

The effect of vitamin K on the wound healing process in rat skin achieved by common wound dressing agents

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Abstract

Background and objective: Vitamin K is a fat-soluble vitamin that plays an important role in the coagulation pathways of living organisms. A popular application of vitamin K has been used to help in the extravascular removal of blood from the skin. The aim of the present study was to test the wound healing effect of vitamin K and some common wound dressing agents available in Erbil city clinical settings such; the moist exposed burn ointment (MEBO) and the Cica silver spray to test their combination with vitamin K injections.

Methods: Six groups of albino rats were used (6 rats in each group). Group M received Mebo ointment, Group Creceived Cica silver spray, Group K received Vitamin K injection, Group MK received Mebo ointment+vitamin K injection. Group CK received Cica silver spray +vitamin K Group, and Group N received no- treatment. The duration of the experiments was as 7, 14, and 21 days post wound surgery. The percentage of wound contraction was measured, and the blood serum was collected to test the level of transforming growth factor β (TGF β) and platelet-derived growth factor (PDGF).

Results: Data presented in our study showed that the best wound contraction percentage (99%) was obtained by the MK treated rats compared to the control group. Similar results were obtained from TGF β and PDGF data in which MK group showed a significant increase in the levels of these growth factors.

Conclusion: The wound healing process is supposed to be due to an increase of TGF β , PDGF, and enhanced fibroblast proliferation and neovascularization of tissues.

Keywords: Wound healing; Rat; Skin; Vitamin K; MEBO.

Introduction

Wound healing remains standing a challenging health problem all over the world. Conductive and organized wound management and treatment are very necessary.¹ many achievements nowadays concentrated on wound care and searching for new and effective therapies and technologies in order for the treatment and management of acute and long-term wounds.² Vitamin-K is an essential vitamin that presents in blood in different forms but it has two main forms, one of them named as phylloquinone or vitamin K1 which have a smaller molecular weight and less effective in the treatment of symptoms and abundant in green leafy vegetables and

some animal cells have ability of converting it to K2 subtypes.³ While the other subtype of vitamin k named as menaquinones or vitamin K2 has a larger molecular weight, more effective in human metabolism and present in many animal source foods such as cheese and red meats. Vitamin k2 is produced in the large intestine by a bacterium called *Bacillus* species such as *Bacillus subtilisnatto* as a result of fermentation of dietary sources.⁴ Many dressing agents such as ointments, creams, and sprays are generally used at governmental and private hospitals in Erbil city for the treatment of burns and wounds. Likewise, in a point of many vital actions of vitamin K that has been reported

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previously⁵ as it stops bleeding after any injury through clot formation. Thus, the present study aimed to test the effect of vitamin K on the wound healing process, and two available dressing agents that are considerably used by the dermatologists and surgeons in Erbil city, named as MEBO ointment and Cica silver spray, and to demonstrate their synergistic or antagonist effect together.

Methods

All chemicals and their obtained places were shown in Table 1. Healthy adult male albino rats weighing between (180 -250 g) with age averaged between (3-4 months), were handled and received humane care depending on the ethical principles of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals⁶ under permission of College of Pharmacy's ethics committee (no. 180507/72). The excision model of in vivo wound healing was followed as described by Zahra et al.⁷ The rats were randomly divided into six groups of 6 rats for each group. The skin of all experimental rats was shaved by using

an electric shaver then disinfected with 70% alcohol. Then anesthetized under sterile conditions by using an intravenous dosage of ketamine (100 mg/kg body weight) and xylazine (20 mg/kg) anesthesia (2:3=0.5 ml) before a full-thickness uniform wound measured 2.00 cm (as shown in Figure 1) in diameter was excised from the nape of the dorsal neck with the assist of a round seal as described by Rawat et al. After making in vivo excision wound model, the animals were confessed to recover from anesthesia and housed separately in disinfected cages. The wound area was calculated by placing a transparent tracing paper over the wound, then put it on 1mm² graph sheets and traced out. The results of wound measurements on different days were considered as percentage wound contraction. The percentage of epithelialization and wound size reduction was calculated by using Wilson's formula as follows:

wound closure percentage % = [(wound area at day 0 – wound area at particular day X) / (wound area at day 0)] × 100⁸.

Table 1: Chemicals of the study.

Materials	Company	Country
MEBO ointment	Julphar	U.A.E
Cica silver spray	Sakura	Italy
Vitamin K injection	Caspian	Iran
Lidocaine injection	Pharma-chem	India
Ketamine	Romvac	Romania
Xylazine	Interchemie	Holland
Hematoxyline	Dako	Denmark
Eosin	Tissue-Tek	Netherland
Platelet-derived growth factor (PDGF) Rat-ELISA kit	Al-shkairate establishment for medical supply	Jordan
Transforming growth factor Beta-1 (TGF-B1) Rat-ELISA kit	Al-shkairate establishment for medical supply	Jordan

The rats were divided into six groups depending on the types of treatment: Group M: Mebo ointment (0.25% w/w) twice a day, Group C: Cica silver spray twice a day, Group K: Vitamin K injections twice a week, Group MK: Mebo ointment (0.25% w/w) twice a day +Vitamin K injection twice a week. Group CK: Cica silver spray twice a day +Vitamin K injection twice a week, Group N: (No- treatment or Control group) this group of rats was untreated. Blood samples of the experimental rats were collected at days 7, 14, and 21 at the end of the experiment in blood collection tubes and allowed them to clot for 1hr at 25°C. Blood samples were then centrifuged at 3000rpm for 15 minutes at 4 °C. After that, the rat serum was collected and preserved at -80°C prior to use. Platelets derived growth factor (PDGF) and transforming growth factor beta (TGF- β 1) levels in rat serum were measured by using sandwich enzyme-linked immunosorbent assay (ELISA) following the method of ⁹. These growth factors as PDGF and TGF β levels were measured by using rat ELISA kits PDGF [Al-shkairate establishment for medical supply (Catalog No.RDEER1240) and TGF-B1 [Al-shkairate establishment for medical supply (Catalog Number RDEER0061) respectively followed the manufactures instructions.

Statistical Analysis

The data are presented as the mean \pm standard error of mean (S.E.M.). The statistical differences among groups were assessed using one-way ANOVA (analysis of variance), (GraphPad Prism 7). A value of $P < 0.05$ was considered significant. Statistical analysis was performed using IBM statistical package for the social sciences (SPSS) statistics 23 software for Windows (SPSS Inc., Chicago, IL, USA).

Results

Figure 2 shows the effects of all experimental treatments on the wound area and the wound closure after several days of surgery. Throughout the experiment, the healing in the vehicle control group wounds was significantly lower than those of treated groups. The findings of the 7th day of the treatment showed that wound contraction was restored in groups (M, K and MK). Consequently, on day 14 of the surgery, the rate of wound contraction was improved, and the highest wound closure percentage was recorded in K group (wound closure= 84%) on day 14 of treatment. However, the lowest results were given by rats of the control untreated group (wound closure= 30%), as shown in Figure 3. Moreover, on day 21, after surgery, all experimental rat groups

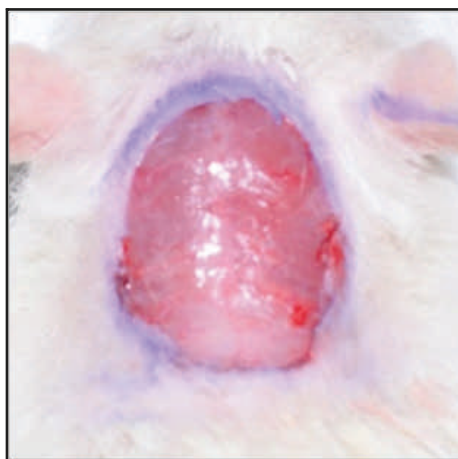


Figure 1: Rat's dorsal neck nape showing a uniform wound measured 2 cm in diameter.

showed restored wound contraction levels. The peak wound closure was given by MK group (wound closure= 99%), and the

second best wound contraction was given by rats in the C group (wound closure= 97%) (Figure 4).

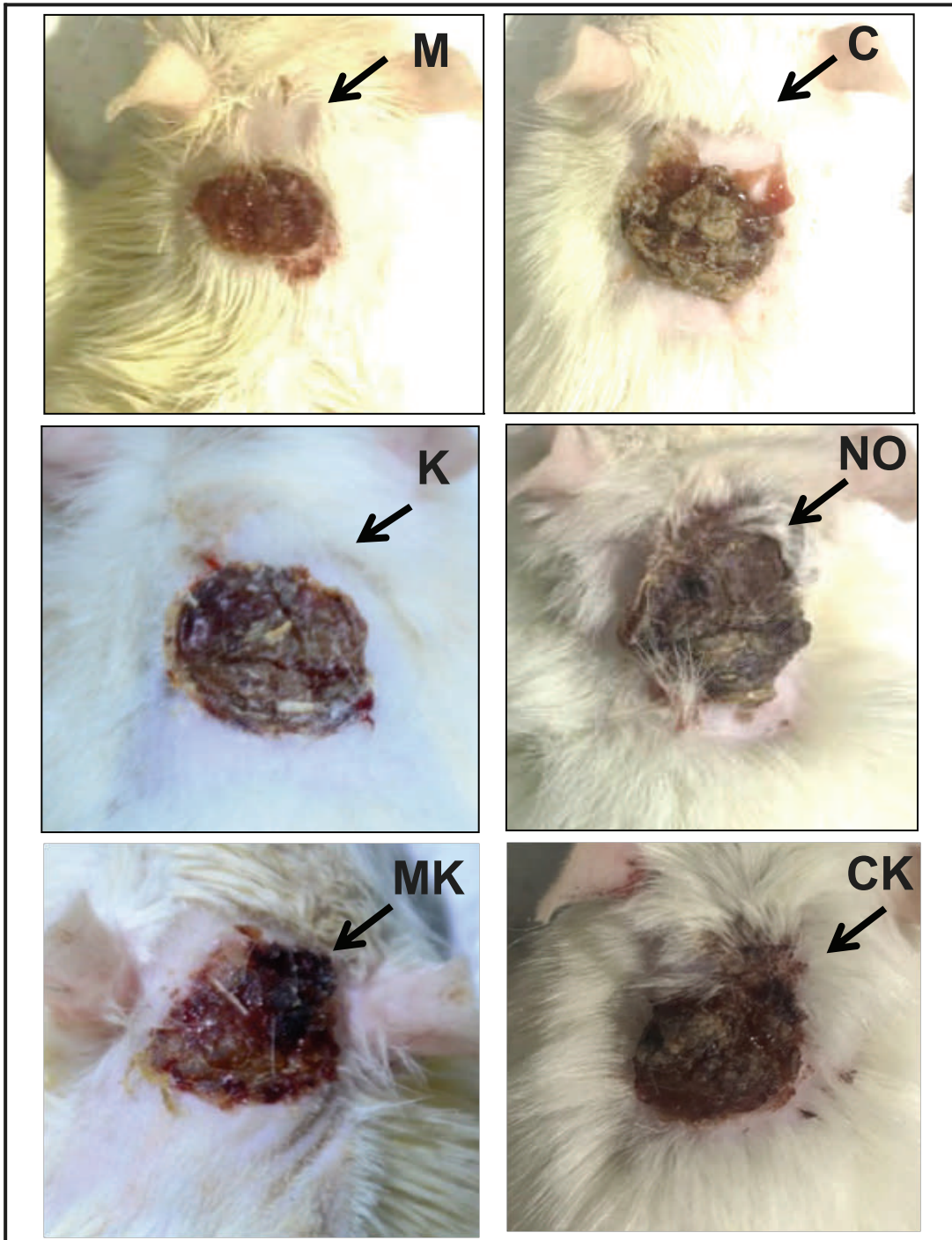


Figure 2: Macroscopic appearance of excision wound healing area on rat skin at day 7 after surgery within different groups of treatments (M) Mebo ointment group; (C) Cica silver spray group; (K) Vitamin K group; (NO) vehicle control group; (MK) Mebo ointment+ vitamin K; (CK) Cica silver spray+ vitamin K (CK).

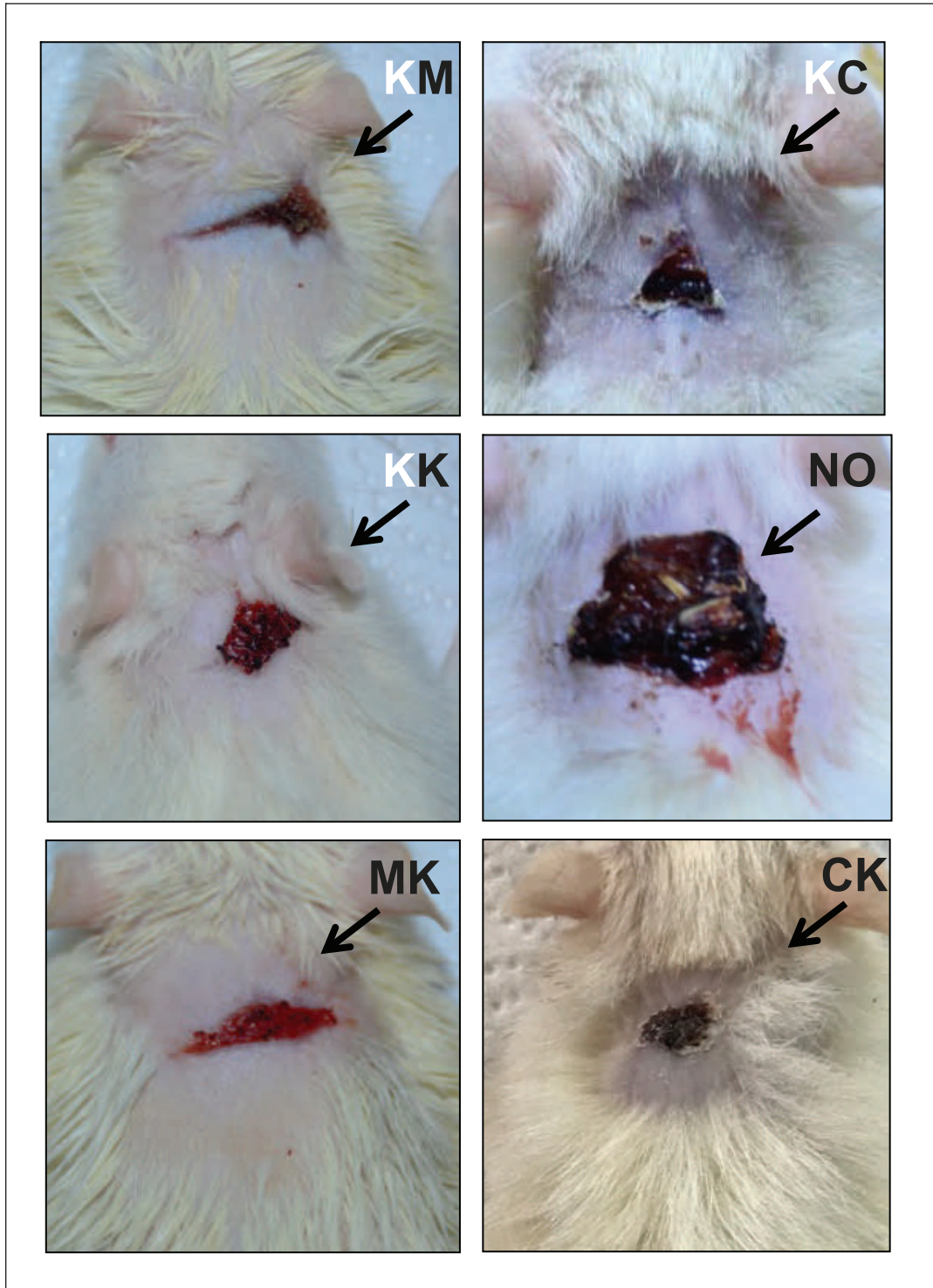


Figure 3: Macroscopic appearance of excision wound healing area on rat skin at day 14 after surgery within different groups of treatments (M) Mebo ointment group; (C) Cica silver spray group; (K) Vitamin K group; (NO) vehicle control group;(MK) Meboointment+ vitamin K; (CK) Cica silver spray+ vitamin K (CK).

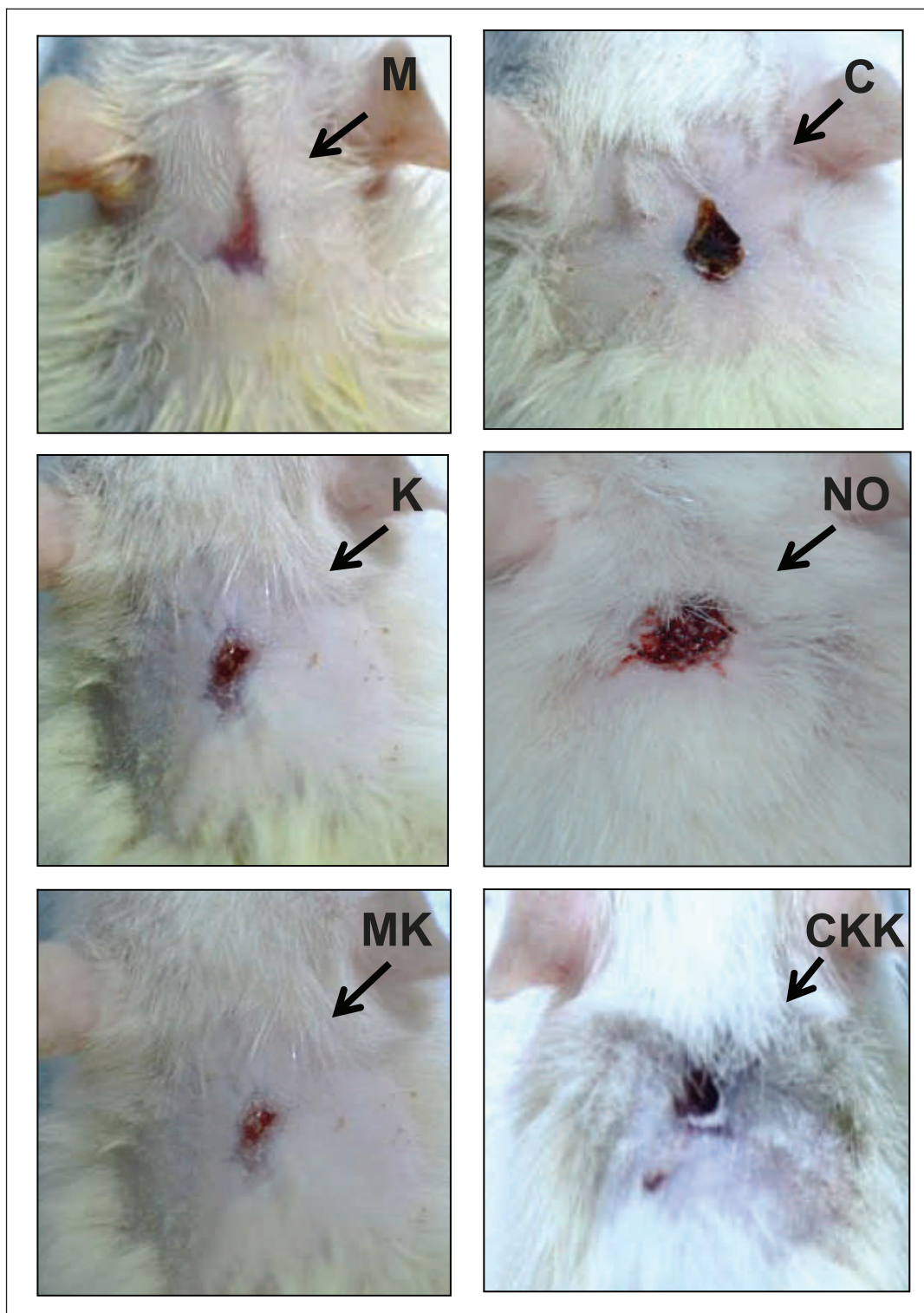


Figure 4: Macroscopic appearance of excision wound healing area on rat skin at day 21 after surgery with different groups of treatments (M) Mebo ointment group; (C) Cica silver spray group; (K) Vitamin K group; (NO) vehicle control group; (MK) Mebo ointment+ vitamin K; (CK) Cica silver spray+ vitamin K (CK).

On the other hand, the concentration of TGF-B1 of the blood samples of experimental rat groups at 7, 14, and 21 days of treatment are shown in Table 2. Significant results were recorded in the

MK group (87.5± 3.87 pg/ml). The second highest significant result of TGF-B1 concentration was marked in CK group (66.5± 1.29 pg/ml) compared to the control group.

Table 2: The expression of TGFβ in different weeks of treatment (concentration in pg/ml); (A) Comparison of means using ANOVA test, (B) comparison of groups using Post Hoc LSD test.

(A)

Groups	N	Mean	SD	P value (ANOVA)
Day 7				
M	6	13.38	3.725	
C	6	11.76	5.88	
K	6	16.9	4.965	<0.001
MK	6	14.42	2.11	
CK	6	33.8	10.88	
N	6	2.5	0.577	
Day 14				
M	6	35.75	4.856	
C	6	35.5	1.29	
K	6	43	2.16	<0.001
MK	6	56.5	1.29	
CK	6	40.5	4.795	
N	6	3.5	0.577	
Day 21				
M	6	48.5	5.802	
C	6	47.25	5.909	
K	6	63.5	12.124	<0.001
MK	6	87.5	3.872	
CK	6	66.5	1.29	
N	6	3.5	1.29	

(B)

	Groups	M	C	K	MK	CK	N
Day 7	M		0.18	0.49	0.84	0.05	0.043
	C			0.51	0.26	0.02	0.002
	K				0.63	0.05	0.03
	MK					0.001	<0.001
	CK						<0.001
	N						
Day 14	M		0.91	0.003	<0.001	0.03	<0.001
	C			0.003	<0.001	0.03	<0.001
	K				<0.001	0.02	<0.001
	MK					<0.001	<0.001
	CK						<0.001
	N						
Day 21	M		0.78	0.003	<0.001	0.001	<0.001
	C			0.002	<0.001	<0.001	<0.001
	K				<0.001	0.01	<0.001
	MK					<0.001	<0.001
	CK						<0.001
	N						

M= MEBO, C= Cika silver, K= vitamin K, N= no treatment, MK= MEBO + vitamin K, CK= Cika silver +vitamin K.

Table 3 shows the concentration of PDGF in blood samples, in which, at day 21 of treatment, all groups showed an increased PDGF level in comparison to 7th and 14th days of the study.

Table 3: The expression of PDGF in different weeks of treatment (concentration in pg/ml); (A) Comparison of means using ANOVA test, (B) comparison of groups using Post Hoc LSD test.

(A)

Groups	N	Mean	SD	P value (ANOVA)
Day 7				
M	6	0.093	0.025	<0.001
C	6	0.126	0.038	
K	6	0.104	0.021	
MK	6	0.106	0.003	
CK	6	0.151	0,095	
N	6	10.5	0.577	
Day 14				
M	6	0.102	0.033	0.389
C	6	0.122	0.05	
K	6	0.124	0.019	
MK	6	0.137	0.09	
CK	6	0.114	0.025	
N	6	0.184	0.069	
Day 21				
M	6	0.209	0.106	0.168
C	6	0.097	0.02	
K	6	0.114	0.019	
MK	6	0.166	0.066	
CK	6	0.386	0.354	
N	6	0.205	0.538	

(B)

	Groups	M	C	K	MK	CK	N
Day 7	M		0.85	0.95	0.94	0.73	<0.001
	C			0.90	0.91	0.88	<0.001
	K				0.99	0.79	<0.001
	MK					0.79	<0.001
	CK						<0.001
	N						
Day 14	M		0.60	0.60	0.37	0.75	0.05
	C			0.96	0.70	0.83	0.13
	K				0.74	0.80	0.14
	MK					0.56	0.24
	CK						0.04
	N						
Day 21	M		0.32	0.40	0.03	0.12	0.97
	C			0.88	0.04	0.02	0.40
	K				0.64	0.02	0.42
	MK					0.06	0.03
	CK						0.02
	N						

M= MEBO, C= Cika silver, K= vitamin K, N= no treatment, MK= MEBO + vitamin K, CK= Cika silver +vitamin K.

Discussion

The topical treatment of wounds is of great significance both in preventing death and invasive infection. In our research, we used multiple common dressing agents that have been used mostly in Erbil city for treating acute wounds and burns on many groups of experimental rat skin. Furthermore, these dressing agents were moist exposed burned ointment (MEBO) and Cica silver spray in addition to vitamin K injection. Each one of them had been used separately and also in combination with vitamin K and compares it with control groups with no treatment. The best significant results of wound contraction in our study were given by MK rat groups at day 21 of treatment among all experimental rat groups except the control group, and this suggests a synergistic activity of both MEBO ointment and vitamin K during in enhancement of wound healing process. A similar result was reported by Tang and coworkers.¹⁰ Whose clinical findings showed that MEBO promotes wound contraction and improves scar quality of wounds. Also, our results agree with another study done by Moustafa on the equine wound model in horses; they found that Mebo significantly accelerated the wound healing process and reduce wound closure in comparison with the control group.¹¹ Also, vitamin K has increased the rate of wound closure, and this result agrees with the result of the previous study have been reported by Hemmati and coworkers.¹² Additionally, all experimental groups of rats showed significant results in the level of TGF-B1 expression and the most significant results of TGF-B1 expression at day 21 of treatment was shown in MK, CK, and K rat groups in which the mean level of TGF-B1 expression was (87.5, 66.5, and 63.5 pg/ml) respectively, so the highest level of TGF-B1 expression was found in MK rat groups in comparison of the control group of rats as shown in (Table 2). Similar results were reported by Abood, whose clinical findings showed that the level of

TGF-B1 and other inflammatory cytokines expression, which plays a key role in the wound healing process, was significantly increased in treated rat groups in comparison with the vehicle control group.¹³ Other similar results were reported by Kandhare and coworkers whose clinical findings showed significant up-regulation in the level of TGF-B1 and other essential growth factors for angiogenesis and collagen deposition in the wound healing process in treated rat groups in comparison with control group.¹⁴ Besides that, the highest significant level of PDGF expression at day 21 of treatment was shown in MK rat groups as the mean was (0.39 ng/ml) which were significantly higher than the mean level of PDGF expression in M group (0.30 ng/ml) than in the CK group (0.26 ng/ml) and the lowest significant result of PDGF expression was shown in C rat groups as the mean of PDGF expression was (0.24ng/ml) in comparison with the vehicle control group as shown in Table 3. Similar results were reported by Tang and coworkers whose clinical findings showed that MEBO ointment increases the messenger ribonucleic acid (mRNA) expression of many growth factors which are correlated with enhancement of fibroblast proliferation and neovascularization in granulation tissue during the proliferative phase which are essential for wound healing process.¹⁰

Conclusion

The application of MEBO ointment showed significant results during the wound healing process when it used separately, but the best result of MEBO ointments was given when it used in combination with vitamin K in treating excisional wound model on rat skin. Cika silver showed faster results of wound healing (at day 7th of the surgery) when applied alone or together with vitamin K but slows down at day 14 and day 21 in comparison to MEBO's action. Therefore, our results provide a scientific basis for co-administration of vitamin K and MEBO in the management of wounds.

Competing interests

The authors declare no competing interests.

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