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Preventive efficacy evaluation of *Urtica dioica* on in vitro fertilization and embryo development in exposed mice

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Nowadays impact of cell-phone RF on human health is subject of many researches. Antioxidant properties of hydroalcoholic extract of Urtica dioica (UD) have been proven to eliminate free radicals and oxidative stress. Purpose of this study was to assess the impacts of cell-phone RF on in-vitro fertility (IVF) rate and role of the UD extract against induced the RF damages. A total number of 54 adult, male NMRI mice were randomly divided into six equal groups randomly; control group, RF-irradiation alone (2hrs a day for 28days), those received only 50mg/kg UD, 50mg/kg UD plus RF-irradiation, only 100mg/kg UD, and 100mg/kg UD plus RF-irradiation. The UD was intraperitoneally injected 1hr before RF-irradiation. IVF performed 1day after the last RF-irradiation. Chromatin integrity in sperm DNA evaluated by acridine orange staining. Finally, frequency and morphology of the embryos were assessed. ANOVA and Tukey's tests applied for statistical analysis. RF-irradiation alone significantly decreased frequency of two-cell embryos (p=0.000) and grade-A embryos (p=0.014); while it significantly increased the dead embryos (p=0.001) and grade-B (p=0.033), grade-C (p=0.017) and grade-D embryos (p=0.008). RF-irradiation alone led to significant genetically damages in DNA chromatin (p=0.000). Injection of 100mg/kg UD increased significantly frequency of the two-cell embryos (p=0.01) and grade-A embryos (p=0.018); where it significantly reduced the dead embryos (p=0.026), grade-C (p=0.026) and grade-D embryos (p=0.038). Cell-phone RF can change number of two-cell mouse embryo as well as embryo's morphology and induces molecular damages in the DNA of the sperm. Moreover, UD extract plays a protective role against RF induced damages.

INTRODUCTION

The increasing use of cell phones by people has raised concerns about their safety (Carrubba et al., 2010). Cell phones emit radiofrequency energy, a form of non-ionizing, radiation and may include biological effects and potential hazards (Carrubba et al., 2010, Fatehi et al., 2017). According to statistics, in 2011, six billion cell-phone subscriptions were registered (Volkow et al., 2011). The waves emitted from the cell-phone (radio waves) are part of the electromagnetic waves spectrum, ranging from 900 MHz to 1.8 GHz (wavelengths of about 1 mm to 1 m) (Michaelson, 1991). Cell-phone today are one of the most common emitters of radiofrequency (RF) waves; they are almost available to half of the various age groups of the people around the world (Michaelson, 1991, Khadrawy et al., 2009). The expansion of mobile networks and the variety of services supported by the providers of such networks, *e.g.* internet access, SMS & MMS services, easy sending of images and videos, banking and information exchange in numerous social networks and cybernetics in recent years has caused the public to be more than welcome to use this device; in a way that today, mobile phone is not only a luxury gadget, but also one of the necessary tools for most people (Khadrawy et al., 2009). Concurrently with the increasing number of cell-phone users in the world, several recent reports have raised serious concerns about the harmful effects of these waves on various processes of growth and development of various organs of the human body (Baste et al., 2008, Phillips et al., 2009, Narayanan et al., 2009, Fragopoulou et al., 2010). The effects of RF waves on biological systems have been investigated since several years ago; however, mechanism of the effect of these waves, research in this field continues (Hyland, 2000). Some of the deleterious effects of RF waves are: impact on fertility standards (Baste et al., 2008), damages on the DNA of brain cells (Phillips et al., 2009), induction of apoptosis in brain tissue (Yilmaz et al., 2014, Zhu et al., 2008, Zuo et al., 2014), decrease in frequency of pyramidal neurons and increase frequency of ischemic neurons of the cerebral cortex

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(Celikozlu et al., 2012), impaired brain and behavioral activities (Hamblin and Wood, 2002, Sienkiewicz et al., 2005), increasing the permeability of the blood-brain barrier (Nittby et al., 2009), inducing oxidative stress by increasing free radicals (increased lipid peroxidation) and altering the antioxidant defense system of the tissues (Meral et al., 2007, Ilhan et al., 2004), induction of brain tumors, headache, depression, inducing the transitional or prominent hot flashes around the ear and disturbance in nervous balance and focus (Edelstyn and Oldershaw, 2002, Khurana et al., 2009). Antioxidant supplements and antioxidant-rich food can reduce oxidative damage by reducing the free radicals and active oxygen levels (Gülçin et al., 2002, Fatehi et al., 2018). The nettle is a plant of the Urtica dioica (UD), herbaceous and persistent with parted branches. Its stems are straight and rectangular and its leaves are covered with biting shards (Jalili et al., 2014). The extract of UD is used to treat diseases of the urinary system (prostate), respiratory tract, digestive tract (stomach) and as a drug for reducing blood sugar. The UD have factors such as alpha tocopherol (active form of vitamin E) and calcium, and Secretin which is the best factor for stimulating the secretion of the digestive glands in the stomach, intestine, liver, pancreas and gallbladder (Yarnell, 2002). The phenolic and caffeic acid compounds in the nettle-secreted material can modulate and down regulation the synthesis of arachidonic acid and its metabolites (Jalili et al., 2014, Bnouham et al., 2003). The phenolic acid also inhibits the synthesis of leukotriene B4 (Yarnell, 2002, Bnouham et al., 2003). Currently, the anti-allergic properties of the UD are reported (Bnouham et al., 2003). This plant has an inhibitory effect on the lipooxygenase-cyclooxygenase enzymes. These two enzymes are responsible for converting arachidonic acid into prostaglandins and leukotrienes (Bnouham et al., 2003). Studies have shown that the decoction of this plant shows antioxidant activities against oxidation of phospholipids, linoleic acids and iron deoxyribose (Matsingou et al., 2001). The antioxidant properties of UD extract have also been proven to eliminate free radicals, superoxide ions, hydrogen peroxidase and the activities of metallic atoms; and the total phenolic compounds has been assessed (Gülçin et al., 2004). Several studies have indicated the use of UD to improve or treat disorders such as: diabetes (Roman et al., 1992, Swanston-Flatt et al., 1989), prostate hyperplasia (Krzeski et al., 1993), and inflammation of rheumatoid arthritis (Mittman, 1990). Therefore, according to various reports on the effects of cell-phones on male fertility; in the present study, the effect of cell-phone RF waves on *in-vitro* fertility (IVF) rate and the role of hydroalcoholic extract of UD on modulation or prevention these cell-phone induced effects was investigated in NMRI mouse.

MATERIALS AND METHODS

Grouping and irradiation

In this study, we used 54 male NMRI mice aged 6 to 8 weeks and of a weight about 30±5 grams. The mice were purchased from the Pasteur Institute (Tehran, Iran). The mice received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication 8523). The Ethics Committee for animal experiments of Ilam University of Medical Sciences, Ilam, Iran, approved the study protocol (Ethical code: IR.MEDILAM.REC.1396.6). The mice were kept in wooden cages under the laboratory standard conditions of 23 to 25°C temperature, 50 to 55% humidity, 12-hour optical cycle, and easy access to water and food for 2 weeks in order to adapt to the environmental conditions. The mice were divided into 6 groups of 9 mice as shown in the table 1. The RF-irradiation was performed under the active mode of the cell-phone (Nokia 1100, Finland) at a distance of 10 centimeters 2 hours a day for 28 days.The frequency of the RF was 900 MHz. The UD extract was injected intraperitoneally to the mouse one hour before the irradiation for 28 consecutive days (Jahanshahi et al., 2009).

Preparation of hydroalcoholic extract

Soaking method was used to prepare UD extract in the Medical Plants Research Center Herbarium. After removing the waste, 500 grams of the stalk and leave of the UD was first grinded and after passing through the sieve in a glass container, a mixture of water and alcohol and a mixture of 70% ethanol and 30% distilled water was added, so that the mixture of alcohol and water filled the surface of the material, and the powdered parts of the plant floated (the ratio of plant and liquid powder was 5: 1). The mixture was macerated for 72 hours at 20 °C temperature in the laboratory environment, away from direct sunlight and light bulbs. After 72 hours, the soaked blend of the UD extract was shaken, then water and alcohol were passed through a Büchner funnel and filtered through Wattman grade 1 filter paper and collected in a balloon. Afterwards, alcohol was poured a few times on the remainder material from the plant powder and then re-dissolved, resulting in a niche solution. The solution was concentrated using a rotary evaporator and a water temperature of 38 °C and a spin of 75 rpm in a balloon. The concentrated solution obtained from this step was placed in an incubator (Memmert Co., Germany) at 37 °C. After about 72 hours, the extract dried, at which the extract of the plant was shaved from their containers and stored in a refrigerator at 4 °C until it was going to be used. Thereafter, extract of UD was analyze by gas chromatography-mass spectrometry (GC-MS) method that our results about main phytoconstituent in different parts this herb was consistent with Pinelli et al. findings (Pinelli et al., 2008). According to the layout done in this study on the extract, 50 and 100 mg/kg of the mouse weight of the extract was prepared and injected daily for each mouse in the appropriate groups (Table 2).

Chromatin assay (DNA damage)

The chromatin integrity in the sperm DNA was evaluated by acridine orange (AO) staining. In this method, first, the smears from each group of sperm samples were exposed to air in the laboratory under sterile conditions. After drying in a fixative methanol solution (3 parts), acetic acid (1 part) was fixed at 4 °C for 14 hours and then washed with AO solution (0.19% in Phosphate citrate buffer pH = 2.5) and stained for 10 minutes. The slides were rinsed gently with distilled water for 5 minutes and dried at laboratory temperature. Then, the smeared slides were examined by a 400× magnification fluorescence microscope (490/530 nm filter; Nikon, Tokyo, Japan). The three stained designs in sperm heads include: the green-sperm expresses double stranded DNA (normal DNA), and sperms with yellow and orange heads shows single stranded DNA (sperm with an abnormal DNA content). At least 100 sperm were counted in each slide to determine the percentage of two-stranded and single-stranded sperm (Tejada et al., 1984).

Superovulation and oocyte collecting

To induce the reproduction and evolution of oocytes in each mouse, PMSG hormone (Folligon; Intervet, Boxmeer, The Netherlands) was injected intraperitoneally as much as 10 units. In order to induce the maturation process, 48 hrs later 5 hCG hormone (Chorulon, Intervet, the Netherlands) units were injected intraperitoneally. 14 hours after the injection of hCG, the fallopian tube of the mice were removed from the site and transferred to the human tubal fluid (HTF) (Irvine Scientific, Santa Ana, CA, USA) that's dropped in mineral oil (Sigma, cat. no.

M8410). Subsequently, using the subtle forceps, the ampulla of tubes was immobilized and the oocytes were poured out through a subtle cut in the uterine tube membrane. The collection of oocytes and cumulus cells around them were transferred to a drop of HTF medium that was dipped under mineral oil. It was then stored in the CO_2 incubator until the sperm was added.

Sperm collecting and IVF

One day after the last radiation, the mice were killed by cervical dislocation and their skin and peritoneum, the tail epididymis and their vasodilator duct were removed. The equal amount of sperm (5 µl) from the swim up sperms at the edge of the drops, were removed by sampler and added to drops containing 100 eggs, each containing 10×10^6 ml of sperm; and was placed in the incubator at 37 °C and 5% CO2 for 1 hour. Roughly 5 hours after adding the sperm, embryos were transferred into Petri dishes containing 5 drops of KSOM medium (Specialty Media, Millipore, Madison, WI, USA) that's appropriate medium for sperm capacitation and IVF. After being washed, the embryos were transferred to the 4 side drops and finally the 5th drop in the middle of Petri dish; free of any impurities. In order to study the quantitative process of IVF, 24 hours after the insemination, frequency of two-cell embryos (and possibly 4 cells) were counted and recorded under stereo-microscope (SMZ-2, Nikon, Tokyo, Japan). Also, for qualitative studies on the IVF process, the two-cell embryos were morphologically examined under the stereo-microscope and divided into four grades as revealed in the table 3 (Bolton et al., 1989).

Statistical analysis

The ANOVA test was applied to compare the averages of the damages within the each group, and Tukey's HSD test was used to compare the six groups. The p-values are two-sided at a significance level of ≤ 0.05. SPSS software (Version 19; SPSS Inc., Chicago, USA) was used for the statistical analysis.

RESULTS

The results are summarized in tables 4-6, as well as figures 2 and 3. As table 4 shows in group II the RF waves of the cell-phone significantly reduced frequency of two-cell embryos (p=0.000) and increased frequency of dead embryos (p=0.001) compared to healthy control group (Fig. 2). Extract injection in group III neither significantly increased frequency of two-cell embryos nor decrease the dead embryos. Moreover, injection of 50 mg/kg in group IV did not have a significant effect on the development of the fetal process and the level of fetal damage caused by RF-irradiation. However, the results showed that injection of 100 mg/kg (group V) resulted in a significant increase in frequency of healthy embryos (p=0.034), but had no effect on frequency of dead embryos compared to groups 1 and 3. Furthermore, the findings showed that frequency of two-cell embryos in group VI increased significantly compared to the group II (p=0.01) and group IV (p=0.031). Although frequency of dead embryos in group VI decreased significantly compare to 2nd group (p=0.026), the reduction was not significant in comparison to the group IV. Qualitative results showed RF-irradiation 2 hours a day for 30 days (group II) significantly decreased number of embryos in grade A compared to the healthy control group (p=0.014). Moreover, it significantly increased frequency of the grade B, C and D embryos (p=0.033, p=0.017, p=0.008, respectively) (Table 5 and fig. 1). The results indicated that injection of 100 mg/kg one hour before RF-irradiation (group VI) resulted in a significant increase and improvement of grade A embryos (p=0.018) as well as a significant decrease in grade C and D embryos compared with group II (p=0.026 and p=0.038, respectively); while, there was no significant effect on the embryos of grade B. Furthermore, no visible effects were seen in the other groups. Qualitative and quantitative assessments showed that RF-irradiation (group II) leads to significant genetically damages in DNA chromatin in comparison with group I (p=0.000). The results showed that injecting of 50 mg/kg of the UD extract (in group V), although modified induction damage to DNA of the sperm (in comparison with group II); but it was not significant. In contrast, the dose of 100 mg/kg (group VI) resulted in a significant decrease in induction damage compared with the groups II (p=0.008) and IV (p=0.034). There was no statistical significant change in the other groups (Table 6 and fig 3).

DISCUSSION

This study showed that cell-phone RF leads to changes in frequency of two-cell NMRI mouse embryos, morphology of the embryos, and induced molecular damages in the DNA structure of the sperm. Additionally, UD extract played a preventive role against the RF induced damages. Male infertility is one of the today's human society's problems, especially those who live in industrial societies (Desai et al., 2009). Male infertility can result from a variety of changes in reproductive health. Problems in reproduction, puberty, mobility, and the ability to fertilize sperm are among the main causes of male infertility (Desai et al., 2009, Agarwal et al., 2011). One of the problems encountered in male reproductive system may be due to exposure to chemical environmental (*e.g.* chemotherapy drugs) or physical factors (*e.g.* radiation (Desai et al., 2009). Radiation can act as a disturbance of the intrinsic system to alter the concentration of hormones such as FSH (Follicle Stimulating Hormone), Luteinizing Hormone, Testosterone and Estrogen in males (Agarwal et al., 2011). Additionally, its destructive effects on the tissues of testicular neurotropic tubes, testicular serotypes, or an epididymal antioxidant system disorder are induced by oxidative stress induction (Agarwal et al., 2011). According to the results of the present study, it was found that cell-phone RF-irradiation 2 hours a day for 30 days leads to significant changes in the frequency of two-cell and morphology of the mouse embryos *in-vitro* and also induced molecular damages in the DNA structure of the sperm of the mouse. Moreover, our results showed that intraperitoneally injections of hydroalcoholic extract of UD into the cells had a protective effect against the cell-phone RF induced damages. Moreover, it ameliorated induced damage to the number and structure of the mouse embryo and maintained the integrity of the sperm. The reproductive system, due to the presence of continuous, repeated, and more fundamental and immature cells, is susceptible to environmental factors such as radiation (Erogul et al., 2006, Kesari et al., 2011). Erogul *et al.* showed that exposure to cell-phone radiation at a frequency of 900 MHz for 25 minutes at a distance of 10 cm reduced the power of sperm motility (Erogul et al., 2006). They also reported that long-term exposure to this radiation leads to genetic and morphological male sex cells. As Kesari *et al.* showed that the electromagnetic waves of mobile phones with frequency of 900 MHz for 35 days resulted in a significant reduction in the antioxidant enzymes of glutathione peroxidase, superoxide dismutase, and histone kinase (Kesari et al., 2011). The results of their work revealed that cell-phone radiation increased the production of reactive oxygen species (ROS), which also affects sperm fertility. The results of their research confirm the findings of our present study. Mammalian sperm membranes contain high levels of unsaturated fatty acids that are sensitive to lipid peroxidation due to free oxidative stress and radical

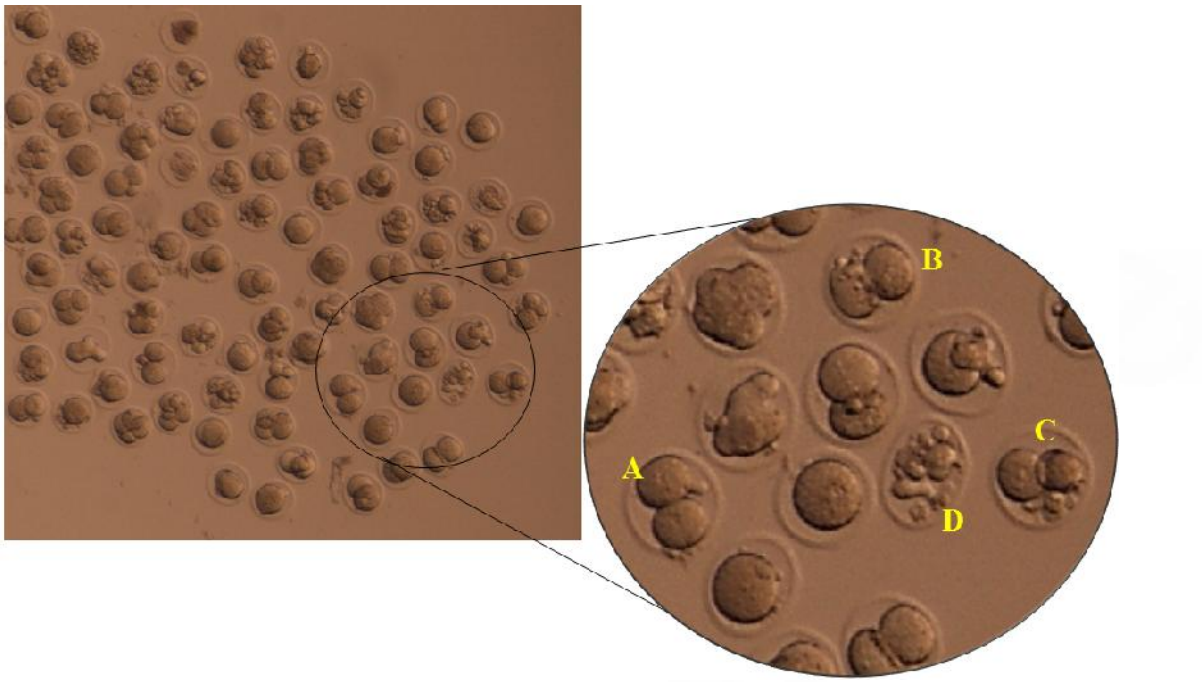

Figure 1 Bolton classification for grading of embryo based on quality of embryos in the culture medium. Based on the classification, grade A has the least damage and morphological changes, and its blastomers are equal in size and volume, while grade D has the highest damage degree in morphology and size of blastomers

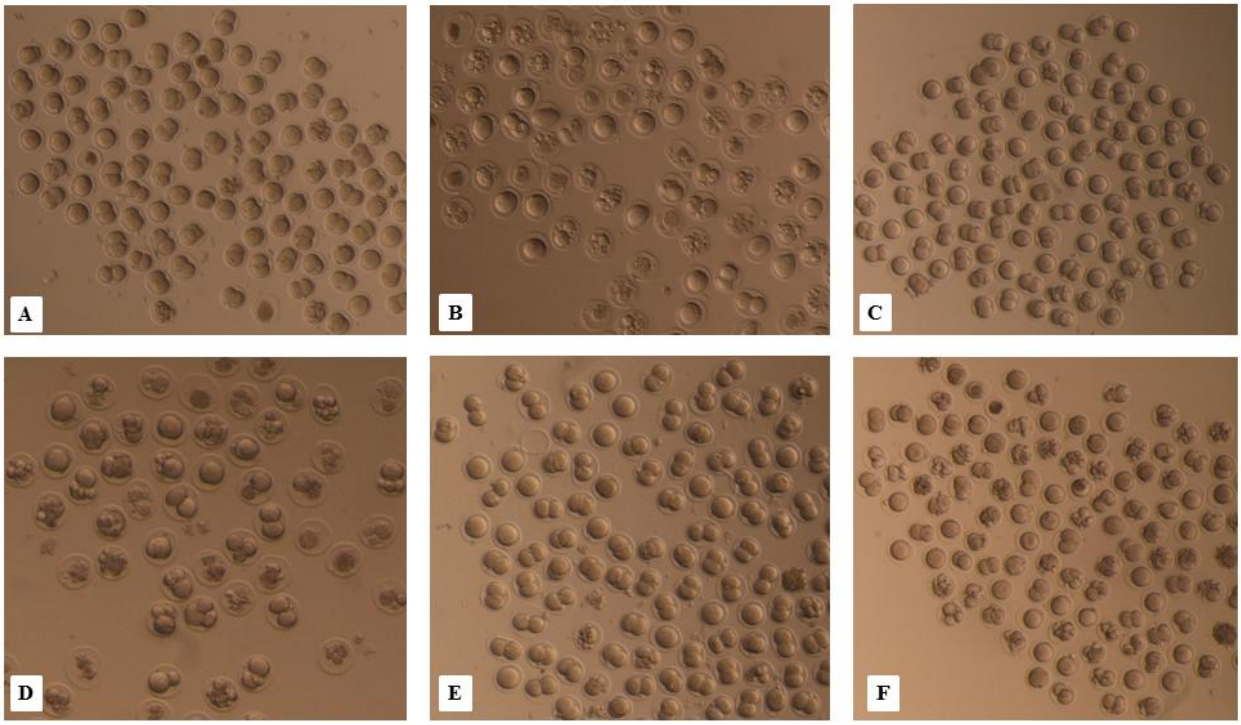

Figure 2 Photomicrographs of the embryos in KSOM medium at the first day after IVF. Image A shows embryos of control group that most of them entered in the two-cell and 4-cell stage with little fragmentation and disturbance. Image B reveals embryos of the group II that RF-irradiation has delayed the development of the embryos in the two-cell phase. Also in this group, fragmentation, darkening of the cytoplasm, degeneration of the zona pellucida and death is obviously seen. Image C exhibits increasing in frequency of embryos in group III. Image D demonstrates neither significant change in the mortality rate nor increasing in frequency of two-cell embryos of the group IV. Image E indicates increasing in frequency of embryos of the group V. Image F shows significant increase in frequency of the two-cell embryos of group VI, compared to the both groups of II and IV

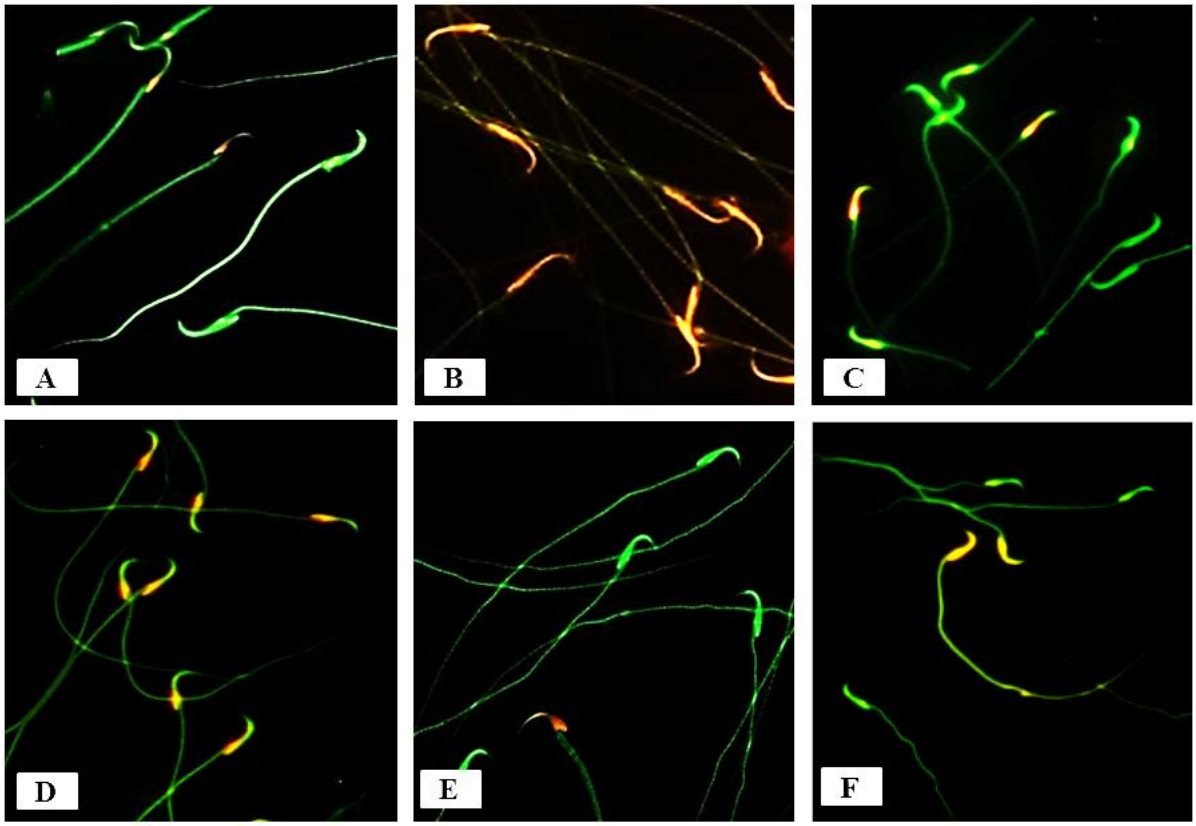

Figure 3 Evaluation of sperm DNA damage using acridine orange fluorescence staining. The results showed that RF-irradiation increased DNA damages. The heads of healthy sperm cells appear in green, often seen in the control group (Image A), the third group (Image C) and the fifth group (Image E). The orange-red head is related to the damaged DNA-sperm, which is very severe in the group II (Image B). Image D is an image for the group IV and part F is belong to the group VI.

Table 1 Mice groups as used in the present study

Groups no.	Groups name	Treatment (The mice received RF or Urtica dioica- UD)
I	healthy control	Neither RF-irradiation nor UD extract injection.
II	RF irradiated	Cell-phone RF 2 hours a day for 28 days.
III	control for UD 50	50 mg/kg of the UD extract.
IV	intervention UD 50	50 mg/kg of the UD extract plus RF-irradiation.
V	control for UD 100	100 mg/kg of the UD extract.
VI	intervention UD 100	100 mg/kg of the UD extract plus RF-irradiation.

Table 2 The main phytoconstituent of stalk and leave of the nettle (Urtica dioica) that analyzed by gas chromatography-mass spectrometry (GC-MS)

Components	Compounds
Acids	Caffeic acid derivative, silicic, citric, fumaric, glyceric, malic, phosphoric, quinic, succinic, threonic
Amines	Acetylcholine, betaine, choline, lecithin, histamine,serotonin, glycoprotein
Flavonoids	-3-O-glucoside, -3-O-rutinoside, -3-O-glucoside, -3-O-rutin, isorhamnetin-3-O-glucoside, 3-O-neohesperidoside, quercetin, kaempferol, p-coumaric acid
Mineral elements	calcium, potassium, phosphorus, magnesium, iron, sodium, zinc
Other constituents	Choline acetyltransferase, scopoletin (Cumarin), bsitosterol, tannin

Table 3 Grades of the mouse embryo and their properties according to Bolton scale.

Grades	Properties
A	Embryos with the same and round blastomer, without any kind of fermentation, smooth cytoplasm and transparent yellow zona pellucida.
B	Embryos of this group have almost dissimilar blastomers and have about 10% fermentation. Also, their cytoplasm also has some granules.
C	Size of the blastomers in this group is completely non-symmetrical and the embryos have more than 50% fermentation. Abnormal granules and large vacuoles can also be seen in their cytoplasm.
D	Blastomers of this gradient are completely non-symmetrical and have almost complete fermentation (more than 80%), and their cytoplasm is occupied by large and numerous vacuoles

Table 4 Quantitative results (mean ± standard deviation) of cleavage divisions of the embryo of study groups 1 day after IVF

Embryo types		Groups no. I	II	III	IV	V	VI
1st Day	two-cell	68.88±1.42	57.12±1.28*	65.77±1.1	59.67±0.97	72.06±1.03*	64.92±0.81#$
	Dead	6.11±0.63	9.42±0.26*	6.00±0.4	9.12±0.25	5.44±0.5	7.67±0.44*

* Significantly different from group I (p<0.05), #: significantly different from group II (p<0.05), $: significantly different from group IV (p<0.05)

Table 5 Qualitative evaluation (mean ± standard deviation) of the two-cell embryos based on the Bolton scale in the present study groups

Groups	Grades A	B	C	D
I	55.68±0.41	17.45±0.58	9.84±0.06	14.7±0.43
II	42.56±1.84*	22.35±0.33*	17.26±1.25*	19.41±0.64*
III	56.26±0.47	16.79±0.52	9.23±0.26	14.06±0.21
IV	45.04±0.87	21.62±0.15	15.72±0.24	18.25±0.54
V	57.65±0.82	16.05±0.28	8.66±0.52	13.54±0.27
VI	52.34±0.29#	20.17±0.3	12.3±0.33#	15.12±0.42#

* Significantly different from group I (p<0.05), #: significantly different from group II (p<0.05).

Table 6 Quantitative comparison (mean ± standard deviation) of sperm with defective DNA in the study groups (from 100 sperm in each rat group).

Variable	Groups I	II	III	IV	V	VI
Damaged sperm DNA	19.45±1.87	36.59±0.05*	18.39±0.28	31.92±1.47	17.51±0.22	25.75±1.14#$

* Significantly different from group I (p<0.05), #: significantly different from group II (p<0.05), $: significantly different from group IV (p<0.05).

damage, which results in rapid loss of intracellular ATP and reduced sperm motility and survival (Agarwal et al., 2011). Therefore, it is possible that changes in frequency of dead embryos caused by damages done to the DNA structure and loss of sperm survival in mice exposed to oxidative stress induced by lipid peroxidation of sperm membranes (Agarwal et al., 2011). Reactive oxygen species (ROS) are involved in the interaction between the sperm and the oocyte, in a way that the phenomenon of lipid peroxidation caused by small amounts of ROS leads to change in the sperm membrane and facilitates sperm-oocyte binding (Erogul et al., 2006, Bayat et al., 2012, Fatehi et al., 2018). With this evidence, the excessive amount of ROS results in failing of the binding of sperm-oocyte, and ultimately fertilization or fertility; or that it is incomplete and leads to fertility failure (Bayat et al., 2012). This factor can justify a reduction in frequency of two-cell embryos in the *in-vitro* environment after IVF in the present study. Additionally, the increased production of ROS from radiation causes inflammatory response (39) (Riehemann et al., 1999). Hydroxyl radicals and superoxide anions result in activating the nuclear factor κB (NFκB) that's a key factor in inflammation and apoptosis (Riehemann et al., 1999). The extract of the UD, due to the presence of caffeic-malic acid, which is a phenolic compound, suppresses the NFκB transfection factor by inhibiting the proteolytic decomposition of the inhibition subunit (IκB) (Riehemann et al., 1999). Therefore, this signaling pathway is probably one of the factors that reduces the amount of DNA damage and decreases frequency of dead embryos in group IV and strongly group VI of the present work. The UD contains compounds such as sterols, flavonoids and polysaccharide (Agarwal et al., 2011, Nahata and Dixit, 2012). These compounds have anti-androgenic effects and prevent the formation of active testosterone by inhibiting the 5-alpha reductase enzyme (Nahata and Dixit, 2012). Thus, through the normal level, the function of Leydig cells and cell lines and the testicular epithelial layers is preserved; consequently, the reproductive capacity and sperm capacity are increased, which can justify increasing frequency of two-cell embryos in groups IV and VI (Nahata and Dixit, 2012). Since the UD contains phytoestrogenic compounds, it leads to a significant reduction in the activity of cytochromes P450 and cytochrome reductase P450 (Özen and Korkmaz, 2003). The P450enzymes are capable of binding to progesterone, which results in the production of androgens from Leydig cells and increases the survival rate of sperm and prevents apoptosis induction. (Özen and Korkmaz, 2003) This route could justify a significant reduction in frequency of dead embryos in group VI. In conclusion, this study suggests the electromagnetic waves of the cell-phone can change frequency of the two-cell NMRI mouse embryo as well as its morphology and induces molecular damages in the sperm DNA structure. Furthermore, UD extract plays a protective role due to scavenging free radicals, resulting it can be utilized against the RF induced damages in reproductive.

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Abbreviations
Urtica dioica, UD ; radio frequency, RF; reactive oxygen species ROS; multimedia messaging service ,MMS; short message service , SMS; in vitro fertilization, IVF; NF-κB , nuclear factor kappa-light-chain-enhancer of activated B cells; cytochromes P450 ,CYPs; the naval medical research institute; NMRI.

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