



The Effect of Citrus aurantium on Uterus Apoptosis and Serum Antioxidants in Rats Exposed to Electromagnetic Fields

Elham Mansouri¹, Arash Khaki^{2*}

Abstract

Objective: In the industrial world, almost everyone is unavoidably exposed to ambient electromagnetic field (EMF) generated from various technical and household appliances. Thus, the aim of this study was to evaluate the diminishing effects of Citrus aurantium on uterus cell injury induced by EMF.

Materials and Methods: In order to examine this, 40 female Wistar rats were selected and kept for 1 week in quarantine, and then, subdivided into two groups of 20 rats and were exposed to 40 Hz of EMF (non-ionizing radiation, 0.1 tesla) for 6 weeks. Group 1 received 3 cc/rat Citrus aurantium juice, and group 2 only received 3 cc Citrus aurantium. The control group only received 3 cc water using gavage method. The materials were processed and apoptosis was observed under a light microscope using TUNEL method.

Results: In the experimental rats, apoptotic cells showed significant increase in the EMF groups. However, in the exposed group that received Citrus aurantium, apoptotic cells and serum malondialdehyde (MDA) were decreased and superoxide dismutase (SOD) was increased ($P < 0.05$).

Conclusion: This study showed that EMF could induce cell injury on uterus tissue, and 3 cc Citrus aurantium juice has antioxidative effects on uterus tissue by reduction of apoptosis.

Keywords: Antioxidant, Apoptosis, Citrus aurantium, Malondialdehyde (MDA), Superoxide Dismutase (SOD), Uterus

Introduction

Magnetic field is produced by a magnetic object with particles or by changing the electric field, and is identified by the force applied to other magnetic materials or movement of the electric charge. Magnetic field is identified at any given point by both parameters of direction and intensity (or resistance), which is known as a vector field and in today's industrial and economic world makes its effects on health, forecasting, and management implications of these developments difficult (1). One of the major issues of concern is exposure to electromagnetic waves due to the increasing use of equipment such as computers, microwaves, cell phones, high voltage power transmission cables, and various industries such as electronic equipment manufacturing industry, steel rolling factories, and industrial

furnaces. Documentary evidence-based epidemiological records showed the relationship between cancers and exposure to extremely low frequency electromagnetic field in children when exposed to electromagnetic fields as carcinogens and focused attention on this issue. Research has shown that the response of the nervous system to electromagnetic waves is due to the fact that the brain tissue has the most ability to guide electromagnetic wave and the influence of electromagnetic waves, relative to the radiation level, is high (2). Most conducted studies were retrospective studies and based on reports by people who were exposed to electromagnetic waves in frequency range of 900-1800 MHz (3). Many studies on animals have attempted to evaluate the effects of electromagnetic fields on reproduction, but the

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¹ Department of Medical Physics, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

² Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

*Corresponding Author: Arash Khaki, Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Tel: +98 9143138399, Email: arashkhaki@yahoo.com

results have been contradictory. Many studies have shown that exposure to electromagnetic fields can have adverse effects on reproduction and embryonic development, and thus, a relationship between exposure to electromagnetic fields and miscarriage is conjectured. Other studies have not reported adverse effects on reproduction or fertility in rats and humans exposed to electromagnetic fields (4). According to documents in Iranian traditional medicine, some of the plants that can be effective in treating infertility are from the citrus family with the scientific name of Rutaceae (5,6). The beneficial parts of the citrus are its flowers, bark, fruit, and juice; they all have medicinal properties. This plant is known as a sedative, hypnotic, appetizer, and has therapeutic effects on tachycardia (7). This plant is native to tropical regions of Asia such as India and China, and grows in Spain, South America, Italy, Palestine, and Iran in the Fars Province. The citrus peel contains essential oil, flavonoids, coumarin, terpenes, vitamin C, carotene, and pectin. Flavonoids have anti-inflammatory, anti-bacterial, and anti-fungal effects (8,9). The purpose of this study was to investigate the effects of antioxidant of citrus aurantium on uterus apoptosis in rats exposed to electromagnetic waves.

Materials and Methods

This study was performed on 40 female Wistar rats, which were 2 months old and weighed approximately 220 ± 10 g, for 6 weeks. The rats were kept in plastic cages under laboratory conditions at 20 ± 2 °C with light controlling for 12 hours light and 12 hours dark at the Department of Anatomy of Tabriz University of Medical Sciences, Iran.

The studied rats were randomly divided into 4 groups.

The first group (the control group) received 3 cc normal saline daily and was treated for 6 weeks.

The second group was exposed to electromagnetic radiation 8 hours daily for 6 weeks in electromagnetic field of 0.1 tesla.

The third group received 3 cc per rat of citrus aurantium daily for 6 weeks orally (7).

The fourth group, in addition to exposure to electromagnetic waves (8 hours per day in an electromagnetic field of 0.1 tesla for 6 weeks), received 3 cc per rat of citrus aurantium daily for 6 weeks orally.

On the 42 day pentobarbital (40 mg/kg) was used for anesthesia through intraperitoneal injection, and then, the peritoneum was opened by transverse abdominal incision. The uterine tissue was removed in both the study group and control group. At the end of the study, the animals were killed by cervical dislocation of the neck.

The superoxide dismutase (SOD) of the blood was measured by Randox kit (Randox Laboratories Ltd., Ardmore, UK) based on mmol per liter at 600 nm wavelength (9).

The malondialdehyde (MDA) in blood was

measured by the reaction of thiobarbituric acid based on mmol per liter.

After preparation of paraffin-embedded blocks of tissue from the uterus of rats in the study group and control group, 5 micron thickness sections were prepared. The sections were placed on glass slides. Sections related to the study and control groups were tested for planned cell death using Apoptosis kit (Roche Diagnostics GmbH, Mannheim, Germany) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method.

A. The tissue slices were deparaffinized using xylol.

B. Paraffin-embedded sections were placed in microwave device of 700 W for 10 minutes.

C. Tissue sections were incubated in phosphate buffer substance (PBS) containing H_2O_2 3% for 10 minutes.

D. Tissue sections were incubated in fluorescein-dUTP substance in the TUNEL reaction mixture for 60 minutes at 37 °C.

E. Tissue sections were washed 3 times in phosphate buffer substance (PBS).

F. Tissue sections were incubated for 30 minutes in anti-fluorescein-pod substance.

G. Tissue sections were washed 3 times in PBS.

H. Tissue sections were impregnated with H_2O_2 -Diaminobenzidine (DAB, Rosche, Germany).

I. Differential staining was performed and tissue sections were stained with hematoxylin (10).

The system produces a field with a frequency of 50 Hz and 80 Gauss, but the flow rate is controlled by a transformer which is joined to a field generator, this means that the tool includes two main parts (8). In the first part, two coils of copper were placed within a distance of about 50 cm of each other and between them was the wooden cylindrical where the rat cages were placed. The second part consisted of a field generator, a transformer that passed through the input and output power of the device, a voltmeter showing the voltage, and an ammeter showing the current intensity (11). The location of the device was the Dr. Khaki laboratory in Tabriz University of Medical Sciences.

To prepare a batch of Citrus aurantium, the juice was prepared daily using a manual fruit juicer and was orally fed to the rats.

To evaluate and compare the results of apoptosis, levels of superoxide dismutase (SOD) in the blood, and malondialdehyde in control and experiment groups, ANOVA and SPSS software (version 19, SPSS Inc., Chicago, IL, USA) were used

Results

Findings of superoxide dismutase (SOD) in blood

The superoxide dismutase (SOD) in the control group was 3.25 ± 0.05 ml, and in the second to fourth experiment groups was 1.15 ± 0.05 , 4.90 ± 0.05 , and 2.05 ± 0.05 ml, respectively. The statistical analysis of comparative Dunnett's test (one-sided) showed a significant difference ($P < 0.05$) between the second,

third, and forth groups and the control group.

Results of malondialdehyde (MDA) in blood

The malondialdehyde (MDA) in blood in the control group was 0.33 ± 0.05 mmol/l, and in the second to fourth experiment groups was 1 ± 0.05 , 0.35 ± 0.05 , and 0.65 ± 0.05 mmol/l, respectively. The statistical analysis of comparative Dunnett's test (one-sided) showed a significant difference ($P < 0.05$) between the second, third, and forth groups and control group.

Results of the apoptosis of uterine tissue

The amount of uterine tissue apoptosis in the control group was 8 ± 0.05 , and in the second to fourth experiment groups was $13 \pm 0.05\%$, $7 \pm 0.05\%$, and $10 \pm 0.05\%$ per 100 microscopic sections, respectively. The statistical analysis of comparative Dunnett's test (one-sided) showed a significant difference ($P < 0.05$) between the second, third, and forth groups and control group.

Discussion

According to Iranian traditional medicine books, some of the plants that can be effective in the treatment of infertility are fenugreek, ginger, nettle, raspberry, banana, cabbage, onions, red and green pepper, licorice, and pumpkin seeds. Following the recognition of the mechanism of action of traumatic factors on vital tissues through oxidation and release of free radicals, accelerated recognition of antioxidants and bioactivity of plant extracts have gained much attention because they are derived from natural resources and are compatible with vital systems. The use of antioxidants and vitamins C, A, and B, through decreasing the damage caused by free radicals, strengthening and protecting blood-testicular barrier, and protection and restoration of sperm DNA, may be useful in the treatment of male infertility (9). The use of medicinal plants to increase fertility and resolve issues such as hormonal imbalance, erectile dysfunction (sexual weakness), oligospermia, low sperm motility, prostatitis, and varicocele can have positive effects, and thus, has been gaining much attention. Mobile phones that produce 900 MHz electromagnetic frequency waves are commonly used in many countries. Radio-frequency electromagnetic radiation (RF-EMR) from these devices could potentially affect sperm development and function. Around 14% of couples from high- and moderate-income countries have difficulty conceiving, and there are unexplained declines in semen quality reported in several countries. Given the ubiquity of mobile phone use, the potential role of this environmental exposure needs to be clarified (10).

The researchers found that intrauterine exposure to electromagnetic waves has toxic effects on the rat ovary, and concluded that wave of microwaves or mobile phones can reduce the number of ovarian follicles in rats by unknown, undocumented mechanisms (12). In another study, toxic alternation were reported in reproductive organs of female rats resulting from radiation. The frequency of 30 Hz was

used in the present test which was a simulation of military radar that the human body is inadvertently exposed to (13).

Pregnant physiotherapists are at an increased risk of miscarriage due to their job and exposure to microwaves (14). The microwaves used in this study were not of very high frequencies, like those that can be in many places such as near a cell phone tower, mobile phones at home and work, and wireless systems. Researchers have found that radiation from cell phones for 2 hours per day for 10 days caused significant changes in reproductive genes and cell division (15,16). Electromagnetic waves can lead to the induction of programmed cell death (apoptosis). Several studies have investigated the apoptotic process in the magnetic field.

In recent centuries, many synthetic and biological drugs have been tested to determine their radio-protective effect. These medications include antioxidants, non-steroidal anti-inflammatory drugs, immune regulators, sulfhydryl compounds, prostaglandins, and lipoproteins. Because many of these synthetic drugs and chemicals in accurate operating doses showed serious side effects before reaching the pre-clinical stage, they were excluded from the studies process. For this reason, in this century, plants, as a source of radio-protective pure compounds, have been under investigation by many researchers. Several studies have shown the beneficial effects of antioxidants such as vitamins C and E, as well as polyphenols, flavonoids, and epigallocatechin against radiation (17).

In this study, exposure to electromagnetic waves caused thinning of the diameter of the uterine wall. The number of ovarian follicles in the exposed group was significantly reduced compared with the other studied groups. Furthermore, in the groups exposed to radiation, increased interstitial fibrosis was observed in the uterine tissue and uterine glands were atrophied. Moreover, superoxide dismutase serum reduction and increased levels of malondialdehyde in the groups receiving electromagnetic waves were evidence of the release of free radicals, production of oxidative stress, and an increase in uterine tissue cell damage. Citrus aurantium, due to its abundance of natural antioxidant, quercetin, and vitamins B and C, causes neutralization and reduction of damaging effects of cells and biochemical resulting from exposure to electromagnetic waves in rats.

Conclusion

Cell damage caused by oxidative stress, in term, causes cell dysfunction and cell death. Exposure to radiation and electromagnetic waves can cause a variety of mammalian cell death in different organs. The uterus has an important role in maintaining, nurturing, and developing the embryo and fetus during pregnancy; therefore, for those living in industrialized societies the daily use of Citrus aurantium juice for its antioxidant properties is recommended.

Ethical issues

We have no ethical issues to declare.

Conflict of interests

We declare that we have no conflict of interests.

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