AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Evaluation of Wound Healing Activity of *Swietenia macrophylla* (Meliaceae) Seed Extract in Albino Rats

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ABSTRACT

The present study was aimed to evaluate the wound healing activity of extract of seeds of *Swietenia macrophylla*. It is well-known plant in Asian traditional medicines. On the basis of traditional use and literature references, this plant was selected for wound healing potential. The ethanolic extract of seeds of *Swietenia macrophylla* was examined for wound healing activity in the form of ointment in the excision wound model using Albino rats. The extract ointments showed efficient response in the excision wound model as comparable to the group treated with standard drug Betadine ointment and control group in terms of wound contracting ability, wound closure time and epitheliallization period. Histological analysis was also consistent with the proposal that *Swietenia macrophylla* seed extract exhibits significant wound healing. *Swietenia macrophyllan* was found to possess significant wound healing property. This was evident by decrease in wound closure ($P<0.005$) and epitheliallization period.

Keywords: *Swietenia macrophylla*, Wound healing, Betadine, ethanolic extract

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Received 11 August 2017, Accepted 31 August 2017

Please cite this article as: Nilugal KC *et al*., Evaluation of Wound Healing Activity of Swietenia macrophylla (Meliaceae) Seed Extract in Albino Rats. American Journal of PharmTech Research 2017.

INTRODUCTION

Wound is the interruption of the continuity in tissue resulting from the opening or break of the skin. Wound may arise due to physical, chemical or microbial agents. Every wound is unique and deserves individual care. The healing of wound is essential in order for the restoration of the tissue continuity and disturbed skin status [1]. Healing is thus a survival mechanism that represents an attempt of maintaining the normal anatomical structure together with function. When healing takes charge from the normal course, the possibilities of non-, under or over healing can be expected. In conjunction with the healing event, treatment is aimed in order to minimize the unsought consequences and abridge the healing time. Skillful surgical techniques however have lessened the undesired consequences in healing process to some extent. Wound repair must occur in a physiological environment for repair and regeneration to take place. Nevertheless, several factors are known to impede the process of wound healing viz. hypoxia, tumors, infection, metabolic disorders and a diet deficient in protein, vitamins, or minerals [2]. Wound healing is a complex and dynamic process with the parallel changes of wound environment with the individual's health status. The knowledge of the physiology of the cutaneous wound healing is trajectory through the phases of hemostasis, inflammation, formation of granulation tissue, and extracellular matrix remodeling. Cutaneous wounds heal by primary union or secondary union whereby secondary healing involves more extensive scarring and wound contraction. The wound healing process can be categorized into four stages. In stage 1, primary wound healing or healing by first intention occurs within hours of repairing a full-thickness surgical incision. In stage 2, if the wound edges are not approximated immediately, delayed primary wound healing takes place. This type of wound is often associated with contaminated wound. The phagocytosis of the contaminated tissue is known to be resolved by the fourth day, and the epithelialization and maturation process start. In stage 3, the secondary healing or healing by secondary intention takes place. Here, a full-thickness wound is allowed to close and heal. A pronounced contraction can be expected in secondary healing. Myofibroblasts from the fibroblastic differentiation is believed to contribute in the wound contraction. Finally, in stage 4, the migration and mitosis replication of epithelial cells are composed in the epithelialization process which traverses the wound. Wounds with partial thickness that involve the epidermis, epithelialization is the predominant method by which healing occurs. Appropriate treatment and care for the wound will not only promote healing but save the hospitalization cost and the patient from other complications such as amputation. Many different methods of treatment are being introduced currently for wound healing yet the outcome is still far from optimal. This condition indicates the need for our field to introduce efficient and safe means of wound healing agent. Some of the traditionally claimed plants with wound healing properties have been screened for the wound healing activity in different pharmacological models but the potential of most still remains unexplored. In addition to this, there are few cases whereby the chemical constituents responsible for wound healing are identified. The present study deals with the screening of *Swietenia macrophylla*, seed extract for wound healing properties in Albino rats. *Swietenia macrophylla,* belonging to the Meliaceae family is commonly known as the big-leaved mahogony. *S.macrophylla* is well known in the timber industry for its wood quality. This economically important timber tree is traditionally used for the treatment of several diseases including diabetes, malaria, skin diseases, fever, hypertension and tuberculosis. It is also used as purgative, astringent, depurative and tonic [3].

Hence the objective of present investigation was to unravel therapeutic potential of ethanolic extract of *Swietenia Macrophyla* formulated ointment in excision wound model by assessing macroscopic and histopathological parameter.

MATERIALS AND METHOD

Collection of plant material

Switenia macrophylla seeds were commercially purchased from an online distributor from Sungai Buloh, Selangor, Malaysia. The seeds of *Swietenia macrophylla* were sent to the Institute of Bioscience, University Putra Malaysia (UPM), Serdang, Malaysia for species confirmation. The seeds were identified as *Swietenia macrophylla* King (Meliaceae) with the voucher number, SK 2227/13.

Preparation of extract

The *Swietenia macrophylla* seeds were cleaned well with dry clean cloth to remove dust or any soil substances. Then, the seeds were air dried under shade and homogenized in blender without adding water and extracted with 95% ethanol using maceration for 6 days, at room temperature. The ethanolic extract was filtered and let to concentrate.

Animals

Healthy adult male Sprague Dawley (200-250 g) albino rats were obtained from AMU Animal House (Malaysia). The rats were divided into three groups. Each group consisted of six rats. Rats were housed in polyethylene cages with adequate ventilation. Cleaning and sanitation of the cages were done on alternate days. Bedding materials were changed every two consecutive days. Wood shavings were used as the bedding materials for experimental rats. Food pellets and water were provided *ad libitum.* The experiments were conducted with approval from the AMU University Animal Ethics Committee (AMU/AEC/HS-FBS/2013/5). The experiment protocols were followed as per guidelines provided by the AMU University Animal Ethics Committee.

Ointment preparation

An ethanolic extract of *Swietenia macrophylla* seed was used for the preparation of the oitment for topical application. Extract based ointment was prepared in concentration of 10% using suitable preservative and stored in refrigerator until further use.

Excision wound model

Excision wound model was used for the study of rate of contraction of wound and epithelization. Animals were lightly anaesthetized with diethyl ether and the back hairs of the animals were depilated by shaving. An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. Excision wounds sized 200 mm^2 and 2 mm depth were made by cutting out layer of skin from the shaven area. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The entire wound was left open. The study comprised three different groups of six animals in each groups as follows and the treatment was done topically in all the cases:

Group I- Control animals: received injury for wound formation but did not receive any ointment or drug treatment

Group II- Treated animals: received injury for wound formation and treatment with Betadine ointment (10% w/w)

Group III- Drug treated animals: received injury for wound formation and treatment with *Swietenia macrophylla* ethanolic extract oitment (10% w/w).

Measurement of wound area

The progressive changes in wound area were monitored on predetermined days i.e., 0, 5, 10, 15 and 20. Later on, wound area was measured using the vernier caliper.

Measurement of wound contraction

Wound contraction was calculated as percentage of the reduction in original wound area size. It was calculated by using following formula:

Percentage wound contraction=(Initial area of wound-Nth day area of wound)/Initial area of wound x 100

Histopathological evaluation

A specimen sample of tissue was isolated from the skin of each group of rat were collected at the end of the experiment to evaluate for the histopathological alterations. Samples were fixed in 10%

buffered formalin, processed and blocked with paraffin and then sectioned into 5 µm and stained with hematoxylin & eosin (HE) and Masson's Trichrome. Photomicrographs were captured at a magnification of 100 X. Re-epithelization, fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neovascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling.

Data and statistical analysis

All the results were expressed as mean ± S.D. Data analysis was performed using SPSS software, version 16.0. Statistical comparisons were made between drug-treated groups and control animals. Data of wound area and percent wound contraction was analyzed using one-way ANOVA, Dunnett's test was applied for post hoc analysis. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1: Mean number (mm²) ± SD of wound contraction. Values are expressed as mean ± SD

Group	Day 0	Day 5	Day 10	Day 15	Day 20
Control	200.00 ± 0.00	181.67 ± 7.53*	150.00 ± 14.14*	101.67 ± 7.53*	30.00 ± 8.94
Standard	200.00 ± 0.00	135.00 ± 13.78*	85.00 ± 13.78*	20.00 ± 10.95*	0.00 ± 0.00
Test	200.00 ± 0.00	146.67 ± 12.11*	78.33 ± 16.02*	31.67 ± 9.83*	0.00 ± 0.00

p<0.05compared to control (n=6 in each group).

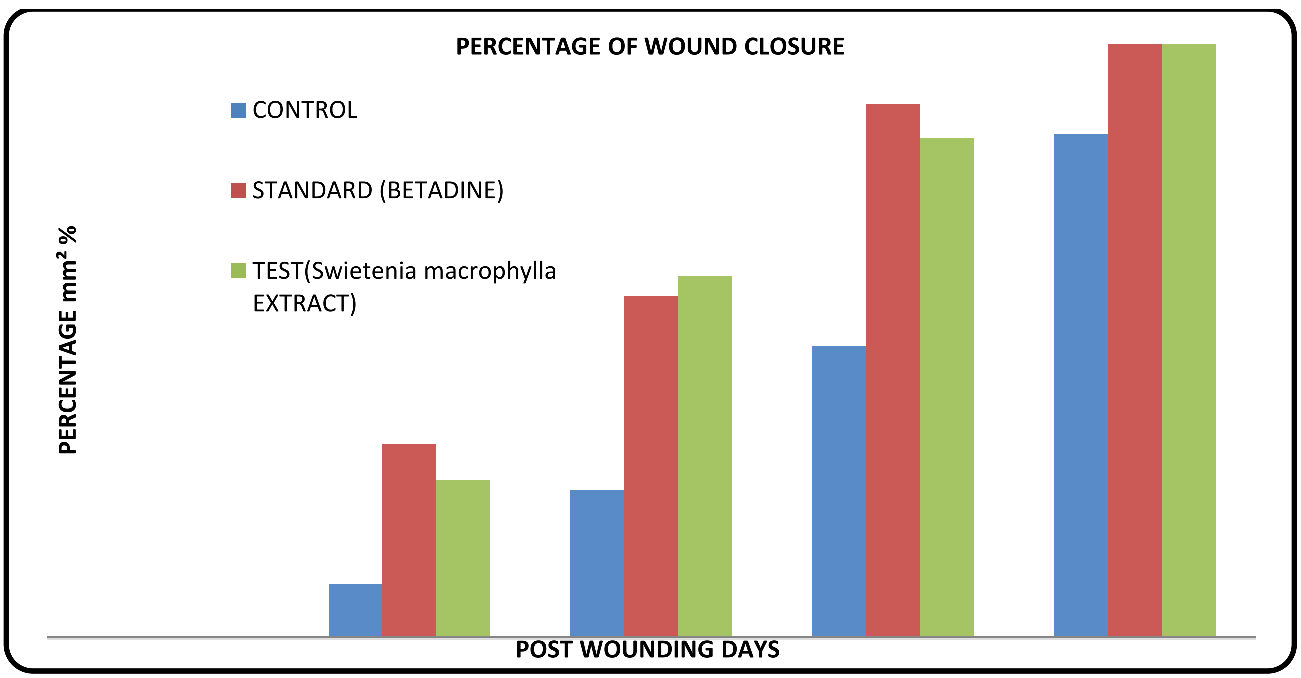

Figure 1: Percentage of wound closure

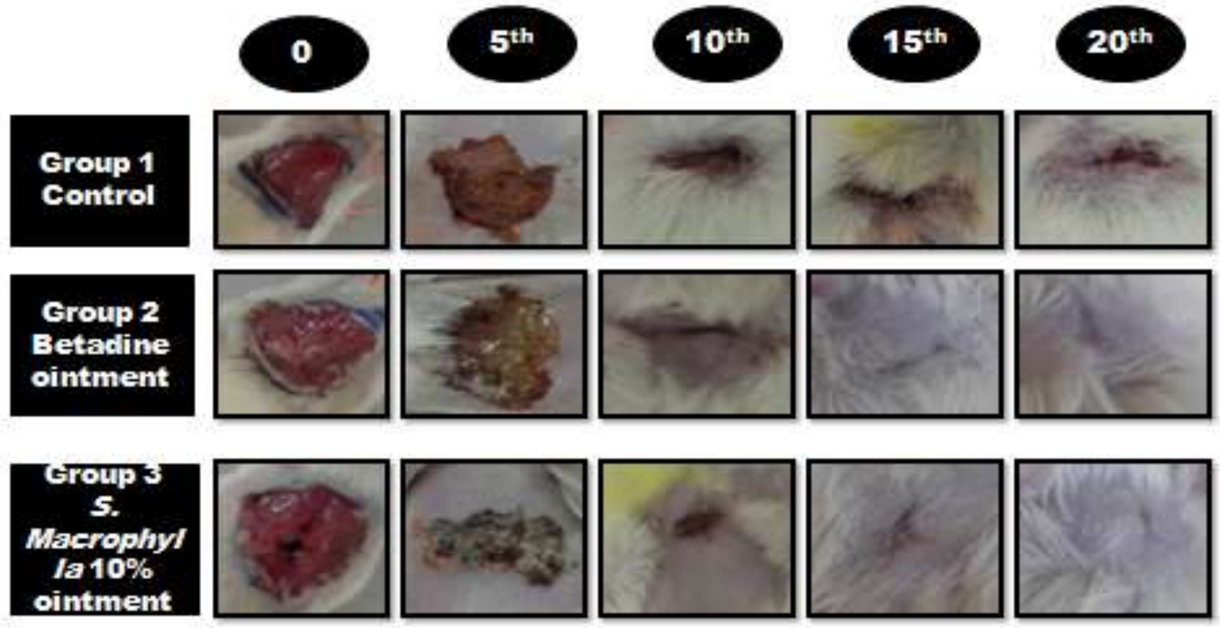

Figure 2: Wound contraction progress

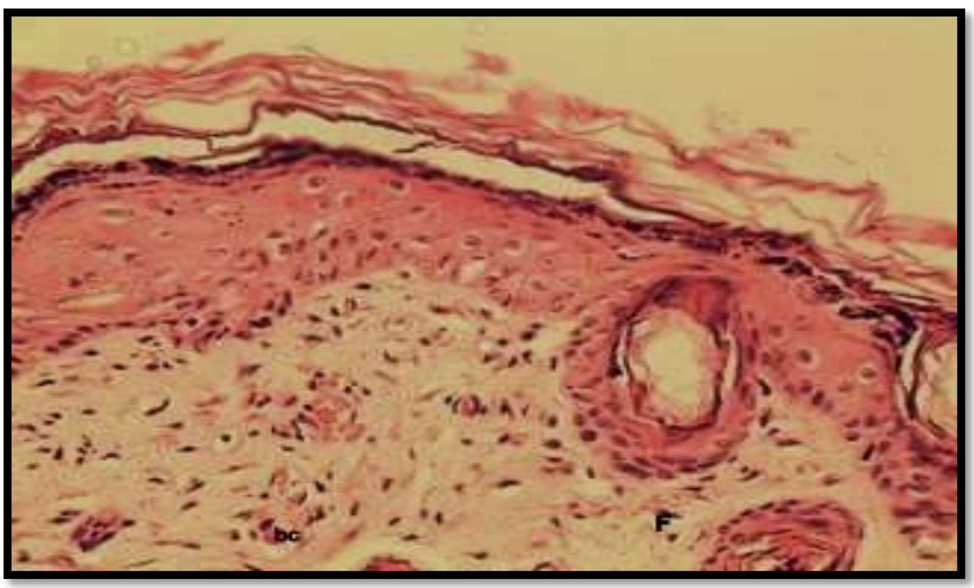

Figure 4: Control group tissue (H&E) exhibited disorganized fibroblasts (F) and lesser blood capillaries (bc).

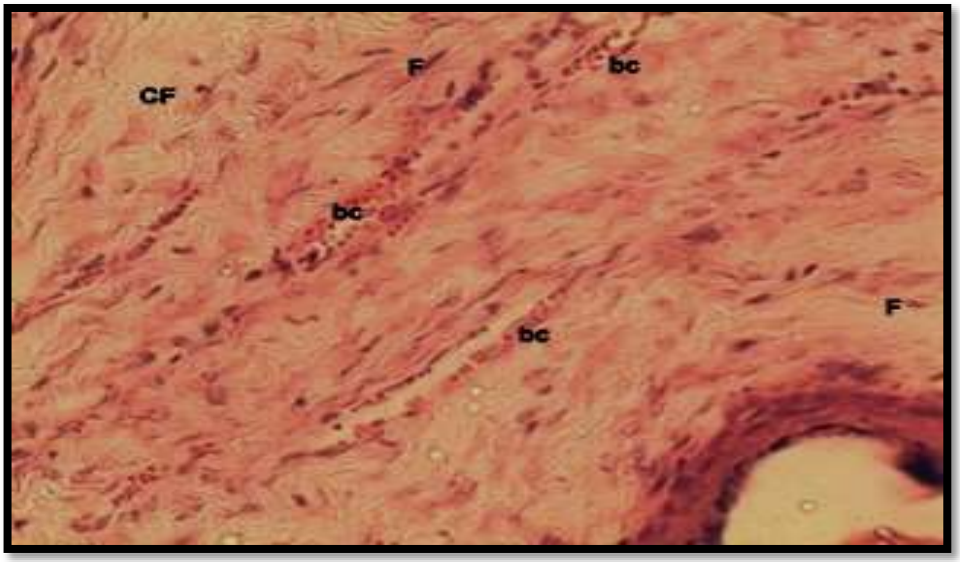

Figure 5: Standard group (betadine ointment) tissue (H&E) exhibited higher collagen fiber (CF) deposition, more blood capillaries (bc) and organized fibroblasts (F).

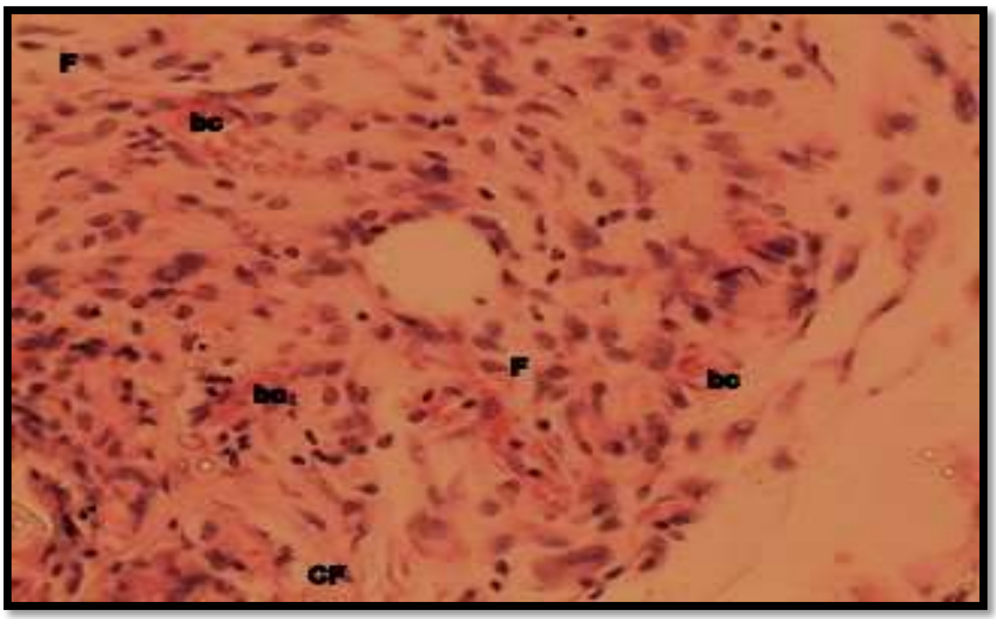

Figure 6: Test group tissue (10% *Swietenia macrophylla* seed extract) (H&E) exhibited higher fibroblasts (F) proliferation, more newly formed blood capillaries (bc) and presence of collagen fibers (CF).

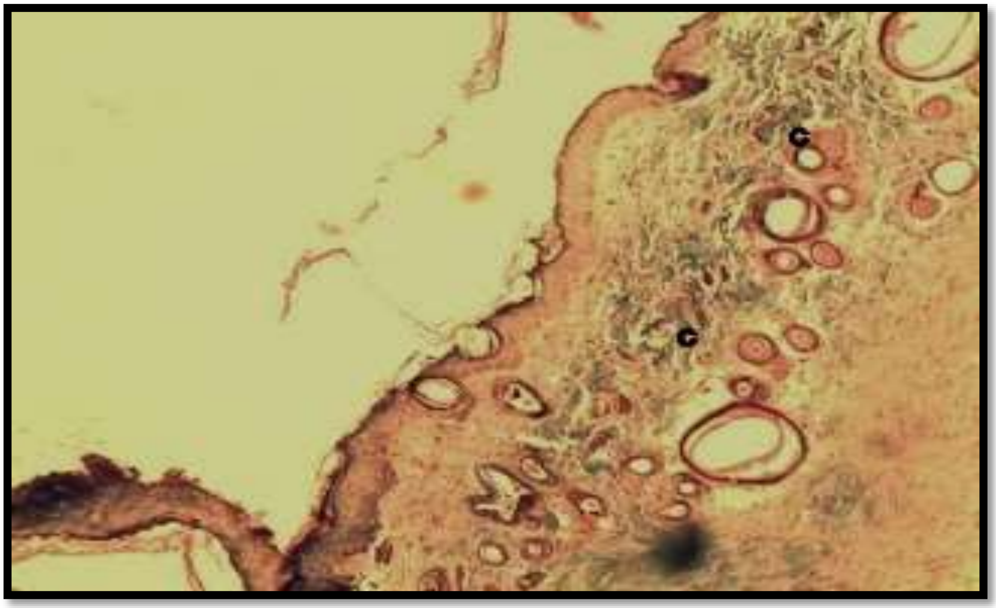

Figure 7: Control group tissue exhibited less collagen (C) deposition (indicated by intensity of blue color).

Figure 8: Standard group (betadine ointment) tissue exhibited high collagen (C) deposition (indicated by high blue color intensity) along with epithelialization demonstration.

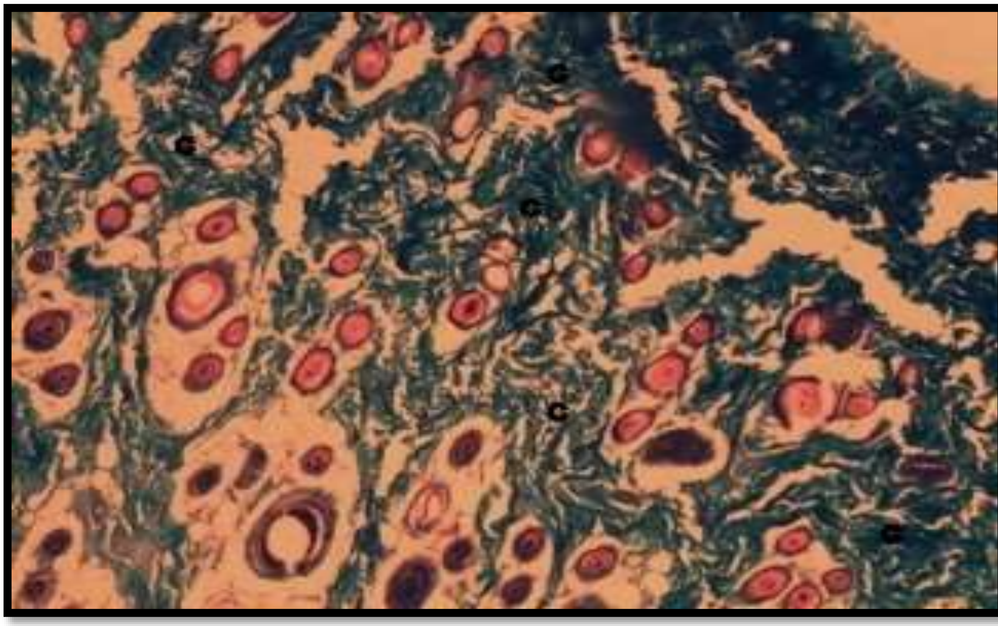

Figure 9: Test group (10% *Swietenia macrophylla* seed extract) tissue exhibited higher collagen (C) deposition (indicated by high blue color intensity).

DISCUSSION

Wounds are referred to as disruption of normal anatomic structure and function. Skin wounds could happen through several causes like physical injuries resulting in opening and breaking of the skin. Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes. The aim in these processes is to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin [4].

The results showed wound healing and repair, accelerated by applying ointment of *S.macrophylla*, which was highlighted by the full closure coverage of the wound area by organized fibroblasts. Excision wound model was conducted in this study. Three groups were segregated viz. the control group, standard group and the test group. The control group of Albino rats was left untreated. Meanwhile, the standard group and the test group of Albino rats received Betadine ointment (Povidone-Iodine) and *S.macrophylla* seed extract ointment respectively.

The enhanced capacity of wound healing with *S.macrophylla* seed extract could be explained on the basis of anti-inflammatory effects of the plant that are well documented in the literature. *S.macrophylla* seed extract suppressed the fMet-Leu-Phe (fMLP) induced superoxide anion generation by human neutrophils [5]. The anti-inflammatory activity of this extract could have aided in the enhanced wound healing as mentioned earlier.

In addition, *S.macrophylla* ethanolic seed extract possesses analgesic activity. This is partially connected to either lipooxygenase and/or cyclooxygenase via the arachidonic acid cascade and/or opiod receptors producing analgesic in thermal and chemical pain models [6]. The chemical pain model can be linked with wound occurrence. Wound can be caused by chemical agents. In order to improve wound healing and repair, analgesic property of *S.macrophylla* seed can be a source for wound and pain management.

The active constituents present in *S.macrophylla* seeds are alkaloids, flavanoids, saponins, phenols, steroids, glycosides, resins, tannins and oils [7]. Tannins, phenols and flavanoids are the major constituents that are responsible for wound healing. The plant *Tinospora crispa* containing the flavanoids possesses wound healing activity [1] as that of *S.macrophylla*. Any one or combination of the observed phytochemical constituents may be responsible for the healing activity exerted by *S.macrophylla* seed extract. The seed of *S.macrophylla* also comprises of limonoids and triterpenoids [8]. Limonoids are known compound that possess antibacterial and antifungal activities.

Limonoids inhibited the plant pathogenic fungi in a study conducted recently [9]. Two limonoids were isolated from *S.macrophylla* viz. 2-hydroxy-3-swietenolide and 2-hydroxy-3-0-tigloyswietenolide which has antibacterial activity against eight bacterial strains [10]. These limonoids have antimicrobial and antifungal properties, hence it can be inferred that the wound healing activity of the seed extract of *S.macrophylla* observed is due partly to its limonoids content, which seems to be responsible for wound contraction.

Antioxidant property *of S.macrophylla* ethanolic seed extract observed in a study showed an established antioxidant activity due the presence of isolated compound swietemacrophyllanin[8]. Increase in total antioxidant status has been shown to be important in recovery from wounds [11]. The presence of swietemacrophyllanin and phenols is likely to be responsible for the free radical scavenging effects observed and further contribute to wound healing process.

Possibly, the constituents like triterpenoids and alkaloids of the seeds of *S.macrophylla* may play a major role in the process of wound healing. Furthermore, triterpenoids and flavanoids are known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization [11].

Through the macroscopic changes and histopathological observation throughout the wound healing period of the present study, the sequential changes and normalization of the repair of skin wound in the Albino rat model were observed and it took 15 days for epithelialization period for the test group and standard group. Meanwhile, the control group took 21 days to complete the epithelialization period. The statistical analysis done has established the values from the control, standard and test groups that were compared to be significant.

Hence, the ointment formulated with *S.macrophylla* seed extract applied on test group has high wound healing effect, on par with the Betadine ointment compared to the untreated control group as it promotes wound contraction and shortens epithelialization period. The percentage values demonstrated in Figure 1 shows the healing capacity observed with the healing duration are parallel with potential of wound healing of *S.macrophylla* seed extract. The *Swietenia macrophylla* seed extract has proved its remarkable rapid wound-healing associated with considerable analgesic [12] and potent anti-microbial effects [10].

CONCLUSION

We conclude that ethanolic extract of *Swietenia Macrophyla* has significant wound healing activity in excision wound model compare to control

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