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# COMPARATIVE STUDY OF THE EFFECT OF ELECTROMAGNETIC FIELDS, INTERFERON-BETA AND TRANSFER FACTOR IN THE RECOVERY OF CHRONIC ULCERS IN TREATED RATS

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## ABSTRACT

Objectives: To construct a device capable of emitting electromagnetic fields (EMF) of ultralow frequency, based on Helmholtz coils and determine whether the frequency of total lymphocytes CD4+, CD8+ is modified in Wistar rats with chronic ulcer. Metodology**:** 1) A tri-axial prototype was designed to generate EMF to a frequency of 120 Hz. 2) Biological tests, 7 groups of Wistar rats, with previous ulcer formation; a control group with application of sterile water for injection (ip). Experimental groups: 1) Application of EMF (120 Hz) every 24 hours for 12 days; 2) Interferon-β and 3) transfer factor, with doses (ip) every 24 hours for 12 days. Peripheral blood samples were taken to measure frequency total of lymphocytes, and biopsies of ulcers. Results: The frequency of total lymphocytes increased significantly (P <0.05) as well as frequency of CD4 + and CD8 +; EMF, INF-β and TF a trend to normal ranges significantly (P <0.05), compared with the negative control group was observed. Ulcers of the negative control rats began scar tissue within 96 hours, whereas with FT and EMF start time of scar tissue was 72 hours and with the INF-β-β, cicatrization started in 120 hours.

**KEYWORDS**: skin ulcers, total lymphocytes, CD3, CD4, CD8 lymphocytes, interferon-β, transfer factor, electromagnetic fields

## INTRODUCTION

The skin ulcers may be developed by alterations in the venous or arterial circulation in patients with metabolic diseases such as diabetes or hypertension or even in patients who are with areas of compression due to disabilities; these ulcers are called "bedsores" and are defined as localized injury areas and tissular necrosis that develops when a soft tissue presents hypoxia. The skin of the ulcers in immunohistochemical studies shows cellular infiltrate mainly from macrophages and CD3+, CD4+, CD8+ lymphocytes and by automated morphometry expresses interferon-gamma (IFN-γ), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), growth factor-beta (TGF-β). In studies by the method of "gel zymography", it was determined that cells secrete, due to hypoxia, metalloproteinases-2 and 9; These kinins are responsible for epidermal necrolysis[1,2,3,4,5] The cells have physical characteristics that make them behave like a bioelectrical structure and therefore behaves like a dipole, based on this investigations have been performed on the effect of ultralow frequency electromagnetic fields (EMF) and concentration and calcium release (Ca2+) from intacelulares deposits, which could initiate a phenomenon of activation or cell proliferation.[6,7,8,9,10] Activation through electromagnetic fields of T cells, possibly results from a signal on CD3 lymphocyte membrane, which is propagated through the phosphorylation of proteins, where the protein kinase C (CK) is the key enzyme. The signal triggers the production of antibodies antiCD3, unlike lectin that interacts directly with the CD3 complex. Calcium ionophores open ion channels, thus increasing intracellular calcium levels since the CK needed calcium ions for activity [11,12,13]. The objective was to determine the effect of EMF on the frequency of CD4, CD8 lymphocytes and the recovery of chronic ulcers in rats.

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## MATERIALS AND METHODS

**The methodology was conducted in two parts**

***First part:***

Design and construction of the electromagnetic field:

***Helmholtz coils***

Two coils arranged in series on the same axis and spaced apart a distance equal to its radius, allow to create in the central region between them a field of approximately uniform magnetic induction. This type of arrangement is known as *Helmholtz coils*.

The intensity ***B*** of the magnetic field in the vicinity of the midway point of the coils axis is:

$$B = \frac{8\mu_0 ni}{r}$$ ; where:

μ = magnetic permeability in vacuum
n = the number of turns of each coil
r = the radius of the coil
i = current intensity

Considering the Biot-Savart law for two turns acting on the same point, we have:

$$B(r) = \frac{I}{c}\oint ds_1 * \frac{r - r_1}{|r - r_1|^3} + \frac{I}{c}\oint ds_2 * \frac{r - r_2}{|r - r_2|^3}$$ ;

Where $B(r)$ is the field produced by the coils, c = 1x $10^{-7}$, $r$ is the position vector to the point $z$ (point where you want to calculate the field), $r_1$ and $r_2$ are the position vectors of current flow.

Correction formula of the Helmholtz coils was deduced for each turn, after a set of them is made with their respective variations in radio and separation.

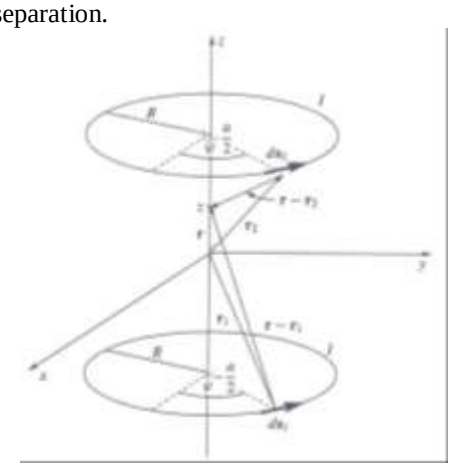

***Figure 1: Helmholtz coils***

R is the radius of the coil, r = 0, r1 = (Rcosω, R senω,-a/2)
r2 = (Rcosω, Rsenω,+a/2) and **a** is the separation between the spires

After using cylindrical coordinates and perform algebraic operations, the following formula is obtained:

$$B(r) = \frac{4\pi I}{c} * \frac{R^2}{\left(\sqrt{R^2 + a^2/4}\right)^3}$$ ;

Where R equals the radius of each coil and (a) is the distance between the coils.

Taking R = a, the equation conventional Helmholtz coils is obtained:

$$B(r) = \frac{4\pi I}{c} * \frac{8}{5^{3/2} R}$$ ;

It is known that the field depends directly on the number of turns and the current supplied, but by varying the size and changing the field size is altered.

With a triaxial arrangement based on Helmholtz coils you can make the manipulation of a magnetic field determined in the x, y, z.

**Required characteristics:**

1. A pair of Helmholtz type coils for each coordinate axis (X, Y, Z).
2. Volume of the uniform field area: 0.01 $m^3$
3. $B_{mín}$ = 20 G

***Table 1: Characteristics of built device***

| **Data** | | **Coil 1** | **Coil 2** | **Coil 3** |
|---|---|---|---|---|
| *r* | [cm] | 30 | 33 | 36 |
| *N* | [turns] | 600 c/u | 168 c/u | 169 c/u |
| $I_{máx}$ | [A] | 1.7 | 9 | 9 |
| $B_{máx}$ | [G] | 30 | 35 | 35 |
| Calibre | [AWG] | 20 | 12 | 12 |
| W | [Kg] | 5.5 c/u | 13.08 c/u | 14.23 c/u |
| L | [Hy] | 0.5652 | 0.0502 | 0.0557 |
| R | [Ω] | 38.7132 | 1.9333 | 2.1027 |
| $X_{120}$ | [Ω] | 429.2639 | 37.8804 | 41.9753 |
| $Z_{120}$ | [Ω] | 431.0061 | 37.9927 | 42.0280 |
| l | [m] | 1142.7 | 727.0635 | 790.7750 |

Where:

*r* = radius — *N* = number of turns

$I_{máx}$ = maximum current — $B_{máx}$ = maximum field

Cal = caliber — W = weight

L = inductance — R= resistance

$X_{120}$= reactance (120 Hz) — $Z_{120}$= impedance (120 Hz)

l = lenght

The following formulas are used for calculation:

$$Z = \left(R_t^2 + X^2\right)^{\frac{1}{2}}$$ [Ω] ---------- Impedance of the coil at a certain frequency

$$X_l = 2\pi fL$$ [Ω] ---------- Inductive reactance

$$Pot = Z * I^2$$ [watt] ---------- Power dissipated by the coil

$$L = \frac{0.393N^2r^2}{6a + 9b + 10c}$$ [Hy] ---------- Inductance in air core coil and several layers.

The base for placing samples (rats) measures 30cm x 40cm and is adjusted so that the sample is placed at the center of the coils.

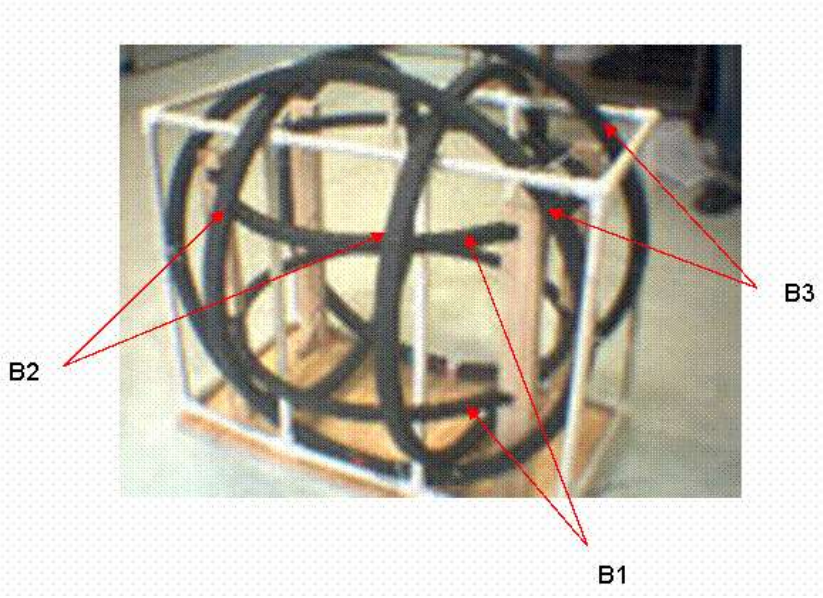


***Figure 2: Prototype triaxial offline ($B_1$=coil 1, $B_2$=coil 2, $B_3$=coil 3)***

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**Operation:**

The prototype produces a pulsating magnetic field in $B_1$ of 0-30 G and 0-35 G in $B_2$ and $B3$ at a frequency of 120 Hz.

According to Helmholtz configuration, there are two variants of connection for each pair of coils, so that the field inside is zero or produces a constant uniform field, the first is used to cancel magnetic fields inside the device and the second to produce the required stimulation field.

If the circulating current has the same direction in each coil of the pair used, the second variant (homogeneous field) occurs; otherwise it presents the first (zero fields).

The prototype can be configured in the previous two forms, depending on their form of connection.

The configuration of the power system of the coil is shown below (figure 3):

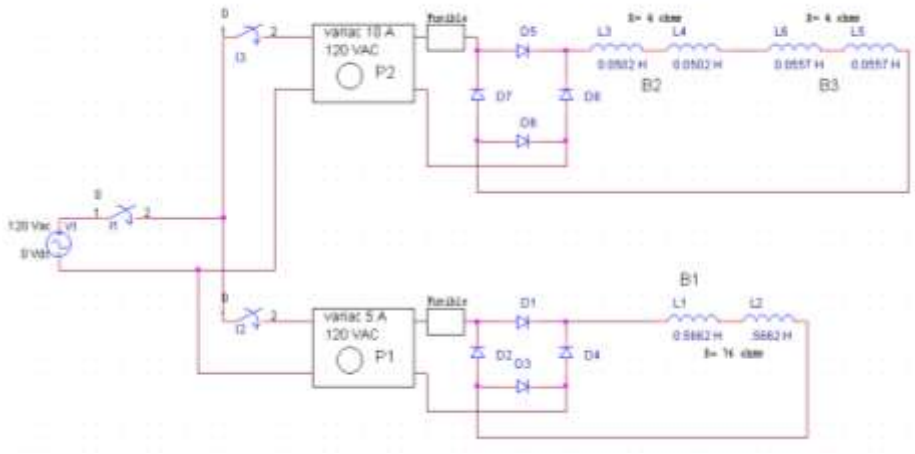


***Figure 3: General diagram of the prototype power***

The variac 1 (5A) corresponds to $B_1$.

El variac 2 (10A) corresponds to $B_2$ and $B_3$ power.

***Figure 4: Variac 5 A***

***Figure 5: Variac 10 A***

It has a main switch for the total power of the prototype and each variac has a switch, so they can be used independently.

**Characterization**

Measurements to each pair, in order to know the magnitude and distribution of the field at every point within the same are presented.

Presents data for characterization of each pair of coils, whose measurements were taken every 2 cm of separation, in the three coordinate axes, and due to their cylindrical morphology, arises in radial form, the field in every position varying RADIUS and height.

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The radius of the coils is took on scale 1:2

**For B1**

N = 600 rotation

I = 0.9 A

With the following data:

p(1,:)=[18.2 18.6 19.3 19.9 20.5 21.6 22.8 24.8 27.8 30.8 36.2 41.2]; % (x=0 y=0 z=0)
p(2,:)=[18.0 18.4 18.7 19.1 19.7 20.1 20.8 21.6 22.4 23.5 23.9 22.7]; % z= 4)
p(3,:)=[18.6 18.6 18.8 19.1 19.3 19.4 19.4 19.4 19.3 18.7 17.4 15.2]; % z= 8)
p(4,:)=[18.7 18.7 18.8 19.0 19.0 18.9 18.6 18.3 17.8 16.8 15.4 13.5]; % z=12
p(5,:)=[18.9 18.9 18.9 18.8 18.7 18.6 18.2 17.6 16.7 15.4 13.9 12.0]; % z=14
p(6,:)=[19.1 19.1 19.1 19.1 19.0 18.9 18.7 18.3 17.4 16.3 14.8 12.7]; % z=16
p(7,:)=[19.4 19.4 19.2 18.9 17.4 17.0 16.2 15.1 14.7 12.9 9.6 6.2]; %z=18
p(8,:)=[18.9 18.9 18.9 18.9 18.6 18.1 17.4 16.6 15.3 12.1 9.8 6.6]; %z=20
p(9,:)=[19.4 19.7 19.7 19.8 19.8 19.6 19.3 18.2 16.7 13.6 9.4 6.6]; %z=22
p(10,:)=[19.7 20.4 20.7 20.9 21.5 21.7 22.2 22.8 22.4 19.3 12.2 4.4]; %z=25
p(11,:)=[19.7 20.5 20.9 21.3 22.0 22.6 23.5 24.5 24.9 21.6 15.2 5.1]; %z=27
p(12,:)=[19.7 20.5 20.9 21.3 22.0 22.6 23.5 24.5 24.9 21.6 15.2 5.1]; %z=27

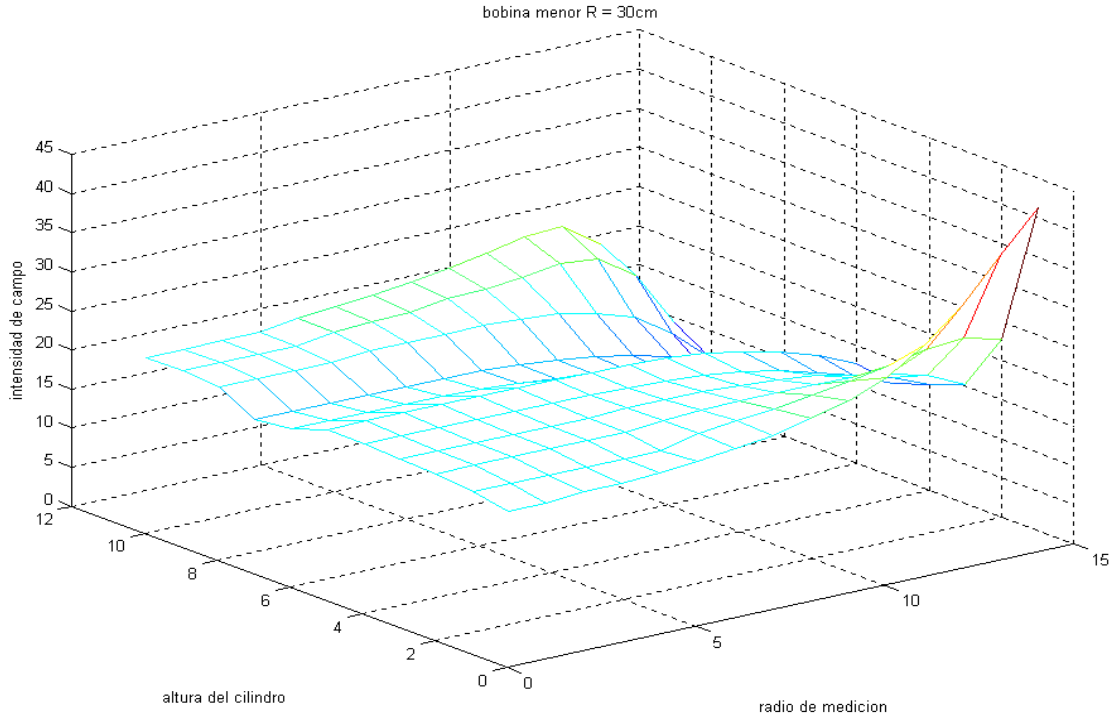


***Figure 6: Field generated in B1***

**For B2**
N = 169 rotation
I = 4A
With the following data:
s(13,:)=[19.6 19.6 19.9 20.1 20.3 20.8 21.2 22.0 22.9 24.0 25.7 27.6 30.1 32.0 29.1 14.1 0.3]; %(z=2)
s(12,:)=[19.6 19.6 19.9 20.1 20.4 20.7 21.1 21.4 21.9 22.6 23.5 24.4 24.8 23.8 19.6 12.0 5.4]; %(z=4)
s(11,:)=[19.5 19.5 19.7 19.9 20.1 20.3 20.3 20.7 20.8 21.2 21.2 21.1 20.3 18.1 14.5 10.1 5.5]; %(z=6)
s(10,:)=[19.7 19.8 19.8 20.0 20.0 20.1 20.3 20.3 20.4 20.1 19.9 19.0 17.7 15.6 12.5 9.2 6.8]; %(z=8)
s(9,:)=[19.6 19.6 19.6 19.7 19.7 19.9 19.9 20.0 19.9 19.6 19.0 18.4 16.9 15.1 12.5 9.8 7.5]; %(z=10)
s(8,:)=[19.5 19.5 19.5 19.5 19.5 19.5 19.5 19.2 19.0 18.4 17.5 16.5 14.7 13.5 11.4 9.2 7.3]; %(z=12)
s(7,:)=[19.3 19.3 19.3 19.3 19.3 19.3 19.2 18.9 18.4 17.7 16.9 15.6 14.3 12.5 10.5 8.8 7.2]; %(z=14)
s(6,:)=[19.2 19.2 19.3 19.3 19.2 19.2 19.0 18.8 18.4 17.8 16.9 15.8 14.5 12.8 10.7 9.0 7.0]; %(z=16)
s(5,:)=[19.2 19.2 19.2 19.2 19.2 19.2 18.9 18.5 18.1 17.4 16.6 15.5 14.4 12.4 10.9 8.4 6.5]; %(z=18)
s(4,:)=[16.0 16.0 16.0 16.0 16.0 16.0 16.0 15.7 15.5 14.9 14.4 13.0 12.1 10.3 8.1 5.6 3.3]; %(z=20)
s(3,:)=[16.0 16.1 16.1 16.3 16.4 16.4 16.3 16.3 16.1 15.9 15.5 14.2 13.3 11.6 8.9 5.8 3.5]; %(z=22)
s(2,:)=[16.7 16.7 16.7 16.8 16.8 17.0 17.0 17.2 17.2 17.4 17.1 16.6 15.6 13.8 8.6 5.0 3.3]; %(z=24)
s(1,:)=[17.0 17.0 17.1 17.2 17.5 17.8 18.1 18.5 18.7 18.9 19.3 19.6 19.3 18.9 17.8 14.4 1.2]; %(z=26)

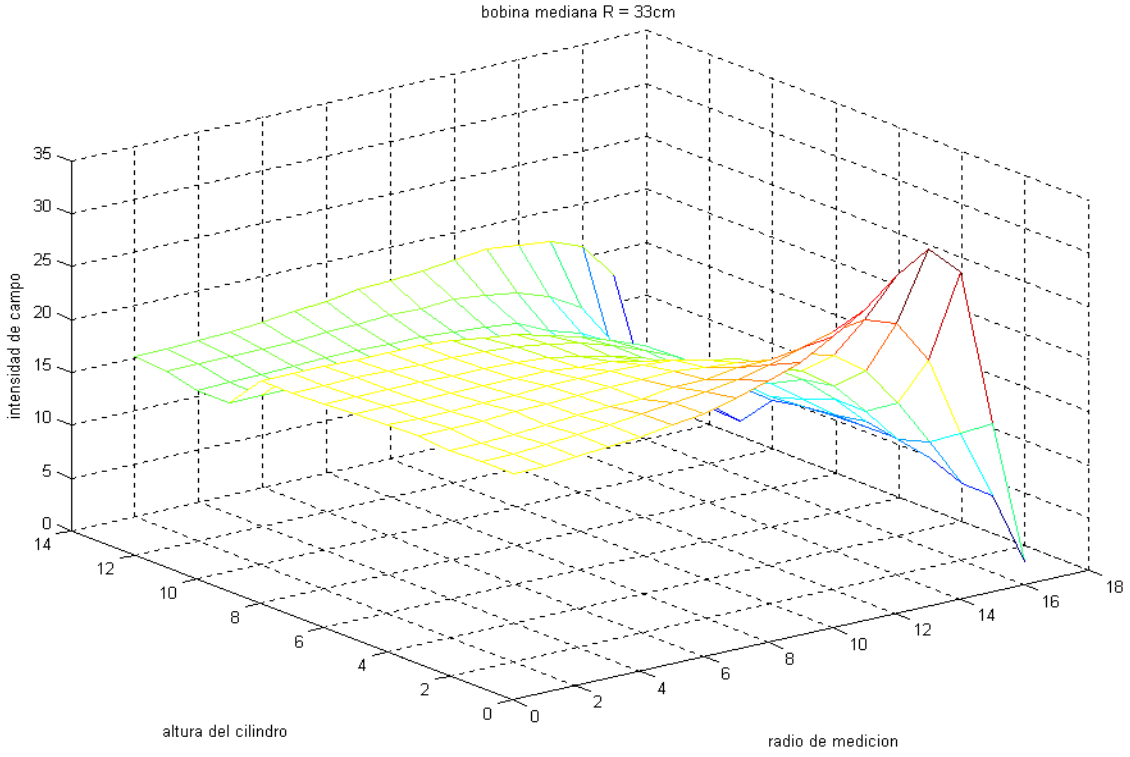


***Figure 7: Field generated in B2***

**For B3**
N = 168 vueltas
I = 4ª

With the following data:

g(1,:)=[17.5 17.5 17.5 17.7 18.1 18.6 18.9 19.4 20.4 21.6 23.1 25.3 28.3 39.2 46.5 5.4]; %z=2
g(2,:)=[17.5 17.7 17.8 17.9 18.1 18.6 19.0 19.6 20.3 21.2 22.6 23.9 25.8 31.3 31.6 5.1]; %z=4
g(3,:)=[18.3 18.3 18.3 18.6 18.6 18.9 19.2 19.6 19.8 20.4 21.1 21.3 21.6 20.7 19.2 9.4]; %z=8
g(4,:)=[18.6 18.6 18.8 18.6 18.6 19.0 19.2 19.4 19.6 19.8 20.1 20.1 19.8 17.9 16.3 8.9]; %z=10
g(5,:)=[18.5 18.5 18.6 18.6 18.8 19.0 19.0 19.2 19.2 19.2 18.9 18.6 18.1 15.3 15.6 8.0]; %z=12
g(6,:)=[18.1 18.1 18.1 18.2 18.4 18.6 18.5 18.5 18.5 18.5 18.1 17.7 17.3 16.6 13.2 7.2]; %z=14
g(7,:)=[14.9 15.9 15.0 15.0 15.0 15.3 15.3 15.4 15.2 15.0 14.9 14.4 13.5 10.1 9.5 3.7]; %z=16
g(8,:)=[15.6 15.6 15.6 15.6 15.6 15.6 15.7 15.7 15.6 15.4 15.3 14.5 14.1 12.0 9.0 4.4]; %z=18
g(9,:)=[15.4 15.6 15.6 15.7 15.9 15.9 15.9 15.9 15.9 15.7 15.6 14.9 14.2 11.2 3.0 3.2]; %z=20
g(10,:)=[15.9 15.9 15.9 16.0 16.0 16.1 16.3 16.3 16.3 16.3 16.3 16.0 15.4 12.9 10.9 4.8]; %z=23
g(11,:)=[15.3 15.3 15.3 15.3 15.3 15.3 15.3 15.2 15.0 14.6 14.2 13.7 12.9 11.6 10.0 7.7]; %z=25
g(12,:)=[16.3 16.0 16.0 16.0 16.1 16.1 16.1 15.9 16.0 16.0 15.7 15.7 14.0 13.5 10.4 3.3]; %z=27

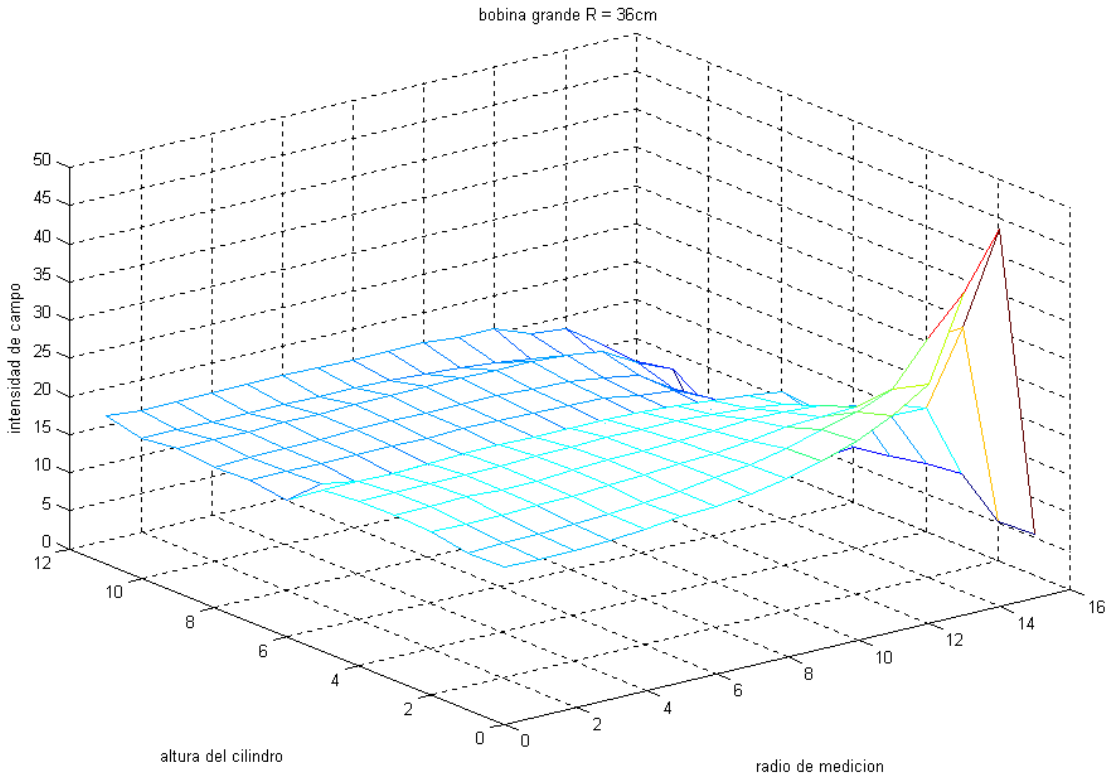


***Figure 8: Field generated in B3***

***Part Two:***
**Use of electromagnetic fields in biological products**
**Treatment with immunomodulators:**
This study was performed to evaluate the frequency of total lymphocytes in rats administered with glycine, factor of transfer and interferon-beta, substances known as immunomodulators and used in the treatment of ulcers by pressure

**Material and Methods**
7 groups of 5 Wistar rats (180 g / body weight) were formed: Negative Group: injectable water, 0.3 ml intraperitoneally. FT Group: leukocyte extract is obtained in the National School of Biological Sciences, IPN: 0.13 and 0.26 µg/kg. Group INF-β: Betaferon (Serono) 6,000,000 UI: 0.13 y 0.26 µg/kg. Electromagnetic Fields Group ultralow frequency: 120 Hz for 15 minutes every 24 hours for 12 days.
NOTE: The doses of INF-β y FT that were applied were calculated based on the concentrations administered in humans.

1. - The animals were weighed daily, food *ad libitum*
2. - Formation of chronic ulcers: All animals were shaved dorsal part of the rat and ammonium hydroxide ($NH_4OH$) (29%) was applied every eight hours for a week.
3. - Determination of the frequency of total lymphocytes: staining technique peripheral blood film (as Wright) (Fig. 9):

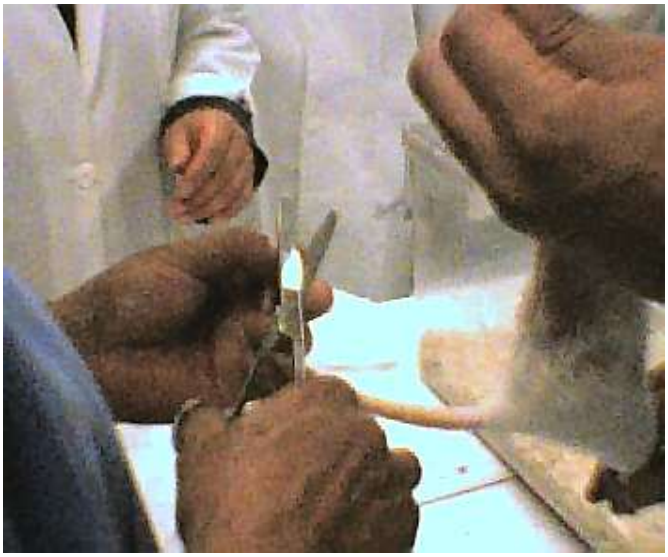

***Figure 9: Cutting the tail of mouse 2 mm***

A) Peripheral blood samples every other day (tail) were taken for a period of 12 days.

a) The extension is allowed to dry, "to the contact with air"
b) The foil is placed with extension up a bridge staining
c) Cover the slide completely with the dye solution
d) Let stand for 2 to 5 minutes
e) Add the buffer solution
f) By slight blows, mixing the dye and the buffer.
g) Rinse the slide into a homogeneous water jet
h) Allow dry it to the contact with air
i) Read the slides: differential counts of total / 100-cell lymphocytes, Olympus, objective 100X

4. - Getting peripheral blood sample (1 ml) to quantify the frequency of CD4, CD8, with the technique of flow cytometry, cell samples were taken before treatment and 12 days after treatment
5. - All animals took them a biopsy to 120 h of treatment to observe the histological changes with two microscopic techniques: hematoxylin-eosin and scanning

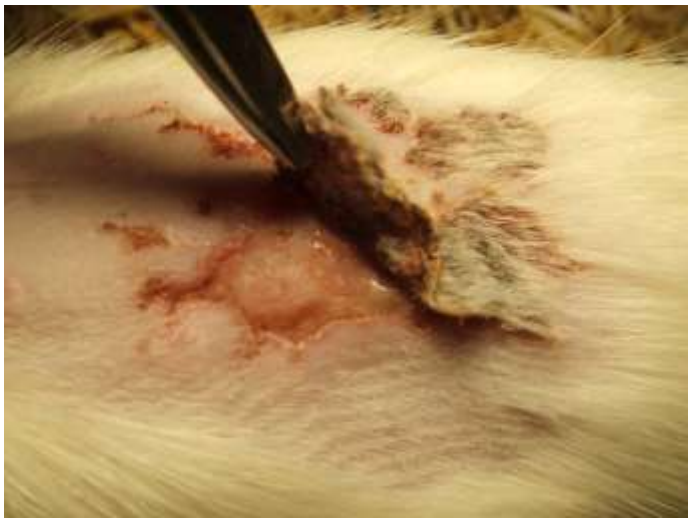

***Figure 10: Take biopsy sample***

Method: Light Microscopy H-E
Fixing the skin sample in 10% formol

1) Mix in paraffin.
2) Histological staining with hematoxylin-eosin.
3) Observation in a Axiphot Zeiss microscope

Method: scanning microscopy:

1) Fixing in glutaraldehyde (3%) for 2 hrs.
2) Post-fixation in $O_sO_4$ (1%).
3) Dehydration in ethanol.
4) Inclusion in Epon 812 resin.
5) Observation under a transmission microscope JEOLJEM-1010

Two fragments of skin of each of the groups had this process:
1) Fixing in glutaraldehyde (2.5%) for 2 hrs.
2) Post-fixation in OSO4 (1%).
3) Dehydration in ethanol.
4) Placing samples in a specimen holder and covered with gold.
5) Observation in high vacuum scanning microscopy JSM-5800 LV (fig. 11).

***Figure 11: Treated rat skin***

## RESULTS AND DISCUSSION

Body weight: on all treatments at the end increased significantly (P <0.001) compared to before treatment. The general state of the rats did not change during treatment (Table 2). Rats treated with EMFs showed drowsiness and decreased alertness during the first two hours after treatment of the first session. The lesions showed different recovery times: In the negative control group, the formation of scar tissue began at 120 h, the FT group at 72 h, the group of INF-β at 96 h, and the group of EMFs at 72 h (Table 2).

***Table 2: Body weight of rats with treated ulcers***

| Substance | Dose µg/kg | No. of Rats | Body weight (g) Zero day: X ± DE | Body weight 12 day X ± DE | Body weight difference % |
|---|---|---|---|---|---|
| Negative control | 0.3 ml water | 5 | 180.8 ± 0.83 | *183.6 ± 1.14 | +1.01 |
| F T | 0.13 | 5 | 181.8 ± 1.48 | *185.2 ± 1.30 | +1.01 |
| F T | 0.26 | 5 | 181.2 ± 1.30 | *186.4 ± 1.51 | +1.02 |
| INF-β | 0.13 | 5 | 181.6 ± 1.14 | *185.2 ± 0.83 | +1.01 |
| INF-β | 0.26 | 5 | 181.8 ± 1.09 | *187.2 ± 0.83 | +1.02 |
| CEM | 120 Hz | 5 | 181.8 ± 1.09 | *187.2 ± 1.34 | +1.02 |

** Statistical significance. (P<0.001). Treatment (Tx)*

The frequency of lymphocytes in normal rats falls within a range of 50 to 80%with an average value of 60% [14]. The negative control group, FT, INF-β (0.13 mg/kg) and EMF were unchanged. However, in rats treated with INF-β (0.26 mg/kg) was observed from the third day a significant increase (P <0.05) in the frequency of total lymphocytes. In the group of rats treated with FT (0.13 and 0.26 µg) and EMF, frequency of total lymphocytes increased significantly (P <0.05) from the 6th day, compared to the negative control (Table 3).

***Table3: Frequency of total lymphocytes with FT, INF-β y EMF***

| Treatment µg/kg | Lymphocyte frequency/100 cells (%) | | | |
|---|---|---|---|---|
| | 3º Day X ± DE | 6º Day X ± DE | 9º Day X ± DE | 12º Day X ± DE |
| Negative control (Water, 0.3 ml) | 53.6 ± 2.07 | 54.6 ± 2.30 | 55.0 ± 1.87 | 54.8 ± 1.92 |
| FT (0.13 µg/kg) | 54.8 ± 0.83 | *62.0 ± 1.58 | *69.0 ± 1.58 | *74.0 ± 2.34 |
| FT (0.26 µg/kg) | 57.8 ± 2.70 | *70.0 ± 1.58 | *74.8 ± 1.64 | *77.2 ± 1.92 |
| INF-β (0.13 µg/kg) | 55.0 ± 2.12 | *66.0 ± 2.73 | *71.8 ± 1.30 | *73.4 ± 2.30 |
| IFN-β (0.26 µg/kg) | *58.0 ± 1.87 | *70.6 ± 1.34 | *73.2 ± 1.92 | *72.4 ± 3.05 |
| EMF 120 Hz | 57.2 ± 1.81 | *68.2 ± 1.52 | *72.5± 1.87 | *76.1± 2.00 |

** Statistical significance: ANOVA, Tukey-Kramer (P>0.05)*
** Significant increase in the frequency of total lymphocytes*

**Frequency of CD4+, CD8+ lymphocytes in treated rats**

The frequency of CD4+ lymphocytes in the negative control, its range was 511.6±14.3 before and 526.3±13.1 after 12 days of treatment, with FT dose of 0.13 µg/kg before and after 12 days treatment no significant occurred. A significant increase (P <0.05) from the third day of treatment in the CD4+ lymphocytes with FT (0.26 µg/kg); INF-β (0.13 and 0.26 µg/kg) and EMF was observed (Table 3).

The frequency of CD8+ lymphocytes in the negative control rats was 169.4±12.3 to 157.2±13.2. Here a non-significant decrease was observed. However, with the two doses of FT, the INF-β and EMFs, a significant decrease (P <0.05) it was observed in the frequency of CD8+ lymphocytes after 12 days of treatment (Table 3).

***Table 3: Frequency of CD4, CD8 lymphocytes concentration***

| Dosage mg/kg | Lymphocytes CD4 0 Day X ± DE | Lymphocytes CD4 12 Days X ± DE | Lymphocytes CD48 0 Day X ± DE | Lymphocytes CD8 12 Days X ± DE |
|---|---|---|---|---|
| Water | 511.6 ± 14.3 | 526.3 ± 13.1 | 169.4 ± 12.3 | 157.2 ± 13.2 |
| FT 0.13 | 502.2 ± 18.2 | 510.7 ± 11.2 | 176.3 ± 11.3 | 144.1 ± 11.7* |
| FT 0.26 | 525.1 ± 12.3 | 545.9 ± 17.6 | 173.2 ± 10.1 | 158.2 ± 13.4* |
| INF-B 0.13 | 512.3 ± 14.4 | 588.5 ± 18.9 | 167.4 ± 15.7 | 147.4 ± 11.5* |
| INF-B 0.26 | 529.2 ± 11.2 | 592.4 ± 17.5 | 176.2 ± 13.4 | 141.5 ± 10.9* |
| EMF 120 Hz | 523.4 ± 17.1 | 589.1 ± 15.1 | 181.4 ± 16.9 | 140.2 ± 12.7* |

** Statistical significance: ANOVA and t Student (P<0.05), (Tx treatment)*

**Biopsy results**

Negative control group: Skin rat granulation tissue was observed with little inflammatory infiltrate tissue and low density of fibrocytes and neoplastic tissue (Figure 12 and 13). The ulcer showed healing tissue 96 hours after the last application of ammonium hydroxide.

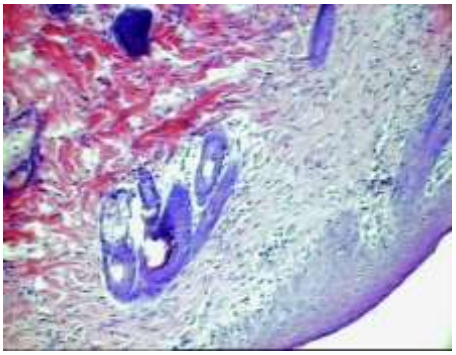

***Figure 12: Control rat skin***
***Little inflammatory infiltrate is observed.***

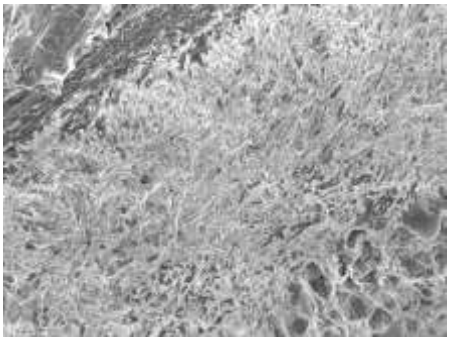

***Figure13: Control rat skin***
***Little inflammatory infiltrate is observed.***

Group of rats treated with transfer factor: after the last application of $NH_4OH$, normal epithelium of epidermis, papillary dermis with little tissue collagen and fibrocytes, and scarce inflammatory infiltrate (Figure 14 and 15) was observed, compared with the negative control; recovery began after 72 h.

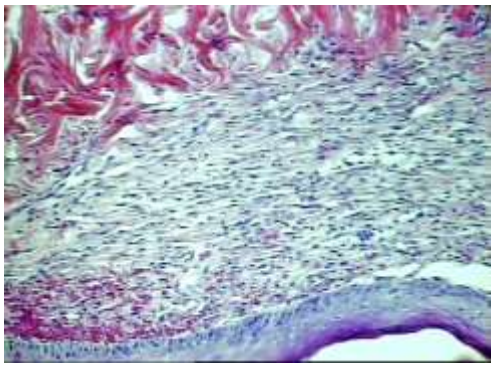

***Figure 14: Rat skin treated with transfer factor It observed slight inflammatory infiltration.***

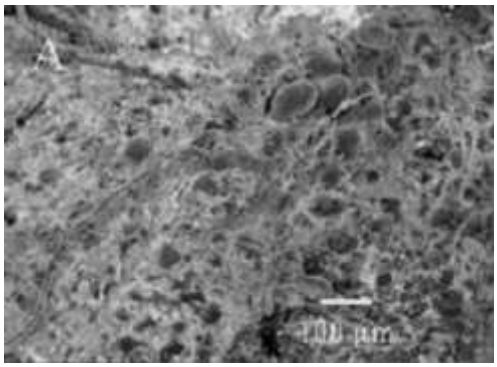

***Figure15: Rat skin treated with transfer factor It observed slight inflammatory infiltration.***

Group of rats treated with interferon-beta: in the area of healing, abundant inflammatory cells were observed, prevailing fibrocytes; in addition, the inflammatory infiltrate shows the formation of multinucleated foreign body cells and extensive destruction of the epithelium of reddish color is observed. The start time of the tissue wound healing was 120 h.

The results and discussion may be combined into a common section or obtainable separately. They may also be broken into subsets with short, revealing captions.

## CONCLUSION

The results on body weight of treated animals do not indicate a significant increase in all groups; data have not been published in the literature. On data analysis of total lymphocytes frequency, a significant increase was observed from the sixth day of treatment. In determining the frequency of CD4 lymphocytes, a tendency to normal values of these lymphocytes it was observed. However, in previous research by Loots et al. (1998), they observed a decrease in lymphocytes T CD4+ and increased infiltration of the lesion of macrophages and CD8+ lymphocytes, as noted in our results[15] with chronic ulcers of patients with diabetes and venous insufficiency. A significant increase was observed in the frequency of CD8+ lymphocytes before treatment, during treatment these values were set in normal ranges, data observed by Oliver et al. (2000) when applied thalidomide to scleroderma patients and observed changes in fibrosis also an increase in the epidermal and dermal infiltrate of cells T CD8+ and plasma levels of interleukin 12 (IL-12) and tumor necrosis factor alpha (TNF).[16] The literature has not reported the use of transfer factor and interferon-β in chronic ulcers. For recovery of dermal ulcers, our results show a faster healing with transfer factor and ultra low frequency electromagnetic fields treatments, compared to interferon-β and untreated rats.

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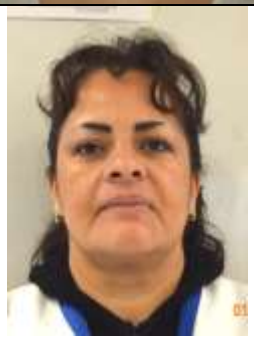
|  | **Norma Hernandez Campos**<br>Technical laboratory "B", and Collaborative research projects. Biochemistry Service of the National Institute of Rehabilitation, Mexico, D.F |  | **Julieta Karina Peñuelas Romero**<br>Chemical Industrial Pharmacist with Master of Science specializing in Toxicology, National School of Biological Sciences of the National Polytechnic Institute. Research in medical sciences at the National Institute of Rehabilitation. |