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Histological Evaluation of the Protective Role of Caffeic Acid on the Testes of Adult Wistar Rats Following Exposure to Radio- Frequency Electromagnetic Radiation

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ABSTRACT

Radiofrequency electromagnetic radiation (RF-EMR) has been extensively reported by previous studies to have deleterious effects on the male reproductive system. On the other hand, research has proven that caffeic acid possess potent anti-inflammatory and antioxidant properties. This research aimed to investigate the potential of caffeic acid in mitigating histological damage to the testes caused by RF-EMR exposure. The study assessed testicular histoarchitecture using Haematoxylin and Eosin, as well as Verhoeff-van Gieson stain. The results revealed improved preservation of normal histoarchitecture in the caffeic acid-treated groups compared to the untreated ones, with well-arranged germinal epithelial cells, and improved elastic fibre expression in the peritubular walls of the seminiferous tubules. Pre-treatment with caffeic acid demonstrated its ability to protect the testes of experimental rats from the harmful effects of RF-EMR. The results indicate that treatment with caffeic acid before and after exposure to phone radiation exhibited protective effects on the testes of the rats.

Keywords: radiofrequency, electromagnetic radiation, testes, anti-inflammatory, antioxidant, CAPE

INTRODUCTION

Infertility stands as a formidable health challenge affecting couples of childbearing ages, with almost half of primary infertility cases attributed to male factors [1,2]. In recent years, a growing concern has emerged regarding the potential association between male infertility and the prevalent use of mobile phones. The widespread practice of storing mobile phones in trouser pockets or attaching them to belts places these devices near the testes, raising apprehensions about their impact on male reproductive health. Mobile phone radiation has been identified as a source of concern, as it has been shown to generate heat and reactive oxygen species in the testes [3], thereby inducing oxidative stress—a well-established contributor to poor sperm quality and infertility [4, 5].

Caffeic acid phenethyl ester (CAPE), a bioactive compound derived from honeybee propolis, has garnered significant attention due to its diverse health benefits. Extensive research has highlighted its anti-hypertensive, anticancer, anti-inflammatory, and antioxidative properties [6, 7, 8]. Notably, CAPE's protective effects extend to various organs, including the heart, liver, and neurons, primarily attributed to its potent antioxidant activity [6,7].

Against this backdrop, our study aimed to delve into the protective role of caffeic acid phenethyl ester on the testes of adult Wistar rats exposed to Radiofrequency Electromagnetic Radiation (RF-EMR). Through differential histological and histochemical analyses, we examined the potential mitigating effects of CAPE on the testicular damage induced by graded exposure to RF-EMR. This investigation holds promise in providing strategies to counteract the adverse impacts of mobile phone radiation on male reproductive health, offering valuable insights for addressing infertility concerns associated with contemporary lifestyle practices.

METHODOLOGY

Animal Grouping

Thirty-six (36) male Wistar rats weighing 180–200 g were obtained from the Department of Anatomy, Ekiti State University, Ado-Ekiti, Nigeria. The rats were

housed in polycarbonate cages and maintained in a well-ventilated room at room temperature on a 12-h light/12-h dark cycle. They had free access to standard rat chow and water. The rats were allowed to acclimatize for 2 weeks, after which they were randomly divided into six (6) groups of six rats each. The ethical approval of this study was obtained from the Departmental Research Committee of the Federal University of Technology, Akure. The research was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals [9].

Experimental Protocol

Infinix 6 cell phone with body dimensions of 157.7 x 75.4 x 8 mm and weighing 70 g, dual-band EGSM900/1.3 GHz was used to generate RF-EMR. The screen size is six inches with vibration and polyphonic alert modes. The modalities of exposure and treatment for the groups are as follow: Group A - Control; Group B - RF-EMR Exposure Call mode + Data mode (3 hours/day); Group C - RF-EMR Exposure Data mode (3 hours/day); Group D - RF-EMR Call mode + Data mode (3 hours/day) + CAPE; Group E- RF-EMR Data mode (3 hours/day) + CAPE; Group F- CAPE pretreatment for 15 days + RF-EMR Exposure Call mode + Data mode (3 hours/day).

Groups D and E were concurrently exposed to RF-EMR and administered CAPE for 30 days. In contrast, group F was exposed to RF-EMR for 30 days after initial pretreatment with CAPE for 15 days.

To automate the duration of the exposure and generate mobile phone radiation at a preset duration, the mobile phone was integrated with Arduino Uno, which is a prototyping board based on ATmega 328 micro-controller. The Arduino GSM Shield was equipped with a programme in the Arduino Integrated Development Environment (IDE).

The rats were continuously exposed to radiation for 15 minutes at 15-minute intervals, totalling 3 hours of radiation exposure per day for 30 days. This procedure was necessary to minimize the heat generated by the phone so that the effects of exposure could be attributed to phone radiation rather than heat emission by the phone. The exposure to the mobile phone was observed every day for a period of 30 days, between 8:00 a.m. and 2:00 p.m.

Caffeic acid was purchased from Loba Chemie Pvt Limited, Mumbai. A dose of 50 mg/kg body weight was prepared and administered according to the experimental design.

At the end of the experiment, the rats were anaesthetized with chloroform and blood was drawn via cardiac puncture into plain specimen bottles. The testes were excised through laparotomy, blotted dry and weighed. Thereafter they were fixed in Bouin's fluid for histological and histochemical procedures.

Histological Evaluation using Haematoxylin and Eosin Staining

The testes of the rats were fixed in Bouin's fluid for 24 hours and then dehydrated in 70%, 90%, and absolute alcohol, as well as xylene for varying durations. The tissues were then infiltrated with molten paraffin wax for 1 hour each in an oven set at 65°C. Serial sections of five microns were made using a rotary microtome, and the tissues were picked up with albumenized slides and allowed to dry on hot plates for 2 minutes.

The slides were dewaxed with xylene and passed through a series of alcohol solutions in decreasing concentrations (absolute alcohol twice, 70% alcohol, and 50% alcohol), followed by water for 5 minutes. Hematoxylin and Eosin (H&E) stain was applied to the slides, which were then mounted in DPX. Photomicrographs were taken at a magnification of 100x on an Omax microscope (USA).

Histochemical Evaluation using Verhoeff-Van Gieson (VVG) Staining

The Verhoeff-Van Gieson staining protocol, as described by (Puchtler and Waldrop) [10] was followed. The slides were deparaffinized and hydrated in distilled water, then stained in Verhoeff's solution for 1 hour until the tissue was completely black. The slides were then rinsed in tap water with 2-3 changes before being differentiated in 2% ferric chloride for 1-2 minutes and washed in tap water.

After treatment with 5% sodium thiosulfate for 1 minute, the slides were washed in running tap water for 5 minutes and counterstained in Van Gieson's solution for 3-5 minutes. The slides were then rapidly dehydrated through 95% alcohol, followed by 2 changes of 100% alcohol. Subsequently, they were cleared in two changes of xylene for 3 minutes each before applying coverslips with resinous mounting medium.

Data analysis

Data of replicate experiments were pooled and expressed as mean ± standard error of mean (SEM). One - way ANOVA was used to analyse the mean values, followed by Tukey post-hoc tests. Significant levels were accepted at $p<0.05$. All statistical analysis was performed using GraphPad Prism.

RESULTS

Histological Evaluation Using Haematoxylin and Eosin Staining

Histological evaluation was performed using Hematoxylin and Eosin staining. The testis from Control Group A showed seminiferous tubules with a regular outline and thin basement membrane. The connective tissue stroma was well demonstrated, with angled interstitial spaces containing Leydig cells and blood vessels. The seminiferous tubules exhibited a progressive array of spermatogonia, primary and secondary spermatocytes, with the luminal spaces filled with the tails of the spermatids (Figures 1A; 2A).

The tubules of rats exposed to RF-EMR in Data mode and Call+Data mode exhibited irregularities, including thickened basement membranes and hyalized seminiferous epithelium with exfoliation of the spermatocytes. The spermatogenic cells within the tubules were disorganized and clumped together. Additionally, there was significant disruption of the connective tissue septa, with oedema observed as widened interstitial spaces. The blood vessels appeared enlarged and congested (Figures 1 B, C; 2 B, C).

The testes of RF-EMR-exposed rats treated with caffeic acid showed regularly outlined and oriented seminiferous tubules with very mild distortion. The spermatogenic cells were progressively arrayed from the basement membrane to the lumen, and the angular interstices were well-defined and contained Leydig cells. The tubules also contained a discreet progressive array of spermatogenic cells (Figures 1 D, E; 2 D, E).

The tubules of rats in Group F (pretreated with caffeic acid before exposure to RF-EMR) had characteristics comparable to the normal Control groups (Figures 1F; 2F).

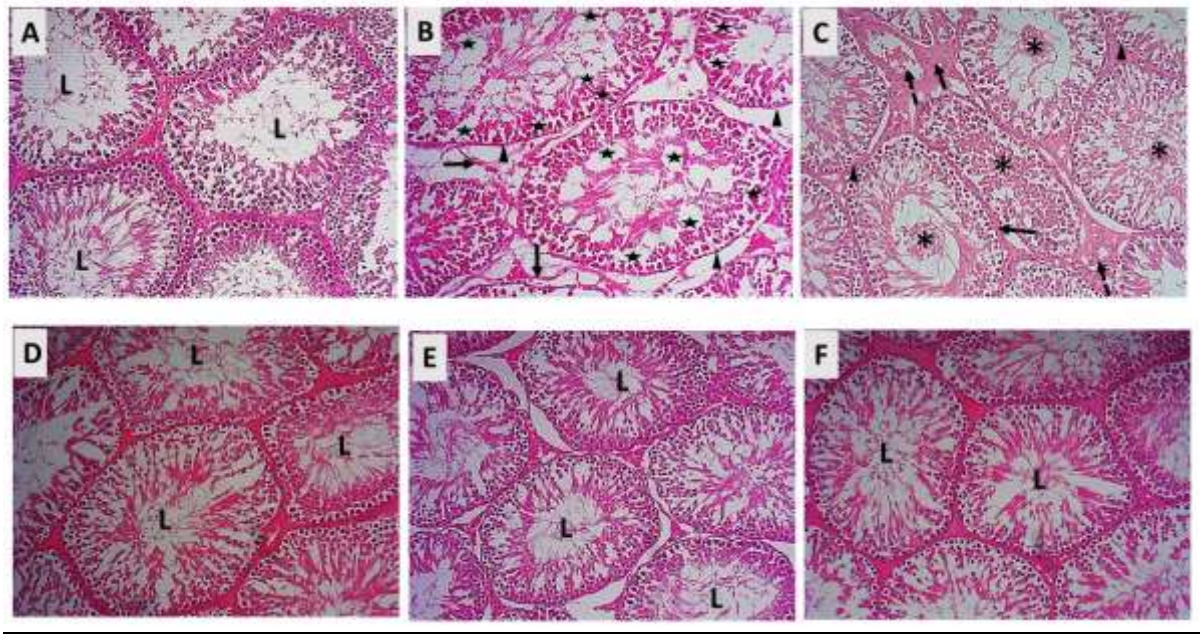

Figure 1: Sections from seminiferous tubules of experimental rats. Arrowheads indicate thickened basement membrane. Block arrows indicate distorted connective tissue with oedema. Stars indicate vacuolated germinal epithelium. Asterisks indicate clumped tails of spermatids and occlusion of the lumen. Broken arrows indicate enlarged and congested blood vessels. L indicates normal lumina. Stain: H&E. Magnification: x100.

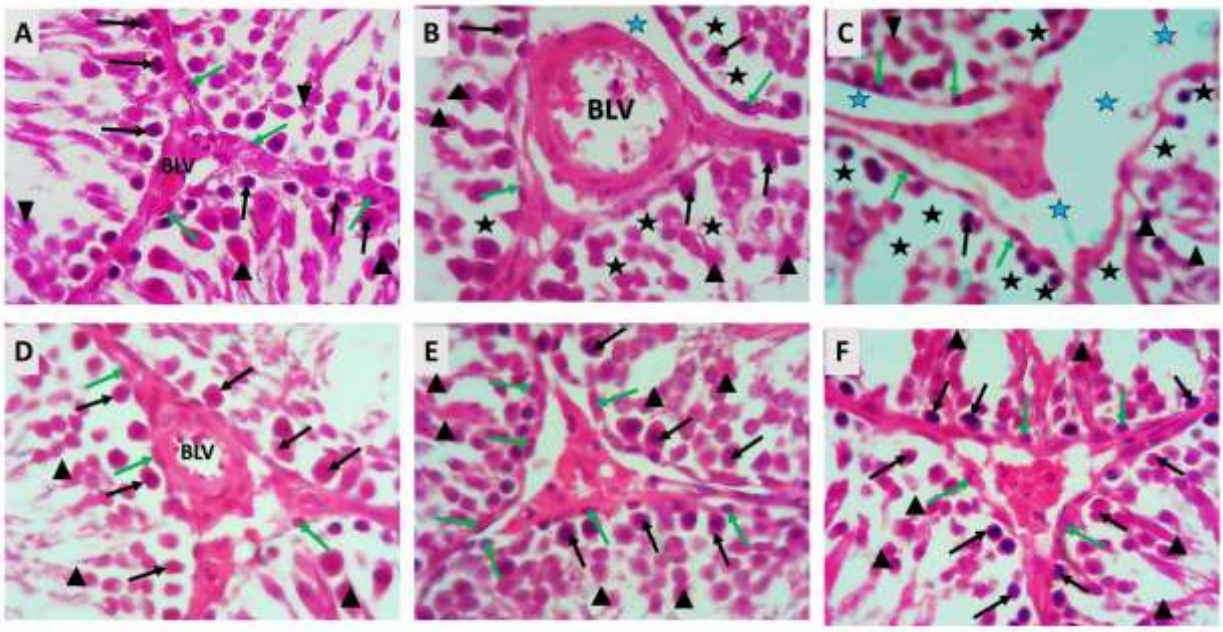

Figure 2: Sections from seminiferous tubules of experimental rats. Green arrows indicate spermatogonia. Black arrows indicate primary spermatocytes. Arrowheads indicate round spermatids. Asterisks indicate vacuolated germinal epithelium. Grey stars indicate widened interstitial spaces. BLV = Blood vessels. Stain: H&E. Magnification: x400.

Histochemical Evaluation

Verhoeff-van Gieson (VVG) stain was used for histochemical evaluation of the testes of experimental rats. The peritubular walls of the seminiferous tubules and the connective tissue around the blood vessels of sections from groups A, D, E, and F rats stained positive for elastic fibres. Sections from group B and C rats that were exposed to radiation exhibited sparse aggregation of elastic fibres around the peritubular walls (Figure 3).

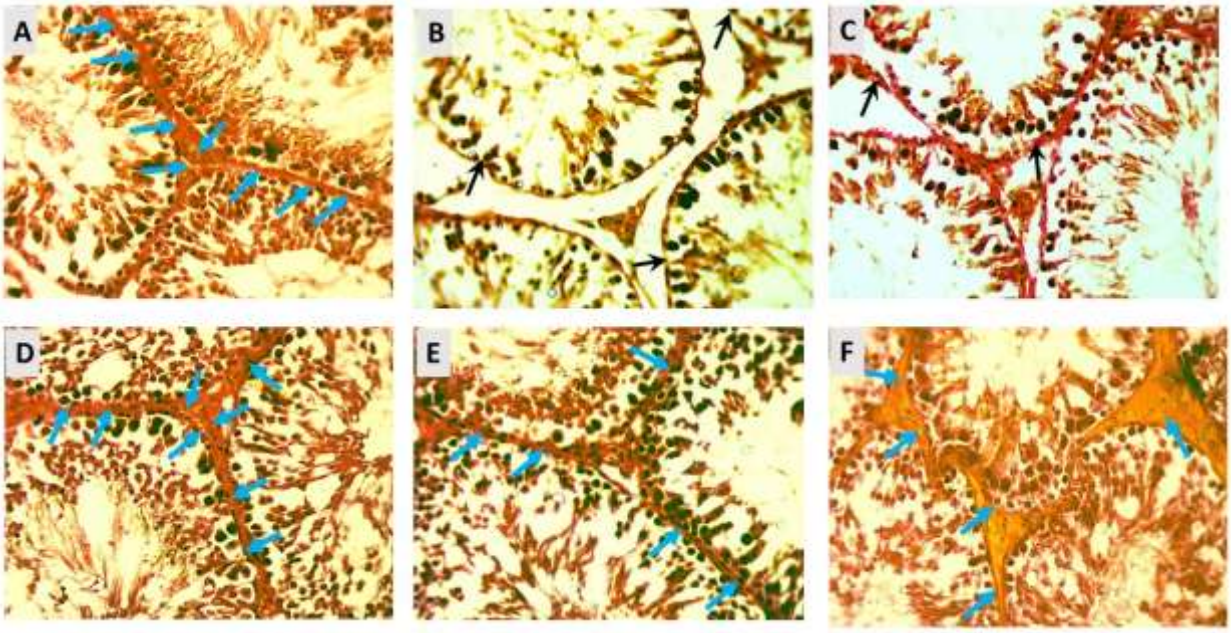

Figure 3: Sections from seminiferous tubules of experimental rats. Blue arrows indicate thick collagen fibres. Black arrows indicate sparse collagen fibres. Stain: VVG. Magnification: x400

DISCUSSION

Undoubtedly, RF-EMR exposure has been extensively reported to have significant detrimental effects on the body. The primary mechanism through which RF-EMR causes physiological damage is by increasing thermal energy (temperature). This rise in temperature subsequently triggers a series of events, including lipid peroxidation, increased generation of reactive oxygen species (ROS), oxidative stress, and cellular apoptosis.

The testes hold great importance, particularly due to the intricate physiological equilibrium required for spermatogenesis, as well as the maturation and preservation of spermatozoa [11]. Significant histoarchitectural damage was observed in the groups exposed to RF-EMR without treatment during histological evaluation. The germinal cell layer exhibited irregularity and sparsity, potentially due to the generation of reactive oxygen species (ROS) caused by disruption of the cell membrane potential by electromagnetic fields (EMF). Electromagnetic radio waves disturb the membrane potential of cells, resulting in an imbalance in the normal redox state within the cells. This disruption leads to the generation of ROS and oxidative stress, which hinders the normal steroidogenic function of Leydig interstitial cells. Consequently, the differentiation of germinal cells into various stages of the spermatogenic lineage is affected [12, 13].

The damage observed in the seminiferous tubules in this study is consistent with previous findings that reported a reduction in the height of the seminiferous tubules due to an increase in apoptotic death of the cells in the germinal epithelium[14, 15]. Specifically, a study that exposed mice to EMF of 1.7 GHz demonstrated derangement in the luminal epithelium of the seminiferous tubules [16]. A mechanism by which EMF induces apoptosis is by disrupting critical stages of the cell cycle. A study on EMF exposure showed disruption in the normal duration of the sub G1 phase of the cell cycle, leading to spermatozoa DNA fragmentation [17]. Similarly, a study involving exposure to a super low frequency EMF of 60 Hz from the 13th day of gestation to 21 days postnatally demonstrated a similar reduction in the number of seminiferous tubules, Leydig cells, and derangement of the germinal epithelial cells [18].

Our study also revealed the destabilizing effects of EMR on the connective tissue surrounding the seminiferous epithelium, known as peritubular myoid cells (PTMC). The PTMC comprises a single cell layer and extracellular matrix proteins [19]. Previous studies have reported that PTMC contains type III collagen fibres and abundant amount of elastic and reticulin fibres which are arranged in a network-like structure around the seminiferous tubule [20, 21,22].

The elastic fibres are a prominent feature of PTMCs and are important for the mechanical support and contractile function of these cells [23], helping in the transport of spermatozoa through the tubules. Our study demonstrated reduced characterization of elastic fibres in the PTMC of testes exposed to EMR relative to the control and caffeic-treated groups. Damage to the PTMCs can disrupt the migration of spermatozoa from the periphery to the centre during spermatogenesis, potentially resulting in a reduced quantity of spermatozoa in the lumen, affecting reproduction negatively. This study observed evidence of such an impact on the testes exposed to EMR.

For this study, caffeic acid phenethyl ester (CAPE), a natural derivative of propolis, was used as a treatment. CAPE has been demonstrated to possess antioxidant [24] and anti-inflammatory properties, making it suitable for this study [25, 26]. The groups that received CAPE demonstrated the retention of PTMCs and preservation of the germinal epithelium.

CONCLUSION

Overall, our data suggest that treatment with caffeic acid before and after exposure to phone radiation can exhibit protective effects on the testes of rats. Further in-depth studies are recommended to elucidate the underlying molecular and cellular mechanisms by which caffeic acid exerts its protective effects on the testes.

Declaration of Interest Statement

We declare that there is no conflict of interest with respect to the authorship and/or publication of this paper.

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