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Formulation and Evaluation of Rutin-Allicin Gel Against Diabetic Foot Ulcer

Imran A. KHAN [1,2] *, Ali RIZWAN [2], Mashhud U. ABID [1], Ashira MANZOOR [1], Maliha K. KHAN [3], Khizar ABBAS [3], & Muhammad O. IQBAL [2]

[1] *Department of Biochemistry, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan*
[2] *Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan*
[3] *Department of Physical Therapy, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan*

SUMMARY. **The purpose of this study was to prepare and evaluate a gel that is effective against non healing or resistant ulcers such as diabetic foot ulcers. A 20% w/v gel of each (rutin-allicin with optimized ratio 2:1) was made using Carbopol-940 in the concentration of 5% and evaluated for diabetic wounds. A total of 60 patients with partial thickness diabetic wound were divided into two equal groups. One group was treated with tetrachlorodecaoxide (TCDO) while the other with rutin allicin gel (RAG), and the results regarding the duration of wound epithelialization, wound contraction, pain relief, and cost of treatment were compared. In patients treated with RAG, healing of diabetic wounds was slightly earlier than those patients treated with TCDO ($p < 0.05$). All the patients of RAG treated group were relieved of pain earlier than those patients who were treated with TCDO furthermore, found cost effective as compared to TCDO. The results suggest that RAG possesses significant wound healing potential in chronic diabetic wounds and can be used as an effective, alternative, and cost effective medicine for wound care with unnoticeable toxicity.**

RESUMEN. El propósito de este estudio fue preparar y evaluar un gel que sea efectivo contra úlceras no curativas o resistentes como las úlceras del pie diabético. Se preparó un gel al 20% p/v de rutina-alicina con una proporción optimizada 2:1) usando Carbopol-940 en una concentración del 5% y se evaluó para detectar heridas diabéticas. Un total de 60 pacientes con heridas diabéticas de espesor parcial se dividieron en dos grupos iguales. Un grupo fue tratado con óxido de tetraclorodecaóxido (TCDO) mientras que el otro con gel de rutina y alicina (RAG), y se compararon los resultados con respecto a la duración de la epitelización de la herida, la contracción de la herida, el alivio del dolor y el costo del tratamiento. En los pacientes tratados con RAG, la curación de las heridas diabéticas fue un poco más temprana que la de los pacientes tratados con TCDO ($p < 0.05$). Todos los pacientes del grupo tratado con RAG se aliviaron del dolor antes que aquellos pacientes que fueron tratados con TCDO; además, RAG es más rentable en comparación con TCDO. Los resultados sugieren que el RAG posee un potencial de curación significativo en las heridas diabéticas crónicas y puede usarse como un medicamento eficaz, alternativo y rentable para el cuidado de heridas con toxicidad imperceptible.

INTRODUCTION

Wound refers to an injury or tissue insult especially skin is cut or broken and the dermis is exposed to invaders badly. Pathologically wound is defined as 'an injury which harms the dermis of the skin [1].

Wound healing is a very complex process consisting of four programmed phases which overlap and compliments eachother. Each phase and the events happen in a fixed and synchronized manner [1]. The first phase begins immediately after tissue insult/ injury, with vascular constraint and fibrin clot formation. Pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) are released from the clot and neighbouring injured cells. In a controlled bleeding scenario, inflammatory cells travel into the wound and triggers the inflammatory phase, characterized by the chronological penetration of lymphocytes,

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* Author to whom correspondence should be addressed. *E-mail:* imranahmadkhandurrani@gmail.com

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neutrophils, and macrophages [2]. Neutrophils are associated with the clearance of cellular debris and invading microbes in the wound area, and generate substances such as proteases and reactive oxygen species (ROS), which is responsible for further damage [3].

Tetrachlorodecaoxide (TCDO) is relatively new, more potent and efficacious topical wound dressing solution. TCDO is chemically rich in oxygen based on the latest concept of direct activation of macrophages by increasing the partial pressure of oxygen in the wound [2-6]. This is a good technique for the healing of pressure ulcers, especially for the chronic ulcers associated with diabetes [7]. TCDO is an aqueous solution containing bio-activated oxygen carrier that potentially breaks the vicious cycle of hypoxia in a wound. Because of its oxygen richness, it fulfills the increased oxygen demand needed for phagocytic activity, without compromising the physiological degree of local hypoxia required for neo- vascularization [8]. Its mitogenic properties on fibroblasts adds extra strength [8]. Decomposition of TCDO does not give rise to any toxic metabolite [9]. The therapeutic effect of TCDO based on three ways, intensifying the wound cleaning, promoting the formation of new tissue granulation and modulating the immunoresponse to injury [8].

Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside, (Fig. 1a) is a flavonol, abundantly found in plants, such as apple, buckwheat, passion flower and tea [10]. Chemically it is a glycoside comprising of flavonolic aglycone quercetin along with disaccharide rutinose. It has shown a number of pharmacological activities, including anticarcinogenic, antioxidant, cytoprotective, vasoprotective, cardioprotective and neuroprotective activities [11].

Allicin (S-allyl 2-propene-1-sulfinothioate, (Fig. 1b) is a polar compound of phenolic and steroidal nature which stimulates immune functions such as proliferation of lymphocyte, release cytokine and phagocytosis and lessen cellular proliferation of virally infected cells [12], induces antioxidant action by hunting reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase and increasing glutathione in the cells [12].

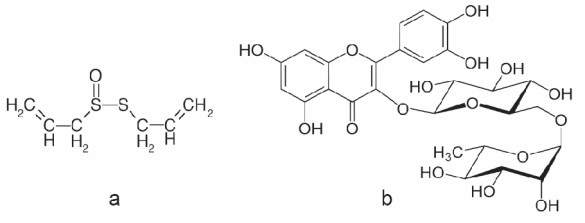

Figure 1. Chemical structure of allicin (**a)** and rutin (**b**).

MATERIAL AND METHODS

Drugs and chemicals

All the drugs used in this study were of pharmaceutical grade. Tetrachlorodecaoxide (10% w/v) was purchased from Brooks Pharmaceuticals Pvt. (Ltd.) Pakistan. The preparation of rutin used here was obtained from LKT Laboratories, Inc. (St. Paul, MN), and allicin were purchased from All sure neutraceuticals, China.

Preparation of gel

Rutin and allicin were dissolved in dimethyl sulfoxide (DMSO) at 10% for rutin and 5% for the allicin. A 20% w/v gel of each (rutin and allicin with optimized ratio 2:1) was made using Carbopol-940 in the concentration of 5%. The concentrations chosen for the two active phytoconstituents were based on the already reported animal studies.

PATIENTS AND METHOD

This comparative study was carried out in the Department of Medicine, Al-Huda Medical Center, Multan, during the period from September to November, 2018. After the approval of the study protocol from the Institutional Ethical Committee (IRC/MIMAS/4/Med./ DFU/18), 60 patients who had partial thickness diabetic ulcers were enrolled and their consent was submitted in the social welfare department (06/EXP/DB/18). Patients who had a previous history of hypertension, renal, cardiovascular and neurological diseases or pregnancy were excluded. After the patients were admitted, the wounds were cleaned and dressed with TCDO and RAG. Dressing with TCDO and RAG was continued on a daily basis until the wounds were fully healed and re-epithelialized. Wound swab cultures were also taken for bacteriological examination. The routine medicines for DM were continued during the study.

Sixty patients with partial thickness diabetic wound were randomized (consecutive sampling method) into 2 groups, one were cleaned and dressed with TCDO (30 patients) and second with RAG (30 patients). Both groups were com-

pared with respect to patient demographics including age, sex, wound size (%) and cost per dressing. Patients were also interviewed for documentation of efficacy of treatment, including time required for healing (epithelialization), pain scores, type of wound, colonization and infection, cost of treatment and mortality between both groups. Topical dressing was done twice a day until healing was complete. At the time of change of dressing details regarding the condition of the wound such as signs of wound infection, condition of surrounding tissues, fluid discharge, smell, necrotic tissue and state of epithelialization was noted on every 3rd day. Subjective factors such as pain and local irritation were recorded regularly. Allergies, inflammation or other side effects were noted in both groups. The patients and attendants were taken in confidence and given information regarding the use of TCDO and RAG under senior medical supervision.

Visual analogue scoring system (1-10) was used to note pain relief. Pain was considered as severe for score 6-10, moderate 3-5 and less than 3 was considered mild. Pain was considered relieved if patient scored 0-1 [16]. On the 21th day, by using Eq. [**1**], the percentage protection was calculated.

$$Percentage\ wound\ closure = \frac{Initial\ wound\ area - n^{th}\ day\ area\ of\ wound}{Initial\ wound\ area} \times 100 \quad [1]$$

Statistical analysis

Data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, USA) and results were expressed as mean ± SD, whereby variables had first been tested for normality through Shapiro-Wilk test. The difference between experimental groups was analyzed using one-way ANOVA followed by Bonferroni test.

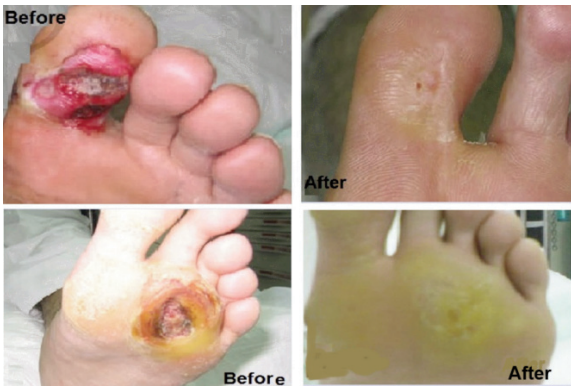

Figure 2. Wound area (cm) of both groups over a period of 21 days.

RESULTS

Demographic characteristics and other parameters of the patients are summarized in Table 1. All patients in the present study survived. The authors found no significant differences in age, total body surface area; type of injury. Of sixty patients, 33 were males and 27 were females (age range, 20-60 years). In PHP treated group, epithelialization begun at 6th day, and in all patients' healing was complete on the 21th day (p value = 0.01), whereas, in TCDO group epithelialization started at 4th day and complete wound healed on the 24th day of the treatment (p value = 0.03). There were no differences in wound infection between both the drugs shown good bactericidal activity. All of the patients who developed wound infection before the start of this treatment still responded well to targeted topical dressing. The price of 50 mL of TCDO was Rs. 380 Pak rupees whereas the price of 200mL of PHP was Rs. 200 Pak rupees. The cost of wound cleaning and dressing per percent body surface area was almost 8 times less as compared to TCDO. Scar formation or the development of contractures. These differences were statistically significant. Wound area (cm) of both groups over a period of 21 days is shown in Fig. 2.

DISCUSSION

Mechanistically production of antioxidant at the injury site is favourable of wound healing site by enhancing cell proliferation and migration [17,18]. Both the phytoconstituents have been reported for excellent antioxidant activity in earlier studies [15]. Antioxidants reported to be an important character in the whole wound healing process [19]. Control of microbial growth and infection at the wounded area is clinically mandatory and all three phytoconstituents, allicin and rutin possess excellent antimicrobial property [12-16], which may further strengthen our claim as wound healer. Antimicrobials are extensively used to control microbial growth in pre and post operated patients for better wound healing

Patients characteristics		TCDO group	RAG group
		Number of cases *(n=30) %age*	Number of cases *(n=30) %age*
Gender of patients	Male	19 (63.3)	14 (46.6)
	Female	11 (36.6)	16 (53.3)
Age	20-30	06 (20)	04 (13.3)
	31-40	15 (49.9)	06(20)
	41-50	06 (20)	19 (69.9)
	51-60	03 (10)	01 (3.3)
Type of DM	DM-1	09 (30)	13 (43.3)
	DM-2	21 (70)	17 (56.6)
	Gestational	0 (0)	0 (0)
Wound thickness, type*	Partial	3 × 3 × 0.7	3 × 2.5 × 0.5
Time required for epithelialization**		24 days(p value=0.03)	21 days(p value= 0.01)
Infection status***	Non infected	20 (66.6)	16 (53.3)
	Mild (≤ 2cm cellulites)	8 (26.6)	11 (36.6)
	Moderate (≥2cm cellulites)	2 (6.6)	3 (10)
	Severe (sepsis)	0 (0)	0 (0)
Bacterial Isolate	MRSA	9 (30)	13 (43.3)
	Candida spp.	3(10)	5(16.6)
	No significant growth	18 (60)	12 (40)
Pain scores	No pain (0)	27 (90)	29 (96.6)
	Mild pain (≤3)	3 (10)	1 (0.4)
	Moderate (3-5)	0 (0)	0 (0)
	Severe (6-10)	0 (0)	0 (0)
Cost per dressing ****	38 PKR	10 PKR	

Table 1. Demographics of patients in both groups & Comparison of results in both groups. *Average wound measurement at present (L × W × D, cm); **Average epithelialization rate (mm/day); *** Not infected (1 mm/day) Infected (0.6 mm/day); **** Average recommended dose/ dressing is 5 mL. PKR = Pakistan rupees. MRSA = Methicillin-resistant *Staphylococcus aureus*.

and its management [17-20]. Anti-inflammatory agents helps to accelerate wound healing [21] and allicin and rutin are well reported for anti-inflammatory potential [21,22]. Among phytochemicals, flavonoids and tannins are known to boost wound healing mechanism mainly due contraction of the wound and enhanced rate of epithelization [23]. Rutin has been reported to be responsible for increased tissue repair in incised wounds [13] and also involved in stimulation of human skin fibroblast collagen [23]. While in the diabetes group new vasculature is the most important factor the 2nd phase of wound healing, TCDO/RAG which stimulates production of macrophages, generate chemotectic impulses which results in wound contraction and interns fibroblasts ends up with collagen formation and mitogenic impulses increase neo vascularization, so quick to respond to diabetic foot ulcers, chronic and resistant wounds [24]. Both TCDO and RAG are aqueous based solutions which moistens the wound, moreover, TCDO /RAG contains bioactivated oxygen carriers which break the vicious cycle of hypoxia in wounds that promotes phygocytic activity [25].

CONCLUSION

The wound healing potential of this preparation may be due to the synergistic effect of phytoconstituents allicin and rutin. Their cumulative

effect may be responsible for quick and effective wound healing. The application of RAG, produced efficient and had a quick response by affecting multiple pathways. Both TCDO and RAG are safe to use.

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