MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (6): 1149-1151, 2009
ISSN: 1680-5593

The Evaluation of Epigallocatechin Gallate and N-Acetylcysteine on Serum Testosterone Levels in Male Guinea Pigs Expose to Cell Phone Microwave

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Abstract: Recently, there has been a few information on the effects of mobile phone-exposed on reproduction. The aim of this study to determine the effects of antioxidants namely EGCG and NAC on the serum testosteron levels in the cell phone-exposed guinea pigs. Three-months-old male Guinea pigs were randomized into 6 groups; sham group, not exposed to mobile phone, NAC administration group (300 mg kg^{-1} i.p. during 7 days), 20 min mobile phone-exposed group with NAC administration, 20 min mobile phone-exposed group, EGCG administration group (25 mg kg^{-1} i.p. during 7 days) and 20 min mobile phone-exposed group with EGCG administration. During the exposure of every guinea pig, external E fields were measured by NARDA EMR 300 and type 8.3 probe. Guinea pigs were exposed to RFR averaged as 11.2±0.5 V m^{-1} for 20 min a day during 7 days. Serum testosterone levels were analysed by ELISA. The serum testosterone levels were found insignificantly increased in mobile phone-exposed group compared to the control and the other groups ($p>0.05$). Likewise, the serum testosterone levels were not found significantly different in the N-Acetyl L-Cysteine (NAC) and Epigallocatechin Gallate (EGCG) administrated groups compared to the sham group.

Key words: Epigallocatechin gallate, N-Acetylcysteine, testosterone, cell phone, guinea pig

INTRODUCTION

Use of mobile and desktop cellular telephones results in exposure to microwaves. Exposure is characterized through the Specific Absorption Rate (SAR) expressed as watt kg^{-1}. The area where the cellular telephone is placed, receives the highest Radio Frequency (RF) exposure (Hardell *et al*., 2006). Electromagnetic Radiation (EMR) or radio frequency fields of cellular mobile phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation and by changing the antioxidant defense systems of human tissues, thus, leading to oxidative stress (Ozguner *et al*., 2005; Kismali *et al*., 2009).

N-Acetyl L-Cysteine (NAC) is a thiol, a mucolytic agent and a precursor of L-cysteine and reduced glutathione. NAC is a source of sulfhydryl groups in cells and scavenger of free radicals as it interacts with ROS such as OH and H_2O_2. NAC can scavenge ROS, increase glutathione levels, undergo autooxidation (and produce H_2O_2) and serve as reducing agent. NAC has been an extensively utilized tool for investigating redox sensitivity of biological or pathological processes (Zafarullah *et al*., 2003). The antioxidant components in green tea include Epigallocatechin Gallate (EGCG), Epicatechin Gallate (ECG), Epigallocatechin (EGC) and Epicatechin (EC). All of these components are catechin-based flavonoids and have antioxidant properties (Graham, 1992; Zhang and Osborne, 2006). The compound Epigallocatechin Gallate (EGCG) is the major catechin found in green tea and has been described as being a more active antioxidant than the other components (Nagle *et al*., 2006). Importantly, total plasma antioxidant activity has been shown to be increased following significant intake of green tea (Zhang and Osborne, 2006). Green tea catechins and polyphenols are effective scavengers of physiologically relevant reactive oxygen and nitrogen species *in vitro*, including superoxide, peroxyl radicals, singlet oxygen, peroxynitrite and hypochlorous acid (Frei and Higdon, 2003). EGCG has protective effects against lipid peroxidations (Higuchi *et al*., 2003; Kismali *et al*., 2009). Plasma malondialdehyde (MDA, an index of lipid peroxidation) level has been shown to be increased in 10 min mobile phone exposed guinea pigs, while EGCG causes a significant reduction of MDA levels (Kismali *et al*., 2009).

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The aim of this study is to determine the effects of antioxidants namely EGCG and NAC on the serum testosteron levels in the mobile phone-exposed guinea pigs.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University. All the animal procedures were performed in accordance with the approved protocol.

In this investigation, 48 each 3-months-old male Guinea pigs divided into 6 groups as mentioned at Table 1.

Animals were placed inside the cage just at the beginning of the experiment in order to reduce the stress factor. Cages, made of transparent plastic with the dimensions of 8×10×18 cm have efficient holes for animals breathing. RF source, Nokia 3210 mobile phone with 0.81 W kg^{-1} digital SAR value was positioned on the cage where the antenna of the mobile phone is above the head of the guinea pig. While, mobile phone is off mode as the sham exposure condition, it was in talking position during the exposure conditions. During the exposure of every Guinea pig, external E fields were measured by NARDA EMR 300 and type 8.3 probe. Measurements were taken for duration of 20 min/2 sec and the data saved to the computer connected to device via fiber optic cable. We had 300 instantaneous data for 20 min exposure. Averages and the statistical deviations of the whole instantaneous data were derived before statistical analysis. Guinea pigs were exposed to RFR averaged as 11.2±0.5 V m^{-1} for 20 min a day during 7 days.

Biochemical analysis: Blood was collected by cardiac puncture into noadditive tubes. Serum testosteron levels of guinea pigs were measured by Enzyme Linked Immunosorbent Assay (ELISA). ELISA kits were purchased from dbc-Diagnostics (Canada).

Table 1: Experimental groups

Group 1	Sham exposed group as controls (All control groups injected intraperitoneally (i.p.) with 1 mL isotonic saline solution in order to prevent the occurrence of stress from injection during 7 days)
Group 2	Sham exposed with N-Acetyl L-Cysteine (NAC) administration group (NAC was treated (300 mg kg^{-1}) i.p. during 7 days)
Group 3	20 min mobile phone-exposed group with N-Acetyl L-Cysteine (NAC) administration (NAC was treated (300 mg kg^{-1}) i.p. Thirtymin before the exposure period during 7 days)
Group 4	20 min mobile phone-exposed group (20 min mobile phone-exposed groups injected intraperitoneally (i.p.) with 1 mL isotonic saline solution before 30 min from exposure during 7 days)
Group 5	Sham exposed with (-) Epigallocatechin Gallate (EGCG) administration group (EGCG was treated (25 mg kg^{-1}) i.p. during 7 days)
Group 6	20 min mobile phone-exposed group with (-)-epigallocatechin-Gallate (EGCG) administration (EGCG was treated (25 mg kg^{-1})i.p. Thirty min before the exposure period during 7 days)

Statistical analysis: Kruskal-Wallis test was carried out in order to find any differences between groups.

RESULTS AND DISCUSSION

The Electromagnetic Field (EMF) emitted from mobile phones can effect reproductive organs. Dasdag *et al.* (2003) reported no effects of cell phone use on the testis of rats, whereas Davoudi *et al.* (2002) observed declining levels of rapid progressive spermatozoa among a small study group of cell phone users. A recent study found a moderate correlation between mobile phone use and semen quality (Fejes *et al.*, 2005). The effect of electromagnetic field on the reproductive organs depends on its frequency and power.

In the 1800 MHz, GSM-like microwave exposed mice had been significantly elevated serum testosterone level (7.85±1.08 ng mL^{-1}) compared with the sham exposed controls (5.12±0.79 ng mL^{-1}). But the differences of serum testosterone level had been found within the physiological range. No microwave exposure related histopathological alterations were found in testicles, epididymes, adrenals, prostates, kidneys, hearts, livers and pituitary glands (Forgacs *et al.*, 2006) (Table 2).

According to Forgacs *et al.* (2006), the effect of GSM-like microwave exposure on serum testosterone level may be associated with its possible effect on melatonin secretion of pineal gland. Some recent human data indicate that prolonged use of GSM mobile phones may lead to reduced melatonin production. It is known that melatonin decreased directly the testosterone secretion in Leydig cells.

Melatonin is an antioxidant and it is a potent and efficient endogenous free radical scavenger. It can also, protect molecules from oxidative damage by stimulating GSH-Px activity which metabolizes hydrogen peroxide to H_2O_2 (Ozguner *et al.*, 2005).

In this study, the serum testosterone levels were found insignificantly increased in mobile phone-exposed group compared to the control and the other groups ($p>0.05$). The reason of insignificantly increased of serum testosterone levels in mobile phone-exposed group may be related with duration of exposure and the power of EMF. The serum testosterone levels were not found significantly different in the N-Acetyl L-Cysteine (NAC) and Epigallocatechin Gallate (EGCG) administrated groups compared to the sham group. NAC and EGCG have antioxidant properties (Zafarullah *et al.*, 2003; Zhang and Osborne, 2006). The antioxidant effect of NAC and EGCG may be lead to reduced the negative effect of mobile phone on melatonin production.

Table 2: Testosterone levels of guinea pigs (ng mL^{-1})

Groups	N	Mean	SE	Min.	Max.
1	6	1.71	0.26	1.11	2.95
2	7	1.87	0.27	0.75	3.02
3	7	1.68	0.54	0.59	4.73
4	6	3.01	0.57	1.09	4.77
5	8	1.64	0.21	0.79	2.39
6	6	1.60	0.32	0.49	2.41

CONCLUSION

To explain the mechanism and the site effect of GSM-like exposure on serum testosterone level further investigation of other parameters like FSH, LH, melatonin levels etc. is necessary.

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