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Influence of Withania Somnifera(L) Dunal Root Extract on Rat Liver Exposed to Electromagnetic Waves from Cell Phone: A Histopathological & Antioxidant Analysis

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Abstract

The radiofrequency electromagnetic radiation produced by cell phones on biological systems has large media protection and general public concern lately. The aim of this study would be to discover the consequences of 4th Generation cellular phone radiation visibility on MDA, anti-oxidants, antioxidant markers, and histopathological variations in the Liver of Wistar Albino Rats. A total of 144 male Wistar Albino Rats weighing 140-150 g were randomly separated into four groups. The group I is kept as the control group, Group II, was subjected to cellphone radiation of 2400 MHz for 3 hours/day for the experimental time period of 6 months, the group III was exposed to 2400 MHz fields for 3 hours/day during the experimental time and administered 250 mg/kg aqueous extract of *Withania Somnifera* root (Aq-*Wsr*) orally for 180 days. Group IV received 250 mg/kg Aq-*Wsr* during the experimental time. The electromagnetic radio frequency meter was placed within the cage to analyze the frequency of electromagnetic radiation emitted from mobile devices. The control group was held under similar circumstances, but the electromagnetic field was not given during the period. All of the rats had been sacrificed at the end of the experiment with 6 rats from each group each month. The liver has been accumulated for histopathological and anti-oxidant studies. Results of the study demonstrated that the LPO was significantly ($p < 0.05$) increased while SOD, CAT, GSH, and GPx were significantly ($p < 0.05$) decreased in the EMR exposed group when compared to the control and Group III. Histopathological observation of irradiated group showed progressive degeneration of hepatocyte during the study period, marked sinusoidal congestion, kupffer cell hyperplasia, hydropic degeneration, marked biliary hyperplasia, periportal inflammation, focal areas of fatty changes, cloudy swelling whereas Group III showed reduced degenerative changes and absence of necrosis in contrast to Group II.The defensive effects of Aq-*Wsr* were witnessed within the liver . It is determined that fourth-generation technology cell phone radiation exposure may induce oxidative stress and inflammation of rat's liver system. Based on these studies, it is very important to raise open public awareness of the potential adverse effects of cell phone radiofrequency electromagnetic radiation exposure.

Keywords: Electromagnetic Radiation (EMR), Withania somnifera, Oxidative Stress, Liver Damage.

Introduction

Over the past ten years, cell phone usage has significantly increased. Researchers have shown that the electromagnetic fields (EMF) that are released into the environment by our cell phones are harmful to people [1]. Currently, cellphones are using 4th

generation (4G) Wi-Fi (2400 MHz) connectivity technology, which offers extremely fast internet speeds. The negative effects of EMF are currently a serious issue for the world community. The use of cell phones has been linked to numerous negative consequences on various body systems, including the reproductive organs, the ear, and the brain [2]. Numerous studies have shown that the electromagnetic frequencies emitted by cellular phones may have detrimental consequences on molecular and cellular levels, including DNA damage, several types of cancer, oxidative stress, lipid peroxidation, an increase in free radicals, and chromosomal issues [3].

EMF can cause ionic equilibrium imbalances and the breakdown of big cell molecules, among other biological effects. According to Song *et al*., (2018), increased reactive oxygen species (ROS) generation is correlated with EMF coverage [4]. These ROS have the potential to harm biological components like lipids, proteins, and DNA. Numerous factors, such as UV light, immunological response, radioactivity, stress, smoking, and physiological redox, can cause free radical production [5]. It has been established that electromagnetic radiation influences the growth or production of ROS, which in turn influences the effect on living things. DNA damage is just one biological effect that ROS causes [6]. Oxidative stress (OS) caused by cellphone radiation has improved in all human organs. Due to the fact that 2400 MHz cell phones are frequently carried through the belt, the liver may be susceptible to EMF from these devices [7]. The hepatic system has been the focus of recent cellphone exposure worries. Given that the Liver is located relatively close to the pocket where the cellphone is frequently stored. In this study, male rats were exposed to the 2400 MHz 4G radiofrequency electromagnetic radiation (RF-EMR) for an extended length of time. The hepatic tissues may be harmed by the wireless electromagnetic radiation exposure. The rats exposed to daily 4G-linked 2400 MHz cellphone radiation for 30 days developed mononuclear cellular aggregates surrounding the bile duct and hepatic artery, as well as blockages inside the portal vein and the central vein from the liver [8]. The amount of harm increased over the course of an EMF exposure. The longer the EMF coverage, the greater the potential damage [9].

Second generation (2G) or third generation (3G) wireless phone radiation exposure causes several physiological changes, according to a large body of prior research. In addition, compared to many other inner organs, the liver can absorb more cell phone radiation from the digestive organs. Therefore, the purpose of this study was to assess the effects of 4G cell phone radiation exposure on oxidative stress, potential liver histopathological effects, and the protective effects of *Withania somnifera* extract against hepatic damage caused by electromagnetic radiation from cell phones in rats.

Materials and Methods

Collection of plants

The root part of *Withania somnifera* was collected from the Vishnu Ayurveda College, Shornur, Palakkad, Kerala.

Aqueous Extract preparation

After appropriate identification and authentication, roots 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 evaporated to dryness. The paste form of the extract attained was stored in an airtight container at $4^{o}C^{20}$.

Experimental Design

The subjects of this research were 144, weighing 150-180 g of Wistar Albino Rats. These rats were kept 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 were achieved in agreement with the Institutional Animal Ethical Committee as per the instructions of the CPCSEA.

The rats were split up into four groups; 36 rats in each Group. the control (Group I) received just a standard diet regime while Group II was in contact with 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 of time and compounded every day and for 180 days with 250 mg/kg aqueous extract of *Withania somnifera* (Aq-*Wsr*). Group IV was given standard diet along with 250 mg/kgBW Aq-*Wsr*. All animals from the control and experimental groupings were

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actually located collectively in polycarbonate cages 30 × 40 × 40 cm (W × L × H). The experimental animals of Group II and III were continually in contact with EMR through their cellphone. The radiofrequency waves were constructed with a cell phone. 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 bottom of the cage was 4.5 cm with the maximal distance from the cage corners being 25.2 cm.

Anti-oxidants and oxidative stress

Lipid peroxidation (MDA) was estimated as a thiobarbituric acid reactive substance (TBARS) according to the method of Okhawa *et al*. [10]. The concentration of reduced glutathione (GSH) was determined using Moron *et al*. [11] and glutathione peroxidase (GPx) were determined using Rotruck *et al*. [12]. Catalase (CAT) activity was evaluated according to the method described by Maehly and Chance [13]. Meanwhile, the activities of superoxide dismutase (SOD) by Misra and Fridovich [14].

Histopathological studies

Preparing of paraffin sections: For histological preparations, animals were sacrificed, and Liver carefully dissected. It had been washed and instantly resolved in 10% neutral buffered formalin for 24h. After fixation, hepatic cells had been dehydrated in ascending series of ethyl alcoholic beverages 70%, 80%, 90%, and 96% for 30 minutes every, then by two total ethyl alcohol alterations for a fifty percent-hour or so each and every. Muscle tissues were actually removed by immersion in xylol for 10 minutes (two modifications), then impregnated in paraffin (three adjustments) at 60°C and embedded in wax tart. Histological segments 4 µm dense were prepared using the microtome and stained with haematoxylin and eosin [15].

Statistical analysis

Data were analyzed using a commercially available statistics software package (SPSS® for Windows, v. 9.0, Chicago, USA). For statistical analysis, One-way ANOVA followed by the Turkeys Multiple Comparison Test was performed. A p-value <0.05 was considered to be statistically significant. Data were expressed as mean ± standard error (mean ± SEM).

Results

LPO level of liver tissues was significantly higher in EMR exposed group in comparison with the control group and group IV ($p<0.05$). The most dramatic increase in the level of the tissues was observed in liver tissue. No significant difference was observed in terms of tissue MDA levels between the control and Group III groups in liver tissue in the 6th month (Figure 1).In the Group I rat, the mean value of LPO was 72.30±0.98nmol MDA·mg^{-1} protein. The values of LPO were increased significantly ($p < 0.05$) in 3 hours per day (99.34±7.31nmol MDA·mg^{-1} protein) EMR exposed rats compared to the control rats. Aq-*Wsr* treated groups (Group III) attenuated oxidative stress demonstrated by the reduction (70.22±1.73nmol MDA·mg^{-1} protein) in the levels of lipid hydroperoxide in the EMR exposed rats. Lipid hydroperoxide level was significantly ($p<0.05$) diminished compared to EMR exposed ones. These trends confirm that EMR-exposed liver lipid peroxide levels were normalized by treatment with Aq-*Wsr*.

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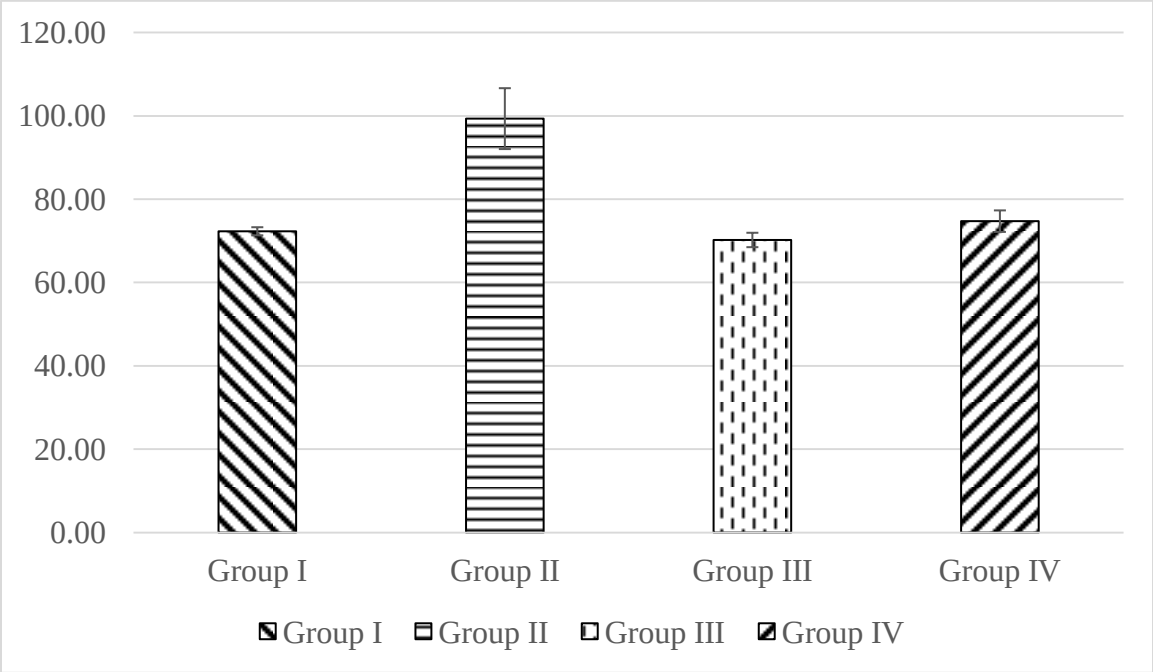
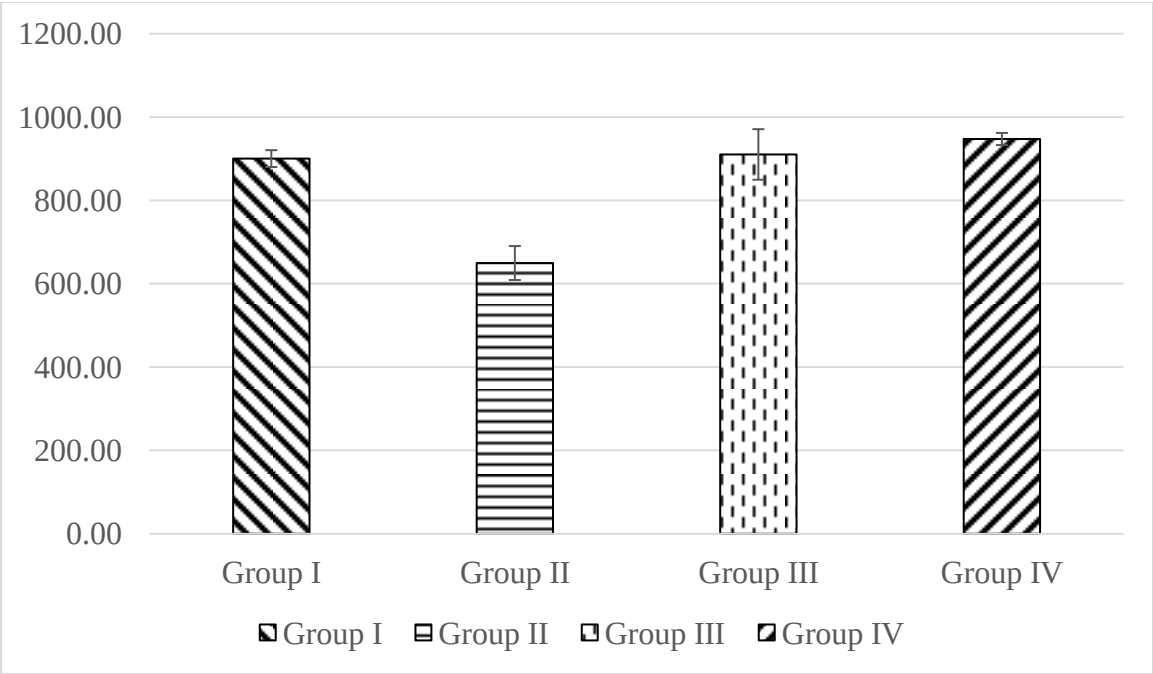
Figure 1: Effect of Aq-*Wsr* on LPO in EMR exposed Wistar Albino rats



Bar diagram showing the mean liver LPO 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 the standard error mean (SEM). The LPO is expressed in nmol MDAmg-1 protein.

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



Bar diagram showing the mean liver GSH 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 the standard error mean (SEM). The GSH is expressed in the U.mg-1 protein.

GSH levels were statistically significantly lower in EMR exposed group than in the control and group III ($p<0.05$). No significant difference was observed in terms of tissue GSH levels between control and Group III

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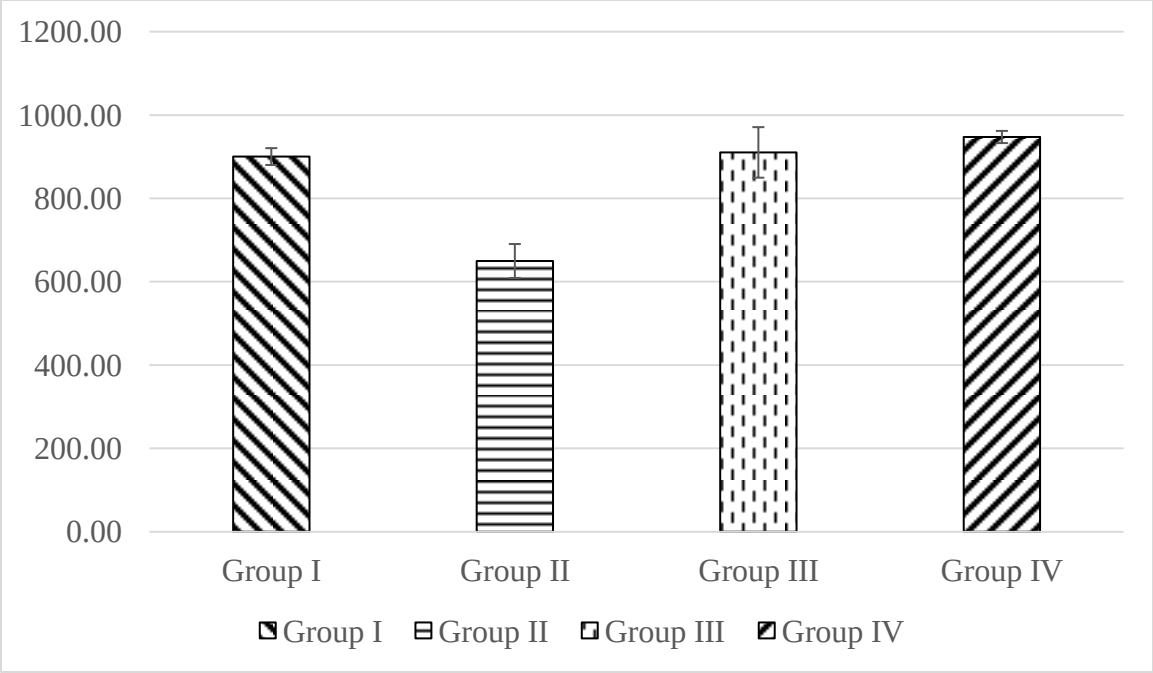
liver tissue (Figure 2). Group II (EMR exposed alone) showed significant ($p<0.05$) lowered levels of glutathione (649.68±40.76U.mg-1 protein) with normal control rats (900.33±20.22U.mg-1 protein); whereas, Group III (EMR exposed with Aq-Wsr treated (250mg/kg BW) and group IV (No EMR but Aq-Wsr treated) showed no significant differences over normal control (Figure 2). Liver GPx level was significantly lower in EMR exposed group than in the control and group II ($p<0.05$). No statistically significant difference was seen between control and Group IV in liver tissue ($p>0.05$) (Figure 3). The decreased activity of GPx noticed in Group II (877.65±94.71mg.g-1 wet tissue) may be the result of EMR exposed alone when compared to control groups (1154.79±7.22mg.g-1 wet tissue), EMR and Aq-Wsr treated (1189.33±18.16mg.g-1 wet tissue), Aq-Wsr treated alone (1244.5±8.11mg.g-1 wet tissue) groups (Figure 3).

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



Bar diagram showing the mean liver GPx 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 the standard error mean (SEM). The GPx is expressed in mg.g-1 (wet tissue).

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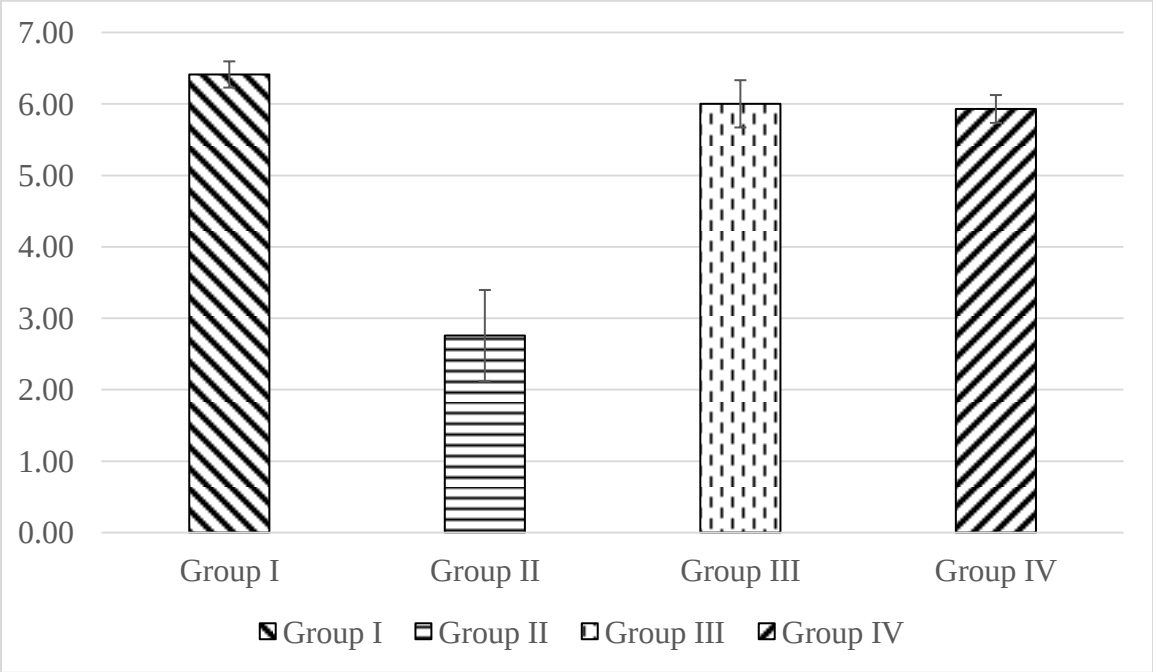
Figure 4: Effect of Aq-Wsr on SOD in EMR exposed Wistar Albino rats



Bar diagram showing the mean liver SOD 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 the standard error mean (SEM). The SOD is expressed in U·mg-1 protein.

Liver SOD activities were significantly lower in EMR exposed group than in the control and sham groups ($p<0.05$). There was no statistically significant difference between control and Group III in liver tissue (Figure 4). The SOD was found to be decreased significantly ($p<0.05$) in EMR-exposed group II (2.76±0.64Umg-1 protein) animals in comparison to the group I (6.41±0.18Umg-1 protein) and group III (6.00±0.33Umg-1 protein) animals. The administration of Aq-Wsr in EMR exposed animals resulted in a notable recovery in the above-mentioned SOD toward the control level. Aq-Wsr treatment (5.93±0.19Umg-1 protein) in the control animals did not affect SOD activities in comparison to the control animals. Liver 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 the control group and EMR exposed with Aq-Wsr treatment group in liver tissue. The data given in Figure 5 indicated that EMR-exposed group II, a significant decrease in CAT activities from control 37.2±0.91 to 27.19±2.56Umg-1 protein after EMR exposure. However, administration of Aq-Wsr 300 mg/kg BW in EMR exposed led to an increase of CAT activities to 37.23±0.86 Umg-1 protein. The extracts in Group IV animals did not show any such significant alteration (35.98±0.09Umg-1 protein) in CAT activities (Figure 5).

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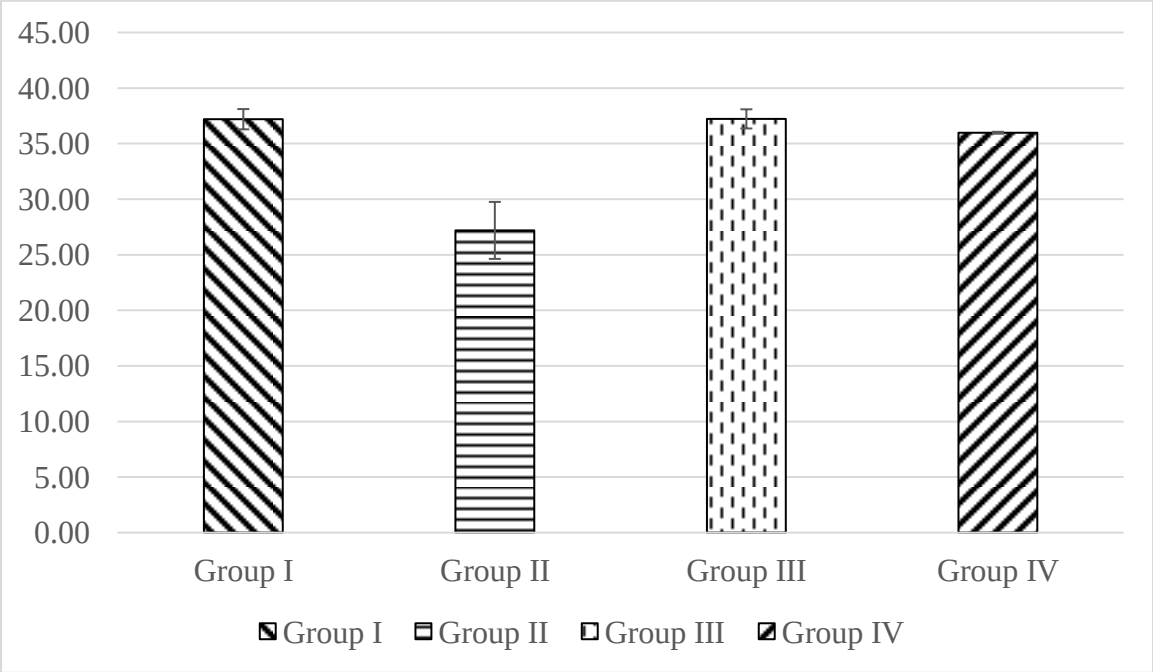
Figure 5: Effect of Aq-Wsr on CAT in EMR exposed Wistar Albino rats



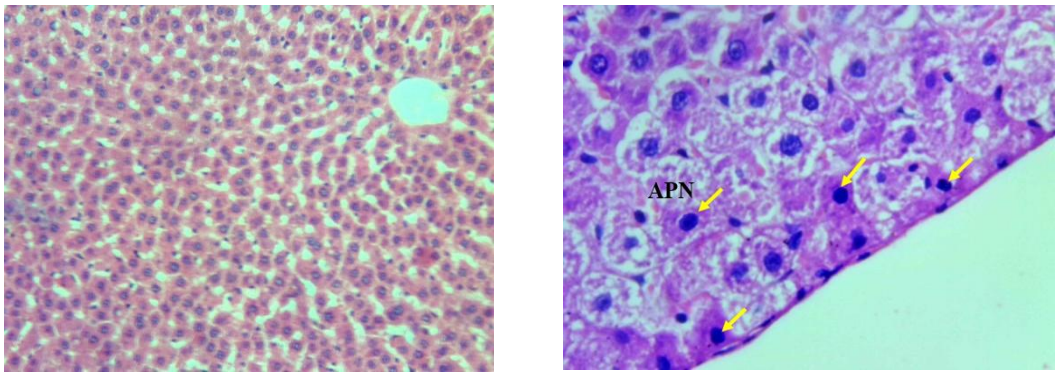
Bar diagram showing the mean liver CAT in all groups. (*) and (§) indicate differences between the Group I & Group II and Group II & Group III groups respectively at the 0.05 level ($p < 0.05$). The error bars indicate the standard error mean (SEM). The CAT is expressed in U·mg-1 protein.

Histo-pathlogical Observation:

All hematoxylin and eosin-stained hepatic specimens were analyzed histopathologically using a light microscope. The liver of the experimental rats in group I (Figure 6a) and Group IV group (Figure 6d) showed regular liver cell properties and architecture whereas the irradiated group showed progressive degeneration of hepatocyte during the study period, marked sinusoidal congestion,, apoptotic nuclei, kupffer cell hyperplasia, hydropic degeneration, biliary hyperplasia,, periportal inflammation, focal areas of fatty changes, feathery degeneration and cloudy swelling.

Figure 6: Histological observation of the liver of control (a), EMR exposed (b), EMR exposed treated Aq-Wsr (c and d) and Aq-Wsr treated alone (e and f).

(a) (b)



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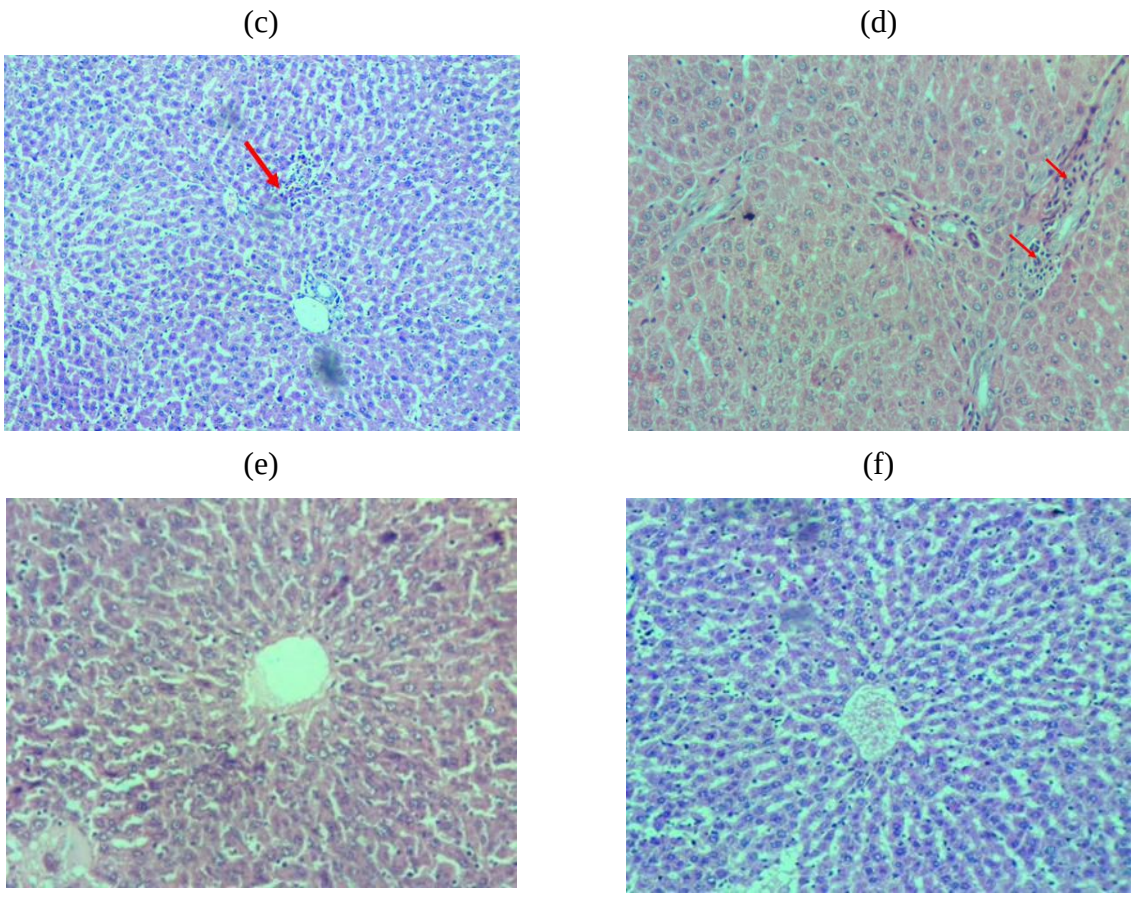

Fig.6.Photomicrograph of 4G exposed liver 400x H&E stain: (a) shows 6th month control group with normal architecture, (b) shows 6th month active radiation group yellow arrows showing apoptotic nuclei (APN),(c and d) shows 6th month drug and radiation treated group red arrow indicating lymphocytic inflammation with mild degenerative changes and (e and f) shows the 6th month drug alone group with a normal liver architecture.

Discussion

The harmful effects of cell phone radiation exposure on a vital organ like the liver is not much studied. Users of mobile phones in various nations and continents are exposed to varied frequencies. EMF exposure depends on the cell phone's frequency [16]. Numerous histological and biological investigations have been conducted to evaluate the adverse effects of electromagnetic radiation on the central nervous system, soft organs, fertility, growth, immune function and tumor formation. In this experiment, rats were exposed to 2400 MHz electromagnetic radiation (EMR) because 2400 MHz frequency 4G linked cellphones are widely utilized in India and many other countries. By either thermal or non-thermal methods, exposure to EMR can harm biotic cells. The transformation and absorption of heat by the electromagnetic energy of the human body might have thermal consequences. Blood flow stabilizes and alleviates increased body temperature [17]. Even when non-thermal effects do not elevate body temperature sufficiently to impair tissue structure, this effect is mitigated by the generation of reactive oxygen species (ROS). ROS are involved in a variety of cellular processes and can be either necessary or very harmful to cellular equilibrium. Their harmful effects originated from membrane phospholipid peroxidation. This results in a change in membrane conductivity and a breach in membrane integrity [18]. Changes in antioxidant levels suggest deteriorating cellular homeostasis, which causes stress and a decline in functional capacity [19]. Antioxidant activity levels improves the estimation and identification of EMR-induced harmful cellular alterations. In the present investigation, oxidative stress markers such as MDA, antioxidant markers such as GSH, and antioxidant enzyme activities of SOD, CAT, and GPx were measured in the liver

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tissue of rats following exposure to EMR (2400 MHz, 3 hours/day for 180 days).

Long-term exposure to 2400 MHz EMR can induce lipid peroxidation and antioxidant suppression in hepatic tissues, according to our research. Liver MDA ranges increased considerably in the EMR group compared to the control, EMR-exposed Aq-*Wsr*-treated, and Aq-*Wsr*-alone groups. However, the activities and levels of GPx, SOD, and CAT decreased significantly in hepatic tissues of irradiated rats . The results of our study supported the idea that exposure to EMR may induce oxidative damage in hepatic tissue. The variations in MDA and GSH levels and GPx, SOD, and CAT activities in the liver tissue of rats exposed to electromagnetic fields represented the pathophysiological consequences of the electromagnetic field. Due to its responsive ultrastructure to oxidative stress, EMR may also act as a stressor on hepatic tissue, based on these findings. Guleken *et al*. (2022) discovered that rats exposed to electromagnetic fields had elevated MDA levels in their liver cells [20]. MDA levels in the liver of rats exposed to 900 MHz RF-EMR throughout adolescence were substantially higher than in the control group [21]. Hasim *et al*., (2021) observed that an elevated MDA level decreased the overall antioxidant capacity of adult rat liver tissue [22]. In contrast, exposure to 900 MHz RF-EMR significantly decreased rat hepatic cell GPx, SOD, and CAT activity and GSH levels compared to control groups [23]. We hypothesized that liver cells have relatively poor enzymatic antioxidant resistance systems capable of producing significant levels of reactive oxygen species (ROS) via blood perfusion and high anaerobic metabolism; hence, the lipid peroxidation alterations are notable. Santini *et al*., (2018) reported a decrease in GPx, SOD, and CAT activity in rat liver tissue exposed to 900MHz RF-EMR, as well as a drop in GSH levels [24]. Increased SOD activity may be an attempt to counteract or prevent excessive chain oxidation of GSH or decreased GSH levels [25].Berköz et al., (2018) examined the effects of EMR on oxidative stress in the liver tissues of newborn rats [26]. There is evidence that cell phone induced EMR may cause oxidative liver injury in prenatal rat liver tissue [27]. The function of SOD, myeloperoxidase, and glutathione peroxidase (GSH-Px) decreased in the liver of rats in response to EMR exposure, according to Alkis *et al*., (2021) [28].

Currently, the primary effect of antioxidants on human health is their ability to scavenge free radicals. The use of antioxidants to mitigate the adverse effects of EMR is the subject of an increasing number of research investigations [29].

The histopathological observations made by Holoyska v et al proved that rats exposed to electromagnetic field of frequency 2.45 GHz and mean power density of 2.8 mW/cm^2 for 3 h/d for 3 week depicted the presence of moderate hyperemia, dilatation of liver sinusoids, and small inflammatory foci in the center of liver lobules that substantiated our study. [30]

In our study we have seen that EMR exposed rat liver tissue lead to feathery degeneration, portal congestion, cloudy swelling, kupffer cell hyperplasia, lymphoplasmocytic inflammation, coagulation necrosis and apoptosis that was substantiated by the study conducted by Ozgur E et al on mobile phone radiation-induced free radical damage in the liver which is inhibited by the antioxidants N-acetyl cysteine and epigallocatechin-gallate.[31].

HM Fahmy et al proved that 2.4 Ghz EMF radiation exposure 24hr daily for a period of 40 days showed significant reduction in SOD,GSH enzymes and increased MDA levels and also altered histological structure such as vacuolated cytoplasm, dilated sinusoids, kupffer cell hyperplasia etc. that was very much in consistent with our present study .[32]

In this current study, there are no liver histological abnormalities in the control group and drug alone treated groups. EMR and powerful waves exposed for a longer period result in larger temperature increases. These waves interacted and formed free radicals that, like ionizing rays, can enhance lipid peroxidation and have detrimental consequences on biological substance. Free radicals attack the cell's lipid membrane configuration, alter their composition, and split the proteins' border, resulting in the damage and death of the cell. The oxidative stress caused by ROS is a key feature of radiation-induced tissue damage [33].

Recent research has shown that long-term exposure to 2400 MHz RF EMR might generate oxidative damage. This stimulation was moderated by an increase in lipid peroxidation and a decrease in both enzymatic and non-enzymatic antioxidants. The

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study also provided evidence that RF-EMR may affect the structure and integrity of liver cells. This study indicates that RF-EMR may act as an environmental stressor and cause oxidative damage in liver tissues. Oxidative stress has been linked to multiple disorders, including liver damage, hypertension, and several types of cancer. Our findings suggested that the use of electromagnetic sources should be controlled to protect human health and the natural system.

Conclusion

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

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