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Effect of Cold Plasma on Histological Compositions of the Rabbits Fracture Bone Tissue

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Abstract

In this research, the rabbits' bones have been fractured, the rabbits were exposed to the cold plasma for five minute times two weeks. The microwave plasma voltage which was used in the search was "175v" and the gas flow was" 2 L" at the room temperature. A Biologic parameters such as alkaline phosphates (ALP), osteocalcin, vitamin D (Vit. D) and calcium (Ca), Triglycerides (TG), Cholesterol (T.chol.), Estradiol and Glucose have studied in its serum. Physiological parameters were measured to prove the effects of plasma on the fracture bone tissue and show the amount of damage and the effect of plasma therapy before and after exposure to plasma.

Keywords: Cold plasma, Osteoporosis, Alkaline phosphates, Calcium, Vitamin D, Glucose.

تأثير البلازما الباردة على تراكيب الانسجة لعظام الارانب المكسورة

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الخلاصة

في هذا البحث، تم تكسير عظام الأرانب وتعريضها للبلازما الباردة. الجهد الكهربي المستعمل لانتاج البلازما المنتجة بالمايكروويف والمستخدم في البحث هو "175 فولت" ، وكان تدفق الغاز "2 لتر" في درجة حرارة الغرفة لمدة خمس دقائق. أذ درست العوامل البيولوجية مثل الفوسفات القلوي (ALP) والأوستيوكالسين وفيتامين د (Vit. D) والكالسيوم (Ca) و (TG))Triglycerides والكوليسترول (.T.chol) و Estradiolو سكر الكلكوز .(Glucose) وايضا قيست المعلمات الفسيولوجية لإثبات آثار البلازما على نسيج عظم الكسر ولإظهار مقدار الضرر ومدى تاثر نسيج العظم بالبلازما قبل وبعد التعرض للبلازما.

Introduction

The direct application of plasma in medicine is to understand the physical, chemical and biological mechanisms of direct non-thermal plasma interaction with living tissue. Engineering research has resulted in many advances in health care. Ionizing radiation and laser are examples of a technological breakthrough that have created a diagnosis and new treatments for the disease. Non-thermal plasma medical techniques also have important therapeutic effects and lead to new medical diagnostic tools. Some of these factors include deactivating pathogens, stopping bleeding without damaging healthy tissue, promoting wound healing and treating cancer[1,2,3]. The fracture on the long bone of the

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rabbits is treated with plasma to improve effect of cold plasma on healing the bone. The microwave plasma is the plasma with "high frequency electromagnetic radiation in the GHz range". Mazhir et al. showed that there was no side effect on the tested animals after exposure to microwave plasma [4]. Plasma is a hot ionized gas consisting of approximately equal numbers of positively charged ions and negatively charged electrons [5]. Microwave plasma or non-thermal plasma is used here. Microwave plasma is a type of plasma that has high frequency electromagnetic radiation in the GHz range. Microwave generated plasma system chooses over other kinds of plasma sources because they are electrodeless plasma, so the task of replacing or cleaning the electrodes and filaments is averted [6]. Microwave discharges produce non-equilibrium plasma since the electrons can respond to the oscillations of the electric field whereas the ions are not able to respond due to their large mass. So most of the microwave energy goes preferentially to the electrons, and then produce plasma far from thermodynamic equilibrium [7]. The aim of this research is to study the effect of plasma on the fracture of rabbits' bone tissues

Methodlogy And Material

The microwave plasma generated from this system was applied on rabbits which shows in Figure- 1 and worked with 2.45 GHz microwaves frequency and Argon gas, 175 V applied voltage, 2 L/min argon gas flow rate and 10 m discharge tube diameter.

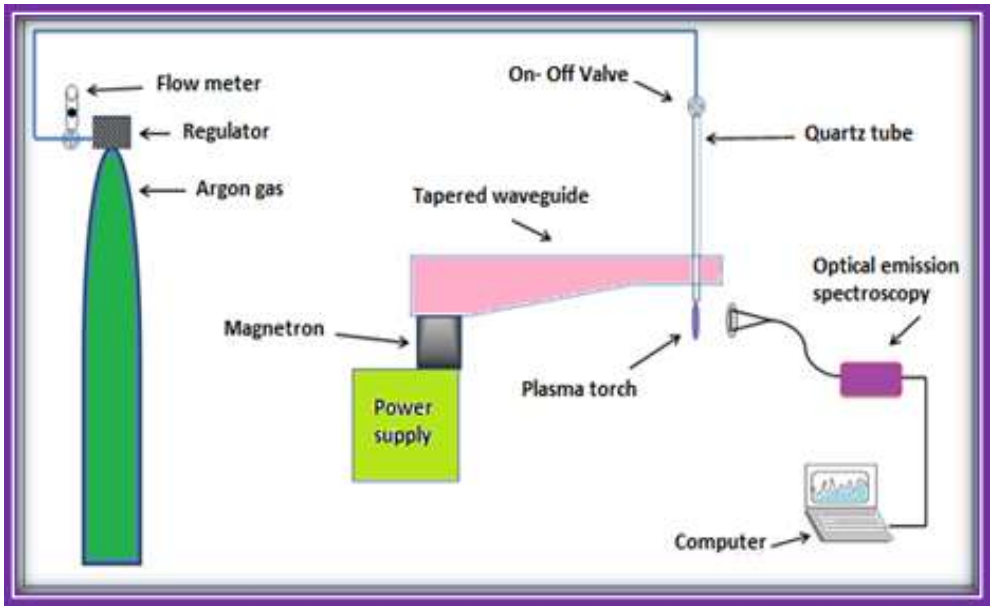

Figure 1-a schematic diagram of the MIPJ system applied on the rabbit

Fracture Bone: It is broken or cracked bone because of the strength exerted against the bone stronger than the bone resistance, which destroys the structure and strength of bone which leads to loss the function of the main bone and leads to pain, sometimes bleeding and the most common sites for fractures of bones are the wrist, ankle, and hip [8].

Histological Composition of the Bone Tissue

Calcium: Calcium is one of the most important minerals in the body and is responsible for, bone stiffness, immune defense [9]. It is a basic base for building strong bones and teeth and gives shape and maintenance to bones and teeth and helps in muscle contraction and causes osteoporosis in adults, short-term calcium deficiency can lead to osteoporosis in the bones where the bones deteriorate and there is an increase in fractures.Lack of calcium in the blood (low blood calcium) can be caused by kidney disease or vitamin D deficiency[10]**.**

$$\text{Calcium Conc. (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 10 \quad \text{……..(1)}$$

Vitamin D: Vitamin D works like a hormone it helps to make calcium available for bone growth. It promotes intestinal absorption of phosphorus and calcium, vitamin fat soluble the excess is stored in the liver and fatty tissues can be synthesized by the body by exposure to UV light from the sun, the

main sources being yogurt, yolks, liver and fish [11]. Therefore, vitamin D is necessary because its deficiency causes rickets and osteoporosis [11,12].

Alkaline Phosphatase (ALP):

Alkaline phosphatase is an enzyme found in all tissues of the body but is mostly concentrated in the bones, liver, kidneys, placenta, and intestines. It is implicated in bone calcification and has an important role in the growth of teeth and bones because it is main for mineralization, mineralization occurs when minerals, such as calcium and phosphorus are deposited into bones and teeth to help them become hard bone . Alkaline phosphatase is a useful parameter for monitoring changes in bone synthesis, the normal level of alkaline phosphatase in the blood is 20 to 140 units / liter, this can alter from laboratory to laboratory [13].

$$\text{ALP Activity (U/L)} = (\text{OD / min.}) \times 2750 \quad (2)$$

(U/L): unit per liter

OD: optical density (amount of attenuation intensity loss which depended on the thickness of sample).

2750: present the number release to the ALP activity equation.

Estradiol: It is sex hormone estrogen and testosterone and the main secretion of estrogen in the ovary and placenta additional small amounts of the adrenal glands are extracted from the male testicles, are important factors for bone metabolism in both men and women. The studies indicate estrogens and androgens act via different cellular mechanisms, the bone-sparing effect of estrogen is antiresorption by inhibition of osteoclast action[14].

Osteocalcin: Osteocalcin is a bone protein (bone matrix) produced by bone cells during bone formation during the late stages of bone formation, bones are constantly remodeled through processes close to bone restoration and formation to maintain the skeleton, dynamic balance disorders such as osteoporosis can lead to bone loss and fractures, bone metabolism can be monitored by measuring the biochemical indicators of bone turnover in the blood, marks provide dynamic and fast procedures for skeletal condition or fracture risk assessment. The osteocalcin measurements are used to assess the rate of bone formation in clinical investigations [15].

Blood Glucose: The relationship between diabetes (high blood glucose levels) and osteoporosis in all clinical and experimental conditions has been documented with a decrease in diabetes in cell proliferation and collagen synthesis during the early stages of fracture healing causing these unknown effects, but insulin has a major role to play in healed fractures through insulin receptors in bone cells and was able to enhance bone stiffness[16].

Physiological Parameters for Fracture bone

A. **Glucose:** By using enzyme-linked immunosorbent assay (ELISA) kits of Glucose. Mix and incubate for 10 minutes at 37^0C and read the absorbance of standard and sample against reagent blank.

$$\text{Glucose Conc. (mg/dL)} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}}\right) \times 100 \quad (3)$$

Where dl = deci liter

B. **Triglycerides:** By using ELISA kits of triglyceride .Mix and incubate for 5 minutes at 37^0C. Measure the change in absorbance of standard and sample against reagent blank.

$$\textbf{Triglycerides Conc. (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 200 \quad (4)$$

C. **Cholesterol:** By using ELISA kits of Cholesterol, and mixing the incubate for 5 min at 37^0C, the absorbance of samples was measured.

D.

$$\textbf{Cholesterol Conc. (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 200 \quad (5)$$

Result and Discussion

Table-1 shows the statistical values for the alkaline phosphates, Triglycerides, Cholesterol and glucose for Fracture group. Figure-2 shows the statistical level value for the fracture group for the alkaline phosphates, Triglycerides, Cholesterol and glucose.

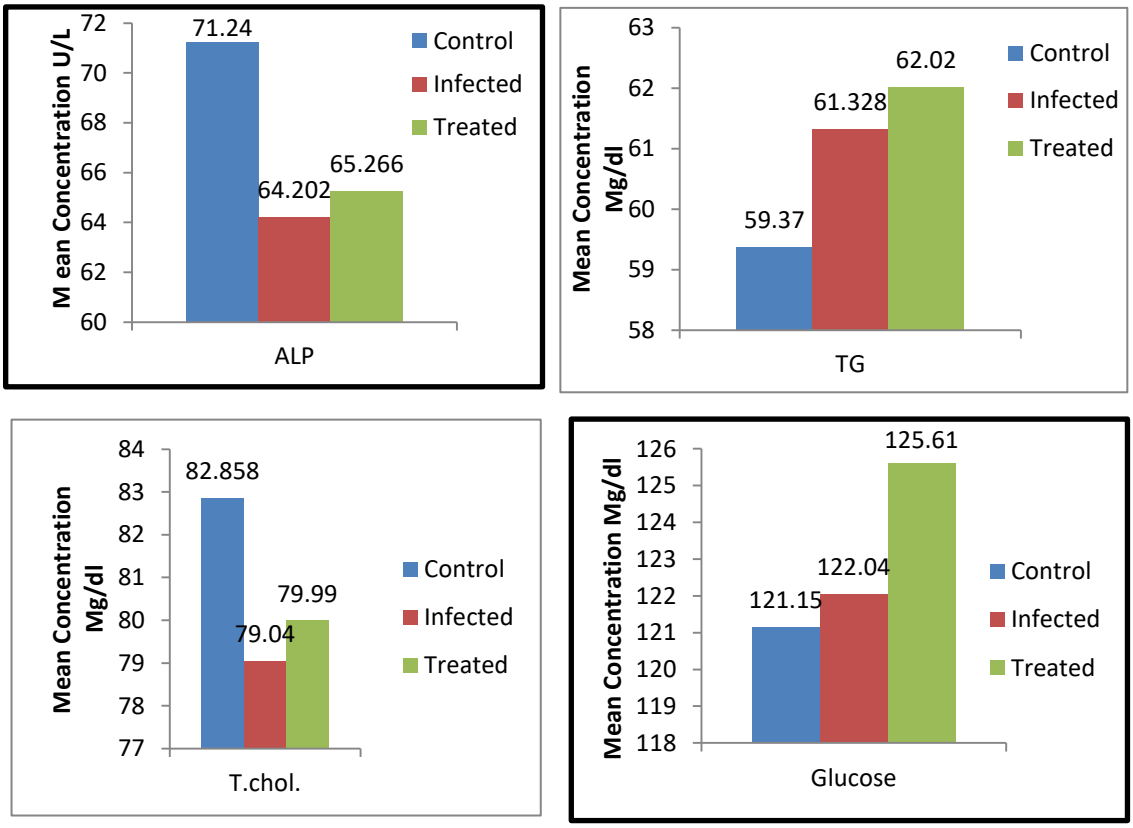

Figure 2-Statistical level of (Alkaline phosphates, Triglycerides, Cholesterol and glucose) for Fracture group.

Table 1-The Statistical value for (Alkaline phosphates, Triglycerides, Cholesteroland glucose)for Fracture group.

Fracture group	ALP U/L (Mean±SD)	TG Mg/dl (Mean±SD)	T.chol. Mg/dl(Mean±SD)	Glucose Mg/dl(Mean±SD)
Control Before OV.	A 71.240±2.342	A 59.37 ±1.67	A 82.858±3.120	A 121.15±3.41
Infected After 6 weeks of OV.	B 64.202±2.489	B 61.328±1.674	B 79.040±1.295	B 122.04±3.41
treatment	B 65.266±1.745	B 62.028±1.674	B 79.990±2.626	B 125.61±1.37
LSD	**3.49**	**1.88**	**2.78**	**3.2**
P-value	**0.001**	**0.068**	**0.075**	**0.071**
Significant	**Sign.**	**No.Sign.**	**No.Sign.**	**No.Sign.**
OV. is the overoctomy LSD is the lest significant Difference. SD Standard diviation				

The results are shown in Table-1 for fracture group indicate that there is significant change (p value <0.05) in the ALP of control group as compared with infected group "rabbits with fractured bones".

There is significant change in ALP for infected group compared with treated group. There are non-significant changes (p value >0.05) in Tchol., TG and glucose that means the cold plasma did not effect on these biological parameters Tchol.,TG and glucose this is clearly shown in Figure-3. From this figure, the value of ALP of treatment group are approximately above the values of infected group but not reached the control group , while for the values of T chol.,TG and glucose of treated group approximately reach to the values of the infected group. This indicated that the plasma doesn't effect on Tchol, TG and glucose.

Table-2 shows the statistical value of the calcium and osteocalcin for Fracture group. Figure-3 shows the statistical level value for calcium and osteocalcin of the fracture group.

Table 2 -The Statistical values for (Ca and Osteocalcin)of fracture group

Fracture group	Ca Mg/dl (Mean±SD)	Osteocalcin Mg/dl (Mean±SD)
Control Before OV.	A 12.994±0.454	A 7.608±0.489
Infected After 6 w of OV.	B 9.418±0.883	A 7.5880±0.5614
treatment	C 11.586±0.466	A 7.1780±0.2429
LSD	**0.71**	**0.51**
P-value	**0.00052**	**0.275**
Significant	**Sign.**	**No.Sign.**

The results in a Table-2 show that there is significant change ($p<0.05$) in the Ca of the control group as compared with infected group. There are significant changes in Ca of infected group compared with treated group. There is non-significant change ($p>0.05$) in osteocalcin of control group as compared with infected and treated group.This can be shown in Figure-3 from this figure the values of Ca and Osteocalcin for the treated group approximately reach the values of control group (same response as osteoporosis group).

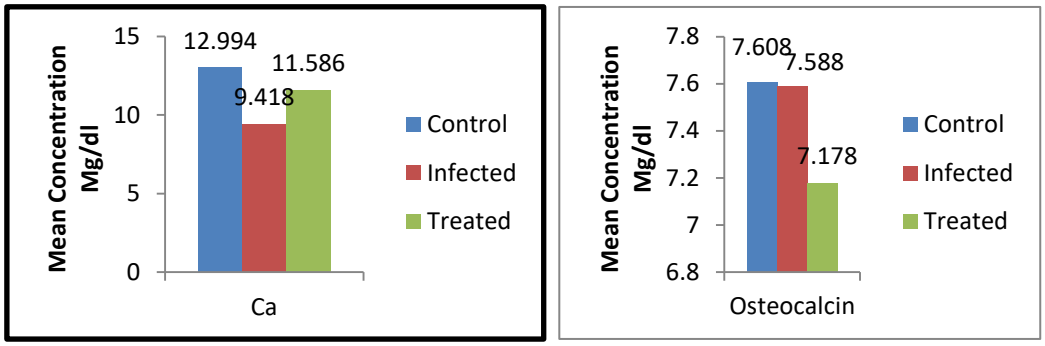

Figure 3-The Statistical levels of Ca and Osteocalcin for Fracture group.

Table-3 represented the statistical value for vitamin D and Estradiol, Figure-3 shows the level of vitamin D and estradiol of the fracture group.

Table 3 -The Statistical value of vitamin D and Estradiol for fracture group.

Fracture group	Vit D (Mean±SD)	Estradiol pg/ml (Mean±SD)
Control Before OV.	A 65.024±8.315	A 26.154±2.245
Infected After 6 w of OV.	A 62.914±8.315	A 25.538±2.232
treatment	A 66.794 ±8.315	A 27.520±1.177
LSD	**9.3**	**2.19**
P-value	**0.766**	**0.295**
Significant	**No.Sign.**	**No.Sign.**

The result from the Table-3 shows that there is non-significant change ($p>0.05$) in the vit. D and estradiol in the control group as compared with infected group and treated group. This can be illustrated in Figure-4 from this figure the values of vit. D and estradiol of treated and infected group approximately the values of control group.

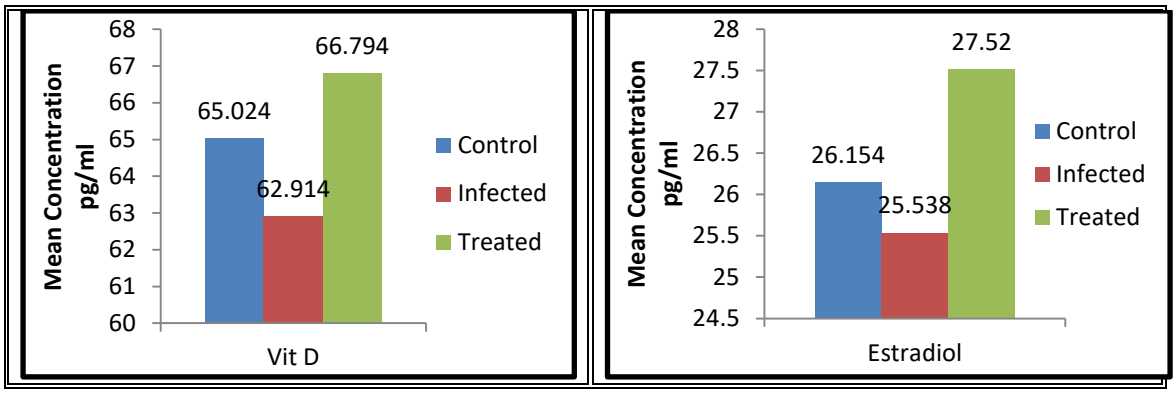

Figure 4-The Statistical levels of vitamin D and Estradiol for fracture group.

Conclusion

The results showed that the fracture rabbits bones infected with the cold plasma. The physiological parameters demonstrated a response to the treatment. The ALP had a significant changing and showed a response to the plasma. The result clarified that TG, T.chol, and Glucose had no response to plasma since the p-value was greater than 0.005. The value of osteocalcin illustrated no response to the plasma in the fractured bone since the p-value was greater than 0.005; while the Ca response to the plasma, Vit. D and Estradiol have no significant change.

References

1. Fridman, G. **2008.** Direct Plasma Interaction with Living Tissue. PhD. Thesis Submitted to the Faculty of Drexel University.
2. Mazhir S N, Abdullah N A, Al-Ahmed H. I, Harb N H, Abdalameer N. **2018**. The Effect of Gas Flow on Plasma Parameters Induced by Microwave. *Baghdad Science Journal*. **15**(2): 205-210.
3. Heinlin, J. **2010.** Plasma-Medizin: Anwendungsmöglichkeiten in der Dermatologie, *JDDG - J. Ger. Soc. Dermatology*, **8**(12): 968–977.
4. Lebedev, Y**. 2010.** Microwave discharges: generation and diagnostics, *J. Phys. Conf. Ser*., **257** (1): 1–12.
5. Mazhir, S N, Hadi F W., Mazher, A. N. and Alobaidy, L. H. **2017.** Texture Analysis of smear of Leukemia Blood Cells after Exposing to Cold Plasma, *Baghdad Science Journal*, **14**(2): 403-410.
6. Mazhir S. **2017.** Studying The Effect of Cold Plasma on Living Tissues Using Images Texture analysis, *Diyala Journal For Pure Science*. **13**(2): 184-202.
7. Ali H Ali, Al-Ahmed H, Mazhir S N. and Noori S A. **2018.** Using Texture Analysis Image Processing Technique to Study the Effect of Microwave Plasma on the Living Tissue. *Baghdad Science Journal*, **15**(1): 87-97.
8. Weiner S. **2000.** Wagner HD. *Bone fractures*, **27**(6): 875–876.
9. General B I N. **2014.** Bone and Vitamins and Minerals. Report of a joint FAO/WHO expert consultation Bangkok, Thailand, 302–357.
10. Pravina P, Sayaji D, Avinash M. **2013.** Calcium and its Role in Human Body. *Int. J. Res*. **4**(2) :659–668.
11. Gallieni M, Cozzolino M, Fallabrino G, Pasho S, Olivi L. and Brancaccio D. **2009.** Vitamin D: physiology and pathophysiology. *Int. J. Artif. Organs*, **32**(2): 87–94.
12. Boonen S., Vanderschueren D, Haentjens P, Lips P**. 2006.** Calcium and vitamin D in the prevention and treatment of osteoporosis - A clinical update. *J. Intern. Med*. **259**(6): 539–552.
13. Krook L, Minor L L. **2007.** Alkaline phosphatase as an enzyme of bone apposition 2. Alkaline phosphatase as an enzyme of bone resorption, **31**(4):177–182.
14. T. V Nguyen T V. **2014.** Sex hormone levels as determinants of bone mineral density and osteoporosis in Vietnamese women and men.
15. Ivaska K. **2005.** OSTEOCALCIN Novel insights into the use of osteocalcin as a determinant of bone metabolism, 1–113.
16. Beam AH, Parsons J R, S. S. Lin S S. **2002.** The effects of blood glucose control upon fracture healing in the BB Wistar rat with diabetes mellitus," *J. Orthop. Res*. **20**(6): 1210–1216.