Original Article

Comparative wound healing activity of different marketed formulation in albino *wistar* rats

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ABSTRACT

The present study was aimed to compare in-vivo topical wound healing activity of different marketed formulation in wistar rats by excision and incision wound models. Three different marketed formulations were chosen to have different formulae. The animals were grouped into; group I (left untreated) considered as control, group II (Povidone Iodine 5% w/w, once daily), Group III (Mupirocin 2% w/w, once daily), group IV (Povidone Iodine 5% w/w + Mupirocin 2% w/w in 1:1 ratio, once daily). The healing effect of the formulations was accessed as excision wound area measurement, Percentage wound contraction and epithelialization period in the excision wound model, however wound breaking strength was accessed in the incision wound model. The results of the present study revealed that, animals treated with Mupirocin 2% ointment shows significant wound healing potential in comparison to control and other treatment groups. Mupirocin showed a significant and faster rate of wound contraction ($p<0.001$) as compared with control with the minimum epithelialization period as compared to other groups. Mupirocin also showed significant tensile strength ($p<0.001$) as compared to control. So the study concludes that mupirocin might be a drug of choice for better healing. Such type of comparative study is also further can be done on a large scale to find out more better significant results.

1. INTRODUCTION

The wound is defined as the disruption of the cellular and anatomic continuity of a tissue. The wound may be produced by physical, chemical, thermal, microbial or an immunological insult to the tissues [1]. The process of wound healing consists of integrated cellular and biochemical events leading to re-establishment of structural and functional integrity with regaining of strength in injured tissues. Wounds generally termed as physical injuries that result in an opening or breaking of the skin [2].

There are many factors that can affect wound healing by interfering with one or more phases in this process, thus causing improper or impaired tissue repair. In individuals with diabetes mellitus, the rate of wound repair is slow [3]. The underlying mechanisms of defective wound repair in diabetic patients are not completely understood, but it is thought that all phases of the healing process are disrupted. Indeed, delayed collagen synthesis, impaired epithelization, and reduced angiogenesis have been observed during the proliferative phase of the healing process [4-7].

Wound healing is impaired in diabetic patients with infection or hyperglycemia and is one of the major contributors to chronic wound healing problems. The diabetic patients with ulcer become at high risk for major complications which include infection and amputation. In traditional medicine plants are generally used for treatment of various acute and chronic diseases and abnormalities in the body. Most of these patients tend to face a tremendous problem when they get an infected wound [8].

Wound healing is the dynamic self-recovery body mechanism from an injury which has been characterized in four different overlapping phase homeostasis, inflammatory, proliferative, and remodeling. These phases and their bio-physiological functions must occur at a specific time, and maintain for a specific duration at an optimal strength [9]. Wound healing is currently a clinical challenge due to inconsistencies encountered in the healing processes [10]. Number of complications are associated that impairs the natural healing process and the healing ability of the body. Mupirocin (Accession no. DB00410) is an antibiotic originally isolated from Pseudomonas fluorescens. It is used topically, and is primarily effective against Gram-positive bacteria. Mupirocin is bacteriostatic at low concentrations and bactericidal at high concentrations. Mupirocin has a unique mechanism of action, which is selective binding to bacterial isoleucyl-tRNA synthetase, which halts the incorporation of isoleucine into bacterial proteins. Because this mechanism of action is not shared with any other antibiotic, mupirocin has few problems of antibiotic cross-resistance [11]. Povidone-iodine (Accession no. DB06812) is a stable chemical complex of polyvinylpyrrolidone (povidone, PVP) and elemental iodine. It contains from 9.0% to 12.0% available iodine, calculated on a dry basis. Human clinical trials showed the product to be superior to other iodine formulations. Povidone-iodine was immediately marketed, and has since become the universally preferred iodine antiseptic and antineoplastic [11,12]. A Number of drugs are available in the market, but under some circumstances or complications, there is a need to find out the drug of choice for fast and better healing therapy [13, 14]. The aim of the present study was to preliminary compare in-vivo topical wound healing activity of different marketed formulation in wistar rats.

2. EXPERIMENTAL WORK

2.1 Materials and Method

2.1.1 Animals

All the rats were healthy and 100- 250 gm of body weight. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 22°C (± 3°C), relative humidity 44-56% and light and dark cycles of 12:12 hours, fed with standard pellet diet and water ad libitum during experiment. The bedding of animals was changed every 3rd day. All the procedures and protocols of the experiments were reviewed and approved by IAEC, NRI Institute of Pharmaceutical Sciences, Bhopal, Madhya Pradesh. (Approval no. 1238/a/08/CPCSEA).

2.1.2 Grouping and treatment

For excision and incision wound models, animals were divided into four groups consisting of six animals in each group as follows: group I (left untreated) considered as control, group II (Povidone Iodine 5% w/w, once daily), group III (Mupirocin 2% w/w, once daily), group IV (Povidone Iodine 5% w/w and Mupirocin 2% w/w in 1:1 ratio, once daily).

2.1.3 Incised wound model

The trunks of the rats were thoroughly shaved with a sterile razor blade, disinfected with methylated spirit (94% ethanol+1% methanol), and 1.5 cm incision wound was created on the skin using a surgical blade under anesthesia (diethyl ether). After complete hemostasis, the wound was stitched by means of interrupted sutures and the wound was left as such and animals were treated daily for 10 days. On the 10th day, all rats were anesthetized and sutures were removed and tensile strength of wound skin was measured using tensiometer [15].

2.1.4 Excision wound model

Excision wound was created by according to the method described by Thakur, et al., 2011 with some modification [16]. Animals were categorized as according to selected evaluation parameters. For excision wound formation, the particular skin area was shaved 1-day prior to the experiment. The rats were anesthetized by administering ketamine and xylazine (50 mg/kg and 5 mg/kg i.p. body weight). A full thickness of the excision wound of the circular area was made on the shaved back of the rats 15 min later the administration of anesthesia. The shaved portion and an excision wound were inflicted by cutting away a 1 cm^2 full thickness of the skin from a predetermined shaved area. Wounds were left undressed to the open environment. The animals were treated for 20 days and wound areas were measured by tracing the wound on transparency sheet with permanent marker by using millimeter based graph paper on day 0, 4th, 8th, 12th, 16th and 20th day for all groups. In this model, % Wound contraction, and period of epithelization were measured.

2.2 Wound healing evaluation parameters

2.2.1 Measurement of tensile strength

On the 10th day, all the animals were anesthetized, sutures were removed and the healed tissue was excised from all animals. Tensile strength of excised tissue was measured with the help of tensiometer [17].

2.2.2 Wound area and epithelization of wound

Wound area of all the groups was measured on every 4th day up to 20th day. Epithelization time refers to the number of days taken by the wounds to appear completely closed with no moist granulation tissue, and the wound was covered with new epithelium [18].

2.2.3 Wound contraction

Wound contraction was expressed as a reduction in the percentage of the original wound size [19]. The percentage wound contraction was determined using the following formula:

$$\% \text{ wound contraction} = \frac{(\text{Initial wound area–Specific day wound area})}{\text{Initial wound area}} \times 100$$

3. RESULTS

3.1 Comparative effect of different marketed formulation on tensile strength of recovered wound

In incision model, the group-III, treated with Mupirocin 2% alone showed significant tensile (P<0.01) strength as compared

with group-II (Povidine Iodine 5%) and Group IV (Mupirocin 2%+ Povidine Iodine 5%) (Table 1).

Table 1. Treatment effect of different marketed drugs on wound tensile strength

Group	Wound breaking strength (kg)
Group-I	0.4132 ± 0.090
Group-II	0.312 ± 0.040*
Group-III	0.600 ± 0.0365**
Group-IV	0.36 ± 0.049*

3.2. Effect of different marketed drugs on Wound contraction and epithelization of wound

In the excision wound model, during the course of treatment it was found that group III (Mupirocin 2%) treated animals showed maximum or complete wound contraction (p<0.001) as compared with group-II (Povidine Iodine 5% treated) (p<0.01) and Group IV (Mupirocin 2%+ Povidine Iodine 5% treated) (p<0.01) as shown in Tables 2 and 3. Mupirocin 2% alone was also found to take minimum time for complete epithilization (Table 4).

Table 2. Treatment effect of different marketed drugs on excision wound area (mm²)

Treatment group	0 day	4th day	8th day	12th day	16th day	20th day
Group-I	364.38±3.10	220.32±4.33	100.32±2.41	50.38±3.41	20.51±1.07	7.88±1.50
Group-II	348.56±3.36	144.59±1.56***	64.33±4.21***	40.66±5.47	17.98±1.89	3.43±0.11**
Group-III	368.87±2.32	110.50±3.28***	56.71±1.52***	18.76±3.47***	2.31±0.32***	0.00±0.00***
Group-IV	340.87±3.46	115.48±2.76***	74.33±4.0***	33.50±5.55	11.66±1.10***	3.10±0.21**

Data represents mean ± SEM (n= 6) * p<0.05, ** p<0.01, *** p<0.001 as compared to control group (One way ANOVA multiple comparison followed by a Tukey Cramer multiple comparison test)

Table 3. Effect of different treatment on % Wound contraction

| Treatment group	0 day	4th day	8th day	12th day	16th day	20th day
Group-I	0	39.53	72.46	86.06	94.37	97.83
Group-II	0	58.51	81.54	88.33	94.84	99.01
Group-III	0	70.04	84.62	94.91	99.37	100
Group-IV	0	66.12	78.19	90.17	96.57	99.09

4. CONCLUSION

The results of the present study revealed that, animals treated with Mupirocin 2% ointment shows improved wound healing potential in comparison to control and other treatment groups. Mupirocin showed a faster rate of wound contraction and epithelization in the excision wound model. It also found to show

significant increment in the tensile strength of the healed wound. So this study concluded that mupirocin is the drug of choice, we conclude from result that such type of comparative study is also option for finding a better treatment option. This study further can be done on a large scale to find out more better significant results as well as to access effect of a particular treatment under certain complication or associated diseases like Diabetes.

Table 4. Epithelzation period in excision wound model in wistar rats

Group	Epithelization period (Days)
Group-I	24
Group-II	22
Group-III	18
Group-IV	22

Days	Group-I	Group-II	Group-III	Group-IV
0				
4				
8				
12				
16				
20				
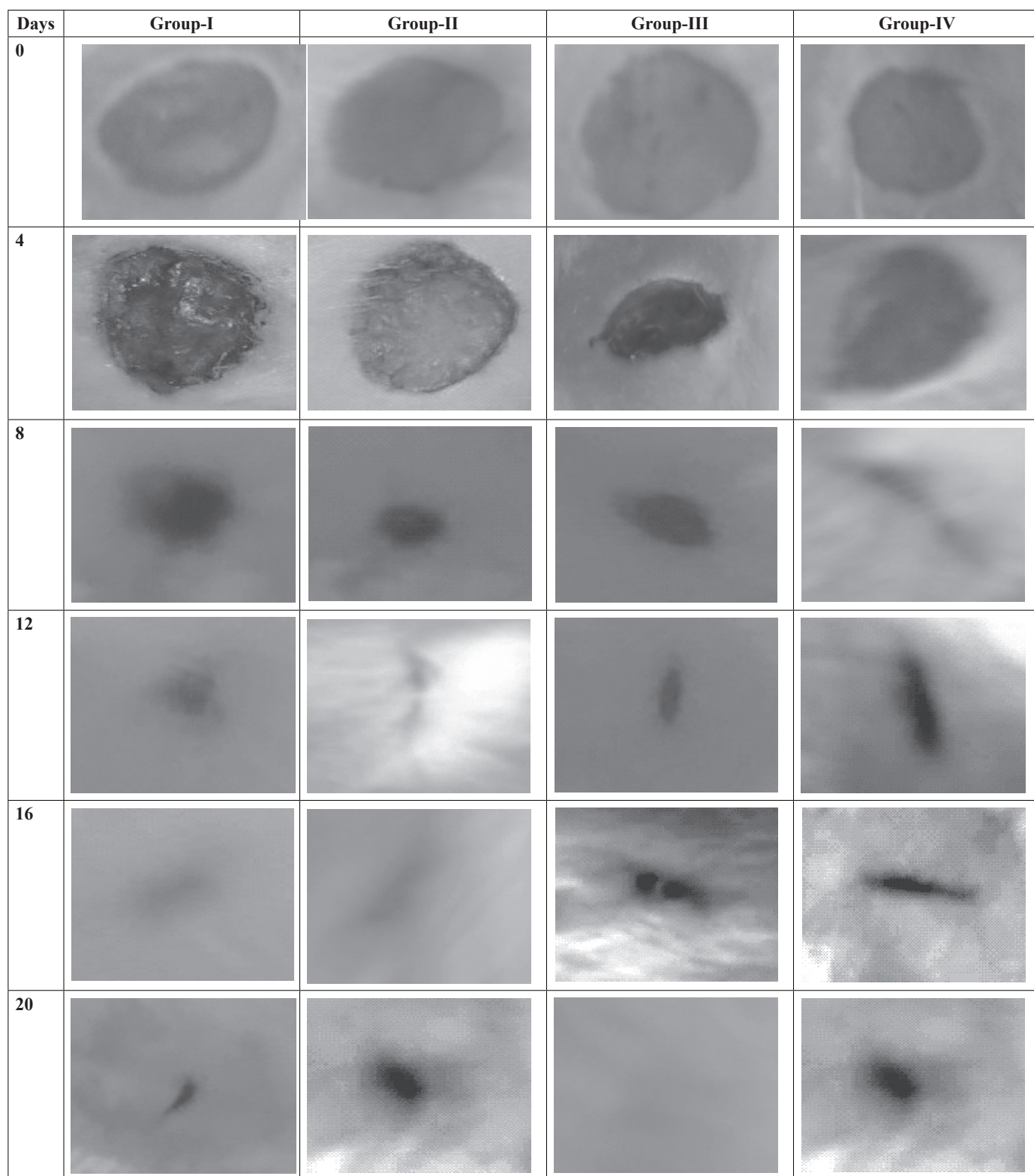
Fig. 1. Photographs of wound repair at different times of treatments in different groups of rats in excision wound model.

Group I; control, group II; Povidone Iodine (5% w/w) treated, group III; (Mupirocin 2% w/w) treated and group IV; (Povidone Iodine 5% w/w + Mupirocin 2% w/w) treated group

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