The Effect of Pulsed Magnetic Field on The Healing of Infected Cutaneous Wounds at Thigh Region in Rabbits

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Summary

Magnetic therapy was applied in the present study, which play two roles; as antimicrobial (*Escherichia coli, Staphylococcus auras, Streptococcus spp, Klebsialla* ,and *Pseudomona*) and as stimulator to tissue repair.24 rabbits were used in this study, they divided into two groups; 1st group(treated group) contains 20 rabbits, 2nd group (control group) contains 4 rabbits, the last subdivided into two subgroup (standard control subgroup and sham-control subgroup, the left side of all animals in control group is standard control subgroup, while the left side is sham-control subgroup). After surgery to all, the wounds were exposed to pulsed magnetic field except standard control subgroup, they healed with conventional treatment, 1st group(treated group) and sham-control subgroup were treated with(600 Gauss,50 Hz) twice daily for 30 minutes during 7 days.

Microbiologically, the bacterial Petri dish were exposed to pulsed magnetic field in present study (600 Gauss, 50Hz) 30 minutes/day for one only, after re-culture of these bacteria at new media, there weren't bacteria growth appeared which were used in present study.

Clinically, the clinical signs of wounds were recorded at 1, 3, 5,7, and 9 days post induce wound ; the sham-control subgroup was healed after 5.5 days after induce wound, 1st treated group was healed after 7-10 day post induce wound, while standard control subgroup was healed after 9 day post induce wound. Biopsies were toke after 3,5,7,and 9 days post induce wound, the histopathological study of sham-control subgroup revealed that show more develop compare with other.

Key word: pulse magnetic field, wound healing, infected wound

تأثير المجال المغناطيسي المتناوب على التئام الجروح الجلدية المخمجة في منطقة الفخذ بالأرانب

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الخلاصة

استخدم المجال المغناطيسي في هذه الدراسة, الذي لعب دورين رئيسيين أولهما كمضاد جرثومي(*Escherichia coli, Staphylococcus auras, Streptococcus spp, (Klebsialla , Pseudomonas*), والدور الأخر كمحفز لنمو والتأم الجروح .

في هذه الدراسة, استخدم 24 أرنب, قسمت الأرانب إلى مجموعتين: مجموعة المعالجة وتتضمن عشرين أرانب ومجموعة السيطرة وتتضمن 4 أرانب والأخيرة قسمت إلى مجموعتين فرعيتين هما مجموعة السيطرة القياسية ومجموعة السيطرة الصورية(الجانب الأيسر من حيوانات مجموعة السيطرة هو مجموعة السيطرة القياسية بينما الجانب الأيمن هو مجموعة السيطرة الصورية).

اخضع الجميع إلى العمليات الجراحية في منطقة الفخذ ,وعرضت الجروح إلى المجال المغناطيسي المتذبذب عدا مجموعة السيطرة القياسية التي شفيت بصورة طبيعية, عرضت المجموعة الأولى (مجموعة المعالجة) ومجموعة السيطرة الصورية إلى المجال المغناطيسي بجرعة (600 گاوس ,50 هرتز) مرتين في اليوم /30 دقيقة ولفترة 7 أيام.

مجهريا بايولوجيا, عرضت الأطباق الحاوية على الجراثيم إلى المجال المغناطيسي المستخدم في التجربة (600 گاوس ,50 هرتز) لمدة 30 دقيقة/يوم لمرة واحدة , وبعد اخذ العينات وزرعها بأطباق جديدة لم يظهر أي نمو للجراثيم المستخدمة في التجربة.

سريريا,سجلت العلامات السريرية بعد 1, 3, 5, 7 و 9 أيام من استحداث الجرح , شفيت مجموعة السيطرة الصورية بعد 5,5 يوم من استحداث الجرح , وشفيت مجموعة المعالجة بعد 7-10 يوم من استحداث الجرح ,بينما مجموعة السيطرة القياسية استمرت إلى أكثر من 9 يوم بعد العملية.

أخذت الخزعة النسجية المرضية بعد 3, 5, 7 و9 يوم من استحداث الجروح, أظهرت الدراسة النسجية المرضية لمجموعة السيطرة الصورية أكثر تطورا من الأخر.

Introduction

Magnetic therapy, is a modern technique in wound healing and surgical or medical application. Magnetic therapy means application the magnetic field on defect vital organ of body as skin wound, fractured bone, injured nerve …ect, by vary level of doses of pulsed magnetic field(pmf). The magnetic field measured by unit called Gauss(G) or Tesla(T) ($T=10^4$ G) [1]

The effect of magnetic field on the body organs depend on three factors; type of magnetic field, type of organ, and type of animals as well as age and body condition [17]. There are two types of magnetic field; 1st static magnetic field, and 2nd pulsed magnetic field, each one have special medical uses [7]. Pulsed magnetic field have be therapeutically for all most 25 years ago. the development of magnetic field by German, they use power line frequency 50Hz-60Hz with 100G, these products have proved to be beneficial in wound healing [9]. Generally, the check point in the effect in the effect of pmf at the wound in three axis: the relationship between pmf and tissue repair, the relationship between pmf and increase neovascularizition and tubulazation, and the relationship between pmf and immune response mechanism [6]. Tepper et al. were applied pmf to endothelial cells culture, they showed demonstrated a

marked increase in proliferation of cells and tubulaztion, they also reported a substantial increase in expression of fibroblast growth factors-2 (FGF-2), a potent stimulation in angiogenesis[20]. There are two ways to the effecting of pmf on immune system , indirect by direct killed of bacteria and tissue debris, therefore cause to stimulate humeral immunity and directly effect on immune body organs, locally by direct effect on WBCs to enhancement to engulf the foreign bodies or systemically by effect on thymus, and lymph nodes [11].

The healing wound is an extremely complex and dynamic tissue which is come way could be regarded as an organ. Normal wound healing occurs in recognizable, usual progressive though overlapping [10]. The phases of wound healing: haemostatic phase, inflammatory phase, proliferation cellular phase, and remodeling phase [22].

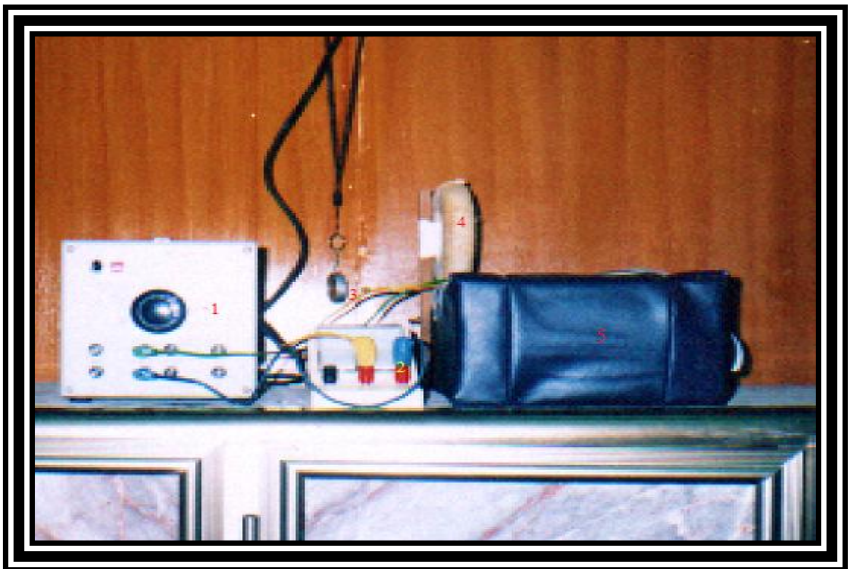
The quality of wound healing is depending on other factor such as bacteria, hot, chemicals …etc, therefore, there are three type of wound ; acute, sub acute, and chronic [8]. The type of bacteria may influence on wound healing. Many chronic wound are colonized with *staphylococcus aurous* [5].Bacteria are present in most wound, the numbers, virulence, and host defense are determined the stage of wound inflammation, therefore most bacteria cause odor, dehydration, local cellulites and death [18]. The patient immune system will significantly influence the effect the bacteria have on wound, there are some factors effect on immune defense such as stress, nutrition, circulation problems metabolic diseases, and immune suppressant drugs [22]. The aim of study uses physical therapy in surgery replace classical chemical medicine, specially in chronic wound healing resistance to antibiotic.

Materials and Methods

Animals, in present study were used 24 rabbits (regardless of genera), healthy, mature(up to 8 months), and live similar condition.

Bacteria, five genera of were used in this study which were obtain from microbiology department, veterinary medicine college, university of Basrah., they are (*Escherichia coli, Staphylococcus auras, Streptococcus spp, Klebsialla* ,and *Pseudomonas),*Surgical instruments, sterile surgical blade, silk suture, as well as local anesthesia (lindocian 2%) to induce skin wound.

Magnetic field apparatus which were used in this study: consist of 5 parts as fellow: power supply, magnetic coil, compass, ammeter, and rabbit bed see figure (1)



Figure(1) Magnetic apparatus(1-power supply2-ammter, 3-compass,4-magnetic coil,5-bed)

Firstly, prepare the animals to surgery, thigh region was cleaned from hair by hair remover cream and wash with antiseptic(povidin-iodine) solution, the area was covered by cotton saturated with 70% ethyl alcohol, the animal were sedated and local anesthesia were injected (20mg/kg b.w. xylazine+ 2% lidocian 1m/cc tissue). Surrounding the wound a longitudinal incision was made in lateral view of thigh superficial layer of skin equal 4 cm. these wounds were exposed to infection with bacteria in treated group, while control group was leaved without infection, the wound were sutured by simple continues suture. Magnetic therapy pmf was applied into treated group and sham-control group as experimental design table(2):

Table(1) Experimental design

total rabbit(24)						
control group(4)		treated group(20)				
stander control (*animals left side*)	sham-control (*animal right side*)	E.coli	Staph.	klebsialla	Strep. spp	Pseudomonas

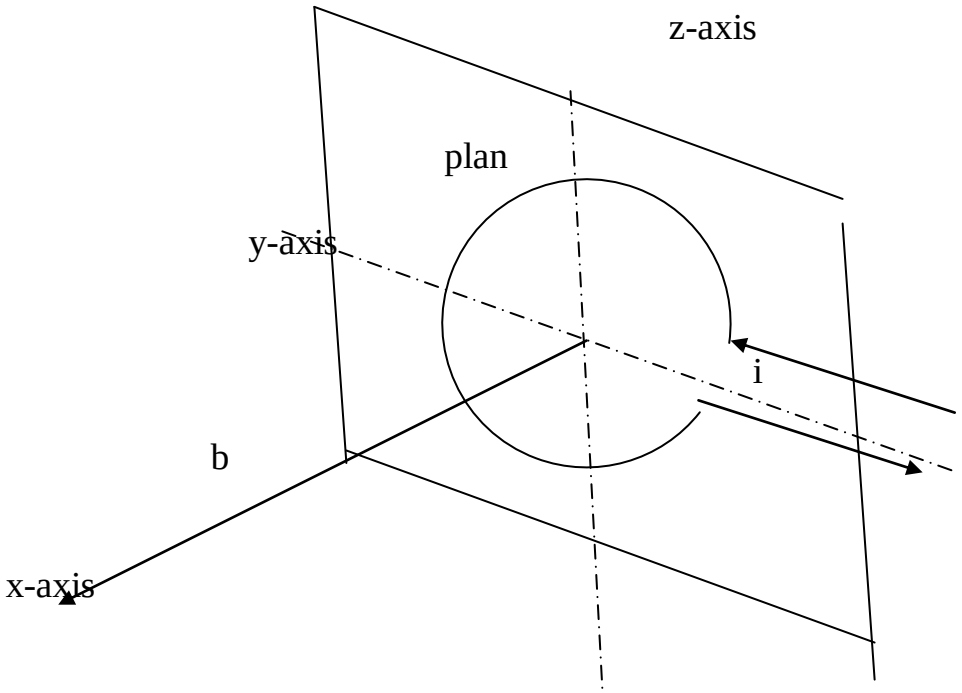
Treated group was exposed to bacteria by dose 10^{-5} according to Martz and Ovington equation to present the probability of wound infection[12]:

$$\text{Infection} = \frac{\text{Dose} * \text{virulence}}{\text{Host Defense}}$$

The wound infected directly by bacteria was leave to 24 hr before pulsed magnetic field application.The magnetic field therapy pmf was calculated by this equation :

$$\beta = \frac{\mu_o}{2} \cdot \frac{ina^2}{(a^2 + b^2)^{3/2}}$$

ß=magnetic dose(flux), μ=12.57*10^{-7} Weber/amp.m, i= current through turn, a=radius of turn, b=axial distance from perpendicular to turn plan figure(2) [24].



figure(2) i= current , x=axis, plan(x,y,z,) plan of magnetic coil, b= axial distance from perpendicular to turn plan

Dose equal of rabbits=600 G,50Hz, the position of magnetic field the angle 90° above the wound, treatment was continue to 7 days/ twice daily/30 minutes at course, as well as the PMF was exposed on bacteria in vetro (bacteria in Petri dish) with single dose of PMF(600 G, 50Hz)/30 minutes, as well as the PMF was applied on bacteria in vitro (culture in Petri dish) with single dose of PMF (600 G,50 Hz), after than bacteria cultured and incubated at 37 C° for 24 hr, as a pulsed magnetic field antimicrobial sensitivity test.

Clinical signs were recorded at 1,3,5,7,and 9 days post induce wound.

Samples from surgical site were taken after 3,5,7,and 9 days post induce wound and made histopathology slide were prepared with routine manner and stained by haematoxylin and eosin.

Results and Discussion

Table (2) Clinical signs of the wound to all group were summarized

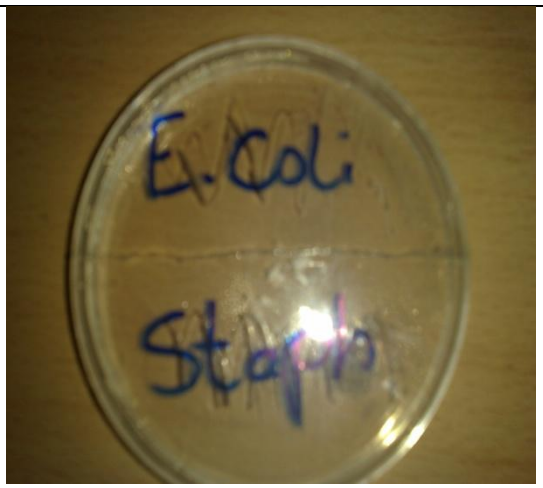
Total rabbits						
Treated group					Control group	
E.coli	Staph.	Strep. spp	Klebsialla spp	Pseudomonas	Stander control	Sham-control*
+	+	++	-	+	-	-
+++	++	++++	+	+	+	+

-no inflammation,+ simple inflammation, ++ moderate inflammation, +++ acute inflammation without exudates, ++++ acute inflammation with exudates, * don't exposed to infection.

Pulsed magnetic field antimicrobial sensitivity test (in verto) summarized by this table(3) and figure (3)

Table (3) PMF antimicrobial sensitivity test

E.coli	Staph.	Strep. spp	Klebsialla spp	Pseudomonas
-	-	-	-	-

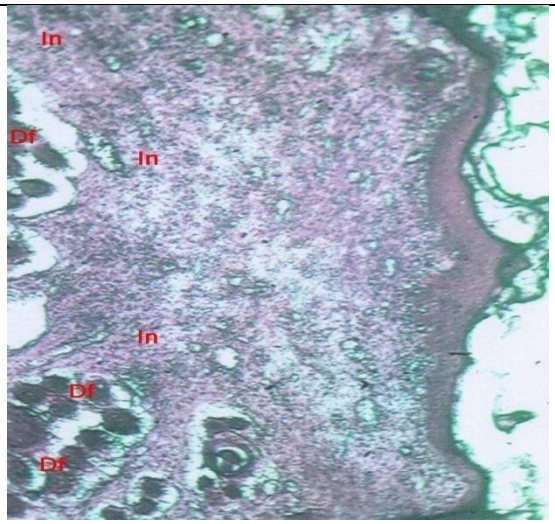


E.coli and *Staph* show inhibition in colonies growth

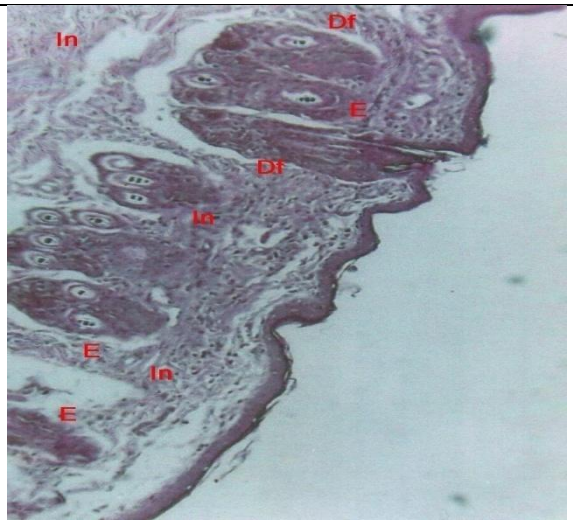
Pseudomonas spp and *Klebsialla* spp show inhibition in colonies growth

Figure (3) anti microbial dish after PMF exposure

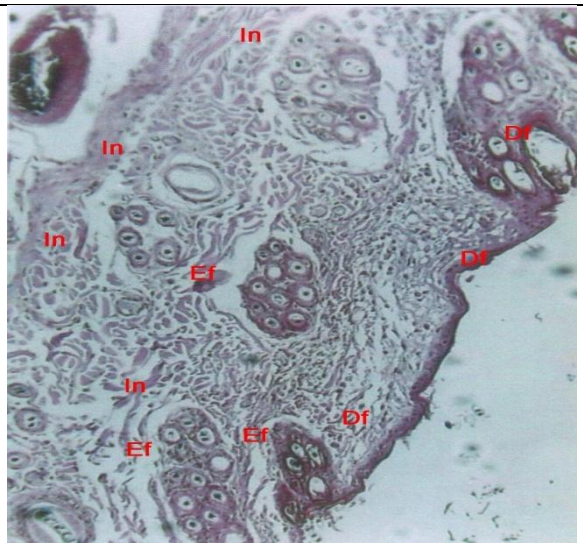
Standard control group was lifted to healing conventionally while other two group treated with pmf, clinical signs were recorded of groups in this table (4)



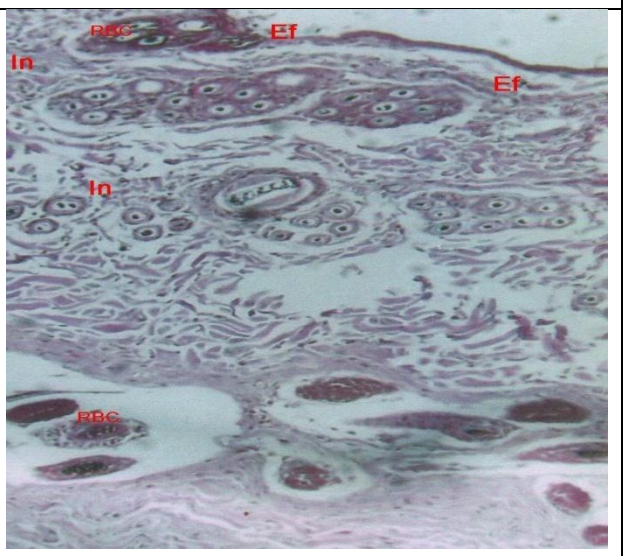
Treated group(*Strep*. Spp.)
Histopathology section of skin show inflammatory cells **In**, dermal fibrosis **Df**.



Treated group (*E. coli*)
Histopathology section of skin show inflammatory cells **In**, dermal fibrosis **Df** and erosion formation **E**.

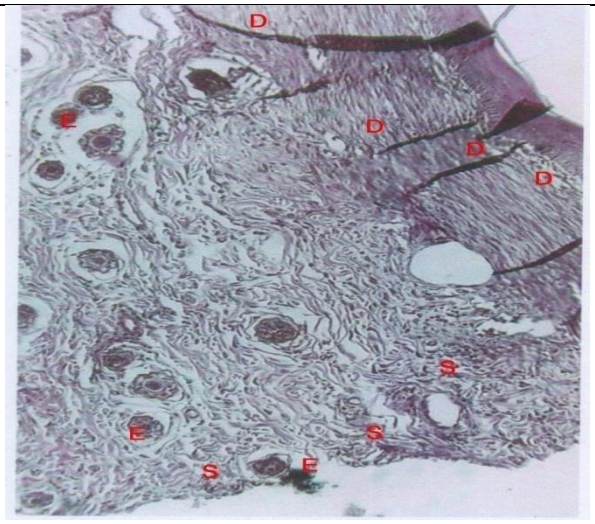


Standard control subgroup
Histopathology section of skin show prominence inflammatory cells **In** , epidermis thickening **Ef** and epidermal fibrosis **Df** .

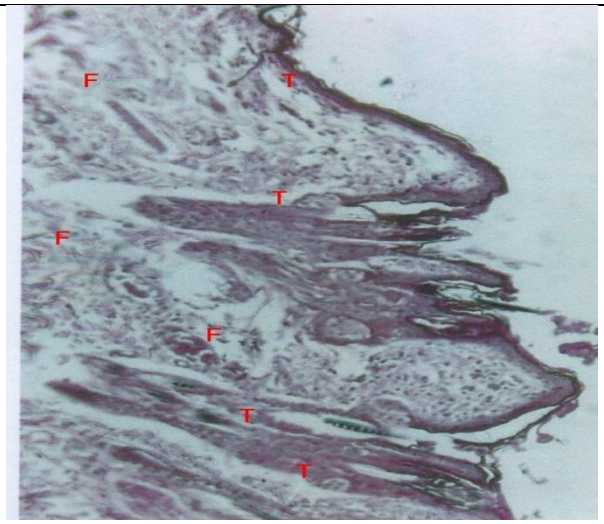


Sham-control subgroup
Histopathology section of skin show inflammatory cells **In** , **RBC**s ,fibrosis **Df** and erosion formation **E**.

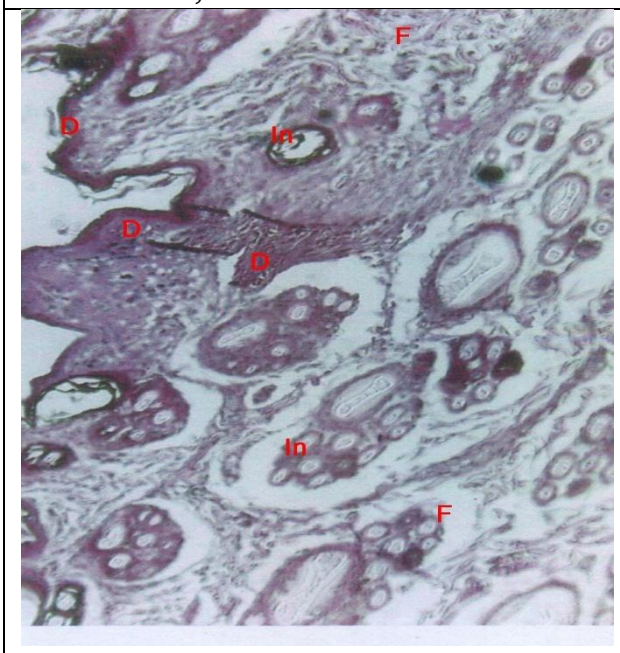
Figure (4) Histopathology section of animals group after (3) days of surgery[25]



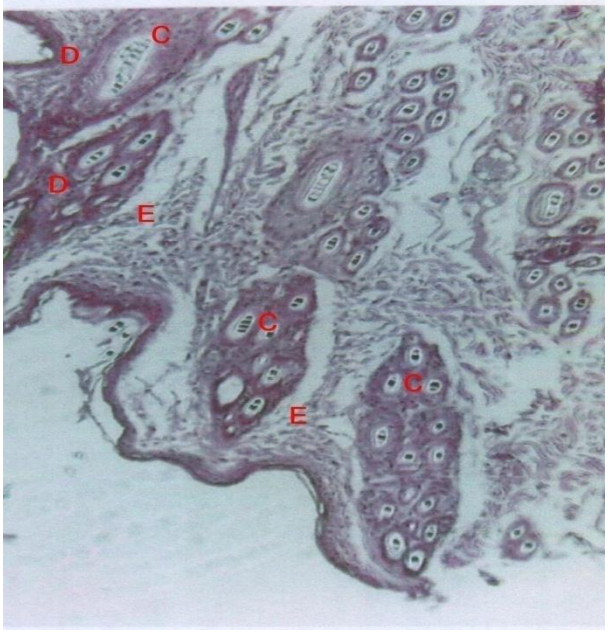
Treated group(*Strep*. Spp.)
Histopathology section of skin show dermal fibrosis **Df**, scar tissue formation **S**, erosion **E**.



Treated group (*E. coli*)
Histopathology section of skin show fibrosis **Df**, epidermis thickening and scar tissue formation **S**.

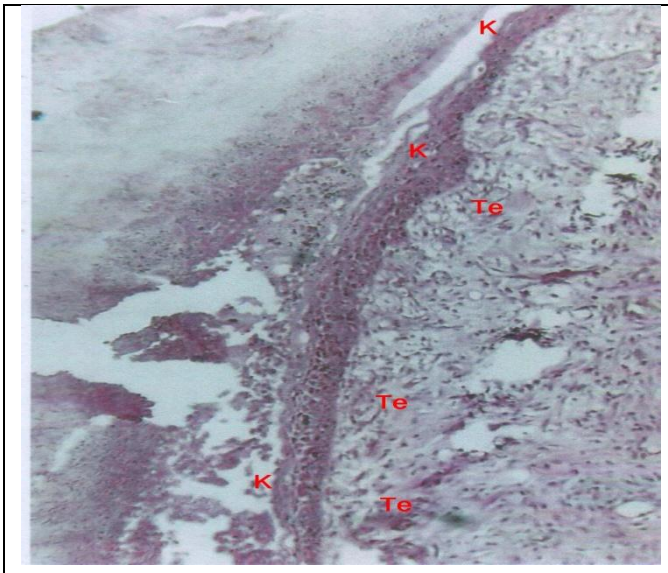


Standard control subgroup
Histopathology section of skin show inflammatory cells **In**, few dermal fibrosis **F,** epidermis thickening **D** .

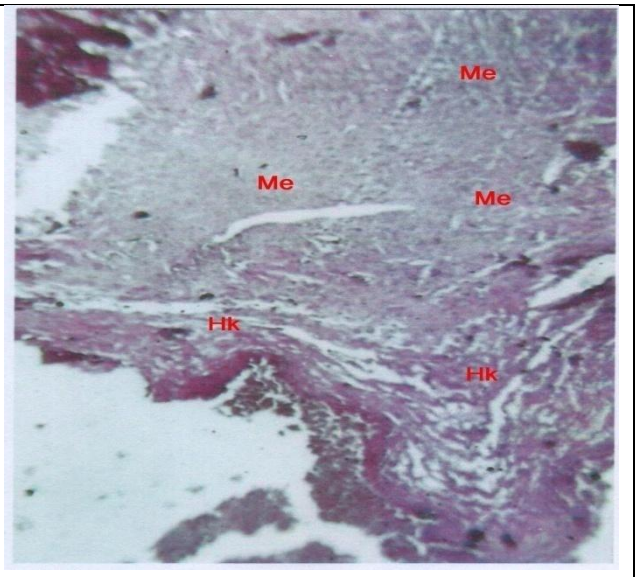


Sham-control subgroup
Histopathology section of skin show dermal fibrosis **Df**, epidermal thickening, collagen infiltration **C**.

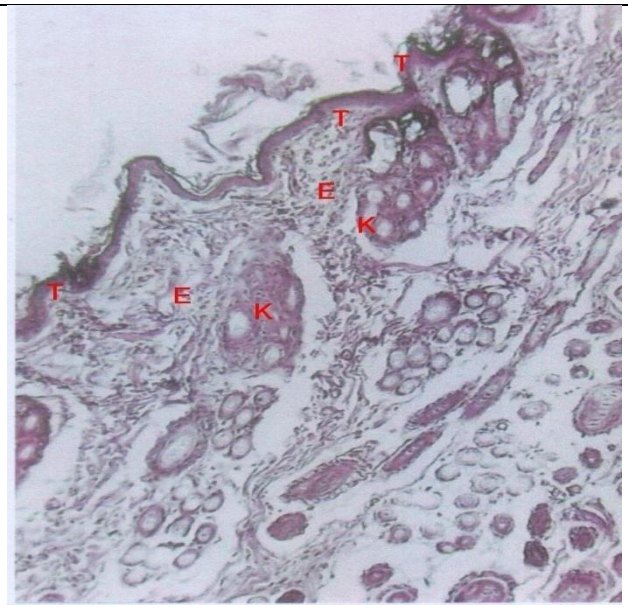
Figure (5) Histopathology section of animals group after (5) days of surgery[25]



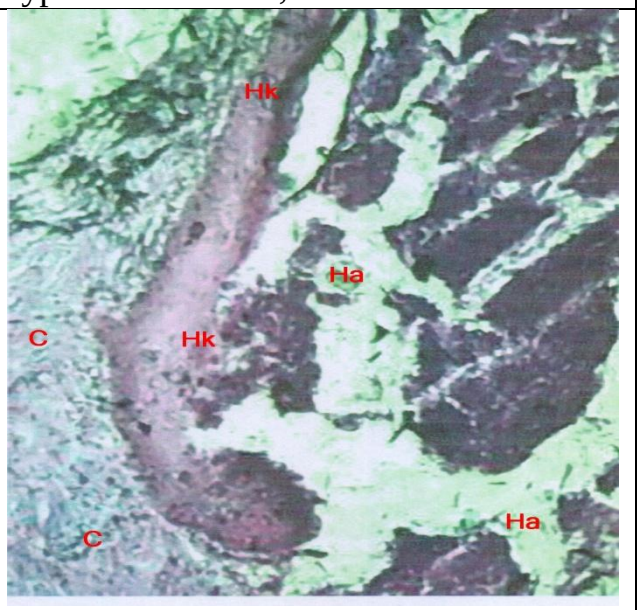
Treated group(*Strep*. Spp.)
Histopathology section of skin show keratosis **K**, and thickening epidermis **Te**.



Treated group (*E. coli*)
Histopathology section of skin show hyperkeratosis **Hk**, microerosion **Me**.

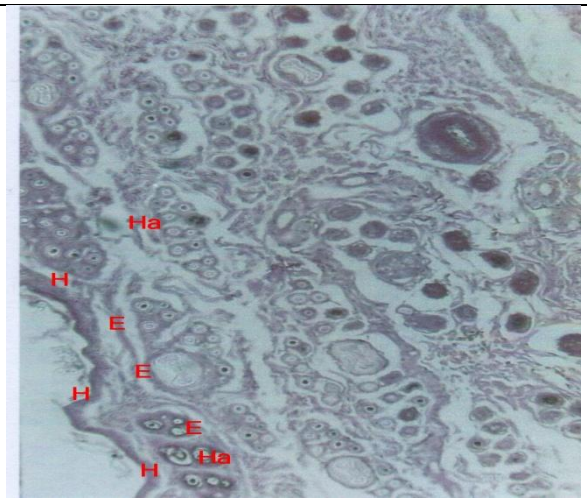


Standard control subgroup
Histopathology section of skin show thickening epidermis **T**, and keratosis **K** in adjacent epidermis **E**.

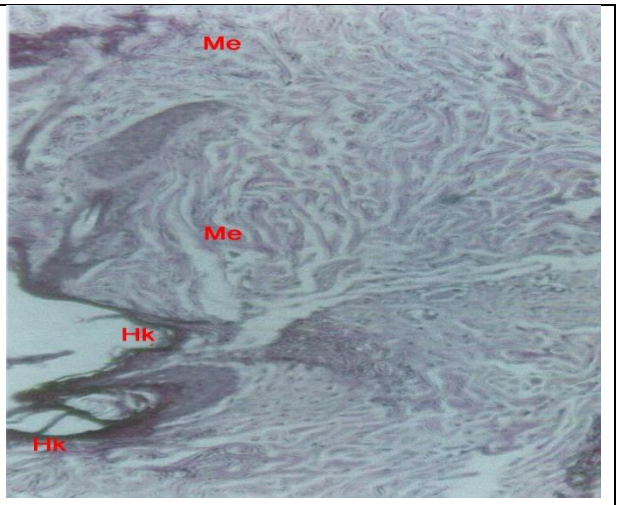


Sham-control subgroup
Histopathology section of skin show collagen infiltration **C** in epidermis, number of hair follicle **Ha**, hyperkeratosis **Hk**

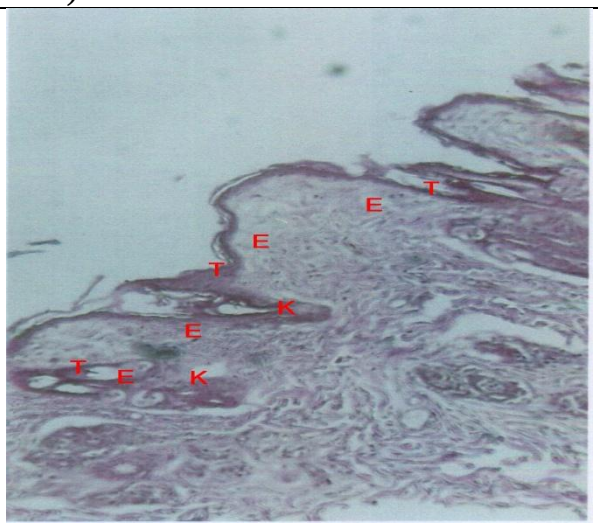
Figure (6) Histopathology section of animals group after (7) days of surgery[25]



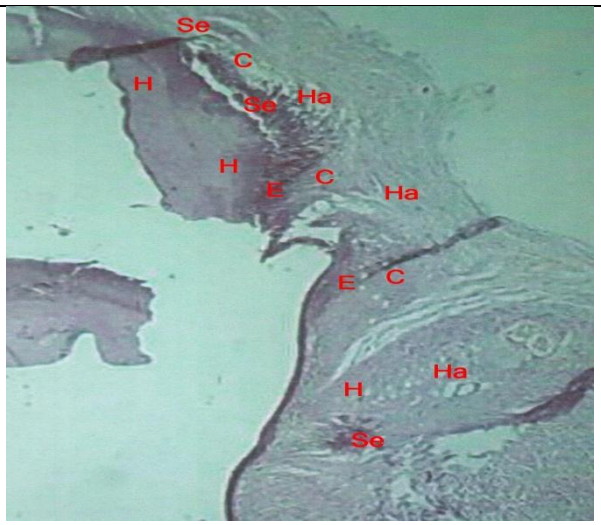
Treated group(*Strep*. Spp.) Histopathology section of skin show hyperkeratosis **H**, Hair follicular **Ha** and thick epidermis **E**(E &H stain 40X)



Treated group (*E. coli*) Histopathology section of skin show hyperkeratosis **Hk** and microerosin **Me** (E &H stain 40X)



Standard control subgroup Histopathology section of skin show thick epidermis **T** and keratosis **K** in adjacent epidermis **E** (E &H stain 40X)



Sham-control subgroup Histopathology section of skin show hyperkeratosis **H**, collagen infiltration **C** in epidermis **E**, number of hair follicles **Ha** and prominence sebaceous gland **Se**(E &H stain 40X)

Figure (7) Histopathology section of animals group after (9) days of surgery[25]

The severity of wound infection is depending on type of bacteria, pathogencity, and host defense, as well as environmental condition, therefore in this present study was show *Streptococcus* spp. was caused sever inflammation while *Klebsialla* spp. Was caused simple inflammation, this results were agree with Scanlon,2005; and Crawford,2008 (in case *Streptococcus* spp.)[19,4], whereas Meakins and Masterson,2005 disagree with this results (in case *Klebsialla* spp.)

they show sever inflammation[13]. This phenomena (severity of inflammation) was regarded to participant check point among (bacteria-host defense mechanism-surgical site). Other bacteria and its severity of inflammation were agree with most researchers.

The effect of pmf on bacteria via two ways, 1st way direct effect of pmf cause death of bacteria, and liberate glycopolysacchrite (wall of bacteria) which enhance macrophages to engulfing as well as which stimulate immune response in body, Murabayashi et al.2004 were show the influence of pmf on T-lymphocytes behaviour and its cytotoxicity[14]. Also Yamaguchi et al.,2005 were examined the effect of pmf on immune function by immunological assay, they show TNF-α and IL-2 production in the spleen are measured after exposure to pmf significantly increase[23]. While Capri et al.,2004 was indicated to neither DNA synthesis nor the capacity of lymphocytes to enter the activation phase and progress into cell cycle don't affect at level (50 Hz, 250G)[3].

Grace,2000 was noted the effect of pmf production of hydroxide that create an alkaline PH and extra-cellular fluid capable of absorbing for more oxygen then acid PH fluid, the potential difference between external and internal cellular fluid allows the nutrition, and ions(Ca^{++},Na^{+},K^{+}) channel open more readily and oxygen uptake and utilization is improve[7]. By same reason Tofani et al.,2001 was showed the effect mainly refer to alteration of cell proliferation rate, changes in the levels of mRNA and protein synthesis[21]. For these reasons the present study show short period in wound healing in treated group and sham-control. As well as this study agree with Athanasiou et al.,2007; Ottani et al.,1988; Patino et al.,1996 they used low frequency magnetic field, they show significant in ratio of wound contraction[2,15,16].

All skin layers (epiderm, derm, subcutaneous, and cutaneous) were affected by PMF, but mostly fibroblasts and inflammatory cells which were fasted resonance to PMF. The activity of fibroblasts clearly show after 5 days of skin incision, after than scar tissue formation in final stage, histopathological epithelial thickening was showed companied with increased inflammatory cells at area, these regarded to acquired energy from PMF either to skin layer or to regional blood flow , these results agree with Henry et al.2003.

While inflammatory cells were early resonance to PMF, due to PMF enhanced local and general immunity in body[23], as well as PMF has ability to killed bacteria which stimulate immune system in area[26]

The difference among groups in present study were depending on previous factors, therefore we conclude there are highly effect on pulsed magnetic field (pmf) on increase wound healing with short period and safety technique accelerator.

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