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# Effects of *Polygonum aviculare* Herbal Extract on Sperm Parameters after EMF Exposure in Mouse

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**Abstract:** Electromagnetic fields with high energy same as ionizing radiation inserts their destructive effects via free radical production. Using antioxidants or herbal plants with antioxidants components could diminish hazardous effects of EMF. *Polygonum aviculare* has a high amount of phenolic and flavonoid and proved that has antioxidants effects. The aim of this study was to evaluate the effects of *Polygonum aviculare* herbal extract on sperm parameters after EMF exposure in mouse. Twenty four male mice, 8 weeks divided to 4 groups (one control and three experimental groups). Control group didn't receive EMF exposure. EMF group mice received 3 mT EMF during 2 months, 4 h daily and 5 days weekly. *Polygonum aviculare* group received 50 mg $kg^{-1}$ herbal extract during 2 months and poly -EMF group received 3 mT EMF during 2 months, 4 h daily and 5 days weekly and 50 mg $kg^{-1}$ herbal extract during 2 months. After 2 months the mice sacrificed with cervical dislocation and sperm obtained from tail of epididymis and motility and morphology of them were analyzed. Sperm analysis results showed that in group with *Polygonum aviculare*, morphology and motility of sperm developed ($p<0.05$). Present results showed that EMF can reduce motility of sperm and treatment of *Polygonum aviculare* after EMF exposure developed sperm quality after EMF exposure.

**Key words:** EMF, *Polygonum aviculare*, sperm parameters, exposure, electromagnetic fields

## INTRODUCTION

Nowadays, all people are somehow related with electromagnetic waves creating several harmful effects in their tissues. These waves affect all body tissues but some of them including reproductive system is of high importance (Vecchio *et al*., 2011). It has been proven that electromagnetic waves result in tissue damage and affect cell natural activities. Electromagnetic fields impose their degenerative effects due to having high energies such as ionization radiations through developing free radicals (Vojtisek *et al*., 2009). The radicals lead to developing of short-chain fatty acid derivations through lipids peroxidation. It can develop some reactions in cells which finally result in amino acid peroxidation, protein-to-protein link, DNA fiber refraction and appearing of different disorders (Motoyama *et al*., 2010). Although, there are antioxidants to remove oxygen active species in body, antioxidants face problems because of imbalance in their production and removing. As a result, accumulation of harmful molecules may cause different diseases including damage of generative cells of testicles as well as change the semen quantity and other indexes such as sperm morphology, motility, vitality each of which are vital for a successful productivity (Duyan *et al*., 2008). Increase of ROS production in seminal fluid by leucocytes or spermatozoa may be a factor for infertility (Lee *et al*., 2009). Hydrogen peroxide is one of the main oxygen free radicals produced by human sperm. Moderate increase of hydrogen peroxide does not affect sperm life rather makes it insite due to decrease of intercellular ATP and acrosomal proteins phosphorylation (Zhao *et al*., 2008). High amounts of hydrogen peroxide lead to lipids peroxidation and finally necrosis. Availability of high amounts of unsaturated fatty acids prepares sperm cell structure to ROS degenerative effects. Accompanied by lack of sperms antioxidants production feature, it leads to degeneration and destruction of these cells by ROS (Moisescu *et al*., 2008). At present, many extractions, compounds and molecules have been extracted from herbs widely used to treat or decrease infertility (Massa *et al*., 2010). Use of new antioxidants may prevent developing of pathologic changes resulted from encountering to electromagnetic waves (Gan *et al*., 2010). In a research conducted in 2010 demonstrated that *Polygonum avicular* contains high amounts of phenolic

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and flavonoid (Mohsenzadeh *et al*., 2010). Flavonoid has been considered as antioxidant compounds. They may have wide biological effects because they have herbal origin. On the other hand, meaningful relation found between antioxidant activity of herbs and phenolic compounds contents has been frequently proved (Chen and Shi, 2009). Accordingly, it has been suggested that phenolic compounds are secondary metabolites in herbs and have healthy effects. They have high antioxidant and peroxidase effects (Avula *et al*., 2007). The present study has used extracts of polygonum avicular found in excess in East Azerbaijan and its strong antioxidant features have been helpful in decreasing degenerative effects of electromagnetic waves in mouse testicle.

## MATERIALS AND METHODS

Mice of BALB/c race taken from animal store of Tabriz University of Medical Sciences were used in this study. The mice were kept in cages similar to their environment. Their food was prepared from ready and compact foods in morning. The required water was provided for them in glass dishes. They were kept in about 22°C temperature and under similar conditions of light cycle (12 h of light and 12 h of dark). After preparing, the 8-week male mice with mean weight of 20±1 g were kept in animal store of anatomy ward for three days (n = 24). The understudy mice were randomly divided into 4 groups. Group I: the mice of the control group were kept for two months in the sent without turning on the set producing electromagnetic waves. Group II: the mice daily received 50 mg $kg^{-1}$ of polygonum avicular extract for eight weeks. Group III: during two months, the mice exposed to electromagnetic waves (3 mT) four hours a day and five days in a week. Group IV: the mice simultaneously received the extract and electromagnetic waves.

**Extraction:** After collecting and determining plant aerial organs, it was dried in an appropriate place in the laboratory far from sun light. The aerial organs are powdered using a mechanical mill. Then, 100 g of the resulted powder is put in a cartouche paper and placed in sooc selle set along with the contents. Extraction operation is done using n-hexane, dichloromethane and methanol solvents for 5 h. After extraction, each of fractions is evaporated and completely dried using a rotary evaporator at 50°C and low pressure. Until consumption, the resulted dry extractions are kept in refrigerator and at a negative temperature.

**Sampling:** The testicles were removed after displacing the mice neck and opening the abdomen. Epididymis tail of the right testicle was used to analyze semen parameters.

**Evaluating of semen parameters:** Sperm samples are taken from epididymis, put in hams f 10 cultivation medium and counted under microscope using neubauer slide. Sperms motility was assessed using microscope. The morphology was studied after painting of papa Nicola. In this study, morphological indexes were used based on WHO criteria. Unnatural morphology of sperms was classified and verified into four categories: coil tail, signs of cytoplasmic node on mid tail, unnatural head and other unnatural forms. All data was analyzed using descriptive statistical methods (Mean±Standard Deviation), SPSS 16 software and variance analysis test and $p<0.05$ was regarded as meaningful.

## RESULTS

The present research studied sperms motility percentage at different groups. In this part, four motility indexes of rapid, moderate, slow and incite were used to evaluate sperm motility. Mean of sperms quick motility in the control, EMF, POLY and EMF- POLY was 19.63±0.85, 0.20±0.20, 30.25±2.14 and 7.09±0.58%, respectively. There was a meaningful decrease of sperms rapid motility in the EMF group in comparison with other three groups ($p<0.05$). A meaningful increase in sperm rapid motility was observed in the POLY group received extracts of polygonum herb in comparison with other groups ($p<0.01$). Percentage of sperms with moderate motility in the control, EMF, POLY and EMF-POLY groups was 14.54±0.73, 4.87±0.90, 16.47±0.73 and 17.54±2.64%, respectively. Moderate motility of EMF group has meaningfully decreased comparing with other three groups ($p<0.01$). There was no difference between POLY and control group considering mean progressive motility. In comparison with the group exposed only to electromagnetic waves, there was significant increase in moderate motility of the group exposed to electromagnetic waves and received extraction ($p<0.01$). There was no meaningful difference in the POLY group in comparison with the control and EMF-POLY groups. No significant difference was observed in EMF-POLY group in comparison with the control and POLY groups considering mean percentage of sperms moderate motility. Sperms percentage with slow motility in the control, EMF, POLY and EMF-POLY groups was, respectively 14.13±1.68, 16.52±1.75, 19.83±3.68 and 30.56±2.43%, respectively. There was no difference between the control and EMF groups considering mean of sperm percentage

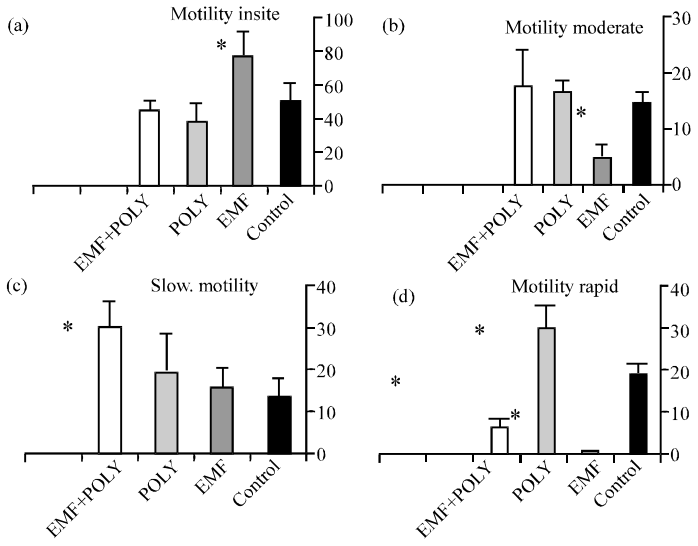


Fig. 1(a-d): Mean percent of sperm motility. *Regarded as significant difference between 3 groups and control group ($p<0.05$)

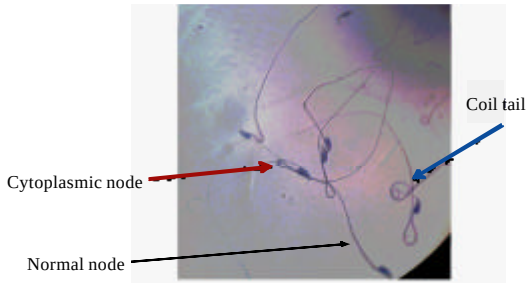


Fig. 2: Sperm photomicrography after papanicolaou colorizing

with slow motility. But, it has been increased in comparison with POLY and EMF-POLY groups ($p<0.05$). There was no difference between EMF group and the control and POLY ones but the difference was meaningful in comparison with EMF-POLY group ($p<0.05$). Insite sperms percentage in the control, EMF, POLY and EMF-POLY groups was 50.67±3.62, 77.20±5.77, 37.51±4.42 and 44.70±2.20%, respectively. The results demonstrate that insite in the MEF group was more than other three groups and the difference is meaningful ($p<0.01$). In comparison with EMF group, percentage of insite sperms has decreased in the POLY and EMF-POLY groups ($p<0.01$) (Fig. 1). Another evaluation of this research includes sperms morphology study (Fig. 2). Outcomes resulted from evaluating the sperms morphology in all four groups demonstrated that percentage of natural morphology in the control and EMF groups is respectively 79.33±2.79 and 26.66±1.58. Percentage of sperms with normal morphology has increased in the EMF group after consuming extracts of polygonum herb. It means that there was a significant difference between POLY and EMF groups ($p<0.01$). There was a meaningful difference between the control and EMF-POLY groups ($p<0.05$) and EMF-POLY and EMF groups ($p<0.01$). The sperms with coil tail was seen in the control, MEF, POLY and EMF-POLY groups with mean of 32.66±7.20, 38.83±13.83, 32.50±7.73 and 42.00±7.97, respectively. There was no significant difference between these groups. Sperms with cytoplasmic node at mid tail were 34.33±70.81, 50.66±5.53, 25.66±2.06 and 35.00±40.23% in the control to EMF groups, respectively. The results demonstrated that EMF and POLY groups had a meaningful increase respectively in comparison with the POLY ($p<0.01$) and EMF-POLY ($p<0.05$) groups. The difference of EMF group to the EMF-POLY one was also meaningful ($p<0.01$). Sperm with unnatural head of the control, EMF, POLY and EMF-POLY groups were 10.66±1.63, 3.66±1.21, 20.16±2.31 and 9.50±1.87%, respectively. Outcomes resulted from the statistical analysis refer to the meaningful difference found between the control group and EMF and POLY groups ($p<0.01$). But there was no significant difference between the control group and EMF-POLY one. There was meaningful difference between POLY and other groups ($p<0.01$). There was no significant difference between the EMF and other groups. Considering other sperm anomalies, there was a meaningful difference between the control group

(19.50±2.88%) with the EMF one (4.50±1.22%) ($p<0.01$). No difference was observed between the POLY group (19.83±1.47%) and EMF-POLY one (17.83±1.47%). Comparison of mean percentage of sperms motility as rapid, moderate, slow and insite progressive motion in four control, EMF, POLY and EMF-POLY groups has been demonstrated in the above diagrams and indicates meaningful difference between the control group and other ones ($p<0.05$).

## DISCUSSION

Present results demonstrate significant decrease in sperm motility and increase in unnatural sperms percentage in those groups exposed to electromagnetic fields (Aziz *et al*., 2004). Demonstrated that use of mobile phone leads to decrease of sperm quality in men and appears as decrease of number of sperms, their motility and morphology. Decrease of sperm parameters depends on duration of mobile phone use. Electromagnetic fields impose their degenerative effects due to having high energies such as ionization radiations through developing free radicals. Increase of oxygen free radicals may have degenerative effects on sperms. It has been known as one of the reasons of men infertility (Nikolova *et al*., 2005). ROS has useful and degenerative effects on sperm activity depending on its concentration, amount and contact place (Barcal *et al*., 2005). Gomez *et al*. (1998) showed that concentration of ROS produced by sperm has a reverse relationship with sperm quality in semen fluid. Sinclair (2000) made it clear that decrease of antioxidant level in plasma of infertile men is a result of increase of ROS production rather than antioxidant insufficiency. At present, outcomes resulted from tests conducted on human sperm parameters demonstrate that consumption of some supplementary such as kinds of vitamins and antioxidants improves parameters of motility, number, morphology and percentage of alive sperms in those subjects with unnatural spermogram (Batlla *et al*., 2007). In this study, we used polygonum avicular extract as an antioxidant and results of the study demonstrate that the drug improves parameters of sperm number and motility in those mice exposed to electromagnetic fields. As expected, percentage of rapid, moderate and slow motility was significantly increased in the EMF-POLY group received polygonum extract in comparison with the EMF group ($p<0.05$). Percentage of insite sperms decreased significantly ($p<0.05$). Therefore, polygonum extract has a positive effect on total motility of sperms. Most researchers, in accordance with this study, found that consumption of herbs with antioxidant effects increases the sperm motility parameters in human and mice. Motility increase rate resulted from consumption of different herbs reported by the researchers is different. In their study, (Gonzales *et al*., 2001) demonstrated that sperm motility significantly increases in healthy men after oral consumption of Lepidium meyenii as a tablet 1.5-3 g $day^{-1}$ for 4 months. Lepidium includes high amounts of phenol compounds which have antioxidant effects. Several studies have been reported considering effects of herbs on mouse sperm. A study conducted by Park *et al*. (2007) demonstrated that gavage consumption of Panax ginseng for 56 days resulted in sperm motility increase in mice. Herbal foods such as soya and ginseng used by human contain phytoestrogen. Generally, phytoestrogen is found in flavonoid and non- flavonoid. Most phytoestrogen are in flavonoid group. Considering that polygonum avicular contains high amounts of flavonoid imposes its useful effects due to this antioxidant (Hsu, 2006). Experimental data suggest that sperm is not active when there are evident anatomical defect on spermatozoa. There are several clinical reports based of relationship found between sperm morphological abnormalities and infertility (Smolarz, 2002). In the POLY and EMF-POLY groups received the extract; meaningful increase was observed in the EMF-POLY group in comparison with the EMF one considering percentage of sperms with normal morphology. There was a difference between the POLY and control group but it was not meaningful ($p = 0.92$). In the EMF group, amount of sperms with cytoplasmic nodes was about 50%. It was about 35% in the EMF-POLY group. Also, sperm with coil tail was about 38% and 42% in the EMF and EMF-POLY groups, respectively.

## CONCLUSION

According to the resulted outcomes, extracts of polygonum avicular as an herb containing antioxidant lead to improve disorders resulted from free radicals developed by electromagnetic fields on semen parameters. The results demonstrate that Polygonum increases the number of sperms with normal morphology. Additionally, it decreases number of sperms with cytoplasmic nodes at mid tail. But, it does not affect sperms with coil tail.

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