

POSSIBLE PROTECTIVE EFFECTS OF AVOCADO PEAR (PERSEA AMERICANA) ETHANOLIC EXTRACT ON THE OVARIAN FOLLICLES OF ADULT FEMALE ALBINO RAT EXPOSED TO ELECTROMAGNETIC FIELDS (EMF) EMITTED FROM CELLULAR PHONES

By

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ABSTRACT

Background: Histological and physiological studies have increased in evaluating the effects of electromagnetic fields on human health. Several studies focused on investigated the effect of low frequency EMF on follicle development, and ovulation process in ovary.

Objective: To study the structural and functional changes in the ovaries of adult female albino rats post-exposure to cellular phone and the possible protective effect of avocado extract.

Material and Methods: The present study was carried out on 50 healthy adult female albino rats. divided into 5 main groups: 1) Group I: Control rats left without treatment, 2) Group II: Control rats receive the avocado extract, 3) Group III: Rats exposed to frequency equals 950 MHz of electromagnetic field for one month, 4) Group IV: Exposed to EMF and treated orally with a daily dose of the avocado extract in same day, 5) Group V: Rats left for spontaneous recovery for one month after exposure to EMF. The animals were sacrificed and the ovaries were dissected. The dissected tissues were processed. Using Hematoxylin and Eosin, and Masson's trichromestain. Immunohistochemical stains were used to detect two proteins: Nuclear Factor Kappa B (NF- κ B) and Proliferating Cell Nuclear Antigen (PCNA).

Results: There was an increase in tunica albugenia (TA) thickness, Blood vessels congestion and collagen deposition in EMF exposed group. Besides, there was an increase in NF- κ B expression in Group III. Avocado reversed the structural and functional changes in ovary. In Group V. There was a reduction but did not reach to the Group I as shown in avocado treatment.

Conclusions: EMF affected negatively on ovary. Avocado as antioxidant, protected the ovary from structural and functional changes resulting from exposure to EMF

Key words: EMF, Avocado, ovary, female albino rats.

INTRODUCTION

Electromagnetic Field (EMF) arises whenever electrical energy is used. We can find examples of EMFs all around us.

In our home from electrical kitchen appliances, from work processes such as radiofrequency heating and drying and even from radio, TV and Telecom

broadcasting masts and security detection devices (*Khdoor et al., 2011*).

Exposure to extremely low frequency magnetic fields may impair fertility of female mammals by reducing the ability of follicles to reach the stage of development which is an essential prerequisite for successful reproduction (*Ahmadi et al., 2016*).

Antioxidant defense systems have developed in organisms to control the formation of free radicals and to prevent the harmful effects of these molecules (*Goraca et al., 2010*). Avocados increase antioxidant absorption from other foods, they are also high in antioxidants themselves (*Chaudhary et al., 2015*). Ethano medicinal uses of avocado pear plant parts including the seeds against varied ailments have been reported in the management of hypertension, diabetes, cancer and inflammation have been reported (*Alhassan et al., 2012*). The ethanol extract of avocado seeds significantly decreased endometrial implant volume. This effect was associated with decreased estradiol levels in both serum and endometrial implants (*Rencher et al., 2018*).

This work aimed to study the structural and functional changes in the ovaries of adult female albino rats post-exposure to cellular phone radiation and the possible protective effect of avocado extract.

PATIENTS AND METHODS

The present study was carried out on 50 healthy adult female albino rats of a local strain weighing from 150 to 180 grams each. All rats were kept in clean properly ventilated cages, width [W] ×

depth [D] × height [H] = 220 × 320 × 135 mm, The first cage was placed 20 female albino rats (control group and Avocado group), the second cage was placed 30 female albino rats (Exposed group and, Exposed +Avocado group and Recovery group) under similar conditions and had free access to laboratory food and water throughout the experiment.

The rats were divided into 5 main groups:

1. **Group I:** Control rats left without treatment.
2. **Group II:** Control rats received the Avocado extract.
3. **Group III:** Rats exposed to frequency equals 950 MHz of electromagnetic field (EMF) for one month.
4. **Group IV:** Rats were be treated orally with a daily dose of the Avocado extract (AVOE) 1 ml/kg body weight (bw) and exposure to a frequency equal to 950 MHz of EMF on the same day that was done for one month.
5. **Group V:** Rats left for spontaneous recovery for one month after exposure to EMF.

Electromagnetic waves exposure: This procedure was done at Histology Department lab, Faculty of Medicine Al-Azhar University, Cairo, Egypt. In the Electromagnetic exposure room (EMER), there were no other metal or ferromagnetic materials around the clean benches that would change the structure of the electromagnetic field. The groups were separated from each other, with the control group isolated far from the source of the EMF.

The EMR-exposed animal group was taken to the exposure room and then exposed to the EMF emitted by a specific transformer (Thalheimer LTS 602 Isolation Transforme, Germany) that was adjusted to emit a frequency equal to 950 MHz of EMF (*El-Hady et al., 2015*). The rats were placed in plastic cages. The control animals were in similar cages for the same period in a separate room.

Preparation of Plant Extract: The dried seeds were ground and pulverized, 1Kg of the powdered seeds was macerated in 5:7:10 distilled water. The homogenate was filtered, and the filtrates were Ultrasonic Rotary evaporated to dryness with a rotary evaporator at reduced pressure. The concentrate was stored at 4°C (*Tan et al., 2019*).

All the rats in group I, group II, group III and group IV, were sacrificed after 30 days. The rats in group V were left for another 30 days for the spontaneous recovery. The ovaries were dissected and immediately fixed in 10% formalin fluid for 24 hours and the formalin fixative was washed from the samples with 70% alcohol. The tissues were dehydrated by passing through different grades of alcohol. The tissues were then cleared to remove the alcohol. The clearing was done for 6 hours using xylene, and impregnation and then embedded. Serial

sections were cut using rotary microtome at 5 microns (5µm) (*Oyedeji et al., 2018*).

Tissues were stained with:

1. Hematoxylin and eosin stains to study the histological structure of the ovary (Mohamed and Mubarak, 2015)
2. Masson trichrome technique: to study the collagen fibers in the ovary (*Naba et al., 2014*).
3. Immunohistochemical technique was for studying expression of Nuclear Factor Kappa B (NF-κB) (*Said et al., 2019*), Proliferating Cell Nuclear Antigen (PCNA) (*Anggorowati et al., 2017*).

Slides were examined under light microscope, with different high-power fields. The image analysis of tunica albuginea, blood vessels, and collagenous deposition were performed using Image J software.

Statistical analysis: Recorded data were analyzed using the GraphPad Prism 8 (GraphPadSoftware, La Jolla, CA, USA). Quantitative data were expressed as mean ± standard error of the mean (SEM). Qualitative data were expressed as percentage. P-value <0.05 was considered significant, for all the experiments, One-Way ANOVA followed by Tukey's post-hoc test was used.

RESULTS

The effect of EMF on the thickness of tunica albuginea (TA) which was a white collagenous connective tissue that acted as a capsule of the ovary. A marked significant increase in the thickness of TA was found in Group III ($P = <0.0001$). A reduction in the thickness of TA was found in the Group IV that reached the Group I level ($P = 0.771$).

In the Group V which underwent spontaneous recovery, there was a

significant increase in TA thickness compared to the control group ($P = 0.0018$). There was not any a significant difference in the thickness of TA between Group V and Group III. ($P = 0.06$). When we compared between Group V and Group IV, there was an increase in TA thickness in Group V but didn't reach to be significant ($P = 0.11$). There was no significant change between the Group I and Group II ($P = 0.15$) (**Figures 1 and 3**).

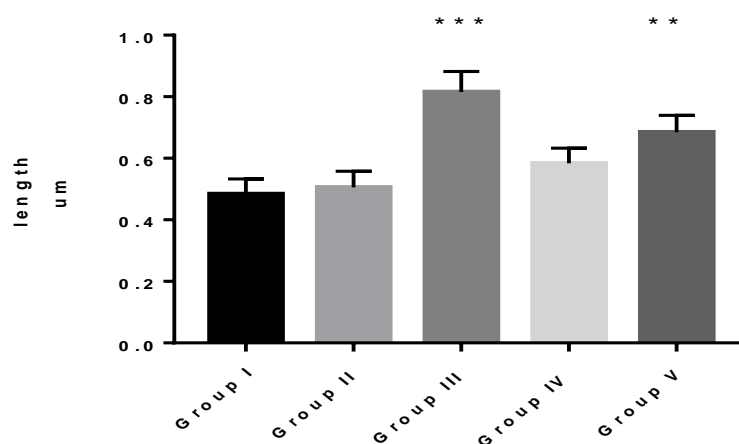


Figure (1): Comparison between the TA thickness in Group I Group II, Group III, Group IV, and. GroupV Data are shown as a length in μm . (p<0.01; ***p<0.0001 vs Group I).**

Table (1): The Mean thickness of tunica albuginea in all studied group

Groups	Group I	Group II	Group III	Group IV	Group V	P .value
Mean thickness of tunica albuginia	0.484± 0.02431	0.5058± 0.02336	0.8157± 0.0384	0.584± 0.02431	0.6843± 0.03175	With group I, II, III, IV, V p<0.0001. With group III, IV p<0.0001 With group III, IV p<0.01

There was a marked significant increase in blood vessels congestion in Group III (P= 0.0004) that was reduced by avocado treatment as shown in Group IV (P= 0.9655). In Group V we found that blood vessels congestion remained significantly increased compared to Group

I (P= 0.0075). We didn't find any significant difference between Group III and Group V (P= 0.6995).

We did not find a significant change between Group I and Group II (P= 0.9999) (Figures 2 and 3).

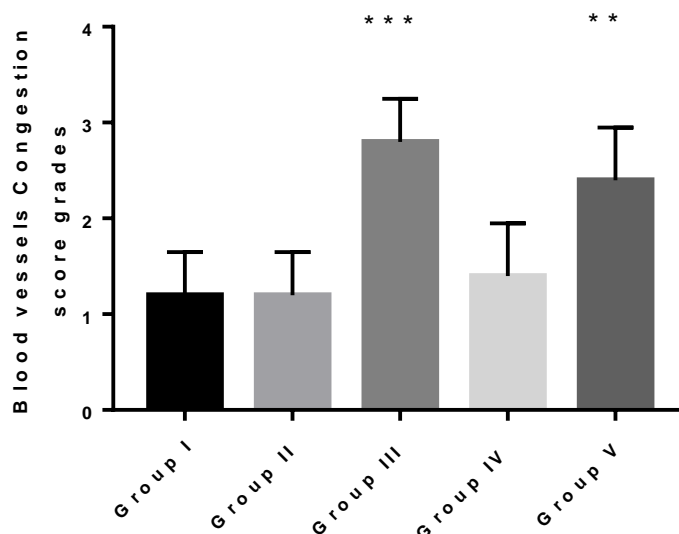


Figure (2): Blood vessels congestion in ,Group II, Group III, Group IV and. Group V. Data are shown as score grades. (**p<0.01; ***p<0.0001 vs Group I)

Table (2): The Mean in blood vessels congestion all studied group

Groups	Group I	Group II	Group III	Group IV	Group V	P .value
Mean blood vessels congestion	1.2± 0.2	1.2± 0.2	2.8±0.2	1.4±0.2449	2.4±0.2449	With group I, II, III, IV, V p<0.0001. With group III, IV p<0.0001 With group III, IV p<0.01

$$\text{Percentage of blood vessels in the ovary} = \frac{\text{Size of all blood vessels}}{\text{Total size of the ovary}} \times 100$$

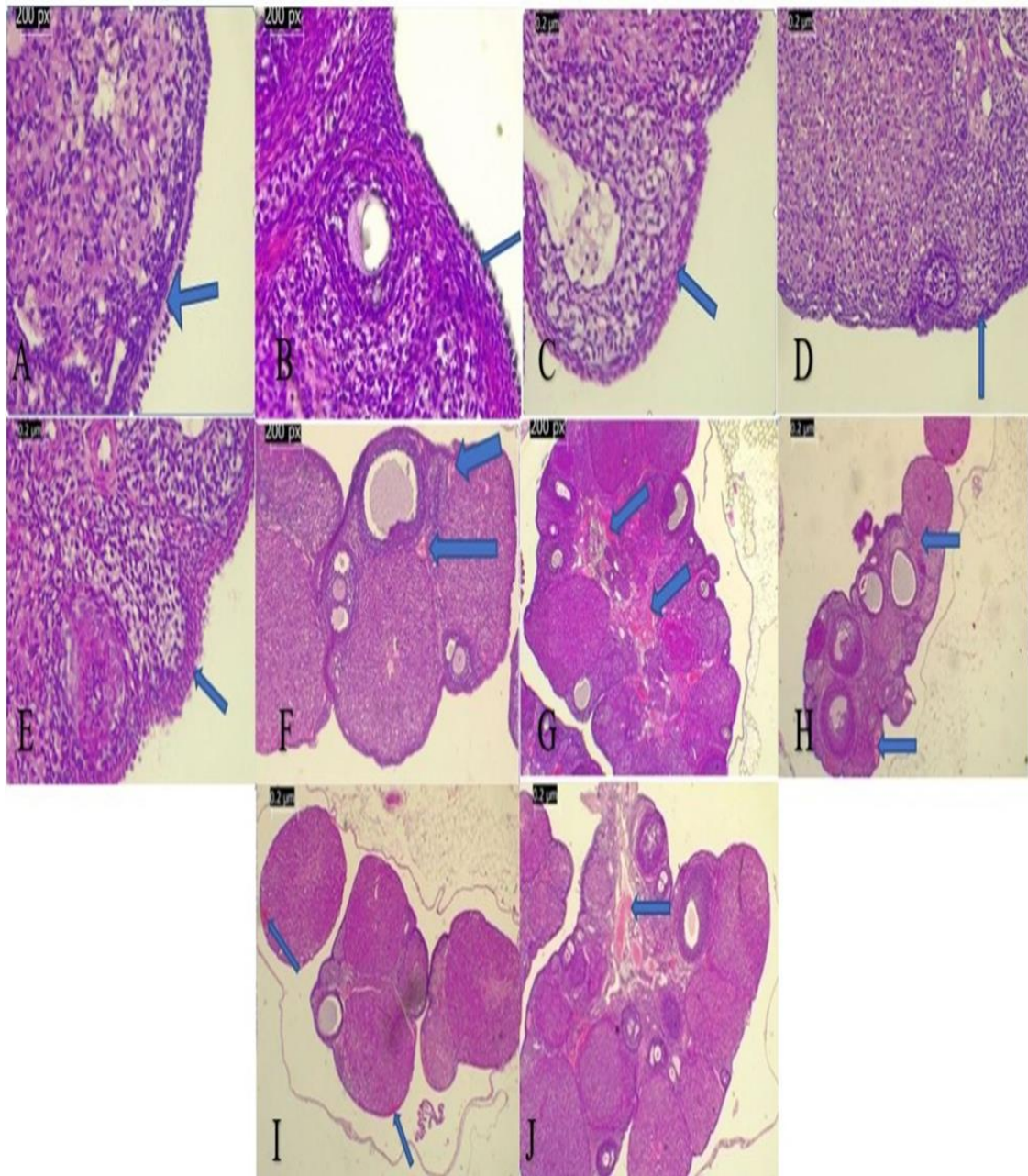


Figure (3): Tunica albuginea (TA) of the ovary: It showed marked increase in TA thickness in group exposed to EMF (Image B) (H & E, 200x). compared to Control group (Image A) (H & E, 200x). In the avocado treated group on top of exposure to EMF there was a significant reduction in TA thickness (Image C) (H & E, 200x). There were no changes in TA thickness between avocado treated group (Image D) (H & E, 200x). and control (Image A) (H & E, 200x). It also showed a significant increase in TA thick in spontaneous recovery group (Image E) (H & E, 200x). Regarding blood vessels, it showed marked blood vessels congestion with irregular walls in group exposed to EMF and spontaneous recovery group (Images G and J respectively) (H & E, 40x). Blood vessels in the ovary of other groups were normally filled with blood with irregular walls. (Control group: Images F(H & E, 40x); group exposed to EMF and treated with avocado; image H(H & E, 40x); avocado treated group: image I (H & E, 40x).

There was a significant increase in collagen deposition in Group III ($P < 0.0001$). This increase was corrected by avocado treatment as demonstrated in Group IV in which collagen deposition reaches to the Group I level ($P = 0.3273$). There was a significant increase in

collagen deposition in Group V ($P = 0.0002$). By comparing collagen deposition in Group V to Group IV, there was a significant increase in Group V ($P = 0.0191$). We didn't find any significant change between the Group I and Group II ($P = 0.9426$) (Figures 4 and 5).

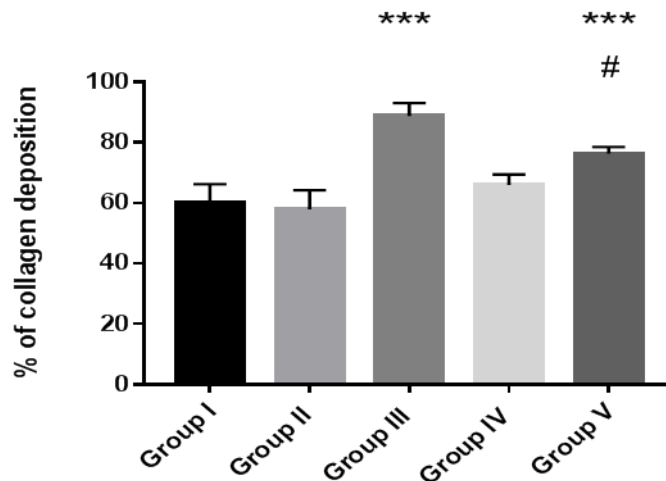


Figure (4): Quantification of collagen deposition in, Group II, Group III, Group IV, and. Group V. Data are shown as a percentage ranging from 0 % and 100 %. (** $p < 0.01$; *** $p < 0.0001$ group; # $p < 0.05$ vs Group IV).

Masson's trichrome stain: to study the collagen fibers in the ovary, increased in Group III, Group V and redacted by effect

of Avocado in Group IV, and on any change between Group I and Group II.

Table (3): The mean area of collagen deposition in all study groups

Groups	Group I	Group II	Group III	Group IV	Group V	P.value
Mean area of collagen deposition	60.12 ±2.715	57.88 ±2.816	88.83 ±1.89	65.95 ±1.589	76.28 ±1.025	With group I, II, III, IV, V $p < 0.0001$. With group III, IV $p < 0.05$ With group III, IV $p < 0.05$

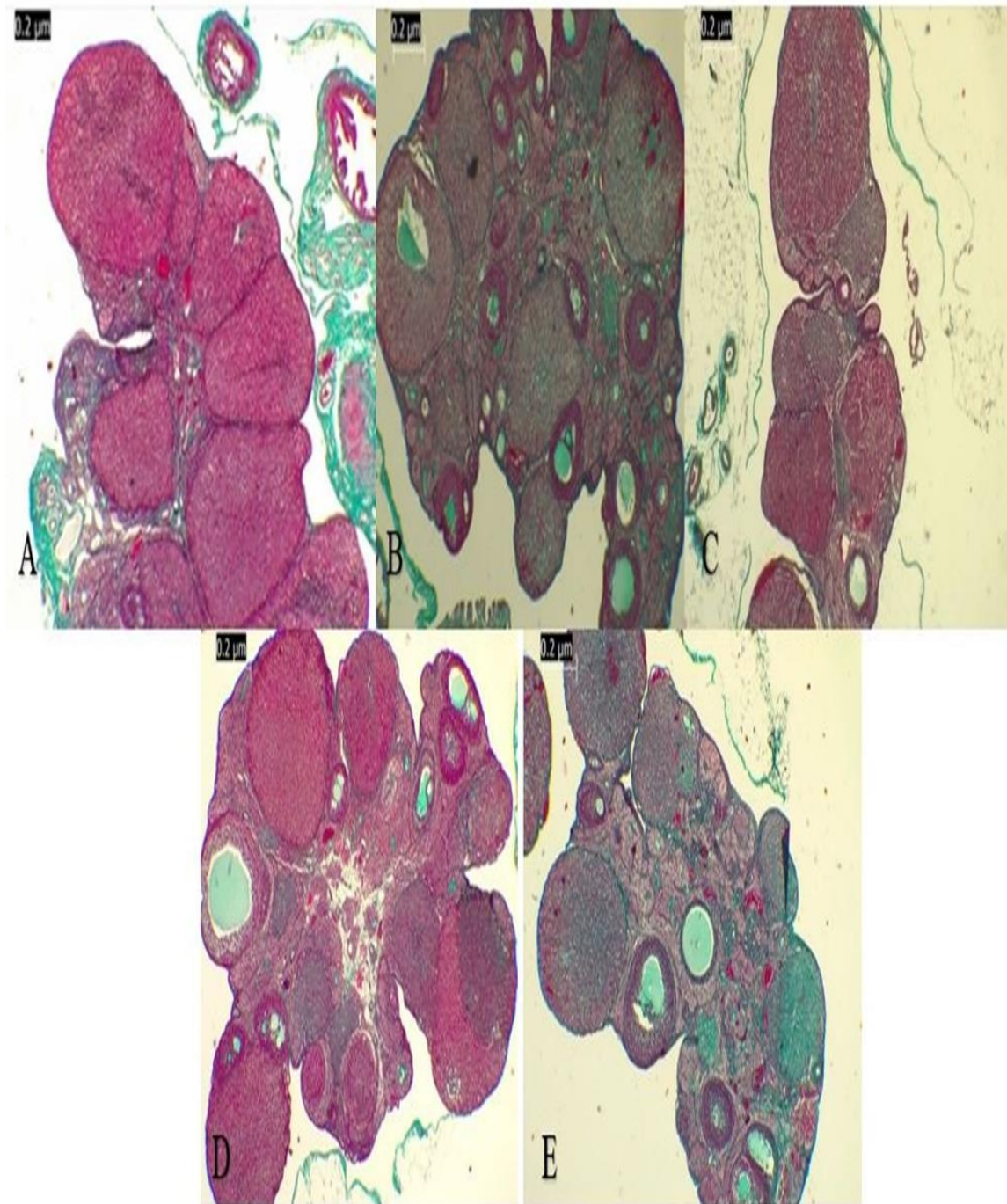


Figure (5): Collagen deposition in the ovary (Masson's trichrome, 40x). It shows marked increase in Collagen deposition n group exposed to EMF (Image B) compared to Control group (Image A) and spontaneous recovery group (Image E). While in the avocado treated group on top of exposure to EMF and avocado treated group (Images C and D respectively) there was no change in collagen deposition compared to control group (Image A).

NF- κ B protein expression in the studied groups. Showed significant increase in Group III compared to Group I ($P = <0.0001$) that was reversed by avocado treatment to the control level as we found in Group IV ($P = 0.3273$). On the other side, spontaneous recovery did correct this increase as shown in Group V

with a significant increase in NF- κ B protein expression compared to the Group I ($P = 0.0002$) but there was a significant reduction compared to Group IV ($P = 0.0191$). There was no significant change between Group I and Group I I ($P = 0.7710$) (Figures 6 and 7).

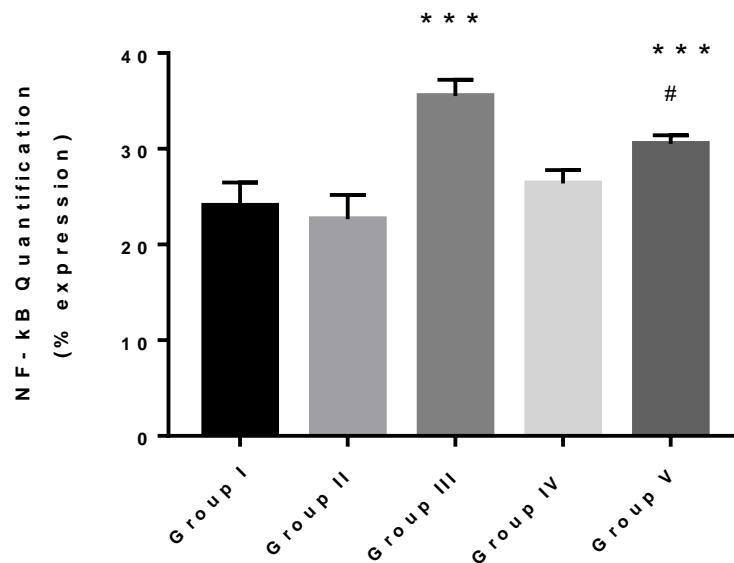


Figure (6): Quantification of NF- κ B protein expression, Group II, Group III, Group IV, and Group V. Data are shown as a percentage ranging from 0% and 100%. (***) $p < 0.0001$ group I; # $p < 0.05$ vs Group IV).

NF- κ B protein in the ovary was increased in Group III, Group V and redacted by effect of Avocado in Group

IV, and on any change between Group I and Group II.

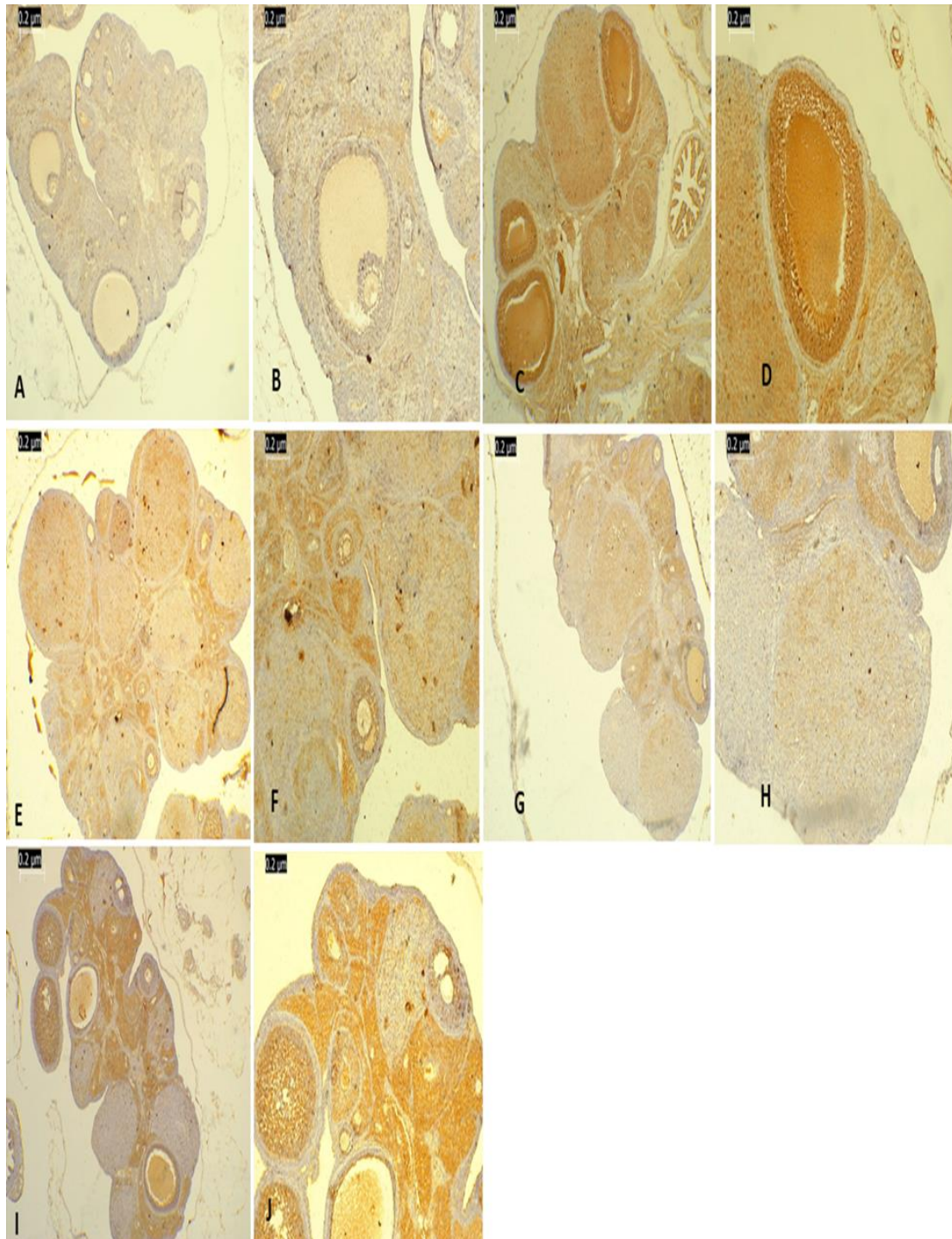


Figure (7): NF- κ B protein expression in the ovary(NF- κ B) Immunohistochemical stain 40x and 100x). It shows intranuclear expression of NF- κ B that took the brown color. It revealed no difference between Control group (Images A & B) and avocado treated group on top of exposure to EMF(Images E&F). Also, there was no change NF- κ B protein expression in avocado treated group (Images G & H) compared to control. On other side, it shows a significant increase in NF- κ B protein expression in EMF exposed group (Images C&D) and spontaneous recovery group (Images I&J) compared to control.

There were no statistically significant differences in PCNA protein expression between all groups (**Figure 8**).

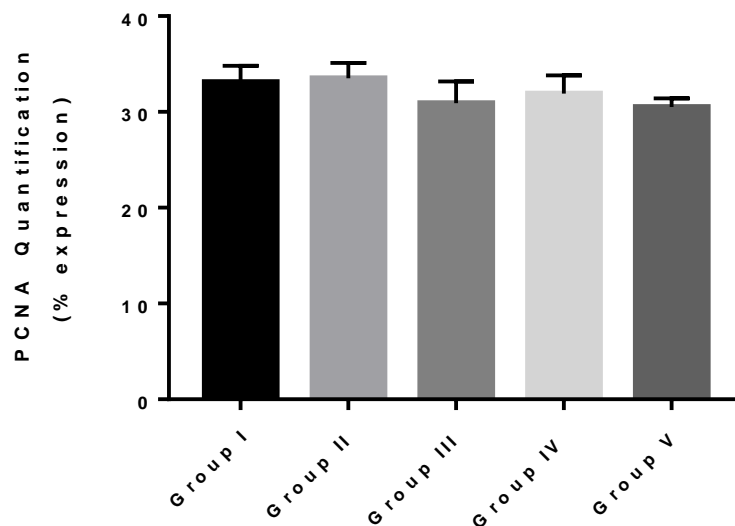


Figure (8): Quantification of PCNA protein expression Group II, Group III, Group IV and Group V. Data were shown as a percentage ranging from 0 % and 100 %.

Our results showed strong nuclear immunostaining for PCNA in all oocytes of small, large follicles, and in proliferating granulosa and theca cells. We measured expression PCNA in all

studied groups. There was some change between the groups but was not statistically significant versus control (**Figure 9**).

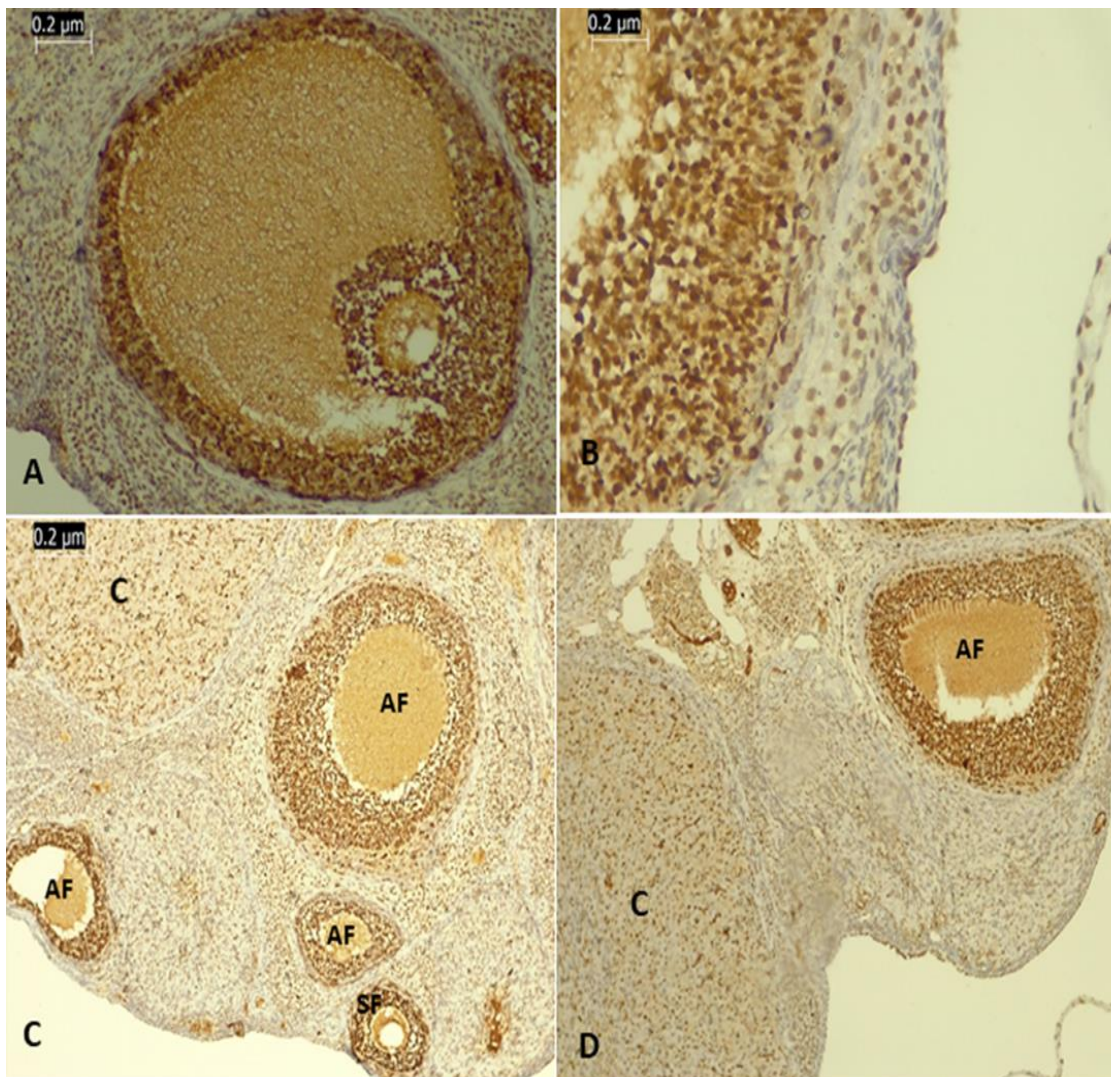


Figure (9): PCNA protein expression in the ovary. (PCNA Immunohistochemical stain 40x and 100x). It shows intra-nuclear expression of PCNA that took the brown color. There no differences between Control group (Images A & B) and the other studied groups in PCNA protein expression. Image A: It shows mature Graafian follicle; Image B: It shows immunostaining of small size follicles that are located beneath tunica albuginea (middle of the photo) and granulosa and theca cells in corpus luteum (left side of the photo); Images C & D: They show Secondary follicle (SF), corpus luteum (C) and atretic follicle (AF) with nuclear immunostaining of granulosa and theca cells.

DISCUSSION

Humans are exposed to electromagnetic waves every day, more than the day before through frequent usage of mobile phones, microwaves, and computers. Some studies reported that EMF exposure induced some change as

stress, headache, tiredness, anxiety, impairment in cognitive functions, and poor concentration (*Behari, 2010*).

Avocado is a high-fat food that has been associated with reduced inflammation and shown to have

beneficial effects on genes linked to cancer (*Bhuyan et al., 2019*).

In this work we investigated the effect of EMF and avocado on ovary. We found that EMF exposure induced increasing in tunica albuginea thickness. Besides increasing in collagen fiber deposition, we found also a marked increase in blood vessels dilation and congestion. These results may affect the normal ovulation process in the ovary. Previous studies had been shown that magnetic fields lead to ovarian tissue changes that include changes in size and shape of the ovary and also change at the level of the ovarian follicles (*Ahmadi et al., 2016*).

In contrary to our results previous studies have reported EMF induce histological and structure change in the ovary, some meta-analyses and investigations have emphasized that EMFs have no negative effects on the female reproductive system (**Merhi, 2012**).

In our study, we found that avocado treatment to the group exposed to EMF induced a significant reduction in the thickness of tunica albuginea compared to the EMF exposed group without treatment. It was found that avocados decrease collagen fiber deposition and blood vessels congestion to the close to the control group, which may be indicated for the Avocado may be an excellent source of B vitamins, which help the body on protection from disease and infection (*Antasionasti, 2017*).

Some other studies' results suggest that avocado can protect different tissues like (liver and thyroid tissue against oxidative damage). Avocado possibly induced this protection through the

antioxidant effects of its bioactive compounds (*Hamouda et al., 2016*).

In our study, the group that received avocado only, there was no significant change in the tunica albuginea thickness and the process of inflammation (fibrosis and congested blood vessels) compare to the control group. This may indicate that Avocado have several antioxidant molecules that protect from environmental hazards beside this group did not exposed to EMF.

Other study reported that the ethanol extract of avocado seeds has promoted ovarian follicle growth and maturation. It also stimulated ovulation which is supported by the observed reduction of endometriosis lesions (*Stilley et al., 2010*).

In the group which underwent spontaneous recovery after EMF exposure, there was an increased tunica albuginea thickness, collagen fiber deposition and congested blood vessels compared to control. At the same time, there was a moderate correction in these changes compared to EMF, may be due to effect the radiation dose on spontaneous recovery, the speed of recovery can range from several weeks to about 2 years (*Coeytaux et al., 2015*).

In our study, nuclear factor- κ B (NF- κ B) expression as a marker for inflammation, showed significant increase in the Group III and Group V. This increase was reversed by avocados shown in Group III. This can be explained by prolonged exposed to EMF about 4 weeks may induce inflammatory process and enhanced cell proliferation that reflected by increase in NF- κ B protein expression.

In another study, the paclitaxel (chemotherapy) induced NF- κ B activation, leading to enhanced cell proliferation. NF- κ B is constitutively active in these cells, resulting in constitutive secretion of pro-inflammatory cytokines (Huang *et al.*, 2014). Elevated NF- κ B signaling has been observed in tumors of the prostate, breast, and ovarian (Rajasekhar *et al.*, 2011).

Proliferating Cell Nuclear Antigen (PCNA) is a protein that shows immunoreactivity in oocytes first appeared in primary follicles, preceding oocyte enlargement, and was observed in all stages of follicle development. (Singh *et al.*, 2019). In our study, we detected strong nuclear immune staining for PCNA in all oocytes of small, large follicles, and in proliferating granulosa and theca cells. We did not find change in PCNA protein expression between all groups.

Previous study had reported no remarkable staining for PCNA either in granulosa cells or in the oocytes. Which is matching with our study (Xu *et al.*, 2011). Another study the effect of low frequency EMF on follicle development in the rat ovary. Their results indicated thinner nuclei of oocytes and zona pellucida in an EMF exposed group compared to a matched control group (Altun *et al.*, 2018).

Identifying the oocyte in rats ovary had be reported in several studies by immunohistochemical technique using antibody directed against Proliferating Cell Nuclear Antigen (PCNA) (Afifi and Reyad., 2013), (Singh *et al.*, 2019).

CONCLUSION

EMF affected negatively on ovary and used avocado as antioxidant for protected of ovary from change in structural and functional changes resulting from exposure to EMF.

Limitations: These study findings needed further assessment on big series.

Conflict of interest disclosure statement: Authors declare that there was not any financial or personal conflict of interest.

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الآثار الوقائية لمستخلص الأفوكادو الإيثانولي (بيرسي أمريكيانا) على بصيالات المبيض في إناث الجرذان البيضاء البالغة المعرضة للموجات الكهرومغناطيسية المنبعثة من الهواتف الخلوية

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خلفية البحث: زادت الدراسات النسيجية والفسولوجية في تقييم تأثيرات المجالات الكهرومغناطيسية على صحة الإنسان، وتأثير التردد الكهرومغناطيسي المنخفض على نمو البصيلات، وعملية الإباضة في مبيض الجرذان.

الهدف من البحث: دراسة التغيرات التركيبية والوظيفية لمبيض إناث الجرذان البيضاء البالغة بعد التعرض للموجات الكهرومغناطيسية الصادرة من الهواتف الخلوية والدور الوقائي المحتمل لمستخلص الأفوكادو.

مواد وطرق البحث: هذه الدراسة هي دراسة تجريبية. تم تنفيذ هذه الدراسة في كلية الطب جامعة الأزهر، قسم الهستولوجي وتنفيذ جميع التجارب علي إناث الجرذان البيضاء البالغة وتركنت في منزل الحيوان لمدة سبعة أيام وذلك للسماح لها بالتأقلم مع البيئة الجديدة، وجميعها تلقى الماء والغذاء وتم تقسيمها الي مجموعات:

(1) المجموعة الأولى: الجرذان الضابطة التي تركنت دون علاج، **(2) المجموعة الثانية:** تتلقى الجرذان الضابطة مستخلص الأفوكادو، **(3) المجموعة الثالثة:** الجرذان المعرضة لتردد يساوي 950 ميغاهرتز من المجال الكهرومغناطيسي لمدة شهر واحد، **(4) المجموعة الرابعة:** تتعرض للمجالات الكهرومغناطيسية وعولجت عن طريق الفم بجرعة يومية من مستخلص الأفوكادو في نفس اليوم، **(5) المجموعة الخامسة:** تركنت الجرذان للشفاء التلقائي لمدة شهر واحد بعد التعرض للمجالات الكهرومغناطيسية.

فحص الوتد: تم استئصال المبيضين والحفاظ عليها للفحص النسيجي والهستوكيميائي.

نتائج البحث: العلاج بالأفوكادو بعد تعرض الموجات الكهرومغناطسية يسبب تخفيضاً في التعبير العامل النووي Kb، وانخفاض ترسب الكولاجين وسمك الغلالة البيضاء بتأثير الأفوكادو والحد من احتقان الأوعية الدموية، علي خلاف مجموعة التعرض التي كانت أكثر تأثير من الموجات الكهرومغناطسية.

الاستنتاج: التعرض الي موجات الكهرومغناطسيه أثر سلباً على المبيض واستخدم الأفوكادو كمضاد للأكسدة لحماية المبيض من التغيرات التركيبية الوظيفية. الناتجة عن التعرض للمجالات الكهرومغناطسية.

الكلمات الدالة: إناث الجرذان البيضاء، موجات الكهرومغناطسيه، الأفوكادو.

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