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RESEARCH ARTICLE

PRE-CLINICAL EVALUATION OF DCL2016 OINTMENT ON SUPERFICIAL BURN WOUND HEALING IN RATS

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ABSTRACT

Objective: To evaluate the efficacy and safety of DCL2016 ointment on superficial burn wound healing in rats.

Materials and Methods: Animals were divided into five groups. Partial burn wound was inflicted on shaven back of anaesthetized rats by exposing it to hot water (72oC for 12sec). The wounds in the five groups of rats were treated topically with Silver sulfadiazine (SSD) 1%, DCL 2016 ointment with lead, DCL 2016 ointment without Lead, Only Lead and the control group was treated with castor oil base. Application of ointment was done till re-epithelization of skinor upto 21 days whichever was earlier.

Results: The percentage of wound contraction was significantly ($p<0.05$) increased in SSD, DCL2016 ointment with lead and only lead treated groups as compared to control groups. The period of re-epithelization was also significantly decreased in these groups as compared to control group. The Hb count was lower in only lead and DCL2016 with lead treated group as compared to control group. Histopathological results showed no signs of microscopic infection and hence showed better healing ability of the ointment. Determination of lead in blood by Atomic Absorption Spectrophotometer (AAS) indicated that the blood lead level in all groups were not significantly different as compared to control group animals.

Conclusion: Incorporation of lead within the said limits with DCL 2016 ointment shows enhanced healing process in superficial burn wounds in rats.

INTRODUCTION

Burn can be defined as tissue damage caused by variety of agents such as heat, chemicals, electricity, sunlight or nuclear radiation (Shrivastava *et al.,* 2008). The most common are burns caused by scalds (lesions produced by moist heat), fire, flammable liquids and gases (Gupta *et al.,* 2016). Burn injuries to skin result in loss of its protective function and act as a barrier for microorganisms leading to high risk of infection (Bingham *et al.,* 1995). Burns are one of the most widespread injuries all over the world. In the United States, more than 1 million burn victims need medical attention every year, but only, 4.5% of them require hospitalization (Demling *et al.,* 2002). Similar situations exist in United Kingdom, where burns comprise 1% of work load in emergency wards as well as 0.014% of hospitalization. Thus, most burns are not severe and could be managed outside the hospital (Wilkinson *et al.,* 1998).

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According to World Health organization (WHO), around 3,00,000 deaths are estimated per year worldwide due to burns (Mathers *et al.,* 2004). Despite the discovery of vast spectrum of antiseptics, burn healing still remains a challenge to modern medicine (Chaudhari *et al.,* 2006). In Ayurveda abundant research has been envisaged to develop better healing agents (Sinha *et al.,* 2004). Several drugs of plant origin like *Tinospora cardifolia* (Meravanige *et al.,* 2012), *Moringa oleifera* (M.Khan *et al.,* 2004), *Bryophyllum pinnatum* (B.rathi *et al.,* 2004),*Allium cepa* (C.Shenoy *et al.,* 2009), *Kaemperia galangal* (Shanbhag *et al.,* 2006), *Aloe ferox* (Y.Jia *et al.,* 2008), *Rubus species* (Suntar *et al.,* 2011), *Ficus religlosa* (Roy *et al.,* 2009), *Hyptis suaveolens* (Shenoy *et al.,* 2009), *Thespia populnea* (Nagappa *et al.,* 2009), *Morinda cardifolia* (SNayak *et al.,* 2009), *Memocylon edule* (Naulkaew *et al.,* 2009), *Trigonella foenum* (Taranalli *et al.,* 2009),minerals like zinc, iron and certain vitamins like Vit-C, Vit-A are described for wound healing process and are found to be effective (Meravanige *et al.,* 2012). The most prevalent topical treatment for partial thickness burns is 1% Silver Sulfadiazine (SSD). SSD is a topical agent of choice for severe burns and is used almost universally today in preference to compounds

such as Silver nitrate and Mafenide acetate (Taddonio *et al.,* 1990 and Beheshti *et al.,* 2013). Silver Sulfadiazine in spite of being effective, causes some systemic side effects consisting neutropenia, erythema multiforme, crystalluria and methemoglobinemia (Gracia, 2011). Topical agents which are used only as antimicrobials include Silver Nitrate, Sulfamylon and a combination of Sulfonamide and SSD. Sulfamylon has broad spectrum activities, but is easily absorbed systemically and can lead to toxic complications. SSD has become the standard topical treatment for burn wounds (Beheshti *et al.,* 2013 and Gracia, 2011).

One of the potential burn dressings is Sucralfate. Sucralfate is basic aluminium complex of sucrose sulfate and a Cytoprotective agent. There are many other topical antimicrobial agents which are used to treat superficial burns such as Pirfenidone (Barrangan *et al.,* 2010), Chlorhexidine, Mafenide acetate, Povidone-Iodine ointment, Phenytoin, Minoxidil gel, gentamicin sulfate, Bismuth impregnated petroleum gauze, Honey, Dakin's solution (0.025% sodium hypochlorite) (Moghimi *et al.,* 2009).

Silver Sulfadiazine 1% cream is soft, white, water miscible cream containing the antimicrobial agent silver sulfadiazine in micronized form. Each gram of Silver Sulfadiazine cream 1% contains 10mg of micronized silver sulfadiazine. The cream vehicle consist of white petroleum, stearyl alcohol, isopropyl myristate, sorbiton monooleate, polyoxy 40 stearate, propylene glycol and water, with methyl paraben 0.3% as preservative. Silver Sulfadiazine cream 1% spread easily and can be washed off readily with water (Jarret *et al.,* 1978). Regardless of its wide spread use SSD is associated with several limitations such as it delayed separation of scar, delayed reepithelization, development of post burn contractures, skin necrosis, erythema burning sensation, rashes, interstitial nephritis. SSD is also contraindicated in patients who are hypersensitivity to sulfadiazine (Purohit *et al.,* 2003). Due to such limitations in modern therapy interest in polyherbal products is growing very rapidly which is has promising results with less side effects. Solanum nigrum*:*

Solanum nigrum (Black nightshade, Sn) is an African paediatric plant belonging to the family of Solanaceae. Solanum nigrum possess some essential pharmacological activities such as hypolipidemic activity, hypotensive activity, anti-cancer activity, anti-convulsant activity, anti-oxidant and anti-inflammatory activity etc (F.O.Atanu at al, 2011). In oriental medicine *Solanum nigrum* (Solanaceae) has been used for the treatment of inflammation and oedema (K.S.Heo *et al.,* 2004). In Egypt the healing effect of this plant in burns and in infections has been mentioned (F.Abas *et al.,* 2006). Iranian traditional medical (ITM) scholars believed that aqueous extract of *S.nigrum* leaves are astringent and restraint, so it has been used as a swelling reliever in ITM burn prescription with Malva sylvestris or other ingredients (Fahimi *et al.,* 2015).

In view of the paucity of information of *S.nigrum* on wound healing the study was intended to explore the influence of *S.nigrum* on the process of wound healing. Hence, the aim of the present study was to investigate the influence of DCL2016 on superficial burn wound in rats.DCL2016 ointment is polyherbal product consisting of *Solanum nigrum* (Decoction-68.97% and Pulp-4.31% was used), *Garcinia indica* (Oil-8.62%), Ricinus communis (17.24%), and lead oxide (0.86%).

MATERIALS AND METHODS

Animals

Healthy male Wistar rats weighing 150-200 g were used for the study. Each rat was housed individually and maintained on normal diet and provided with water *ad libitum*. The study was conducted after obtaining the approval of Institutional Animal Ethical Committee.

Chemicals

DCL 2016 ointment (Table-1), Castor oil base and ketamine were procured from Ayurvedic Sanshodhan Vibhag (Nashik, India), Diazepam and Silver sulfadiazine were obtained from Surya Hospital Pharmacy (Nashik, India). DCL2016 ointment with lead, DCL2016 ointment without lead, and only lead were the different forms of this ointment used for topical application in burn wound rats in our study.

Study design

Group 1- Control groups, Animals were treated with Castor oil base (n=6).
Group 2- Animals were treated with DCL2016 ointment with lead (n=6).
Group 3- Animals were treated with DCL2016 ointment without lead (n=6).
Group 4- Animals were treated with only lead ointment (n=6).
Group 5- Animals were treated with 1%Silver sulfadiazine (n=6).

The ointment were applied topically daily from the 1stday wound till 21st day or till complete epithelization, whichever was earlier.

Burn wound

Animals were anesthetized using a combination of ketamine (100mg/kg, i.p) (Kahkeshani *et al.,* 2013) and Diazepam (3mg/kg, i.p) (Valsh *et al.,* 2012). Depilator cream was used to shave the back of the animal. Shaven back of the animal was exposed to hot water at 72^{0}C for 12 sec and were housed individually on standard pellet diet with water *ad libitum*. The following parameters were observed in the study.

- ***Percentage of Wound contraction*:** It was monitored by noting the progressive changes in wound. Tracings were taken on 3rd, 7th, 14th and 21st day on transparent paper. The tracings were transferred to 1mm^2 graph sheet, from which the wound surface area was evaluated. The calculated surface area was then used to calculate the percentage of burn wound contraction by using following formula, (Bairy *et al.,* 2012)

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

- ***Re-epithelization:*** It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind (G.Meravanige *et al.,* 2012)
- ***Determination of Hb-count and lead in blood by Atomic absorption spectrophotometer (AAS):*** At the

end of the experiment, the rats were anesthetized their blood sample was withdrawn from carotid bleeding. Hb count was determined by Sahli's haemoglobinometer (T.Srivastava *et al.,* 2014). The plasma was separated from whole blood using high speed centrifuging machine (Remi Research Centrifuge machine, R-24) at 3000rpm for 10 minand analysed for presence of lead using Atomic absorption spectroscopy (using Inductive couple plasma method).

- ***Histopathological evaluation:*** After complete epithelialization, the rats were sacrificed and the wound bed was dissected out, preserved in 10% formalin. Then it was embedded in paraffin wax and cut into 5µm thick sections and stained with Hematoxylene (H)-Eosin (E) stain and observed under the compound microscope at 40X magnification (S.Ghosh *et al.,* 2012).

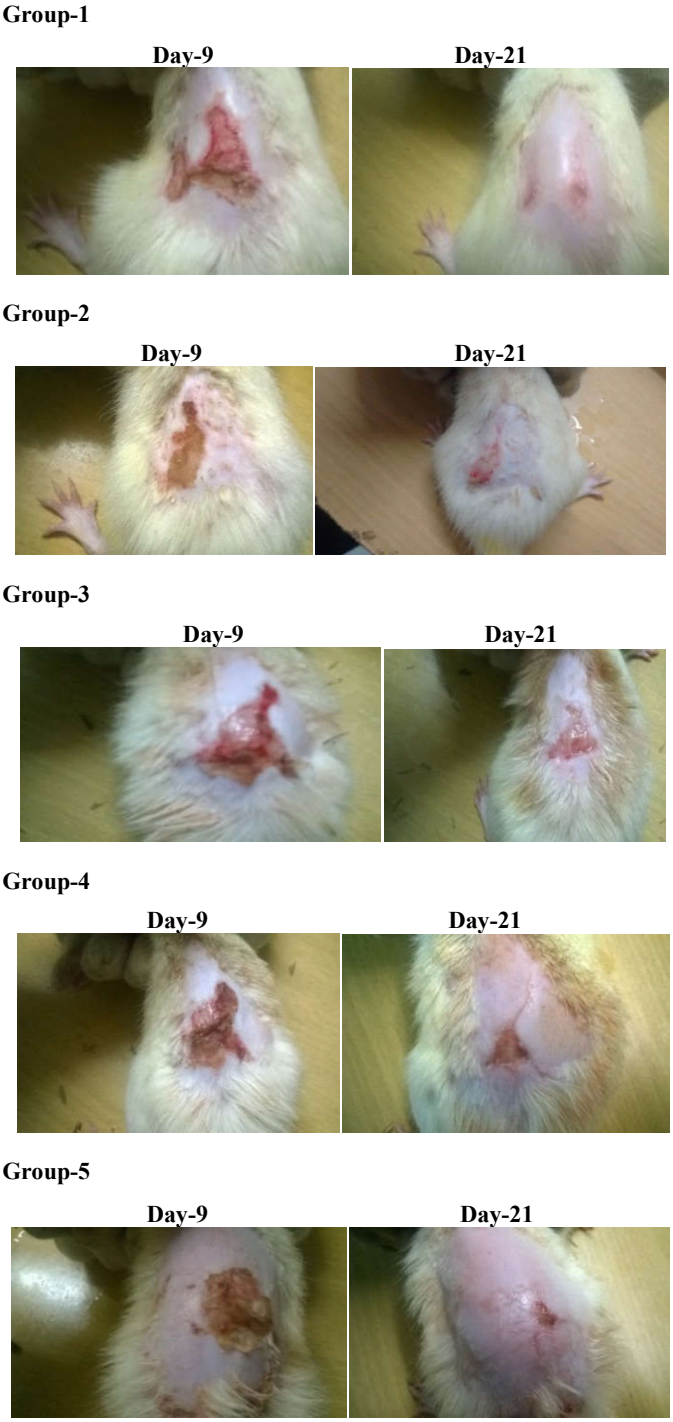


Figure 1. Re-epithelization of burn wound skin tissue

Statistical Analysis

The data were expressed as Mean±SEM and was subjected to one way ANOVA followed by Dunett's test (*p<0.05 is considered significant).

RESULTS

a) ***Percentage of Burn wound contraction*****:** Percentage of burn wound contraction was significantly increased in Group DCL2016 ointment with lead, Plain lead and 1%Silver sulfadiazine as compared to Control Group (Fig-2)

b) ***Re-epithelization*****:** Similarly the period of epithelization was significantly ($p<0.05$) decreased in same groups as compared to control group (Fig-1 and Fig-3).

c) ***Determination of Hb-count and lead in blood by Atomic absorption spectrophotometer (AAS):*** The Hb count was lower in plain lead and DCL2016 with lead treated group as compared to control group (Fig-4). The blood lead level in all groups was not significantly different from the control group (Fig-5).

d) ***Histopathological evaluation*****:** Histopathological evaluation of burn wound samples of all groups were evaluated based on parameters like thickness of epithelium, infiltration of inflammatory cells, thickness of granulation tissue and appendages and vascularity. The study revealed that only lead ointment showed high thickness of epithelium which indicated good healing of burn wound, similarly infiltration of inflammatory cells and formation of granulation tissue (macrophages, fibroblast) was more in case of lead containing ointments as compared to Control group which showed better signs of wound healing. Vascularity was better observed in SSD group as compared to other groups whereas no changes in appendages formation were found in any groups. (Fig-6).

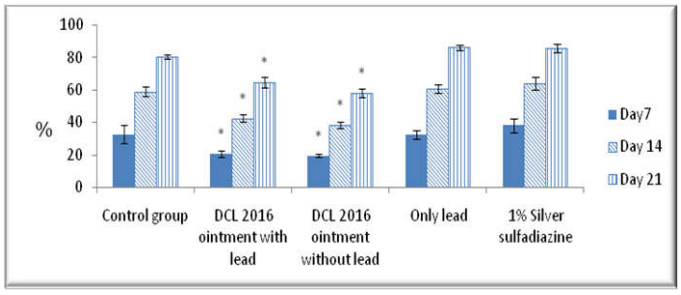


Figure 2. Effect of DCL2016 ointment on percentage of burn wound contraction in rats on day 7, day 14 and day 21. All the data were subjected to one way ANOVA followed by Dunnett's test. P<0.05 is considered significant

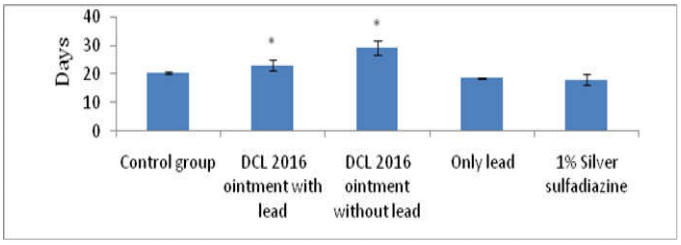


Figure 3. Effect of DCL2016 ointment on period of epithelization of burn wound in rats. All the data were subjected to one way ANOVA followed by Dunnett's test. P<0.05 is considered significant

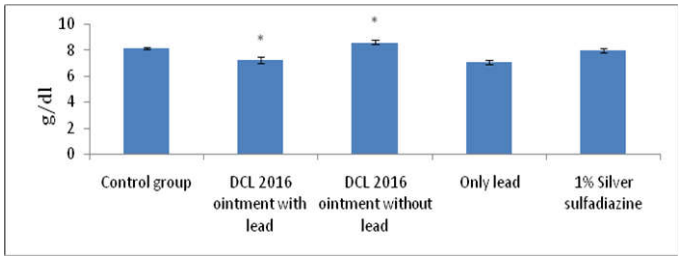


Figure 4. Effect of DCL2016 ointment on Hb count of burn wound rats. All the data were subjected to one way ANOVA followed by Dunnett's test. P<0.05 is considered significant

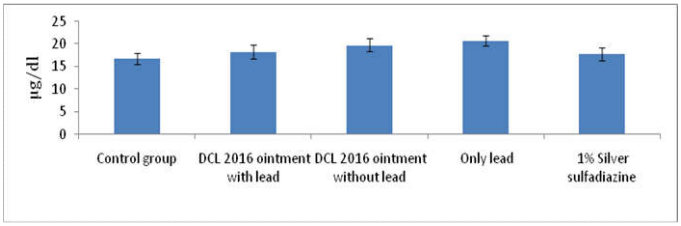
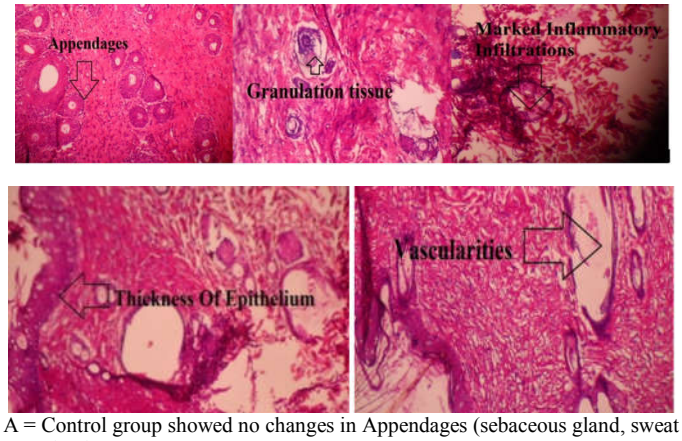


Figure 5. Effect of DCL2016 ointment on level of lead in blood of burn wound rats by AAS.All the data were subjected to one way ANOVA followed by Dunnett's test. P<0.05 is considered significant



A = Control group showed no changes in Appendages (sebaceous gland, sweat gland).
B = DCL2016 ointment with lead group showed abundant granulation tissue.
C = DCL2016 ointment without lead group showed marked infiltration of inflammatory cells.
D = Only lead group showed marked Thickness of epithelium as compared to control group and showed better sign of wound healing.
E = 1% Silver sulfadiazine group showed better Vascularity as compared to control group and showed better sign of wound healing.

Figure 6. Microphotographs of burn skin tissue stained with H and E (40X)

DISCUSSION

Burn wound healing is a complicated process occurring in injured tissue to restore its construction and return the damaged tissue to its normal situation as soon as possible (Midwood *et al.,* 2004). Burn wound healing process involves three phases viz, Inflammatory phase (it involves migration of neutrophils and monocytes into surrounding tissues which is characterized by vasodilation, fluid extravasation and edema. Cells involved in the phase are neutrophils, monocytes and macrophages), Second phase is proliferative phase (This phase is characterized by wound closure and revascularization. Cells involved in the phase are kerationocytes, fibroblast, macrophages, and lymphocytes) (Matthew *et al.,* 2015). Third phase in healing process of superficial burn is remodeling phase which is characterized by wound maturation and scarring of injured tissue. Cells involved in third phase are elastin, collagen, and fibroblast (Keast *et al.,* 1988). Healing process not only re-establishes the structural and functional integrity but also regains the strength of an injured skin tissue (Thang *et al.,* 2001). Oxidants are inhibitory factors to wound healing due to their cell damage ability. Studies of topical application of compounds with free radical scavenging properties on patients or animals have shown significant improvement in wound healing and protect tissue from oxidative damage. Antioxidants could also play an important role in promotion of wound healing (Pathak *et al.,* 2013). The present study was designed to investigate the influence of polyherbal product combined with lead on experimentally induced superficial burn wound in Wistar rats. The results demonstrate and confirm the efficacy and safety of DCL2016 ointment on burn wound healing in rats.

The most common topical product used for treatment of burn injuries is Silver sulfadiazine. The silver ion binds to the organism DNA and consequently releases the sulphonamides that kill the microbes. The antimicrobial efficacy of this agent is the main reason for its widespread use in burn wounds. However delayed re-epithelization, delayed separation of scar wound healing, development of post burn contractures are some of the most important clinical adverse effect of silver topical agents which limits their long term use especially on broad wound. Therefore the concurrent use of *S. nigrum* cream may be suggested during the early stages of burn treatment (Kiran *et al.,* 2008). Healing of burn wound involves infiltration of inflammatory cells, granulation tissue formation, restoring the thickness of epithelium, appendages formation (sebaceous and sweat glands), synthesis of extracellular matrix proteins, formation of collagen and remodeling (Shuid *et al.,* 2005). Lysosomal enzymes from neutrophils, free radicals, leukotrienes and prostaglandins released during this process can cause tissue damage. Free radicals act on important components of cells such as lipids, proteins, carbohydrates and DNA resulting in cellular damage and cell death. Free radicals have unfavorable effects on wound healing, granulation tissue formation, along with collagen and cartilageneous tissue (Keskin *et al.,* 1999). The healing effect of DCL2016 ointment may be due to its several mechanisms such as re-epithelization, destruction of free oxygen radicals, inflammation reduction and control of infection through the antioxidant, anti-inflammatory and anti-microbial property of the plants used in the cream. Thus in conclusion DCL 2016 formulation was found to enhance the healing process both histopathologically and statistically. Thus on account of its antimicrobial, antioxidants, and anti-inflammatory effects, DCL2016 can be used as an adjunct to existing healing therapies in future.

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