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Wound Healing Activity of Oil Extract of *Tectona Grandis* Leaves in Wistar Rats.

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ABSTRACT

Frontal leaves of *Tectona grandis* are used traditionally for the treatment of variety of wounds. Scientific data is not available regarding the wound healing activity of oil extract of frontal leaves of *Tectona grandis*. Hence this study was undertaken to investigate the effect of topical application of oil extract of frontal leaves of *Tectona grandis* on incision and excision wound models in rats. 4 groups (control, vehicle control - coconut oil, standard - aloe vera, test - oil extract of *Tectona grandis* leaves) of wistar rats were used each for excision and incision wound model. Parameters studied were wound breaking strength in incision wound model and percentage of wound contraction and period of epithelisation in excision wound model. Results showed a significant increase in the breaking strength ($p<0.001$) in incision wound model; decrease in period of epithelisation ($p<0.01$) and increase in wound contraction rate ($p<0.001$) in excision wound model in the test group when compared to control group. Therefore, this study ascertains the wound healing activity of *Tectona grandis* leaves and it substantiates the use of frontal leaves of *Tectona grandis* in Ayurveda for treatment of wounds.

Keywords: excision and incision wound model, wistar rats, *Tectona Grandis* leaves, oil extract

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INTRODUCTION

Wound is a discontinuity in a normal tissue structure that results in a variety of cellular and molecular changes. Wound healing is a complex process that includes inflammation, granulation tissue formation, epithelisation, collagen synthesis and tissue remodelling [1, 2].

Many dietary modification and nutritional and herbal supplements have proved to improve quality of wound healing by influencing repairative processes or by limiting the damaging effects of inflammation. Some of these factors [3] help in reversing delayed wound healing due to drugs like corticosteroids [4], anticancer [5] and non-steroidal anti-inflammatory drugs [6].

Various medicinal plants have been used for centuries as a remedy for treating human diseases [7,8]. The plant, *Tectona grandis* (family verabinaceae) is grown throughout India and is also known as Indian teak. *Tectona grandis* (TG) is a large deciduous tree 10-12 meter tall. Leaves of TG are opposite, elliptic or obovate in shape, rough and glabrous above [9]. According to ancient Indian literatures, leaves of TG are having cooling property and are used commonly as hemostatic. They are useful in treatment of inflammations, leprosy, skin diseases, pruritus, stomatitis, ulcers and hemorrhages.

The phytochemical screening of alcoholic extract of TG leaves reveals the presence of alkaloids, flavonoids, tannins [10]. It is also shown to have nitric oxide scavenging activity [11]. Hydro alcoholic extract of TG leaves improves incision, excision and burn wound healing in rats [12]. It has been found that topical application of methanolic extract of this leaves have antifungal and antibacterial activity [13,14] **.**

In ayurvda practice, oils are used as one of the mode of application in treatment of the wounds [15]. Traditionally prepared oils are used as a base for external applications of medicine. There is no scientific data available on the wound healing activity of oil extract of frontal leaves of *Tectona grandis* which is prepared according to traditional methods. Hence this study was undertaken to evaluate the effect of topical application of oil extract of leaves of TG on incision and excision wound models in wistar rats.

MATERIALS AND METHODS

Experimental animals

Healthy inbred wistar rats of either sex, weighing between 200 - 250 g were used. The experimental protocol was approved by Institutional Animal Ethics Committee and animals were maintained under standard environmental condition of temperature 23±2C, humidity 50± 5% and 10 -14 hours light and dark cycle respectively in the animal house approved by Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were provided with standard rat feed and water; ad libitum. The animals were housed individually in polyprophylene cages containing sterile husk as bedding after making wounds till the completion of wound healing.

Drugs used

Injection ketamine, aloe vera gel and coconut oil were obtained from the pharmacy of Kasturba Hospital. Fresh frontal leaves of TG were collected locally from Udupi district, Karnataka and they were authenticated by department of Ayurveda, KMC, Manipal.

Traditional method of preparation of oil extract

Procedure followed in this study for the preparation of medicated oil extract is recognized by Drugs and Cosmetic Act [16] and it is also included in the Ayurvedic Formulary of India (AFI) [17]. Tender leaves of TG plant were collected in the month of December and dried under shade for 15 days and powdered. One part of coarse powder leaves was added to sixteen parts of water and boiled to reduce the volume to one forth. The decoction is strained using a muslin cloth. Some fresh tender leaves were grounded to make a fine paste. Coconut oil is taken in a vessel and heated for some time. Then one part fine paste of leaves were added to four parts of coconut oil and sixteen parts of above prepared decoction. This mixture is boiled on mild fire with frequent stirring to avoid paste to adhere to the vessel and boiling continued till all the water evaporates.

Well-cooked oil should not have any residual moisture. The oil is strained while warm through muslin cloth and allowed to cool [15].

Wound Induction

Incision wound model

Animals were fasted for twelve hours prior to the experiment. Rats were anaesthetized by using intraperitoneal injection of ketamine (50mg/ kg body wt) [18,19]. Two Para vertebral straight incisions of 6 cm were made through the entire thickness of the shaved skin one on each side of vertebral column with the help of a sharp blade. After mopping the wounds dry, wounds were closed by using 4-0 silk thread and straight round body needle. The interrupted sutures were place at equidistance points of 1 cm each [20]. Wounds were then mopped with cotton swabs soaked in 70% alcohol. The animals were housed individually.

Excision wound model

After fasting for 12 hours rats were anaesthetized by using intraperitoneal injection of ketamine (50 mg/kg body wt). A round seal of 2.5cm diameter was impressed on the dorsal of thoracic region 5 cm away from the ears on shaved back of the rat. Full thickness skin from demarked area was excised to get a wound measuring 500 mm^2 [21]. After achieving full haemostasis by mopping the wound with cotton swab soaked in warm saline, animals were placed in their individual cages.

Drug treatment

4 groups of animals (n=6) were used each for incision and excision wound models. All the drugs were applied topically. Control groups received distilled water, vehicle control groups received coconut oil, standard groups received aloe vera gel and test groups received oil extract of *TG leaves*. Day of wounding is considered as day 0. In case of incision wound model, drugs were applied once daily from the day 0 till day 9 post wounding day. In excision wound model, animals were treated once daily from 1 to 21 post wounding day [18].

Parameters assessed:

Incision wound model

Removal of the sutures was done on 7^{th} post wounding day. The wound breaking strength was estimated on 10 day by continuous, constant water flow technique. The breaking strength was expressed as minimum weight of water necessary to bring about gaping of wound [6].

Excision wound model

Two physical attributes of healing namely wound contraction rate and epithelisation period were studied . To monitor wound contraction the progressive changes in wound area will be followed planimetrically .Wound area were traced on a transparent paper on day 0, 4, 8,12 and 16. The tracings were then transferred to 1mm graph sheets and the wound area was measured. Percentage of wound contraction was calculated using the following equation:

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size (500 mm}^2\text{)} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Epithelisation period was monitored by noting the number of days required for the eschar to fall off leaving no raw wound behind [21,22].

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Stastical Analysis

Data was analyzed using SPSS 16. The results were expressed as mean ± SEM. Differences between the experimental groups were compared using one-way ANOVA followed by Bonferroni's post hoc test. Values of p<0.05 were considered statistically significant.

RESULTS

In incision wound model (Table 1), topical application of oil extract of TG showed significant increase in the wound breaking strength (p<0.001) when compared to control group and vehicle control group. Standard group also showed significant improvement and test group results were comparable to that of standard group.

Table 1: **Mean wound breaking strength in incision wound model**

Group (n=6)	Wound breaking strength (gms)
Control(C)	172±13.15
Vehicle control (VC)	183.23± 6.94
Standard (SD)	258.35 ± 20.75 *
Oil extract of TG	308.33 ± 14.70***

Control vs SD * p< 0.05; Control vs TG,*** p<0.001

In excision wound model (Table 2), TG treated animals showed significant reduction in wound area when compared to control and vehicle control group on day 8, 12 and 16 (p<0.001) and there was no significant difference between test group and standard.

Table 2: Mean wound contraction rate in excision wound model

Groups (n=6)	Percentage of wound contraction			
	Day 4	**Day 8**	**Day 12**	**Day 16**
Control	13.72 ±2.77	58.96 ±2.63	81.66 ±1.656	91.33 ±0.571
Vehicle control	18.88 ± 2.66	70.36 ± 3.14	8 1.55 ±1.679	93. 55 ± 0.899
Standard	22.36±3.77	75.28± 1.64*	88.23 ± 1.514*	98.4 ±0.280*
Oil extract of TG	24.8 ± 2.83	76.36 ±1.37***	92.23 ±1.19***	96.76 ± 1.06***

Control vs TG on 8th, 12th, 16th day ***p<0.001; Control vs SD on 8th, 12th, 16th day ***p<0.05

Period of epithelisation (Table 3) was shorter in test group when compared to that of control and vehicle control group (p<0.05) but there was no significant difference between test and standard group.

Table 3: Mean Period of epithelisation for excision wound model

Groups	Period of epithelisation(days)
Control	19.33 ± 0.49
Vehicle control	19 ± 0.36
Standard	15 ± 1.09**
Oil extract of TG	15.33 ± 0.61**

Control vs TG ***p<0.05; Control vs SD **p<0.05

DISCUSSION AND CONCLUSION

Wound healing is a process of restoration of damaged tissue to its normal state and wound contraction is the process of shrinkage of the area of the wound. The results of the present study showed that the oil extract of *Tectona grandis* leaves when applied topically improved the wound breaking strength and wound contraction rate in incision and excision wound models respectively.

The above mentioned prohealing actions of the extract maybe due to the presence of phytoconstituents like alkaloids, carbohydrates, glycosides phytosterols, saponins, proteins aminoacids and flavonoids. The flavonoids [23], and triterpenoids[24] are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties, which could be responsible for improved wound contraction rate and shortened period of epithelisation.

Traditionally prepared oil extracts have principally three components namely, a liquid which may be aqueous decoction of one or more herbs, a fine paste of the herbs and a vegetable oil. Normally crude sesame oil (SO) is used but occasionally castor oil and coconut oil is also used.

In the present study coconut oil is used as a vehicle because it is also used in treating wounds traditionally. Various studies proved that coconut oil itself has wound healing activity [25]. Therefore, using coconut oil as base to prepare oil extract potentiates the wound healing activity of TG. Moreover, properly prepared oils have long expiry (about one year) without any separate preservatives which helps in the easy storage of the medicinal extract. The oil preparation by using above mentioned procedure is home based, economical and convenient. In this study, aloe vera gel is used as a standard drug for wound healing as it is known to have wound healing and anti- inflammatory activity [26].

This study ascertains that oil extract of TG leaf extract possess good wound healing activity when applied topically in incision and excision wound models and it substantiates the use of TG leaves in folklore medicine for treatment of wounds. This study also support that oil prepared in traditional method can be used as a wound healing agent. Further phytochemical studies are suggested to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity.

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