

## Evaluation of Treatment with a Pulsed Electromagnetic Field on Wounds Healing and Clinic Pathologic Variables of Rats Subjected to Nicotine Treatment

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**Abstract:** The electrotherapy through electromagnetism is a non-invasive technique and can be applied as co-adjuvant in the resolution process of surgical wounds, accelerating healing. Our aim was to study the influence of electromagnetic therapy on the resolution of surgical cutaneous wounds in rats subjected to treatment with nicotine. The application was carried out during 21 days, once a day, in a total of 21 sessions, with each session lasting 30 min. The results obtained from the assays suggest a significant benefit to the healing process of surgical wounds, accelerating the healing process. This could be an alternative therapy to similar alterations without adverse collateral effects, particularly in post-surgical patients with impairment to anti-inflammatory therapy.

**Key words:** Electromagnetic therapy, wounds, rats, nicotine

### INTRODUCTION

Reports on the use of pulsed electromagnetic waves used on the treatment of recumbent ulcers in humans demonstrate that the wounds tend to decrease in size<sup>[1]</sup>. The electromagnetic field has been used for treatment of various types of wounds and other conditions during the last 10 years and has been reported as efficient in the treatment of wounds, skin transplant, diabetes mellitus, myocardial ischemia and brain ischemia<sup>[2]</sup>. The use of electromagnetic therapy in osteotomized rats has been reported and it was observed that regeneration in bone and wound treatment derived from burns was more rapid when electromagnetic therapy was used. It was also observed a decrease of the microbial flora, accelerating the healing process of these wounds<sup>[3,4]</sup>.

Rats have been used as a model for studies in the transplant of organs and tissues, as well as in studies of tissue regeneration. Surgical wounds have a fundamental importance in the programming and execution of the majority of procedures in plastic surgery, being able to be used in the reconstruction of substance loss and in other surgical techniques, such as otoplasty and lipaspiration, becoming a resource which should be further studied, in the attempt to obtain the aimed success<sup>[5]</sup>.

The viability of the cutaneous wounds is directly related to its vascularization, being necrosis a frequent

finding, which could be derived from extrinsic and intrinsic factors, being blood flow an intrinsic factor most responsible for its losses<sup>[6,7]</sup>. Tissue damages observed in cutaneous wounds are due, in its majority, to a post-systemic perfusion, when oxygen returns to the tissues with critical energy levels, unleashing biological reactions and giving origin to other toxic species, with potential for tissue damage<sup>[8,7,9,5]</sup>. Nicotine activates the brain receptors which release catecholamins, noradrenalin and reduces the synthesis and release of vessel-dilating prostaglandins. Besides that, brain receptors produce a cutaneous vasoconstriction, decreasing the skin temperature and coagulation time and increasing the plaque adhesively. Lesions in the arterial intima, calcification and fibrosis, on average, are related to nicotine, causing irreversible lesions<sup>[10-13]</sup>. A poor healing of wounds is attributed to nicotine, since it inhibits the maturity, the proliferative ability of the precursor erythrocytes, as well as for going to re-epithelization by the catecholamins released, decreasing the blood flow by its vessel-constrictor effect<sup>[14,15]</sup>. Campos<sup>[5]</sup> highlights that nicotine presents a noxious effect to randomized cutaneous wounds in rats, when such drug is used in the pre-surgical period, in dosages of 2 mg kg<sup>-1</sup> twice a day for four weeks. He also highlights that the use of nicotine in the post-surgical period increases the

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necrosis area, when compared to the use only in the pre-surgical period.

Forest *et al.*<sup>[16]</sup>, in a series of protocols about the use of nicotine with varied dosages and intervals, reached significant results in relation to the area of necrosis in wounds, when administered the dosage of 2 mg kg<sup>-1</sup> twice a day, during four weeks in the pre-surgical period. The author concluded that the effect of the treatment with nicotine caused a significant decrease in the capillary blood flow, distal perfusion and area of survival of the wounds in rats. Schastny *et al.*<sup>[17]</sup> consider electromagnetic stimulation as a non-invasive and biologically adequate technique and that the efficiency of this therapy can be of 75-90%, depending on the type of use. The main clinical application of the non-ionizing and non-thermal electromagnetic therapy were described in osteoarthritis<sup>[18,19]</sup>, in the regeneration of tissues<sup>[20,23]</sup>, in the healing of wounds<sup>[24,27,2]</sup> and in adjuvant therapy in the treatment for non-binding of fractures<sup>[28-31]</sup>. Scardino *et al.*<sup>[2]</sup> highlight a significant increase of the epithelization in open wounds (10 to 15 days after surgery). In surgical wounds in rats<sup>[24]</sup> used a low frequency (50 Hz, 8 mT) electromagnetic field therapy immediately after surgery and after 12 h. The group treated showed a total healing of the wound 42 days after surgery, demonstrating a cellular organization, formation and maturity of the collagen and a precocious neo-vascularization.

Using the same animal model, Patiño *et al.*<sup>[23]</sup> observed an improvement on the time of healing with electromagnetic fields of 20 mT with 35 min of exposition twice a day and concluded that the Pulsatile Electromagnetic Field (PEMF) should be considered a practical instrument for clinical use. In a study of patients with pressure ulcers in stage II (ulcers without healing resolution between 3 and 12 weeks) and in stage III (ulcers without healing resolution with over 168 weeks) exposed to electromagnetic therapy in addition to conventional treatment, Itoh *et al.*<sup>[26]</sup> highlighted that there was a decrease on the time of healing of these wounds, with the average healing for ulcers in stage II of 1 to 6 weeks (mean of 2.33) and in ulcers of stage III of 1 to 22 weeks (mean 8.85). The same event was observed by Ieran *et al.*<sup>[25]</sup> and Salzberg *et al.*<sup>[22]</sup>, where the authors highlighted electromagnetic therapy as an excellent therapeutic adjuvant in the management of patients with ulcers. Yen-Patton<sup>[32]</sup> evidenced the effect of the low frequency electromagnetic field use *in vitro*, which is capable of increasing the growth of endothelial cells and the angiogenesis, as well as increasing the tumorous activity in the Kupffer cells of the liver.

The purpose of this study is to obtain information about the effect of the electromagnetic field in the treatment of ischemic surgical wounds in nicotinized rats, through a histological evaluation of tissue fragments in the transition area (necrosis and healthy) characterizing inflammation, vascular neoformation, conjunctive neoformation, as well as the presence of vasculitis, edema and thrombosis and time of resolution/healing. For the treatment of surgical wounds, efforts must be made in order to both minimize problems which can interfere in its resolution process and to accelerate its healing, with the purpose to allow the patient return to full function within the least period of time possible, minimizing the time of inactivity and promoting the fast return to daily activities.

## MATERIALS AND METHODS

**Animals:** Were used 22 male animals, from the species *Rattus norvegicus albinus*, Wistar lineage, with mean age of 3 months and weighing 200±50 g, proceeding from the Central Animal House of the University Research Centre of Bahia. All animals were subjected to a period of acclimatization of 15 days. During this period they were maintained for 12 h in the light and 12 h in the dark, with temperature continually maintained at 21°C, with standard diet of specific ration and water, without restriction of consumption. The animals were maintained individually in cages of 15x30x45 cm, being handled according to the protocol submitted to the Animal Experimentation Ethics Committee. This Committee analyzed and approved this research protocol under No. 012/2004, referring to this experiment, with respect to the Federal Law No. 6.638 from 8th May 1979, which establishes rules for the academic-scientific practice of animal vivisection. The body weight of all animals was registered at the beginning and at the end of the experiment, using a precision balance.

**Nicotine administration:** The animals were divided in two groups, chosen randomly, being the GI the experiment group and GII the control group, containing 11 animals each. All animals had a dosage of 2 mg kg<sup>-1</sup> of nicotine administered sub-cutaneously, twice a day, for a period of four weeks before surgical proceeding. The drug used in the experiment was nicotine [nicotine Sulphate L-1 Metil-2 (3-Piridil) -Pirrolidina Sulphate, degree II, Sigma, USA], diluted in saline solution at concentration of 2.5 mg mL<sup>-1</sup>. The confirmation of effectiveness of the nicotine on the animal was performed through the observation of shaking in the animal's tail, after application of the drug, as described by Gomita *et al.*<sup>[33]</sup>.

**Surgical wound:** The animals were anesthetized through the use of 40 mg Kg<sup>-1</sup> of sodium pentobarbital (Nembotal®) immobilized in the decubital ventral position, in a standard board measuring 40x45 cm, tensioning the four paws. After immobilization, a trichotomy of the animal's back was performed, from the neck to the tail, enough to contain a rectangle measuring 10 cm length by 4 cm width, with margin around 1 cm, according to the methodology suggested by McFarlane *et al.*<sup>[34]</sup> for making an ischemic wound of skin. After determining the incision area, anti-sepsis of the trichotomised area was realized using topical Povidine (Iodine-Povidine Prep Solution). The surgical technique was realized using scalpel number 3 and lamina number 15, making an incision on the skin and on the panniculus adiposus, surrounding the rectangle on its sides and tail part, preserving from cutting the cranial base, through which the vascular panniculus of the wound crosses. The dissection of the wound was performed right below the adiposus panniculus, in an individualized and scarcely vascularized surface, until the base of the wound, in the direction tail-skull, after easily identifying the dominant vascular pediculus. After dissected, the wound was positioned in a surface and sutured using a nylon single-string thread of 5.0 in a sequence of simple and separated stitches and the animals were re-conducted to their respective individual cages.

**Electromagnetic therapy:** The stage of therapeutic treatment begun soon after making an ischemic wound of the skin and its fixation (day 1), when the treatment was initiated using electromagnetic therapy only on animals of the experiment group, using the equipment described below. The application was performed for 21 days, once a day, in a total of 21 sessions, being each session of 30 min. The animals were exposed to the therapy chamber in groups of two. The animals of the control group were subjected to the same procedures carried out with the experiment group; however the equipment was turned off. Only the coolers were turned on in order to maintain the temperature, so that a simulation of the application was made. An apparatus generator of continuous and pulsating magnetic field was used, fed by an alternated current of 60 Hz of frequency and an intensity of 7.2 mT (1Gauss = 10<sup>-4</sup> Tesla) with sinusoidal waves and modulated impulses of 60 msec (miliseconds) and mean time of 450 msec, connected to the alternate current of 110 volts, adapted to individual chambers.

**Therapy chamber:** For the treatment with electromagnetic field, a therapy chamber was developed, assembled and instrumented, in which the animals were subjected to treatment. In this chamber, the generation of magnetic

field is made by three units of the electromagnetic therapeutic apparatus Kenkobio®, of Nikken from Brazil. A hall and a temperature sensor, connected to an Analogical to Digital Converter (ADC), realized the measurements and the digitalization of these physical parameters. A Personal Computer (PC), processing a data acquisition and control programme written in VisualBasic®, developed specifically for this application, realized the acquisition of data and controls the actioning of the Kenkobio® units. The duration of each treatment session is determined by the internal PC clock and can be programmed according to need. In the study of magnetic field generators, different tests were carried out. Initially, the mechanical characteristics of the magnet Kenkobio® sets were determined. Afterwards, the electric parameters of functioning (current, tension, impedance) were measured and finally the study of the magnetic characteristics was carried out. For the determination of these characteristics, were investigated: the form of the generated field, the distribution of the field lines on the elements, the dependency between the field and the temperature, the dependency between field and distance in relation to the surface of the magnetic element and the hysteresis curve. The therapy chamber was assembled in transparent acrylic with 21 cm height, 26 cm width and 60 cm length. The frontal, back, base and superior parts all have 1 cm thickness. The other parts have 0.3 thicknesses. The chamber can be divided in four parts.

A central passage with 1 cm width, 12 cm height and 58 cm length, which receives a sliding structure in the shape of a drawer, meant for the rats confinement. Two lateral spaces and a superior space were meant for the three sets of electromagnetic elements. In order to assure the control of the chamber's internal temperature, 16 coolers of the same type used for refrigeration of microprocessors were used. Eight of these coolers were installed in the frontal face and six at the back face, all-working as exhausters.

**Macroscopic evaluation:** A drawing of all the wound area was performed, defining precisely the transition area of the necrosis with the healthy region. These measures were taken in two distinct moments after the period proposed by the therapeutic protocol using electromagnetism (10 and 20th days of treatment). For that, a sheet of vegetal paper was positioned at the back of the animal, with the limit demarcated with a pencil. These drawings were subjected to an analysis for evaluation of the necroses area, using for that a semi-automatic system of computerized morphometrics, through the programme Sigma Scam (Jandell Scientific, California, USA), in which these demarcated areas were transformed in numerical data, expressed in cm<sup>2</sup>.

**Microscopic evaluation:** After the sacrifice of the animals by excessive CO<sub>2</sub> inhalation, fragments of tissue derived from the area of transmission (necrosis and healthy tissue) were collected. The following parameters were evaluated: neo-vascularization, vasculitis, conjunctive neo-formation, inflammation, edema, presence of thrombosis, micro-abcessus and polymorphonuclear leukocytes classified according to its intensity in weak, moderate and intense.

**Hemogram and serical biochemistry:** Hemograms of the animals were realized before and after being submitted to treatment with electromagnetic therapy (control and experiment groups), where the following parameters were observed: red blood corpuscle ( $\times 10^8 \text{mm}^3$ ), hemoglobins (g%), hematocrites (%), VGM ( $\mu\text{m}$ ), HGM (pg), CHGM (%), leucocytes ( $\times 10^3$ ), basophilous ( $\times 10^3$ ), eosinophilous ( $\times 10^3$ ), neutrophilous ( $\times 10^3$ ), lymphocytes ( $\times 10^3$ ), monocytes ( $\times 10^3$ ) and platelets count ( $\times 10^3 \text{mm}^3$ ). The total and fraction proteins (albumin and globulin) and blood serical dosages from the animals were evaluated after being submitted to treatment with electromagnetic therapy (experimental group) and compared to the non-treated animals (control group). The following parameters were evaluated: glucose ( $\text{mg dL}^{-1}$ ), uric acid ( $\text{mg dL}^{-1}$ ), urea ( $\text{mg dL}^{-1}$ ), creatinine ( $\text{mg dL}^{-1}$ ), AST (U/l) and ALT (U/l).

**Splenocytes proliferation assay:** For the lymph-proliferation assay, the concanavalin A (ConA), a mitogen, was used as unspecific stimulator, in optimum and sub-optimum dosages previously determinated. Splenocytes from healthy rats, treated (five animals) or not (five animals) through electromagnetism following the protocol suggested in this study, were obtained through the technique of cellular divulsion and dispersion and placed in 96 well-plates culture at a density of  $2 \times 10^5$  cells/wells in the presence of 0.5  $\mu\text{g mL}$  Con A (sub-optimal dosage) and 2.0  $\mu\text{g mL}$  Con A (optimal dosage) in a total volume of 200  $\mu\text{L}$  of complete RPMI medium (RPMI 1640 added of 10% fetal bovine serum, L-glutamin, sodium piruvate, non-essential aminoacids and gentamicin). The mixture, together with the controls (cells in a medium without mitogen, with or without treatment by electromagnetism), were incubated for five days in a humid atmosphere at 37°C and with 5% CO<sub>2</sub> during 18 h with 0.4  $\mu\text{Ci}$  of [<sup>3</sup>H] thymidine (1.0 Ci/mmol; Amersham, Bucks, UK). Cells were removed and the incorporation of [<sup>3</sup>H] thymidine determined in a  $\beta$  radiation counter. The tests were performed in triplicates and the proliferative response expressed by stimulation index (IE= average of proliferation after stimulation/average of proliferation of the control medium).

**Statistical analysis:** The information processed included calculations of means and Standard deviation of the parameters studied, comparison between means using the t Student test and evaluation of non-parametric data<sup>[35]</sup>. The level of rejection of the null hypothesis was fixed in 0.05 or 5% ( $\alpha < 0.05$ ).

## RESULTS AND DISCUSSION

The form of the field in relation to the geometry of the magnetic elements was determined. In this study, the magnetic field was measured in two directions, width and length. It was observed that the field is neither uniform nor homogeneous and that the intensity of the field varied with distance from the edge of the element. In the central region, alongside the length of the element, the field falls practically to zero, despite the fact that the Gaussians used for numeric adjustment of the data obtained did not present this behavior. For each element, the maximum value of the magnetic field is found in the mean position of the width and at a quarter from its total length.

Measurements of the temperature and of the magnetic field were performed for 22 min. A tendency to decrease the value of the field as the temperature increases was observed. The explanation for this behavior lies in the fact that, very probably, there is a thermo resistive sensor (PTC) of protection, internal to the apparatus, in series with the electro-magnets. The increase of the temperature of the magnetic element increases resistance to PTC and, as a result, decreases the current of the magnetic set, decreasing the field. The decrease observed is not a limiting factor for applications using this equipment. Especially if the dissipation of heat, produced through a joule effect, is done efficiently, keeping the temperature of the elements close to constant. In this test, the variation of temperature was of approximately 91%, whereas the field variation did not exceed 38%. It should be highlighted that during this test the refrigeration system was kept turned off and the results pointed to the imperative need to refrigerate the magnetic elements of the therapy chamber.

With the help of a hall sensor, placed on the surface of the magnetic element, the higher values of the field were searched, for each one of the other four elements. From the results obtained, for the five cells, we calculated the mean value for the magnetic field for the elements of the set ( $B = 479 \pm 15$  Gauss).

The magnetic field generated was determined indirectly. An element was chosen randomly and the measure of the magnetic field intensity was taken in relation to the distance between the hall cell and the surface of the electromagnetic element, the value of the

Table 1: Rats treated with nicotine (2 mg kg<sup>-1</sup> live weight) subjected to treatment with electromagnetic therapy by an alternated current with frequency of 60 Hz and intensity of 7.2 mT during 21 days, once a day, being each session of 30 min on the necrosis area of the surgical wound, taken at two distinct periods

	First measurement (cm <sup>2</sup> ) 10th day of treatment		Second measurement (cm <sup>2</sup> ) 20th day of treatment	
	Group (A)	Control Group (B)	Group (C)	Control Group (D)
Animal # 1	2.838	8.994	0.384	8.368
Animal # 2	1.246	11.005	0.001	4.833
Animal # 3	0.318	4.371	0.001	0.439
Animal # 4	2.459	6.250	0.558	5.716
Animal # 5	7.427	11.164	2.044	3.030
Animal # 6	4.273	12.829	1.337	4.245
Animal # 7	5.314	10.762	2.972	4.720
Animal # 8	8.321	12.555	4.418	5.415
Animal # 9	1.591	6.585	0.488	1.507
Animal # 10	9.848	7.741	5.230	1.851
Animal # 11	12.782	11.621	3.481	8.718
Mean	5.1288	9.4434	1.9013	4.4402

A<B (p<= 0.01) #A = 11#B = 11, W = 89, p<= 0.01512. C<D (p<= 0.02) #A = 11#B = 11, W = 91, p<= 0.02155. (Wilcoxon's rank sum test)

magnetic field for a distance x = 0mm was determined by extrapolation. This calculation resulted on a value of 549±23 Gauss for the mean magnetic field (RMS), generated by a given element of a given set. This difference between the results of both tests is due to the fact that the element is conditioned in a plastic protection, with probably more than 1 mm thickness, which would make impossible the direct measure of the field on the surface of the magnet.

With the help of an electronic system developed by Moreira *et al.*<sup>[30]</sup>, it was possible to establish the hysteresis loops to link to different tension and current values applied to the magnets set. From the drawing of these loops, 32 points were determined with the help of an oscilloscope. The experimental data (dots) were taken at the maximum of each loop. From these data, using the applicative Matlab, the best fit of the experimental set was made.

The curve λ versus i showed a linear characteristic, leading to conclude that on the Therapeutic Electromagnetic Apparatus Kenkobio®, the magnetic field has a higher concentration on the free space than on the nucleus of the material. It is then possible to assert that this is a magnet clearly designed to generate non-confined field lines, which can penetrate the free space around the elements. That is, the rats treated inside the chamber were actually exposed to daily dosages of magnetic field. Since the system developed is fully automated, during the therapy sessions the values of the magnetic field were recorded, in the superior central part of the chamber, as well as its internal temperature of the chamber.

The variation of the internal temperature and electromagnetic field of the chamber was on average ±1.72°C during the whole period of the treatment (21 days of treating the rats) and the electromagnetic field in the center of the chamber varied from ±25.04 Gauss.

Regarding the initial healing area, it was histologically observed, besides the presence of a scab, an intense inflammatory infiltrate. In the intermediary phase, the evolution of the re-epithelization process and the increase in the number of collagenous fibers in the deep dermis was also observed.

The necrosis area of the surgical wound was taken in two distinct periods. The first measurement was made at the 10th day of treatment, whose mean observed in the experiment and control groups were 5.13±4.00 and 9.44±2.83 cm<sup>2</sup>, respectively. At the 20th day of treatment the means found were 1.90±1.86 and 4.44±2.64 cm<sup>2</sup>, respectively. A significant decrease of the necrosis area was observed when the animals were subjected to magnetic therapy at days 10th (p<0.01512) and at day 20th (p<0.02155), (Table 1).

In the control group there was coagulation necrosis in the muscle tissue, variable intensity edema in 81.8% of the cases, inflammatory infiltrates ranging from discrete to moderate intensities. There was conjunctive neo-formation in 100% of the cases. In the deeper areas there was vascular neo-formation ranging from discrete to moderate intensities with discrete and moderate mononuclear inflammatory infiltrate and, in 81.8% of the animals, there were micro-abscess focuses. In 63.6% of the cases, muscle regeneration with the presence of cytoplasm cells ample suggestive of mastocytes was observed. Vasculitis was observed in 54.5% of cases and venous thrombosis in 63.6%.

In the experiment group, a decrease of the percentage of thrombosis (36.4%) was observed in the pathological anatomy findings. There was also a reduction in the percentage of micro-abscess focus presence (54.5%) and an increase on the number of mastocytes (81.8%), characterizing the effective healing process. The results of the blood tests collected from the animals did not present a significant difference between the groups

studied. The blood cells and plaques counting from the animals before being submitted to electromagnet-therapy (experiment group) in comparison to the non-treated animals (control group) did not present significant differences, suggesting that treatment by electromagnet-therapy does not lead to alterations on the hemogram and platelets count. The same fact could be noted in relation to total proteins and fraction and blood serical dosages in the animals of both groups studied the Experiment Group (treatment with electromagnet-therapy) and the Control Group (non-treated animals). No alterations were observed in the value of the serical markers studied (glucose, uric acid, urea, creatinine AST and ALT). The electromagnet-therapy in post-surgery in random surgical wounds of rats treated with nicotine did not alter the renal or hepatic functions, serical dosage of glucose and total protein and its fractions, as well as the hemogram and the platelet count, suggesting its potential use in animals with alterations in these systems, without harmful collateral interference of the electromagnetic field effect.

In order to have access to the possible effects of the treatment by electromagnet-therapy on cellular proliferation, splenocytes from rats were submitted to stimulus with unspecific mitogens, the concanavalin A (conA-in sub-optimal and optimal dosages) and, on the presence or not of LPS (1,0 µg mL). To consider as a positive stimulus the cpm (counting per minute) it was analyzed in relation to the pure medium, generating an index of proliferation (cpm/medium) which should be higher than 2.5. In the assays realized, there was no difference between the values of cpm on both groups studied, the Experiment Group (treatment with electromagnet-therapy) in comparison to the Control Group (non-treated animals) suggesting that the treatment with electromagnet-therapy in nicotized animals does not stimulate cellular proliferation of splenocytes from rats in response to the conA and LPS stimulus, if compared to the medium stimulus.

From the studies carried out using the unities from the Electromagnetic Therapeutic Apparatus Kenkobio®, it was observed that the field generated by this apparatus is neither uniform nor homogeneous, it varies with position. On the other hand, this magnet was clearly designed to generate non-confined field lines, lines which can penetrate free space, exposing the rats treated to a daily dosage of magnetic field.

Another important aspect about the functioning of these devices is the fact that there is a clear decrease of intensity of the field in relation to temperature, which suggests the need to maintain the elements as ventilated as possible. The direct measure of intensity of the magnetic field, on the surface of the element, shows that, in this position, the mean RMS field, on the five elements is of 479±15 Gauss. On the other hand, the indirect

determination has as a result 549±23 Gauss, as claimed by the manufacturer.

The treatment chamber can be considered athermal, once the variation of the mean temperature observed during the whole experiment showed a very small variation (<2°C), being not considered as therapeutic. Caponi<sup>[37]</sup> highlights that there is only considerable thermal effect when the intensity is superior to 1000 Gauss and the frequency at the order of MHz.

The necrosis area of the surgical wound was taken at two distinct periods. The first measurement was made at the 10th day of treatment, whose mean observed in the Experiment and Control Groups were 5.13±4.00 and 9.44±2.83 cm<sup>2</sup>, respectively. These data were significantly different where  $p < 0.01512$ . At the 20th day of treatment the means found were 1.90±1.86 and 4.44±2.64 cm<sup>2</sup> for the Experiment and Control groups, being observed a difference between the groups ( $p < 0.02155$ ). In these two periods, it was possible to verify that the treatment by electromagnetic therapy reduced considerably the observed necrosis area.

The conjunctive tissue has a structural function (it is present in the whole body) and it has a repairing and defensive function (response in defense and repair to infectious, physical, chemical and mechanical aggressions, such as, for example, traumas and surgeries), being composed by cells and intercellular substances. The cells, which belong to the conjunctive tissue, are of six types: Fibroblasts, macrophages, plasmatic cells, sebaceous cells, adipose cells and pigment cells. The intercellular substance is constituted by an extra-cellular matrix, which is formed by collagenous fibers, (reticulin and elastin). The fundamental substance is composed by carbohydrates and proteins. This richness of intercellular substance makes the electromagnetic therapy act in a more intense and specific manner, due to the paramagnetism of the water and the proteic anions of the collagenous, reticulin and proteoglycosaminoglycans<sup>[37]</sup>.

For McKinney<sup>[38]</sup>, the factors which influence the healing process also influence the final form and appearance of the scar. The degree of ischemia, the oxigenation and apart the angiogenic factors of growth determine the specific healing process, since these processes appear since the moment when a wound is established until the final moment of its remodeling, passing through tissue reposition. The complexity involved in the combination of biochemical and biological matrixes in the repairing process includes three events: hemostasia (because the vessels are open), inflammation (because there is a lesion) and actual repairing, since its structures were damaged or destroyed and a reposition of tissues by regeneration or healing should occur.

According to the data exposed in this study, it was observed that the use of electromagnetic treatment

chamber in the post surgical period in surgical wounds of rats reduced the necrosis area significantly, observing an increase of vascularization, therefore accelerating the healing process, which suggests its potential for use in decreasing the healing period of these wounds. Also, the healing of necrosis areas suggests a real action of the magnetic field on the repairing of the wounds, possibly by the stimulation of the conjunctive tissue activities. From these assertions, one can conclude that the electromagnetic therapy is an efficient and valid therapeutic proposal for the healing process of wounds, having applicability in clinical practice.

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