PROTECTIVE EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE) ON LIVER AND KIDNEY OF RATS AFTER EXPOSURE TO 900 MHZ ELECTROMAGNETIC FIELD

Nagla Ahmed El Nabarawy*, Mohamed Ali El Desouky**

*Consultant of Clinical Toxicology, National Egyptian center of Environmental & Toxicological Research (NECTER), Cairo University
**Lecture of Biochemistry, Faculty of Science, Cairo University

Abstract: The use of mobile phones is one of the fastest growing technological developments in present days. The 900 MHz EMR emitting mobile phones are commonly used in many countries. There is evidence that exposure to the radiofrequency radiation from mobile telephones or their base station could affect people's health. This article describes the PROTECTIVE EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE) ON LIVER AND KIDNEY OF RATS AFTER EXPOSURE TO 900 MHZ ELECTROMAGNETIC FIELD [Nagla Ahmed El Nabarawy, Mohamed Ali El Desouky. PROTECTIVE EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE) ON LIVER AND KIDNEY OF RATS AFTER EXPOSURE TO 900 MHZ ELECTROMAGNETIC FIELD Journal of American Science 2011; 7(12):937-944]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: CAFFEIC ACID PHENETHYL ESTER (CAPE); LIVER; KIDNEY; RAT

1. Introduction

The use of mobile phones is one of the fastest growing technological developments in present days. The 900 MHz EMR emitting mobile phones are commonly used in many countries. There is evidence that exposure to the radiofrequency radiation from mobile telephones or their base station could affect people's health.[1-4]

The close proximity of the antenna of this device to the abdominal organ's when carried on the belt has raised concerns about the biological interactions between electromagnetic radiation (EMR) and the kidney.[1,4,5]

It has been reported that mobile phones induce free radical formation in other tissues.[6-8] Researchers found that electromagnetic fields increase free radical activity in cells.[9] In vivo animal studies have shown that oxidative stress (OS) develops in response to cell phone radiation.[10-14] Radiofrequency Electromagnetic waves (RF-EMW) might disturb reactive oxygen species (ROS) metabolism by increasing its production or by decreasing antioxidant enzyme activity. Chronic exposure to RF-EMW decreases the activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and thus decreases the total antioxidant capacity.[10,13,15-17]

It has been demonstrated in lots of studies that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues.[7,8] ROS have been implicated in tissue injury.[18] There are some suggestions that ROS and/or free radicals are involved in the action of Electromagnetic Field (EMF) on biological system.[6,19]

The continuously produced ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT). Under some circumstances, these endogenous antioxidant defenses are likely to be perturbed because of overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure adequately replenish antioxidants in tissue.[18]

Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress mediated lipid peroxidation (LPO) in renal tissue. Increased urinary enzyme activity is generally regarded as an indicator of renal tubular dysfunction.[20]

The antioxidant treatments in the animal and human could be beneficial in preventing or reducing some complications of low frequency EMF.[19] Caffeic Acid Phenethylster (CAPE), a flavonoid like compound, is one of the major components of honeybee propolis. It has been used as a folk medicine for many years in Middle East countries. CAPE, with no harmful effects on normal cells has several biological and pharmacological properties: antioxidant, anti-inflammatory, anti-carcinogenic, antiviral, and immunomodulatory activities.[21-27] CAPE was shown to inhibit lipoxygenase (LPO) activities and suppress ROS – induced LPO in tissues.[22-24] Therefore, CAPE treatment protects the kidney from ischemia-reperfusion injury [28], and from diabetic oxidative damages.[29] It was shown in previous studies that CAPE preserved heart doxorubicin and liver from cisplatin-induced oxidant injury.[30]

The aim of the present study is to investigate the possible harmful effects of 900 MHz EMR emitted by mobile phones on the kidney and liver of rats and its treatment with CAPE.

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MATERIAL AND METHODS

Chemicals

All Chemicals were obtained from sigma chemical Inc. (St. Louis, Mo, USA) and the organic solvents from Merck chemical Inc. (Darmstadt, Germany). Reagents were all of analytical grade.

Animal Models:

Forty-eight male Wister albino rats (8 week old, 150-180 gm weight) obtained from the breeding unit of Faculty of Medicine, Cairo University were used in the study. The study was approved by the Animal Ethical Committee of Cairo University. All the guiding principles in the care and use of laboratory animals were strictly adhered throughout the entire study. They were kept in an environment of controlled temperature (24-26°C) and controlled photoperiod (12h of light and 12h of dark) for one week before starting the experiment. They were housed individually in stainless-steel cages. All animals were fed commercial diet.

Experimental design:

The rats used in the study were randomly grouped (16 each) as follows: 1) Group I, control sham-operated rats, 2) Group II: 900 MHz EMF exposed rats, and 3) Group III: 900 MHz EMF exposed +CAPE treated rats. The latter group were injected daily with CAPE intraperitoneally (i.p.) administered in a dose of 10 µM/mL/kg/day.

The 900 MHz EMF was applied to study groups (II, III) for 60 days (1 h/day). The EMR exposure time was at 10:00-11:00 am in each day. Group III received CAPE dose for 30 days before the daily EMF exposure. Control rats (group I) were also placed in the exposure device with the same environmental room conditions as the exposure groups, but without exposure to EMR (Exposure Device Off). In control rats, isotonic saline solution (an equal volume of CAPE) was also administered i.p. At the end of study, the rats were anesthetized with a cocktail of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) administered i.p. before sacrificing process. The rats were then sacrificed and kidneys and livers were dissected out and removed for biochemical and histopathological examination.

Exposure device

A 900 MHz pulsating wave electromagnetic field was generated by a radiofrequency (RF) source simulating the actual sources of mobile communications. It consists of an RF signal generator (Model: 2025, Aeroflex Inc., UK). Two monopole antennas set in parallel with the help of a T-Junction, a power amplifier (Stealth Microwave, Model: SM 0520-36, SSB technologies, Inc.) to supply a power intensity of 0.02 mW/cm² at the rat location, and field survey meter (Narda EMR 200, frequency 0-4 GHz, Germany) to measure the power intensity of electromagnetic fields at different distance from the generator.

The exposure system is composed of a container designed in such a way that the animals were not restrained. The animals were left for a period to adapt and direct themselves spontaneously towards the antenna as the air opening are concentrated in the cover of the container around the antenna. The antenna was placed at the center of container and the rats were exposed to the RF fields at a frequency of 900 MHz and a power density of 0.02 mW/Cm² for 1 hour. The average SAR was 1.075 W/kg as calculated by the Finite-Difference-Time-Domain (FDTD). All measurements were done in Faculty of Science, Cairo University.

Biochemical determinations:

The removed liver and kidney tissues were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (-30°C) until processing (maximum 10 h). The liver was cut into small pieces with a scissors on ice and homogenized (for 2 min at 5000 rpm) in four volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) by using a glass Teflon homogenizer (Tempest Virtisheer, Model 278069; The Virtis Company, Gardiner, NY). Lipid peroxidation (LPO) analyses were carried out on the homogenate. The homogenate was then centrifuged at 5000 × g for 60 min to remove debris. The clear upper supernatant fluid was taken, and CAT, GSH-Px and XO activities, and protein concentration were measured at this stage. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3, v/v). After centrifugation at 5000 × g for 30 min, the clear upper layer (the ethanol phase) was taken, and used in the SOD activity and protein assays. All preparation procedures were performed at +4°C.

Measuring markers of oxidative stress:

1. Malondialdehyde (MDA) measurement:

MDA levels in liver and kidney homogenate were measured with the thiobarbituric-acid reaction by the method of Draper and Hadley.[31] The quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1,1,3,3 tetramethoxy-propane. The values of MDA were expressed as nmol/g of protein.

2. SOD activity determination

Total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al.[32] The principle of the method is based on the inhibition of NBT reduction by the xanthine–
xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged at 4000 g. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as units per milligram liver protein.

3. Determination of Nitric Oxide (NO)

NO was measured by Griess reaction [33]. Reduction of nitrate to nitrite was accomplished by catalytic reaction using cadmium. The nitrite produced was determined by diazotisation of sulfanilamide and coupling to naphthylethylenediamine. Absorbance of this complex was measured at 540 nm. Results were given as nano moles per mg protein (nM mg⁻¹ prot.).

4. Catalase (CAT) activity determination

CAT (EC 1.11.1.6) activity was determined according to Aebi’s method.[34] The principle of the method was based on the determination of the rate constant (k) or the H₂O₂ decomposition rate at 240 nm. Results were expressed as k (s⁻¹) per gram protein in liver tissue.

5. Glutathione peroxidase (GSH-Px) activity determination

GSH-Px (EC 1.6.4.2) activity was measured by the method of Paglia and Valentine.[35] The enzyme reaction in the tube, which contains NADPH, reduced glutathione, sodium azide and glutathione reductase, was initiated by addition of hydrogen peroxide, and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was given as units per gram protein in liver tissue and all samples were assayed in duplicate.

**Protein determinations**

The protein content in the liver was measured by the method of Lowry et al.[36] with bovine serum albumin as the standard.

**Statistical Analysis**

Data were analyzed using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL). Data were expressed as mean±SD. Comparison between two groups was done using Mann-Whitney test. The three groups were compared using Kruskall-Wallis test followed by Scheffe test on ranks for pairwise comparison. Statistical significance was set at the 0.05 levels.

3. Results

Biochemical parameters in control, 900 MHz and 900 MHz EMF+ CAPE in rats.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 16)</th>
<th>Irradiated group (n = 16)</th>
<th>Treated group (n = 16)</th>
<th>p value</th>
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<tbody>
<tr>
<td><strong>Kidney Tissue</strong></td>
<td></td>
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<tr>
<td>MDA (nmol/gm tissue)</td>
<td>8.823±1.978a</td>
<td>21.265±2.523b</td>
<td>13.310±2.615c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO (µmol/gm tissue)</td>
<td>4.681±1.408a</td>
<td>10.491±1.173b</td>
<td>5.089±1.604a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH-Px (U/gm protein)</td>
<td>1.087±0.013a</td>
<td>0.085±0.016b</td>
<td>1.166±0.032c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAT (U/gm tissue)</td>
<td>1.029±0.227a</td>
<td>0.697±0.266b</td>
<td>0.882±0.198a</td>
<td>0.001</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>0.051±0.010a</td>
<td>0.037±0.005b</td>
<td>0.046±0.005a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Liver Tissue</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MDA (nmol/gm tissue)</td>
<td>10.943±3.357a</td>
<td>18.951±7.488b</td>
<td>12.381±11.089a</td>
<td>0.022</td>
</tr>
<tr>
<td>NO (µmol/gm tissue)</td>
<td>5.381±2.118a</td>
<td>11.309±3.481b</td>
<td>5.357±3.397a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH-Px (U/gm protein)</td>
<td>0.346±0.011a</td>
<td>0.192±0.023b</td>
<td>0.296±0.005c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAT (U/gm tissue)</td>
<td>0.735±0.179</td>
<td>0.963±0.441</td>
<td>0.867±0.120</td>
<td>0.114</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>0.079±0.034</td>
<td>0.084±0.008</td>
<td>0.090±0.008</td>
<td>0.261</td>
</tr>
</tbody>
</table>

MDA = Malonaldehyde, GSH-Px = glutathione peroxidase, CAT = Catalase, NO = Nitric Oxide, SOD = superoxide dismutase, CAPE: Caffeic acid phenethyl ester

Data as mean±SD, (Kruskall-Wallis test) p < 0.05 is significant. Groups with different superscript letters are significantly different.

In kidney tissues, irradiated rats showed significantly higher levels of markers of increased oxidation MDA (p < 0.001) and NO (p < 0.001) compared to control group. Meanwhile, antioxidant enzymes; GSH-Px, CAT and SOD were significantly lower in irradiated group (p < 0.001, p = 0.004, p < 0.001, respectively) compared to control group. The CAPE-treated group showed insignificantly different levels of NO (p = 0.707), CAT (p = 0.915) and SOD (p = 0.617) compared to the control group. However, MDA and GSH-Px levels were significantly higher in treated group compared to controls (p < 0.001 for both comparisons).
In liver tissues, irradiated rats showed significantly higher levels of MDA & NO (p = 0.031 and p < 0.001, respectively) compared to control group. The level of the antioxidant enzyme GSH-Px was significantly lower in irradiated group (p < 0.001) compared to control group. Meanwhile, levels of CAT and SOD were comparable in the three groups (p = 0.114 and 0.261, respectively). The CAPE-treated group showed insignificantly different levels of NO (p = 0.979) compared to control group, an efficient protective role on kidney and liver from oxidative stress.

However, GSH-Px levels were significantly lower in treated group compared to controls (p < 0.001).

**Histopathological Findings:**

**Liver:** (Fig. 1)
- **Radiated Group:** Sections revealed plate biopsy showing liver cells with mild degenerative changes.
- **Treated Group:** Sections revealed liver tissue with no disturbed architecture. There is no evidence of hepatitis or malignancy.

**Kidney:** (Fig. 2)
- **Radiated Group:** Sections revealed kidney tissue with normal glomeruli. The tubules show focal hyaline casts with edematous stroma infiltrated by lymphocytes degeneration. No malignancy.
- **Treated Group:** Sections revealed kidney tissue with no histological abnormality.

Fig. 1: Liver section showing degenerated hepatocytes with ballooning. The portal tracts are dilated with chronic inflammatory cells and areas of spotty necrosis (H&E, x40).
Fig. 2: Kidney section showing acute glomerulonephritis, the glomeruli show hypercellularity, infiltration by neutrophils and monocytes. The tubules contain red cell casts with evidence of degeneration (H&E x40).

**DISCUSSION:**

This study demonstrated a significant effect of exposure to EMR with a frequency of 900MHz on rat kidney and liver. Kidney and liver tissues showed evidence of oxidative stress with significantly higher levels of oxidative markers; MDA and NO and significant reduction of the antioxidant enzymes; GSH-Px, CAT and SOD. This is further confirmed by histopathological examination of liver and kidney tissues showing degenerative changes and lymphocytic infiltration. These findings suggest generation of ROS as a result of exposure to EMFs. All of these biochemical changes were absent in the non-exposed negative control rats.

Our study found a significant increase in NO levels after the exposure to EMR indicating a possible action of EMR pulses on induced nitric oxide synthase under experimental conditions. Furthermore, the positive correlation between NO and MDA levels may suggest that NO behaves as an oxidant radical. Nitric oxide reacts with superoxide anion three times faster than the dismutation of superoxide anion by SOD[6]. The inhibitory effect of CAPE on production and its ability to scavenge free radicals and the peroxynitrite anion may be responsible for the protective effect of CAPE on renal and liver tissues. The reduced MDA and NO levels after CAPE treatment indicate that CAPE has the intracellular antioxidant enzyme SOD converts superoxide radicals to hydrogen peroxide [40,41]. Catalase and GSH-PX, detoxify hydrogen peroxide to water [42]. Reactive oxygen species act as subcellular messengers in complex processes as mitogenic signal transduction gene expression and regulation of cell proliferation when they are generated or when defence system are impaired [41, 43]. Some of the studies suggest that ROS are envolved in the action of EMF on biological system [6,19]. It was found in many studies that ROS are involved in oxidative damage of cellular macromolecules as lipids, proteins, and nucleic acids in tissues [6, 37-39]. SOD, CAT, and GSH-PX protect cells from ROS.

Treatment by antioxidant in animal and human could be beneficial in preventing some complications of low frequency EMF [19].

In this study we used the antioxidant, anti-inflammatory caffeic acid phenethyl ester (CAPE) for protection against oxidative stress induced by EMR. We found that CAPE treatment of exposed rats reversed the above-mentioned oxidative effects. CAPE-treated group showed comparable levels of NO, CAT and SOD in kidney tissues as the control group. In liver tissues, CAPE-treated group had NO levels comparable to control group.

Our findings agreed with Hepsen et al [44] who found that CAPE with concentration of 10um completely blocks the production of ROS in human neutrophils and in the xanthine /xo system. It has been reported that CAPE suppresses lipid peroxidation,
displays antioxidant activity and inhibits lipooxygenase activities.

Our results are concordant with that of Ozguner et al.[18] They reported change in activities of antioxidant enzymes with NO, MDA and NAG levels in rat kidneys exposed to 900MHz EMF. Similar results were also reported by Oktem et al.[12] They found that tissue MDA and urine NAG levels increased and SOD, CAT, and GSH-Px activities were reduced in the EMR-exposed rats.

Koyu et al.[41] reported decreased activities of GSH-Px and SOD in the liver of rats exposed with 900 MHz EMF. This suggests increased lipid peroxidation in the liver of exposed rats. Meanwhile the elevated enzyme activity of GSH-Px in the exposed with 900 MHz EMF+CAPE groups reflected that enzyme played important role in clearing away excessive free radical. These findings are similar to the results of other investigators studying antioxidant enzymes in relation to risk factors in subjects and animals exposed to EMF [6,19].

The role of increased oxidative stress in the pathophysiology of adverse effect of EMR was previously demonstrated in rabbits [7] and in human erythrocytes [19]. Moustafa et al. [19] suggested that acute exposure to radiofrequency fields of cellular phones may enhance lipid peroxidation and reduce the activation of SOD and GSH-Px.

Similar effects have been demonstrated in cardiac tissue, where increased levels of MDA and NO and decreased levels of myocardial SOD, CAT and GSH-Px activities were the result of exposure to 900 MHz mobile phones. CAPE exhibited a protective effect on oxidative heart impairment in rats.[45]

In conclusion, this study showed that exposure to 900 MHz EMF is accompanied by biochemical evidence suggestive of oxidative stress in kidney and liver tissues. These effects are reversible with co-administration of caffeic acid phenethyl ester (CAPE). CAPE may be protective against tissue damage induced by EMF through suppression of oxidative stress.

References:
Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes. Bioelectromagnetics; 24(3):182-188.


