Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 9 (3): 116-126, 2015
ISSN 1819-3455 / DOI: 10.3923/rjmp.2015.116.126

Promotion of Wound Healing by Leaf Extracts of *Sesbania aegyptiaca* in Albino Rats

[1,2]Nata Rajan Livingston Raja and [1]Krishnan Sundar
[1]Department of Biotechnology, Kalasalingam University, Krishnankoil, 626 126, Tamilnadu, India
[2]Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, 626126, Tamilnadu, India

Corresponding Author: Krishnan Sundar, Department of Biotechnology, Kalasalingam University, Krishnankoil, 626126, Tamilnadu, India Tel: +91 4563 289029 Fax: +91 4563 289322

ABSTRACT

The wound-healing activity of *Sesbania aegyptiaca* leaf extract ointment was evaluated in albino rats. The animals were divided into 4 groups of five each. In Group 1 control animals 'simple ointment' was applied on the wounded area twice daily. In Group 2 standard Nitrofurazone ointment was applied on the wounded area twice daily. In Group 3 (Test 1) *S. aegyptiaca* ointment (5%) and Group 4 (Test 2) *S. aegyptiaca* ointment (10%) was applied on the wounded area twice daily. Wound healing parameters like percent wound contraction and histopathological changes were examined in an excision wound model. The application of *S. aegyptiaca* ointment accelerates the healing wound contraction. Histopathological examination of granulation tissue showed much advanced phase of healing, late fibroblastic phase consisted of the presence of thrombus; minimal number of inflammatory cells, minimal to marked amount of fibrous granulation tissue, minimal to moderate amount of vascular granulation tissue and minimal to moderate reepithelialisation. The results of the study suggest that *S. aegyptiaca* ointment significantly enhanced and accelerated the rate of wound healing process in wounded rats.

Key words: *Sesbania aegyptiaca*, histopathological, excision, inflammatory, wound

INTRODUCTION

Wounds are the foremost case of physical disabilities and uncomforted for human beings (Kumarasamyraja *et al*., 2012). Wound is a disorder of the cellular, functional and anatomical continuity of a living tissue. Wound may be formed by varies measures like thermal, physical, chemical, microbial or immunological offensiveness to the skin and tissues. Wound healing is a complex and energetic process with the wound location shifting with the altering health status of the individual (Kerstein, 1997) and in which the skin repairs itself after tissue gets damaged (Nguyen *et al*., 2009). The route of wound healing consists of incorporated cellular and biochemical measures leading to re-establishment of structural and functional reliability with recapture of strength in injured tissues. A number of plants and herbs have been used experimentally to treat skin disorders, including wound injuries, in traditional medicine (Abdulla *et al*., 2009). Wound healing potential of medicinal plant is helpful to progress new wound healing topical formulations for human use.

In India, many of medications based on plants have been used for the treatment and healing of various kinds of diseases (Biswas *et al*., 2004). In addition, Indian folk medicine comprises of

several prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, scabies, venereal disease and ulcers (Mukherjee *et al*., 2000). Most of the world's population still depends upon traditional medicines for various dermatological diseases (Verma *et al*., 2011). Herbal medicines are making remarkable response, not showing any serious side effects compared to expensive allopathic drugs that have undesirable effects (Priya *et al*., 2002) and increasing number of patients are opting alternative remedy to treat various kinds of illness. These factors may be encouraged to extensive attention in medicines derived from plants.

Sesbania aegyptiaca is an important Indian medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine. The genus *Sesbania* belongs to the family *Leguminosae* and its subfamily is *Papilionoideae*. *Sesbania aegyptiaca* is a narrow-crowned, deep-rooting single or multi stemmed shrub or small tree, 1-7 m tall. Leaves paripinnate, long, narrow; leaflets in many pairs, rounded or oblong, usually asymmetric at the base, often glaucous; stipules minute or absent (Orwa *et al*., 2009). *Sesbania aegyptiaca* juices and extracts have astringent property. This property is used for reducing fevers and for promoting fluid discharge and subsequent drying of mucous membranes, hence leading to healing of wounds (Watt and Breyer-Brandwijk, 1962). Fresh *S. aegyptiaca* roots and leaves are used to treat scorpion stings, boils and abscesses. The leaves are used in some countries as a tea and are considered to have antibiotic, antihelminthic, anti-tumour and contraceptive properties.

In the present study leaf extracts of *S. aegyptiaca* are evaluated for wound healing effects in Albino rats' models and also examined to redevelop and recreate the disrupted anatomical continuity and functional condition of the skin through histo-pathological studies.

MATERIALS AND METHODS

Drugs and chemicals: Nitrofurazone U.S.P (FURATOP) was obtained from GOPISH Pharma Limited, Solan. All substances were prepared immediately before use and the reagents used were of analytical grade.

Plant materials: The leaves of *S. aegyptiaca* leaf used in this study were collected from Krishnankoil, Srivilliputur (Tamil Nadu, India). The plant was authenticated by Dr. Stephen, Department of Botany, American College, Madurai, India.

Extract preparation: *Sesbania aegyptiaca* leaves were shade dried and coarsely powdered. The powdered materials were extracted with methanol. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at -4°C until ready for use. The yield of the extract was 46.4% (w/w). In each experiment, the extract was diluted with water to desired concentration.

Animals: Adult male albino rats weighing about 150-250 g were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water *ad libitum*. The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for the purpose of Control and Supervision of Experimental Animal (CPSCEA).

Phytochemical screening: A Preliminary phytochemical screening of *S. aegyptiaca* leaf extract was conducted to determine the presence or absence of glycoside, tannin, saponin, polyphenol, reducing sugar, protein, flavonoids and steroids by standard methods (Kokate, 1997).

Formulation of ointment

Preparation of simple ointment: The following ingredients (Wool fat-2 g Hard Paraffin-2 g Cetostearyl alcohol-2 g White Soft Paraffin-34 g) were mixed and heated gently with stirring then cooled. The base was then packed in a wide mouth container (Lazarus *et al*., 1994). This is shown in the following Table 1.

Required quantities of emulsifying wax, liquid paraffin and white soft paraffin were weighed and melted. To this, an adequate quantity of *S. aegyptiaca* leaf extract (5, 10 g) is added separately and stirred well until a homogeneous mass were obtained. The ointment was then packed in a wide mouth container and used for the wound healing studies in rats (Panda, 2010).

Evaluation of *Sesbania aegyptiaca* ointment preparation

Physicochemical parameters: Preliminary evaluation of formulations at different concentrations was carried out: Color and odor were examined. The stability of the prepared formulations was tested at two different temperatures i.e., 4 and 37°C for 3 months and the pH of various formulations was also determined (Chhetri *et al*., 2010). For this one gram of ointment was dissolved in 100 mL of distilled water and stored for 2 h and the pH was determined using a digital pH meter.

Wound healing activity of *S. aegyptiaca* ointment

Excision wound model: The Albino rats were divided into four groups of five in each. Excisions of wounds were made as described (Morton and Malone, 1972). Animals were anaesthetized with inhalational anesthetic ether and placed in operation table in its natural position. Hairs were removed from the dorsal thoracic central region of anaesthetized rats. A circular wound of about approximately 2 cm was made on depilated ethanol-sterilized dorsal thoracic region of rats and all the preparations were applied topically by following manner:

Group 1: Control-Simple ointment applied on the wounded area twice daily
Group 2: STD-Nitrofurazone ointment applied on the wounded area twice daily
Group 3: Test1-*S. aegyptiaca ointment* (5%) applied on the wound twice daily
Group 4: Test2-*S. aegyptiaca ointment* (10%) applied on the wound twice daily

The wound healing area of each animal was measured on 1st, 4th, 8th, 12th and 16th of post-wound days and calculated as percent reduction in wound area (Devender *et al*., 2011):

$$\text{Wound contraction } (\%) = \frac{\text{Healed area}}{\text{Total area}} \times 100$$

Table 1: Preparations of leaf extract *S. aegyptiaca* ointment

Working formula	Ingredients	Quantity (g)
Emulsifying ointment base	Emulsifying wax	75
	White soft paraffin	125
	Liquid paraffin	50
S. aegyptiaca ointment (5%)	*S. aegyptiaca* leaf extract	5
	Emulsifying ointment	q.s-100
S. aegyptiaca ointment (10%)	*S. aegyptiaca* leaf extract	10
	Emulsifying ointment	q.s-100

Histopathological examination: From the healed wound, a specimen sample of tissue was isolated from each group of rats for histopathological examination. Slides containing paraffin sections were placed on a slide holder and deparaffinized with xylene for 30 min and the excess of xylene was removed by blotting. The tissue was rehydrated successively with 100, 90 and 80% isopropyl alcohol for 2-3 min each and kept in a water bath for 3 min. The excess water was blotted the tissue was kept into Hematoxylin stain for 1-2 min followed by tap water for 1-2 min. The slides containing tissue sections dipped into 1 M HCl followed by Scott's water (Sodium Bicarbonate 3.5 g, Magnesium Sulfate 20 g, distilled water 1 L) for 1 min each. The thin sections of the tissue were stained with Eosin I bluish solution and observed for any histological changes under Leica microscope.

Statistical analysis: All the data are presented as Mean±SEM Dunnett's Multiple Comparison Test and one way Analyses of Variance (ANOVA) were performed.

RESULTS

Preliminary phytochemical screening of the extracts of *Sesbania aegyptiaca* leaf extract revealed the presence of flavonoids, saponins, polyphenols, anthraquinone glycosides and carbohydrates as shown in Table 2.

Preliminary evaluation of *S. aegyptiaca* leaf extract formulations at different concentrations and the measurement of pH of each formulation values are presented in Table 3.

Excision wound model: A significant increase in the wound-healing activity was observed in the animals treated with the *S. aegyptiaca* leaf extract compared with control and standard treatments. In this model, the increased percentage of wound contraction, the rate of wound closure was observed on the post wounding days were showed in Fig. 1-5. The *S. aegyptiaca* leaf extract (10%) treated animals showed a more rapid decrease in wound size (Fig. 5) compared with the control rats, which received simple ointment preparation. The increased percentage of wound contraction values are shown in Table 4.

Histopathological evaluation of wound healing activity: Histopathological studies showed inflammatory phase consisted of the presence of thrombus; moderate to marked presence of

Table 2: Phytochemical screening of the extracts of *S. aegyptiaca*

Test	*S. aegyptiaca*
Saponin	+
Reducing sugar	+
Polyphenol	+
Flavonoids	+
Protein	+
Volatile oil	-
Tannins	+
Glycoside	+
Steroid	+
Anthraquinone	+

Table 3: Physicochemical evaluation of formulated *S. aegyptiaca* formulations

Physicochemical parameters	F1 (5%)	F2 (10%)
Colour	Dark green	Dark green
Odour	Characteristic	Characteristic
pH	7.2	7.3
Storage (4, 37°C)	Stable	Stable

Table 4: Evaluation of *S. aegyptiaca* ointment on wound healing by excision wound in rats

Post wounding days	Wound area (mm^2) and wound contraction (%)							
	Simple ointment	(%)	Nitrofurazone ointment	(%)	*S. aegyptiaca ointment* (5%)	(%)	*S. aegyptiaca ointment* (10%)	(%)
0	361±2.34	-	361±2.12	-	324±1.86	-	361±2.46	-
4	169±1.82	53	196±1.34	46	100±3.43	69	49±0.82	86
8	121±1.32	66	36±0.96	90**	25±0.84	92**	09±0.18	98**
12	64±0.74	82**	09±0.16	97**	09±0.16	97**	00	100**
16	25±0.82	93**	01±0.00	99**	01±0.00	99**	00	100**

Data is expressed as Mean±SEM from five observations as compared to Control group and analyzed by one way Analyses of Variance (ANOVA). The p-value is less than 0.0063, (**p<0.05). By Dunnett's Multiple Comparison Test, this difference is considered to be statistically significant

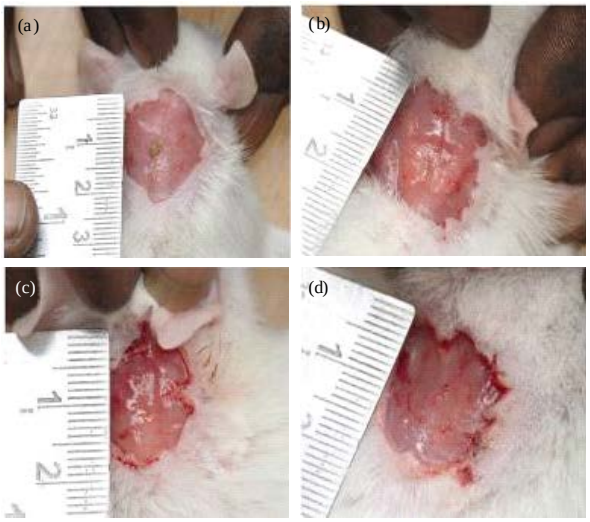

Fig. 1(a-d): Wound healing effect of *Sesbania aegyptiaca* ointment on excision wound model, (a) Rats were untreated, (b) Treated with standard drug, (c) *Sesbania aegyptiaca* leaf extract ointment (5 and 10%) and (d) 0th day after wound creation

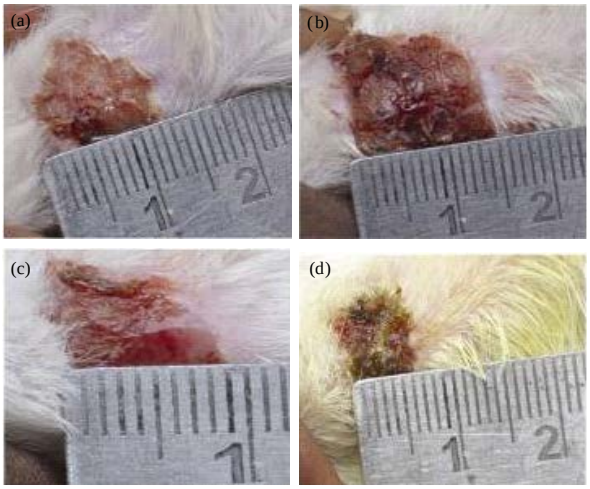

Fig. 2(a-d): Wound healing effect of *Sesbania ointment* on excision wound model, (a) Rats were untreated, (b) Treated with standard drug, (c) *Sesbania aegyptiaca* leaf extract ointment (5 and 10%) and (d) 4th day after wound creation

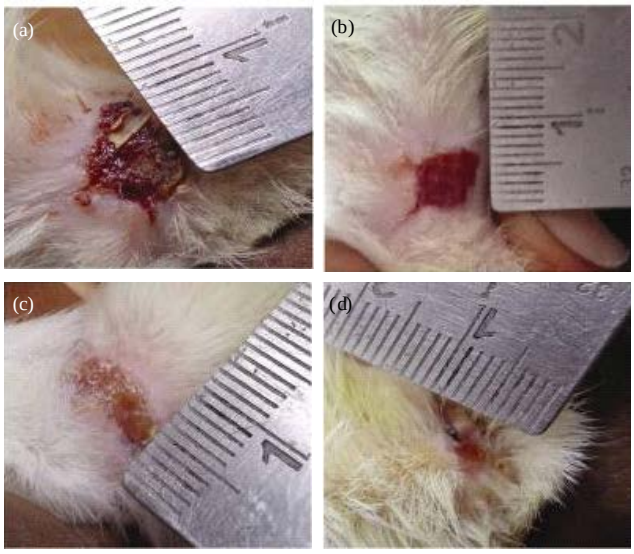

Fig. 3(a-d): Wound healing effect of *Sesbania aegyptiaca* ointment on excision wound model, (a) Rats were untreated, (b) Treated with standard drug, (c) *Sesbania aegyptiaca* leaf extract ointment (5 and 10%) and (d) 8th day after wound creation

Fig. 4(a-d): Wound healing effect of *Sesbania ointment* on excision wound model, (a) Rats were untreated, (b) Treated with standard drug, (c) *Sesbania aegyptiaca* leaf extract ointment (5 and 10%) and (d) 12th day after wound creation

inflammatory cells, predominantly the polymorphonuclear cells and a moderate number of mononuclear cells, mild to moderate amount of fibrinous exudates and absence of reepithelialisation (Fig. 6) and late fibroblastic phase consisted of the presence of thrombus, minimal number of inflammatory cells, minimal to marked amount of fibrous granulation tissue, minimal to moderate amount of vascular granulation tissue and minimal to moderate reepithelialisation (Fig. 4a, b). The score used for histopathological evaluation were shown in Table 5.

Table 5: Histopathological evaluation of wound healing activity

Animal	Reepithelialisation	Granulation tissue		Fibrinous exudate	Thrombus	Wound contraction	Inference
		Vascular	Fibrous				
GM 1	+	-	+++	-	-	+	Late fibroblastic phase
GM 2	-	-	-	-	-	-	No significant pathological changes
GM 3	-	-	-	+++	++++	-	Inflammatory phase
GM 4	-	-	-	+++	++++	-	Inflammatory phase
GM 5	+	-	+++	-	-	+	Late fibroblastic phase
GM 6	+	-	+++	-	-	+	Late fibroblastic phase

Score used for histopathological evaluation post wounded rats: Minimal, +: Mild, ++: Moderate, +++: Marked, ++++: Massive/complete (+++++)

Fig. 5(a-d): Wound healing effect of *Sesbania aegyptiaca ointment* on Excision wound model, (a) Rats were untreated, (b) Treated with standard drug, (c) *Sesbania aegyptiaca* leaf extract ointment (5 and 10%) and (d) 16th day after wound creation

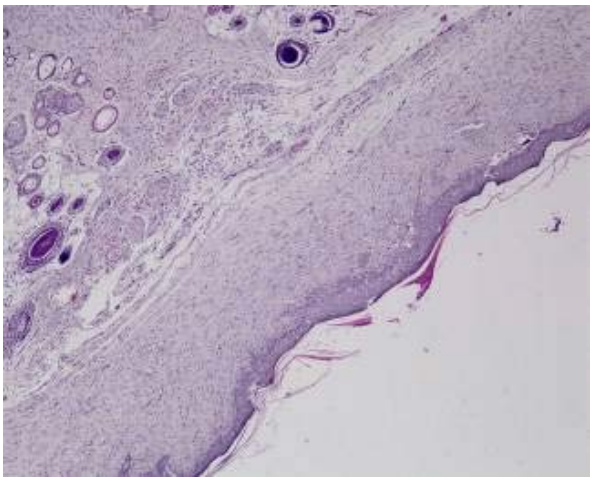

Fig. 6: Hematoxylin and Eosin stained sections of the Granulation tissue contains minimal to marked amount of fibrous granulation tissue, minimal to moderate amount of vascular granulation tissue, minimal to moderate reepithelialisation and minimal number of inflammatory cell on initial day after wound creation (Captured at 40X)

DISCUSSION

Research on plant constituents are of greater importance as they have given rise to many useful drugs like tubocurarine, the most powerful muscle relaxant, morphine from poppy, cocaine from coca. The traditional Indian medicine-Ayurveda, describes various herbs, fats, oils and minerals with anti-aging as well as wound healing properties. Several studies specify that medicinal plant products are most potential agents for wound healing and mostly chosen because of the absence of unnecessary side effects and their effectiveness (Jagetia *et al*., 2003). Additionally, more than 70% of wound healing pharma products are plant based, 20% are mineral based and remaining contain animal products as their base material (Kumar *et al*., 2007). The present study was undertaken to evaluate, whether, *S. aegyptiaca* leaf extract mediate wound healing in experimentally induced wounds in rats.

Wound healing is the process of repairing the injury to the skin and other soft tissues. Several factors delay the wound healing process including bacterial infection, necrotic tissue and intervention with blood supply, lymphatic blockage and diabetes mellitus; in general if the factors possibly will be altered by any agent having an increased healing rate of wound could be achieved (Chithra *et al*., 1998). Appropriate healing of wound is crucial for the reappearance of disrupted anatomical continuity and disturbed functional condition of the skin and soft tissues. Cutaneous wound repair is accompanied by a controlled and definable progression of biological proceedings early with wound closure, contraction of the collagen lattice formation, cellular proliferation, progressing to the repair and remodeling of injured tissue (Shetty *et al*., 2006). Research works on acute wounds in an animal model shows that wounds heal in four phases. It is implicit that chronic wounds have to also leave through the same basic four phases (Kerstein, 1997). The 4 phases of wound healing are (a) hemostasis or coagulation which prevents blood loss (b) inflammation (c) proliferation or granulation and (d) tissue remodeling or maturation and collagen deposition.

In this study, a topical ointment from the leaf extracts of *S. aegyptiaca* was prepared and evaluated for wound healing activity using an excision wound model in albino rats. Topical application of *S. aegyptiaca* leaf extract ointment enhanced wound contraction and closure, with reepithelialisation was observed starting from 4th post-wounding day. The results suggested that treatment with fresh leaf extract of *S. aegyptiaca* ointment may have a valuable influence on the various phases of wound healing process such as fibroplasias, collagen synthesis and wound contraction, resulting in more rapidly wound healing in the damaged skin and soft tissue. Histopathological studies also evident that presence of thrombus and minimal number of inflammatory cells, minimal to marked amount of fibrous granulation tissue promoted the wound healing process by enhancing epithelial cell proliferation and cell collagen formation (Fig. 7-9). Collagen is the family of protein, which provide structural support and it is the main component of tissue such as fibrous tissue and cartilage. The collagen synthesis is stimulated by various growth factors. Growth hormone is also known to promote the proliferation of fibroblasts and form the granulation tissue (Kiran and Asad, 2008). The findings of the present study confirmed that the leaf extract of *S. aegyptiaca* ointments possess wound healing effects against experimentally induced wound models. These data corroborate with the earlier observations on (Zahra *et al*., 2011; Nayak *et al*., 2007) in healing the experimentally induced wounds. Therefore, the wound healing effects of the leaf extract of *S. aegyptiaca* may be due to its phytochemical constituent's like flavonoids, tannin, saponins and anti-oxidant properties.

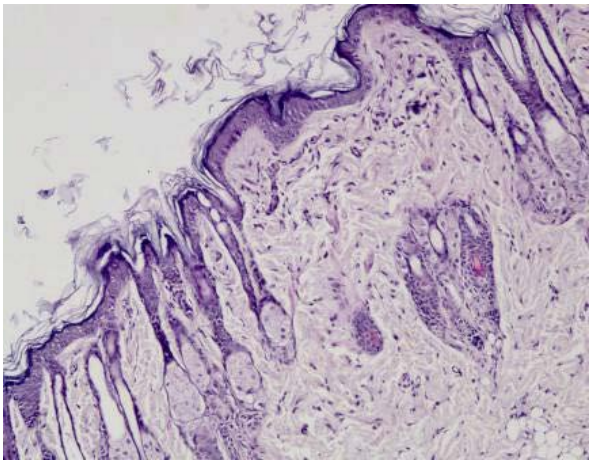

Fig. 7: Hematoxylin and Eosin stained sections of the Granulation tissue showed only wide scar, vascular granulation tissue and not showed any significant pathological changes on 4th day after wound creation (Captured at 40X)

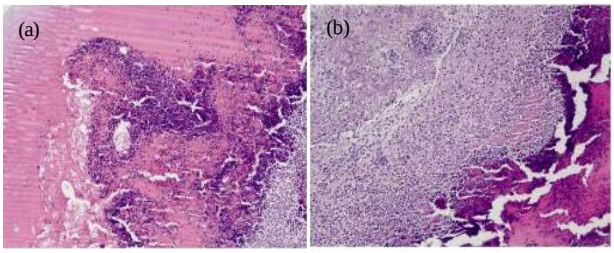

Fig. 8(a-b): Hematoxylin and Eosin stained sections of the granulation tissue both (a, b) contains inflammatory phase consisted of the presence of thrombus; moderate to marked presence of inflammatory cells, predominantly the polymorph nuclear cells and a moderate number of mononuclear cells, mild to moderate amount of fibrinous exudates and absence of reepithelialisation on 8th day after wound creation (Captured at 40X)

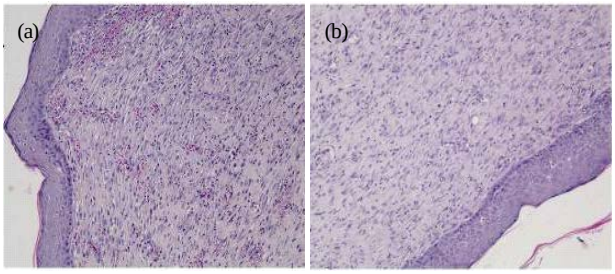

Fig. 9(a-b): Hematoxylin and Eosin stained sections of the granulation tissue both (a, b) contains late fibroblastic phase consisted of the presence of thrombus; minimal number of inflammatory cells, minimal to marked amount of fibrous granulation tissue, minimal to moderate amount of vascular granulation tissue and minimal to moderate reepithelialisation on 12th day after wound creation (Captured at 40X)

CONCLUSION

Natural products like plants are the more potent healers of wounds because they enhance and promote the repair mechanism in a natural way. This study highlights the importance of conventional medicines that are still being used by people of ethnic origin and recognizes the importance in wound healing. Apparently very little is known about *S. aegyptiaca* and its various medicinal properties. Further studies are needed to isolate the active principle and evaluate the efficacy of the plant in the treatment of chronic and diabetic wounds.

ACKNOWLEDGMENTS

The authors thank the Management of Kalasalingam Anandam Ammal Trust, the Vice Chancellor, Kalasalingam University and the Principal, Arulmigu Kalasalingam College of Pharmacy for providing facilities to carry out this work successfully.

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