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Wound Healing Effect of *Ficus Dalhousiae Miq* Stem Bark Ethanolic Extract In Excision And Incision Wounded Wistar Rats.

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ABSTRACT

The aim of the present study was to evaluate the wound healing effect of Ficus Dalhousiae stem bark ethanolic extract (FDSBE) by excision and incision experimental models. Wistar albino rats weighing 150 - 200 gm were utilised for the study. The preliminary phytochemical screening of the methanolic extract of stem bark showed the presence of various phytoconstituents namely alkaloids, flavanoids glycosides, saponins, sterols and tannins. The study was intended to evaluate the effects of topical application,three different concentrations 5%.10% and 15% of FDSBE on the rate of wound closure and the histology of wound area. Wounds dressed with the extract and standard ointment healed significantly earlier than those with vehicle. The beneficial effect of FDSBE was evident by the progressive decrease in wound area in excision wound model ($p<0.001$) and decrease in tensile strength($p<0.01$) in incision model Histological analysis of the wound area after 10 days showed that wounds dressed with the extract had less scar width when compared to control. The tissue contained less inflammatory cells and more collagen and angiogenesis, compared to wounds dressed with vehicle which was also supportive for the wound healing potential of the extract.

Keywords: FDSBE, Albino rats, wound healing.

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INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against various diseases. Based on the usage of natural products in traditional medicine, a large number of modern medicines have been extracted from natural sources. The use of herbal therapies for caring of wounds and injuries has been popular since ancient civilizations. In contrast to only 1-3% of modern drugs used for the treatment of wounds and skin disorders ,almost one third of all traditional medicines are in use for this treatment[1].

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha .Medicinal components from plants play an important role in conventional as well as western medicine. Wounds are physical injuries that result in breakage of the skin. Wound healing is a natural response to tissue injury, consisting of a multifactorial and complex cascade of events involving various cellular, molecular and biochemical processes ,resulting in the healing of the wound and the restoration of the intact fuctional barrier [2,3]. Inflammation, cell proliferation, angiogenesis, epithelialisation, wound contraction, and matrix remodelling is the sequence of several basic wound healing processes[4]. The wound healing process is generally categorised into three integrated and overlapping phases inflammatory phase, which consists of the establishment of homeostasis and inflammation ,the proliferative phase which consists of granulation, contraction and epithelialisation and remodelling phase which eventually determines the strength and appearance of the healed tissue[5]. Flavanoids and other biomolecules promote the process of wound healing by influencing one or more phases of the healing process [6,7]. Ficus Dalhousiae is a small tree belonging to family Moraceae[8]which is reported to possess the following medicinal uses: Fruit is used as cardio tonic. Leaves and bark are used in affections of the liver and skin diseases [9]. Ficus dalhousiae is endemic to peninsular region and is a rare species [10]. The current study was undertaken to evaluate the wound healing activity of *Ficus Dalhousiae Miq* stem bark ethanolic extract in wistar rats.

MATERIAL AND METHODS

Experimental animals

Thirty healthy adult male Sprague Dawley rats,8 weeks old and weighing 200 to 250 g were obtained from the Experimental Animal House.The rats were divided randomly into 5 groups of 6 rats each.Each rat was housed separately (one rat per cage) and the animals were maintained on standard pellet diet and tap water.The study was approved by the Ethical Committee for animal experimentation ,Ethics no.1534/A/11/CPCSEA.

Experimentally Induced Excision Wounds

The rats were inflicted with excision wounds as described by Mughrabi et al.[11] The animals were anaesthesized with 0.09ml of Ketamine by i.m injection(30mg/kg,100mg/ml) prior to creation of the wounds.The skin was shaved using an electrical clipper, disinfected with 70% alcohol,injected with 1ml of lignocaine HCl s.c injection(2% 100mg/5ml).An area of uniform wound 2.00 cm in diameter was excised using a circular stamp and sterile scissors,from the depilated dorsal neck of all the rats.Incision of the muscle layer was avoided during the procedure to avoid any effect on the tension of the skin .The wound area was measured immediately by placing transparent paper over the wound and tracing out the area.This was subsequently placed on a $1mm^2$ graph sheet.The area of the counted squares was calculated in square millimetres and the area was recorded as described by Zahra et al[12].The animals were divided randomly in five different groups of 6 each in the treatment schedule. The ointments were applied once daily as per schedule i.e the negative control group of animals was treated with ointment base, whereas 5%, 10%, 15% extracts ointments were used for 3 test groups, the reference drug (5% povidone iodine) used for standard group and the wound area was determined on days 0, 2, 4, 8, 12 & 16 using transparent paper and a marker. Change in wound area was calculated, giving an indication of the rate of wound contraction. The day of scar falling without any residual raw wound were considered as period of epithelialization.

Experimentally Induced incision Wounds

One mid dorsal incision (2cm long) was made through the full thickness of the skin on the middle of the vertebral column under anaesthesia induced by ketamine hydrochloride (100mg/kg body weight,ip) and xylazine (15mg/kg body weight,im).Wounds were closed with interrupted sutures,1 cm apart.[13]All the sutures used in the experiments were non-absorbable braided non-capillary and siliconized. The animals were randomly distributed into five groups, 6 in each group; i.e the 3 test, the reference drug and the negative control groups. The ointments were applied once daily as per schedule i.e the negative control group of animals was treated with ointment base, whereas the 5%, 10%, 15% extracts ointments were used for 3 test groups, the reference drug (5% povidone iodine) used for standard group, till 9 days. All the sutures were removed on the 9th post wound day. On Day 10 all the animals were killed under anesthesia. The Tensile Strength (in Newton) of one linear paravertebral incised skin was measured using Tensiometer as shown in the Figure-5 and average value was taken as the tensile strength and the other paravertebral incised skin was taken carefully and send for histopathological studies.

Histological Evaluation of Healed Wounds

Skin tissue samples of all groups were immediately fixed after dissection,in 10% buffered formalin,dehydrated and processed using a paraffin tissue processing machine for paraffin sectioning.The wound tissues were cut 5µm thick perpendicular to the wound,stained with standard haematoxylin and eosin (H& E) and examined using a light microscope and captured using a digital camera.The evaluated parameters were epithelialisation,inflammatory cell infiltration,fibroblast proliferation,neovascularisation,and collagen deposition,which were assessesed individually by using a numerical scale.Stained sections were examined under a light microscope and were graded in a blind fashion on 3 slides per animal,using the modified 0 to 4 numerical scale as described by other researchers[14,15].The scores were 0 for abscence,1 for occasional presence,2 for light scattering, 3 for abundance,and 4 for confluence of cells or fibres.

RESULTS

Results Of Phytochemical Screening

The phytochemical screening of the ethanolic extract of *Ficus Dalhousiae Miq* stem bark showed the presence for sterols, alkaloids, tannins, phenols, flavonoids and reducing sugars.

Results of Excision Wound Model (EWM)

In the excision wound model *Ficus Dalhousiae Miq* stem bark extract presented promising effects as shown in the Table-1. The beneficial effect of FDSBE were evident from the day of first measurement as the progressive decrease in wound area was observed. On the 2nd day of wounding, the wound area shrinkage in 10% FDSBE treated group was more when compared to the other test groups as shown in the Figure-1.

Table 1 Improvement in wound size of animals on different experimental days in excision model

Group	Day2	Day4	Day 8	Day16	Period of epitheliazation
-ve Control	4.91±1.02	4.75±0.96	3.79±0.45	2.45±0.67	27±0.39
Standard 5% P.I	4.89±1.14	4.57±0.79	2.07±0.46	1.10±0.45*	17±0.46
5%FDSBME	4.25±0.68	4.24±0.87	2.25±0.35	1.67±0.29^{ns}	19±0.49
10%FDSBME	4.76±0.96	4.56±0.56	2.18±0.38	1.17±0.56*	17±0.34
15%FDSBME	4.34±0.78	4.67±0.65	2.34±0.34	1.25±0.60*	17±0.39

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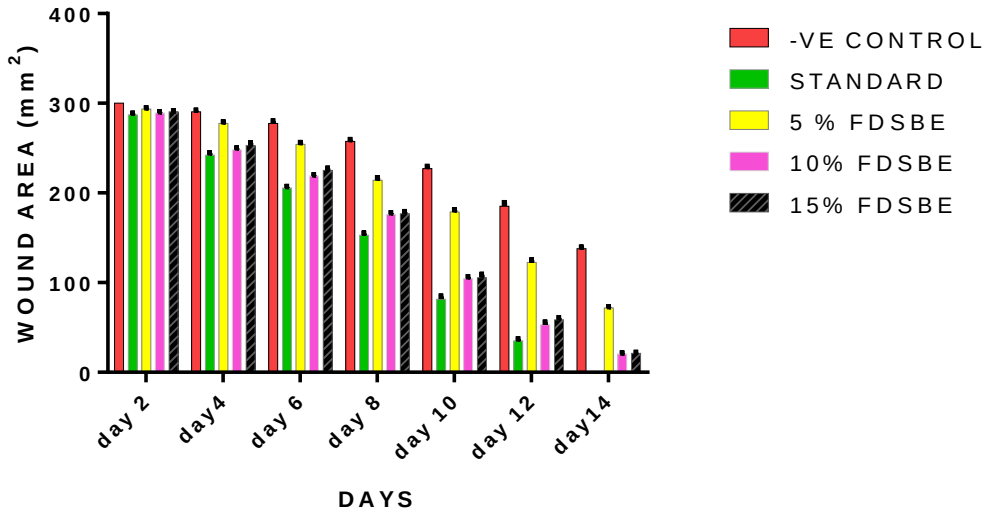

Figure I: Graph showing the effects of Excision wound healing

Results of Incision Wound Model (IWM)

From the result obtained it was evident that the ethanolic extract of *Ficus Dalhousiae Miq* Stem Bark Extract has shown wound healing activity in the incision wound model too, as shown in Table-2. The results of the measurements of tensile strength of the animals treated with the 10% FDSBE was the next to the highest value of the standard group as shown in the Figure-2. The P value of the test treated groups was also in the range of <0.05-0.01, which is considered as pharmacologically significant range.

Table 2 Tensile strength of wounds in different experimental groups

TITLE	-VE CONTROL	STD 5% P.I	5% FDSBME	10% FDSBME	15% FDSBME
MEAN/SD	14.57±0.97	29.57±1.27**	21.58±1.29^{ns}	28.14±1.46**	28.13±1.48**

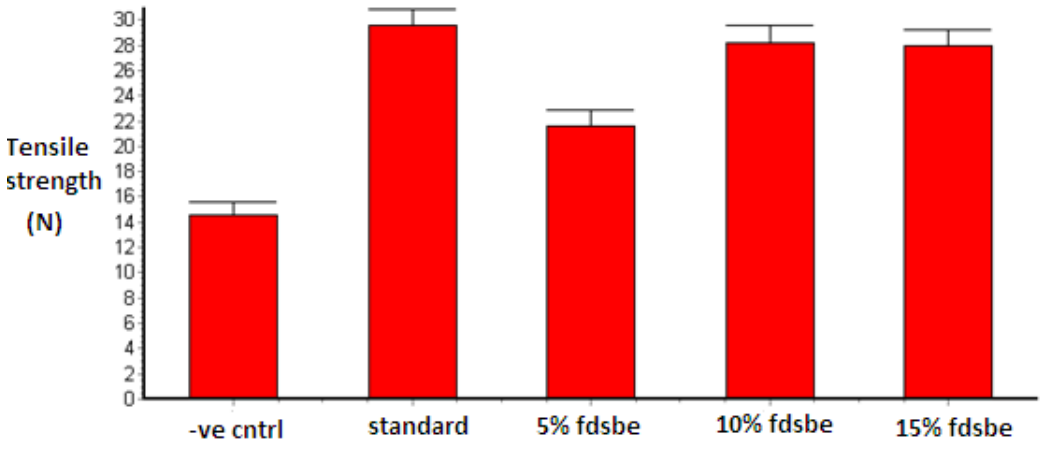

Figure II: Graph showing the effects of Incision wound healing model

Histopathological Examination

In the histopathological examination, the phases in the wound healing process were observed which showed that proliferation phase was the predominant stage of action of the *Ficus dalhousiae Miq* Stem bark

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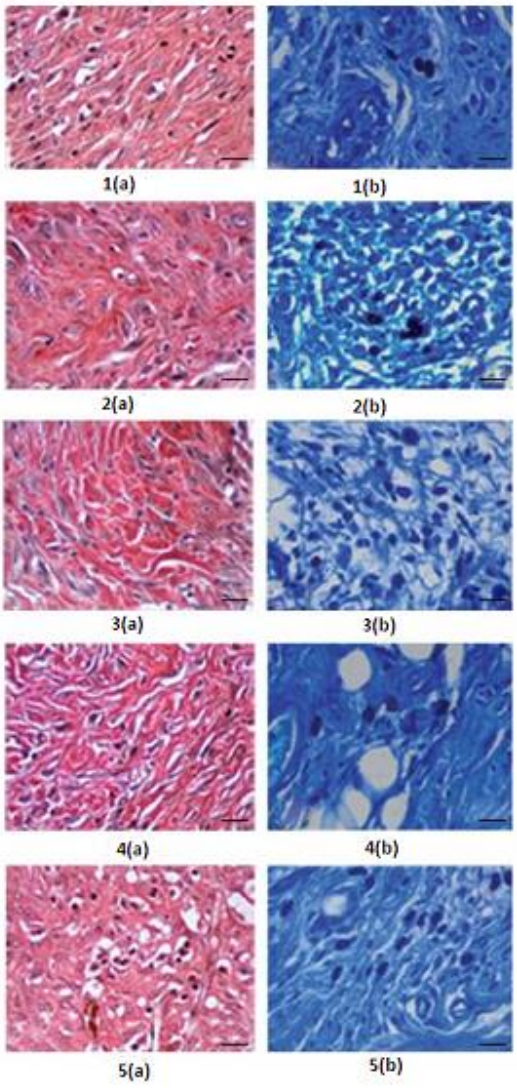
extract. The best re-modeling, particularly, re-epithelization were detected with the 10% extract group. On the other hand, faster keratinization characterized with minor intraepithelial cornification was seen in 15% extract groups.The microscopic view of slides of various groups showed that the major effect of *Ficus dalhousiae Miq* Stem bark extract starts after the inflammatory phase of the wound healing. It shows that *Ficus dalhouseae Miq* Stem bark extract helps in tissue growth.



Histopathological view of each group at 10th day of post wounding. The slides were prepared by eosin and toliudine blue for each group representative and shown as (a) and (b) respectively in the figure. The original magnification was 40x and 100x for (a) and (b) respectively. The data is representative of 6 animals per group.

1 = -ve control
2 = 5 % FDSBE
3 = 10 % FDSBE
4 = 15 % FDSBE
5 = 5 % Povidone Iodine

FDSBE = Ficus Dalhouseae Stem Bark Ethanolic Extract

DISCUSSION

Wound healing process begins with the restoration of damaged tissue as closely as possible to its natural state and wound contraction is the course of shrinkage in wounded area. The healing primarily depends on the repairing ability of the tissue in addition to type and degree of damage and general health status of the tissue. The collagen is the main constituent of extra cellular tissue, which is responsible for support and strength. The phytochemical analysis of stem bark of *Ficus Dalhousiae Miq* revealed the presence of flavonoids and phenolic acid derivatives which have therapeutic uses due to their anti-inflammatory, anti-fungal, antioxidant and wound healing properties.

Moreover, flavonoids and their derivatives are known to decrease lipid peroxidation by improving vascularity and by preventing or slowing down the progress of cell necrosis. Hence, any drug that inhibits lipid peroxidation is supposed to increase the viability of collagen fibres by increasing the circulation and the strength of collagen fibres, by encouraging the DNA synthesis and preventing the cell damage. Flavonoids are also known to endorse the wound healing process primarily due to their antimicrobial and astringent

properties, which appears to be responsible for wound contraction and elevated rate of epithelization. Therefore, wound healing potential of *Ficus Dalhousiae Miq* may be attributed to the phytoconstituents present in the aerial parts, which may be either due to their individual or additive effect that speeds up the process most probably the proliferation phase of wound healing.The measurements of the progression of wound healing induced by the extracts, reference drug, negative control groups were observed in this studies.

In the excision wound model, the 10% ointment of extract treated groups of animals showed 56.5% contraction of the wounds on day 6. The same extract demonstrated 80.6% contraction on the day 12, and healed completely on day 14 which was close to contraction value of the reference drug povidone iodine(5%). However, the 5% concentration of extracts presented no significant results. Further, upon increasing the concentration to 15% the extract does not increase the contraction value.

In the incision wound model tensile strength of the animals treated with 10% extract demonstrated the highest value (38.9%) on day 10. Topical application of the 10% extract on the incision wound model demonstrated a remarkable improvement in wound tensile strength compared to other groups.Phases in wound healing processes (inflammation, proliferation and remodeling) were observed and recorded successfully within the experimental groups for incision wound models by the Histopathological examination. The negative control groups demonstrated delayed wound healing processes compared to the other groups. In comparison with the negative control groups, faster re-modeling were noticed in extracts treated groups. Weak foreign body reaction, superfluous process in wound healing, characterized with a few foreign body giant cells, which generally localized in peripheral sides of some hair follicles were detected in all groups except for the reference drug standard group [16].

CONCLUSION

In conclusion, the present study demonstrated that the *Ficus Dalhousiae Miq* stem bark extract promotes wound healing activity in animals as a preclinical study. The 10% *Ficus Dalhousiae Miq* stem bark extract showed remarkable wound healing activity and it may be suggested for treating various types of wounds in humans. Further studies with purified constituents compared to the crude extracts may be needed to comprehend the complete mechanism of wound healing activity of stem bark of *Ficus Dalhousiae Miq*.

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