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# A comparative study on the increased radioresistance to lethal doses of gamma rays after exposure to microwave radiation and oral intake of flaxseed oil

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***Background:*** Mobile phones, use electromagnetic radiation in the microwave range. On the other hand, there is only one report on radioprotective effects of flaxseed oil. The aim of this study was to investigate the effect of irradiation of rats with microwaves and/or treatment with flaxseed oil on the induction of adaptive response to a subsequent lethal dose (LD) of gamma rays. ***Materials and Methods:*** Eighty male rats were randomly divided into 6 groups of 13-15 animals. The animals in the 1st to 5th groups received microwave exposure, microwave+flaxseed oil (dissolved in olive oil), flaxseed (continued after LD), flaxseed, and olive oil. At day 5, all animals were whole-body irradiated with a previously reported LD 50/30 of 8 Gy gamma radiation. The 6th group (controls) received the same LD 50/30, but there was not any other treatment before or after the LD. ***Results:*** No death event was observed during days 1-9 after LD irradiation in either group. At day 10, death events started in the 4th group. Thirty days after irradiation of the animals, the survival fractions for the control group, as expected, was 53.3% while there was no death event in the 1st group (survival rate of 100% in microwave-pretreated animals). The survival fractions for the 2nd to 5th groups were 69.2%, 92.3%, 46.1%, and 61.5%, respectively. ***Conclusion:*** While these findings open new horizons in radiation protection, the radioresistance induced by microwave radiations emitted by a mobile phone may interfere with the outcome of any subsequent therapeutic application of photons or radioisotopes.
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***Keywords:*** *Microwave, adaptive response, nonionizing radiation, survival, rat.*

## INTRODUCTION

There is substantial evidence that low-dose ionizing radiation (LDR) can produce stimulatory or adaptive responses, which increase the resistance to detrimental effects of subsequent high-dose radiation exposures. This phenomenon was firstly reported by Olivieri *et al.* [1] but later adaptive response was shown in various test systems including plant cells [2], insects [3], Chinese hamster V79 cells [4, 5], cultured human lymphocytes [6-11], embryonic and HeLa cells [12], occupationally exposed persons [13, 14], cultured animal lymphocytes [15], and *in-vivo* studies on laboratory animals [16-19]. There are also reports indicating lack of radioadaptive response in cultured human lymphocytes [20-22]. Furthermore, long-term follow up studies indicate that lack of radioadaptive response is not a temporary effect and, in contrast with the early reports, does not depend on

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transient physiological factors [23, 24].

Although there is substantial evidence about the induction of adaptive response with low doses of ionizing radiation, doubt still persists concerning whether it is possible to induce such a response after exposure to adapting doses of non-ionizing radiations such as microwaves. Mobile phones, as wireless communication devices with drastically widespread use and increased popularity, use electromagnetic radiation in the microwave range [25].

Cell phones use pulsed electromagnetic radiation in the radiofrequency (RF) region to transmit information, which can be done at different frequencies. Common Global System for Mobile Communication (GSM) mobile phones emit electromagnetic radiation with a carrier frequency at 900 MHz, "modulated" by human voice (speaking emission). GSM cell phones use frequencies within four different frequency bands; 850 MHz (824.2 - 848.8 MHz Tx; 869.2 - 893.8 MHz Rx), 900 MHz (880-2 - 914.8 MHz Tx; 925.2 - 959.8 MHz Rx), 1800 MHz (1710.2 - 1784.8 MHz Tx; 1805.2 - 1879.8 MHz Rx), 1900 MHz (1850.2 - 1909.8 MHz Tx; 1930.2 - 1989.8 MHz Rx), where Tx and Rx refer to transmit and receive, respectively. In Islamic Republic of Iran, GSM mobile phones use only two frequencies; 900 and 1800 MHz.

We also studied the effect of oral intake of flaxseed (linseed) oil on the survival of animals after receiving a lethal dose (LD) of ionizing radiation. Flaxseed, the richest source of a lignan called secoisolariciresinol diglucoside (SDG) [26, 27], has an exceptionally high concentration of α-linolenic acid (57% of total fatty acids). Animal and epidemiologic studies have shown that due to these characteristics, flaxseed has protective effects against breast cancer [28]. Based on these findings, it can be hypothesized that flaxseed might be effective in cancer prevention and its treatment [29]. It has been shown that SDG metabolites may serve as beneficial health agents due to their antioxidant activity [27, 30]. Also these metabolites have weak oestrogenic or anti-oestrogenic effects and they can induce phase 2 proteins and/or inhibit the activity of certain enzymes [27]. There is only one report indicating radioprotective effects for flaxseed [31].

The aim of this study was to investigate the effects of irradiation of rats with microwaves, as well as treatment with flaxseed oil, on the induction of adaptive response to a subsequent LD of gamma rays.

## MATERIALS AND METHODS

### Animals

Eighty male adult Sprague Dawley rats (200-250 g) obtained from the animal house of Shiraz University of Medical Sciences (SUMS) were randomly divided into 6 groups of 13 animals (1st to 5th groups) and 15 animals (6th group). The animals were kept in special cages with controlled temperature, humidity, lighting in university animal house and fed with standard pellete and water ad libitum. Animals were transferred to their cages ten day before the experiment to allow them to adapt to *their new environment*. All laboratory animals used in this study, received humane care in compliance with the SUMS Regulations on Animal Care and the study was approved by SUMS ethics committee.

### Grouping

The animals in the 1st group received microwave exposure for 3 hours each 12 hours (6 hours a day) for 4 days before exposure to the lethal dose of gamma radiation. Animals in the 2nd group received the same microwave irradiation plus daily doses of 50 mg flaxseed (TruNature, USA) dissolved in 0.5 mL of pure olive oil for the same period (4 days). Treatment with flaxseed continued after LD 50/30 for 7 days (days 6 to 12). The 3rd group treatment was the same as the 2nd group (flaxseed before and after exposure to the LD) but there was no microwave exposure. The 4th group

treatment was the same as the 3rd group but treatment with flaxseed did not continue after the LD. Animals in the 5th group received daily doses of 0.5 mL pure olive oil for the first 4 days (before the LD). The 6th group (controls) received the same LD but there was no other treatment before or after the LD.

### RF Exposure

A GSM mobile phone simulator (made at the School of Engineering, Shiraz University) was used for microwave irradiation. Emitted power (circular space distribution) of the generator was fixed at 2 W during exposure. The frequency, maximum power output and other cardinal exposure parameters of the simulator was the same as common GSM mobile phones.

### Gamma Irradiation

At day 5, all animals were whole-body irradiated with a previously reported LD 50/30 of 8 Gy gamma radiation [32] emitted by a therapeutic Theratron 780 C Cobalt-60 source (90 cm SSD, 54.68 cGy/min dose rate, 35×35 $cm^2$ field, 14.63 min irradiation time). 7-8 animals were irradiated in a group at one time.

### Survival Study

After gamma irradiation, the animals were monitored for 30 days. During this interval, the number of the living animals as well as death events were daily controlled/recorded by an expert group.

### Statistical Analysis

Survival rates were analyzed by the Kaplan-Meier test. P-values < 0.05 were considered statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 16 (SPSS, Chicago, IL).

## RESULTS

The observed survival rates of the animals in different groups are plotted in figure 1. No death event was observed during days 1-9 in any group. At day 10, death events started in the 4th group and the survival fraction decreased to 84.6%. At day 15, the survival fractions in the 1st and the 6th (control) groups were 100%, and 84.6% for all remaining groups. At day 20, the survival fractions in the 1st and group was still 100%, while it was 76.2%, 84.6%, 69.2%, 61.5%, and 86.7% for the 2nd to 6th groups, respectively.

At day 25, the survival fractions in the 1st and group was unchanged (100%), while it was 9.2%, 76.9%, 61.5%, 61.5% and 66.7% for the 2nd to 6th groups, respectively. Thirty days after irradiation of the animals, the survival fractions for the control group, as expected, was 53.3% while there was no death event in the 1st group (survival rate of 100%). The survival fractions for the 2nd to 5th groups were 69.2%, 92.3%, 46.1%, and 61.5%, respectively. The animals pretreated with microwave (P=0.009) or flaxseed oil that was continued after irradiation of the animals with the lethal dose (P=0.039) showed a statistically significant difference in survival compared to the controls. Oral intake of either flaxseed (P=0.615) or olive oil (P=0.686) before irradiation with an LD of gamma radiation, however, did not induce a significant level of radioresistance. Microwave irradiation and oral intake of flaxseed before irradiation, however, did not induce a

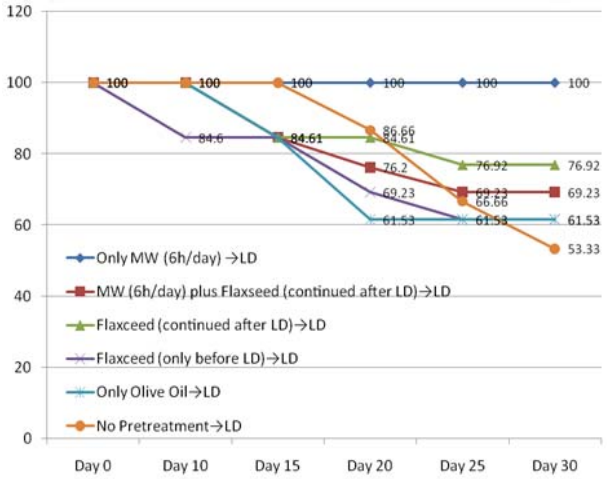


**Figure 1.** Survival rate of the animals in all groups every 5 days until day 30 after irradiation of the animals with LD 50/30.

significant level of radioresistance (P=0.653). Table 1 shows the significance of the differences observed in survival rates of each group compared to that of the controls.

**Table 1.** The significance of the differences observed in survival rates of each group compared to that of the controls.

| Compared Groups | | Significance (P -Value) [Kaplan-Meier] |
|---|---|---|
| Group 1 MW→ LD | Group 6 0→ LD | **P=0.009** |
| Group 2 MW + Flax $_{cont}$→ LD | Group 6 0→ LD | P=0.653 |
| Group 3 Flax $_{cont}$→ LD | Group 6 0→ LD | **P=0.039** |
| Group 4 Flax→ LD | Group 6 0→ LD | P=0.615 |
| Group 5 Olv→ LD | Group 6 0→ LD | P=0.686 |
| All 6 Groups | | P=0.041 |

MW: Microwave LD: Lethal Dose 50/30
Flax: Flaxseed Oil
Flax $_{cont}$: Flaxseed Continued After Receiving LD
Olv: Olive Oil

The mean (±SD) weight of the control animals (only treated with LD 50/30) surviving after 30 days was 311.1 ± 47.9 g while it was 309.8 ± 37.8, 318.6 ± 28.1, 308.1 ± 34.8, 333 ± 42.9, and 304.6 ± 37.3 in the 1st (MW), 2nd (MW + flaxeed), 3rd (flaxeed continued after irradiation), 4th (flaxeed stopped before irradiation) and 5th (only received olive oil) groups, respectively. These differences were not statistically significant.

## DISCUSSION

Results obtained in this study clearly indicate the possibility of the induction of survival adaptive response as increased resistance to lethal doses of gamma rays by pre-exposing the animals to microwave radiation. Survival fractions, thirty days after irradiation of the animals with LD 50/30, for the animals pre-treated with microwave radiation was 100% while it was 53.3% for the animals that had no pre-treatment before LD 50/30 (control group). This finding is in line with the only report that recently indicated the possibility of the induction of adaptive response after pre-treatment with microwave radiation [33]. In this study, Sannino et al. showed that pre-exposure of peripheral blood lymphocytes collected from human volunteers to non-ionizing RF radiation (900 MHz, at a peak specific absorption rate of 10 W/kg for 20 h) increased their resistance to a challenge dose of mitomycin C (100 ng/ml at 48 h). In this light, in spite of the fact that there were some basic differences between our study and the above- mentioned investigation, both studies confirm the potential of RF radiation for induction of adaptive response. Due to the cardinal differences between our study and that conducted by Sannino *et al.* [33], namely the type of the challenge dose (LD of gamma radiation in our study versus chemical agent of mitomycin C in Sannino's study and the endpoint (survival, 30 days after LD 50/30 in our study versus induction of micronuclei in Sannino's study [33], these studies can be compared thoroughly.

Our study also showed that oral intake of flaxseed oil before irradiation with an LD of gamma radiation cannot induce a significant level of radioresistance. Thirty days after irradiation of the animals with LD 50/30, the survival fractions for the control group (no pretreatment bofore the LD) was 53.3% while it was 61.5% in either animals pretreated with oral doses of flaxseed oil or olive oil. In this light, our results cannot confirm the previously radioprotective effect of flaxseed reported by Bhatia *et al.* [31]. However, we found significant increased survival in animals that were treated with daily oral doses of flaxseed oil after irradiation with the LD dose. Thirty days after irradiation of the animals with LD 50/30, the survival fractions for the animals treated with daily doses of flaxseed after the LD either with pretreatment with microwave radiation or without it were 76.9% and

69.2%, respectively. This possibly is due to bioeffects other than the radioprotective effects of flaxseed oil. It can be hypothesized that treatment of the animals with flaxseed after receiving a lethal dose of gamma radiation possibly reduces the gastrointestinal symptoms of the lethal dose. According to Bhatia's report, the radioprotective effects of flaxseed oil may be associated with omega-3 essential fatty acids and phytoestrogenic lignans, which are known free radical scavenging and singlet oxygen quenching agents. They also reported that flaxseed oil may have prophylactic potential against liver degenerative changes which are produced by radiation [31]. On the other hand, flaxseed oil suppress oxygen radical production by white blood cells [34].

It has also recently been shown that flaxseed oil supplementation diet protects animals against bacterial infection [35]. It is well documented that total body irradiation may lead to two major components of the acute radiation syndrome; i.e. bone marrow insufficiency and possible infection due to immunosuppression [36]. As infection plays an important role in the lethality of high doses of ionizing radiation, the higher survival rate in animals treated with oral doses of flaxseed after LD may be due to controlling the infection. Further studies are needed to clarify the accurate mechanisms by which flaxseed increases the survival rate of irradiated animals.

## CONCLUSION

The results obtained in this study clearly show that pre-exposure of laboratory animals to adapting or conditioning doses of microwave radiation can induce an adaptive response observed as significant increased survival fraction at a specific time after exposure to a lethal dose of ionizing radiation. Altogether these findings are in line with the previous report of Sannino et al. who reported the possibility of induction of adaptive responses by adapting doses of microwave radiation. Based on these findings, the radioresistance induced by pre-exposure to microwave radiation emitted by common source of radiofrequency radiations such as mobile phones may interfere with the outcome of any subsequent radiation-based therapeutic procedures (teletherapy, brachytherapy and targeted therapies).

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