

EVALUATION ON WOUND HEALING PROPERTIES OF ROOT EXTRACT OF *FLEMINGIA STROBILIFERA* (L.) R. BR. IN ALBINO RAT MODEL

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Abstract

Flemingia strobilifera (L.) R. Br. is medicinally important plant which belongs to the family Fabaceae. In this study, the evaluation on wound healing properties of 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. have been conducted in albino rat model. Male albino rats were divided into 3 groups contains 3 replicates each and anesthesia was administered by an intramuscular injection of xylazine and ketamine. The back side skin of the rats were shaved and an excision of 1 cm diameter on the shaved skin were prepared. In this experiment, group 1 was treated with ethanolic root extract, group 2 by standard treatment (Tetracycline ointment) and group 3 as the control. The rate of epithelialization and complete wound closure was daily recorded. A better healing pattern was observed in rats treated with ethanolic root extract and standard treatment (Tetracycline ointment) compared with the untreated control. The epithelialization period of the group 1 and 2 were much shorter than the group 3. Specimens of healed skin at a thickness of 5 μ from each rat were taken at the 20th day of treatment and were fixed in 10% buffered formalin solution for histological studies. These skins were stained with hematoxylin and eosin (H & E) and assessed for histological changes. 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. group was well developed granulation tissues in all layers of skin. According to the results of this experiment, it may be concluded that 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. has the prospective outcome as a new therapeutic agent for wound healing.

Keywords: *Flemingia strobilifera* (L.) R. Br., wound healing, albino rat, a new therapeutic agent

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Introduction

Flemingia strobilifera (L.) R. Br. (Fabaceae), which is known as Wild hops in English and Gaung-own-sar or Say-laik-pya or Pa-lan-phyu in Myanmar. *Flemingia strobilifera* (L.) R. Br. is ethano medicinally used in India and Philippines. It is well known for its medicinal effects and is being used for the treatment of various ailments such as to relieve rheumatism, body pain, fever and indigestion.

Plants and their extracts have immense potential for the management and treatment of wound. The phytomedicine for wound healing are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents (Omale and Isaac, 2012).

Morphological Characters of *Flemingia strobilifera* (L.) R. Br.

Wound healing is a biological process that is initiated by trauma and often terminated by scar formation. Thus, healing is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and function (Suruse, 2011). The process of wound healing occurs in different phases such as coagulation, epithelialization, granulation, collagen formation and tissue remodeling. Animal wound healing models are important biological tools to understand basic process of tissue repair and to develop and validate strategies for treatment of wounds (Pandey, 2012).

Wound healing potential of *Flemingia strobilifera* (L.) R. Br. root has not been experimentally evaluated so far, hence the present investigation was undertaken to study the wound healing property of 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. on excision wound models(Figure 4).

Materials and Methods

Plant Material

Flemingia strobilifera (L.) R. Br. roots were collected from Bago Region (N- 17⁰ 16' 19.4" and E- 109⁰ 28' 15.2") from June 2017 to January 2018. It was identified taxonomically. Herbarium samples have been deposited in the Department of Botany, University of Yangon.

Preparation of Extract

The roots of *Flemingia strobilifera* (L.) R. Br. were collected and dried in shade. The roots were then powdered and extracted with 70% ethanol for a period of 36 hrs in a Soxhlet extractor. The extract was then concentrated and dried.

Experimental Animals

Male albino rats weighing 150-200 g were used. They were housed in standard cages at room temperature ($25 \pm 2^\circ\text{C}$) and provided with food and water. The animals were deprived of food for 24 hrs before experimentation, but had free access to drinking water.

Wound healing studies

Wound healing property of 70% ethanolic root extract was studied on excision wounds using male Albino rats. Animals were divided into 3 groups of 3 each. Group 1 was treated with ethanolic root extract, group 2 by standard treatment (Tetracycline ointment) and group 3 as the control (without root extract). All treatments were made by topical application of ethanolic root extract once a day.

Excision wound model

Hairs were removed from the back side skin of the rats using a blade. Then, an excision of 1 cm diameter on the shaved skin was prepared. The albino rats were divided into three groups and each groups performed three animals. Each rat was anaesthetized with Ketamine hydrochloride and Xylazine and the hair on the back was scrapped off with a pair of curve scissors. This area was disinfected with methylated spirit. An excision of about 1 cm in width area and it was made on the disinfected area of the skin surface (Figure 1 to 3). The wounds Group 1 animals were treated by ethanolic root extract. Group 2 animals were treated on tetracycline ointment. Group 3 animals were kept in their cage without treatment (control). Drugs were topically applied once a day till complete epithelialization, starting from day of excision. Number of days required for falling of scab without any residual raw wound, gave the period of epithelialization (Kodati *et al.*, 2011)



Figure 1. Cleaned and shaved area of albino rats



Figure 2. Excision wound model on albino rat model



Figure 3. Measure the wound size after wound creating

Results

The morphological characteristics of *Flemingia strobilifera* (L.) R. Br. Showing its habit and root were shown in Figure 4 and 5.



Figure 4. Habit



Figure 5. Root

Appearance of excision wound in day 1



Figure 6. 70% ethanolic root extract



Figure 7. Standard drug (Tetracycline ointment)



Figure 8. No Treatment

Appearance of excision wound after 20 days

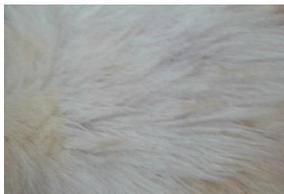


Figure 9. 70% ethanolic root extract



Figure 10. Standard drug (Tetracycline ointment)



Figure 11. No Treatment

Histological Reports - after 20 days evaluation of wound healing activity of rat skin

Specimens of skin from healed wounds from each rat were taken at the 20 days of treatment and were fixed in 10% buffered formalin solution for histological studies. Specimens of the healed skin were made at a thickness of 5 μ and were stained with hematoxyline and eosin (H&E) and assessed for histological changes. The microscopic slides were photographed.

Histological Section of the Skin Tissue from 20 Days Excision Wound Model

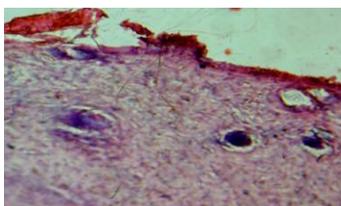


Figure 12. Normal albino rat skin tissue (10X)

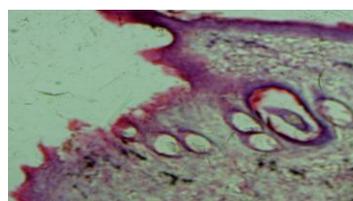


Figure 13. 70% ethanolic root extract treated (10X)

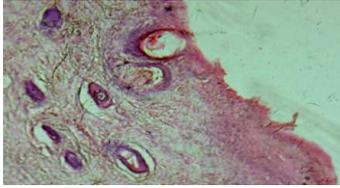


Figure 14. Standard drug (Tetracycline ointment) treated skin (10X)

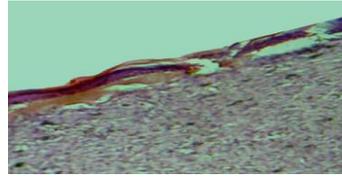


Figure 15. Control (Untreated) skin (10X)

Table 1. Histological report after 20 days of wound healing on Albino rat model

Type of skin	Epidermis	Dermis	Subcutaneous tissue	Histological Diagnosis
Normal Skin Figure 12	The papillary pattern of epidermal outline is thick and composed of squamous epithelium cells, normal keratin layer, a lot of sebaceous glands and hair follicles are also noted.	Granulation tissues consist of fibroblast and collagen tissues are noted.	Small blood vessels and part of muscular layer are also noted.	Normal skin
70% ethanolic root extract treated skin Figure 13	The papillary epidermal out line is well developed and composed squamous epithelium cells and thin layer of keratin. Well developed sebaceous glands and hair follicles are also seen.	Granulation tissue is composed of thin layer of fibroblast and collagen tissues.	Normal blood vessels and muscular layers are well developed.	Well developed granulation tissues in all layers of skin

Type of skin	Epidermis	Dermis	Subcutaneous tissue	Histological Diagnosis
Standard drug (Tetracycline) Figure 14	The papillary epidermal outline is thin and composed of squamous epithelium. Thin keratin layer is also noted. Loss of some sebaceous and hair follicles are present.	Granulation tissue is composed of thick layer of fibroblast and collagen tissues.	Scanty of small blood vessels and muscular layers are also noted.	Appearance of re-epithelialization was nearly normal but vascularization and granulation tissue formation is reduced.
Untreated (control) Figure 15	The papillary pattern of epidermal outline is thin and composed of squamous epithelium cells. In some areas, keratin layer is thin and scanty in site of disrupted areas. Some sebaceous glands and hair follicles are lost.	Granulation tissues consist of fibroblast and collagen tissues are noted.	Small blood vessels and part of muscular layer are also noted.	Normal degree of re-epithelialization of rat's skin.

Discussion and Conclusion

In this study, ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. showed wound healing activity in albino rats. The results of excision wound model were shown in figure (12 to 15) and table (1). According to this findings, the ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. and standard drug (Tetracycline ointment) exhibited significant wound healing activity as compared to control. It is observed that the wound closure time was faster in both treated groups than in the control. Sebaceous glands and hair follicles were more developed in albino rat treated with ethanolic root extract than standard drug (Tetracycline).

The histological result of wound healing was agreed with Min Htun Min Latt (2014) who stated that sebaceous glands and hair follicle were well developed.

It is concluded that 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. showed better wound healing property, which support the traditional use of *Flemingia strobilifera* (L.) R. Br. for treatment of wounds.

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