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Research Paper

Studying the effect of Magnetic Field and Moringa leaves Extract on the healing of wound in rats

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ABSTRACT : The present study has revealed the effect of electromagnetic field and moringa oleifera leaves extract on wound healing in rats in order to improve the healing process of an experimental excised wound on back of rats. Thirty five healthy Spraque-Dawley female rats weighting (150-200 g) were divided equally into seven groups, an experimental excised wound on back of rats (1.5cm X 1.5cm) were carried out. Control group G1, groups G2,G3andG4 exposed to extremely low frequency magnetic field of 50HZ and magnetic flux density 2mT for 1hr,1.5hr and 2hr respectively . groups G5,G6 andG7 treated with leaf extract moringa with concentration of 12.5%,25% and50% respectively. Wound area were measured daily on day 0,3,7,10,14,17 and 21 and photographic for wound were taken, Blood samples were collected at day 21 for all groups for measuring the changes in some hematological parameters . showed that group G2 exposed and treated with electromagnetic field for 1hr had faster rates of wound healing with shorter days of contraction where the number of RBCs ,HB and WBCs increased at $p \le 0.05$ since observed over control and after that the treated group with moringa leaves extract G5 of concentration of 12.5%. magnetic field of 1hr (G2) accelerate the wound healing better than all groups and moringa leaves extract of concentration of 12.5% accelerate wound healing also relative to the other concentration .

KEYWORDS: Rats ; Wound Healing ;Electromagnetic field ;Moringa oleifera extract ;wound area ;Blood.

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1. INTRODUCTION

Extremely low frequency magnetic field of 50HZ and magnetic flux density 2mT have been used in medicine to enhance drug delivery and increase bone regeneration [1]. Becker pioneered promising research along these lines in the 1960s. He discovered an electromagnetic skin circuit while studying amphibians, as well as changes that occurred during limb regeneration [2]. Shoman, H.M et al(2020) indicates that the exposure to EMF may cause changes on physical properties of hemoglobin such as viscosity and conductivity and red blood cells morphological structure[3]. Also Aida ,salama et al (2020) showed that exposure of animal to electromagnetic field change the the absorption of spectrum of hemoglobin[4]. Neacsu IV et al (2005)indicated that 100HZ MF for 10 min/day for 5, 10 and 20days shows A significant increase in RBCs count, Hb content and The total leucocytes number increase at 10 days of treatment[5]. Salem A, et al (2005)was showed that expose to SMF 128mT 1h/day for 30 days

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found significant increases in RBCs count and Hb content and A significant increase in WBCs count[6]. Hashem MA and El-Sharkawy NI (2009) investigated that 50 Hz 2mT 4h /day for 30 days asignificant increases in RBCs count, Hb content, and Hct value and A significan increased in the phagocytosis % and phagocytic index[7]. Purushothaman G et al(2013)investigated that EMF 202 μ T A significant increase in RBCs, and Hb and A significant increase in WBCs count[8]. Al-Uboody WSH,(2015)indicated that 1200 MHz 6 h/day for 45 days a significant increase in RBcs count and a significant increase in WBCs count, lymphocytes, monocytes and acidophils[9].

The use of medicinal plants as an alternative to chemically synthesized pharmaceuticals in the treatment of ailments is now widely acknowledged [10]. Any plant that has been identified and proven to be antioxidant, antibacterial, and antiproliferative can be employed as an ointment delivery mechanism in wound treatment [11]. Moringa oleifera is a plant of the Moringaceae family. It is native to India but is spread all over the world. It is also known as the drumstick tree or horseradish tree. M. oleifera is distinguished by its nutritional and medicinal properties. Quercetin, kaempferol, vitamins, carotenoids, B-sitosterol, caffeoylquinic acid, zeatin, tannin, flavonoids, alkaloids, polyphenol, phenolic acid, oxalates, isothiocyanates saponins, glycosylates, and phytates are all found in Moringa [12]. Ariegoro et al. (2019) studied the effects of Moringa oleifera on some haematological parameters of rats[13]. They concluded from their results on Red blood cell, Packed Cell Volume, haemoglobin and lymphocyte of the animals that Moringa oleifera possesses nutritional benefits. Mun'im A ,et al (2016) , Bhattacharya A et al(2014) and Kashyap P, Kumar S (2022) showed an increased level of hemoglobin, red blood cell count, hematocrit, and total iron content in Sprague Dawley female rat blood after 6 days received Moringa leaves extract[14][15][16]. Onyekwelu K (2018) indicated that Moringa leaves extract can increase the level of hemoglobin, hematocrit, red blood cell, and total white blood cell count on days 8 and 15 of Wistar rats at normal conditions [17]. Hisham M. Osman et al (2012) indicated the positive effect of moringa extract on some blood parameters and body weights of the experimental animals [18]. M. oleifera showed an antimicrobial effect and significantly improved wound contraction, reduced epithelization period, increased antioxidant enzymes activity, and reduced capillary density [19]. This plant has been recommended by the World Health Organization (WHO) and the United Nations (UN) as an alternative to dietary supplementation in meeting nutritional requirements[20].

The skin is the biggest organ in the body and serves as a barrier against external forces. Cutaneous wound healing is a complex procedure involving the interaction between different cells in the injured tissue, including inflammatory cells, fibroblasts, keratinocytes, and endothelial cells [21]. Wound healing is a natural process in which the body replaces normal structure functions and heals tissue damage. Hemostasis, inflammatory, proliferative, and remodelling stages are all part of the skin wound healing .

The aim of this study was role out the effect of electromagnetic field and moringa oleifera leaves extract on wound healing in rats in order to improve the healing process of an experimental excised wound on the back of rats.

2. MATERIALS AND METHODS.

Thirty five healthy Sprague-Dawley female rats weight(150-200 g) were divided equally into seven sub groups control group G1, G2,G3and G4 exposed to extremely low frequency magnetic field of 50HZ and magnetic flux density 2mT for 1hr,1.5hr and 2hr respectively . groups G5,G6 and G7 treated with leaf extract moringa with concentration of 12.5%,25%and 50% respectively , moringa extraction used topically for groups G5,G6and G7.

Animal Preparation and wound measuring :

The animals were housed individually in plastic cages. Animals were fed with standard pellet diet and water *ad libitum*. The rats were used after acclimatization to the laboratory environment for a 7 day period.

For the experiments, the rats were anaesthetized with ketamine hydrochloride (50 mg/kg) and xylazine (5mg/kg). Excision wound was inflicted on the back of rats as described by Murthy et al (2013)[22] where about (1.5cm*1.5cm) full thickness of skin was cut away from pre-determined area for all groups rats, simple ointment base was administered to the control group. Magnetic field and moringa extraction were administered topically on the wound sites till the wounds were maximum healed for 21days.

Sampled rats in each group were euthanized while healed wound sites were measured .Wound contraction was measured daily with a caliper (Vernier) on days 3, 5, 7, 10, 14, 17 and 21 representing the inflammatory, proliferative and maturation phases of wound healing respectively. Blood samples were collected on the last day(day21) for analysis.

Electromagnetic field exposure device:

A magnetic field always produce when there is an electric current flowing .a static of magnetic field is formed in the case of direct current ,and a time varying magnetic field is exists from alternating current .

A magnetic field is describing by fundamental vector quantities magnetic field strength H(unit: A/m) and magnetic flux density B(unit Tesla).

These quantities are relate to each other by the equation :

В=μ Н

Where μ is the magnetic permeability of the medium.

H is the force with the field acts on an element of current

A homogenous magnetic field generator was designed and constructed in Cairo University in the electronic center which animals can be housed and exposed to the magnetic field(fig 1). A cylindrical solenoid consisted of coil placed on wooden rack .the coil is 320 turns from electrically insulated 2mm thick cupper wire was wounded in a homogenous way around a cupper cylinder of 2mm thick 40cm diameter and 40cm length. The cylinder wall is earthed to eliminate the effects of electric field .The magnetic field generator should be temperature controlled by using air ventilation. The ends of the coil are connected to variac fed from the mains (220V and 50HZ) through stabilizer. The magnetic field strength inside magnetic chamber(where animals is housed) was adjusted by changing the voltage across the coil by the variac to produce magnetic field of flux density 2mT in the area where the animals housed [23].

During the exposure , the rats lived under normal condition of food ,drinking ,lighting and temperature. Control rats(G1)kept away from MF and lived in the same condition of ventilation and food .



Fig(1)the image shows the animal which were exposed continuously as a group in plastic cage on the shelf within the solenoid.

Preparation of moringa extraction:

Plant collection, preparation and phytochemical screening:

Fresh leaves of M. oleifera were collected. The leaves were oven-dried at 40 $^{\circ}$ c to constant weight and pulverized with an electrical grinder in pest physiology department ,plant protection institute (sharqia branch). The powdered plant part was screened for secondary metabolites using standard procedures [24][25]. Plant extraction Successive solvent extraction was carried out with the soxhlet apparatus using solvents of increasing polarity n-hexane as shows in fig (2 and 3). The extract were concentrated under reduced pressure using a rotary evaporator. The dried extracts were stored at 4 $^{\circ}$ C in a refrigerator.





Fig2: shows the moringa leaves ,fig 3:shows soxhlet apparatus.

Extracts and standards use:

Three types of ointment formulations were prepared with n-hexane leaf extract with concentration12.5% (w/w) ,25% (w/w) and 50% (w/w) . The 50% (w/w) ointment where 0.5 g of extract was incorporated into 0.5 g of simple ointment base B.P.the25% (w/w) ointment where0.25 g of extract was incorporated into 0.75g of simple ointment base. The12.5% (w/w) ointment where0.125 g of extract was incorporated into 0.875g of simple ointment base. The control group used Simple ointment base B.P on it is surface.

Collection of blood samples:

The rats were anaesthetized with isoflurane and blood samples were taken from the animals by draining the blood from eyes into tubes containing heparin at day 21, using capillary tubes .the tubes sealed and gently checked

ready for performing measurement and analyzed using Automated hematology analyzer (Sysmex XP-300). These blood samples were immediately used for the analysis of hemoglobin (Hb), erythrocytes (RBC) and leukocytes (WBCs) count.

3. RESULTS AND DISCUSSION

The wound of all animals were detected in 0.3,7,10,14,17and 21 days respectively . wounds of all groups exhibited gradual and consistent contraction .At day 7 Granulation tissue was detected in all animals starting . On the last day of the study, the process of contraction and epithelialization was maximum in treatment group G2 (1hr magnetic field) and in group 5 (12.5 concentration Moringa extract). after surgery The total wound area decrease at day3 in rats belonging to both the control group and the treatment group. Granulation tissue was detected in all animals starting at postoperative day 7 fig (5 and 11).

Fig(4) showed Photographs of the wound and table(1) showed the wound healing area of three groups treated with magnetic field (groups 2, 3 and 4). Animals on group two (G2) showed increase in percentage wound and decrease in epithelization period as compared to control group (G1). Also showed the best result with of wound closure of all groups on day 21(table2).Fig(10) showed Photographs of the wound and table(4) showed the wound healing area of three groups treated with moringa (groups 5, 6and 7). Animals on group two (G5) showed increase in percentage wound and decrease in epithelization period as compared to control group (G1) also showed the best result with of wound closure of moringa groups on day 21(table5).

(measuring wound area and blood parameter for ELF, MF groups)

A) measure wound area for magnetic field groups:



We measure the length and width of all groups at days 0,3,7,10,14and21 day . Fig(4) Digital photos taken on days 0, 3, 7,10, 14, and 21 after wounding that were created on healthy control animals and wounds treated with magnetic field mf1hr , mf 1.5hr and 2hr . Table(1)wound area for magnetic field groups (MF1hr,MF1.5hr and MF2hr)and the control on day0,3,7,10,14,17and 21.

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Days		Wound area(cm2)		
	Control	MF1hr	MF1.5hr	MF2hr
0	2.25±0.0001	2.25±0.0002	2.25±0.0001	2.25±0.0001
3	2.25±0.0001	2.25±0.0001	2.25±0.0001	2.25±0.0001
7	2.1±0.150000	2.025±0.106066	1.81±0.014142	2.035±0.304056
10	1.166±0.548483	1.1±0.141421	1.7±0.388909	1.625±0.68252
14	0.68±0.27784	0.6±0.565685	1±0.0006	1.2±0.68252
17	0.433±0.208167	0.26±0.084853	0.9±0.070711	0.952±0.5121
21	0.233±0.11547	0.07±0.042426	0.5±0.070711	0.678±0.306066



figure(5)wound area of MF1hr , MF1.5hr,MF2hrand control on post-wounding day 0, 3, 7, 10, 14 ,17and 21. Data are expressed as mean ± SD.

Table(2) area contration of wound for magnetic field groups compared to controlat day 21.

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Groups	area contraction (cm ²) at day21
Control(C1)	0.222+0.11547
	0.23310.11347
Magnetic field 1hr(G2)	0.07±0.042426
Magnetic field 1 5hr(G3)	0 5+0 070711
Magnetie neia 1.5m (65)	0.520.070711
Magnetic field 2hr/G/	0.678+0.806066
	0.070±0.000000

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Figure (6)Histogram illustrates the contraction area measurements ±SD for control and magnetic field groups at day21.

For higher period of exposure the area of wound was higher than control area for 1.5hr or 2hr where long exposure to magnetic field can destroy the RBC membrane so healing is poor in this situation[9][26].

B)Blood sample for magnetic field groups:

Table(3) ,figure(7,8,9) shows the changes in blood parameter (RBCs , WBCs count and HB content) after exposure to 2mT,50HZ electromagnetic field for one hours ,one and half hours and two hours .there was a statistically significant in total erythrocyte and leukocyte in G2(exposure of mf for 1hr) \leq 0.05.the changes in other groups are non-significant .

Table (3) also shows the effects of EMF after 1hr,1.5hr and 2hr of exposure ,it clearly shows that prolongation of exposure for 1.5hr and 2hr does not make any appreciable changes and all blood parameters were turn to be statistically insignificant .so the wound healing is not appear on G3,G4.

The changes of RBCs, WBCs count and HB content in control and magnetic field groups are appear in figure(7),(8),(9) respectively.

Table (3) blood parameters(RBCs , WBCs count and HB content) of control group and treatment group with magnetic field at day21.

At day 21	RBCS(10 ⁶ μl)	HB(g/dl)	WBCS(10 ³ µl)
Control(G1)	6.71±0.57376	12.53±0.46188	22.4±8.229216
Mf 1hr(G2)	7.05±0.113137	14.1±1.131371	27.1±2.545584
Mf1.5hr(G3)	6.905±0.586899	13.2±1.414214	24.4±4.101219
Mf2hr(G4)	6.82±0.084853	13.45±1.06066	23.7±2.262742



Figure (7)Histogram illustrates changes in RBCs count ±SD for control and magnetic field groups at day21.



Figure (8)Histogram illustrates changes in HB count ±SD for control and magnetic field groups at day21.

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Figure (9)Histogram illustrates changes in WBCs count ±SD for control and magnetic field groups at day21.

(measuring wound area and blood parameter for moringa groups)

A) measure wound area for moringa groups:

Fig (11) shows that area contraction in moringa leave extract of concentration 12.5% are smaller than other concentration 25%,50%.which is appear also from table (4,5).

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Fig(10) Digital photos taken on days 0, 3, 7,10, 14, and 21 after wounding that were created on healthy control animals and moringa groups(12.5%,25%,50%).

Days	Wound area(cm2)			
	Control	MO12.5%	Mo25%	Mo50%
0	2.25±0.00	2.25±0.00	2.25±0.00	2.25±0.000
3	2.25±0.000	2.25±0.0001	2.25±0.0002	2.25±0.0001
7	2.1±0.150000	1.383±0.500833	1.95±0.396863	2.25±0.086603
10	1.166±0.548483	0.633±0.340343	1.683±0.596518	1.9±0.086603
14	0.68±0.277849	0.49±0.220681	0.66±0.431084	1.23±0.152753
17	0.433±0.208167	0.27±0.251197	0.533±0.087178	1.033±0.152753
21	0.233±0.11547	0.20±0.248462	0.425±0.106066	0.69±0.327872

Table(4)wound area for moringa groups (12.5%,25% and 50%) and the control on day0,3,7,10,14,17 and 21.



figure(11)wound area o of moringa 50%,moringa 25%,moringa 12.5% and control on post-wounding day 0, 3, 7, 10, 14, 17 and 21. Data are expressed as mean ± SD.

Table(5) area contration of wound for moringa groups compared to control atday 21.

Groups	Area contraction(cm ²) at day21
Control(G1)	0.233±0.11547
Moringa 12.5%(G5)	0.2±0.248462
Moringa 25%(G6)	0.425±0.106066
Moringa 50%(G7)	0.69±0.327872



Figure (12)Histogram illustrates the contraction area measurements ±SD for control and moringa group at day21.

B)Blood sample for moringa groups:

Table(6) ,figure(13,14,15) shows the changes in blood parameter (RBCs count , ,WBCs count)and HB content after treatment rats by using moringa leaves extraction of concentration 12.5%,25% and 50% .the data shows that moringa extraction are increase the RBCs , WBCs count and HB content .the increase in blood parameter are more clear on concentration of 12.5%. there was a statistically non-significant in total erythrocyte and leukocyte in all moringa groups .

Table (6) are also show high concentration of moringa extract (25%,50%) not make the appreciable changes in blood parameter so the goal of wound healing is not achieve in this concentrations.

Figure (13,14,15) shows the changes in blood parameters(RBCs, WBCs count and HB content)in moringa extract groups and groups respectively.

At day 21	RBCS(10 ⁶ μl)	HB(g/dl)	WBCS(10 ³ μl)
Control(G1)	6.71±0.57376	12.53±0.46188	22.4±8.229216
MO12.5%(G5)	6.97±0.306105	13.3±0.793725	29.67±6.671082
MO25%(G6)	6.76±1.046247	12.95±1.626346	26.73± 5.90827
MO50%(G7)	6.7166±0.257164	12.93±0.635085	23.9± 4.03687

Table (6) blood parameters(RBCs ,HB,WBCs) of control group and treatment group with moringa at day21 .

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Figure (13)Histogram illustrates changes in RBCs count ±SD for control and moringa groups at day21.



Figure (14)Histogram illustrates changes in Hb count ±SD for control and moringa groups at day21.



Figure (15)Histogram illustrates changes in WBCs count ±SD for control and moringa groups at day21.

The present work was aimed to study the biological effect of magnetic field and Moringa leaves extraction on wound healing area .In this study also we defined the changes in some blood parameters after exposing rats to 2mT magnetic field 50HZ and also after topical treating with moringa leaves extraction on the excised wound .The use of magnetic field of about 2mT at 1hr increased the wound healing due to increase the number of red blood cells and HB count[5][6][27] in which it increase the blood flow to the wound area which is necessary to increase wound healing .The result explained that the number of WBCs increased also which decrease the inflammation and increase wound healing .This result is in good agreement with Marek Glinka et al (2012)which assured that ELF-MF could be a useful supplement in the complex treatment of prolonged wound healing [28]. We also notice that when healing is complete(21 day) the number of WBCs decreased to reach the normal level . Also we notice that at long exposure time 1.5hr or 2hr, the magnetic field can destroy the RBC membrane so healing is poor in this situation[9][26].

At concentration 12.5% Moringa extract are also increased the blood flow due to increasing the number of red blood cell and WBCs ,but at higher concentration as 25% and 50% caused retard wound healing due to less interference by components of M. oleifera [29].

Healing and regenerative changes were more noticeable in group 2 (exposed to magnetic field 2mt for 1hr), and moderate in group 5(treated group use concentration of 12.5%) relative to the control , where area of wound for the two groups after 21 day became smaller than the other groups. there was a statistically significant increase in RBCs and WBCS count at P 0.05 and non- significant result in another groups.

It is obvious that the increase in RBCS count due to the increase in the population of erythrocyte precursor cells in the bone marrow .the rise in total leukocyte count is increase due to increase observed in granulocytes population in the peripheral blood and their precursors in the bone marrow or due to release of adherent granulocytes from vascular endothelial walls into the [30] .Based on our result obtained it seems that ELF-MF could be a useful supplement in the complex treatment of prolonged wound healing which may be due to the activation of endogenous enzymatic antioxidant system .

4. CONCLUSION

magnetic field of strength 2mT,50HZ for 1hr accelerate wound healing better than all group where moringa leaves exrtract of concentration of 12.5% accelerate wound healing also relative to the other concentration. Healing and regenerative changes were more noticeable in group 2.

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