

Effect of Green Tea Extract in Reducing Genotoxic Injuries of Cell Phone Microwaves on Bone Marrow

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Article information

Article history:

Received: 16 May 2012

Accepted: 22 Aug 2012

Available online: 17 May 2013

ZJRMS 2013; 15(11): 39-44

Keywords:

Balb/C

Cell Phone

Green Tea

Micronucleus Test

Polychromatic erythrocyte

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Abstract

Background: Green tea (*Camellia sinensis*) extract is rich source of natural antioxidants specially catechin that is quickly absorbed into the body and it has cancer protective, anti microbial and anti inflammation effects. In this study has been studied role of green tea extract against genotoxic damage induced by cell phone microwaves on bone marrow polychromatic erythrocytes of adult male Balb/C mouse.

Materials and Methods: In this experimental study 40 mouse were divided into five groups, control animals were located under natural condition, sham -exposed animals were prepared by experimental condition without cell phone waves radiation. Experimental 1 group that irradiated at cell phones for 4 days (3 hours/day) and experimental 2 groups were injected intraperitoneal 100 mg/kg green tea extract for 5 days and experimental 3 group that irradiated at active mobile phones for 4 days (3 hours/day) and were injected intraperitoneal 100 mg/kg green tea extract for 5 days. After treatment period micronucleus test was evaluated in polychromatic erythrocytes on bone marrow. The quantitative data was analyzed by ANOVA and Tukey test with using of SPSS-13 software at the level of $p < 0.05$.

Results: Based on this study, treatment with extracts of green tea decreased micronucleus frequency in bone marrow polychromatic erythrocytes of Balb/C mouse that irradiated at cell phone microwave (0.92 ± 0.129), ($p < 0.001$).

Conclusion: Cell phone microwaves (940 MHz) increased micronucleus on bone marrow polychromatic erythrocytes of male Balb/C mouse, but green tea had inhibitory effect and it decreased the average number of micronucleus.

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Introduction

Tea is the second-most consumed beverage in the world (water is the first). It has been used medicinally for centuries in India and China. The tea shrub (genus *Camellia*, family Theaceae) is a perennial evergreen with its natural habitat in the tropical and subtropical forests of the world. Cultivated varieties are grown widely in its home countries of South and South East Asia, as well as in parts of Africa and the Middle East [1]. The plant *Camellia sinensis* yields a variety of white, green, black and oolong tea. Tea is manufactured in four basic forms. Green tea is prepared in such a way as to preclude the oxidation of green leaf polyphenols. During black tea production, oxidation is promoted so that most of these substances are oxidized. Oolong tea is a partially oxidized product. White tea is made from new growth buds and young leaves that have been steamed to inactivate polyphenol oxidation and then dried [2]. Human research is very promising using green tea as treatment for various cancers. Since 1970s, epidemiological studies of cancer have shown that in parts of the world where green tea is consumed, the incidence of solid tumor cancers such as breast, lung, and gastrointestinal cancers is lower and mouse-model testing of green tea's cancer prevention properties has shown they protect against solid tumors [3]. Studies have

indicated that tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox active transition metal ions and hence tea may reduce the risk of a variety of illnesses, including different types of cancer, heart disease, liver disease and coronary heart diseases [4]. The fresh tea leaves contain four major catechins such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) [5]. EGCG makes up about 10-50% of the total catechin content and appears to be the most powerful of the catechins with antioxidant activity about 25-100 times more potent than vitamins C and E [5]. Tea catechins and polyphenols are effective scavengers of reactive oxygen species in vitro and may also function indirectly as antioxidants through their effects on transcription factors and enzyme activities [4] and anti-viral activity as well as their ability to serve as an anti-plaque forming agent, anti-carcinogenic agent, anti-cardiovascular disease agent, hypotensive agent, hypocholesterogenic agent and blood sugar reductive agent [6]. Catechins have been reported to have many pharmacological properties such as effects of antioxidative, antimutagenic, antitumor, induction of apoptosis and inhibitory effects of angiotensin, converting enzyme hyaluronidase, telomerase, α -amylase and

glucocyltransferase activity [7]. Numerous in vitro studies reveal that EGCG scavenges a wide range of free radicals including singlet oxygen, superoxide anions, peroxy radicals and also the most active hydroxyl radicals, which initiates lipid peroxidation [8]. Radio frequency (RF) energy is a type of nonionizing radiation that produced by cellular phone and it is not strong enough to cause ionization of atoms and molecules. Cellular phones emit low levels of RF in the microwave range while being used. Several experimental studies demonstrated that exposure to electromagnetic fields had adverse effects on the reproductive system [9]. Statistically significant changes were observed in the rapid progressive, slow progressive and no-motility categories of sperm movement [9]. This is especially important as virtually everybody in the world is now exposed to this kind of physical environmental pollutant that is, together with electromagnetic fields from overhead power lines. Sometimes referred to as electrosmoke. Effects on the genetic material (DNA) are considered very important since damage to the DNA can among other effects lead to the development of cancer. It is assumed that Radiofrequency Radiation (RFR) does not induce genetic damage in cells after in vitro or in vivo exposure. But suspicion remains, especially because a number of studies indeed show RFR induced genetic damage in a number of cell types and for particular conditions of exposure [10]. RF radiation exposure from cell phones is associated with tumor risk, specifically risk of glioma, meningioma, acoustic neuroma and parotid gland tumors in the long-term (≥ 10 -year) users [11]. Decreases in cell growth rate and survival were found in hamster ovarian cells exposed to radiofrequency radiation over brief time periods but at high specific absorption rates, whereas increased DNA fragmentation and cell death and altered reproductive frequency were seen in fruit flies exposed to cell phone radiation [11]. An excess of malignant tumors was found in animals exposed for 1 to 2 years to radiofrequency radiation at levels comparable to current standards, while increased levels of DNA damage via "strand-breakage" have been reported in rat brain cells and in human fibroblasts and rat granulosa cells after exposure to cell and cordless phone radiofrequency radiation [12]. Non-thermal genotoxic effects of Radiofrequency-Electromagnetic Field (RF-EMF) is convincingly demonstrated by a substantial number of published studies that have been performed with a variety of different test systems. Some studies used more than one test system which will be assigned here to the three principle endpoints of a genotoxication effect on chromosomes, DNA fragmentation and gene mutations [13]. The increasing use of mobile phones and other wireless technologies in our everyday life increased public, governmental and scientific attention to the issue of whether or not adverse effects are induced after exposure to radiofrequency electromagnetic fields of cell phones. In this study has been studied about protective role of green tea extract against genotoxic damage induced by cell phone waves on bone marrow polychromatic erythrocytes of adult male Balb/c mouse.

Materials and Methods

Source of Chemicals: Bovin serum albumin (BSA) were purchased from Sigma chemical Co (St Louis, Mo, USA) and all solvents used for analytical and extraction purposes were purchased from Merck chemical Co (Darmstadt, Germany). All other chemicals were used of analytical and reagent grade.

Animals: 40 adult male Balb/C mice (Razi Institute, Mashhad, Iran), weighing 25-30 g were housed in polypropylene cages (38×22×16 cm) under controlled temperature ($23\pm 1^\circ\text{C}$), humidity 60-70% and ventilated room with a 12/12 hr light/dark cycle as well as free access to food (Javane Dame Khorasan Co.) and water. The animals were bred and kept at the animal room (Department of Biology, Islamic Azad University, Mashhad branch, Iran).

Plant: Plant materials used in this study consisted of the dry green tea leaves purchased from Refah tea (Refah Lahijan Company, Iran).

Extraction procedures: Green tea leaves powdered by mechanical grinder and then fifty grams of green tea leaves powder were extracted with 400 ml of solvent 80% (Vol/Vol) ethanol/water solution at solid material to solvent ratio of 1:10 for 24-48 hours and the mixture was subsequently filtered. After centrifugation, the combined extract was evaporated in a rotary evaporator and freeze-dried and stored at 4°C . Ethanol was used to extract catechin, because it has been reported to be an ideal solvent for the high recovers of tea catechins.

Experimental procedure: In this study 40 male adult Balb/C mouse (25-30 g) were randomly divided into five equals groups. Control animals were located under natural condition and sham -exposed animals were prepared by turning off the cell phone (T-128 China) for four days (3 hours/day). Experimental 1 group that irradiated at 940 MHz of two active cell phones (T-128 China) for four days (3 hours/day) and experimental 2 group were injected intraperitoneally 100 mg/kg/day green tea extract for 5 days and experimental 3 group that irradiated at 940 MHz of two mobile phones for four days (3 hours/day) and were injected intraperitoneally 100 mg/kg/day green tea extract for 5 days. After treatment period, all mice were anesthetized by chloroform and accessories explain both feet off and bone marrow were avoid slowly injection dissolved by fetal bovine serum (GIBCO) and were collected in tube. The tube containing the suspension was placed in centrifuged for 10 min (Kakusan, Japan) with a round 900 rpm and the supernatant was discarded and the remaining drops more slowly shook up till obtained uniform cell solution, then with a suction Pasteur pipette some of the remaining solution put on clean glass slides and was given expansion. After 48 hours fixed them in methanol for 5 minutes and after drying slides were colored with Mai Granvald - Giemsa (Merck, Germany) at Schmid method. In a proper coloring, polychromatic erythrocytes can be observed as well as pink. These cells lack nuclei and micronucleus can be seen to form a small core of purple in the cytoplasm (Fig. 2). In each slide number of

micronucleus were counted in 1000 erythrocyte cells by optical microscope (Nikon, Japan). Executives at all stages of research were committed to ethics about working with animals.

Statistical analysis: The quantitative data was analyzed by ANOVA and Tukey test with using of SPSS-13 software at the Level of $p < 0.05$.

Results

Significant difference were not seen ($p > 0.998$) in the average number of micronucleus between control (3.43 ± 0.605) and shame- exposed groups (3.47 ± 0.253) that were at the two inactive cell phones and this shows clearly that the inactive phone is not very effective on chromosomal damages. However, the waves emitted from activate cell phones increased the average number of chromosomal aberrations and micronucleus frequency in polychromatic erythrocytes of mouse bone marrow. Statistical analysis comparing the experimental 1 group exposed to cell phone waves (5.64 ± 0.308) with control and shame-exposed groups was shown significant increase ($p < 0.001$) in the chromosomal aberrations (Fig. 2). But animals treated with extract of green tea alone (0.76 ± 0.125) (Fig. 3) and using both green tea and mobile phone (Fig. 4) showed a significant decrease (0.92 ± 0.129) in rate induced damage of cell phone microwaves ($p < 0.001$). However, were not showed any significant difference between experimental 2 and 3 ($p > 0.930$), (Fig. 5).

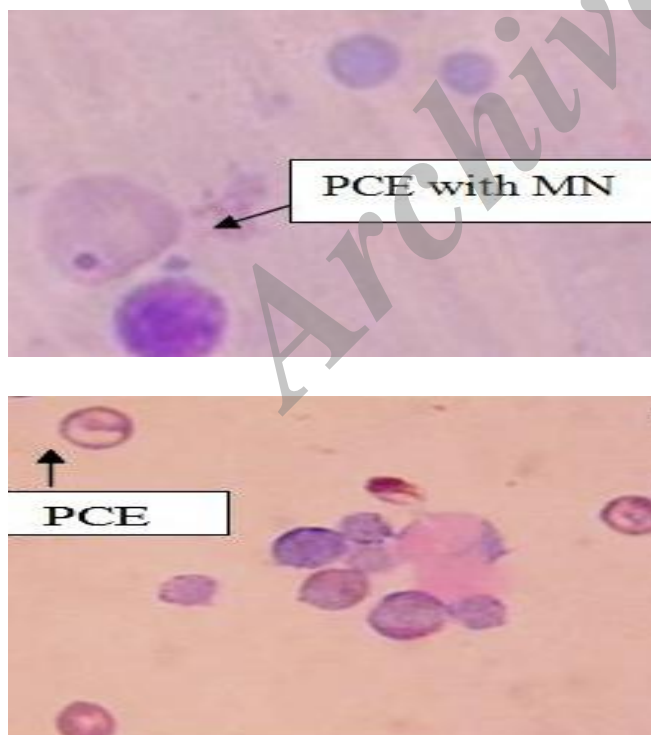


Figure 1. View polychromatic erythrocyte in expanding of bone marrow of adult male Balb/c mice, stained: Mai Granvald – Giem

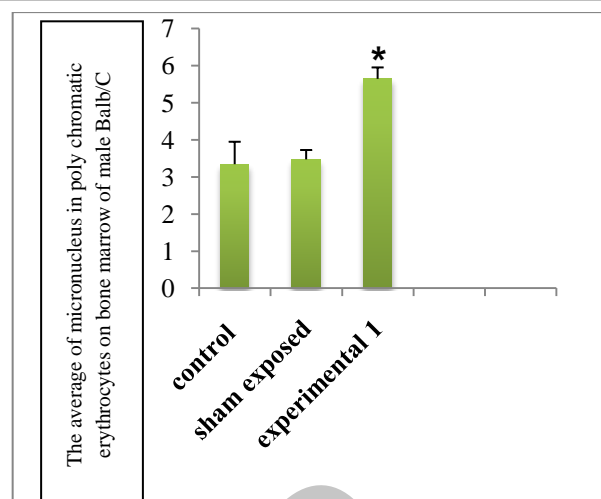


Figure 2. Experimental-1 group that exposure to waves of cell phones indicated significant increase in micronucleus frequency on bone marrow erythrocytes (* $p < 0.001$)

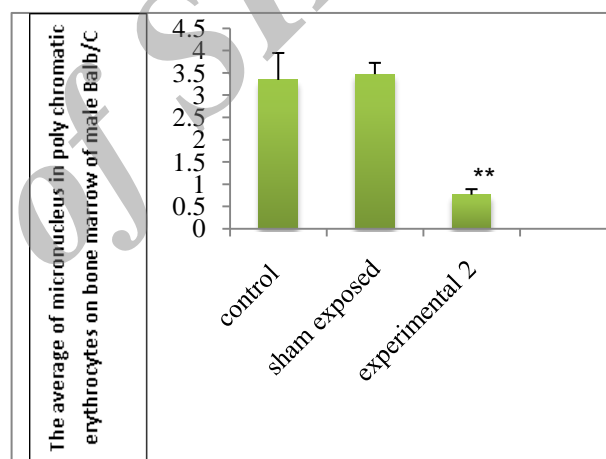


Figure 3. Experimental-2 group that were injected intraperitoneal 100 mg/kg green tea extract indicated significant decrease in micronucleus frequency of bone marrow erythrocytes (** $p < 0.001$)

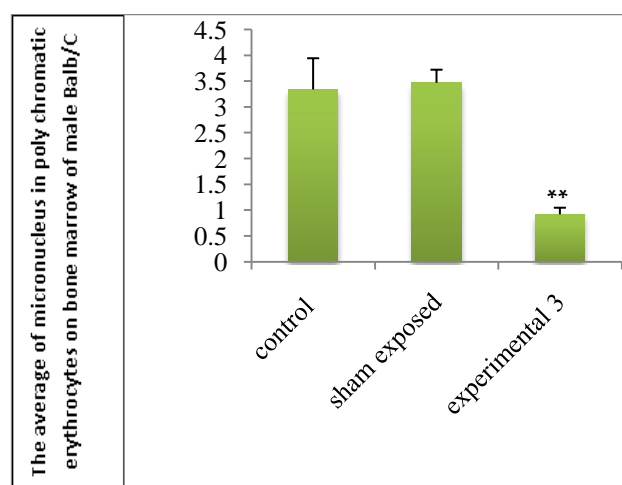


Figure 4. Experimental 3 group that both receiving intraperitoneal 100 mg/kg green tea extract and irradiated, indicated significant decrease in micronucleus frequency of bone marrow erythrocytes (** $p < 0.001$)

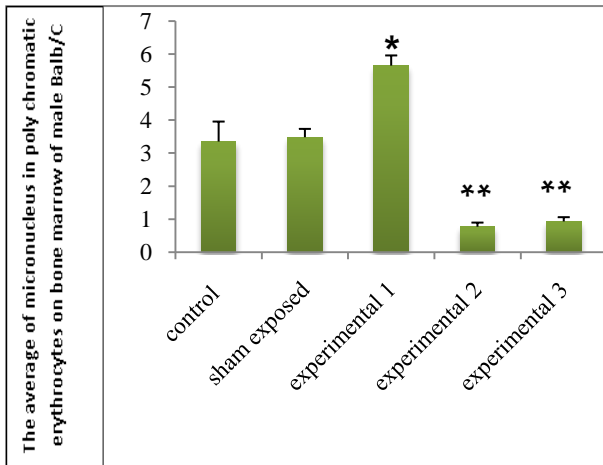


Figure 5. Comparison frequency of micronucleus in polychromatic erythrocytes of experimental groups with control groups. 5.643 ± 0.87 = Mean \pm SD) \cdot (0.001 *significant increase in micronucleus frequency in experimental 1, ($*p < 0.365$) ± 0.92 = Mean \pm SD) \cdot 0.001 < significant decrease in micronucleus frequency in experimental 3, (** $p < 0.001$) (**significant decrease in micronucleus frequency in experimental 2, (** $p < 0.001$)) (** { No significant difference between experimental 2 and 3 ($p > 0.930$)}

Discussion

The results of research related to chromosomal and genetic damages of electromagnetic radiation is very contradictory. In this study, micronucleus had increased in mice exposed to mobile phone waves during the specific time. So, the waves emitted from activate cell phones increased the average number of chromosomal aberrations and micronucleus frequency in polychromatic erythrocytes of mouse bone marrow. But animals treated with extract of green tea alone and using both green tea and mobile phone showed a significant decrease in rate induced damage of cell phone microwaves. Our results conform with results of Desia experiences. He indicated that the number of micronucleus has increased tripled in bone marrow of rats exposed to 910 MHz cell phone waves [14].

The cellular and molecular modifications dependent on duration of exposure, tissue penetration and heat generation, which are in turn related to the intensity and frequency of them [15]. Cell responses may depend upon the signal characteristics, such as waveform (sinusoidal, square), modulation scheme and moreover upon the biological status and type of cells exposed [15]. Studies show that the micronucleus frequency of the Chinese Hamster ovary cells had been placed at different intensity electromagnetic waves for two hours has increased according to the severity of waves [16]. According to Suzuki, electromagnetic field induction increase micronucleus in erythrocytes of male Balb/C mice that it conforms with our results too [17]. While Vijayalaxmi report shows that electromagnetic waves radiation for 30 minutes do not significant increase in number of micronucleus polychromatic erythrocytes of Balb/C mice [18] and Vijayalaxmi report is opposite of our study. It seems the main reason for this paradox is related to

different study conditions. Numerous proposals has been presented on how effects of electromagnetic fields on biological systems that pointed out Mashevich comments about effect of electromagnetic waves on aneuploidy and mutation [19]. In addition, increasing risk of DNA damage and cell proliferation has also been reported [20]. Some also were expressed that cause of genetic and epigenetic damages of cell phones creation are free radicals, changes in gene transcription, changes in proteins folding and production heat shock proteins [21]. In the present experience, green tea inhibited chromosomal damages induce by cell phone waves are conforms with Katiyar results, he has stated that green tea inhibited the inhibitory effects of ultraviolet radiation on DNA repair and prevents cancer spreading [22]. Green tea prevents UV-induced immune suppression through increased levels of IL-12 and as IL-12 has antitumor activity and the ability to repair UVB-induced DNA damage [23]. Green tea catechins inhibit the neuraminidase activity (Neuraminidase is an antigenic glycoprotein enzyme found on the surface of the influenza virus) [24]. Green tea has a strong inhibitory effect on deletion mutations in mitochondrial DNA of human blood lymphocytes to [25]. Studies effect of green tea on liver and testis cells of Pigs exposed to electromagnetic waves 900 MHz cell phone had been shown that electromagnetic can change levels of minerals such as iron, manganese and zinc in tissues but green tea return them to normal level [26].

All of these findings confirm our results. Effect of green tea catechin on enzyme activities and gene expression of antioxidative system in rats liver were exposed microwave radiation indicate that mice treated with catechin are similar to control group [27]. Decreased induce DNA oxidative damage in human lymphocytes has been observed [27, 28]. Antioxidants efficiently of green tea inhibit LDL oxidation and reduce the biological consequences, such as macrophage uptake [29]. About mechanism of green tea effects on reduction of chromosomal damage is presented different proposals. Some believe that the anticarcinogenic potential of green tea catechins studied extensively with human cancer cells in vitro have correlated their cytotoxic effects with the induction of apoptosis, activation of caspases, inhibition of protein kinases, modulation of cell cycle regulation and inhibition of cell proliferation [30].

Another study has been proposed that connection green tea catechin to specific proteins, including fatty acid synthase (FAS), laminin, 67-kDa laminin receptor (67LR), vimentin, glucose-regulated protein and starts apoptosis pathway [31]. Epigallocatechin gallate acts against urokinase (an enzyme often found in large amounts in human cancers) inhibits ornithine decarboxylase (a rate-limiting enzyme closely associated with tumor promotion) and blocks type 1, 5-alpha reductase. Urokinase breaks down the basement membrane of cell junctions that may be a key step in the process of tumor cell metastasis as well as tumor growth. Epigallocatechin gallate attaches to urokinase and prevents these actions [32].

According to the experiences described can be concluded that green tea by destroying free radicals, oxidative stress, inhibition DNA damage, inhibition of binding carcinogenic agents to relevant receptors, preventing the formation of irritating factors, creating and disseminating cancer cells and induce apoptosis in damaged cells, cell cycle control and inhibition of cell proliferation, gene transcription changes, changes in protein folding and heat shock proteins or by blocking certain enzymes to be effective in reduction of chromosomal damages induced by cell phone, which is consistent with our results. In this study, It seems that green tea with destruction free radicals and prevention DNA damage have prevented of micronucleus formation and chromosome injuries. The findings of this study revealed that cell phone waves have genotoxic effects and they increase chromosomal damages, but concomitant conception of green tea reduces waves illness effects. So green tea has inhibitory properties against genotoxic effects induced by physical and chemical environment factors. Because of widespread

using of electromagnetic equipments in human environment and increase destructive effects of waves emitted from this instruments (physical environmental pollutants), it seems to predict the daily consumption of green tea to be effective in reducing of chromosomal damages (Fig. 5).

Acknowledgements

Authors are thankful to Islamic Azad University of Mashhad for the financial assistance and support.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of interest

The authors declare no conflict of interest.

Funding/Support

Islamic Azad University Tabriz branch, Tabriz-Iran.

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Please cite this article as: Zahedifar Z, Baharara J. Effect of green tea extract in reducing genotoxic injuries of cell phone microwaves on bone marrow cells of mouse. *Zahedan J Res Med Sci (ZJRMS)* 2013; 15(11): 39-44.