treatment of EMW exposed group with Vit E exhibited more improvement in the level of the hormones.

In recovery group, level of T3 and T4 in circulation was also decreased while the level of TSH increased and this group showed insignificant difference in T3, T4 and TSH levels in comparison to EMW exposed and showed high significant difference as compared to groups I, III & IV.

Morphometric results

The mean area % of collagen deposition for all groups was represented in (Table 2, Histogram 2). There was significant increase in mean area% of collagen deposition ($P \leq 0.02$) in group II as compared with groups I, III & IV. But area % of collagen deposition was increased in groups III & IV without significant difference as compared to control group ($P > 0.05$). Also, area % of collagen deposition was insignificantly decreased in group IV as compared to group III ($P > 0.05$). There was high significant increase in mean area% of collagen deposition ($P \leq 0.02$) in group V as compared with group I and there was significant increase in mean area% of collagen deposition ($P \leq 0.05$) in group V as compared with group IV, but there was insignificant increase in mean area% of collagen deposition ($P > 0.05$) in (group V) as compared with group III .

The mean area % of Ki-67 immuno-expression for all groups was represented in (Table 3, Histogram 3).

The area percent of Ki-67 immunoreactivity has highly significantly increased in EMW exposed group as compared to control group ($P < 0.02$). In the EMW +Vit E treated group the area percent of Ki-67 immunoreactivity has markedly improved with no significant difference when compared to control group ($P > 0.05$) and it has highly significantly decreased when compared to EMW exposed group ($P < 0.02$). Also, the area percent of Ki-67 immunoreactivity has highly significantly decreased in Spirulina+EMW treated group as compared to EMW exposed group ($P < 0.02$) but it has increased with no significant difference when compared to control group ($P > 0.05$) and it has insignificantly increased when compared to VitE+ EMW treated group ($P > 0.05$). The

area percent of Ki-67 immunoreactivity has highly significantly increased in recovery group as compared to control, vit E+EMW treated and Spirulina+EMW treated groups ($p \leq 0.02$).

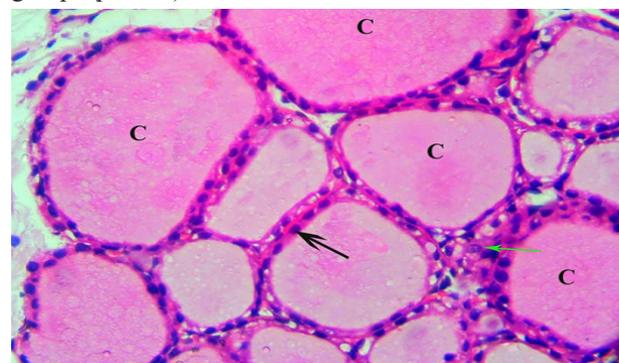


Fig. 1A: A photomicrograph of section in the thyroid gland of the control group showing the thyroid follicles of variable sizes and with regular outline, the follicles lined with follicular cells with rounded nuclei (black arrow) and filled with homogenous colloid (C), and parafollicular cells are seen (green arrow). (H&E X400)



Fig. 2A: A photomicrograph of section in the thyroid gland of EMW exposed group showing loss of normal architecture of follicles, some follicles of scanty amount of colloid (c), some follicles are degenerated and others show desquamation of follicular cells in the lumen (black arrow), disrupted and fused thyroid follicles with degeneration of basement membrane (yellow arrow), darkly stained parafollicular cells (red arrow) .(H&E X400)

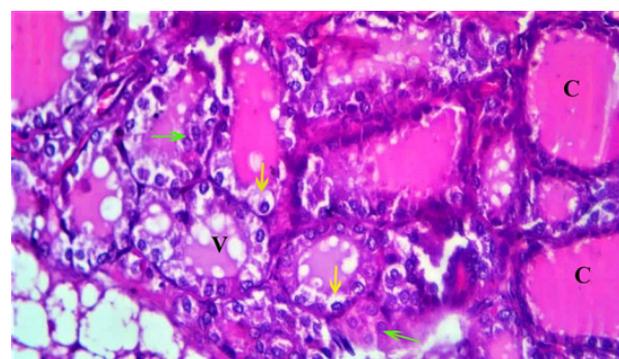


Fig. 3A: A photomicrograph of section in the thyroid gland of EMW exposed group showing some follicles filled with colloid (C) and other follicles show vacuolation in the colloid (V), Some of the follicular cells exhibited apparent cytoplasmic vacuolation (yellow arrow), Both follicular cells and the interfollicular cells revealed moderate hyperplasia with desquamation of follicular cells in the lumen (green arrow). (H&E X400)

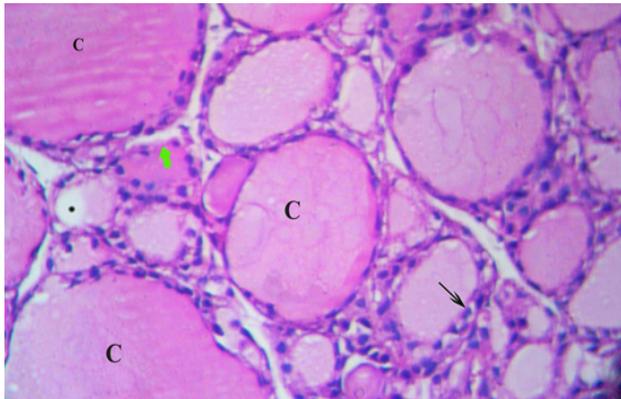


Fig. 4A: A photomicrograph of section of thyroid gland of EMW exposed+Spirulina treated group showing some follicles lined with follicular cells with flattened nuclei with homogenous colloid (C) , other follicles are degenerated with interrupted follicular cells (green arrow), and others with scanty colloid (asterisk), some follicular cells have vacuolated cytoplasm (black arrow). (H&E X400)

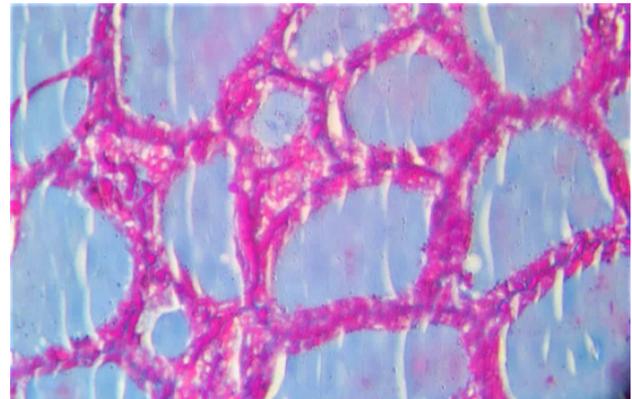


Fig. 1B: A photomicrograph of section in the thyroid gland of the control group showing a minimal amount of connective tissue between the follicles. (Masson X400)

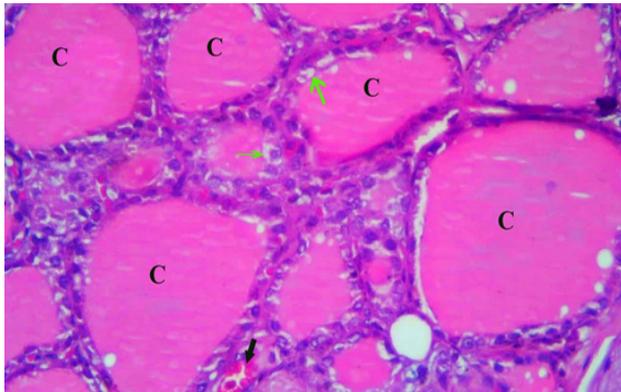


Fig. 5A: A photomicrograph of section of thyroid gland of EMW exposed + Vit E treated group , thyroid follicles appeared filled with homogenous colloid (C) and lined with nearly normal follicular cells, some follicular cells have vacuolated cytoplasm (green arrow) and there is congested blood Capillaries (black arrow). (H&E X400)

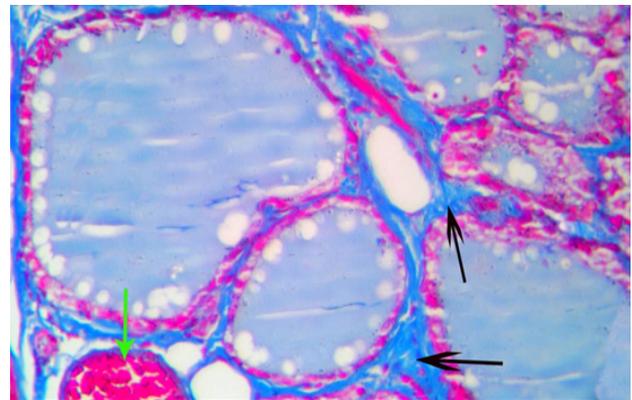


Fig. 2B: A photomicrograph of section in the thyroid gland of EMW treated group showing an increased connective tissue in between the thyroid follicles (Black arrows). with a congested blood vessel (green arrow). (Masson X400)

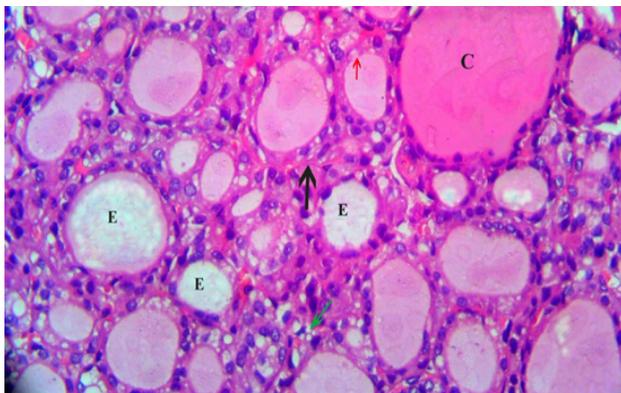


Fig. 6A: A photomicrograph of section of thyroid gland of recovery group showing empty follicles (E), other follicles filled with colloid (C), Vacuolation in interfollicular tissue (green arrow) and some areas of fibrosis (Black arrow) and some follicles show interrupted follicular cells with vacuolation (Red arrow). (H&E X400)

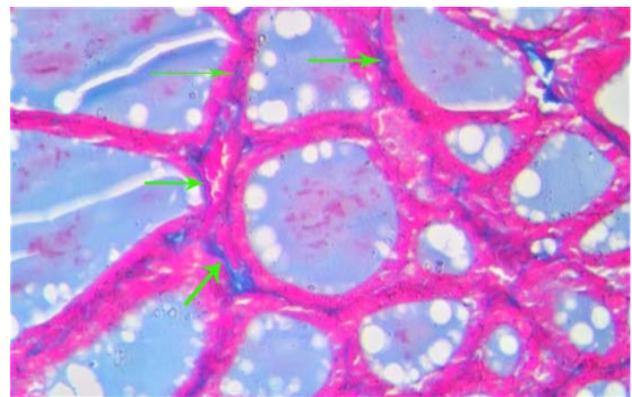


Fig. 3B: A photomicrograph of section of thyroid gland of EMW exposed+ Spirulina treated group , there is thin collagen fibers (green arrows) between the thyroid follicles. (Masson X400)

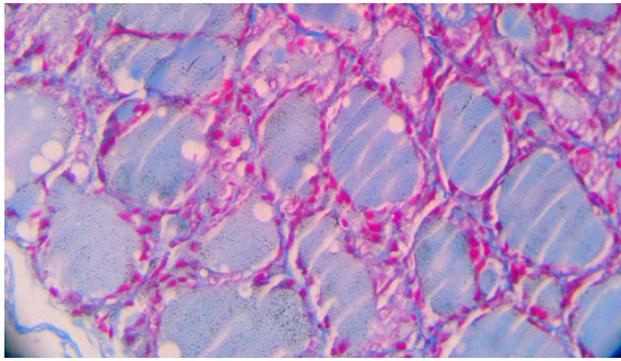


Fig. 4B: A photomicrograph of section of thyroid gland of EMW exposed + Vit E treated group, connective tissue present in minimal amount between the thyroid follicles (Masson X400)

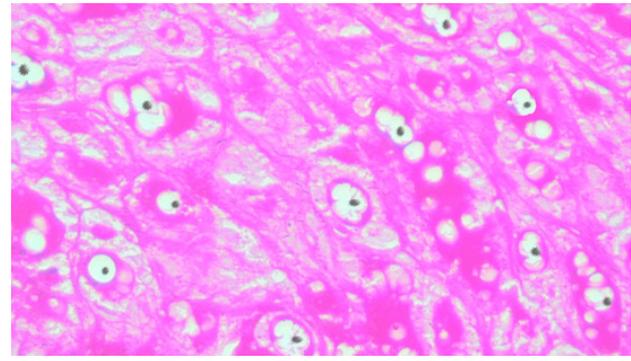


Fig. 2C: A photomicrograph of section in the thyroid gland of EMW exposed group showing a marked decrease in the amount of PAS positive central colloid, some follicles are completely empty (asterisk). A positive PAS reaction is also observed in the cytoplasm of follicular cells. (PAS X400)

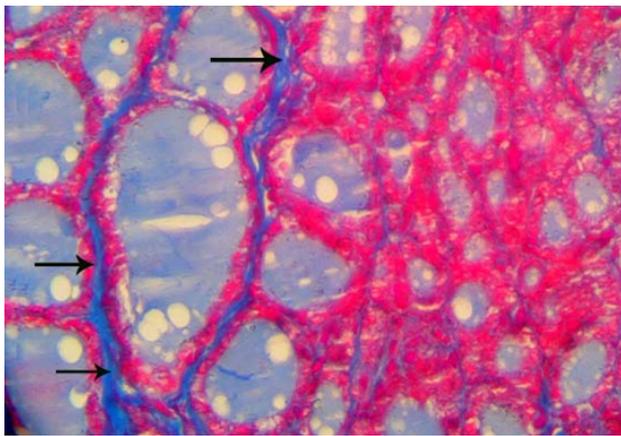


Fig. 5B: A photomicrograph of section of thyroid gland of recovery group, showing an increased amount of the connective tissue (Black arrows) in between the thyroid follicles. (Masson X400)

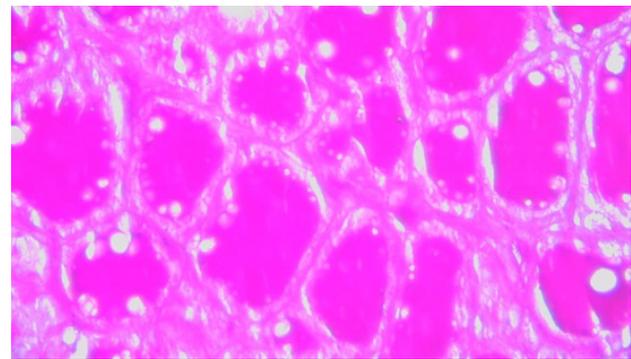


Fig. 3C: A photomicrograph of a section in the thyroid gland of EMW exposed+ Spirulina treated group showing some thyroid follicles with a reduced positive PAS reaction in the colloid with increased peripheral vacuoles, also the basement membrane of the thyroid follicles shows moderate reaction. (PAS X400)

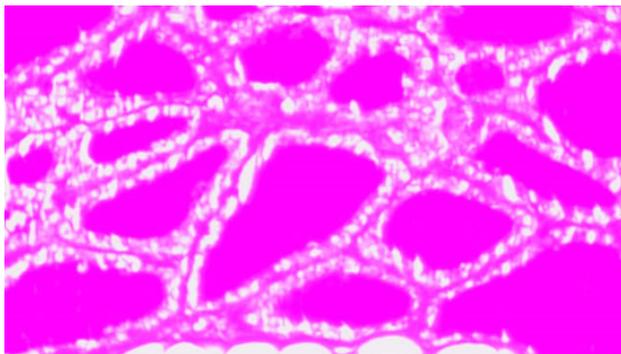


Fig. 1C: A photomicrograph of section in the thyroid gland of the control group showing a strong PAS positive reaction in the colloid with few marginal vacuoles and in the basement membrane of the thyroid follicles which show a moderate reaction. (PAS X400)

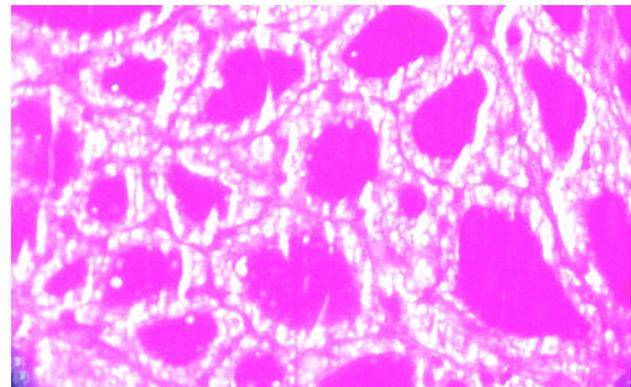


Fig. 4C: A photomicrograph of a section of the thyroid gland of EMW exposed + Vit E treated group showing some thyroid follicles with positive PAS reaction in the colloid with increased peripheral vacuoles, also there is a PAS positive reaction in the basement membrane of the thyroid follicles. (PAS X400)

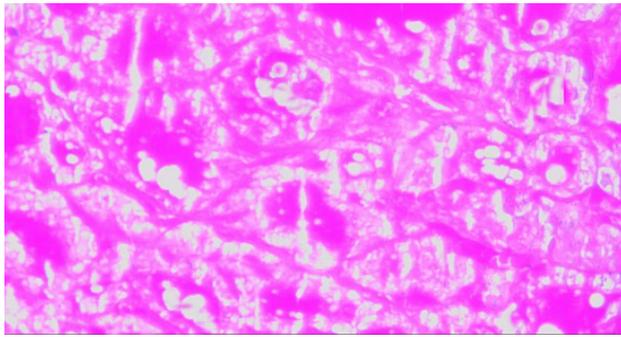


Fig. 5C: A photomicrograph of a section in the thyroid gland of recovery group showing a marked decrease in the amount of PAS positive central colloid, some follicles are completely empty. A positive PAS reaction is also observed in the cytoplasm of the follicular cells. (PAS X400)

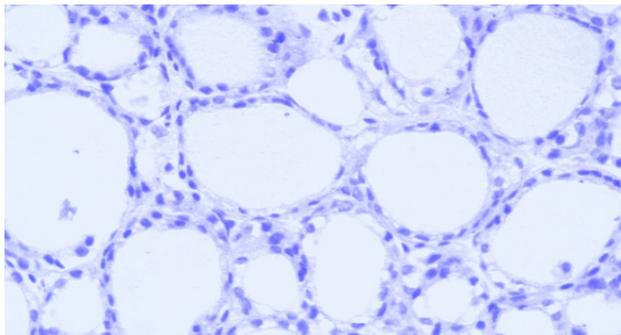


Fig. 1D: A photomicrograph of a section in the thyroid gland of the control group showing a negative immunostaining for Ki-67. (Ki-67 X400)

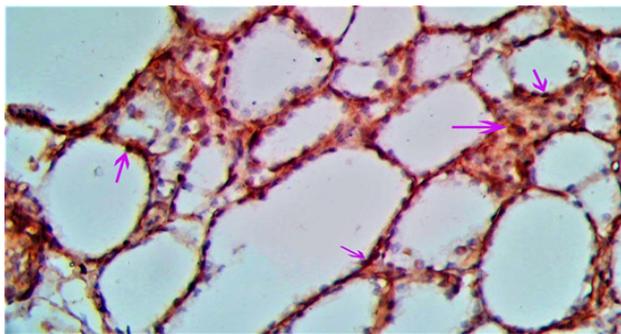


Fig. 2D: A photomicrograph of a section in the thyroid gland of EMW exposed group showing a strong positive nuclear immunostaining for Ki-67 protein (violet arrow). (Ki-67 X400)

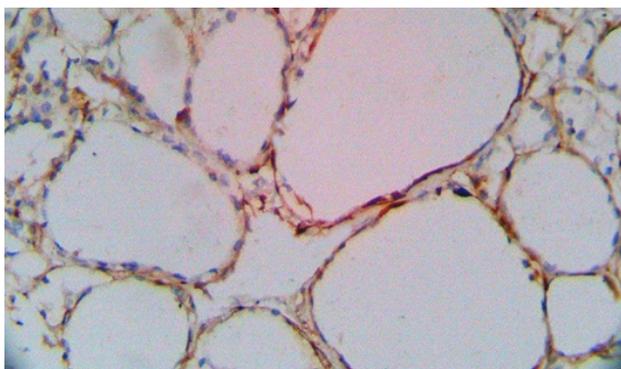


Fig. 3D: A photomicrograph of a section in the thyroid gland of EMW exposed+ Spirulina treated group showing a weak positive nuclear immunostaining for Ki-67. (Ki-67 X400)

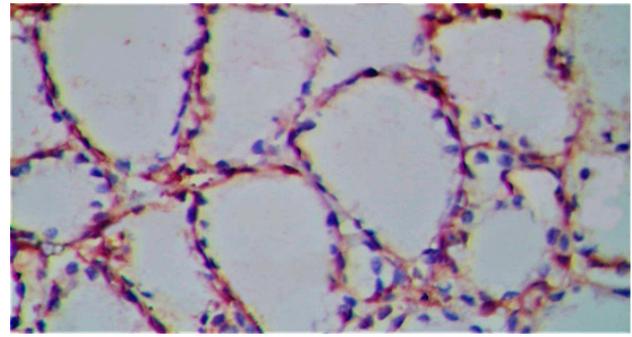


Fig. 4D: A photomicrograph of a section in the thyroid gland of EMW exposed + Vit E treated group showing a very weak positive nuclear immunostaining for Ki-67. (Ki-67 X400)

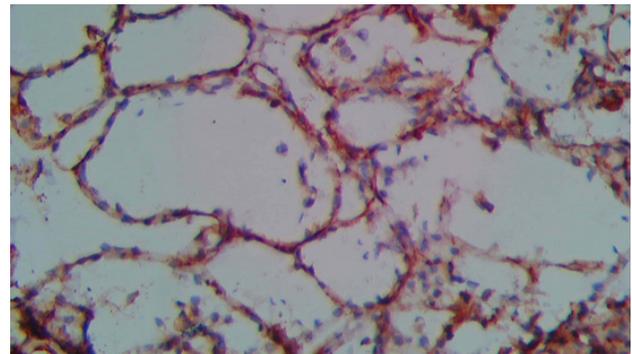


Fig. 5D: A photomicrograph of a section of the thyroid gland of the recovery group showing a positive nuclear immunostaining for Ki-67 protein. (Ki-67 X400)

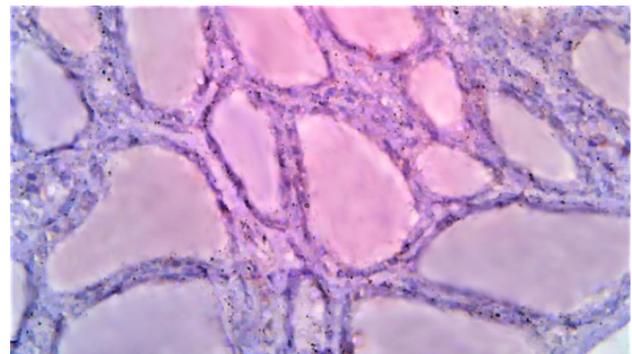


Fig. 1E: A photomicrograph of a section in the thyroid gland of the control group showing negative immunostaining for TGF beta1. (TGF β 1 X400)

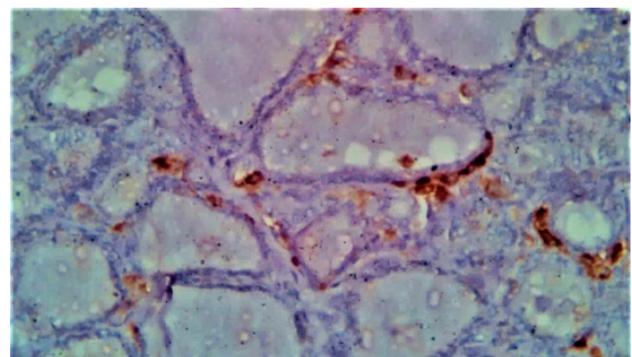


Fig. 2E: A photomicrograph of a section in the thyroid gland of EMW exposed group, TGF β 1 expression is more intense in the follicular epithelium and colloid. (TGF β 1 X400)

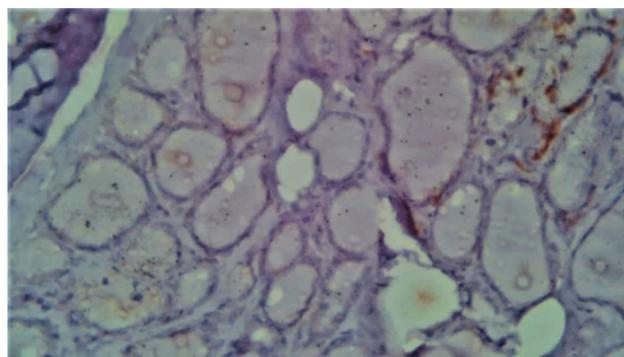


Fig. 3E: A photomicrograph of a section of thyroid gland of EMW exposed+ Spirulina treated group ,TGF β 1 expression is less intense in the follicular epithelium. (TGF β 1 X400)

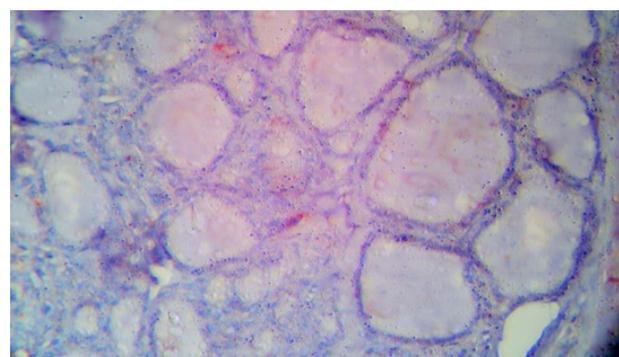


Fig. 4E: A photomicrograph of a section of thyroid gland of EMW exposed + Vit E treated group, TGF β 1 expression is weaker than that expressed in spirulina treated group and nearly similar to the control group. (TGF β 1 X400)

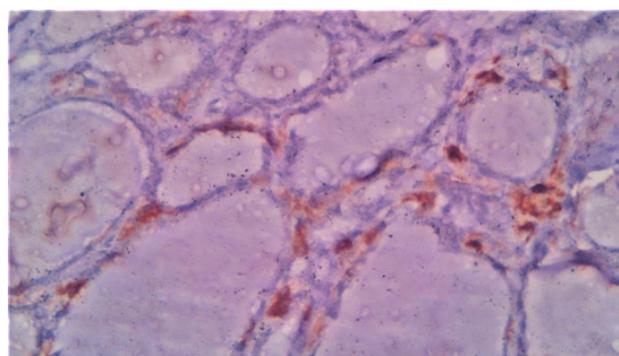


Fig. 5E: A photomicrograph of a section of thyroid gland of the recovery group, TGF β 1 expression is stronger in the follicular epithelium but lesser than that of the EMW treated group. (TGF β 1 X400)

Table 1: Showing mean values of T3, T4 & TSH \pm SD in the 5 groups

Mean \pm SD	Group I	Group II	Group III	Group IV	Group V
T3 (ng/dl)	58.7 \pm 6.4	7.4 \pm 1.9	49.03 \pm 1.95	55.7 \pm 3.2	9.2 \pm 4.1
T4 (μ g/dl)	5.8 \pm 0.45	0.95 \pm 0.13	3.9 \pm 0.31	4.8 \pm 0.79	1.19 \pm 0.21
TSH(ng/ml)	1.07 \pm 0.25	17.6 \pm 2.56	3.8 \pm 1.17	1.8 \pm 0.3	16.7 \pm 0.47
Significance \leq 0.01	With groups II & V	With groups I,III & IV	With group II	With group II & V	With groups I,III & IV

Table 2: Showing mean values of area percent of collagen fibers deposition \pm SD in the 5 groups

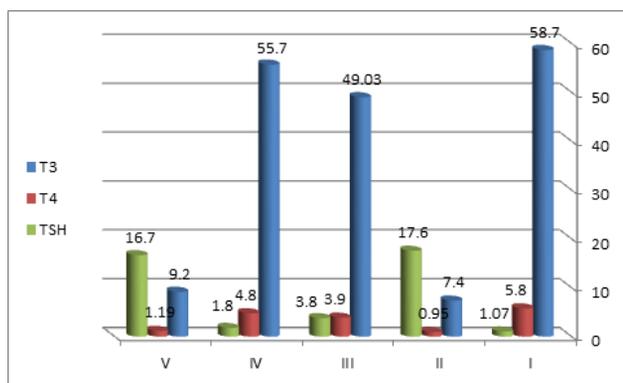
Mean % \pm SD	Group I	Group II	Group III	Group IV	Group V	F test
Masson%	3.14 \pm 3.82	27.65 \pm 9.62	10.14 \pm 0.974	5.50 \pm 3.16	20.30 \pm 3.71	12.194
Significance \leq 0.05	With groups II & V	With groups I,III & IV	With group II	With groups II & V	With groups I & IV	

Table 3: Showing mean values of area percent immunoreactivity of Ki-67 \pm SD in the 5 groups

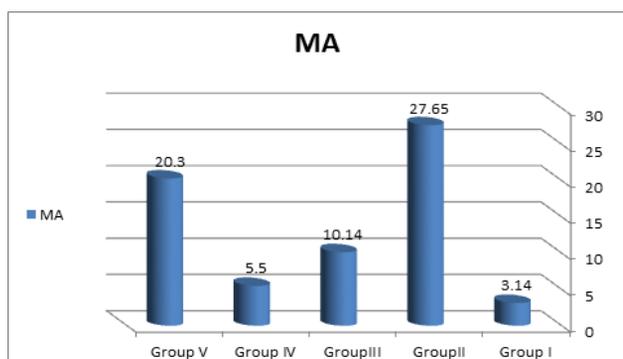
Mean % \pm SD	Group I	Group II	Group III	Group IV	Group V	F test
MA	1.02 \pm 0.75	26.36 \pm 2.16	7.26 \pm 2.33	3.04 \pm 1.46	20.19 \pm 4.02	64,31
Significance \leq 0.05	With groups II & V	With groups I,III & IV	With group II & V	With groups II & V	With groups I,III & IV	

Table 4: Showing mean values of area percent immunoreactivity of TGF \pm SD in the 5 groups

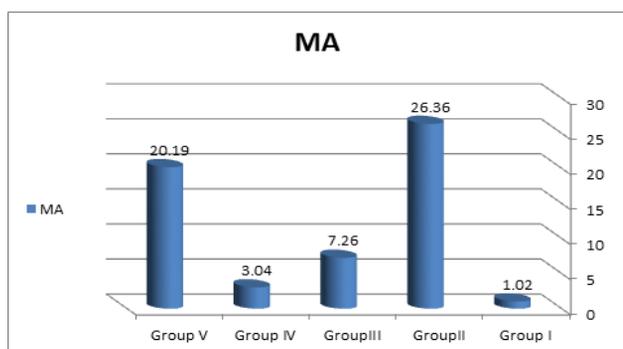
Mean \pm SD	Group I	Group II	Group III	Group IV	Group V
MA	2.89 \pm 1.46	27.83 \pm 10.5	5.48 \pm 0.86	3.44 \pm 2.3	26.1 \pm 0.51
Significance \leq 0.05	With groups II & V	With groups I,III & IV	With groups II & V	With group II & V	With groups I,III & IV



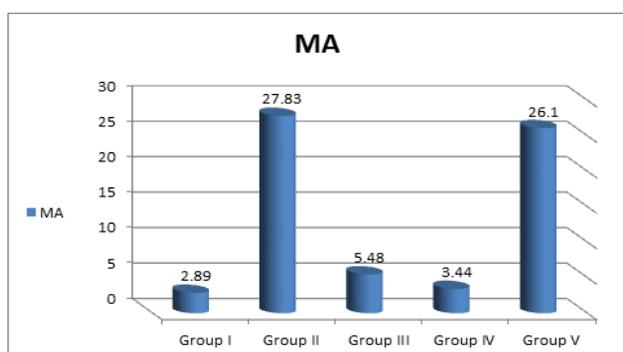
Histogram 1: Showing mean values of T3, T4 & TSH in the 5 groups



Histogram 2: Showing mean values of area percent of collagen fibers deposition in the 5 groups



Histogram 3: Showing mean values of area percent Ki-67 immunoreactivity in the 5 groups



Histogram 4: Showing mean values of area percent immunoreactivity of TGF in the 5 groups

DISCUSSION

The thyroid gland is one of the most affected crucial organs and may be an aim for any sort of electromagnetic waves. Since cell phones are source of EMW and usually located near thyroid gland during their use^[20].

Spirulina platensis has antioxidant, immunomodulating, anti-microbial and incorporates a radio protective impact^[10,21].

Consumption of many natural antioxidants such as, vitamin E,C and their derivatives before or after radiation exposure can protect cells against radiation induced damage^[22]

This study was designed to study the possible protective effect of spirulina and vit E on EMW induced thyroid gland damage and their reversibility.

In the current study the biochemical assay of rats after EMW exposure showed that the levels of T3 and T4 in the circulation was significantly decreased, while the level of TSH was significantly increased compared to the control group. Whereas the treatment of EMW exposed group with spirulina exhibited an improvement in the level of the hormones. And the treatment of EMW exposed group with vit E exhibited more improvement in the level of the hormones. While the recovery group showed results nearly similar to EMW exposed group

This is in agreement with Mohamed and Elnegris^[23] who assessed that exposed group to EMW showed significant drop in T3 and T4 levels together with significant rise in TSH level as compared to control and EMF exposed+ vitamin E treated groups. While in recovery group, there were deteriorations in these results as this group showed somewhat significant difference in T3, T4 and TSH levels in comparison to exposed and control groups.

Similarly Ebrahim^[24] showed that there was a significant increase ($P<0.05$) in serum TSH and a significant decline ($P<0.05$) in the level of the thyroid hormones T4 and T3 in irradiated rats as compared to control group. While, Spirulina pre-treatment of the irradiated rats significantly improved the level of thyroid hormones as the decrease of T3 and T4 and the increase of TSH were significantly lower ($P<0.05$) in the irradiated animals cured with Spirulina before irradiation compared to their relative levels in irradiated rats.

Some investigators^[25] clarified that the elevation in TSH levels following irradiation was due to stimulation of the hypophysis. Also Madanat *et al*^[26] settled that the decrease in blood level of thyroid hormone leads to stimulation of anterior pituitary to emit more TSH to prevent occurrence of hypothyroidism.

EMW can cause stress which can affect thyroid functions through an increment in endogenic cortisol generation, since rise of glucocorticoid emission represents an impediment to the conversion of T4 into T3^[27]. Whereas another investigators^[28] clarified that the change in the

exocytotic and endocytotic processes were behind low Free-T3 and Free-T4.

However Koyu *et al*^[29] explored the impact of 900 MHz electromagnetic radiation on the rat TSH, T3 and T4 and the results discovered a diminishment in the serum levels of TSH, T4 and T3.

The improvement of thyroid physiological status after EMF exposure cessation requires a longer period of time because the decreased processes of exocytosis and endocytosis at the apical membranes of follicular cells were the main reason behind the low levels of T3 and T4 after recovery period^[30].

In the present study, the histological H&E examination of the thyroid follicles in group II showed degeneration and disorganization. Some follicles appeared with interrupted follicular wall, Desquamated epithelial follicular cells in the lumen were seen. Also congested blood vessels and cellular infiltration were present.

These findings are in agreement with some authors^[31] who explained the flaking of epithelial lining of some follicles by indirect mechanisms of this vascular damage.

Other studies showed that electromagnetic waves were appeared to apply their impacts on biological systems through generation or release of reactive oxygen species (ROS). ROS, as a medium, causes numerous biological effects including DNA damage and mutation stimulation^[32].

Also some researchers^[33] stated that EMW may alter biological systems by intensifying free radicals, which appear mainly to upgrade lipid peroxidation, and by changing the antioxidant defense systems of human tissues, thus leading to oxidative stress.

In the present work group II showed thickened C.T septa with increased collagen fibers deposition as morphometrically confirmed. Also congested blood vessels were seen, This is in assertion with Jung *et al*^[18] who found that follicles in the control rats showed little Masson's trichrome staining but more connective tissue in the interfollicular spaces seen in the thyroid of irradiated rats. Also Mohamed & Elnegris^[23] stated that Little aggregate of collagen fiber was perceived in connective tissue septa in control group While, Numerous collagen fibers were noticed in the connective tissue septa in EMF exposed group.

In this study, the PAS reaction of the thyroid tissue sections of EMW exposed group showed marked decrease in the amount of PAS positive central colloid, some follicles are completely empty.

This is in agreement with Jung *et al*^[18] who found that PAS-positive density of the colloid in irradiated rats was decreased significantly as compared to other groups.

In our study, inspection of TGF β 1 immunoexpression of the thyroid glands of EMW exposed group revealed dark brown color in the cytoplasm and nuclei of the majority of follicular cells.

The TGF- β 1 immunoreactivity was increased in irradiated rats and was more severe on the day 7 after exposure to radiation^[18].

Also in this study, strong Ki-67 immunoreactivity was shown in thyroid gland sections of the EMW exposed group.

The abundance cellular levels of ROS induced by ionizing radiation can be harmful and elicit oxidative stress. Oxidative stress is defined as "a serious imbalance between the generation of ROS and antioxidant resistances in favour of ROS, causing extreme oxidative damage^[34].

In the current work, examination of group III that received spirulina during EMF exposure showed that the histological structure of the gland was improved in comparison to group II. As, some thyroid follicles restored their normal configuration. They were lined by cuboidal cells with rounded nuclei. Some follicles were expanded with colloid that showed peripheral vacuolation. Some follicles had irregular outline and other follicles were disrupted with vacuolated colloid. In the interfollicular spaces moderate amount of collagen fibers was detected. PAS reaction of the thyroid tissue sections showed a moderate reaction. immuno histochemical results revealed weak Ki-67 expression, also Examination of TGF β 1 immunoexpression expressed weak positive reaction in some of the follicular cells in comparison with EMW exposed group.

This concurred with other researchers^[35] who reported that spirulina administration for 15 days before irradiation provided female rats a substantial protection against the harmful impacts of radiation. Other researchers^[36] stated that this was owing to its free radical scavenging and powerful antioxidant activity.

The antioxidant defensive role of spirulina could be due to the presence of Phycocyanin, a biliprotein pigment which fortifies the antioxidant systems to diminish the early radiation reaction^[37].

The blue-green algae Spirulina has antioxidant and antiapoptotic properties, which make it reduces the cell injuries, improve the enzyme functions, and inhibit the oxidation process in the body. Also, its content of vitamins, superoxide dismutase, minerals and the C phycocyanin which prevents oxalate-mediated lipid peroxidation and restrains damage in many tissues^[38].

Also Madanat *et al*^[26] stated that the administration of spirulina pre-irradiation has significantly improved the radiation-induced oxidative stress.

In the current work, examination of group IV that received vitamin E during EMF exposure showed obvious improvement in the tissue structure of the gland. The thyroid follicles were more or less near to the control group. Rounded thyroid follicles were more or less restored also, the follicular lumina of the thyroid tissue filled with thyroglobulin which has become more homogeneous. This agreed with Mohamed & Elnegris^[23] who stated

that some thyroid follicles in group III that treated with vitamin E during EMF exposure retained the appearance of controlled follicles. They were lined by cuboidal cells with rounded nuclei some follicles were expanded with colloid that showed peripheral vacuolation.

Minimal collagen fibers were perceived in inter follicular connective tissue septa. This was in agreement with Mohamed & Elnegriss^[23] who stated that minimal collagen fibers were seen in interfollicular connective tissue septa in EMF exposed+ vitamin E treated group.

PAS reaction of the thyroid glands showed a similar reaction to that of group I.

The immunohistochemical results revealed weak Ki-67 expression nuclei of the epithelial cells lining the thyroid follicles. TGF β 1 immunoexpression also revealed a faint brown color in the cytoplasm of some of the follicular cells in comparison with both the control and EMW exposed groups.

The protective effects of vitamin E reported that vitamin E consumption diminishes change of antioxidant capacity after mobile phone exposure in pregnant and fetal mice^[39].

Mohamed & Elnegriss^[23] evaluated that vitamin E improves histological structure and function of the thyroid gland. Also other researchers^[40] stated that vitamin E is a powerful antioxidant that is able to inhibit cell damage by holding sulfhydryl groups of proteins binding to the membrane and eradicating free radicals.

The current study assumed that withdrawal of EMW exposure in the recovery group (group V) resulted into minimal functional and histological improvement. While Mohamed & Elnegriss^[23] stated that the thyroid of recovery group showed nearly normal thyroid follicles that lined with flattened or low cuboidal epithelium. some follicles were involuted.

In the present study, connective tissue septa contained countless collagen fibers in Masson stained thyroid sections of recovery group with congested blood vessels were also noticed.

This agrees with Mohamed & Elnegriss^[23] who stated that moderate amount of collagen fibers could be perceived in the interfollicular spaces.

In our study, PAS reaction of the thyroid gland of recovery group revealed decrease in the amount of magenta coloured colloid. The immunohistochemical results revealed moderate KI-67 expression and TGF β 1 immunoexpression revealed a dark brown color in the cytoplasm and nuclei of some follicular cells.

Similarly, other authors^[41] found that in the recovery group some thyroid follicles were seemingly normal, few follicles were interrupted with congested blood capillaries, and vacuolated acidophilic colloid filled thyroid follicles. There were moderate amount of collagen fibers.

CONCLUSION

The thyroid gland is sensitive to EMF exposure which leads to changes in the thyroid gland structure & function. These changes need a longer period of time to be improved after EMF exposure cessation. The consumption of Spirulina platensis during the period of EMF exposure is considered to have a protective effect against EMF exposure. Meanwhile, the consumption of vit E has a more protection against EMF than Spirulina.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربي

التأثير الوقائي للسبيرولينا مقابل فيتامين هـ على الضرر المسبب بالموجات الكهرومغناطيسية للغدة الدرقية في الجرذان البيضاء: دراسة نسيجية وكيميائية مناعية

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مقدمة: يتزايد وبسرعة الاستخدام الواسع للعديد من التطبيقات الكهربائية والاتصالات السلكية واللاسلكية والتي تنتج مجالات كهرومغناطيسية و من أهم الأعضاء التي تتأثر بالأنواع العديدة من الموجات الكهرومغناطيسية الغدة الدرقية. **هدف الدراسة:** تهدف هذه الدراسة إلى استكشاف آثار التعرض للمجالات الكهرومغناطيسية على الغدة الدرقية والتأثير الوقائي للسبيرولينا بـلاتنسيس وفيتامين هـ.

المواد والطرق: تم فصل خمسين جرذاً سليماً من الذكور البيضاء البالغين إلى خمس مجموعات متساوية. مجموعة التحكم، المجموعة المعرضة للموجات الكهرومغناطيسية وفيها تعرضت الفئران للتأثير الكهرومغناطيسي بشكل فردي لمدة 4 ساعات يومياً لفترة شهر واحد. المجموعة التي تعرضت للموجات الكهرومغناطيسية مع اعطاء سبيرولينا حيث تلقت الجرذان سبيرولينا بجرعة 300 مجم / كجم من وزن الجسم. المجموعة المعرضة للموجات الكهرومغناطيسية وعولجت بفيتامين هـ حيث تعرضت الفئران للمجال الكهرومغناطيسي مثل المجموعة الثانية وتعطى فيتامين هـ عن طريق الفم بجرعة 200 مجم / كجم من وزن الجسم ومجموعة التعافي التي تركت بها الفئران لمدة شهر واحد بعد شهر واحد من التعرض للمجال الكهرومغناطيسي منخفض التردد. ثم تم فحص أنسجة الغدة الدرقية من حيث التغيرات النسيجية المرضية والكيميائية المناعية

النتائج: أظهرت المجموعة التي خضعت للتعرض للموجات الكهرومغناطيسية عدم ترتيب وتحلل لبصيلات الغدة الدرقية أيضاً العديد من ألياف الكولاجين موجودة داخل الحواجز الليفية عند صبغها بصبغة الماسون و اظهرت انخفاضاً واضحاً في مقدار (PAS) ملون شيف حمض البيروديك الموجبة في المادة الغروانية المركزية مع نشاط مناعي قوي لـ Ki-67 والتعبير المناعي لعامل النمو المحول-بيتا. سبيرولينا بـلاتنسيس وفيتامين هـ يقللان من تأثير الموجات الكهرومغناطيسية على الغدة الدرقية لكن فيتامين هـ كشف عن زيادة واضحة في مقدار ملون شيف حمض البيروديك الموجبة في المادة الغروانية المركزية وانخفاض كبير في ألياف الكولاجين وانخفاض في التعبير المناعي لعامل Ki-67 والتعبير المناعي لعامل النمو المحول-بيتا مقارنة بمجموعة السبيرولينا.

الخلاصة: استخدام سبيرولينا بـلاتنسيس خلال فترة التعرض للأشعة الكهرومغناطيسية يعتبر له تأثير وقائي بينما استخدام فيتامين هـ له حماية أكثر من سبيرولينا