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The Effect of Curcumin on Biochemical Parameters in 4G-Cell Phone-EMR exposed Male Wistar Rats

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ABSTRACT

Introduction:

Currently, male infertility is the major concern worldwide.Literature is available regarding the negative effects of cell phone radiation on testicular function. The aim of the present study is to document the effect of Curcumin on biochemical parameters in 4G-cell phone-irradiatedmale Wistar rats.

Materials and methods:

Four-week-old male Wistar rats were split up into four groups, each with six rats. The experiment was done for a period of two months. Control group with no cell phone; CUR-2 group received 100 mg of Curcumin/kg body weight/day; R2 group was exposed to 4G-EMR for two hours per day (5 minutes every half hour from 8 AM to 8 PM); the R+CUR-2 group was exposed to 4G-EMR for two hours per day(5 minutes every half hour from 8 AM to 8 PM) and received 100 mg of Curcumin/kg body weight/day. Serum testosterone, superoxide dismutase (SOD) and malondialdehyde (MDA) in testicular tissue homogenate were measured after the experiment.

Results:

EMR from4G-cell phonealtered the level of serum testosterone, SOD and MDA in the R2 group as compared with control, CUR-2 and R+CUR-2 groups.Nevertheless, Curcumin effectively shielded those parameters from cell phone-EMR in R+CUR-2 group.

Conclusion:

Curcumin can safeguard the testis from the 4G-cell phone radiation inWistar rats.

INTRODUCTION

The World Health Organization (WHO) defines infertility as the incapacity to conceive despite a year of continuous, unprotected sexual activity. Approximately 50% of individuals globally are infertile, with men making up the majority of these cases. In addition to physiological factors, a variety of external variables also affect infertility. Alcohol intake, smoking, and electromagnetic radiation exposure are a few of the outside influences that contribute to lower fertility. Human exposure involves both ionizing and non-ionizing radiation (1). Numerous electronic devices emit electromagnetic radiation (EMR), which is hazardous to human health. One of these devices is the growing concern for public health worldwide due to mobile phones (2). In males and animal models, EMR from cell phones may deteriorate sperm quality, namely sperm count, motility, viability, and normal morphology (3). Reactive oxygen species (ROS) production, the heating effect, and detrimental effects on the reproductive system are associated with increased exposure to EMR (2). The antioxidant defense system of cells is crucial in preventing ROS. In addition to several antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase, the antioxidant system consists of low molecular weight antioxidant compounds like glutathione (4). Oxidative stress is a disorder driven on by an excess of oxidants compared to antioxidants due to an imbalance between oxidants and antioxidants. Exposure to radiofrequency electromagnetic radiation (RF-EMR) has been linked to detrimental biological consequences because it increases the generation of ROS, which compromises the body's defense mechanisms(5).The primary male hormone involved in reproduction is testosterone. Exposure

to cell phone-EMR causes changes in the concentration of testosterone leading to a negative impact on reproduction.The ROS generated in rats exposed to 3G radiation was the primary factor responsible for the changes in sperm mitochondrial activity, sperm membrane integrity and sperm viability (1).

Plants and herbs contain phenolic chemicals that have anti-oxidant qualities. Curcumin (CUR) is a potent antioxidant that scavenges oxygen-free radicals and stops lipids from peroxidizing (6). In tropical parts of south and southeast Asia, Curcuma longa Linn, a perennial rhizomatous monocotyledonous plant of the Zingiberaceae family, is widely grown. Since 1280, it has been utilized as a spice, coloring, and medication.Protein, fiber, carbs, curcuminoids, essential oils and resins are all present. About 70% of the curcuminoids complex is composed of curcumin, with demethoxycurcumin making up 17%, bis-demethoxycurcumin 3%, and cyclocurcumin (curcumin IV) 10%. The most potent component of curcuminoid, curcumin, is a phenolic molecule with a striking orange-yellow color (7). It possesses antibacterial, antiprotozoal, antidiabetic, and antitumor activities (4). Few studies documented its protective capacity on testis from the damaging effects of hyperthermia and heat produced by lamps (8, 9).It has the ability to scavenge reactive oxygen products, including superoxide radicals, hydroxyl and nitrogen dioxide (10).Treatment with curcumin results in elevated levels of antioxidant, antiapoptotic, and testosterone in heat-induced scrotal stress (11).Hence, the present study aimed to show the effect of CURon the oxidative stress and testosteronein male Wistar rats exposed to 4G cellphoneradiations.

Material and Methods:

Animals:

Four-week-old male Wistar albino rats weighing between 110 and 130 grams were used in the study. They were purchased from India's Biogen Laboratory Animal Facility,Bangalore. The Institutional Animal Ethical Committee approved the protocols for this study's use of animals in research (permission letter No. 04/IAEC/MG/09/2021-II, dated September 07, 2021). The rats were used and cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals. They were placed in Polycarbonate cages and given commercially balanced food and unrestricted access to drinking water after a week of acclimatization. They were exposed to a temperature range of 22–24°C with a 12-hour light and dark cycle.

Cell phone-EMR Exposure Setup:

A common commercial brand of 4G Android smartphones with a peak power density of 2W/kg and a whole-body SAR value of 1.6W/kg, as provided by the manufacturer, were employed in this investigation. The average power density of the phone was found to be 195.6 mW/m2 when the electromagnetic field values of the inner side of the rat's cage were measured using a Cornet Electrosmog RF meter. The phone was suspended from the top of the rat's cage in a wooden box with a bottom that measured about 14 x 7 x 5 cm. As a result, the animals were free to move around and arrive at the cage's center, which was 7.6 cm from the bottom and the ceiling (12). It was ensured that there were no other cell phones, metal objects, or cell phone towers in or close to the testing room in order to prevent scattered or interfering radiation. The phone inside the cage was turned on and left in auto-answer mode when a video call was placed from another cell phone of the same brand and model.

Procurement of curcumin and its preparation:

The products that were purchased were 0.5% w/v carboxymethyl cellulose (Product No. C5678) and curcumin (Curcuma longa, or turmeric) (Product No. C1386) from Sigma-Aldrich Chemicals Private Limited in Bangalore, India. To achieve a concentration of 100 mg/mL, the CUR was dissolved in 0.5% w/v sodium carboxymethyl cellulose (CMC) salt with water (13).

Grouping of animals:

Figure 1: The effect of Curcumin on the level of serum testosterone inWistar rats exposed to 4G cell phone-EMR.

At random, four groups including six rats each were assigned. An identical arrangement, but without an EMR, was used to handle the control and Curcumin groups in a separate room.

Control group: With no cell phone-EMR.

CUR-2 group: Orally treated with 100mg of curcumin /kg body weight/day for two months.

R2 group: Exposed to cell phone-EMR for 2 hours/day for two months (5 minutes/every half an hour from 8 AM to 8 PM).

R+CUR-2: Exposed to cell phone-EMR for 2 hours/day for two months (5 minutes/every half an hour from 8 AM to 8 PM) and orally treated with 100mg of curcumin /kg body weight/day for two months.

Twenty-four hours after the trial ended, the rats were anesthetized with an intraperitoneal injection of 45 mg/kg ketamine hydrochloride. After drawing blood samples through the left ventricle, the serum was separated and kept in aliquots at -80°C until it was needed to estimate the amount of testosterone. Then,the testes were removed via a trans-abdominal incisionand used for the preparation of tissue homogenates for the estimation of Superoxide dismutase (SOD) and Malondialdehyde (MDA).

Analysis of serum testosterone:

Using Enzyme-Linked Immunosorbent Assay (ELISA) kits from Bioassay Technology Laboratory in Shangai, China, the amount of testosterone in serum wasdetermined. As directed by the manufacturer, the serum testosterone levels were ascertained. The testosterone assay demonstrated a lower limit of detection of 10ng ⁄ L, an intra-assay coefficient of variation of less than 8%, an inter-assay coefficient of variation of less than 10%, and a functional sensitivity of 5.25ng ⁄ L.

Preparation of tissue homogenate:

A portion of the testis was isolated, weighed, and cleaned with phosphate buffer saline (PBS) to get rid of extra blood, following the manufacturer's instructions. Using a glass tissue homogenizer set on ice, the tissue was chopped and homogenized in phosphate buffer saline (PBS). Using a chilled centrifuge, the homogenates were filtered and centrifuged for approximately 20 minutes at 4ºC (2000-3000 RPM). The supernatant was then stored at -20ºC in aliquots until it was needed for biochemical experiments.

Estimation of SOD and MDA:

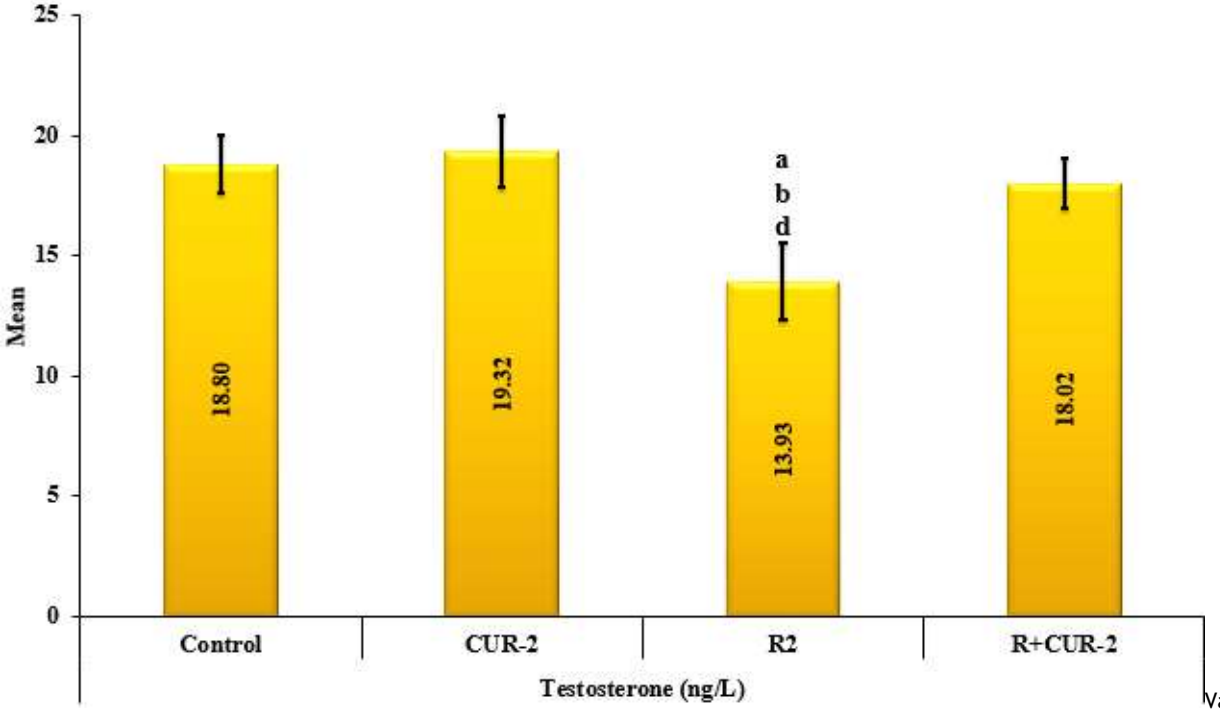
According to the manufacturer's instructions, the levels of SOD and MDA were determined from tissue supernatant using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Bioassay Technology Laboratory, Shangai, China). The absorbances were measured at 450 nm using a microplate reader (Bio-Rad, California, USA) after their reactions were stopped by the addition of an acidic stop solution.The manufacturer specified the following ranges for those parameters' measurements: 0.05–20 ng/ml (minimum–maximum) for SOD with functional sensitivity of 0.022 ng/ml; 0.05–10 nmol/ml for MDA with functional sensitivity of 0.01 nmol/ml. The intra-assay coefficient of variance was less than 8% and the inter-assay coefficient of variation was less than 10% for each of them.

Statistical analysis:

The Statistical Package for the Social Sciences (SPSS) software version 16.0 was used for the analysis of the data, which were entered into MS Excel 2010. The variables were calculated as mean ± standard deviation, and the parameters were compared between the groups using one-way ANOVA and a post hoc test (Tukey test), with a p value of less than or equal to 0.05 being considered significant.

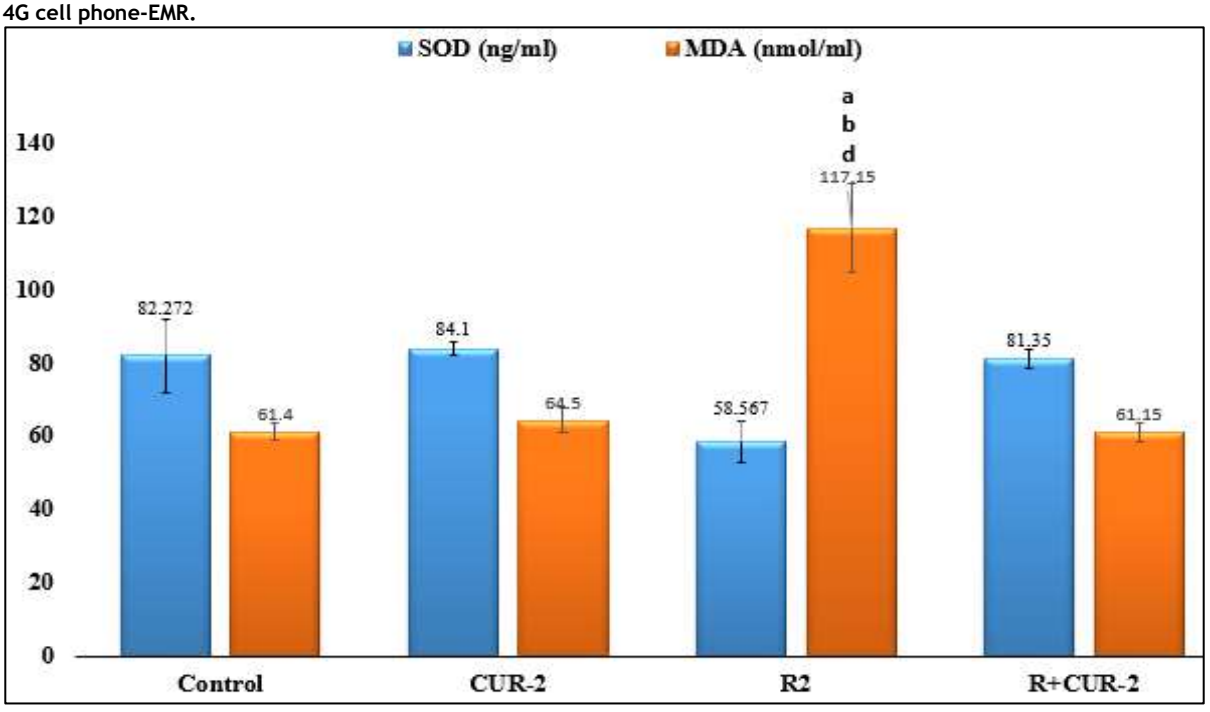
Results:

Cell phone-EMR induced a significant reduction in the levels of serum testosterone and SOD and elevation in the MDA levelin R2-rats as compared to control and CUR-2 groups ($p<0.001$). But *Curcumin*significantly increased the levels of serum testosterone and SOD and decreased the MDA levelin R+CUR-2 when compared with R2 group ($p<0.001$) (Figure 1 &2).



Values are expressed as mean ± SD. Different superscript letters show significant differences between groups. a- different from the control group; b- different from the CUR-2 group; d- different from the R+CUR-2 group.

Figure 2: The effect of Curcumin on the levels of SOD and MDA in testicular tissue homogenate of Wistar rats exposed to 4G cell phone-EMR.



Values are expressed as mean ± SD. Different superscript letters show significant differences between groups. a- different from the control group; b- different from the CUR-2 group; d- different from the R+CUR-2 group.

DISCUSSION

Depending on the EMR apparatus's power density and proximity to the patient, electromagnetic waves can breach the tissues and cause ROS either directly or indirectly (14). Since EMR penetrates 4-5 cm deeper into the tissues, the testis is the organ most vulnerable to it.The adverse effects of EMR are also determined by the type, mode, dose, duration of exposure, and undeveloped testis (15).Although ROS are typically produced by living cells, including spermatozoa, throughout their metabolic processes, excessive synthesis might trigger a cell-mediated defense response that uses endogenous antioxidants (16). Because OS is caused by an excess of ROS leading to diminished sperm function (14).

In the present study, Cell phone-EMR induced a significant reduction in the levels of serum testosterone and SOD and elevation in the MDA level in R2-rats as compared to control and CUR-2 groups ($p<0.001$). However,*Curcumin* significantly increased the levels of serum testosterone and SOD and

decreased the MDA level in R+CUR-2 as to the levels of those variables observed in control and CUR-2 groups.The findings of the current investigation are in good agreement with those of Ozguner et al. who found that acute exposure to 900 MHz radiofrequency fields from cell phones increased lipid peroxidation and lowered SOD activityCompared to the control group, the EMR group exhibited a substantial decrease in both the diameter of the seminiferous tubules and the mean height of their epithelium (17). Similar to the findings of the R2 group in this study, Okechukvu et al. reported that one month of exposure to cell phone radiation caused an insignificant decrease in the serum testosterone level (18). Likewise, rats exposed to 900 MHz RF-EMR for four hours per month can experience lower levels of inhibin-B and testosterone (19). Prolonged exposure to mobile phone radiation causes hypoplasia of Leydig cells, hypospermatogenesis, and decrease in sperm motility, count, and blood testosterone levels. Groups exposed to 4G saw more severe effects than those exposed to 3G-cell phones (2).

ROS primarily target intracellular molecules, particularly polyunsaturated fatty acids, and transmembrane proteins. Because of this, ROS easily interact with them, oxidizing these molecules with alcohol, lipidic aldehydes, and peroxides as byproducts, increasing the permeability of cell membranes resulting in the oxidative breakdown of unsaturated fatty acids in cell membranes.The sperm plasma membrane's unsaturated fatty acids are extremely sensitive to MDA, which can cause lipid peroxidation and rupture the membrane (16).In a previous experiment, rats were given an EMR at a frequency of 2.104 GHz (4G LTE) for four weeks using an EMR device almost similar to the setup utilized in this work. Leydig cell count, germ cell count, and Johnsen biopsy score were all considerably lower in the results(20). Kesari et al. reported that RF-EMR from a cell phone can impact spermatozoa's ability to fertilize in rats by lowering SOD, glutathione peroxidase, histone kinase, and MDA levels (21).

There is evidence that CUR can shield the testis from damage caused by toxins in animals. CUR's anti-inflammatory and antioxidant properties underpin its beneficial effects (7). The non-thermal impact of EMR is explained by the production of reactive oxygen species, which leads to oxidative stress (3). Studies have shown that CUR has radioprotective properties and is a more potent antioxidant that can combat tissue damage due by free radicals (7). Even when an animal has a higher scrotal temperature, CUR is a powerful medication that can reverse the changed parameters. Curcumin-loaded iron particles, according to Afshar*et al*., have the ability to dramatically increase blood testosterone levels by increasing the volume of the testis, the length of the seminiferous tubules, the characteristics of the sperm, and the stereological parameters in mice that have chronic scrotal hyperthermia (8). Comparable outcomes with testicular hyperthermia were reported by Khorsavi *et al* (9).As an antioxidant, CUR enhanced serum levels of prolactin, follicle stimulating hormone, testicular weight, and sperm motility, thereby protecting the testis from the damaging effects of compact fluorescent lamps (22). Similarly, Curcumin administration dramatically alleviated the testicular damage caused by scrotal hyperthermia in a dose-dependent manner through its antioxidative, anti-apoptotic, and androgen production properties, suggesting that it may be used to prevent male infertility (11). Testis weight index and testis volume dropped in the 4G-LTE-EMR group and slightly increased in the Crocin and 4G-LTE-EMR+Crocin groups, according to the findings of a study by Vafei*et al*. (23). This difference may be caused by a shorter exposure period. In this study, however, CUR could protect the treatment group from the OS caused by 4G cell phone radiation.

CONCLUSION

EMR 4G cell phone had negative effects on level of serum testosterone by altering the antioxidant status of testis. Such reduced hormone could be marker of spermatogenesis impairment.*Curcumin*, on the other hand, successfully safeguarded the testis from 4G cell phone-EMR. Even while it might not be feasible to completely avoid exposure to cell phone-EMR, regular consumption of *Curcumin* can counteract it and preserve reproductive health.

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