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Experimental Evaluation of Wound Healing Activity of *Tinduka Twak Kwatha* (Diospyros malabarica (Desr.)Kostel in Wistar Albino Rats

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ABSTRACT

Background: Burn wounds is the fourth most common type of injury worldwide. Approximately 90% of burns occur in low-to-middle income countries. *Tinduka* (*Diospyros malabarica* (Desr.) Kostel) has been classically as well as traditionally used for burn healing but scientific evidence remains unclear. **Aim:** The study was carried out to evaluate the wound healing activities of *Tinduka twak kwatha* in Wistar albino rats. **Materials and methods:** Partial thickness burn wound model were selected for the current study using 3 groups with 6 rats in each group. Group I was assigned as control, Group II was treated with Standard-Silver sulphadiazine, Group III was assigned as trial group which was treated with *Tinduka twak kwatha.* All drugs were applied topically at the burn site. The parameters observed were wound contraction, eschar fall day, complete cure day and histopathological observation. **Results:** The percentage of wound contraction in *Tinduka twak kwatha* group is statistically significant increase on 8th and 16th day, highly significant increase on 12th, 20th, 24thday and better on 28th day. Significant decrease in duration of complete cure day was found in kwatha group. Good regeneration was observed in histological study in kwatha group. **Conclusion:** *Tinduka twak kwatha* was effective in burn wound healing.

Key Words: *Complete cure day, Eschar Fall Day, Partial Thickness, Tinduka (Diospyros malabarica (Desr.)Kostel), Wound Contraction*

INTRODUCTION

The World Health Organization has described burns as “destruction of some or all layers of the skin, when they come in contact with hot liquids (scalds), hot solids (contact) or a flame (flame burns) or due to lightning and radiation injury”. Burn is the fourth most common type of injury all over the world. Approximately 90 % of burns occur in low-to-middle income countries. It is probably the most devastating of all kinds of wounds and it imposes a serious burden on physical, mental and socio-economic conditions of the victim.

Suppose proper care of burn wound management was not taken, healing processes are usually delayed by bacterial infections. Since the ages, many herbal remedies have been in practices for the local management of burn wounds to smooth the progress of healing process. The literatures have emphasized the usage of locally available drugs in the management of any kind of ailments. *Tinduka* is one such drug mentioned in classics, which are abundantly available in nearby places of Coastal belts. *Acharya Bhavamishra* has mentioned the utility of *Tinduka twak kwatha* in the management of all types of burn wound[1]. Also in the management of burnt wound, *Tinduka twak kwatha* usage has been practiced by folklore practioners of nearby places of Coastal belts. Research studies on the source of Tinduka, *D.malabarica* had concluded that the drug possess antimicrobial and antioxidant activity[2, 3].

Though *Tinduka* has been classically as well as traditionally used for burn healing but scientific evidence remains unclear. Thus the study was undertaken to evaluate the burn wound healing activities of *Tinduka twak* in *kwatha* form.

MATERIALS AND METHODS

Drug:

Test drug: *Tinduka twak kwatha*

Standard drug: Silver sulfadiazine

Selection of the animal:

The experimental protocol was approved by the animal ethical committee (Ethics No.: IAEC/CPCSEA/SDMDG01 dated 14-3-2017).Wistar albino rats were selected for the study. The rats were used after acclimatization to the laboratory environment for seven days. The animals were fed with normal rat diet ad libitum and water throughout the study. The housing provided had the following conditions- controlled lighting of 12:12 light and dark cycle, temperature of 25±3° C and relative humidity of approximately 50-55%.

Inclusion criteria:

Healthy Wistar albino rats of either sex weighing 200g-250g were taken for the study.

Exclusion criteria:

Rats subjected to other experiments and diseased and pregnant.

Animal grouping:

The rats of either sex weighing 200gm -250 gm were selected randomly and grouped into 3 groups of 6 rats each. (Table no.1)

Table 1 Grouping of animals

Group	Group Name	Treatment	No. Of animals
Group I	Control (C)	No treatment	6
Group II	Standard (S)	Silver sulfadiazine	6
Group III	Test drug (T)	*Tinduka twak kwatha*	6

Methodology:

The partial thickness burn wound model was employed as per Bairy et.al[4]. The rats were subjected for mild ether anaesthesia and the hairs on nape of the neck were shaved. The burn wound was created by pouring hot molten wax at 80°C into a metal cylinder of 400mm^2 circular opening placed on shaven back of the rat at the nape of the

neck. Wax was allowed to solidify in the metal cylinder. After 8 minutes the metal cylinder containing solidified wax adhering to the layers of skin was gently removed. Then wound area was marked using marker pen. Once the animals recovered completely from anaesthesia, they were kept individually in cages. External application of standard drug and test drugs was started to the respective groups from 1st day of wounding. All the drugs were applied topically once a day by sterile gauze on the burn wounds till complete closure of wound. Apart from the drugs under investigation no local/systemic chemotherapeutic cover was provided to animals. Wound contraction was traced on every fourth day till complete closure of wound. Time taken for fall of eschar and complete healing was noted in days.

Assessment criteria for burn wound healing:

All the three experimental groups were inspected daily and the healing was assessed based on the physical parameters like percentage of wound contraction, eschar fall day, complete cure day, histopathology of skin of healed burnt area of each rats.

Statistical Analysis:

The data were expressed in Mean±SEM. Statistical analysis were carried out by one way ANOVA followed by Dunnett's multiple comparision"t-test" as Post hoc test using Graph Pad Prism instal version 3. (Table no.2) (Table no.3)

Table 2 Results of Wound contraction

	Control	Standard	Tinduka twak kwatha
Day 4	16.00± 2.59	27.97± 3.32	26.00± 7.11
Day 8	25.82± 6.20	39.77± 2.52	47.62 ± 2.78*
Day 12	33.25± 4.95	47.45± 1.24	59.45 ± 2.50**
Day 16	41.79± 5.22	66.71± 6.36*	68.12 ± 4.62*
Day 20	43.34± 7.35	79.21± 5.12**	96.33 ± 0.80**
Day 24	75.84± 10.15	98.29± 1.03**	98.47 ± 1.00**
Day 28	97.78± 1.43	99.85± 0.14	99.69 ± 0.30

***statistically significant, ** statistically highly significant**

Table 3 Results of Eschar fall day, complete cure day and Hydroxyproline concentration

	Control	Standard	Tinduka twak kwatha
Eschar fall day	19.28± 0.52	17.71± 1.14	16.2 ± 0.73
Complete cure day	30.42± 0.75	26.57± 0.99	23.4 ± 1.43**

***statistically significant, ** statistically highly significant**

RESULTS AND DISCUSSION

Wound contraction:

The observation of wound contraction on every 4th day is tabulated in the table no.2. The data obtained were compared with control group statistically. On 4th day, the data showed non-significant increase in percentage wound contraction in standard group, *Tinduka twak kwatha* group (Figure no.1).

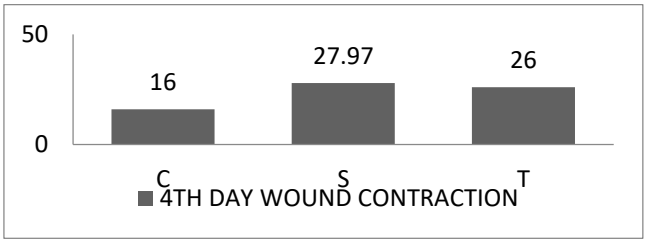


Figure 1 4th day wound contraction

On 8th day, non-significant increase in percentage wound contraction in standard group and significant increase in percentage wound contraction in *Tinduka twak kwatha* group (Figure no.2) was observed.

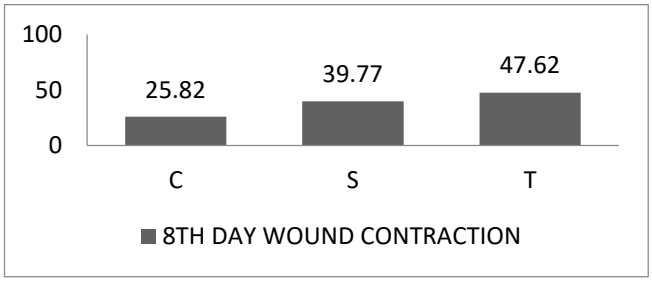


Figure 2 8th day wound contraction

On 12th day, non-significant increase in percentage wound contraction in standard group and very significant increase in percentage wound contraction in *Tinduka twak kwatha* group (Figure no.3) was observed.

Figure 3 12th day wound contraction

On 16th day**,** significant increase in percentage wound contraction was observed in standard and *Tinduka twak kwatha* group (Figure no.4).

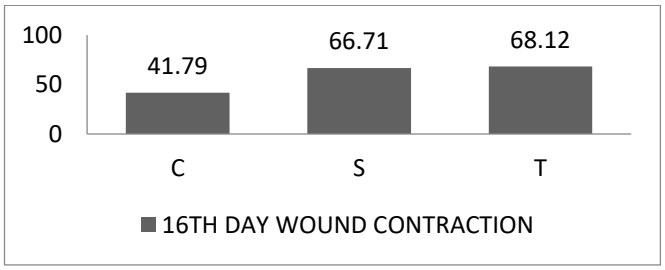


Figure 4 16th day wound contraction

On 20th day, very significant increase in percentage wound contraction was observed in standard group and *Tinduka twak kwatha* group (Figure no.5). On 24th day, very significant increase in percentage wound contraction was observed in standard group and *Tinduka twak kwatha* group (Figure no.6). On 28th day, non-significant increase in percentage wound contraction in standard group and *Tinduka twak kwatha* group (Figure no.7) was observed. (Table no.2).

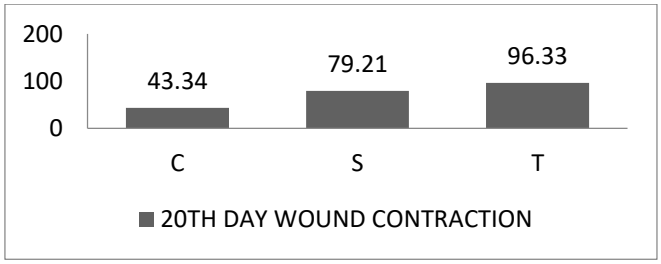


Figure 5 20th day wound contraction

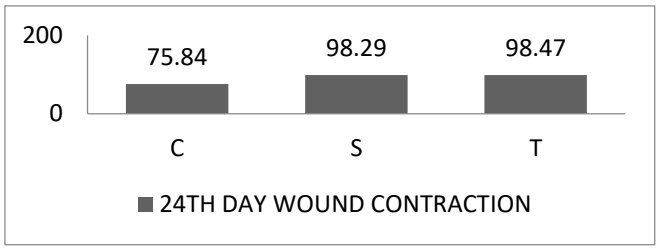


Figure 6 24th day wound contraction

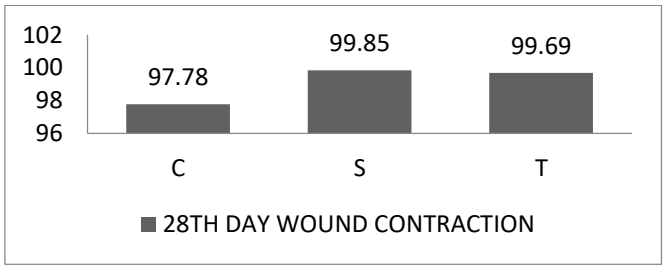


Figure 7 28th day wound contraction

Eschar fall day:

The data showed non-significant decrease in eschar fall day in standard and test group compared to control group.(Table no.3) (Figure no.8)

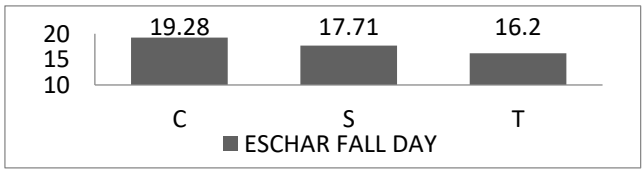


Figure 9 Eschar fall day

Complete cure day:

The data shows there was very-significant decrease in complete wound healing day in *Tinduka twak kwatha* and non-significant decrease in standard group when compared to control group. (Table no.3) (Figure no.9)

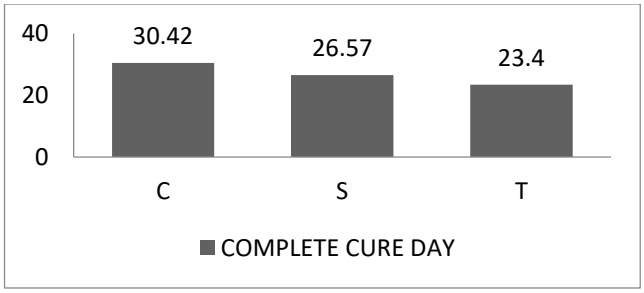
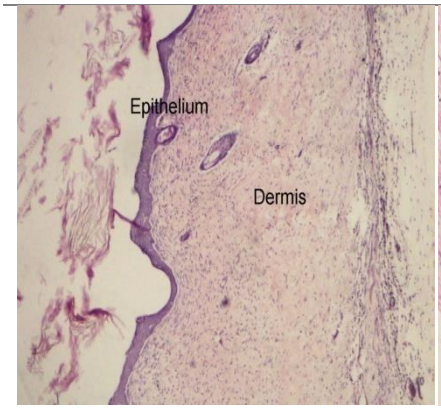


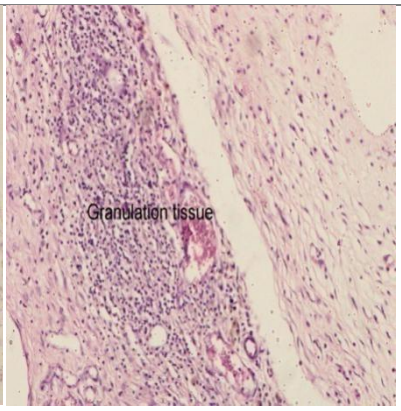
Figure 9 Complete cure day

Histopathological Observations:

In control group, severe inflammatory changes were observed. In standard and *Tinduka twak kwatha* group good regenerations were seen.(Figure no.10-12)

Any injury to bark of plant, it recovers by tissue regeneration. Literature emphasis the application of *bark* powder or decoction of *Tinduka* on the skin surface for wound healing. Through *lokhapurushasamya vaada*theory it can be inferred that bark of *Tinduka* is having action on skin layer of body when applied and there enhances capacity of tissue regeneration of injured skin tissue [5].





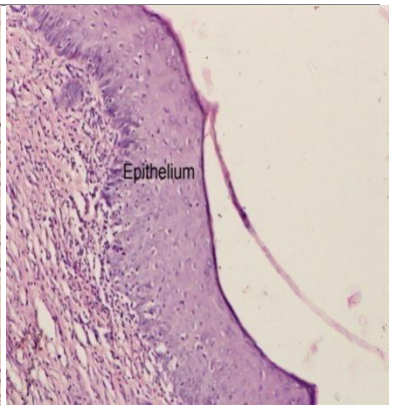


Figure 10 Control group **Figure 11 Standard group** **Figure 12 Test group**

Histopathology observation of control, standard and test group

In the pathogenesis of *agnidagdha vrana*(burn wound), mainly *pitta* and *rakta* is aggravated due to *kshatoshma*[6]. *Drava* and *snehamsha shoshana* in the burn area is due to excessive increase of *ushna guna* of *pitta* in the area which in turn increase *ruksha guna* in that area leading to indirect *vata prakopa*. Because of *ashrayaashrayee sambandha*, *rakta* also gets vitiated[7]. Hence, as per *tridosha siddhantha*, *sheeta* and *snigdha guna dravya* has to be used for pacifying *pitta dosha* and thus making wound bed free from vitiated *dosha* which proper healing of wound. In the classical literature mentioned that *Tinduka* is having action on *rakta* and *pitta*. The bark of *Tinduka* is having *kashaya* as *pradhana rasa*, *tikta* and *madhura rasa* as *anurasa*[8]. Usually drugs with *rasa* such as *kashaya*, *tikta* and *madhura* are having *pitta shamana* property[9]. *Kashaya rasa* exhibits *sandhanakara*, *ropana*, *shodhana*, *lekhana*, *shoshana* action[10,11]. These properties responsible for cleansing wound area, scraping out the dead tissue, removes *kleda* from

site of wound and thereby cause shrinkage of wound surface which leads to wound closure. *Tikta rasa* possess *vishaghna, krimighna, twak mamsa sthirikarana, lekhana, kledashoshana* property[12]. Because of these properties it will detoxify the burn wound area, destroys pathogens, provide stability to wound bed and granulation tissue, scrap out the tissue debris, removes excess fluid accumulation. *Madhura rasa* having *twachya, sthairyakara* and *kshatasandhanakara* property[13]. Due to these properties it stabilizes the granulation tissue and helps for union of tissue.

Though *panchamahabhuta* predominant of *Tinduka twak* is not directly mentioned in the classics, it can be inferred through *rasa*. By the rasa we can consider that the drug is having predominance of *prithvi* and *vayumahabhuta*. *Prithvi mahabhuta pradhanadravya* is having *upachaya* and *sanghata karma*[14]. By this it will nourish growing granulation tissue as well as helps in union of tissue. *Vayu mahabhuta* is having *virukshana karma*, by this drug does *drava shoshana* in the wound area, thus causing shrinkage of wound surface thereby initiating *vranaropana*[15].

Effective wound closure was observed from 8th day of post wounding in *Tinduka twak kwatha* group. Wound contraction was in its peak till complete healing compared to control. Eschar fall and complete cure was earlier in this group. This suggests that epithelialisation rate is better in *kwatha* form. In *Tinduka twak kwatha* the active principles are gets extracted in more concentration, thus have more effective role in *ropana karma*. *Parisheka* with *kwatha* cleanses the wound area and provides barrier protection from *krimis*. Good regeneration of epithelial tissue in *kwatha* group. All this results indicative of better healing capacity of *kwatha* in proliferative and remodeling phase.

The bark of *Diospyros malabarica* has preliminary phytochemical such as alkaloids, steroids, carbohydrate, tannin, flavanoids, saponins, triterpenoids, phenols, Quinone[16]. Each of these bioactive agents will have specific action on different stages of wound healing. Saponins content may enhance the synthesis of pro-collagen. Flavanoids have antimicrobial, antioxidant activity. More recent research has found that tannin has property to form complexes with several biomolecules and thereby has efficacious in precipitating poisonous materials in burned wound. Also, tannin does inhibition of formation of reactive oxygen species and growth of microorganism, in addition it promotes growth of epithelium. Alkaloids, steroids, terpenoid etc have anti-inflammatory activity. Recent researches on the source of *Tinduka* concluded that *Diospyros malabarica* (Desr.) Kostel drug possess astringent property, anti-inflammatory, antimicrobial and antioxidant activity.

CONCLUSION

Tinduka has been classically as well as traditionally used for burn healing. In the current study, Tinduka kwatha group showed statistically significant result towards wound healing parameters compared to control and standard. The

current study certainly encourages further study on this drug in different perspective clinically and thus helps in better utility in future treatment..

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