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Science Department of Physics

The Effect of Magnetized Nutrition on Bone Density

A Thesis Submitted to the Council of the College of Science University of Diyala in Partial Fulfillment of The Requirements for the Degree of M.Sc. in Physics

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بسم الله الرحمن الرحيم

وفوق كل ذي علم عليم

صدق الله العظيم

Dedication

To my wonderful and greatest Mother and father that support, and encouragement to raise me to be the person I am today…..

To my greatest Husband Saif

To my greatest sister manar and my Brother Thoualfeqar…..

To my lovely Ali, Karam and yuser…..

With my love and regard for all support that provides to me in all my life.

Nour

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Nour

CERTIFICATION

We certify that this thesis entitled ***"The effect of magnetized nutrition on bone density"*** was prepared by ***(Nour Abdalrazaq Hassan)*** under our supervision at the University of Diyala / College of Sciences / Department of Physics as a partial fulfillment of the requirements for the Degree of Master of science in Physics.

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Publications research

- **Evaluation the Effect of Magnetized Water on The Bone Density And Osteoporosis In The Experimental Rats By DXAScan**

Nour Abd Alrazaq Hassan (BSc)[1], **Tahseen Hussain Mubarak** (PhD)[2] **and Nameer Fadhel Gheaeb** (PhD)[3]

- **Evaluation The Effect of Magnetized green tea on The Bone Density And Osteoporosis In The Experimental Rats By DXA-Scan.**

Nour Abd Alrazaq Hassan (BSc)[1], **Tahseen Hussain Mubarak** (PhD)[2] **and Nameer Fadhel Gheaeb** (PhD)[3]

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List of Symbols and Abbreviations

Symbol	Description
i	Current
H	Magnetic field
r	Radial
B	Density of magnetic flux
Jf	Electric current density
m	Magnetic moment
n	Individual loops
V	Voltage
l	Wire length
Φ	Flux
v	Velocity
EF	Electric Field
dA	Area
dt	Time
Wb	Weber
DC	Direct current
AC	Alternating current
V-I	Voltage-Current

Symbol	Description
EMF	Electromagnetic field
q	Static charged partical
BMSCs	Bone marrow stromal cell
hBMSCs	Human bone marrow stromal cell
J	Current density
PEMF	Pulsed electromagnetic filed
ALP	Alkaline phosphates
FACS	Florescence activated cell sorting
CaP	Calcium phosphate
TCP	Tricalciume phosphate
OPG	Osteoprotegerin
CT	Computed tomography
QCT	Quantitative Computed tomography
VAT	Visceral adipose tissue
λ	Wave length
SAT	Subcutaneous adipose tissue
DXA	Dual X ray absorptiometry
BMC	Bone mineral content
BMD	Bone mineral density

Symbol	Description
BA	Bone area
aBMD	Areal bone mineral density
FM	Fat mass
LSTM	Lean soft tissue mass
FFM	Fat free mass
STM	Soft tissue mass
XRD	X- ray diffraction
FTIR	Furrier Transform Infra Red
AFM	Atomic Force Microscope

List of Scheme

Abstract

Magnetized nutrition had effects on regeneration and healing of bone. The amount of this effect depends on the power density and frequency of the electromagnetic field. The properties of the nutrition change when exposed to the magnetic fields. Electromagnetic field easy to penetrate the wall of the cell and increase the speed of deployment.

This study used to estimation the impact of magnetized nutrition on bone density and simultaneous action bone turnover process in rats, and effect on the Healing of Bones fractures in less time.

Eighty males and females adult Wister rats (Rathus Norvegius) were used in this study. Magnetized nutrition is prepared by the electromagnetic system was made in physics labs by student which consist of a iron tube wrapped around a 7000-cylinder roll wire and supplied with a constant current at 220 volts.

In the experiment, a Gauss meter was used to measure the magnetic flow. The DXA scan technique was used to measure bone mineral density (BMD), bone mineral content BMC, T-score and Z-score was used, In addition the dental X-ray machine was used to estimate the healing of bane fracture.

A comparison of bone mineral density, bone mineral content, T-score and Z-score. It was concluded that after 21 days of continuous consumption of magnetized nutrition's leads to intensification of bone regeneration and construction, in addition to the healing of the fractured bone which was obtained after 14 day of continuous consumption of magnetized nutrition`s.

Spss analysis was used to analyze the results and the analysis showed that the results were highly significant, where The collected information to a statistical analysis. The results obtained are presented here

based on SPSS Statistics generates tables in one-way ANOVA analysis and Tukey post hoc test.

Keywords: *Electromagnetic field, Bone density meter in rats, Osteoporosis, Bone regeneration.*

Chapter One

Introduction and previous studies

1.1 Introduction

During the last contracts there was an increasing interestin the bio effects of the electromagnetic fields interaction. The biological effects of synthetically alternative current with 50 Hz standard frequency have been much debated in the context of the biological allergy to extremely low frequency magnetic field (ELF-MF) **[1]**. All living organisms are incessantly exposed to electromagnetic fields from synthetically and domestic sources. It seems clay now that electromagnetic exposure an inductions biological changes, although the precise effects are not yet well known **[2]**. In recent decades, many scientific research have confirmed that magnetic fields of extremely low frequency (ELF; frequency <300 Hz) can influence the biological systems. Data reported in the literature regarding direct effects induced by ELF-MF on cell functions are controversial and the interaction mechanisms of electromagnetic fields with biological systems are still partially understood **[3]**.

Magnetic fields quantities have been characterized by both strength and direction. The strength of a magnetic field is measured in units of tesla in the SI units, and in gauss in the cgs system of units, 10,000 gauss are equal to one tesla. The measurements of the Earth's Introduction magnetic field are often quoted in units of nano tesla (nT), also called a gamma **[4]**.

Electromagnetic fields composed of waves that transport the energy through the space characterized by wave length and frequency, the two of which are inversely correlated. The shorter the wavelength, the greater the frequency **[5]**.

Osteoporosis is an important systemic commotion, affecting mainly Caucasian women, with a variety and multi factorial etiology. A large variety of animal species, including rodents, rabbits, dogs, and primates, have been used as animal exemplars in osteoporosis research. Among these, the laboratory rat is the favored animal for most researchers. Its skeleton has been studied large-scale, and although there are several limitations to its similarity to the human

condition, these can be over come through detailed know ledge of its specific rates or with certain techniques. The rat has been used in many experimental protocols leading to bone loss, including hormonal interventions (ovariectomy, orchidectomy, hypophysectomy, parathyroidectomy), immobilization, and dietary manipulations. In addition, several methods of bone mass evaluation are assessed, such as biochemical markers, densitometry, histomorphometry, and bone mechanical testing, that are used for monitoring and evaluation of this animal model in preventive or therapeutic strategies for osteoporosis **[6]**.

1.2 Literature review

Yacout, et.al. (2015) conducted that treatment water properties could be changed to become more energized, active, soft and high pH toward slight alkaline and free of germs **[7]**.

Alhassani and Amin. (2012) Several reports are available on the application of water magnetization on broiler production **[8]**.

Rona. (2004) found that using magnetic drinking water for chickens resulted in shortening of fattening period of broiler chickens, an increase in growth rate by 5-7 %, improving meat quality, flavor and tenderness **[9]**.

Mak and Zhang. (2001) They mechanical forces have been known to affect molecular signaling and molecules in bone cells via mechanic transduction **[10]**.

Yoshida, et. al. (2009) found that the conversion of mechanical loads to bioelectric signals (i.e., pressure generated potentials also known as piezoelectricity) in bone has been suggested to control repair and remodeling **[11]**.

Guzelsu and Walsh. (1990) stated that the signals are attributed to electrically-generated kinetic behavior where mechanical forces generate

electrical signals due to the motion of ion-carrying extracellular fluid in the bone matrix. The effect is known as streaming potential **[12]**.

Sun, et. al. (2007) showed that a direct current (DC) 0.1 (V/cm) stimulus 30 (min/day) for 10 days enhanced expression of osteogenic factors for hBMSC differentiation into the osteogenic cell lineage by reducing the Ca^{2+} wave frequency typically found in the differentiation process**[13]**.

Tsai, et. al. (2009) found Increased cell numbers were observed at late stages of osteogenic culture with this same PEMF exposure. The production of ALP, an early marker of osteogenesis, was significantly enhanced at 7 day when exposed to PEMF treatment in both basal and osteogenic cultures as compared to untreated controls. Furthermore, the expression of a key osteogenic regulatory gene *RUNX2/CBFA1* and ALP, was also partially modulated by PEMF exposure, indicating that osteogenesis in hBMSCs was associated with the specific PEMF stimulation **[14]**.

Tsai, et. al. (2007) reported similar results when they isolated hBMSCs from adult patients and cultured them in osteogenic medium for up to 28 days. Using aPEMF stimulation of 7.5 (Hz), greater cell numbers were observed compared with controls. The production of ALP was significantly enhanced at 7 day on both basal and osteogenic cultures as compared to untreated controls. Also the expression of early osteogenic genes RUNX2/CBFA1 and ALP was indicative of PEMF stimulation. ALP accumulation produced by the hBMSCs, along with Ca^{2+} deposits reached their highest levels at 28day **[15]**.

Kim, et. al. (2015) EMF alone, and in combination with nano magnetic particles (MPs), has also been used to promote the differentiation potential of hBMSCs. investigated the effect of both EMF and MPs on hBMSCs by treating them with 50 μg/ml of Fe3O4 MPs and/or an exposure of 45 Hz, 1 mT intensity EMF The cells were exposed to EMF twice every 8 (h/day) for 7 days. The treatment with MP, and/or then exposure to EMF did not cause cytotoxic effects. The strong expression of osteogenic markers OSTEOCALCIN, OSTEOPONTIN, and OSTEONECTIN was observed in the cells treated with

MPs, EMF alone, MP alone, or a combination of MP and EMF, as compared with controls. The quantitative RT-PCR revealed that mRNA expression levels of OSTEOCALCIN,OSTEOPONTIN, OSTEONECTIN,COLLAGEN I (COL1A1),COLLAGEN III (COL3A1),BONE MORPHOGENETIC PROTEIN 2 (BMP2),BONE SIALOPROTEIN (IBSP), and RUNX2 were significantly increased in cells treated with MPs, than those exposed to EMF**[15]**.

De Mattei, et. al. (1999); Lohmann, et. al. (2000); Schwartz, et. al. (2008); Sun, et. al. (2009); Trock DH, et. al. (1993); Tsai, et. al. (2007); Tsai, et. al. (2009) The Frequencies used thus far for stimulating and enhancing osteogenesis have varied from 7.5 to 75 (Hz), and have revealed that not only frequency, but also the direction of the EMF makes a difference in the results. The hBMSCs exposed to positive 30/45 (Hz), 1 (mT) and negative 7.5 (Hz), 1 (mT) EMF for osteogenic differentiation reported increases in ALP mRNA expression **[17- 18- 19- 20- 21- 14- 15]**.

Poon, et. al. (1995) The indication that the effect of EMF on osteogenic differentiation is significantly dependent on the direction of the EMF exposure. It is important to point out that the effects occurring at 7.5, 15, 45, and 75 (Hz) are harmonic waves and these pulsed patterns going from lower to higher order harmonics cause a decrease in relative energy states **[22]**.

Bianco, et. al. (2013) Results suggest that EMF promotion of bone ECM deposition in vitro is more far more efficient in osteoblasts differentiated from hBMSCs than from cells of other tissues **[23]**.

Sun, et. al. (2009) have investigated the effect of PEMF on the proliferation and differentiation potential of human hBMSCs. EMF stimulus was administered to cells for 8 h per day during the culture period. The EMF applied consisted of 4.5 (m sec) bursts repeating at 15 (Hz), and each burst contained 20 pulses. Results showed 59% more viable hBMSCs were obtained in the EMF-exposed cultures at 24 (h) after plating and 20–60% higher cell densities were achieved during the exponentially expanding stage. Many newly divided cells appeared from 12 to 16 (h) after the EMF treatment; however, cytochemical

assays and immunofluorescence analysis showed multiline age differentiation of EMF-exposed hBMSCs to be similar to that of the control group, which used only standard growth media **[20]**.

Saino, et. al. (2011) tested the effects of EMF on hBMSCs seeded on gelatin cryogel disks and compared with control conditions without EMF stimulus. Treatment with EMF (at 2 (mT) intensity and 75 (Hz) frequency) increased the cell proliferation and differentiation, as well as enhanced the biomaterial surface coating with bone ECM proteins Using this approach, the gelatin biomaterial, coated with differentiated cells and their ECM proteins, has the potential to be used in clinical applications as an implant for bone defect repair. for example, under the appropriate culture conditions, PEMF enhances the osteogenic effects of BMP-2 on hBMSCs. Thus, PEMF could potentially be used clinically to stimulate bone formation from transplanted hBMSCs **[24]**.

Schwartz, et. al. (2009) they specific suggests investigating whether the effects of PEMF on osteogenic cells were substrate dependent, and could also regulate osteoclastic bone resorption. treated hBMSCs and human osteosarcoma cell lines (MG63 cells, SaOS-2 cells) capable of osteoblastic differentiation with BMP-2, then cultured them on calcium phosphate (CaP) or tricalcium phosphate (TCP) to test their response to a 15 Hz PEMF at either 4.5 (m sec) bursts or 20 pulses repeated for 8 (h/day). Outcomes were determined to be a function of the decoy receptor, osteoproteger in (OPG), and RANK ligand (RANKL) production both of which are associated with the regulation of osteoclast differentiation. Results suggested that when osteogenic cells were cultured on CaP, PEM decreased cell number and increased production of paracrine factors associated with reduced bone resorption such as OPG. RANKL was unaffected, indicating that the OPG/RANKL ratio was increased, further supporting a surface-dependent osteogenic effect of PEMF. Moreover, effects of estrogen were surface-dependent and enhanced by PEMF, demonstrating that PEMF can modulate osteogenic responses to anabolic regulators of osteoblast function Therefore, PEMF shows promising results when used in conjunction with

complex 3-D cell culture systems as a strategy for tissue engineering approaches **[25]**.

1.3 Aim of the study

- Estimate the impact of magnetized nutrition on living bone density and bone turnover process in rats by means of analysis bone density.
- Study the impact of electromagnetic field on magnetized nutrition.
- Study of the effect of magnetic nutrition on the healing of broken bones.