Histological and Immunohistochemical Study on the Effect of 900 MHz Mobile Phone Radiation on the Adrenal Cortex of the Adult Male Albino Rat and the Possible Protective role of Green Tea Extract

Original Article

### Hanaa S.E. Mousa

Department of Medical Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt

#### ABSTRACT

**Background and Objectives:** Mobile phones emit electromagnetic waves that have adverse effects on many organs. Adrenal cortex is an important gland in the human body. In this study, the effect of mobile phone radiation on the adrenal cortex and the possible protective role of green tea extract were investigated.

**Materials and Methods:** Twenty- seven adult male Wistar albino rats were used. They were divided into three groups; group I [the control group], group II [the mobile phone group], and group III [the mobile phone group received green tea extract (GTE)]. Serum adrenocorticotrophic hormone (ACTH), and corticosterone and tissue malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were measured. Gene expression of NF- $\kappa$ B was measured. Histological examination was done using H&E, Masson trichrome, and caspase 3. Morphometric and statistical analyses were performed.

**Results:** Adrenal MDA was elevated and CAT and SOD were decreased in group II, while in group III significantly decreased MDA and increased the activities of CAT and SOD. NF- $\kappa$ B was highly increased in group II and, moderately increased in group III. Histological examination showed distortion of histological architecture, congestion and increased vacuolation , a significant increase in collagen fibers and caspase 3 immunoreaction in group II. In group III, there were restoration of histological architecture and few areas of congestion and vacuolation and moderate increase in collagen fibers and caspase 3 immunoreaction.

**Conclusions:** Mobile phone radiation caused adverse effects on the function and structure of the adrenal cortex and the intake of green tea significantly prevented this effect.

#### Key Words: Adrenal gland, Electromagnetic waves, Green tea extract and Mobile phone radiation.

ACTH: adrenocorticotrophic hormone, CAT: Catalase, ELISA: enzyme-linked immunosorbent assay, FCC: Federal Communication Commission, GTE: green tea extract, IARC: International Agency for Research on Cancer, iNOS: Inducible nitric oxide synthase, MDA: malondialdehyde, NF-Kb: nuclear factor kappa B, One-way ANOVA: One-way analysis of variance test, RF: radiofrequency, RF-EMW: radiofrequency electromagnetic waves, ROS: Reactive oxygen species, SAR: specific absorption rate, SD: standard deviation, SOD: Superoxide dismutase.

Revised: 11 July 2021, Accepted: 03 October 2021

**Corresponding Author:** Hanaa S.E. Mousa, Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt, **Mobile:** +201017330763, **E-mail:** hana7aih@gmail.com.

**ISSN:** 1110-0559, Vol. 05, No. 01

#### **INTRODUCTION**

Mobile phones have become an essential part in our daily life. They emit radiofrequency electromagnetic waves (RF-EMW). These waves have an electrical field and a magnetic field; both have harmful effects on the tissues, but the magnetic field has more detrimental impact due to its higher ability to penetrate the living tissues. The rate of absorption of these radio frequency electromagnetic waves by the human body is called Specific Absorption Rate (SAR) value. The unit of SAR is

watt/kg. The Federal Communication Commission (FCC) has determined 1.6 watt/kg as the maximum allowed SAR of any mobile phones or tablets<sup>[1, 2]</sup>.

Electromagnetic radiation of the mobile phones is a non-ionizing radiation, but it has thermal and nonthermal effects on body organs. The thermal effects can induce damage in different organs such as the eye and the brain, whereas the mechanisms are for nonthermal effects are not exactly known yet<sup>[3, 4]</sup>. The harmful effects following the use of cell phones include impaired male fertility, DNA damage in the liver and lung, and cellular ultrastructure changes in the spiral ganglia. The International Agency for Research on Cancer (IARC) has considered the radiofrequency electromagnetic fields as a possible carcinogen for humans (Group 2B). Studies proved that there is an association between exposure to mobile phone radiation and increased risk of glioma meningioma and acoustic neuroma<sup>[5, 6]</sup>.

Personal non-commercial use only. JMH copyright © 2017. All rights eserved

The adrenal gland functions mainly in regulating the body response to various strssors. It is composed of the cortex and medulla. The cortex secretes the steroid hormones that regulate body homeostasis and chronic stress responses, whereas the medulla synthesizes adrenaline and noradrenaline; which modulate the acute stress response<sup>[7, 8]</sup>.

Green tea (Camellia sinensis) is a common beverage, which is popularly consumed in many countries such as Egypt and Algeria. Green tea contains a wide variety of antioxidants namely polyphenolic flavonoids and the catechins<sup>[9]</sup>. The catechins fight the various oxidants both directly through eliminating the reactive oxygen and nitrogen species; and indirectly by activating the antioxidant enzymes<sup>[10, 11]</sup>. Green tea polyphenols are effective in preventing cancer through removing reactive oxygen species or scavenging transition metals<sup>[12]</sup>.

The intake of green tea has many beneficial health effect due to its antioxidant, antimicrobial, anticancer, and anti-inflammatory properties. Regular intake of green tea has been proved to reduce the risk of many skin, metabolic, cardiovascular and neurological diseases<sup>[13, 14]</sup>.

Many studies investigated the harmful impact of mobile phone radiation on the endocrine system. In this study, we investigated the effect on mobile phone radiation on the adrenal gland and the potential protective role of the green tea extract.

#### **MATERIALS AND METHODS**

#### 2.1. Experimental animals:

Twenty- seven adult male Wistar albino rats aged 2 - 3 months and weighing between 250 - 280 g were used as experimental animals in the present investigation. The rats were obtained from the animal house of Faculty of Medicine, Zagazig University, Egypt. They were kept for 15 days under observation before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in metallic cages with well-aerated covers with a 12 h light/dark cycles. Each cage contains 3 rats. The rats were housed under good ventilation at normal atmospheric temperature ( $25 \pm 5$  °C). They received water and standard balanced diet. All animal procedures were in accordance with the recommendations for the proper care and use of laboratory animals stated by the Institutional Animal Ethical Committee.

#### 2.2. Preparation of the Green Tea Extract (GTE):

The crude aqueous extract of green tea was prepared according to El-Beshbishy<sup>[15]</sup> by soaking 15 g of instant green tea leaves in 1 L of distilled water whose temperature did not exceed 90 °C, for 5 min to obtain soluble polyphenols dissolved in the aqueous extract. The solution was filtered to obtain the final 1.5 % (w/v) green tea extract. This solution was substituted in the place of water as the only source of drinking fluid.

#### 2.3. Experimental Design:

Rats were divided into three equal groups designed as the following:

Group I (Control group): They received water and normal food daily.

**Group II (mobile phone group):** They were put in cage; they were free in motion and subjected to 4 G mobile phone signal [GSM 900 MHz; specific absorption rate (SAR) 1.18 W/kg] for 4 weeks. The mobile phones were just above the cages of each study group (approx. 10 cm away from the rats). The mobile phones used in the study were in the standby position for 5 hours and called intermittently (six times a day for 10 min); so the total daily duration of exposure was 6 hours.

Group III (mobile phone group received green tea extract): They were exposed to the same dose of mobile radiation as that of group II Rats were fed as usual; but they were orally given aqueous green tea extract as the sole drinking fluid during the 4 weeks at a concentration of  $1.5 \% (\text{w/v})^{[15]}$ .

#### 2.4. Body weights:

Each animal were weighed in the beginning and at the end of the experiment.

#### 2.5. Collection of blood samples:

At the end of the experiment period, blood samples were collected using capillary tubes from the retro-orbital venous plexus and centrifuged for 10 min at 5000 rpm to obtain clear serum.

### 2.6. Measurement of serum ACTH and corticosterone:

Serum adrenocorticotrophic hormone (ACTH) and corticosterone levels were measured using

enzyme-linked immunosorbent assay (ELISA) kits (USCN Business Co., Ltd., Wuhan, China; DRG International, USA, Cat. No. EIA-5186, respectively) according to the manufacturers' instructions. Absorbances were measured at 450 nm.

# 2.7. Collection of tissue samples for biochemical analysis, gene expression, histological and immnuohistochemical examination:

All rats were anesthetized with ether inhalation and the adrenal glands were carefully dissected. The left adrenal glands were used for measurement of malondialdehyde (MDA), antioxidant enzymes; catalase (CAT), and superoxide dismutase (SOD), and gene expression of NF- $\kappa$ B while the right ones were assigned for histological and immnuohistochemical examination.

## 2.8. Measurement of Malondialdehyde (MDA), and Catalase (CAT), and Superoxide dismutase (SOD):

Half of the left adrenal glands were homogenized in ice-cold phosphate buffer (KCl 140 mmol/L, phosphate 20 mmol/L, pH7.4) and centrifuged at 10,000 X g for 15 min at 4 °C. The supernatant was used for measuring adrenal malondialdehyde and antioxidant enzymes using Biodiagnostic kits (Biodiagnostic, Giza, Egypt).Adrenal malondialdehyde (MDA) level was measured according to the method of Odukoya *et al.*<sup>[16]</sup>. Catalase (CAT) and superoxide dismutase (SOD) were measured according to Weydert and Cullen<sup>[17]</sup>.

#### 2.9. qRT-PCR relative gene expression of NF-kB:

Total RNA was extracted from the other half of the left adrenal glands a Qiagen RNA isolation kit (RNeasy, Qiagen Ltd, Crawley, West Sussex, UK) according to the manufacturer's protocol. The total RNA was quantified by the measured absorbance at 260 nm in a spectrophotometer. After that, RNA was converted into complementary DNA (cDNA) by reverse transcriptase (QuantiTect Reverse Transcription Kit, Qiagen, # 205310, Germany). This extracted RNA was used for nuclear factor kappa B (NF-kB) according to Pfaffl<sup>[18]</sup>.

### 2.10. Histological and immnuohistochemical examination:

The right glands from each group were used for evaluation of the histological changes. Half of the glands were fixed in bouin and the other half were fixed in 10 % formalin. Then, all specimens were dehydrated with ascending grades of ethanol (70, 90, and 100 %). Dehydration was followed by clearing the samples in two changes of xylene. Samples were then impregnated with two changes of molten paraffin wax, then embedded and blocked out to paraffin blocks. Sections of the adrenal glands sections (4  $\mu$ m thickness) were cut using a microtome and mounted on a glass slide. Sections fixed in bouin were used in H&E and Masson trichrome<sup>[19]</sup>, and those fixed in 10 % formalin were used for immunohistochemistry. Immunohistochemical expression of caspase-3 was performed using streptavidin–biotin complex immune-peroxidase system according to Ramos-Vara *et al.* (2008)<sup>[20]</sup>.

#### 2.11. Morphometric analysis:

The thickness of the zona fasciculata, the area percentage of collagen fibers, and the area percentage of immune reaction to caspase 3 were measured within 9 fields for each rat using image analysis software (National Institute of Health; NIH, Bethesda, MD, USA)<sup>[21]</sup>.

#### 2.12. Statistical analysis:

Data obtained were presented as mean  $\pm$  standard deviation (SD). SPSS 19 was used for all statistical analysis. Data were analyzed using One-way analysis of variance test (one-way ANOVA) followed by Tukey's post hoc multiple comparisons test for comparative analysis among the groups. Values for  $p \le 0.05$  were considered statistically significant<sup>[22]</sup>.

#### 2.13. Scaling of histological findings:

Grading of histological and immunohistochemical findings was performed using the data obtained from the morphometric analysis and histological examination.

#### RESULTS

#### 3.1. Body weight:

There was no significant change in the final body weight of rats in different groups (Table 1, Figure 1).

#### 3.2. Serum ACTH and corticosterone levels:

Serum ACTH was significantly elevated in group II when compared to the control group. In group III, its level was markedly decreased, but still higher than that of the control group. Serum corticosterone was

significantly elevated in group II when compared to the control group. In group III, its level was markedly decreased (Table 2, Figure 2).

#### 3.3. Level of MDA, CAT, and SOD activity:

MDA level was increased significantly in group II, but it was decreased in group III. SOD and CAT

activity were decreased significantly in group II, but they were increased in group III (Table. 3, Figure 3).

Table 1: Statistical analysis of Initial body weight (g) and Final body weight (g) of the experimental rat groups using one-way ANOVA test:

Group	Group I	Group II	Group III	Б	р
Parameter	Group I	Group II	Group III	г	I
Initial body weight (g)	$273.7\pm4.04$	$277.8\pm10$	$275.4\pm10.7$	0.28	> 0.05
Final body weight (g)	$331.1\pm21.14$	$335.3\pm30$	$333.1\pm16.4$	0.07	> 0.05

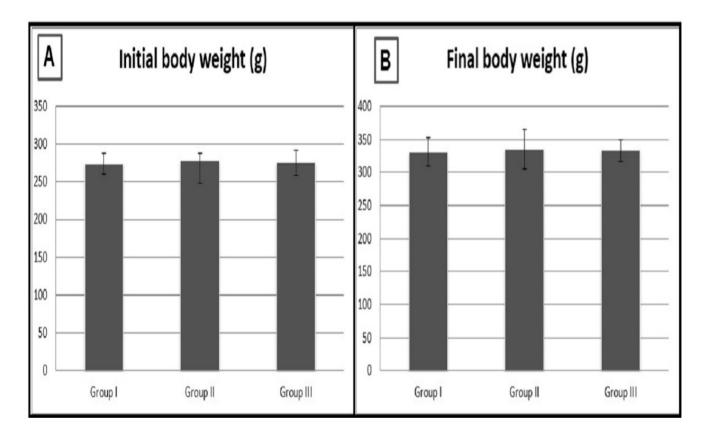


Figure 1: A: Bar chart showing the initial body weight (g). B: Bar chart showing the final body weight (g).

Table 2: Statistical analysis of serum ACTH (pg/dl) and corticosterone (µg/dl):

Group	· Group I	Group II	Group III	Б	D
Parameter	Group I	Group II	Group III	г	I
Serum ACTH (pg/dl)	$12.7 \pm 1.6$	$21^{a}\pm 2$	$16.4^{ab}\pm2$	44.58	< 0.001*
Serum corticosterone (µg/dl)	$20.9\pm1.7$	$35.7^{\text{a}}\pm4.8$	$26.8^{\text{ab}}\pm4.03$	35.22	< 0.001**

a: Significant from group I.

b: Significant from group II.

\*\*: Highly significant one-way ANOVA test.

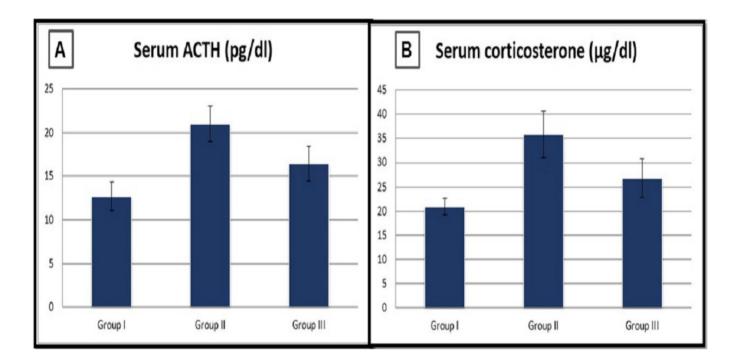


Figure 2: A: Bar chart showing the serum ACTH (pg/dl). B: Bar chart showing the serum corticosterone (µg/dl)

Table 3: Statistical analysis of the MDA, CAT, and SOD activity in the adrenal glands in different groups:

Group	- Group I	Group II	Group III	F	D
Parameter	Group I	Group II	Group III	г	I
MDA levels (mmol/g)	$1.5\pm0.14$	$2.59^{\rm a}\pm 0.4$	$2.06^{ab}\pm0.56$	15.6	< 0.001**
CAT (U/min)	$6.89\pm0.5$	$4.35^{\rm a}\pm 0.8$	$5.6^{ab}\pm0.54$	33.03	< 0.001**
SOD (U/mg protein)	$1.63 \pm 0.18$	$0.94a\pm0.25$	$1.35^{ab}\pm0.34$	18.07	< 0.001**

a: Significant from group I.

b: Significant from group II. \*\*: Highly significant one-way ANOVA test.

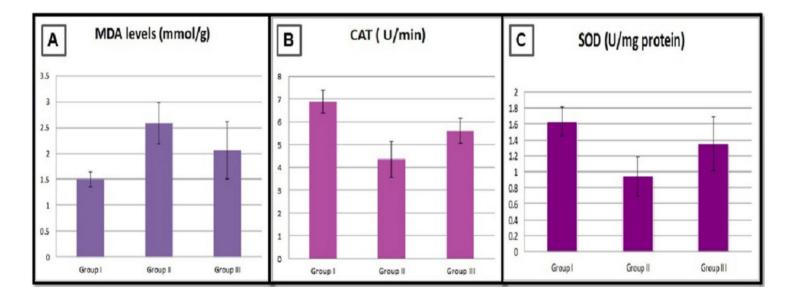


Figure 3: A: Bar chart showing MDA. B: CAT. C: SOD activity in the adrenal glands in the different groups.

#### 3.4. qRT-PCR relative gene expression of NF-kB:

Gene expression of NF-κB was significantly elevated in group II in comparison with to group I; the expression of the gene reduced markedly in group III. There was a significant difference between group I and group III (Table. 4, Figure 4).

Table 4: Statistical analysis of the gene expression of NF- $\kappa B$  in different groups:

Group	Cuoun I	Group II	Crean III	F	р
Parameter	Group I	Group II	Group III	г	r
Gene	$1.14\pm0.2$	$2.07^{\text{a}}\pm0.4$	$1.52^{ab}\pm0.2$	26.08	< 0.001**
expression of NF-kb					

a: Significant from group I.

b: Significant from group II.

\*\*: Highly significant one-way ANOVA test.

#### 3.5. Histological results:

H&E stained sections of group I showed that the adrenal cortex consisted of three zones; the outer zona glomerulosa formed of arches of cells, the middle zona fasciculata formed of cords of cells, and the inner zona reticularis formed of anastomosing cords of cells (Figures 5 A and 6 A).Group II showed disturbed architecture of the adrenal cortex, pyknotic

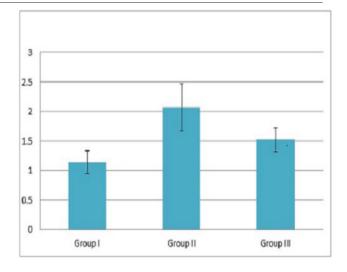
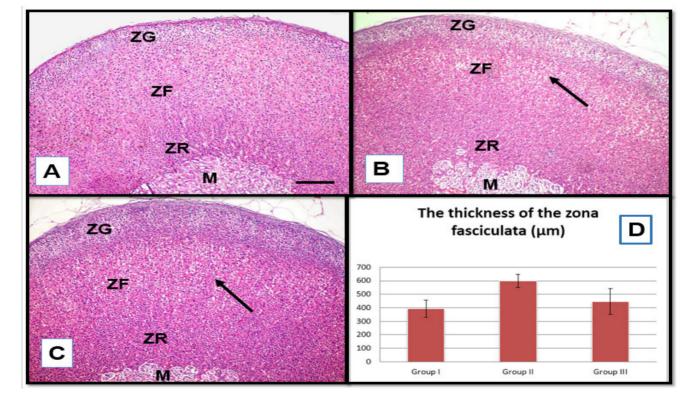


Figure 4: Bar chart showing comparison on mean values of qRT-PCR relative gene expression of NF- $\kappa$ B.

nulcei, increased vacuolation, and congested dilated blood sinusoids (Figures 5 B and 6 B). In group III, there was restoration of the normal histological structure of the adrenal cortex, vacuolation and few congested blood sinusoids. Some nuclei were apoptotic, while other nuclei were pale (Figures 5 C and 6 C). The thickness of the zona fasciculata of adrenal cortex was measured in all groups and statistically analyzed (Table. 5, Figure 5 D).



**Figure 5:** Photomicrographs of sections stained by H&E. A: Group I showing zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis (ZR), and medulla (M). **B:** group II showing loss of normal architecture of all zones and increased vacuolation (arrows). **C:** Group III showing restoration of architecture and areas of vacuolation (arrows). (H&E X 100, scale bar=  $100 \mu m$ ). **D:** Bar chart showing the thickness of the zona fasciculata of adrenal glands ( $\mu m$ ) in the different groups.

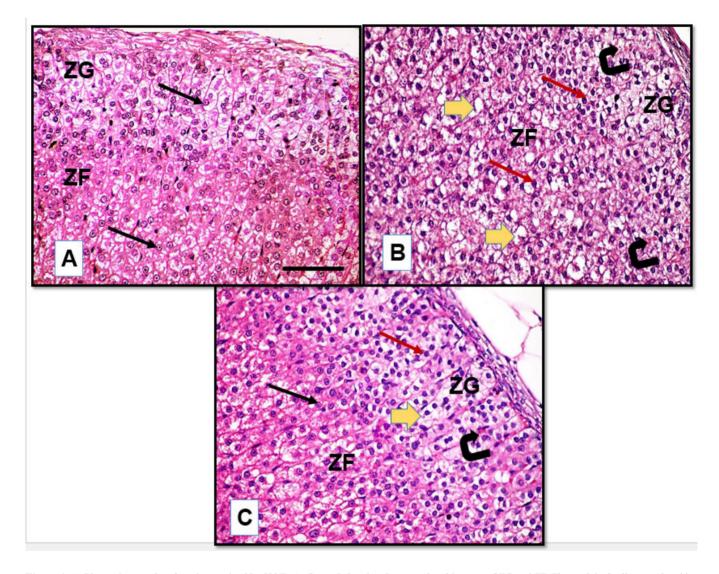
Table 5: Statistical	analysis of serum	ACTH (pg/dl) and	l corticosterone ( $\mu$ g/dl):

Group	Chann I	Crear II	Crown III	Б	р
Parameter	· Group I	Group II	Group III	г	r
The thickness of the zona fasciculata (μm)	392.2 ± 62.8	$598.7^{\mathrm{a}}\pm48.9$	$446.1^{ab}\pm96.5$	19.8	< 0.001**

a: Significant from group I.

b: Significant from group II.

\*\*: Highly significant one-way ANOVA test.



**Figure 6: A:** Photomicrographs of sections stained by H&E. **A:** Group I showing the normal architecture of ZG and ZF. The nuclei of cells are pale with prominent nucleolus (Black arrows). **B:** Group II showing loss of the normal architecture of ZG and ZF. The nuclei of cells are pyknotic (red arrows). Increased vacuolation (thick yellow arrows) and congestion (curved arrows) were apparent. **C:** Group III showing partial restoration of architecture of ZG and ZF. Some nuclei are pale (black arrows), others are pyknotic (red arrows). There are few areas of congestion (curved arrows) and vacuolation (thick yellow arrows) and vacuolation (thick yellow arrows). (H&E X 400, scale bar = 50 µm).

Masson trichrome stained sections of control group revealed few collagen fibers in the capsule and between cells (Figure 7 A). While in group II, increased collagen fibers were evident in the capsule and between cells (Figure 7 B).

Moderate amount of collagen fibers in the capsule and between cells were detected in group III (Figure 7 C). The area percentage of collagen fibers was measured in all groups and statistically analyzed (Table. 6, Figure 7 D).

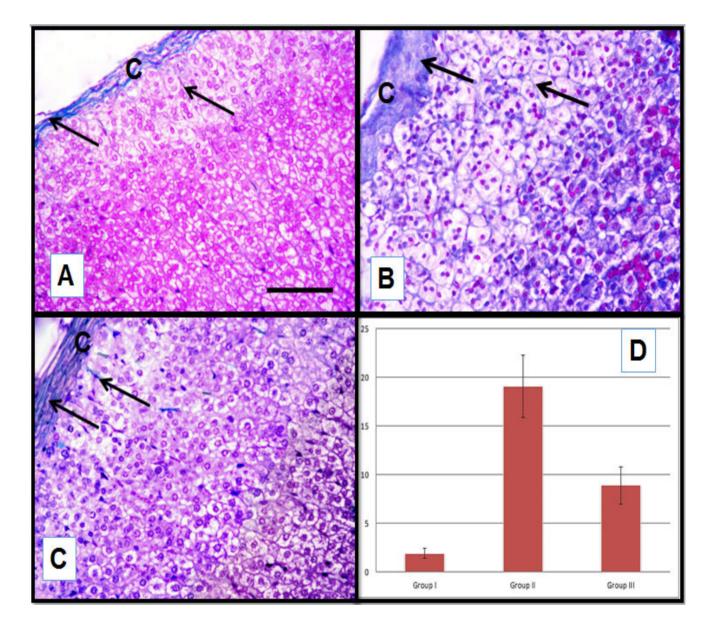
**Table 6:** Statistical analysis of the gene expression of NF- $\kappa$ B in different groups:

Group	Group I	Group II	Group III	F	Р
Parameter	Group I	Oroup II	Oroup III	r	1
Area percentage of the collagen fibers	$1.9\pm0.5$	$20.1a \pm 2$	$8.4ab \pm 2.1$	269.9	< 0.001**

a: Significant from group I.

b: Significant from group II.

\*\*: Highly significant one-way ANOVA test.



**Figure 7:** Photomicrographs of sections stained by Masson trichrome. **A:** Group I showing few collagen fibers in the capsule (C) and between cells (arrows). **B:** Group II showing excess amount of collagen fibers in the capsule (C) and between cells (arrows). **C:** Group III showing moderate collagen fibers in in the capsule (C) and between cells (arrows) (Masson trichrome X 400, scale bar =  $50 \mu m$ ). D: Bar chart showing the area percentage of collagen fibers in different groups.

Examination of caspase-3 immunostained sections revealed few areas of weak positive caspase-3 immunoreaction in the cytoplasm of cells of zona glomerulosa and zona fasciculata in group I (Figure 8 A). Strong positive immunoreaction for caspase-3 was detected in group II (Figure 8 B). Moderate immunoreaction for caspase-3 was detected in group III (Figure 8 C). The area percentage of the immune reaction to caspase 3 was measured in all groups and statistically analyzed (Tazble. 7, Figure 8 D).

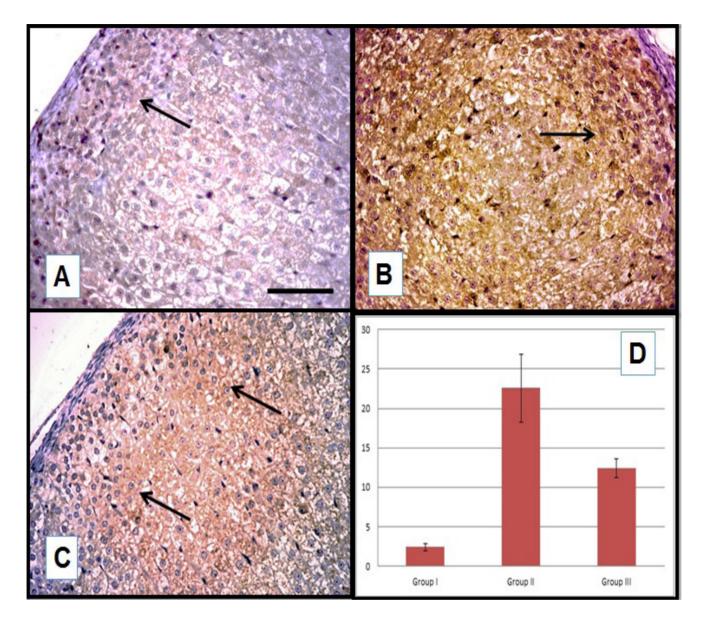


Figure 8: Photomicrographs of sections stained by immunohistochemical stain to caspase 3. A: Group I showing weak cytoplasmic reaction in some cells (arrows). B: Group II showing strong cytoplasmic reaction (arrows). C: Group III showing moderate cytoplasmic reaction (immunohistochemical stain to caspase 3 X 400, scale bar = 50  $\mu$ m). **D:** Bar chart showing the area percentage of the immune reaction to caspase 3.

Table 7: Statistical analysis of area	percentage of immune re	eaction to caspase 3 in t	he adrenal gland in d	ifferent groups:

Group	Crown I	Group II	Crown III	F	D
Parameter	Group I	Group II	Group III	г	I
Area percentage of immune reaction to caspase 3	2.4 ± 0.5	22.6a ± 4.3	12.4ab ± 1.2	136.9	< 0.001**

a: Significant from group I b: Significant from group II

\*\*: Highly significant one-way ANOVA test.

## The thickness of the zona fasciculata of adrenal glands:

The thickness of the zona fasciculata of adrenal glands was significantly elevated in group II when compared to the control group. In group III, its thickness was markedly decreased, but still higher than that of group I (Table. 5, Figure 5 D).

#### 3.6 Scaling of Histological Findings:

Scaling of histological and immunohistochemical changes are listed in (Table 8).

 Table 8: Scaling of histological and immunohistochemical changes:

	Group I	Group II	Group III
Diameter of zona fasciculata	normal	++ Increased	+ Increased
Vacuolation	-	+++	++
Congestion	-	+++	+
Fibrosis	-	+++	++
Apoptosis	-	+++	++

#### DISCUSSION

The effects of radiofrequency electro-magnetic waves on the human health are an important area of research in this era. The mobile radiation is proved to generate reactive oxygen species (ROS) in the living tissues inducing several pathological and biochemical insults<sup>[23]</sup>. In this study, the effect of mobile phone radiation on the adrenal gland and the possible protective role of green tea intake were investigated.

There was no significant alteration in the body weight of rats of group II when compared to group I. This agreed with Lee *et al.*<sup>[24]</sup> who detected no significant change in the body weight in rats exposed to mobile radiation. Serum ACTH and corticosterone were significantly increased in group II in comparison with group I; this agreed with Shahabi *et al.*<sup>[25]</sup> who reported increased level of ACTH and corticosterone upon exposure to mobile electromagnetic radiation. On the other hand, Sarookhani *et al.*<sup>[2]</sup> detected no change in the level of cortisol upon exposure to mobile radiation.

The significant increase in MDA level in group II and decrease in CAT and SOD activity agreed with Guney *et al.*<sup>[26]</sup> who detected similar results in the uterine tissue exposed to EMR. The histological changes detected in H&E stained sections in group II were due to the release of ROS which cause lipid peroxidation and disruption of cell membrane

and tissue damage<sup>[27]</sup>. Hanafy *et al.*<sup>[28]</sup> detected profound histological changes in the form of altered architecture and congestion in the heart, lung, liver and kidneys of rats exposed to mobile phone radiation.

Nuclear factor kappa B (NF-kB) is a transcriptional factor of the inducible expression of several genes, such as inducible nitric oxide synthase (iNOS), which participate in the inflammation and host defense mechanisms. Cytokines and ROS activate NF- $\kappa$ B and consequently activate the target genes, such as iNOS<sup>[29]</sup>. In this study, there was significant increase in the gene expression of NF- $\kappa$ B denoting the inflammatory effect of mobile radiation. This disagreed with Szilágyi *et al.*<sup>[30]</sup> who concluded that there was insignificant increase in the level of cytokines in the 3D skin model following exposure to the radiofrequency of mobile radiation.

Masson trichrome stained sections showed increased deposition of collagen fibers in the capsule and intracellularly. This is in agreement with Ayata *et al.*<sup>[31]</sup> who reported skin fibrosis in rats exposed to mobile phone radiation. They attributed the skin fibrosis to the inflammation induced by the oxidative stress. The strong reaction to capase 3 in group II agreed with Oral *et al.*<sup>[32]</sup> who detected increased expression to caspase 3 in the cytoplasm of endometrial cells in rats exposed to a 900-MHz mobile phone.

Green tea extract (GTE) had no effect on the body weight; this finding agreed with Baladia *et al.*<sup>[33]</sup> who concluded that intake of green tea or its extract doesn't significantly affect the body weight. On the hand, Li *et al.*<sup>[34]</sup> reported that green tea extract reduced body weight in obese canines. GTE reduced serum ACTH and corticosterone in group III. This agreed with Zhu *et al.*<sup>[35]</sup> who detected that green tea polyphenols decreased the serum ACTH and corticosterone in mice subjected to forced swim test.

Green tea extract (GTE) contains catechins that inhibit the lipid peroxidation through a single electron transfer followed by deprotonation in the peroxylradicals in the phospholipids bilayers. Polyphenols also decrease the oxidation level besides direct role as antioxidants. Additionally, green tea contains minerals such as selenium, zinc, and manganese; these minerals act as co-factors in antioxidant enzymes<sup>[36, 37]</sup>. Thus in group III, GTE significantly reduced the level of MDA and increased CAT and SOD activity; this agreed with Heikal *et al.*<sup>[38]</sup> who found that green tea ameliorated the oxidative stress in the kidney induced by cyromazine and chlorpyrifos. Decreased expression of NF-κB was attributed to the powerful antinflammatory effect of  $\text{GTE}^{[39]}$ . This agreed with Park *et al.*<sup>[40]</sup> who reported that green tea extract prevented the activation of NF-κB in obese rats.

The restoration of the normal histological structure of the adrenal cortex in group III agreed with Elhalwagy *et al.*<sup>[41]</sup> who reported that green tea extract protected against fenitrothion insecticide induced- damage in the kidney and liver. The collagen fibers in the group III are markedly decreased. This denotes the antifibrotic activity of green tea extract. Kim *et al.*<sup>[42]</sup> and Tsai *et al.*<sup>[43]</sup> reported the antifibrotic properties of green tea on the liver fibrosis. Regarding its effect on apoptosis, GTE markedly reduced the immune reaction to caspase 3 in group III. This agreed with Ogaly *et al.*<sup>[44]</sup> who found that green tea extract has decreased the apoptosis induced by deltamethrin in rat brain.

#### CONCLUSION

Radiofrequency electromagnetic waves (RF-EMR) emitted from mobile phones has a harmful effect on the structure and function of the adrenal cortex and that the regular intake of green tea significantly prevented the oxidative damage and enhanced apoptosis induced by exposure to the mobile radiation.

#### **CONFLICT OF INTEREST**

There is no potential conflict of interest among the authors.

#### REFEREANCES

- 1. Al Shadidi, Z. A., & Al Shaddadi, M. (2021). The biological effects of radio waves on human body tissues. Journal of Genetic and Environmental Resources Conservation, 9(1), 183.189-
- Sarookhani, M. R., Rezaei, M. A., Safari, A., *et al.* (2011). The influence of 950 MHz magnetic field (mobile phone radiation) on sex organ and adrenal functions of male rabbits. African journal of biochemistry research, 5(2), 6568-. https://doi. org/10.5897/AJBR.9000240
- Ilhan, A., Gurel, A., Armutcu, F., *et al.* (2004). Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. Clinica chimica acta, 340(1162-153 ,(2-. https://doi.org/10.1016/j. cccn.2003.10.012
- 4. Demirel, S., Doganay, S., Turkoz, Y., *et al.* (2012). Effects of third generation mobile phone-emitted

electromagnetic radiation on oxidative stress parameters in eye tissue and blood of rats. Cutaneous and ocular toxicology, 31(2), 8994-. https://doi.org/ 10.310915569527.2012.657725/

- Shrestha, M., Raitanen, J., Salminen, T., *et al.* (2015). Pituitary tumor risk in relation to mobile phone use: A case-control study. Acta Oncologica, 54(8), 11591165-. https://doi.org/10.3109028418/ 6X.2015.1045624
- Zuo, W. Q., Hu, Y. J., Yang, Y., Zhao, X. Y., Zhang, Y. Y., Kong, W., & Kong, W. J. (2015). Sensitivity of spiral ganglion neurons to damage caused by mobile phone electromagnetic radiation will increase in lipopolysaccharide-induced inflammation in vitro model. Journal of neuroinflammation, 12(1), 113-.
- Koko, V., Djordjeviæ, J., Cvijiæ, G., *et al.* (2004). Effect of acute heat stress on rat adrenal glands: a morphological and stereological study. Journal of experimental biology, 207(24), 42254230-. https:// doi.org/10.1242/jeb.01280
- 8. Fox, J. (2012). Oxford Textbook of Endocrinology and Diabetes (2nd edn).
- Chengelis, C. P., Kirkpatrick, J. B., Regan, K. S., *et al.*, (2008). 28-Day oral (gavage) toxicity studies of green tea catechins prepared for beverages in rats. Food and Chemical Toxicology, 46(3), 978-989. https://doi.org/10.1016/j.fct.2007.10.027
- Sang, S., Hou, Z., Lambert, J. D., & Yang, C. S. (2005). Redox properties of tea polyphenols and related biological activities. Antioxidants & redox signaling, 7(111714-1704 ,(12-. https://doi. org/10.1089/ars.2005.7.1704
- Mandel, S.A., Youdim, M.B. (2012). In the rush for green gold: can green tea delay age progressive brain neurodegeneration? Recent Pat. CNS Drug Discov. 7 (3), 205–217. https://doi. org/10.2174157488912803252005/
- 12. Baranei, M., Taheri, R. A., Tirgar, *et al.*, (2021). Anticancer effect of green tea extract (GTE)-Loaded pH-responsive niosome Coated with PEG against different cell lines. Materials Today Communications, 26, 101751. https://doi. org/10.1016/j.mtcomm.2020.101751
- Upaganlawar, A., Gandhi, C., & Balaraman, R. (2009). Effect of green tea and vitamin E combination in isoproterenol induced myocardial infarction in rats. Plant foods for human nutrition, 64(1), 7580-.

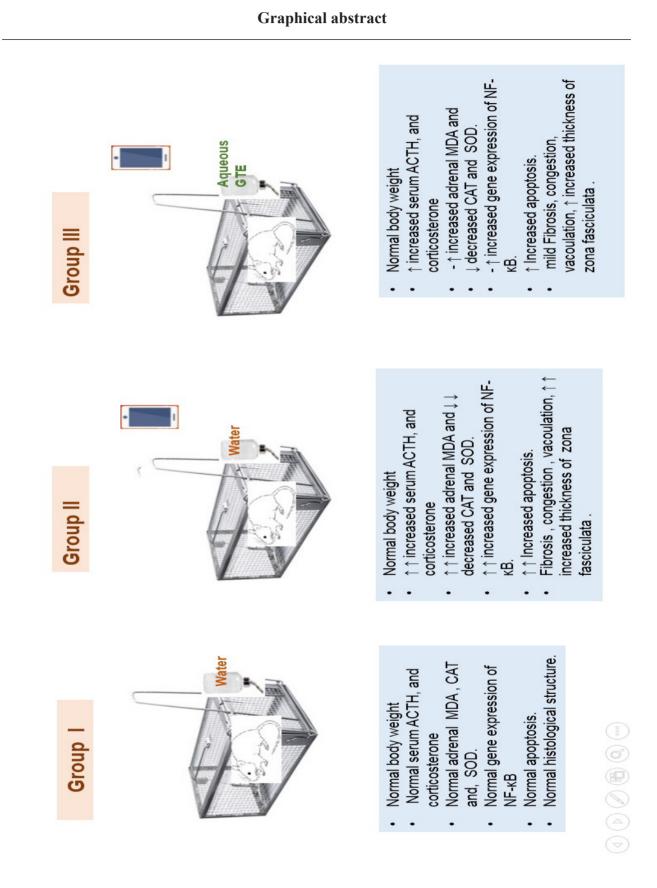
- Naganuma, T., Kuriyama, S., Kakizaki, M., et al., (2009). Green tea consumption and hematologic malignancies in Japan: the Ohsaki study. American journal of epidemiology, 170(6), 730738-. https:// doi.org/10.1093/aje/kwp187
- El-Beshbishy HA (2005) Hepatoprotective effect of green tea (Camellia sinensis) extract against tamoxifen-induced liver injury in rats. J Biochem Mol Biol 38: 563–570. https://doi.org/10.5483/ BMBRep.2005.38.5.563
- Odukoya, O., Sofidiya, M., Ilori, O., *et al.*, (1994). Malondialdehyde determination as index of lipid peroxidation. Int. J. Biol. Chem, 3, 281.285-
- 17. Weydert, C. J., & Cullen, J. J. (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nature protocols, 5(1), 51.66-
- Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. Nucleic acids research, 29(9), e45-e45. https://doi. org/10.1093/nar/29.9.e45
- Suvarna, K. S., Layton, C., & Bancroft, J. D. (Eds.). (2019). Bancroft's theory and practice of histological techniques E-Book. Elsevier Health Sciences. p.126175-.
- Ramos-Vara, J. A., Kiupel, M., Baszler, T., et al., (2008). Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. Journal of Veterinary Diagnostic Investigation, 20(4), 393413-. https:// doi.org/10.11772%F104063870802000401
- Jensen, E.C. (2013): Quantitative Analysis of Histological Staining and Fluorescence Using Image J. Anat Rec., 296: 378–381. https://doi. org/10.1002/ar.22641
- 22. Field, A. (2013). Discovering statistics using IBM SPSS statistics. Sage, New Delhi. p. 395420-.
- Adebayo, E. A., Adeeyo, A. O., Ayandele, A. A., et al. (2014). Effect of radiofrequency radiation from telecommunication base stations on microbial diversity and antibiotic resistance. Journal of Applied Sciences and Environmental Management, 18(4), 669674-. https://doi.org/10.4314/jasem. v18i4.16
- 24. Lee, J. S., Ahn, S. S., Jung, K. C., *et al.* (2004). Effects of 60 Hz electromagnetic field exposure on testicular germ cell apoptosis in mice. Asian Journal of Andrology, 6(1), 2934-.

- Shahabi, S., Taji, I. H., Hoseinnezhaddarzi, M., *et al.*, (2018). Exposure to cell phone radiofrequency changes corticotrophin hormone levels and histology of the brain and adrenal glands in male Wistar rat. Iranian journal of basic medical sciences, 21(12), 1269–1274,https://dx.doi.org/10.2 20382%Fijbms.2018.29567.7133
- 26. Guney, M., Ozguner, F., Oral, B., *et al.*, (2007). 900 MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C. Toxicology and industrial health, 23(7), 411420-. https://doi. org/10.11772%F0748233707080906
- 27. Balci, M., Devrim, E., & Durak, I. (2007). Effects of mobile phones on oxidant/antioxidant balance in cornea and lens of rats. Current eye research, 32(1), 2125-. https://doi.org/10.108002713680601114948/
- 28. Hanafy, L. K., Karam, S. H., & Saleh, A. (2010). The adverse effects of mobile phone radiation on some visceral organs. Research Journal of Medicine and Medical Sciences, 5(1), 9599-.
- 29. Lee, J. K., Choi, S. S., Won, J. S., & Suh, H. W. (2003). The regulation of inducible nitric oxide synthase gene expression induced by lipopolysaccharide and tumor necrosis factor-α in C6 cells: involvement of AP-1 and NFκB. Life sciences, 73(5), 595609-. https://doi.org/10.1016/S00245-00317(03)3205-
- Szilágyi, Z., Németh, Z., Bakos, J., *et al.*, (2020). Evaluation of inflammation by cytokine production following combined exposure to ultraviolet and radiofrequency radiation of mobile phones on 3d reconstructed human skin in vitro. International Journal of Environmental Research and Public Health, 17(12), 4401. https://doi.org/10.3390/ ijerph17124401
- 31. Ayata, A., Mollaoglu, H., Yilmaz, H. R., *et al.* (2004). Oxidative stress deviated skin damage in an experimental mobile phone model can be prevented by melatonin. The Journal of dermatology, 31(11), 878883-. https://doi.org/10.1111/j.13468138.2004-. tb00620.x
- 32. Oral, B., Guney, M., Ozguner, F., *et al.* (2006). Endometrial apoptosis induced by a 900-MHz mobile phone: preventive effects of vitamins E and C. Advances in therapy, 23(6), 957973-.
- Baladia, E., Basulto, J., Manera, M., *et al.* (2014). Effect of green tea or green tea extract consumption on body weight and body composition; systematic review and meta-analysis. Nutricion hospitalaria, 29(3), 479490-. https://doi.org/10.3305/ nh.2014.29.3.7118

- 34. Li, Y., Rahman, S. U., Huang, Y., *et al.* (2020). Green tea polyphenols decrease weight gain, ameliorate alteration of gut microbiota, and mitigate intestinal inflammation in canines with high-fatdiet-induced obesity. The Journal of nutritional biochemistry, 78, 108324. https://doi.org/10.1016/j. jnutbio.2019.108324
- 35. Zhu, W. L., Shi, H. S., Wei, Y. M., *et al.* (2012). Green tea polyphenols produce antidepressant-like effects in adult mice. Pharmacological research, 65(1), 74-80. https://doi.org/10.1016/j.phrs.2011.09.007
- 36. Skrzydlewska, E., Ostrowska, J., Farbiszewski, R., *et al.* (2002). Protective effect of green tea against lipid peroxidation in the rat liver, blood serum and the brain. Phytomedicine, 9(3), 232.238-https://doi.org/10.107800119-7113-0944/
- Cabrera, C., Artacho, R., & Giménez, R. (2006). Beneficial effects of green tea—a review. Journal of the American College of Nutrition, 25(2), 7999-. https://doi.org/10.108007315724.2006.10719518/
- Heikal, T. M., Mossa, A. T. H., Marei, G. I. K., *et al.* (2012). Cyromazine and chlorpyrifos induced renal toxicity in rats: the ameliorating effects of green tea extract. J Environ Anal Toxicol, 2(146), 2161.
- Nichols, J. A., & Katiyar, S. K. (2010). Skin photoprotection by natural polyphenols: antiinflammatory, antioxidant and DNA repair mechanisms. Archives of dermatological research, 302(2), 7183-. https://dx.doi.

org/10.10072%Fs004033-1001-009-

- Park, H. J., Lee, J. Y., Chung, M. Y., *et al.*, (2012). Green tea extract suppresses NFκB activation and inflammatory responses in diet-induced obese rats with nonalcoholic steatohepatitis. The Journal of nutrition, 142(1), 5763-. https://doi.org/10.3945/ jn.111.148544
- Elhalwagy, M. E., Darwish, N. S., & Zaher, E. M. (2008). Prophylactic effect of green tea polyphenols against liver and kidney injury induced by fenitrothion insecticide. Pesticide Biochemistry and Physiology, 91(2), 8189-. https://doi.org/10.1016/j. pestbp.2008.01.006
- 42. Kim, H. K., Yang, T. H., & Cho, H. Y. (2009). Antifibrotic effects of green tea on in vitro and in vivo models of liver fibrosis. World journal of gastroenterology: WJG, 15(41), 5200. https:// dx.doi.org/10.37482%Fwjg.15.5200
- 43. Tsai, C. F., Hsu, Y. W., Ting, H. C., *et al.* (2013). The in vivo antioxidant and antifibrotic properties of green tea (Camellia sinensis, Theaceae). Food chemistry, 136(31344-1337, (4-. https://doi. org/10.1016/j.foodchem.2012.09.063.\
- 44. Ogaly, H. A., Khalaf, A. A., Ibrahim, M. A., *et al.* (2015). Influence of green tea extract on oxidative damage and apoptosis induced by deltamethrin in rat brain. Neurotoxicology and teratology, 50, 23-31. https://doi.org/10.1016/j.ntt.2015.05.005



الملخص العربى

دراسة هستولوجية وهستوكيميائية مناعية عن الدور الوقائي لمستخلص الشاي الأخضر على تأثير إشعاع الهاتف المحمول 900 ميجاهرتز على قشرة الغدة الكظرية لجرذ الذكر البالغ الأبيض

هناء سعيد السيد موسى قسم الهستولوجيا الطبية وبيولوجيا الخلية للطب – جامعة الزقازيق

**الخلفية:** الإشعاع الناتج من أجهزة الهاتف المحمول هو نوع من الموجات الكهر ومغناطيسية التي ثبت تأثير ها الضار على العديد من الأعضاء. تعتبر قشرة الغدة الكظرية من الغدد الهامة في جسم الإنسان، لذلك قمنا في هذاالبحث بدر اسة تأثير إشعاع الهاتف المحمول على قشرة الغدة الكظرية والدور الوقائي المحتمل لمستخلص الشاي الأخضر.

**المواد والطرق:** تم استخدام سبعة و عشرين ذكور جرذان ويستار البيضاء في الدراسة. تم تقسيمهم إلى ثلاث مجمو عات؛ المجموعة الأولى (المجموعة الضابطة)، المجموعة الثانية (مجموعة الهاتف المحمول)، والمجموعة الثالثة (مجموعة الهاتف المحمول مع مستخلص الشاي الأخضر). تم قياس ACTH والكور تيكوستيرون في السيروم ومستوى المالوندهايد والكاتلاز وسوبر أوكسيد ميوتاز في الأنسجة. تم تقييم التعبير الجيني لـ NF-k B. استخدمت صبغة الهيماتوكسلين

والإيوسين والماسون ثلاثية الألوان وصبغة الكاسباز 3. تم إجراء التحليلات المور فومترية والإحصائية للنتائج. النتائج: زاد مستوى المالوندهايد في أنسجة الغدة الكظرية وانخفضت الكاتلاز وسوبر أوكسيد ميوتاز في المجموعة الثانية، بينما قلل مستخلص الشاي الأخضر في المجموعة الثالثة من المالوندهايد بشكل كبير وزاد من أنشطة الكاتلاز وسوبر أوكسيد ميوتاز. زاد NF-k B بدرجة كبيرة في المجموعة الثانية وبدرجة متوسطة في المجموعة الثالثة. أظهرت المقاطع المصبوغة بالهيموكسيلين والإيوسين تغييرًا في البنية النسيجية واحتقانًا في الأوعية الدموية وزيادة في الفجوات في الخلايا. أظهرت مقاطع الماسون ثلاثية الألوان زيادة كبيرة في ألياف الكولاجين في المجموعة الثانية ومناطق قليلة من الاحتقان الأوعية الدموية والفجوات في الخلايا، كما وجد زيادة متوسطة في المجموعة الثانية النسيجية ومناطق قليلة من الاحتقان الأوعية الدموية والفجوات في الخلايا، كما وجد زيادة متوسطة في ألياف الكولاجين وتفاعل

**الخلاصة:** تسبب إشعاع الهاتف المحمول في آثار ضارة على وظيفة وتركيب قشرة الغدة الكظرية، كما أدى تناول الشاي الأخضر إلى تقليل هذا التأثير بشكل كبير.