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Influence of *Withania somnifera*(L) Dunal Root Extract on Rat Kidney Exposed to Electromagnetic Waves from Mobile Phone: A Histopathological and Antioxidant Analysis

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ABSTRACT

This study is about to discover the consequences of 4G mobile phone emission on MDA, anti-oxidants, antioxidant marker, and histo-pathological variations in the kidney of rats.144 male rats were separated into four groups. The Group I is considered as the control group, Group II was subjected to mobile radiation 2400 MHz frequency range for 3 hours/day for the period of 6 months, Group III was as same as Group II but treated with 250 mg/kg aqueous extract of *Withania somnifera* root (Aq-*Wsr*) orally for 6 months. Group IV was given 250 mg/kg Aq-*Wsr* alone for 6 months. The animals were euthanized and the kidneys cells have been evaluated for antioxidant and histo-pathological parameters. Results of the study demonstrated that the LPO were significantly raised ($p < 0.05$) while SOD, CAT, GSH and GPx were significantly lowered ($p < 0.05$) in the EMR exposed group when compared to the Group I and Group III. In histo-pathological observation, intertubular congestion, haemorrhage, necrotic changes in cortex and medulla, cast formation within the proximal and distal tubules have elevated significantly inside the EMR group compared to the control group ($p <.05$). Contrarily, the total number of glomeruli in the cortex of EMR group of subjects decreased compared to the Control group ($p< 0.05$). The defensive effects of Aq-*Wsr* were witnessed within the kidney of Group III rats. From the study, it is very significant to raise open awareness of promising harmful consequences of cell phone radiofrequency EMR exposure.

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INTRODUCTION

Cellphone applications have radically enhanced more than a decade. Scientists apprised that electromagnetic fields (EMF) launched to the surroundings from our mobile phone has lethal consequences on the human population (Azab, 2017). Presently cell phones have been employing 4th technology (4G) Wi-Fi communication technology (2400 Megahertz (MHz)) that provide extremely substantial internet speed. The harmful reactions of EMF really pose an international community concern at the moment. Quite a few harmful consequences of mobile phone custom have been accounted on diverse systems body parts such as the brain (Hard-

ell, 2011), ear (Colletti *et al.*, 2011) and reproductive organs (Falzone *et al.*, 2011). All kinds of research have established that this electromagnetic frequency from a mobile phone may instigate many injurious outcomes at the cellular and molecular levels, such as DNA damage, tomour, lipid peroxidation, increased free radicals, oxidative stress and chromosomal problems (Chauhan *et al.*, 2016).

EMF has several biochemical effects, like the deprivation of big biomolecules and ionic metals stability disparity. EMF coverage is assigned to higher reactive oxygen species (ROS) production (Kivrak *et al.*, 2017). These ROS can begin damage to elements of the cell, such as DNA, protein, and lipids. Free radical production may occur in several techniques, including immune reply, radioactivity, UV light, stress, physiological redox, and tobacco cigarettes (Okano, 2008). It was demonstrated that electromagnetic radiation affects biotics by ROS production. ROS directs to various biological factors, for example, DNA damage (Moustafa *et al.*, 2001). Exposure to cellphone radiation leads to oxidative stress (OS) that has become improved in all organs of the human. The Kidney might absorb EMF from 2400 MHz mobile phones as, most of the time, they may be taken through the waist belt (Oktem *et al.*, 2005). Recent issues on cellphone contact are primarily dedicated to the renal system. As the cellphone is often held in a trouser pocket which is very adjacent to the Kidney and reproductive organ. The 2400 MHz 4G radio frequency electromagnetic radiation (RF-EMR) has been showing to male rats for a prolonged time in this particular experiment.

The coverage of wireless EMR may harm the spleenic, hepatic, renal tissue. Hasan and Islam claimed that mononuclear cells accumulate seen around the hepatic artery and bile duct with blockage inside the central vein and portal vein from the hepatocytes after everyday contact of 4G linked 2400 MHz cellphone emission to rats entire body for 30 days (Hasan and Islam, 2020). With EMF contact time, the quantity of destruction elevated. The coverage of damage enhanced together with the length of EMF coverage (Lee *et al.*, 2010).

Most of the earlier scientific studies done with 2G, or 3G wireless mobile phones emission contact revealed numerous biological modifications. Besides this, cell phone energy emission might be received more by pelvis parts of the body, especially the Kidney, than the reproductive organs. Therefore, these studies focused on assessing the impact of a 4G cell phone emission contact on oxidative stress and possible histopathological effects on the Kidney of the Wistar Albino rat model and the protective effect of the aqueous extract of *Withania somnifera* against renal damage due to electromagnetic radiation from a cell phone in rats.

MATERIALS AND METHODS

Collection of plants

The root of *Withania somnifera* was gathered from Vishnu Ayurveda College, Shornur, Palakkad, Kerala, identified and authenticated in the Centre for Medicinal Plant Research Arya Vaidyasala, Kottackal, Malappuram District, Kerala.

Aqueous Extract preparation

After appropriate identification and authentication, parts of the gathered plant were cleaned, shade dried, and coarsely powdered. 200g of powder in 1200 ml of water was boiled. The substances were reduced to 1/3, and the extract was allowed to get dry. The paste form of the extract attained was stored in an airtight container at 4ºC.

Experimental Design

The subjects of this research were 144, weighing 150-180 g of Wistar Albino Rats. These rats were placed in an air-conditioned room (20-25°C) and subjected to a 12/12 h daylight/darkness cycle with free access to food and water. All the procedures was achieved in agreement with the Institutional Animal Ethical Committee as per the instructions of the CPCSEA.

The rats have split up into four groups; 36 rats each Group. Group I (the control) received just a standard diet regime while Group II contacted cell phone rays 2400 MHz fields for 3h/day throughout the experimental period (6 months). Group III was exposed to 2400 MHz fields for 3h/working day through the experimental period and compounded every day and for 180 days with 250 mg/kg aqueous extract of *Withania somnifera* (Aq-*Wsr*). Group IV received a standard diet along with 250 mg/kg Aq-*Wsr* throughout during the experimental period. All animals from the control and experimental groupings were located collectively in four different polycarbonate cages of 30 × 40 × 40 cm (W × L × H) with 9 rats in each cage for each Group. The experimental animals were continuously in contact with EMR through the cellphone. The radio frequency waves as constructed using a cell phone kept inside the cages in auto-answer mode. A 2400 MHz EMR near-area indicate for the GSM process was utilized. The cellular phone was put into the cage middle, even though the extended distance involving the cellphone from the base of the cage was 3 cm along with the maximal distance from the cage corners was 28.2 cm.

Antioxidants and oxidative stress

Lipid peroxidation (MDA) was estimated as thiobarbituric acid reactive substance (TBARS) according to the method of Ohkawa *et al.* (1979). The concentration of reduced glutathione (GSH) was determined using the procedure done by Moron *et al.* (1979) and glutathione peroxidase (GPx) were determined using the procedure done by Rotruck *et al.* (1973). Catalase (CAT) activity was evaluated according to the method described by (Maehly and Chance, 1954). Meanwhile, the activities of superoxide dismutase(SOD) was determined using the procedure done by Misra and Fridovich (Misra and Fridovich, 1972).

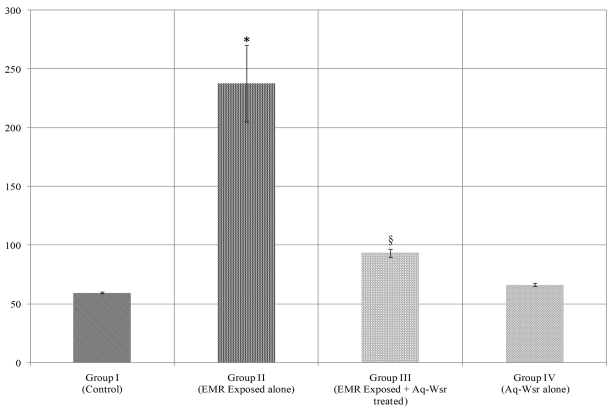

Figure 1: Effect of Aq-*Wsr on* LPO in EMR exposed Wistar Albino rats

From Figure 1 the mean kidney volumes in all groups. (*) and (§) indicate differences between the Group I & Group II and Group II & Group III groups respectively at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM). The LPO is expressed in nmol MDA mg^{-1} protein.

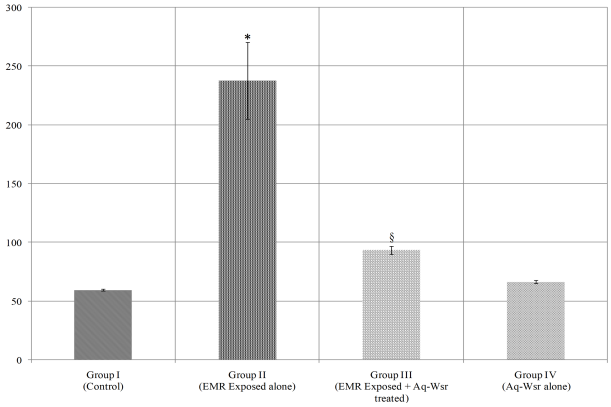

Figure 2: Effect of Aq-*Wsr on* GSH in EMR exposed Wistar Albino rats

From Figure 2 the mean kidney volumes in all groups. (*) and (§) indicate differences between the Group I & Group II and Group II & Group III groups respectively at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM). The GSH is expressed in U mg^{-1} protein.

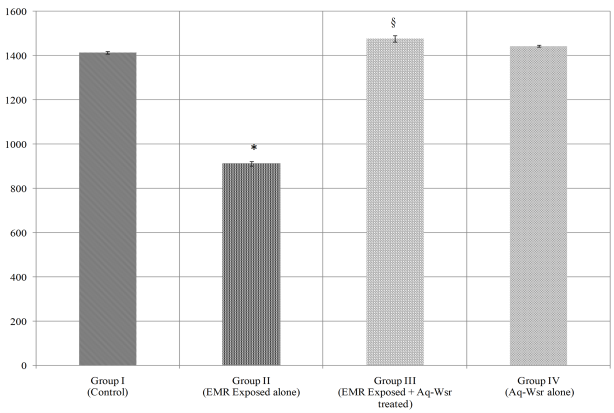

Figure 3: Effect of Aq-*Wsr on* GPx in EMR exposed Wistar Albino rats

From Figure 3 the mean kidney volumes in all groups. (*) and (§) indicate differences between the Group I & Group II and Group II & Group III groups respectively at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM). The GPx is expressed in mg g^{-1} (wet tissue).

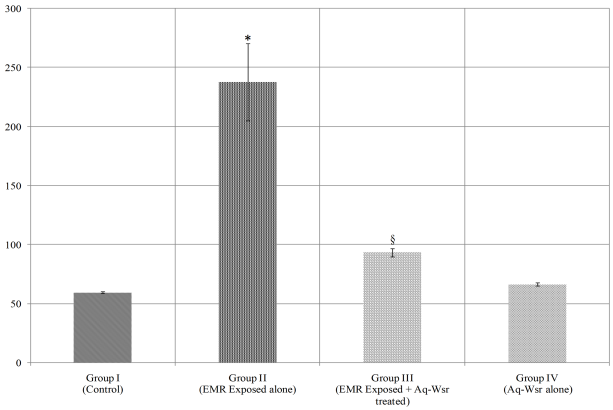

Figure 4: Effect of Aq-*Wsr on* SOD in EMR exposed Wistar Albino rats

From Figure 4 the mean kidney volumes in all groups. (*) and (§) indicate differences between the Group I & Group II and Group II & Group III groups respectively at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM). The SOD is expressed in U mg^{-1} protein.

Histopathological studies

Preparing of paraffin portions

For histological preparations, animals were sacrificed and both kidneys were dissected out. It had been washed with normal saline and directly resolved in 10% neutral buffered formalin for 24 h. After fixation, kidney cells had been dehydrated in

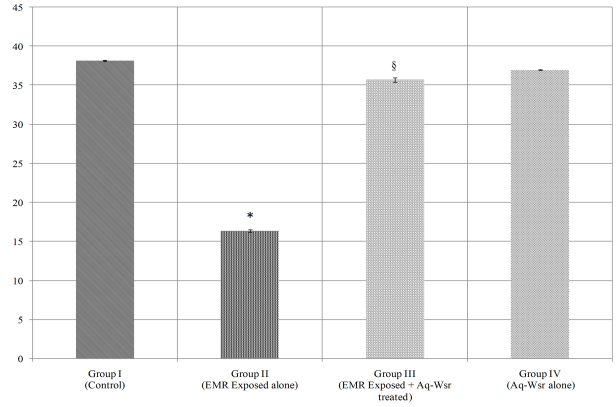

Figure 5: Effect of Aq-*Wsr on* CAT in EMR exposed Wistar Albino rats

ascending series of ethyl alcoholic beverages 70%, 80%, 90%, and 96% for 1 hour each and then immerse in absolute ethyl alcohol for 3 hours. Muscle tissues were removed by immersion in 3 alterations of xylol for 20 minutes, then impregnated in paraffin wax at 60°C for two hours and embedded in the wax tart. Histological sections 4 μm dense were organised using the microtome and stained with hematoxylin and eosin (Harris *et al.*, 1987).

From Figure 5 the mean kidney volumes in all groups. (*) and (§) indicate differences between the Group I & Group II and Group II & Group III groups respectively at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM). The CAT is expressed in U mg^{-1} protein.

Statistical analysis

Data were processed through an available statistics software package (SPSS® for Windows, v. 9.0, Chicago, USA). A p-value <0.05 was considered to be statistically significant. Data were expressed as mean ± standard error (mean ± SE).

RESULTS

LPO level of kidney tissues was significantly increased in EMR exposed Group compared to the control group and group IV ($p<0.05$). The most dramatic increase in the MDA level was observed in group II Kidney tissue. No significant difference was observed in tissue MDA levels between the control and Group III group (Figure 1). In the control rats, the mean value of LPO was 59.45±0.77 nmol MDA mg^{-1}protein. The values of LPO was increased significantly (237.63±32.69 7 nmol MDA mg^{-1}protein) ($p < 0.05$) in 3 hours per day EMR exposed rats compared to the control rats. Aq-*Wsr* treated groups (Group III) attenuated oxidative stress demonstrated by the reduction (93.4±3.39 nmol MDA mg^{-1}protein) in the levels of lipid hydroperoxide in Group III. Lipid hydroperoxide level was significantly ($p<0.05$) diminished compared to EMR exposed ones. These trends confirm that EMR exposed kidney lipid peroxide levels were normalised by treatment with Aq-*Wsr.*

GSH levels were significantly lower in EMR exposed Group than control and Group III ($p<0.05$). No significant difference was observed in tissue GSH levels between control and Group III in kidney tissue (Figure 2). Group II (EMR exposed group) showed significant ($p<0.05$) lowered levels of glutathione (343.75±10.96 U mg^{-1} protein) compared with normal control rats (867.47±16.21 U mg^{-1} protein); whereas GroupIII (EMR exposed with Aq-*Wsr* treated (250 mg/kg bw) and Group VI (Aq-*Wsr* treated without EMR) didn't show any significant difference over normal control (Figure 2). Kidney GPx level was significantly lower in EMR exposed Group than control and group IV ($p<0.05$). No statistically significant difference was seen between control and Group III in GPx levels in kidney tissue ($p<0.05$) (Figure 3). The decreased activity of GPx noticed in Group II (912.26±10.41mg g^{-1} wet tissue) is the result of EMR exposure alone when compared with control groups (1414.98±5.82 mg g^{-1} wet tissue), EMR+Aq-*Wsr* treated (1477.18±15.03 mg g^{-1} wet tissue), Aq-*Wsr* treated alone (1443.42±5.26 mg g^{-1} wet tissue) groups (Figure 3).

Kidney SOD activities were significantly lower in EMR exposed Group than control and Group IV ($p<0.05$). There was no statistically significant difference in SOD levels between control and Group III in kidney tissue (Figure 4). The SOD was found to be decreased significantly ($p<0.05$) in EMR exposed group II (2.62±0.22 Umg^{-1} protein) animals in comparison to the Group I (4.48±0.06 Umg^{-1} protein) and group III (3.93±0.26 Umg^{-1} protein) animals. The administration of Aq-*Wsr* in EMR exposed animals resulted in a notable recovery in the above-mentioned SOD towards the control level. Aq-*Wsr* treatment (5.13±0.09 Umg^{-1} protein) in the Group IV animals did not affect SOD activities in comparison to the control animals. Kidney CAT activities were significantly lower in EMR exposed Group than control and Group III groups ($p<0.05$). There was no statistically significant difference between Group I (control) and Group III. The data in (Figure 5) indicated that EMR exposed Group II showed a significant decrease in CAT activities from 38.18±0.08 (control) to 16.36±0.17 Umg^{-1} protein (EMR exposed). However, administration of Aq-*Wsr* 250 mg/kg bw in EMR exposed led to an increase of CAT activities to 35.72±0.29 Umg^{-1} protein. The extracts in Group IV animals did not show

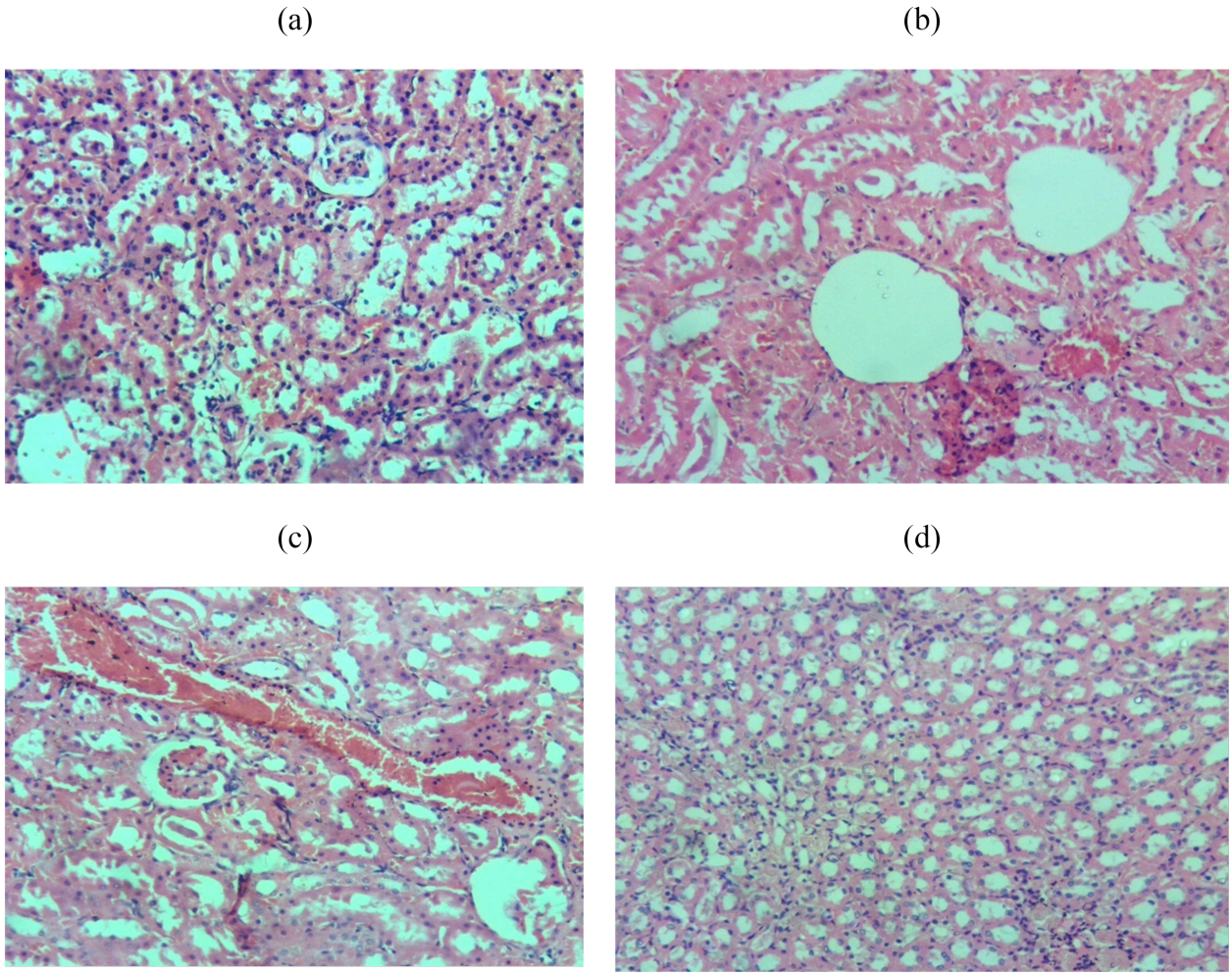

Figure 6: Histological observation of the Kidney of control (a), EMR exposed (b), EMR exposed and treated with Aq-Wsr (c) and Aq-Wsr alone treated (d)

any such significant alteration (36.99±0.06 Umg^{-1} protein) in CAT activities (Figure 5).

Figure 6(a) shows the 4^{th} month control group with normal kidney tubules (H&E x100); Figure 6(b) shows the 4^{th} month EMR exposed group with marked glomerular atrophy and tubular degeneration (H&E x100); Figure 6(c) shows the 4^{th} month EMR + Aq-*Ws*r treated group with occasional congestion and haemmorhage (H&E x100); Figure 6(d) shows the 4^{th} month Aq-*Ws*r alone treated group without EMR with normal kidney tubules and glomerular architecture (H&E x100).

All hematoxylin and eosin-stained renal specimens were analysed histopathologically using a light microscope. The kidneys of the experimental rats in Group I (Figure 6a) and Group IV group (Figure 6d) showed regular kidney tubules and glomerular architecture. Moreover, increased crumbling was noted, such as glomerulosclerosis, tubular defects, and hydrophic deterioration of tubule cells in Group II (Figure 6b). The administration of Aq-*Wsr* condensed these deterioration outcomes in Group III (Figure 6c).

DISCUSSION

The destructive consequences of cell phone energy emission are explicated in an important organ like the Kidney in our body. Mobile phone consumers in several nations and continents are contacted by various frequencies of electromagnetic radio waves. EMF emission based on the frequency of the cell phone (Meo *et al.*, 2011). Numerous histological and biological experiments have been executed to analyze the destructive outcomes of EMR on people health relating to the CNS, growth, the renal system, fertility, tumor development, and immune function. The male wistar rats were contacted to 2400 MHz EMR in this research because 2400 MHz frequency 4G connected cellphones are considerably applied in India as well as many other parts of the globe. Exposure to EMR can injure biotic cells by stimulating cell plasma membrane alterations, which could be

described in the phrase of thermal or non-thermal mechanisms. Thermal effects can take place with the alteration and assimilation of temperature by the human body's electromagnetic energy. Amplified body heat is stabilized and alleviated by blood movement (Kesari *et al.*, 2014). Even though non-thermal outcomes do not increase the body heat adequately to harm the structure of tissues, their effect is intervened by the production of reactive oxygen species (ROS). ROS are engaged in numerous cellular events and can be indispensable or enormously toxic to cellular homeostasis. Their cytotoxic effects developed from the peroxidation of membrane phospholipids. This generates alteration in the trans-membrane potential, thereby affecting the conductivity of the membrane and loss of membrane solidarity (Sepehrimanesh *et al.*, 2014). Changes in antioxidant levels show weakening cellular homeostasis leading to stress and reduced performing ability (Gecit *et al.*, 2014). This facilitates in estimating and identifying the effects of EMR stimulated hazardous changes to human beings. In the present study, we have assessed a number of oxidative stress markers such as MDA, antioxidant markers such as GSH, in addition to antioxidant enzyme activities of SOD, CAT and GPx in rat kidneys after contacted by EMR (2400 MHz, 3 hours/day during 180 consecutive days).

In this study, it was found that long-term chronic exposure to 2400 MHz EMR can trigger lipid peroxidation and antioxidant repression in renal tissues. MDA ranges of Kidney increased significantly in EMR Group compared to control, EMR exposed with Aq-*Wsr* treated and Aq-*Wsr* alone treated groups. However, GPx, SOD and CAT activities and GSH levels exhibited a significant decrease in renal tissues of the EMR exposed Group. The outcome of our study established the hypothesis that EMR exposure might instigate oxidative injury in renal tissue. The alterations in MDA and GSH levels and GPx, SOD and CAT activities in exposed rats reflected pathophysiological effects of the electromagnetic field in kidney tissue. These indicate that EMR may perform as a stress or on kidney tissue as well due to its receptive ultra structure leads to oxidative stress. Ozguner *et al*., the study reported the raised MDA levels on renal cells of exposure to EMR in rats. MDA levels increased significantly in kidney and bladder tissue of rats exposed to the effect of a 900 MHz RF-EMR compared to the control group (Ozguner *et al.*, 2005). Ragy noted an amplified MDA level and a depleted total antioxidant capacity in kidney tissue of adult rats (Ragy, 2015). On the other hand, exposure to 900 MHz RF-EMR remarkably lowered GPx, SOD and CAT activities and GSH level in rat renal cells evaluated to control groups (Ragy, 2015). We assume that the renal cells have comparatively weak enzymatic antioxidant resistance systems proficient in making high ranges of ROS via blood perfusion and high anaerobic metabolism and as a result, the changes in lipid peroxidation is remarkable. Ozturk *et al.* Stated a decline in GPx, SOD and CAT activity and a decrease in GSH activity in rat kidney tissue exposed to 900 MHz RF-EMR (Ozturk *et al.*, 2003). Raised SOD activity may be a comeback intended to equilibrium or restrain high chain oxidation of GSH or decreased GSH level. Özorak et al. inspected the consequences of EMR on oxidative stress in the newly born rats' renal tissues (Ozturk *et al.*, 2003). It has been noted that cell phone and wifi provoked EMR may trigger oxidative renal damage in newborn rats (Ozturk *et al.*, 2003). Ozgur *et al*. reported that EMR exposure provokes lipid peroxidation, with the declined function of SOD, myeloperoxidase and glutathione peroxidase(GSH-Px) in the Kidney of guinea pig (Ozgur *et al.*, 2010). Currently, the main result of antioxidants on man's health transpires through their radical scavenging mechanism. Growing records of research studies are converging on the injurious consequences of EMR and on the use of antioxidants to minimize these (Ozgur *et al.*, 2010).

The histopathological changes induced by EMR released from a cell phone have been investigated in this study by investigating pathological injuries in the Kidney. Histopathological analysis in the present experiment has shown that the kidneys in the rats in the Control group showed a standard anatomical mammalian renal histology. Images from our EMR exposed groups demonstrated improved damage such as tubular defects, cellular cast formation, glomerulosclerosis, and hydropic degeneration of tubule cells. The administration of Aq-Wsr again decreased these degenerative outcomes of EMR. From this standpoint, these histopathological alterations in the tubules might be recognized as the decline in glomerular blood flow and filtering system and the restriction in peritubular capillary wall space in interstitial connective tissue due to oxygen radicals within the renal cells via the relief of biologically active fat molecules. This restriction can lead to inadequate nutrition and oxygenation of the proximal and distal tubules and accordingly leads to deterioration (Khayyat, 2011). It can be determined that vacuolization caused by an increase in toxicity brought on by repeated EMR release in SER and begins with a rise in the occurrence of vacuoles in cell organelle. This organelle takes part in a cell detoxification, the regression there of along with its expression in the form of vac-

uolizations (Khayyat, 2011). Additionally, mononuclear infiltration can probably be related to the massing from the mononuclear and polymorphonuclear cells that happens with chemotaxis.

Within this study, No histological alterations in the renal tissues of the control group were observed. In histopathological observations, the EMR treated rats without drug administration showed greater glomerulosclerosis, tubular defects, necrotic changes and hydrophic deterioration of tubule cells in comparison to the EMR treated rats along with drug administration. Comparable findings have been produced by reporting that EMR emission triggers more atrophied glomeruli, infiltration of leukocytes among the tubules in the renal, and vacuolation of tubules (Forgács *et al.*, 2006). Chauhan *et al.* reported that microwave energy emission coverage brings about shrivelled glomeruli and abnormal renal tubules (Chauhan *et al.*, 2016). EMR and elevated-power waves initiate greater heat.

These waves interracted jointly and created free radicals that can makes elevated lipid peroxidation and display their harming outcomes on cellular material by ionizing rays. Free radicals injure the lipids in the cell and alter their arrangement and split proteins composition resulting in cell death. The oxidative stress encouraged by ROS is an important aspect of tissue damage due to contact with energy emission (Markov, 2013).

In the recent study, we demonstrated that long-term exposure to 2400 MHz RF EMR is able to induce oxidative stress. This stimulation was arbitrated by rising in lipid peroxidation and the decline in enzymatic and non-enzymatic antioxidants.

The study also presented proof that RF-EMR might harm the renal cell architecture and integrity. The outcomes of this study are marked that RF-EMR can trigger oxidative injuries in kidney tissues.

Oxidative stress is identified to cause numerous diseases such as renal failure, hypertension and various kinds of cancer. Our results recommended that using electromagnetic sources should be restricted to safeguard the human health and ecological system.

CONCLUSIONS

In conclusion, we have confirmed that Aq-*Ws*r has a defensive role against nephrotoxicity induced by EMR exposure. According to our free radical antioxidant and its markers evaluation, which were supported by histopathological analysis, administration of Aq-*Ws*r decreased the effects of EMR on rat kidney tissues, thus declining kidney damage.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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