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Effects of Green Tea on Mineral Levels of Liver and Testis of Guinea Pigs Electromagnetic Field Emitted by Mobil Phones

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Abstract: It was reported that the effects of green tea on the mineral levels of testis and liver of Guinea pigs exposed to a 900 MHz electromagnetic field. Four experimental groups labeled as controls (Group A), irradiated (Group B), irradiated receiving green tea extract (Group C) and green tea only (Group D) were formed with seven randomly chosen animals of both sexes in each group. After exposure for one month, the animals were sacrificed by decapitation and testis and liver samples were collected for biochemical analysis. In female Guinea pigs irradiation with and without green tea as well as green tea alone caused significant changes of the iron levels in liver, but no significant changes of manganese, copper, zinc and the copper/zinc ratio. In males, irradiation caused significant increases of manganese and a decrease of the iron levels in liver and of manganese, copper, zinc in testis. Combined with green tea, electromagnetic radiation resnlted in changes of manganese, iron, copper and copper/zinc ratio in liver and of manganese only in testis. Green tea alone changed the levels of hepatic iron, zinc and copper/zinc ratio and of testicnlar concentrations of iron and zinc. The highest levels of copper were found in the liver tissue of the irradiated animals that were also treated with green tea. From present findings we can state that testis tissue is more sensitive to electromagnetic radiation than liver tissue, showing greater changes in trace mineral metabolism. Green tea brings the trace element levels to near normal values; supporting the idea that green tea as a supplement has a protective effect against the damaging effects of electromagnetic radiation.

Key words: Green tea, mineral levels, tissues, electromagnetic radiation

INTRODUCTION

Mobile telephones are communication devices that work in the ultra high frequency range (Ferreira *et al*., 2006; Croft *et al*., 2002; Dasdag *et al*., 2003). Its use has increased exponentially all over the world, leading scientists to become interested on the biological effects of electromagnetic field (EMF) and its possible consequences on human health. Several *in vitro* studies have shown detrimental effects of mobile telephone radiation on brain, kidney and heart tissues and on hematological parameters, nucleic acid damage and oxidative stress (Ferreira *et al*., 2006; Croft *et al*., 2002; Dasdag *et al*., 2003; Guney *et al*., 2007; Syldona, 2007; Karger, 2005).

Tea is the most consumed drink in the world after water. Green tea is a non-fermented tea, richer in antioxidant flavonoids than black tea. Recent human studies suggest that green tea may contribute to a reduction in the risk of cardiovascnlar disease, some forms of cancer, oral health and has other physiological functions that include anti-hypertensive and anti-fibrotic properties, body weight control, antibacterial and antiviral activity, solar ultraviolet protection, increases bone mineral density

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and protects the nervous system (Hodgson *et al*., 2000; Cabrera *et al*., 2006). Consumption of green tea led to an increase of antioxidant activity and reduced lipid peroxidation in rats (Frei and Higdon, 2003; Rietveld and Wiseman, 2003).

Tea is rich in catechins, teaflavins and tearubigins, which are thought to contribute to the health benefits of tea. The flavonoids found in green- and black tea prove antioxidant effect *in vitro* and most probably are so also *in vivo*. As antioxidants, catechins and teaflavins show *in vivo* and *in vitro* activities that are comparable to those of α-tocopherol and higher to that of ascorbate (Nakagawa and Yokozawa, 2002; Guo *et al*., 1999; Hodgson *et al*., 2000). Specifically, green tea shows pharmacological effects that include antioxidant- and iron-chelating activities (Srichairatanakool *et al*., 2006). The iron and copper chelating ability of tea polyphenols may contribute to their antioxidant activity by preventing redox-active transition metals from catalyzing free radical formation (Record *et al*., 1996; Rice-Evans *et al*., 1997).

Trace elements function as activators of enzyme systems or as constituents of organic compounds. For example, zinc and copper play a role in quenching of free radicals through reduction of the peroxidation ratio and breaking the free-radical production chain (Costanzo *et al*., 1995). Other elements are co-factors of several antioxidant enzyme systems (Fridovich, 1995). The levels of trace elements can vary considerably depending on age, sex, diet, geographical and climatic conditions, or genetic factors. The concentration of an element may also change by physical or chemical factors (Shen *et al*., 2005; Dede *et al*., 2003). An initial rise of trace element concentrations has been reported after the application of radiation (Dede *et al*., 2003; Aladzhov and Logofetov, 1990; Chatterjee *et al*., 1994).

As a known source of electromagnetic radiation, mobile telephones have become an element of concern in recent times. These devices are generally carried in trousers' pockets, prompting scientists to question their possible role as mutagens altering testicular function and structure (Ozguner *et al*., 2005; Dasdag *et al*., 2003).

In the present study the effects of green tea on liver and testicular tissue levels of iron, copper, zinc and manganese were studied in Guinea pigs exposed to an EMF originating from a commercially available mobile telephone.

MATERIALS AND METHODS

A total of 56 Guinea pigs (28 males, 28 females) weighing 600-800 g were used in this study. This study was conducted in Physiology and Biochemistry Laboratories of Faculty of Veterinary Medicine, at April-July 2008. Animal handling and maintenance was done following the recommendations of the Ethics Committee, Yuzuncu Yil University. The animals were housed in 60×90×45 cm cages kept under normal conditions and standard diet for 1 week. After this time twenty-eight animals of both sexes were chosen at random and evenly divided into four groups, as follows:

- **Group A (n = 7):** Controls, maintained under standard conditions, normal diet
- **Group B (n = 7):** Animals exposed to an electromagnetic field daily for one month (see below for method of exposure)
- **Group C (n = 7):** Animals exposed to an electromagnetic field daily receiving an oral dose of 100 mg/kg/day of green tea extract for one month
- **Group D (n = 7):** Animals receiving 100 mg/kg/day green tea extract orally for one month

Animals receiving radiation were exposed to a 900 MHz field (217 Hz pulse rate, 2 W maximum peak power, SAR (Specific Absorbtion Rate) 0.95 W kg^{-1} (watts per kilogram) from a mobile telephone that was kept on standby for 23 h 40 min and functioning at normal full power for 20 min a day. The mobile phones were placed 0.5 cm under cages. Groups A and D were kept in a separate room to avoid any chance of exposure to EMF radiation.

At the end of the 30 day experimental period the animals were sacrificed by decapitation and the testis and liver were removed and washed twice in cold saline and stored at -80°C in glass containers until needed for analysis. To determine the concentrations of iron, copper, zinc and manganese, the samples were digested by adding 1 mL of concentrated nitric acid at room temperature and then diluted to 5 mL with doubly distilled water and then analyzed for mineral elements by means of a Unicam 929 Atomic Absorption Spectrophotometer (UK) following literature procedures (Hazelrigg *et al*., 2004; Greenberg *et al*., 1996; Longbottom *et al*., 1994).

The results are expressed as Mean±SD. Duncan's test was used for statistical analysis. Differences were considered significant at $p<0.05$.

RESULTS

In a given line, significant differences between means ($p<0.05$) are identified with different lower case letters. If letters are the same, the difference between values is non significant.

Table 1 shows the element levels in liver of female Guinea pigs. No significant differences were seen in the concentrations of manganese, copper, zinc and of the copper/zinc ratio. The iron levels in groups B-D were significantly lower than those of the controls.

Exposure to EMF with and without administration of green tea caused the manganese levels to increase relative to controls. The iron concentration was decreased in animals exposed to EMF, but the decrease was less pronounced in animals exposed to radiation receiving green tea extracts. Irradiation and green tea treatment (Group C) resulted in higher copper levels (Table 2). Green tea alone resulted in a decrease of zinc levels. Consequently, the copper/zinc ratio was lowest in the controls and higher in the irradiated and green tea treated animals (Group C).

Table 3 shows the mineral levels in testis for all groups. Irradiation with or without green tea administration resulted in decreased manganese levels relative to controls, but treatment with the tea extract did have a normalizing effect of the manganese concentration. The iron levels of tea-ouly group were higher than in all other groups. The lowest iron was seen in the irradiated animals and it was near

Table 1: Element levels in liver of female guinea pigs subject to electromagnetic radiation, with or without green tea extract supplementation

Elements	Group A	Group B	Group C	Group D
Manganese (mg L^{-1})	0.37±0.05	0.38±0.05	0.34±0.022	0.34±0.00
Iron (mg L^{-1})	5.40±0.25^{a}	3.90±0.06^{b}	4.00±0.14^{b}	4.40±0.27^{b}
Copper (mg L^{-1})	2.90±0.37	2.60±0.12	2.40±0.39	2.50±0.73
Zinc (mg L^{-1})	3.00±0.53	2.90±0.32	2.90±0.20	2.70±0.21
Copper/zinc ratio	0.90±0.03	1.00±0.11	0.80±0.03	0.93±0.19

Data were expressed as Mean±SD, Differences were considered significant at $p<0.05$, There are no differences between groups with same letter(s), Group A: Controls, Group B: Guinea pigs kept under influence of an electromagnetic field for 1 month, Group C: Same as Group B, treated with Green tea extract, Group D: Treated with Green tea, no electromagnetic radiation

Table 2: Element levels in liver of male guinea pigs subject to electromagnetic radiation, with or without green tea extract supplementation

Elements	Group A	Group B	Group C	Group D
Manganese (mg L^{-1})	0.35±0.13^{a}	0.45±0.01^{b}	0.40±0.01^{c}	0.36±0.013^{a}
Iron (mg L^{-1})	5.60±0.12^{a}	4.90±0.24^{b}	4.00±0.27^{c}	4.10±0.19^{c}
Copper (mg L^{-1})	2.20±0.22^{a}	2.30±0.13^{a}	3.30±0.25^{b}	2.40±0.15^{a}
Zinc (mg L^{-1})	3.90±0.29^{a}	3.60±0.01^{a}	3.70±0.21^{a}	2.80±0.15^{b}
Copper/zinc ratio	0.55±0.02^{a}	0.65±0.02^{b}	0.90±0.02^{c}	0.84±0.02^{c}

Data were expressed as Mean±SD, Differences were considered significant at $p<0.05$, There are no differences between groups with same letter(s), Group A: Controls, Group B: Guinea pigs kept under influence of an electromagnetic field for 1 month, Group C: Same as Group B. treated with Green tea extract, Group D: Treated with Green tea, no electromagnetic radiation

Table 3: The minerals levels of testis of guinea pigs subject to electromagnetic radiation, with or without green tea extract supplementation

Elements	Group A	Group B	Group C	Group D
Manganese (mg L^{-1})	0.18 ± 0.01^{a}	$0.12 \pm 0.01b^{c}$	0.14 ± 0.01^{b}	0.17 ± 0.01^{ab}
Iron (mg L^{-1})	1.90 ± 0.22^{ab}	1.50 ± 0.14^{a}	1.60 ± 0.10^{ab}	2.20 ± 0.25^{b}
Copper (mg L^{-1})	0.37 ± 0.06^{a}	0.21 ± 0.04^{bc}	0.23 ± 0.03^{ab}	0.33 ± 0.03^{a}
Zinc (mg L^{-1})	5.20 ± 1.80^{a}	3.80 ± 0.07^{b}	5.20 ± 0.37^{ab}	7.50 ± 0.26^{c}
Copper/zinc ratio	0.05 ± 0.02^{a}	0.06 ± 0.01^{b}	0.05 ± 0.01^{ab}	0.05 ± 0.01^{ab}

Data were expressed as Mean±SD, Differences were considered significant at $p<0.05$, There are no differences between groups with same letter(s), Group A: Controls, Group B: Guinea pigs kept under influence of an electromagnetic field for 1 month, Group C: Same as Group B. treated with Green tea extract, Group D: Treated with Green tea, no electromagnetic radiation

normal in those that were irradiated and treated with the tea extract. Copper was lowest in the irradiated group. The copper level of the irradiated animals receiving green tea was near those of the controls and tea-ouly groups. Zinc was higher in the tea-ouly group. Exposure to EMF resulted in the lowest zinc levels, which were near normal in animals that were irradiated and received tea supplements. The copper/zinc ratio was higher in the irradiated animals relative to controls.

DISCUSSION

The biological effects of electromagnetic radiation including that from mobile telephones has been linked to reactive oxygen species that lead to increased free radical production and lipid peroxidation in some tissues (Ferreira *et al*., 2006; Croft *et al*., 2002; Dasdag *et al*., 2003; Guney *et al*., 2007; Syldona, 2007; Karger, 2005).

It has been suggested that tea consumption can disrupt the homeostasis of some trace elements, particularly iron, increasing the risk of anemia in humans and animals. Green tea consumption significantly reduced the serum, liver, spleen, kidney, femur and heart iron stores (Hamdaoui *et al*., 1997, 2005; Greger and Lyle, 1988). As such, green tea could be relevant for the clinical management of iron overload and oxidative stress (Srichairatanakool *et al*., 2006). Rats fed diets containing green or black tea or tea infusions experienced minimal changes in tissue iron levels or in iron absorption (Greger and Lyle, 1988).

In this study, in liver tissues of female and male Guinea pigs, the iron concentrations were higher in the controls than in all other groups. Irradiation with or without green tea administration resulted in lower iron concentrations. The same effect was observed in testis, although green tea minimized the effect. These differences between liver and testicular tissues are interesting, especially because liver iron is lowered by administration of green tea alone. This is consistent with the suggestion that green tea has iron chelating properties and may also disrupt redox processes in which iron is involved (Srichairatanakool *et al*., 2006; Frei and Higdon, 2003).

Record et al reported that black and green teas seemed to have a noticeable effect on tissue manganese levels. In particular, a lower level of brain manganese was associated with green tea consumption (Record *et al*., 1996). Although we observed no statistical differences of manganese in liver tissues of female Guinea pigs, in males increased after EMF exposure. Green tea alone did not change liver manganese levels. In testis the manganese levels were highest in the controls. Irradiation resulted in lower manganese levels with and without tea extracts administration, but it should be noted that green tea did have a normalizing effect.

Zinc is involved in stabilization of the cell membrane and in prevention of oxidative damage caused by free radicals (Dede *et al*., 2003; Zowczak *et al*., 2001). Polyphenol extract was shown to chelate metals *in vitro* but the type of polyphenol-containing beverage supplied affected the level of zinc (Record *et al*., 1996). In another study, the serum, kidney, heart and femur levels of zinc in rats administered with green tea increased in a dose-dependent fashion (Hamdaoui *et al*., 2005). It was found that zinc is reduced in liver tissue of male but not female Guinea pigs green tea treated alone.

Treatment with green tea increased the zinc levels in testis and the opposite happened after exposure to EMF. The reduction of zinc and manganese levels in testis after irradiation suggest that there might be a risk of damage to the reproductive system of males due to exposure to low levels of electromagnetic radiation such as the one emanated from mobile telephones (Underwood, 1977; Bedwal and Bahuguna, 1994).

In addition, certain trace elements such as copper and zinc are believed to play an important role in carcinogenesis. Several studies have shown elevation of copper and decrease of zinc in various malignancies (Gupta *et al*., 2005, 1993). Copper is an essential element that contributes to important intracellular metabolic events and copper ion levels are elevated in a number of malignancies (Gupta *et al*., 1993; Han *et al*., 2003). Also, the zinc and copper levels of animals were drastically changed by irradiation (Dede *et al*., 2003; Aladzhov and Logofetov, 1990; Chatterjee *et al*., 1994). Present results are consistent with these reports. Lower zinc levels in testis may be evidence of damage to testis due to electromagnetic radiation. This is consistent with the results of Gupta *et al*. (2005) who reported that the tissue zinc level of malignant gallbladder tissue was significantly lower than that of benign gallbladder tissue. In the case of copper irradiation lowered its levels in males. Concurrent green tea administration brought them to values near those of controls and of animals receiving tea extract without irradiation in testis tissue.

Hamdaoui *et al*. (1997) reported that tea infusion significantly increased copper levels in whole blood, but not in liver. Contrary to this, Greger and Lyle (1988) found that instant or black tea elevated liver copper levels. Copper is a cofactor of SOD and CAT that has a protective effect against X-ray-originated lipid peroxidation. The activity of these enzymes was increased after irradiation (Russanov *et al*., 1979). There are reports on use of radiation for both therapy and diagnosis causing an initial increase of serum copper concentrations, followed by elevation of ceruloplasmin values, which is considered to be an indicator of radiation cell damage and disease caused by irradiation. It also may be capable of scavenging reactive oxygen species (Healy and Tipton, 2007; Gupta *et al*., 2005).

A mutual antagonism related to the absorption of zinc and copper has been reported (Zowczak *et al*., 2001). Some authors have reported a strong correlation between the stage of various malignancies and elevated serum copper levels and copper/zinc ratio on the one hand and decreased serum zinc levels on the other (Gupta *et al*., 1993; Han *et al*., 2003; Russanov *et al*., 1979). In this study, we observed no significant differences in the copper/zinc ratio in liver of female Guinea pigs but in liver of males the lowest values of this ratio were seen in the controls. This ratio was increased by green tea treatment. In testis, the copper/zinc ratio was higher in the animals under irradiation compared to controls. The groups treated with green tea treated also had increased values in levels similar to those of the irradiated animals.

In conclusion, administration of green tea appears to be useful in reinforcement of antioxidant systems that protect the organism against cell damage by free radicals induced by electromagnetic fields. In most instances, the trace element levels of animal tissues that were exposed to EMF were changed but in those that were also administrated a green tea extract had values that were similar to non-radiated controls. These findings thus support the premise that green tea administration has a positive effect on tissue mineral status.

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