

The Inhibitory Effect of *Camellia sinensis* Extract on Decreasing Inductive Teratogenicity of Low Frequency Electromagnetic Field in Liver and Spleen of Balb/C Rat Embryo

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Article information	Abstract
<p>Article history: Received: 28 July 2011 Accepted: 29 Oct 2011 Available online: 7 Jan 2012</p> <p>Keywords: Camellia sinensis Liver Spleen Electromagnetic field Balb/C rat</p> <p>*Corresponding author at: Department of Animal Developmental Biology, Islamic Azad University, Mashhad branch, Mashhad, Iran. E-mail: baharara@yahoo.com</p>	<p>Background: Many studies have an emphasis on Reactive Oxygen Species (ROS) formation by electromagnetic field. <i>Camellia sinensis</i> is enriched with antioxidants and the antioxidants can neutralize the effects of ROS. In this study, the effect of <i>Camellia sinensis</i> extract on decreasing the inductive teratogenicity of the electromagnetic field (frequency 50Hz and intensity 50G) in liver and spleen of Balb/C embryonic rat is examined.</p> <p>Materials and Methods: Twenty-four heads of pregnant female rat (Balb/C) were divided into four groups: control group, experimental test group (off-device), empirical group1 (electromagnetic field 50 Gauss), empirical group2 (treated using <i>Camellia sinensis</i> extract + electromagnetic field 50 Gauss), in this empirical-experimental study. Then, liver and spleen tissue cross sections of 19-day embryos were prepared for histological assessments after weight and Crown-Rump length were measured. Resulting quantitative data was analyzed using ANOVA statistical tests and Tukey test with the significance level ($p < 0.05$).</p> <p>Results: In examination of tissue sections, mean lymphocyte number of spleen in empirical group 1 showed a significant difference ($p = 0.001$) comparing to the experimental control group, whereas mean lymphocyte number of spleen in empirical group 1 showed a significant difference ($p = 0.001$) comparing to empirical group 2. Mean number of liver hepatocytes empirical samples 2 showed a significant decrease ($p = 0.004$) comparing to the experimental control group, and mean number of liver hepatocytes empirical samples 2 showed a significant decrease ($p = 0.042$) comparing to empirical samples 1.</p> <p>Conclusion: Consumption of <i>Camellia sinensis</i> could compensate for the inductive impairments in many cells electromagnetic fields, but it is not recommended in pregnancy period cause of changing the number of some cells.</p> <p>Copyright © 2012 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

In modern routine life of today, human is inevitably exposed to a variety of adverse and harmful physical, chemical and environmental factors among the vicious effects of which are reactive oxygen species production and DNA mutation [1]. It sets the scene to the outbreak of many human disorders such as cancer, aging, ischemia, and arteriosclerosis [2]. Of the effective physical factors is the electromagnetic field. In the advanced world of today, there is a bulk of electromagnetic-inducing devices including home electrical devices, radio, TV, and mobile phones in humans' life [3].

Some studies have showed that very low frequency electromagnetic fields induct chromosomal impairments in polychromatic erythrocytes of male rat's (Balb/C) marrow [4]. According to the studies, there is a significant

increase in tumor necrosis factor production in T lymphocytes of spleen under waves with a frequency 8-15 GHz, and intensity 1W; the waves affect T cells and reinforce their propagation power [5].

Some studies indicate that mobile phone waves have no deep effect on general structure of spleen tissue, yet enhance the number of cells [6]. Studies demonstrate that antioxidants prevent from undesirable effects of these destructive factors on the body by destroying and reducing the reactive oxygen species and *Camellia sinensis* is also enriched with Katchin natural antioxidants "powerfully removing the reactive oxygen species in laboratory environments" [7].

There have been many experiments carried out regarding anticancer effects of *Camellia sinensis* including the study of the size of tumors and

vasculogenesis in bladder cancer of rats treated for 14 to 24 weeks using N-Butyl 1, (4-hydroxybutyl) nitrosamine and *Camellia sinensis* indicating the effects of polyphenols in inhibiting cancer progress [8]. Furthermore, *Camellia sinensis* has the following qualities; antiallergic effects, inhibiting platelet formation in brain, reducing glucose, decreasing blood triglyceride and cholesterol, stimulating fat catabolism in liver and increasing energy consumption in body [9]. Using Kachin *Camellia sinensis*, we might reduce destructive oxidative effects of microwave on the heart tissues [10].

Studying the effect of *Camellia sinensis* Kachin on enzymatic activities and antioxidative systems genes expression in the liver of rats that at the same time of receiving *Camellia sinensis*, they were exposed to microwave (2.45GHz for 15min in 16 days) has indicated that the extent of the impairments imposed to the liver in the group was same as the control group and there is significant difference [11].

Some studies have showed that the genotoxic effects resulted from the peripheral blood cells being exposed to UV-A waves significantly decrease 60 min after taking *Camellia sinensis* [7]. As implied, the electromagnetic fields have considerable effects on embryonic body and growth and *Camellia sinensis* can create a desirable balance in body for its antioxidant qualities. So, cause of the lack of previous studies in the regard, present study is aimed at the examination of *Camellia sinensis* extract effects on reducing the teratogenic impairments of the embryonic rat's (Balb/C) liver and spleen induced by electromagnetic fields (50 Hz and 50 Gauss).

Materials and Methods

This empirical-experimental study was conducted at Animal Incubation Research Laboratory, Department of Biology; Islamic Azad University of Mashhad in 2010-2011. Twenty-four heads of rat (Balb/C) were bought from Razi Serology Inst., Mashhad and kept in animal breeding room (temperature $23\pm 1^{\circ}\text{C}$, humidity 65-70% and 12-h light-dark cycle) in polycarbonate cages with stainless wire cap washed and disinfected twice a week. They were fed by standard readymade diet. Sufficient water was provided for them in glass bottle. To make sure of the rats' maturity, 2.5-3-month animals (25-30 g) were used. Fecundation of male and female rats was carried out using monogamy and the day of observing vaginal cap in females was considered as the null day.

Electromagnetic field generator system (50 Hz) existing in animal research lab, Department of Biology, Islamic Azad University of Mashhad (designed and constructed by Baharara and Ashraf) was used. The low frequency was used to create the same conditions existing in houses, offices and factories. Some studies have also shown that an increase of reactive oxygen species is related to the genotoxic function of electromagnetic waves on DNA and the hematopoietic stem cells [12].

Accordingly, liver and spleen were examined as a part of hematopoietic system in this study. The time of wave-exposure was also determined 9-19-day of pregnancy,

because the emergence of hematopoietic system (regardless of gallbladder) with liver emergence gets started from 10-embryonic day (counting a 21-day pregnancy) and continues to birth and even thereafter [13]. So, the treatment period is set between 9-19-day of pregnancy.

Here, *Camellia sinensis* extract (100mg/kg) is used. Studying hydroalcoholic extract of *Camellia sinensis* (50, 100, and 200mg/kg doses) effect on blood glucose and weight of diabetic male rats induced using streptozotocin, Mehdizadeh et al showed that the best dose for physical activities of laboratory models is 100mg/kg [14]. In the studies of effect of *Camellia sinensis* on liver and heart tissue 100 mg/kg was mainly used [15].

Since in most papers injected extract dose was considered 100 mg/kg, so we also made use of it in our experiments. Hydroalcoholic extract of *Camellia sinensis* was prepared using Maceration assay.

To prepare the required amounts, suitable amount of hydroalcoholic extract of *Camellia sinensis* dried powder was weighted and suspended in distilled water and administered using intraperitoneal injection. Before each injection, rats were weighted and the injection was carried out in mg/kg. Respective pregnant rats were randomly divided into the following groups:

Control group: pregnant rats kept in animal breeding room under normal conditions
Experimental test group (Sham exposed): pregnant rats were placed in electromagnetic field generator device (off status) under laboratory conditions 4h (8-12am) a day between 9-19 days.

Empirical group1: pregnant rats were exposed to electromagnetic field (50Hz, 50Gauss) under laboratory conditions 4h (8-12am) a day between 9-19 days.

Empirical group2: pregnant rats were exposed to electromagnetic field (50Hz, 50Gauss) under laboratory conditions 4h (8-12am) a day and intraperitoneal injection of *Camellia sinensis* extract (100mg/kg) between 9-19 days.

The ethical principles are accurately and completely met in all steps of maintenance, treatment and anatomizing the animals. At least 6 pregnant rats were considered for each group. All pregnant rats were anesthetized on day 19 and the fetus was taken out. After morphological assessments, they were weighted using digital scale (Sartorius, Germany with accuracy of 0.01g) and their Crown-Rump lengths were measured using caliper.

Then, their livers and spleen were completely dislodged and stabilized in formalin solution (%10), then draining and molding were carried out, and 6μ serial incisions were prepared using Microtome (Germany), dyed using Harris hematoxylin-eosin technique and the permanent slide was prepared.

In observing the traverse cross sections using optical microscope, first general status of the tissue, then the number of hepatocytes and megakaryocytes of liver and the number of nucleus-devoid red globules, lymphocytes and megakaryocytes of the spleen were counted. Results of empirical 1 and 2, experimental test and control groups were compared and experiential qualitative data was

analyzed using SPSS-16 Software and One-Way ANOVA and Tukey test with the level of significance $p < 0.05$.

Results

Results indicated the antibacterial effect of all herbal. Statistical comparison of the results from control and experimental test samples showed no significant changes ($p > 0.05$). So, empirical group was compared to experimental test group.

Statistical comparison of the embryonic Crown-Rump length in the empirical 1 showed a significant increase comparing to experimental test group ($p = 0.008$). Also, the comparison between embryonic weight in the empirical 1 and experimental test showed a significant increase ($p = 0.002$).

The examination of tissue cross sections of liver and spleen shows that mean liver megakaryocytes number in the empirical 1 has significantly increased comparing to experimental test ($p = 0.001$) (Fig.1).

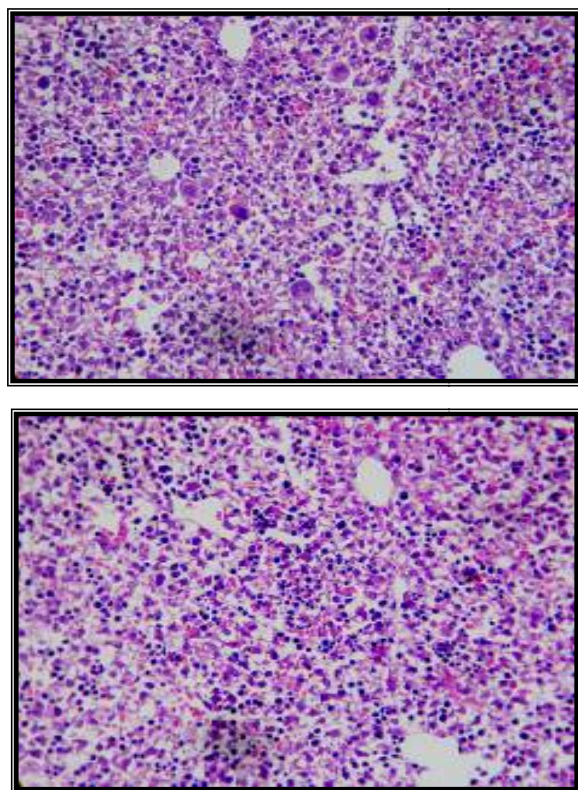


Figure 1. Longitudinal cross section of 19-day embryonic experimental test (bottom) and empirical 1 (top), megakaryocyte number increase in empirical 1 is visible in these cross sections ($p = 0.001$). Magnification $\times 400$, H&E staining

In embryonic spleen of the empirical 1, a significant increase was observed in the number of megakaryocytes and red globules comparing to experimental test ($p = 0.001$), whereas the number of lymphocytes was observed to have a significant decrease ($p = 0.001$) (Fig. 2).

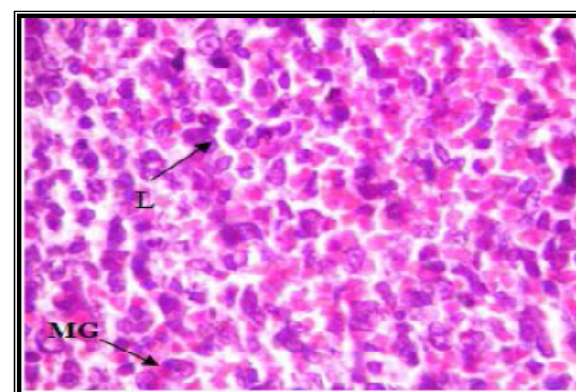
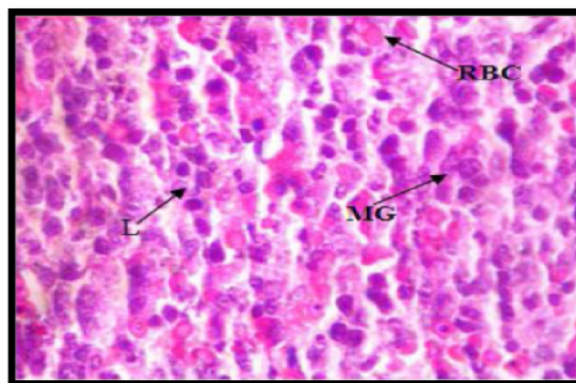


Figure 2. Mid-Parabola cross section of 19-day embryonic experimental test (top) and empirical 1 (bottom). Magnification $\times 1000$, H&E staining RBC: red globule, MG: megakaryocyte, L: lymphocyte

Statistical comparison of embryonic weight in the empirical 2 showed a significant increase ($p = 0.013$). Liver and spleen tissue cross sections showed that mean liver megakaryocytes number in experimental group 2 has significantly increased ($p = 0.001$), whereas mean liver hepatocytes number in empirical group 2 has significantly increased ($p = 0.004$).

In spleen, mean embryonic liver megakaryocytes number in empirical group 2 has significantly increased ($p = 0.001$) comparing to experiential test. Also, mean embryonic red globules number in empirical 2 showed a significant increase comparing to experimental test ($p = 0.001$).

In assessing liver and spleen tissue cross sections in empirical group 2, mean embryonic liver hepatocytes number decreased significantly comparing to empirical 1 ($p = 0.042$), whereas in spleen, mean embryonic red globules number in empirical 2 (comparing to empirical 1) and mean embryonic lymphocytes number in empirical 2 (comparing to empirical 1) had a significant increase ($p = 0.001$) (Table 1) (Fig. 3&4).

Table 1. Statistical comparison of quantitative data between control, experimental test, empirical 1 (treated under electromagnetic field), empirical 2 (treated simultaneously by electromagnetic field and *Camellia sinensis* extract) groups

Group	Index	control	Laboratory control	empirical 1	empirical 2	p-Value
	Mean of weight (g)	0.18±1.05	0.19±1.14	0.06±1.37	0.2±1.33	0.001***
	Mean Crown-Rump length (mm)	2.09±19.95	1.65±20.85	1.52±23.14	2.27±21.44	0.001***
	Mean liver megakaryocyte number	1.87±3.34	1.69±3.24	1.67±4.23	1.91±4.11	0.001***
	Mean liver hepatocyte number	6.96±20.6	7.36±21.72	7.24±21.08	7.38±18.98	0.005**
	Mean spleen megakaryocyte number	1.07±1.42	1.09±1.67	1.06±2.19	1.26±2.13	0.001***
	Mean spleen lymphocyte number	7.53±41.43	7.07±40.97	7.77±34.29	7.33±40.11	0.001***
	Mean spleen RBC number	5.61±22.87	6.41±24.52	6.52±28.91	12.88±38.55	0.001***

In the table, Mean±SEM is represented.

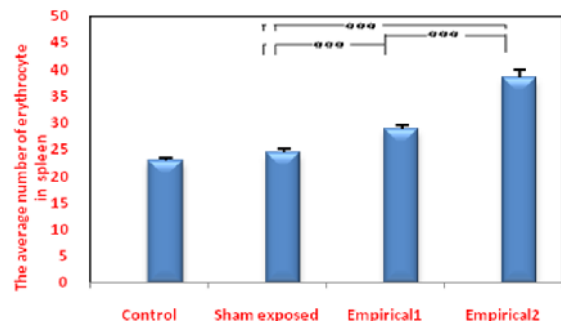


Figure 3. Statistical comparison of mean red globules number in control and empirical groups*** is significant at 0.001 level.

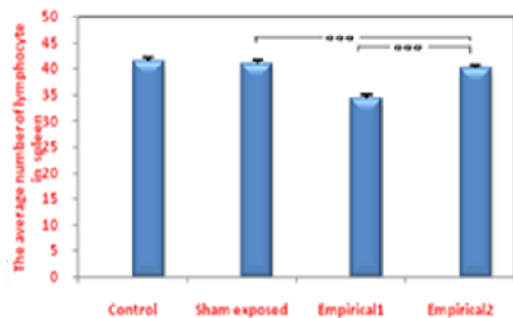


Figure 4. Statistical comparison of mean lymphocytes number in control and empirical groups*** is significant at 0.001 level.

Discussion

Here, no morphological abnormalities and/or changes in the structural and total conditions of the given tissues were observed in 19-day embryos. However, the comparison between the embryos in empirical group 1 (exposed to the electromagnetic field) and experimental test group showed an increase in weight, Crown-Rump length, mean liver and spleen megakaryocytes number, yet the increase was less in empirical group 2 (treated simultaneously by electromagnetic field and *Camellia sinensis* extract). Also, the effect of *Camellia sinensis* extract injection on the number of spleen lymphocytes was observed, because *Camellia sinensis* extract had compensated for the decrease resulted from the

electromagnetic field exposure and recovered the normal cell number.

Some previous studies have showed that if the pregnant rats are exposed to electromagnetic field (100Hz with complete sinusoidal wave, 120Gauss) for 10h (on 5, 6, and 7 days of pregnancy), the weights of embryo, placenta, and (Crown-Rump) CR will increase [16] which correlates with the results of the study.

Whereas some studies do not demonstrate the correlation such as Negishi et al who exposed the rats to electromagnetic fields (intensities 7, 70, 350 μ T) for 22h a day during pregnancy (on 8-15 embryonic days) and assessing the amount of growth and development and organogenesis on 20-embryonic day, and observed some differences regarding liver and kidney weight decrease and or lower male and female embryonic weights in 70 μ T [17]. Furthermore, some studies have demonstrated that using vitamin A and very-low-frequency electromagnetic field has a delaying effect on growth and development and results in the decrease of embryonic weight, motor-organ length, and cartilage cells density in chicken embryo [18]. It is implied in the study that various factors are involved in creating biological effects of the electromagnetic fields the most important of which include: physical features of the field, exposure duration, type of tissue and growth and development stage of the experimental sample [19].

Here, the count of liver and spleen cells demonstrated an increase in the red globules and megakaryocytes in embryonic empirical 1. The result correlates with Forgacs's study on male rats exposed to microwave (1800 MHz). In this study, no changes were observed in tissues of sexual organs, but an increase in the number of red globules is shown [20]. Also, some studies indicate that increase of free radicals is related to the genotoxic function of electromagnetic waves on DNA and hematopoietic stem cells which confirms the effect of the waves on liver and spleen as a part of hematopoietic system [21]. These findings are also consistent with results of the report on the effects of low frequency electromagnetic waves and microwave effects on the hematopoietic system of laboratory rats [21].

Nafisi et al exposed Wistar rats to the low and high frequency electromagnetic fields (respectively, 110KHz, and 10 Hz, 700 mG) for 2 days (2h a day) and reported a significant decrease in high frequency [22] correlating with the results of the study. Amara has reported the effect of electromagnetic field on increasing the number

of red and white globules, hemoglobin, hematocrit and proteins. Also, experiences have showed that in rats exposed to fixed electromagnetic field for 30days, the indices were significantly increased except hematocrits, yet their weights were decreased [23]. In addition, in rats (Wistar) with 5 consequent days of 1-h-exposure to fixed electromagnetic field (128mT), an increase of hematocrit, hemoglobin, transferring concentration of plasma, and hemoglobin concentration of globules, red and white globules, yet a decrease in iron concentration in plasma were observed [24].

In the two above studies, red globules increase correlates with the present study, but white globules increase and weight decrease do not go together with the results. It seems that the variety of the type of animal, growth and development stage, treatment length and type of field are factors inducing the differences. In some studies, it is proposed that the waves induct the Extracellular-signal-Regulated Kinase (ERK) waterfall activity by activating a molecular mechanism, and stimulation of transcription and other cellular processes.

Also, it is observed that the waves enact their effects on biological systems through producing or increasing Reactive Oxygen Species (ROS). As a mediator, ROS is involved in many biological effects including the DNA damage and mutation induction [25]. Among the other results of the study is lower weight increase in the group exposed to the electromagnetic field and simultaneously receiving *Camellia sinensis* extract comparing to the group exposed to the electromagnetic field per se (probably cause of the extract injection).

In an experiment, Saetan showed that adding 130mg *Camellia sinensis* powder to male rat drinking water (fed by 50% sucrose and 50% butter) has resulted in a weight loss in two days [26]. The weight loss might be cause of the decrease in the body fat concentration which was also observed in Sprago rats 14 days after treatment using *Camellia sinensis* [26].

A comparison of lymphocytes between control and test groups demonstrates that *Camellia sinensis* is capable of compensating for the cell number reduction under electromagnetic field, because *Camellia sinensis* leads to an increase in plasma antioxidant level, plasma peroxides level, and a decrease in inducting oxidative destruction of DNA in human lymphocytes and improves antioxidative processes [27] corresponding with the results of Kiliçalp's study. He showed that mineral materials level such iron, manganese, and zinc changed in liver and testicle cells of pigs exposed to electromagnetic waves (900MHz), but *Camellia sinensis* recovered the normal levels of the materials [28]. Researchers assessed the effect of EGCG (Epigallocatechin-3-Gallat) on propagation and stages of cellular cycle of human liver cell line (Hepg2) and the results showed that EGCG inhibits Hepg2 propagation

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using apoptosis induction and by blocking cellular cycle progression stages in G1 phase [29].

According to the studies, it can be said that *Camellia sinensis* can be effective in reducing chromosome impairments inducted by electromagnetic field through destructing ROS, inhibiting oxidative stress, inhibiting DNA destruction, preventing cancerogenesis factors from being connected to respective receptors, preventing from the emergence of stimulating factors and distribution of cancer cells, inducting apoptosis in damaged cells, controlling cellular cycle, changing genes transcription, changing the proteins' bending, producing thermal shock proteins and or blocking certain enzymes [30].

In some of the other studies, destruction of ROS by Katchin is introduced as the major factor [31]. Oral consumption of *Camellia sinensis* (2000mg/kg) in 28 days produced no harms and or intoxication in liver cells of the rats [32]. It seems that differences in growth and development stages and animal type as well as the method of consumption are the reasons for differences in results, but embryonic rats (exposed to *Camellia sinensis* in organogenesis stage) showed conditions of weak intoxication [33].

Also, in a report, the relationship between *Camellia sinensis* consumption by pregnant mothers and giving birth to infants with growth and development abnormalities of spinal cord was demonstrated. Probably, taking *Camellia sinensis* enriched with Katchin and its epimers results in the reduction of folic acid balance in body cause of blocking the activity of dihydrofolate reductase enzyme, so it is not recommended for pregnant women and women loving to have baby [34].

Results of the study indicate that *Camellia sinensis* extract has prevented from an increase in liver and spleen megakaryocytes, embryonic weight and length or a decrease in spleen lymphocytes as a result of treatment with electromagnetic fields which inducts apoptosis, activates caspases, inhibits protein kinaz, controls cellular cycle, and inhibits cellular propagation, accordingly it is recommended for the community of radiologists, TV and radio, and telecommunication staff more than the other individuals in the society. However, its ascending effect on red globules and descending effect on the number of hepatocytes sre also demonstrated. So, its consumption in pregnancy is not recommended (cause of inducting changes in cells) and requires further studies.

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