

Investigation of the Effect of Extremely Low-frequency Electromagnetic Fields Exposure and Curcumin During the Embryonic Period on Catalase and Superoxide Dismutase Activities of Plasma and Liver Tissue of Juvenile Wistar Rats

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ABSTRACT

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To investigate the effectiveness of extremely low-frequency electromagnetic field (ELF-EMF) and curcumin on the enzyme level of Catalase (CAT) and Superoxide Dismutase (SOD) in the plasma and liver of neonates that had been irradiated during pregnancy. Five males and twenty female rats were mated, and vaginal plaques were observed. The pregnant rats were then divided into six groups. During pregnancy, the EMF group was exposed to ELF-EMF for 30 min/day, the curcumin group received a single intraperitoneal dose of 50 mg/kg curcumin, and the EMF + Curcumin group was exposed to ELF-EMF and injected intraperitoneally with curcumin. The DMSO group was injected intraperitoneally with DMSO (curcumin solvent), and the sham group was placed in the solenoid under the same conditions as the EMF group but without ELF-EMF exposure. The sixth group was the control group. After birth, the offspring were breastfed by their mothers until the end of the lactation period (28 days). Once the offspring reached 4 weeks of age, they were sacrificed, separated by sex, and divided into six experimental groups according to their mothers' groups assignments. CAT and SOD levels in the plasma and liver were assayed. Data analysis showed curcumin and stress changed serum SOD ($P = 0.021$) and CAT tissue ($P = 0.033$) levels based on gender. Additionally, ELF-EMF exposure reduced serum catalase activity ($P = 0.002$). This change was sex-dependent, and in the presence of curcumin, the mean values returned to the normal level. ELF-EMF (1.5mT) exposure during pregnancy had a potentially sex-dependent effect on serum CAT activity in juveniles.

Keywords: Radiation exposure; Diferuloylmethane, Superoxide dismutase, Catalase, Embryonic period

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INTRODUCTION

Studies conducted by various researchers, including Kivrak *et al.* in 2017, have shown that non-ionizing electromagnetic fields (EMFs) can penetrate living tissues *in vitro* and *in vivo*, potentially altering their biological environment [1]. Low-intensity EMFs have been found to influence cell survival [2], differentiation [3], apoptosis [4], gene expression [5], and cause phenotypic abnormalities in mouse embryos [6]. Electromagnetic fields can disrupt the transport of Na^+ and Ca^{2+} ions across cell membrane [7, 8], stimulate protein synthesis [9], and cause changes in membrane structure [8, 10]. These membrane alterations may influence free radical generation and modulate the activities of antioxidant enzymes in various cells and tissues. The primary endogenous antioxidant defense system consists of superoxide dismutase (SOD) and catalase (CAT), which play a critical role in detoxifying reactive oxygen species. These enzymes work to eliminate superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) generated during normal metabolic processes [11, 12]. Their activity is essential for maintaining cellular homeostasis and preventing oxidative damage.

Research on the therapeutic effects of herbal extracts has gained significant attention worldwide in recent years [13, 14]. Although herbal remedies have been used for centuries, rigorous scientific studies on the therapeutic and prophylactic properties of pure extracts and isolated plant compounds have increased in the past few decades. Many of these compounds have demonstrated antioxidant, anti-inflammatory, and anti-cancer properties. Among these, turmeric (*Curcuma longa*) is of particular interest because of its active compound, curcumin (diferuloylmethane). Curcumin exhibits a wide range of biological activities, including anti-tumor effects *in vitro* and *in vivo* [14-16]. It has broad therapeutic potential due to its multifunctional effects, including antioxidant, anti-inflammatory, antiarthritic, anti-amyloid, and anti-ischemic properties [17, 18]. Curcumin has also shown significant protective and therapeutic effects in oxidative liver diseases through various cellular and molecular mechanisms [19]. As a potent antioxidant, curcumin scavenges free radicals, quenches singlet oxygen, and acts as a metal-chelating agent [20]. Studies have shown that, upon absorption, curcumin is rapidly distributed across various tissues, including the brain, kidney, heart, colon, lung, spleen, and liver, at detectable levels [21]. After oral administration, the highest

concentration of curcumin was observed in the rat liver (70 nM/mL), highlighting its potential for liver-related therapeutic applications [22].

Curcumin is a highly pleiotropic molecule that targets various enzymes, cofactors, growth factor receptors, and other key biomolecules [23, 24]. These properties make it a promising candidate for treating a range of diseases [25, 26]. Furthermore, newborns exhibit higher levels of enzymatic and non-enzymatic antioxidants, as well as increased oxygen consumption, compared to adults [27]. Most studies on the effects of high-frequency electromagnetic radiation (1000–2450 MHz) have focused on SOD and CAT activity in rat tissues [1, 28–30]. This led us to hypothesize that curcumin, with its ability to penetrate various body tissues, could serve as a potential modulator to mitigate the effects of EMF exposure, particularly during pregnancy.

Therefore, this study was designed to evaluate the effects of extremely low-frequency electromagnetic fields (ELF-EMF; 50 Hz, 1.5 mT) and the protective role of curcumin on the antioxidant enzymes CAT and SOD in the liver and blood plasma of juvenile rats exposed to EMF during gestation.

MATERIALS AND METHODS

Animals and Experimental Groups

Twenty female and five male Wistar rats were housed together for mating over a 48-hour period. For mating, four female rats and one male rat were placed in each of five groups. After observing vaginal plaques, indicating successful mating, the pregnant rats were monitored and subjected to interventions starting from that day.

Adult female Wistar rats were used for pregnancy and exposure to extremely low-frequency electromagnetic fields (ELF-EMF). The pregnant rats were divided into six experimental groups:

- EMF Group:** Pregnant rats were exposed to ELF-EMF (50 Hz) for 30 minutes daily throughout the gestation period (21 days).
- Curcumin Group:** Pregnant rats received a single intraperitoneal injection of 50 mg/kg curcumin.
- Curcumin + EMF Group:** Pregnant rats were exposed to ELF-EMF (50 Hz) for 30 minutes daily during pregnancy and received a single intraperitoneal injection of 50 mg/kg curcumin.
- DMSO Group:** Pregnant rats were injected intraperitoneally with the same volume of curcumin solvent (DMSO in normal saline).
- Sham Group:** Pregnant rats were placed in the solenoid under the same conditions as the EMF group but without exposure to ELF-EMF.
- Control Group:** Pregnant rats were kept in standard conditions without any exposure or intervention.

After birth, the first-generation offspring were breastfed by their mothers until the end of the lactation period (28 days). At this point, the offspring were separated by sex and divided into six experimental groups, mirroring the grouping of their mothers. Each group consisted of six males or six females, resulting in a total of 72 offspring (36 males and 36 females). Sample size adequacy was determined using Cochran's formula and based on previous studies [31, 32], ensuring statistical power and balanced distribution across all groups (EMF, Curcumin, Curcumin + EMF, DMSO, Sham, and Control).

At the end of lactation, the offspring were anesthetized with intraperitoneal injections of ketamine (75 mg/kg) and xylazine (10 mg/kg). Blood samples were collected via cardiac puncture and centrifuged at 2500 rpm for 10 minutes to separate the serum. Liver

tissues were excised, frozen in liquid nitrogen, and stored at -80°C for subsequent analyses.

Preparation of Tissue Homogenates

One hundred milligrams of liver tissue were cut into small pieces, homogenized in 1 mL of cold phosphate-buffered saline (pH 7.4), and centrifuged at $10,000 \times g$ for 20 minutes at 4°C . The transparent upper solution was then divided into small volumes in microtubes and stored at -70°C .

Curcumin, according to the weight of the rats (200 g), was dissolved in 16 μL /mL DMSO, diluted in 50 μL /mL normal saline, and then injected into the animals with an insulin syringe.

Given that the early days of pregnancy are the most sensitive period, and considering curcumin's persistence in the body, particularly in the liver, curcumin was administered immediately after the vaginal plaque was observed.

Enzyme Assay

SOD and CAT activities were evaluated in the serum and liver tissue using a commercial kit according to the manufacturer's protocol (Zell Bio, Germany). SOD and CAT activities in the serum were expressed in U/mL, while their activities in liver tissue were expressed in U/g of tissue. All assays were performed in duplicate.

Electromagnetic Field Generator

The ELF-EMF was generated using an alternating sinusoidal current in a solenoid (coil). The magnetic field lines inside the solenoid were nearly parallel to its axis (Fig. 1).

Exposure to the Extremely Low-Frequency Electromagnetic Field (50 Hz)

The rats with a vaginal plaque were placed in the rat holder (a polyxy glass limiter in the center of the solenoid) with a length of 16 cm in the solenoid, where the field strength was 1.5 millitesla for half an hour per day throughout the entire period of pregnancy (Fig. 1).

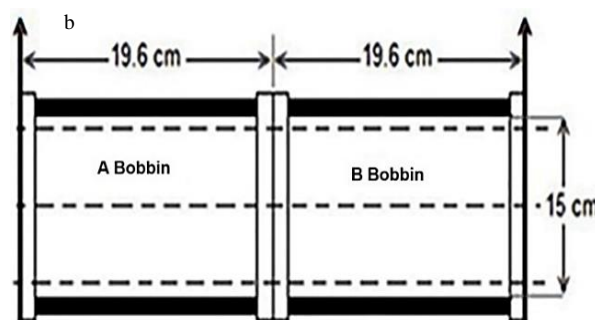
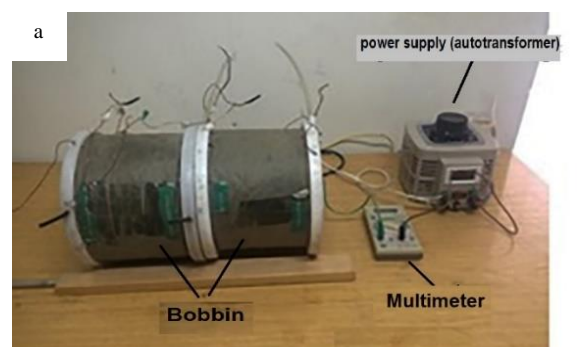


Fig. 1 Electromagnetic Field Generator. A solenoid generating an extremely low-frequency electromagnetic field (ELF-EMF): a) the internal diameter of each solenoid is 15 cm; b) the effective length is 15.6 cm; the number of turns in solenoid A (N_A) is 1756 and in solenoid B is 1732 [25].

Ethical Considerations

This study was conducted in strict adherence to international and institutional guidelines for the ethical treatment of laboratory animals, including the Helsinki Declaration and the guidelines set by the Committee for the Care and Use of Laboratory Animals. Approval for the study was obtained from the Ethics Committee of Arak University of Medical Sciences University of Medical Sciences (Ethics Code: IR.ARAKMUY.REC.1396.52). The animals were housed under controlled conditions, including a temperature-regulated environment (22 ± 2 °C), a 12-hour light/dark cycle, and ad libitum access to standard laboratory chow and water. Special care was taken to minimize stress and ensure animal welfare throughout the experiment. Pregnant rats were housed individually in appropriately sized cages to prevent overcrowding and facilitate natural maternal behaviors. Pain and discomfort were minimized by administering appropriate anesthetics (ketamine at 75 mg/kg and xylazine at 10 mg/kg) during blood sampling and tissue collection. Animals were closely monitored daily for signs of distress, illness, or abnormal behavior. The study was designed to use the minimum number of animals necessary to achieve statistically significant results, as determined by Cochran's formula, ensuring the ethical use of resources.

Statistical Analysis

All results were expressed as mean \pm standard error ($M \pm SE$). The normality of data distribution and homogeneity of variances were assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Statistical analyses were performed using two-way ANOVA, followed by Tukey's post hoc test. When the data did not follow a normal distribution, the Kruskal-Wallis test (a non-parametric equivalent to ANOVA) was applied. All statistical analyses were conducted using SPSS (version 6.07), and differences were considered significant at $P < 0.05$. Sex (male, female), group, and the interaction of sex and group were the variables.

RESULTS

Serum Superoxide Dismutase (SOD) Activity

Two-way ANOVA, followed by the Tukey test, revealed no significant differences in serum SOD activity among the groups (Fig. 2, 3a, and 3b). However, a significant difference in serum SOD activity was observed between male and female groups based on gender ($P = 0.021$; Fig. 3c).

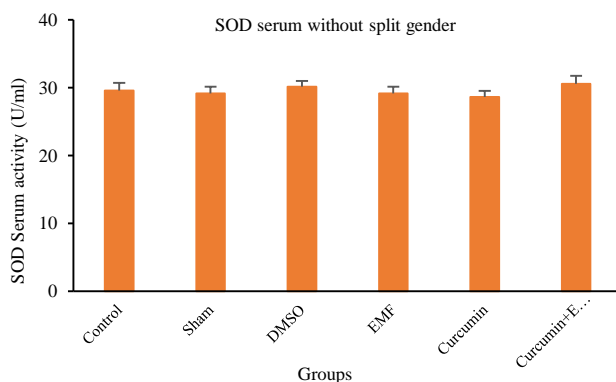


Fig. 2 SOD activity in the serum of different treatment groups. Values expressed as mean \pm SEM ($n = 12$; $P > 0.05$).

Tissue SOD Activity

The normality of the data distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests ($P < 0.05$). Although ELF-EMF exposure resulted in an increase in liver SOD activity in

the EMF group compared to the sham group, the Kruskal-Wallis test revealed that the differences in tissue SOD enzyme activity among the groups were not statistically significant (Fig. 4a-4c).

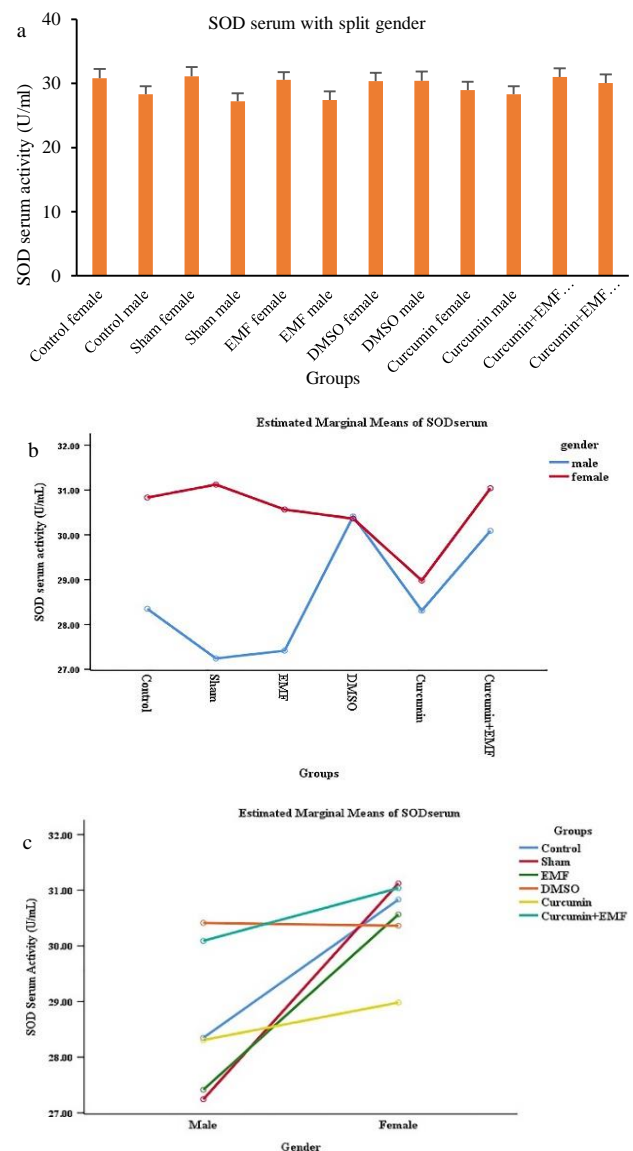


Fig. 3 SOD activity in the serum among groups (a) with regard to gender, (b) estimated marginal means of groups, and (c) estimated marginal means of gender. Values expressed as mean \pm SEM ($n = 6$; $P = 0.021$).

CAT Serum Activity

The normality of the data distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests ($P < 0.05$). The Kruskal-Wallis test indicated significant differences in CAT serum activity among the groups ($P = 0.002$; Fig. 5a and 5b). According to the Mann-Whitney test, the mean rank of CAT serum activity was significantly higher in the sham male group compared to the EMF male group (Table 1). Curcumin treatment increased CAT enzyme levels in the curcumin + EMF group compared to the EMF group in males. However, this effect was not as pronounced in females, as there was no significant difference between the EMF and curcumin +EMF groups in females ($P > 0.05$).

CAT Tissue Enzyme Activity

The normality of the data distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests ($P > 0.05$). Two-way ANOVA, followed by the Tukey post hoc test, showed no significant differences in CAT tissue enzyme activity levels among

the groups (Fig. 6a and 6b). While ELF-EMF exposure reduced catalase activity in liver tissue of both males and females compared to the sham group, this reduction was not statistically significant. However, a significant difference in CAT activity was observed between the male and female groups ($P = 0.033$; Fig. 6c and 6d).

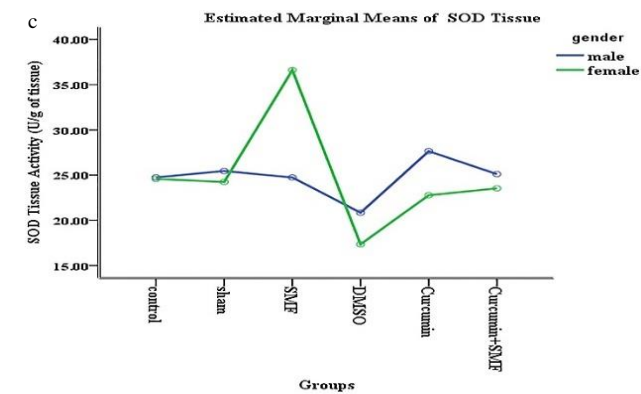
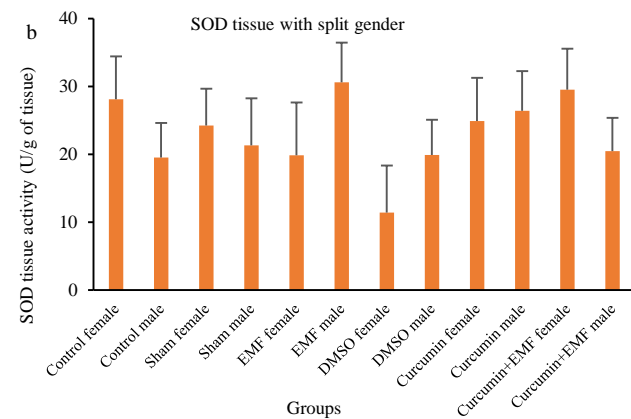
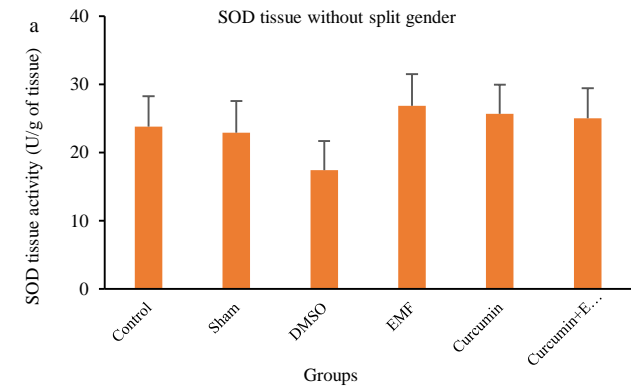


Fig. 4 SOD activity in liver tissue in among groups (a) without split gender, (b) with split gender, and (c) estimated marginal means of groups. Values expressed as mean \pm SEM ($n = 6$; $P > 0.05$).

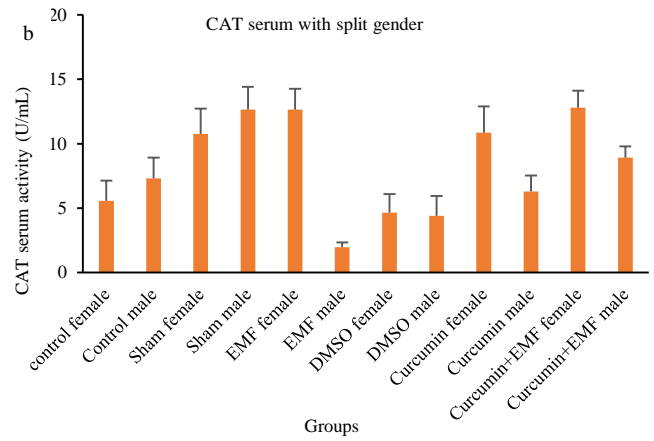
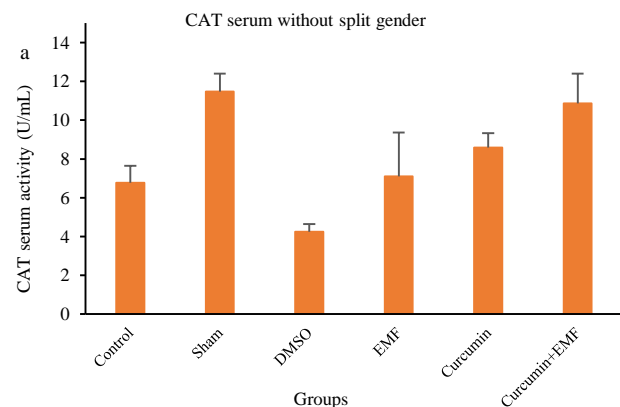
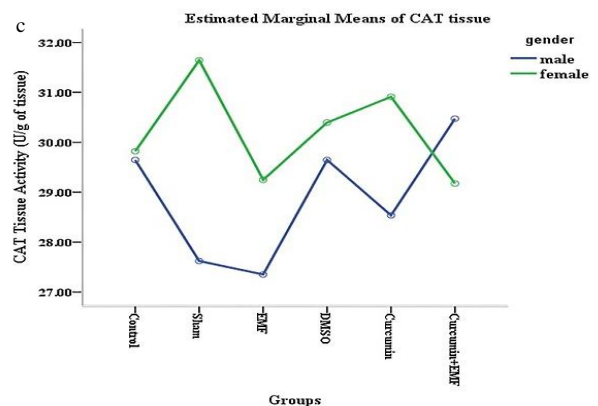
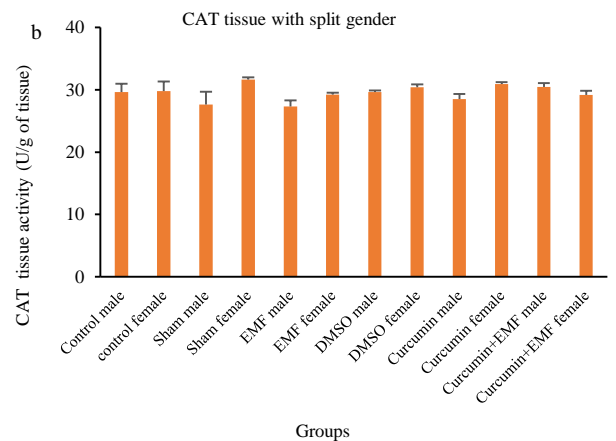
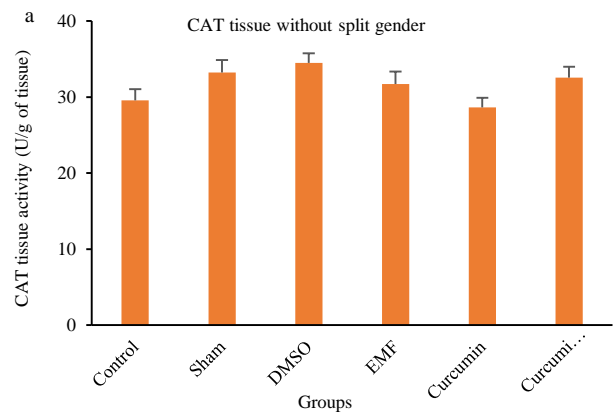


Fig. 5 CAT serum activity among groups (a) without regard to gender and (b) with regard to gender. There is a significant difference in CAT serum activity between males and females in both the curcumin and EMF groups. Values expressed as mean \pm SEM ($n = 6$; $P = 0.002$).



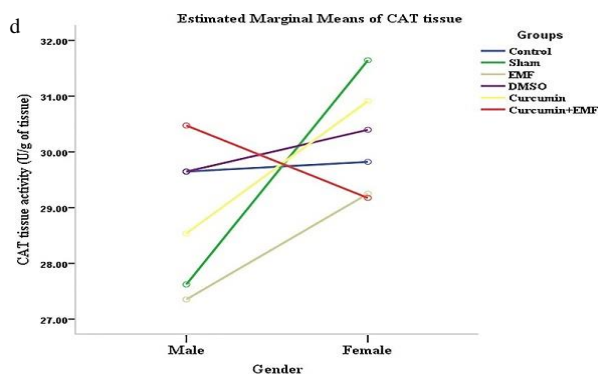


Fig. 6 CAT tissue activity among groups (a) without regard to gender, (b) regarding gender, (c) estimated marginal means of groups, and (d) estimated marginal means of gender d. Values expressed as mean \pm SEM (n = 6; P = 0.033).

DISCUSSION

Exposure to ELF-EMF in juvenile rats (aged 4 weeks) during maternal pregnancy significantly decreased serum catalase (CAT) activity in the male group. However, differences in tissue CAT and

serum superoxide dismutase (SOD) activity were observed between males and females, indicating a gender-dependent effect. Hormonal and genetic factors likely influence this disparity, as estrogen, which is abundant in females, possesses inherent antioxidant properties. Estrogen scavenges free radicals through its phenolic hydroxyl group, enhancing antioxidant capacity and potentially leading to higher baseline levels of antioxidant enzymes such as CAT and SOD [33].

Previous studies support these findings. Lee *et al.* reported that ELF-EMF exposure increased SOD activity in the brain homogenates of mice [34]. In contrast, Ghanbari *et al.* demonstrated that EMF exposure reduced SOD activity, leading to oxidative stress [25]. Another study emphasized that ELF-EMF exposure affected the antioxidant capacity in female rats' brains, with age-dependent effects: increased antioxidant activity in young animals and decreased activity in older ones [27].

In this study, ELF-EMF (1.5 mT, 50 Hz) increased SOD activity in liver tissue, though this change was not statistically significant. These findings align with evidence that EMFs induce oxidative stress across various tissues, altering antioxidant indicators in the blood [1, 3, 35].

Table 1 Comparison of the mean ranks of CAT serum activity between groups

groups	Control male and sham male	Sham male and EMF male	Sham male and curcumin male	Curcumin male and curcumin+EMF male	Sham male and curcumin+EMF male	Sham male and Sham female	Control male and control female	Curcumin male and Curcumin female
Mann-Whitney U	4.20	6	5.70	1.06	5.13	1.80	- 0.20	-5.38
P value (2-tailed) *	0.032	0.008	0.019	0.720	0.029	0.421	1	0.035

* Significance level (P < 0.05)

Animal studies have shown that ELF-EMF exposure reduces antioxidant defenses in the heart, kidneys, liver, and plasma [36, 37]. The present study highlights the novel observation that ELF-EMF exposure causes a significant sex-dependent reduction in serum CAT activity in males, with no notable changes in females. Interestingly, Kivrak *et al.* reported an opposite trend, where EMF exposure increased CAT activity [1]. While a decrease in CAT activity in liver tissue was observed, no significant differences were detected between the sham and EMF groups. However, significant gender-based differences in CAT tissue activity were evident, suggesting non-uniform effects between male and female groups. The observed reduction in serum CAT activity in males following ELF-EMF exposure highlights the heightened vulnerability of males to oxidative stress. Females generally exhibit higher baseline levels of antioxidant enzymes due to estrogen, which possesses receptor-independent antioxidant properties. Estrogen enhances reactive oxygen species (ROS) scavenging and upregulates antioxidant enzyme activities. Lacking this hormonal advantage, males may experience greater oxidative damage under stress conditions such as ELF-EMF exposure [33, 38]. Genetic differences and variations in antioxidant gene expression further contribute to these sex-dependent responses. Histopathological evidence supports these findings, with oxidative stress responses differing between sexes. Lifestyle factors, such as diet and micronutrient intake, may also influence antioxidant capacity and modulate outcomes [39, 40].

Curcumin supplementation effectively normalized diminished CAT activity in male serum caused by ELF-EMF exposure. It significantly increased serum CAT enzyme levels in males, demonstrating a sex-specific protective mechanism. This aligns with prior research showing curcumin's protective effects against

oxidative stress, lipid peroxidation, and DNA damage induced by ionizing radiation [41-43]. Similarly, lotus seedpod procyanidins have been shown to counteract oxidative stress from low-frequency EMFs by scavenging free radicals and stimulating antioxidant enzyme activity, such as SOD and CAT [43].

Curcumin's phenolic structure allows it to directly scavenge ROS, including hydroxyl radicals, hydrogen peroxide, and peroxyxynitrite. It also indirectly enhances antioxidant defenses by activating the Nrf2-Keap1-ARE pathway, which upregulates cytoprotective enzymes like SOD, CAT, glutathione peroxidase, and heme oxygenase-1 (HO-1). These mechanisms counteract oxidative stress induced by ELF-EMF exposure [1]. The findings of this study suggest curcumin's therapeutic potential in restoring antioxidant enzyme levels, particularly during critical periods like pregnancy.

Pregnancy represents a critical period of heightened sensitivity to environmental factors, including EMFs, due to the rapid development of major fetal organs [44]. Chronic or excessive EMF exposure during this time may result in structural abnormalities, cognitive impairments, or long-term health risks. Findings from this study emphasize the protective role of curcumin as a dietary supplement for pregnant rats exposed to EMFs. Curcumin not only scavenges ROS but also upregulates antioxidant enzymes, helping mitigate oxidative stress, especially in male offspring, who appear more susceptible to oxidative damage [45]. These results provide a foundation for exploring curcumin as a natural, accessible strategy to protect fetal development.

This study highlights the sex-dependent effects of ELF-EMF exposure on oxidative stress and the potential of curcumin as an antioxidant intervention. Discrepancies in the findings of previous research may stem from variations in oxidative stress levels and the

body's compensatory antioxidant defenses during the early stages of exposure. In the initial phases, SOD and CAT activity may increase as part of the response to reactive oxygen species, neutralizing ROS like H₂O₂ to protect the body [46, 47]. However, prolonged exposure may overwhelm these defenses, resulting in enzyme inactivation and oxidative damage [1, 48]. Oxidative stress induced by ELF-EMF exposure is a critical mechanism underlying its biological effects, contributing to lipid peroxidation, protein oxidation, and DNA damage via ROS production. Curcumin's antioxidant properties directly and indirectly mitigate these effects, stabilizing cellular membranes and enhancing enzymatic activity. These findings underscore curcumin's potential as a protective agent during pregnancy, especially under environmental challenges like EMF exposure. Future research should focus on optimizing dosages, long-term safety, and broader efficacy across diverse populations. Understanding the intensity and duration of radiation's impact on antioxidant enzyme activity during the embryonic period will be crucial for designing effective interventions and guidelines for maternal and fetal health.

CONCLUSION

This study highlights the sex-dependent effects of ELF-EMF exposure on antioxidant enzyme activity in juvenile rats exposed during maternal pregnancy. ELF-EMF exposures significantly reduced serum CAT activity in males but not in females, likely due to hormonal and genetic differences. Curcumin supplementation effectively restored CAT activity in males, highlighting its potential as a protective antioxidant. These findings emphasize the need for further research on EMF exposure effects and the role of antioxidants like curcumin in mitigating oxidative stress, particularly during critical developmental periods.

Suggestions

Future studies should investigate the use of higher field intensities (>1.5mT) and the appropriate curcumin dose. It is possible that with increasing field intensity or continuous exposure from the gestation period (from observing vaginal plaques) to 4 weeks of age, the enzymatic changes in liver tissue may become significant.

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Data Availability

The data that support this study will be shared upon reasonable request made to the corresponding author.

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This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors report no conflict of interest.

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