Research Article

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Effects of Low Electromagnetic Waves on Thymus Size, Testis and Body Weight and Therapeutic Role of Vitamin C in Mice

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ABSTRACT

Results of several studies on different effects of electromagnetic waves reveal that change of field intensity, even milli tesla has various biological effects, therefore investigation on electromagnetic effects can be done in very wide range. In this research, amount of these effects on biomass, thymus, testis and therapeutic role of vitamin C in improvement of changes caused by electromagnetic field, have been studied. In this experimental study, 24 adult male mice (Balb/C) were divided into 3 groups. The control group consist of 8 mice were kept in normal conditions. The group exposure to Electromagnetic field consist of 8 mice, which were exposed to ELF (50Hz, 4Mt) for 15 days (4 hours per day), and the group that exposure to Electromagnetic field supplemented with vitamin C, contains 8 mice that were exposed to ELF (50Hz, 4Mt) for 15 days (4 hours per day) and 0.5 cc vitamin C were injected intraperitoneally 7 times. This injection was done 3 times a week. After 15 days, mice were anesthetized by chloroform, following careful dissection, organs were splitted over then this samples were dissected and measured. Data was analyzed using one way ANOVA test.

The results show electromagnetic waves lead a significant reduction in body weight and receive appropriate dose of vitamin C may have therapeutic role which reduce damages caused by 50 Hz/4 mT on Balb/C male adult mice. According to this research exposure to electromagnetic waves reduces thymus and testis weight significantly which may root in reduced cell division and inhibition of apoptosis, so simultaneous deliver if vitamin C didn't show significant effect on thymus and testis.

Introduction

During last years, increased use of devices producing electromagnetic wave, has attracted researcher's attention to possible effects of them on human health. Electromagnetic waves have different shapes, they are classified according to their frequencies and wavelength [1]. Today, effects of electromagnetic waves with low frequency (ELF) have been attracted researcher's attention [2,3]. Electromagnetic waves with different intensities influence on prevalence of embryo evolution disorders, infertility, neuronal/sleep disorders, gastro-intestinal, heart disorders and several neoplasms including hematopoietic, lymphoma [4,5]. Thymus is behind of sternum between lungs, often it contains 2 parts. Each part of thymus is divided to smaller parts. Cortex contains compacted lymphocytes. Medulla constructed from sporadic epithelial and lymphocytes. Epithelial cells in thymus produce its hormones, possibly it incorporates in T lymphocyte

evolution; however, its effect is not clear. Thymus is large in childhood but during growth it will be replaced by connective and adipose tissues. During maturation, thymus will be smaller, but it incorporates in T cell evolution. Thymus produces many hormones like thymosin, thymic humoral factor, thymopoietin, mentioned hormones induce proliferation and differentiation of T cells. Some evidence reveals that thymus hormones can delay aging [6].

Testis produces hormones and spermatozoon. Testes are wrapped by tunica albuginea. Tunica albuginea thickens at posterior of testis and constructs mediastinum which is a fibrous connective tissue diffuses to gland and divides it to pyramid structures called testis lobules. These walls are not complete, often there are connections between them. Each lobule contains 1-4 seminiferous tubules. They locate in a fragile connective membrane which is full of blood vessels, lymphocytes, neurons and leydig cells. Seminiferous tubules produce male reproductive cells, spermatozoon. While leydig cells produce androgenic hormones, sperms are produced in seminiferous tubules. Seminiferous tubules are connective tissues contain a basal and a germ epithelial layers. Fibrous tunica properia encompasses seminiferous tubules and has several fibroblast layers. Innermost layer attached to basal layer is constructed from semi-muscular flat cells. These cells function as smooth muscles. Leydig cells occupy most part of space between seminiferous tubules. Epithelial seminiferous tissue has 2 cells; sertoli or supportive cells and cells which construct spermatogene antecedent cells. Spermatogene antecedent cells are distributed in 4-8 layers, they produce spermatozoids. Process of producing spermatozoids is called spermatogenesis. This includes cells divisions via mitosis/meiosis and final differentiation of spermatozoids which is called spermiogenesis [7].

Vitamin C or L-ascorbate is a vital micronutrient for many species like human, monkeys and few number of other mammals, especially guinea pigs and some bird/fish species. Amount of vitamin C is a critical index for commercial value of fruits and vegetables. Vitamin C is a solid, white, water soluble, safe component which has s circular ester, it is hydrolyzed in aqueous medium and convert to acid [8]. This vitamin which attacks to electrons in body reactions, is most important antioxidant in neutralizing free radicals and inhibiting oxidative stress [9]. Antioxidant mechanism of action: free radical/reactive oxygen species production is an inevitable issue in metabolism process. These compounds because of high potential in damaging biological macro molecules like fats, DNAs function as major agents of ageing and cause several diseases. Organisms have various mechanisms to defend and neutralize these reactive species including defensive anti-oxidant immune systems. These systems have enzymes like superoxide dismutase, catalase. Glutathione peroxidase and macro molecules like albumin, ceruloplasmin, ferritin and micro molecules like carotene, alpha-tocopherol, ascorbate, methionine, uric acid, bilirubin, and reduced glutathione (GSH) [10].

There are several reports about role of this vitamin in prevention DNA damages and occurrence cancer or heart diseases [11]. Large amount of vitamin C aggregates in ovaries and other endocrine tissues. In ovaries, vitamin C aggregation occurs in granulosa, luteal and theca cells, these cells relate closely to fertility. Studies on luteal granulosa cells demonstrated that vitamin C can increase progesterone production/concentration [12]. this relation is strong negative feedback, where high levels of progesterone reduces vitamin C concentration and its metabolism is inhibited [9]. Fritze, et al. [13] exposed rats to 900 Hz electromagnetic waves, they observed that these waves increased Hsp7z mRNA replication at brain cortex [13]. In Louis studies, pregnant CD-1 mice exposed to 2.45 GHz waves for 100 minutes from 1-17 pregnancy days, rats were dissected at 18th day and their embryos were evaluated in terms of disorders. Results showed that weight of experimental embryos was less than control [14]. In another study, few Spagu-Dawley rats were exposed to microwaves from 6-20 pregnancy day. Weight of test group was less that control [15].

Occonnor exposed mice to 2450 MHz and found that weight of embryos was reduced significantly because of maternal thermal stress [16]. Kolomiytseva, et al. [17] investigated muridae, they reported that unionizing electromagnetic waves caused lipid storage in adipose tissue and weight gain. Results of several studies on different effects of electromagnetic waves reveal that change of field intensity, even milli tesla has various biological effects, therefore investigation on electromagnetic effects can be done in very wide range [18]. In this research, amount of these effects on biomass, thymus, testis and therapeutic role of vitamin C in improvement of changes caused by electromagnetic field, have been studied.

Material and Methods

This experiment was done in research electrophysiology laboratory of biochemistry-biophysics department of Mashhad Azad University. In order to evaluate effects of 50 Hz electromagnetic waves on biomass, testis, thymus and therapeutic effect of vitamin C on mature male mice, 3-2.5 month Balb/C mice with average weight 20-25 grams were used. Mature mice have been stored in animal room with 60-70% humidity, 23±1 centigrade temperature and 12 hours light/12 hours overnight. Room light was adjusted alternatively by automatic electrical timer and temperature was controlled by radiators and cooler at winter and summer respectively. Cages were cleansed every other day. For smoothness of cage, wood chips were used. Mice feed was special prepared pellets (Khorasan Javaneh dam company), water delivered by flasks. Mice were purchased from Razi Vaccine and Serum research Institute of

Mashhad, for being adapted with new environment and eliminating stress of changing environment, mice were kept one week in animal room before experiment. Electromagnetic producing machine includes 35 cm diameter and 60 cm length PVC tube equipped with 1900 copper coils which coiled 3 times around tube. This machine could produce 0.5-4 mT/25-100 Hz electromagnetic field. In order to evaluate therapeutic role of vitamin C, commercial 250 Osveh vitamin C tablets (chewable scored tablets) were used. In order to inject tablet, it was dissolved in 50 cc physiologic serum. Intra-protaneal vitamin C was injected by disposable syringe.

Study groups in this 15 day research were 15 day old (24 mature 2.5-3 month mice) distributed in 3 groups:

1) **Control:** 8 mice stored in animal room with normal conditions,
2) **Exposed to electromagnetic waves group:** in this group, 8 mice exposed to 50 Hz/4 mT electromagnetic waves for 15 days, 4 hours per day(12-16 hours),
3) **Exposed to electromagnetic waves receiving vitamin C:** in this group, 8 mice exposed to 50 Hz/4 mT electromagnetic waves for 15 days whom were treated with 0.5 cc intra protaneal vitamin C 7 times. This injection was done weekly, 3 times per week. During experiment, mice were weighted 3 times weekly by 0.01 resolution scale and their weights noted. In order to be sure about weighting, this process repeated 3 times. After required time, mice were anesthetized by chloroform, following careful dissection, organs splited over. Then these organs exposed to physiologic serum for 3 seconds, dried on paper for 3 seconds. Organs were located separately on glass to weight. Statistical analysis was done by Spss16 and plots were illustrated by Excel. Data were represented by mean±SEM. Comparison between control and test was done by statistical T-test and $P<0.05$ was considered statistical significant difference (Figures 1-4).

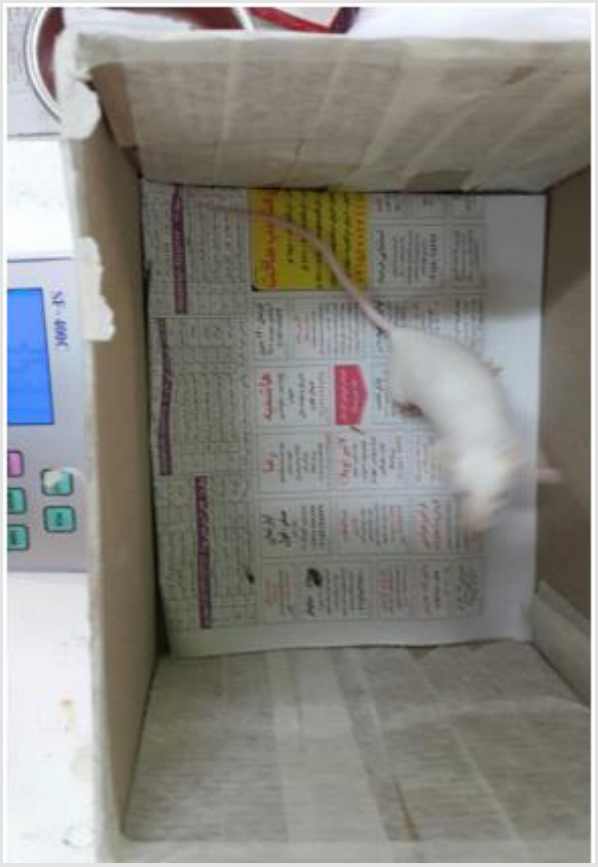

Figure 1: Weighing of mice.

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Figure 2: Dissection of mice.

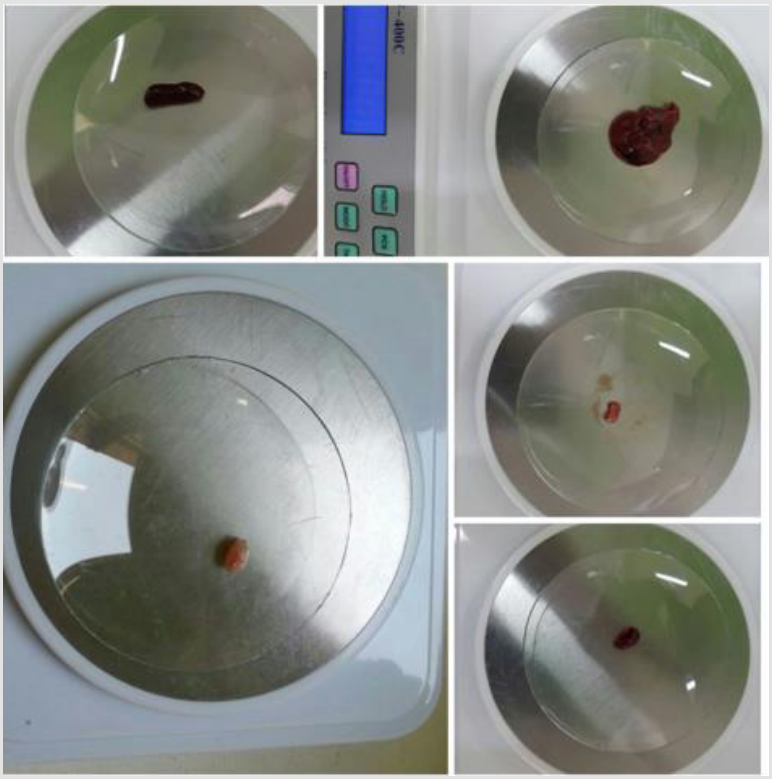

Figure 3: Weighing mice organs.

Figure 4: Intra-protaneal vitamin C injection.

Results

Evaluation of Thymus Weight

Mean weight of thymus in control group is 0.055 ±0.0120 and in exposed electromagnetic fields is 0.115±0.0066, there is significant difference P<0.01 between them. Mean weight of thymus in exposed electromagnetic receiving vitamin C group is 0.0131±0.0081 which is not significant difference to exposed field group.

- **Diagram 1:** comparison weight of thymus between control, electromagnetic field exposed and electromagnetic field exposed receiving vitamin C groups.

++ refers to significant difference between control and electromagnetic field exposed groups with P<0.01.

NS refers to un-significant statistical difference (Figure 5).

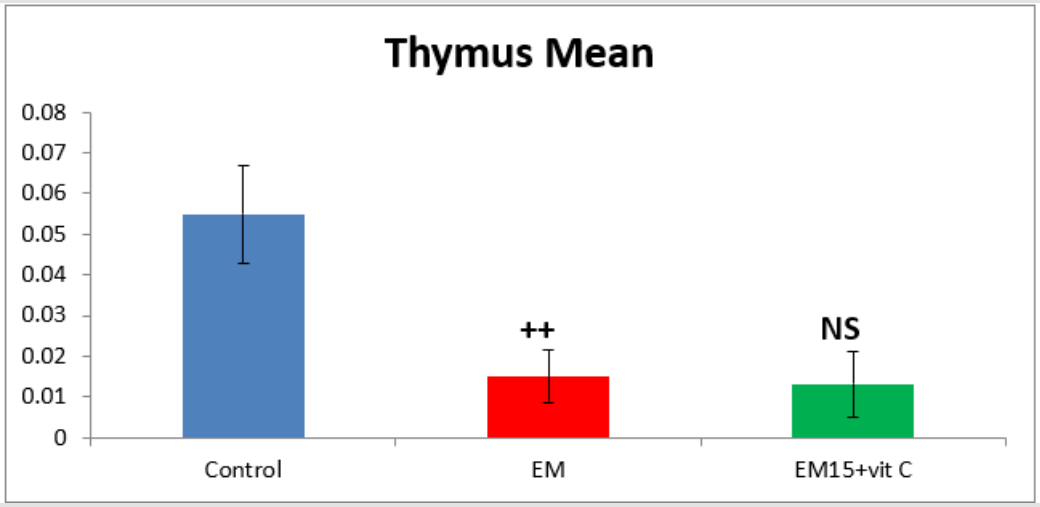

Figure 5: Evaluation of thymus weight.

Evaluation of Testis Weight

Mean weight of testis in control group is 0.0916 ±0.0079 and in exposed electromagnetic fields is 0.045±0.0104, there is significant difference P<0.01 between them. Mean weight of testis in exposed electromagnetic receiving vitamin C group is 0.068±0.0102 which is not significant difference to exposed field group.

- **Diagram 2:** comparison weight of testis between control, electromagnetic field exposed and electromagnetic field exposed receiving vitamin C groups.

++ refers to significant difference between control and electromagnetic field exposed groups with P<0.01.

NS refers to un-significant statistical difference (Figure 6).

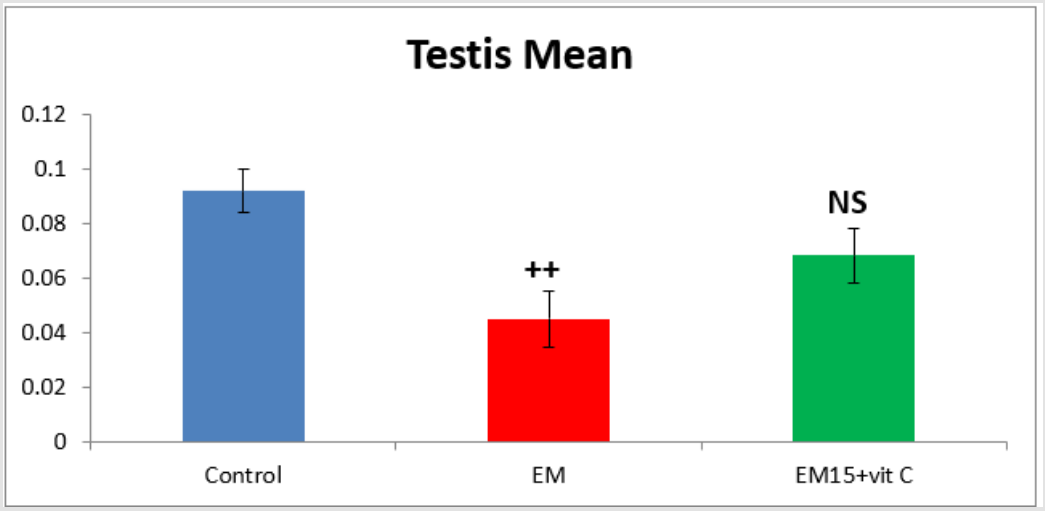

Figure 6: Evaluation of testis weight.

Evaluation of Body Weight

Mean weight of body in control group is 33.615±0.8700 and in exposed electromagnetic fields is 23.542±0.4068, there is significant difference P<0.001 between them. Mean weight of testis in exposed electromagnetic receiving vitamin C group is 26.422±0.8551 which is significant difference to exposed field group with P<0.05.

- **Diagram 3:** comparison weight of body between control, electromagnetic field exposed and electromagnetic field exposed receiving vitamin C groups.

++ refers to significant difference between control and electromagnetic field exposed groups with P<0.001.

*refers to significant statistical difference between electromagnetic field exposed and electromagnetic field Balb/c male mice exposed receiving vitamin C groups with P<0.05 (Figure 7).

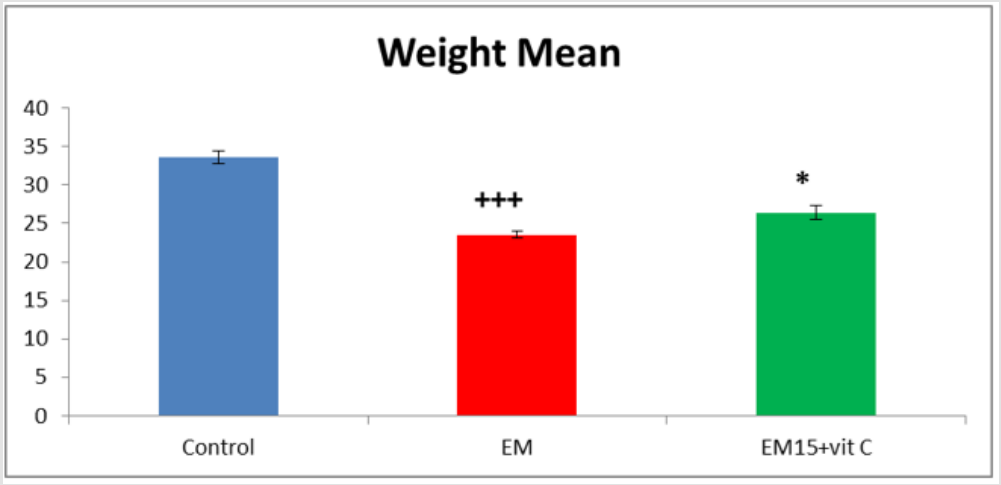

Figure 7: Evaluation of body weight.

Discussion and Conclusion

Analysis the Results of 50 Hz/4 Mt Electromagnetic Wave Effects on Thymus Weight of Mature Balb/C Male Mice

According to this research, 50 Hz/4 mT electromagnetic waves reduce thymus weight of mature Balb/C male mice which shows significant relation.

Analysis the Results of 50 Hz/4 mT Electromagnetic Effects on Thymus Weight of Mature Male Balb/C Mice Receiving Vitamin C

According to this research, 50 Hz/4 mT electromagnetic waves did not change signifantly thymus weight of mature male Balb/C mice receiving vitamin C.

Analysis the Results of 50 Hz/4 mT Electromagnetic Wave Effects on Testis Weight of Mature Balb/C Male Mice

According to this research, 50 Hz/4 mT electromagnetic waves reduce testis weight of mature Balb/C male mice which shows significant relation. Studies on total weight of testis and epididymis showed significant weight reduction after exposure to electromagnetic field which is in agreement with current study and reveals the discharge of sperms at seminiferous tubules or epididymis; because a semi-muscular layer surrounds seminiferous layer in mice which has erectile property. Electromagnetic waves produce electrical current in animal body, this current changes cellular function directly/indirectly, therefore contraction/sperm discharge will be increased. In addition, local secretion of oxytocin after exposure to electromagnetic waves has been demonstrated. This protein factor increases contractile property in seminiferous tubules [19].

Analysis the Results of 50 Hz/4 mT Electromagnetic Effects on Testis Weight of Mature Male Balb/C Mice Receiving Vitamin C

According to this research, 50 Hz/4 mT electromagnetic waves did not change signifantly testis weight of mature male Balb/C mice receiving vitamin C.

Analysis the Results of 50 Hz/4 mT Electromagnetic Wave Effects on Body Weight of Mature Balb/C Male Mice

According to this research, 50 Hz/4 mT electromagnetic waves reduce body weight of mature Balb/C male mice which shows significant relation.

In Louis experiments, CD-1 pregnant mice were exposed to 2/45 GHz waves from 1-17 day of pregnancy for 100 min/day, they were dissected at 18th day and fetus were analyzed in terms of abnormalities. Louis results showed that experimental fetus weight was lower than control [20]. In another study, few Spagu-Dawley rats were exposed to microwaves from 6-20 pregnancy day. Weight of test group was less that control [21]. Occonnor exposed mice to 2450 MHz and found that weight of embryos was reduced significantly because of maternal thermal stress [22]. Some researchers investigated neonate weight of female physiotherapists and concluded that their weights were less than control. They also explained thermal stress of electromagnetic waves for this weight reduction, increased temperature not only kills fetus but also delays fetus development. This phenomenon explains less weight of test neonates than control in mentioned study [23].

Dasdage, et al. [24] investigated mobile phone waves on rats which showed decreased weight of exposed fetus [24]. In another study, invertebrate embryos exposed to low frequency electromagnetic waves lowered fertility in females and inhibited embryo development at bi cellular phase [25]. In another study, it was reported that exposure of embryonic cells to electromagnetic waves decreased cell cleavage and in harsh cases, stopped cleavage, the reason was chromosomal damage induction by electromagnetic waves [26]. It was reported that exposure of Inner Cell Mass to electromagnetic waves inhibited mitosis and pluripotency in these cells. Reason of this was free radical production in these cells [27]. mIn study by Rahbarian and Sadughy, it was demonstrated that development percentage of fetus exposed daily to 50 Hz and 200 Gauss electromagnetic waves was reduced significantly in comparison to fetus exposed to 10 Gauss waves [28].

Balanejad reported inhibitory effects of low frequency electromagnetic waves with 400 Gauss intensity on angiogenesis at chorioallantotic membrane of chick embryo. Balanejad believes that high intensity electromagnetic waves can reduce weight of chick embryo in early phases of growth [29]. Huuskonen claimed that exposure of pregnant wistar rats to low frequency electromagnetic waves leads to sever body weight and occur of abnormality in motor organs, in addition, high intensities lead to mortality of rats [30]. Canseven exposed embryonic cells of guinea pigs to 50 Hz with1,2,3 T electromagnetic waves for 5 days (4 to 8 hours/day) and investigated their development. Results showed that electromagnetic radiation degenerated embryonic cells by DNA damage, influencing on membrane enzymes and changing its permeability [31]. Cieslar studied development phases of embryonic heart cells of rats (*in vitro*) exposed to 50 Hz with 78.3 Gauss electromagnetic waves for 30 minutes. Results showed that length, diameter and size of heart in samples exposed directly to electromagnetic waves decreased significantly in comparison to control [32].

In a study Valles showed that low frequency/high intensity electromagnetic waves could affect cell cleavage and mitotic spindle orientation, even they could inhibit cell division by damaging mitotic spindles [33]. Low frequency electromagnetic waves decrease adrenal weight and inhibit sympatho-adneral system in hypertension rats [34]. According to Jelodar and Beizai studies, leakage waves of microwave oven reduced growth, increased T3, T4, cortisol, triglyceride and HDL levels [35].

Analysis the Results of 50 Hz/4 mT Electromagnetic Effects on Body Weight of Mature Male Balb/C Mice Receiving Vitamin C

According to this research, 50 Hz/4 mT electromagnetic waves increase body weight of mature Balb/C male mice which shows significant relation.

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