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Evaluation of the Wound Healing Potential of *Trichodesma zeylanicum* (Burm. f.) Formulation in Excision Wounds in Albino Rats

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Author's contribution

This work was carried out by the author FN.

Original Research Article

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ABSTRACT

Aim: The study aimed at evaluating in vivo wound healing effect of herbal ointment formulated with 15% w/w *Trichodesma zeylanicumm* methanolic root extract, plant commonly used as traditional medicine.
Methodology: The wound healing potential of *T. zeylanicumm* was assessed using excision wound model and various biochemical parameters; L-hydroxyl, Hexose amine, Malondialdehyde and Ascorbic acid. Treatments were administered daily topically to three groups of albino rats: Ointment base only, 15% w/w *T. zeylanicumm* methanolic ointment and Neosporin ointment for 20 days.
Results: The results showed wound closure was slow in albino rats treated with 15% w/w *T. zeylanicumm* methanolic extract ointment but increased gradually to 76.95% on day 17, while there was steady increase for Neosporin ointment treated group (from 36.01% on day 5 to 92.89% on day 17). Wound contraction ability of the herbal ointment was significantly greater $p<0.01$ on 13th day. The Neosporin ointment treated group had short epithelialization time (19.33±1.53) compared to herbal ointment treated group (21.33±3.06). The levels of mucopolysacharide content in the herbal ointment treated group were significantly decreasing $P<0.05$(from 2.03±0.11 to 1.17±0.13 on 4th to 12th day respectively), while collagen content levels were significantly increasing $P<0.05$ in the herbal ointment treated group (2.59±0.10 and 2.63±0.1 on 8th and 12th day). However, the levels of mucopolysacharide and collagen contents were significantly higher in the

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Neosporin ointment than in the herbal ointment treated group. For the blood plasma samples, the malondialdehyde levels were significantly much higher in the herbal ointment treated group (1.74±0.13) compared to Neosporin ointment treated group (1.55±0.08). The ascorbic content levels were significantly higher in the Neosporin ointment sample (0.92±0.26) compared to herbal ointment treated group (0.64±0.14).
Conclusion: The results showed that *T. zeylanicumm* methanolic root extract has wound healing potential; however, further clinical and toxicological experimentation is needed to scientifically validate its use as a topical ointment.

Keywords: 15% w/w T. zeylanicumm methanolic ointment; excision wound model; Neosporin ointment; wound closure; biochemical parameters.

ABBREVIATIONS

ANOVA: Analysis of variance; PDAB: Para Dimethyl Amino Benzaldehyde; DTC: dithiocarbamate, TCA: Trichloroacetic acid.

1. INTRODUCTION

Wound is an injury, especially one in which the skin or another external surface is torn, pierced, cut or otherwise broken with disruption of normal continuity of structure [1]. Wound healing is an important biological process involving tissue repair and regeneration. Wound healing can be classified into any of the three types – healing by first intention, healing by second intention, healing by third intention, depending on the nature of the edges of the healed wounds [2,3].

There are four distinct stages involved in wound healing namely: inflammatory stage, debridement stage, proliferation stage and maturation/remodelling stage. When injury occurs, the vascular integrity of the injured area is disrupted leading to extravasations of blood into the surrounding tissue or plasma when the damage is minor. The inflammatory stage is directed at preventing further loss of blood by platelet adhesion/accumulation at the site leading to coagulation that results to the formation of thrombus. The debridement stage occurs from the third to the sixth day after injury and involves the appearance of neutrophils to clear contaminating organisms. The proliferation or repair stage is characterized by endothelial budding in the nearby blood vessels forming new capillaries that penetrate and nourish the injured tissue. The maturation stage commences from the tenth day to several months depending on wound severity during which the number of capillaries decreases and wound changes from pink to white [3,4].

Currently, research on wound healing agents is one of the developing areas in modern biomedical science and many traditional health practitioners across the world have valuable information of many lesser known wild plants for treating wounds and burns [5].

In Malawi, many plants are used in the treatment of wounds, and *Trichodesma zeylanicumm* leaves and roots have extensively been reported by the local communities to treat wounds. The roots are also used for treating diarrhoea while leaves are also eaten as vegetables. Ngonda, [6] reported that *T. zeylanicumm* powdered roots have anti-oxidant and free radical scavenging properties. According to Gurib-Fakim et al. [7], *T. zeylanicumm* powdered roots are applied externally on wounds and skin as analgesic. And in 1984, Msonthi isolated

squalene and other known phytosterols compounds from the leaves and recommended the plant as a good source of steroidal hormone precursors because of the high yield. The plant has also been reported to contain the low toxic alkaloids supinine [8].

Trichodesma zeylanicumm belongs to the family Boraginaceae, and it is a densely bristly-hairy annual herbal plant that can grow up to 1 metre. Leaves are narrowly elliptic, while flowers becomes nodding, in terminal 1-sided bracteates inflorescences. Sepals are bristly hairy enlarging in fruit. Corrolla (7-9mm), are scarcely exserted from the sepals, lobes pale blue to lilac or pinkish [9].

This study was aimed at investigating the wound healing effect of methanolic extract of *T. zeylanicumm* powdered roots in albino rats in order to understand the usefulness of the plant in the treatment of wound infections.

2. METHODS

2.1 Collection and Identification of Plant Material

The fresh plants were collected and authenticated by Mr. Patel of National Herbariums and Botanical Gardens of Malawi with voucher number 18930. The root parts were washed with water, shade dried powdered in a mechanical grinder and kept in air tight polythene bag until use.

2.2 Preparation of the Plant Extract

The powder (50g) of the root parts was initially be de-fatted with petroleum ether (60-80ºC), followed by 250ml methanol by Soxhlet extraction method for 72hrs. Solvent elimination under reduced pressure afforded the petroleum ether and methanol extract of which methanol extract was further be used for the study. The extract was dried in vacuum desiccators to obtained constant weight. The extracts were then kept in sterile bottles, under refrigerated conditions at appropriate temperature, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and determines the concentration in mg/ml.

2.3 Ointment Preparation for Topical Application

Alcohol free extract was used for the preparation of the ointment for topical application. A 15% w/w *T. zeylanicumm* methanolic extract ointment was formulated using soft white paraffin base. Neosporin® ointment (neomycin and polymyxin B sulfates and bacitracin zinc ointment, USP Antibiotic, DIN 00666122, GlaxoSmithKline Inc.) was used as control formulation.

2.4 Experimental Animals

Albino rats of either sex, weighing about 220±40g were used in the study. The albino rats were housed in cages maintained under standard housing conditions (12 hours light-dark cycle; 25±3°C; 60-65% humidity).

2.5 Wound Models

The study was carried out using ether-anesthetized rats and their back shaved.

2.5.1 Excision wounds

A circular skin piece of full thickness was removed from the predetermined dorsal area. The wound traced on 1 - mm² graph paper on the day of wounding and subsequently on alternative days until healing is completed. Rats were left undressed to the open environment- to monitor wound contraction and epithelisation time [10]. Standard drugs (Neosporin® ointment), simple ointment base, 15% w/w *T. zeylanicumm* methanolic extract ointment were applied every day till the wound was completely healed.

2.5.1.1 Chemicals and reagents

All the chemicals and reagents were obtained from Merck Chemicals (PTY) LTD, Germany, Associated Chemical Enterprises, SAARCHEM (PTY) LTD and British Drug House (BDH). A standard drug (Neosporin® ointment, neomycin and polymyxin B sulphates and bacitracin zinc ointment, USP. DIN 00666122) was purchased from GlaxoSmithKline Inc., Montreal (Quebec).

2.5.1.2 Measurement of wound area

The progressive changes in wound area were measured planimetrically by tracing the wound margin on a graph paper every alternative day. Changes in healing of wound i.e. the measurement of wound on the graph paper were expressed as unit (mm²). The wound contraction expressed as percentage reduction of original wound size.

$$\%\text{wound contraction} = \Delta\text{healed area/Total area} \times 100$$

2.5.1.3 Methods

18 albino rats used for excision wound model and the ointment applied topically. Animals will be divided into the following groups:

Group 1: Ointment base applied and served as vehicle control
Group 2: 15% w/w *T. zeylanicumm* methanolic ointment will be applied once daily
Group 3: Neosporin ointment will be applied once daily

Six animals will be in each group. Treatment started on the day of operation and continued till the 20th day of healing. On 2, 5, 7, 9, 13, 15, 17, and 20th day. The wound area of each rat traced on a graph paper and measured with the help of planimeter.

2.5.1.4 Biochemical analysis of wound tissue

The animals were anaesthetized on 4, 8, and 12 day after treatment.

2.5.2 Collection of granulation tissue

Granulation tissues from both control and treated rats were collected, washed well in cold saline (0.9% NaCl) to remove blood tissues and stored for analysis of various parameters. Granulation tissues were lyophilized for collagen and hexose amine analysis.

2.5.3 Collection of blood sample

Blood sample collected by cervical decapitation and sterile syringe rinsed with EDTA were used to collect blood. Plasma was separated for malondialdehyde and ascorbic acid estimation.

2.6 Biochemical Parameters

2.6.1 L-Hydroxy Proline

Samples of varying concentrations were used for analysis. Hydroxyl proline was oxidized by adding 1ml of chloramines T to each tube. The contents mixed thoroughly by shaking and allowed to stand for 20min at room temperature. 1ml of 70% perchloric acid was added to each test tube then destroyed the Chloramine T. The contents mixed and allowed to stand for 5min and finally, 1ml of PDAB (Para Dimethyl Amino Benzaldehyde) solution was added and the mixture shaken well. The color developed was read spectrophotometrically at 557nm. The collagen content was calculated by multiplying the hydroxyl proline content by the factor 7.46 and expressed as mg/100mg of dry weight of the sample [11].

2.6.2 Hexose Amine

Samples of varying concentrations were used for analysis. The solution treated with 1ml of freshly prepared 2% acetylacetone in 0.5M Na_2CO_3 in capped tubes and kept in boiling water bath for 15min. After cooling in tap water, 5ml of 95% ethanol and 1ml Ehrlin's reagent (dissolving 2.0g of p-dimethylaminobenzaldehyde in 50ml of 95% ethanol and 50ml of concentrated hydrochloric acid) was added and mixed thoroughly. The purple red color developed read after 30min at 530nm [12].

2.6.3 Malondialdehyde

2ml of 0.67% thiobarbituric acid reagent was mixed with 0.1ml sample and 0.9ml of 10% TCA and kept in boiling water bath for 20min. The tubes cooled after centrifugation and the absorbance of the supernatant read at 532nm [13].

2.6.4 Ascorbic acid

0.5ml of plasma was added to 0.5ml of ice cold 10% TCA and mixed thoroughly and centrifuged for 20min. 3500g supernatant (0.5ml) mixed with 0.1ml of DTC (dithiocarbamate) reagent and incubated at 37°C for 3hrs. Then 0.75ml of ice cold 65% H_2SO_4 added and allowed to stand at room temperature for 30min. The yellow color developed read at 520nm. Ascorbic acid was used as standard [14].

2.7 Statistical Analysis

The Microsoft Excel was used in the analysis and all experimental measurements were carried out in triplicate. The value expressed as mean±standard deviations and The Dunnett one way analysis (ANOVA) was used to determine the significant differences among all columns against control and the *p*-value <0.05 was considered as significant.

3. RESULTS

During the initial stages of the study, the wound closure from Day 0 [Fig. 4(a,b)] was slow in Albino rats treated with 15% w/w *T. zeylanicumm* methanolic (methanol was used as solvent because it has greater extraction power for natural substance with low molecular weight) extract ointment but increased considerable to 15.35% on day 5 [Fig. 5(a,b)], 22.56% on day 7, 64% on day 13 and 76.95% on day 17 [Fig. 6(a,b)]. Similarly, the negative control, base ointment, increased to 12.85% on day 5 and 7, 36.01% on day 9 and 46.21% on day 17 (Fig. 1). However, there was significant increase for the Neosporin ointment treated sample in wound closure percentage from 36.01% on day 5, 46.21% on day 9, 55.55% on day 13 and 92.89% on day 17 (Fig. 1). Wound contraction ability of the ointment containing 15% w/w *T. zeylanicumm* methanolic extract was significantly greater $P<0.001$ on 13th day compared to the control, Neosporin ointment, and showed some significant wound healing potential from the fifth day onwards comparable to the standard control. However, the Neosporin ointment treated rats had the short epithelialization time (19.33±1.53) compared to 15% w/w *T. zeylanicumm* methanolic extract (21.33±3.06) and negative control of base ointment (25.66±2.08) [Table 1]. The results shows that percentage closure of wound area was significantly increasing by the curative effect.

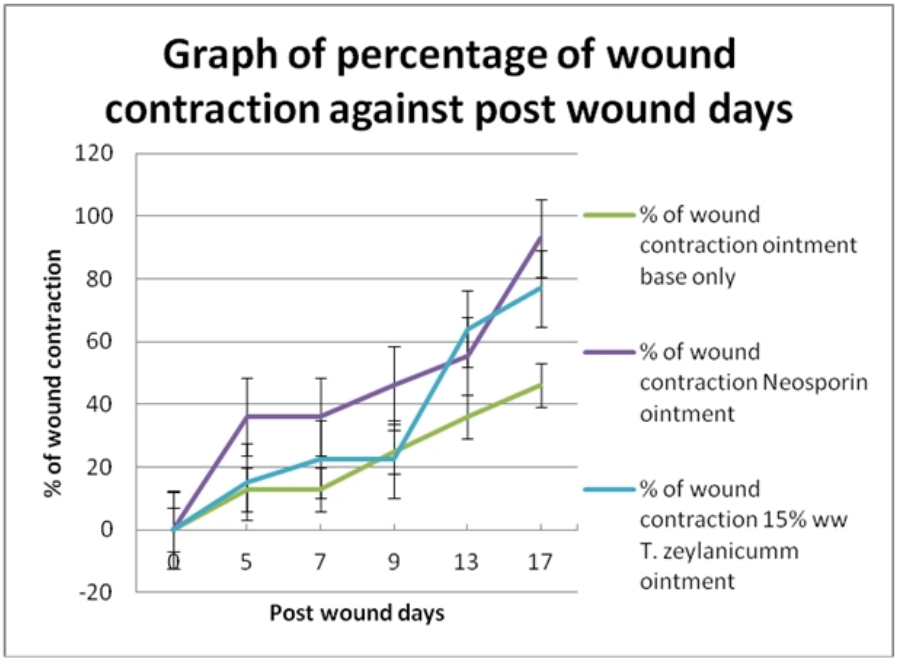

Fig. 1. Graph of percentage of wound contraction against post-wound days

Table 1. Percentage of wound contraction against post-wound days

Post wound days	Wound area (mm²) and percentage of wound contraction		
	Ointment base only	Neosporin ointment	15% w/w *T. zeylanicumm* methanolic extract
0	353.2±3.14	353.2±3.14	490.6±2.543
5	307.8±0.785 (12.85%) ***	226.0±2.543 (36.01%)	415(15.35%) ***
7	307.8±2.543 (12.85%) ***	226.0±6.154 (36.01%)	379.9±2.543 (22.56%) ***
9	265.4±6.154 (24.86%) ***	190.0±2.543 (46.21%)	379.9±0.785 (22.56%) ***
13	226.0±4.521 (36.01%) ***	157.0±3.14 (55.55%)	176.6±6.154 (64%) ***
17	189.98±3.14 (46.21%) ***	25.126%±2.543 (92.89%)	113.04±2.543 (76.95%) ***
Epithelization period (Days)	25.66±2.08	19.33±1.53	21.33±3.06

****P<0.01 compared to control. Values are mean±SEM (n=6)*

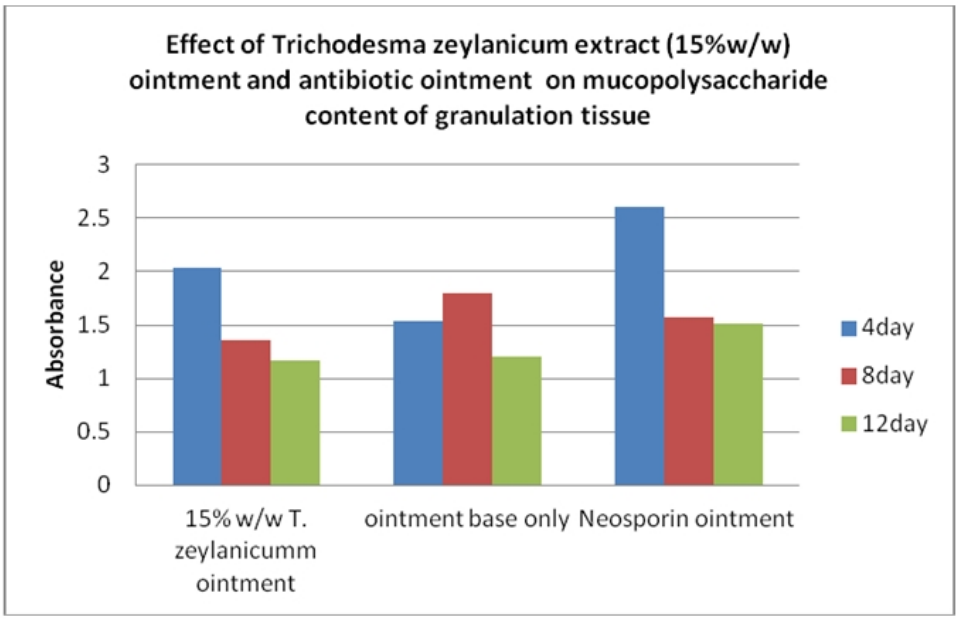

Fig. 2. Graph of effect of *Trichodesma zeylanicum* methanolic extract and Neosporin ointments on mucopolysaccharide content of granulation tissue

It was observed from the study that the levels of mucopolysacharide content in the albino rat topical treated with 15% w/w *T. zeylanicumm* methanolic extract and base ointment were significantly decreasing $P<0.05$ (from 2.03±0.11 to 1.17±0.13 and from1.53±0.34 to 1.2±0.12 on 4th to 12th day respectively) [Table 2]. However, comparatively, the levels of mucopolysacharide content were significantly higher in the Neosporin ointment than in the sample topical treated with 15% w/w *T. zeylanicumm* methanolic extract and base ointment only (Fig. 2).

Table 2. Effect of *Trichodesma zeylanicum* methanolic extract and Neosporin ointments on mucopolysaccharide content of granulation tissue

Treatment	Mucopolysaccharides content in granulation tissue on post wound day(Mean±SEM)		
	4TH	8TH	12TH
15% w/w *T. zeylanicumm* methanolic extract	2.03±0.11***	1.36±0.29	1.17±0.13***
Ointment base only	1.53±0.34***	1.8±0.21***	1.2±0.12***
Neosporin ointment	2.61±0.16	1.57±0.26	1.51±0.03

****P<0.05 compared to control. Values are mean±SEM (n=6)*

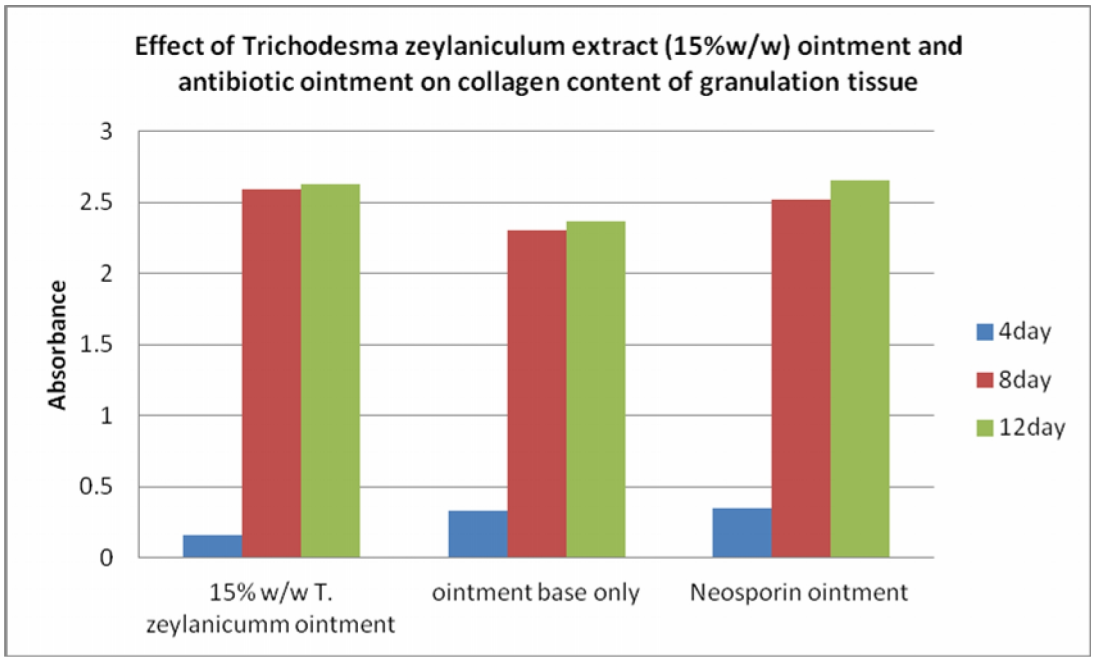

Fig. 3. Graph of effect of *Trichodesma zeylanicum* methanolic extract and Neosporin ointments on collagen content of granulation tissue

The levels of collagen content were significantly increasing $p<0.05$ for topical treatment group of 15% w/w *T. zeylanicumm* methanolic extract (2.59±0.10 and 2.63±0.1 on 8th and 12th day) [Table 3]. However, the levels of collagen was slightly higher in the Neosporin ointment sample compared to the sample treated with 15% w/w *T. zeylanicumm* methanolic extract and base ointment only(Fig. 3).

Table 3. Effect of *Trichodesma zeylanicum* methanolic extract and Neosporin ointments on collagen content of granulation tissue

Treatment	Collagen content in granulation tissue on post wound day(Mean±SEM)		
	4TH	8TH	12TH
15% w/w *T. zeylanicumm* methanolic extract	0.16±0.12***	2.59±0.10***	2.63±0.14***
Ointment base only	0.33±0.13	2.3±0.16	2.37±0.12
Neosporin ointment	0.35±0.21	2.52±0.12	2.66±0.21

****P<0.05 compared to control. Values are mean±SEM (n=6)*

For the blood samples, it was observed that malondialdehyde levels were significantly much higher in the sample of topical treatment group of 15% w/w *T. zeylanicumm* methanolic extract (1.74±0.13) compared to control, Neosporin ointment sample (1.55±0.08). However, ascorbic content levels were significantly higher in the Neosporin ointment sample (0.92±0.26) compared to sample of topical treatment group of 15% w/w *T. zeylanicumm* methanolic extract (0.64±0.14) [Table 4].

Table 4. Estimation of malondialdehyde and ascorbic acid from blood samples

Treatment	Estimation of Malondialdehyde and Ascorbic acid from blood samples (Mean±SEM)	
	Malondialdehyde	Ascorbic acid
15% w/w *T. zeylanicumm* methanolic extract	1.74±0.13***	0.64±0.14***
Ointment base only	1.41±0.14***	0.56±0.12***
Neosporin ointment	1.55±0.08	0.92±0.26

****P<0.05 compared to control. Values are mean±SEM (n=6)*

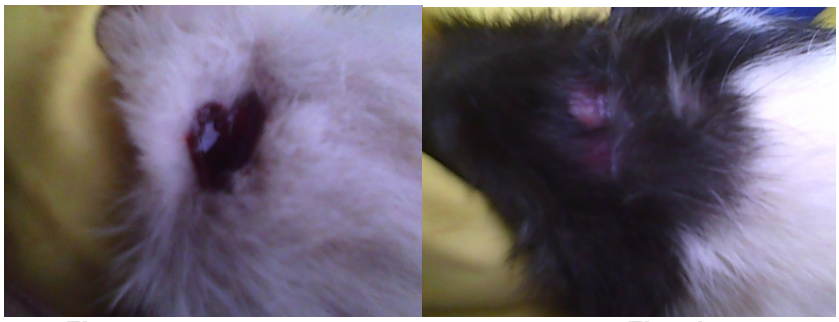

Fig. 4a. **Fig. 4b.**

Figs. 4a & 4b shows excision wound at the back of the rat on day 0 of the experiments

Fig. 5a. Fig. 5b.

Figs. 5a & 5b shows the wound healing in albino rats after treatment

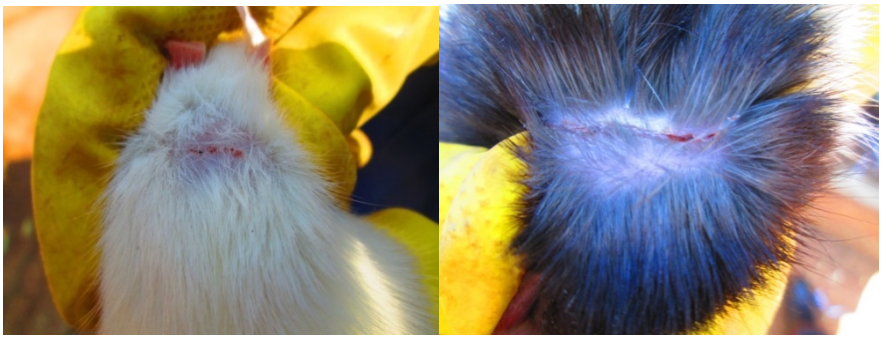

Fig. 6a. Fig. 6b.

Figs. 6a & 6b shows the wound that have almost healed

5. DISCUSSIONS

The results showed that decrease in levels of mucopolysacharide content is associated with an instant increase in the levels of collagen. The mucopolysacharides are made up of repeated disaccharides containing uronic acid and hexosamine. And they are the first components of the extracellular matrix to be synthesized during wound healing and form the template for collagen and elastin deposition. Therefore, increase in mucopolysacharide contents levels of the treated wound might have contributed in matrix synthesis, formation of new tissues which lead to acceleration of wound healing.

The study also found out that there were high levels of malondialdehyde in blood plasma compared to ascorbic acid levels. Malondialdehyde is one of the most frequently used indicators of lipid peroxide. Malondialdehyde is a highly reactive three carbon dialdehyde produced as by product of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. Lipid peroxidation is a well established mechanism of cellular injury in both plants and animals and is usually used as an indicator of oxidative stress in cell and tissues [15]. Therefore, measurement of malondialdehyde is widely used as an indication of lipid peroxide.

The cytokine cascades are activated after an injury with stimulation of phygocytic cells that result in the formation of oxygen free radicals and lipid peroxidation. Production of free radicals at or around the wound bed may contribute to delay in wound healing through the destruction of lipids, proteins collagen, proteoglycan and hyaluronic acid. Agents that demonstrate a significant anti-oxidant activity may therefore, preserve viable tissue and facilitate wound healing [16]. The topical treatment group of 15% w/w *T. zeylanicumm* methanolic extract showed an elevation in lipid peroxidation level which indicates the decrease scavenging capacity of the wounded tissues.

Ascorbic acid is also reported to have free radical scavenging activities and inhibition of lipid peroxidation. Ascorbic acid has been reported to have an unparalleled stimulatory effect on collagen type synthesis and is an important modulator of collagen product and also act as a co-factor in hydroxylation of proline and lysine residue in procollagen [17, 18, 19], therefore it increases collagen deposition. In this study it was observed that ascorbic acid levels were significantly lower in the topical treatment group of 15% w/w *T. zeylanicumm* methanolic extract compared to the Neosporin ointment topical treated group, indicating an increase in lipid peroxidation.

6. CONCLUSION

The results showed that *T. zeylanicumm* methanolic root extract has wound healing potential; however, it can be suggested that further clinical and toxicological experimentation is needed to scientifically evaluate the 15% w/w *T. zeylanicumm* methanolic ointment for possible bioactive effect and assess the potential accumulation of low toxic alkaloid supinine in wound healing.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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