

Experimental Evaluation of Wound Healing Activity of Various Dosage Forms of *Tridax Procumbens*

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Abstract

Introduction: *Tridax procumbens* Linn. (Tridax) is a common weed found all over India. It is commonly known as Peet Bhringraj, Jakhamjod and Kambarmodi etc. It is traditionally being used since centuries for wound healing and haemostasis and also for the treatment of diarrhea, dysentery, bronchial catarrh etc.

Need of the Study: Tridax is traditionally being used in its crude form. Despite of the marked utility of Tridax in wound management, in Ayurvedic literature neither there is much description of Tridax nor its dosage forms are mentioned. Exploring the various dosage forms

Objective: Thus the study is designed to find out an effective Ayurvedic dosage form of Tridax that is more effective and on the same hand is holistic in approach as per principles of Ayurveda.

Materials and Method: An animal experiment is conducted on Albino rats using excision wound model. Five dosage forms of Tridax were prepared such as Ash, Kalka, Oil, Ghana and Ethanolic extract and were compared with control to evaluate rate of contraction and period of epithelialization.

Results and Conclusion: The results were outstanding. Different dosage forms of Tridax exhibited excellent wound healing property. Few dosage forms exhibited typical behavior in different stages of wound healing. Hence Tridax can be seen as a potent drug to develop a good wound healing agent of future.

Keywords: *Tridaxprocumbens*, wound healing, dosage forms, herbal medicine.

Introduction

Tridax procumbens Linn. (Tridax) is a common weed found all over India up to 2400 m above sea level

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and in all hot countries. The plant is native of tropical America and naturalized in tropical Africa, Asia and Australia. In English it is popularly called 'coat buttons'. It is known for its various medicinal properties. Native healers of India are using this herb since ages for various treatment purposes. Bapalalvaiddya in Nighantu Adarsha has mentioned Tridax by the name 'Pardesi Bhringraj'⁽¹⁾. Shodhal Nighantu has mentioned Tridax by the name Avanti⁽²⁾.

Tridax is known by different names in various parts of India, such as, Kambarmodi, Jakhamjod, Ekdandi, Brahmadandi, Dagdipala, Tan tani, Man modi in Maharashtra; Moliamehandi, Pathhargada, Raktrodhi, Raktashoshi in Rajasthan⁽³⁾; Tumbi, Vettu-

kkaya- thalai in Tamilnadu; Raavanaasuraaditalakaai, Kampu Chemanti in Andhra; Gabbu sanna savanthi, nettu gabbu savanthi, Tikki kasa, Tikki toppala, Gayad toppala, Kulae puduga, Kulae buskad, Hochod tasad, Robrobpuru in Karnataka; Vranaropini, Raktarodhi, Tikki kasa, Dandotpala, Sanjivani, Peet Bhringraj in north India⁽⁴⁾.

It is traditionally known for its various medicinal properties, especially wound healing, haemostatic, Antiseptic, pain relieving. In tribal region, application of Tridax fresh leaves paste for haemostasis and wound healing is a common practice. Tridax has a good application for wound management in traditional medicine from centuries.

It is also used in dysentery, diarrhea, bronchial catarrh, sore throat etc. It is eaten as vegetable in some parts. It is also fed to cattle as a nutritive feed. In Kenya the leaves are chewed followed by a drink of water by the Masai for stomachache and malaria and in Tanganyika a decoction of the whole plant in water is taken for persistent low backache and a root decoction for infantile diarrhoea. Traditionally local Yoruba population of Western states of Nigeria uses the leaf of the plant as treatment to reduce blood pressure, conjunctivitis, diarrhoea and dysentery to wound healing and related inflammatory conditions⁵.

Need of the Study: One cannot always use the herb in its native form due to different reasons like undesirable test, smell, physical form, mal absorption etc. Also, drug cannot be obtained fresh in all seasons of the year. Hence, man started converting drug into some processed form that possesses desirable durability, good test, smell, form, potency, prolonged shelf life etc. This science then developed and called Bhaishajya Kalpana Vigyana.

Tridax is traditionally being used in its crude form. The most frequently used form of Tridax for medicinal purpose is Paste, fresh juice and rarely decoction. A few research works has been carried out to evaluate the wound healing activity of Tridax. But most of the times organic solvent extracts of Tridax were used. Ayurvedic

system of drug manufacturing does not support the use of extracts derived using toxic and carcinogenic solvents. In Ayurvedic system all the things used are in edible form as a holistic approach. But Despite of the marked utility of Tridax in wound management, in Ayurvedic literature neither there is much description of Tridax nor its dosage forms are mentioned. Thus the study is designed to find out an effective Ayurvedic dosage form of Tridax that is equally or more effective than solvent extracts and on the same hand is holistic in approach as per principles of Ayurveda.

Method And Material

Materials: For experimental study, the materials required are,

Albino rats: 36

Diethyl Ether, Scissors, Mosquito forceps, Povidone iodine, BP blades, BP handles, surgical gloves, Sterile surgical gloves, sterile gauze pads, sterile eye towels, scientific measuring scale, Rat cages, feeding bottles, standard rat feed, Tridax fresh leaf paste, Tridax Ghana solution, Tridax oil, Tridax Ash, Tridax Ethanolic extract etc.

Method:

Animal Ethics committee approval: Proper approval of animal ethics committee of Apollo college of veterinary medicine, Jaipur was taken for the preclinical study.

Selection of animals: Healthy albino rats of either sex weighing between 150-200 gm were selected randomly for study. The rats were housed in the animal house of Apollo College of veterinary medicine, Jaipur. The rats were fed with standard rat pellets and water ad-libitum.

Grouping of animals: Thirty six Albino rats were selected randomly from the animal house and were divided into six groups each containing six albino rats. The rats were housed in individual cages and kept in a well ventilated room under hygienic conditions.

Table I: Table showing grouping of animals

Group	Group drug	No. of rats	Purpose
C	No drug (control)	6	To serve as prophylactic control
A	Tridax Ash (Bhasma)	6	To serve as prophylactic effect
K	TridaxPaste (Kalka)	6	To serve as prophylactic effect
O	Tridax Oil (Taila)	6	To serve as prophylactic effect
G	Tridaxaqueous extract (Ghana)	6	To serve as prophylactic effect
E	Tridax Ethanolic Extract	6	To serve as prophylactic effect

Experimental Study: The wound healing property of the trial drugs, 5 dosage forms of *Tridax procumbens* was analyzed by Excision Wound Model. This study included following stages.

- Pre operative stage
- Operative stage
- Post operative stage

Pre Operative Stage: The selected albino rats were fasted overnight and water was given ad-libidum. Each rat was hydrated with 5 ml of water and was kept in individual cages.

The part to be operated was shaved with scissors and razors and painted with povidone iodine 5% solution.

The animals were anesthetized using Diethyl ether inhalation anesthesia in a semi-aseptic condition. A stage 3 anesthesia was achieved.

Operative Stage: This was conducted as per the method developed by Morton and Mallon. The anesthetized animals were secured to the dissection tray in prone position. A circle of 15 mm in diameter was marked with surgical marker pen on dorsal region approximately 5 cm away from the ears of the anesthetized rat. Full skin thickness from the marked area was excised in a circular fashion with the help of forceps and surgical scissors. The approximate area of wound thus formed was 177 mm square. After achieving full haemostasis rats were placed in individual cages.

Post operative stage: The control group was dressed with 5% povidone iodine solution, while other trial groups were applied with respective drugs locally once a day. Such as

Tridax Ash (Bhasma)–Dusting locally

Tridax Paste (Kalka)–Painting Locally

Tridax Oil–Local application

Tridax Aqueous extract (Ghana)–1gm/ml solution local application

Tridax ethanolic extract–50mg/ml solution local application

The rats were observed till the recover from anesthesia. Each post operative day, the wounds were cleaned with normal saline water and applied with trial drugs. The rats were given normal feed and water.

Assessment Criteria: Wound contraction and epithelialisation were the parameters employed to study excision wound model and this was achieved using planimerty.

1. Wound Contraction: The factor which contributes mainly to wound healing (closure) is contraction. This was done by tracing the wound margins and measuring the area of wound. Later the percentage of contraction was calculated arithmetically from the measured area. This measurement was done till the wound completely closes.

2. Period of Epithelialisation: It was measured in terms of days required for the falling of the scar. Falling of scar leaving no raw area behind was taken as the end point of complete epithelialization and the time was noted in all animals.

Observations and Results

To monitor the change in wound shape, the wound

diameters in two perpendicular planes were measured. The area of the oval was calculated as per the formulae 'A = $\pi \times r_1 \times r_2$ ' where A stands for the area of the ellipse, $\pi = 3.14$, r_1 = longer radius and r_2 = shorter radius. The

observations of percentage of wound closure were made on 3rd, 6th, 9th, 12th, 15th, 18th and 21st post wounding day. The wound was also observed for the period of epithelialisation in term of days.

Table II: Table showing comparative closure of wound area on 3rd post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 7.5456	< 0.0001	A > C extremely significant
2	Control C & O	t = 3.4374	= 0.0064	O > C very significant
3	Control C & K	t = 13.2686	< 0.0001	K > C extremely significant
4	Control C & G	t = 2.4709	= 0.0331	G > C significant.
5	Control C & E	t = 0.0703	= 0.9453	Not significant.

Table III: Table showing comparative closure of wound area on 6th post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 11.2320	< 0.0001	A > C extremely significant
2	Control C & O	t = 0.1733	= 0.8659	Not significant
3	Control C & K	t = 9.6743	< 0.0001	K > C extremely significant
4	Control C & G	t = 5.3336	= 0.0003	G > C extremely significant.
5	Control C & E	t = 1.8691	= 0.0911	Not quite significant.

Table IV: Table showing comparative closure of wound area on 12th post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 3.5979	= 0.0049	A > C very significant
2	Control C & O	t = 0.2116	= 0.8367	not significant
3	Control C & K	t = 7.1164	< 0.0001	K > C extremely significant
4	Control C & G	t = 2.2653	= 0.0469	G > C significant.
5	Control C & E	t = 2.8618	= 0.0169	E > C significant.

Table V: Table showing comparative closure of wound area on 18th post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 3.6576	= 0.0044	A > C very significant
2	Control C & O	t = 3.6576	= 0.0044	not significant
3	Control C & K	t = 3.6576	= 0.0044	K > C very significant
4	Control C & G	t = 3.6576	= 0.0044	G > C very significant.
5	Control C & E	t = 0.6458	= 0.5329	Not significant.

As represented in above tables, gross findings about wound contraction are as follows.

- All the dosage forms of Tridax showed statistically significant results compared to control group in wound contraction.
- In first 3 days Kalka and Ash were extremely significant in contracting wound. Though in two cases Oil failed in wound contraction, statistically oil was very significant. Ghana was also significant where Ethanolic extract was not significant.

3. On 6th post wounding day Ash, Kalka and Ghana were extremely significant whereas Alcoholic Extract and Oil were insignificant.
4. On 9th post wounding day Kalka was extremely significant in wound contraction, whereas other dosage forms were not significant.
5. On 12th post wounding day Kalka was extremely significant, Ash was very significant, Extract and Ghana were significant But Oil was not significant.
6. On 15th day the wounds of group Kalka and Ash were completely contracted and healed with extremely significant results. Ghana also showed extremely significant results whereas Oil and Extract were not significant.
7. On 18th day wounds of all the group were healed except that of Extract group that was also non significant in contraction.

Period of epithelialisation: Falling of scar crust leaving no raw area behind was taken as the end point of complete epithelialisation

Table VI: Table showing comparative epithelialization time of wound.

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 12.7279	<0.0001	A < C extremely significant
2	Control C & O	t = 1.3815	=0.1972	not significant
3	Control C & K	t = 12.4581	<0.0001	K < C extremely significant
4	Control C & G	t = 10.1419	<0.0001	G < C extremely significant.
5	Control C & E	t = 2.6769	=0.0232	E < C significant.

As clear from above table, followings were the results about epithelialisation of wounds.

1. All the dosage forms of Tridax significantly reduced the period of epithelialisation as compared to the control group.
2. Ash was the best in reducing period for epithelialisation with statistically extremely significant results. It was followed by Kalka and Ghana with extremely significant results. Extract was also significant whereas Oil was statistically insignificant in epithelialisation.
3. It was observed with group Kalka that, on 18th day the healed scar showed hair growth whereas no other group showed hair growth on scar even after 21 days after wounding.

Discussion

As revealed from this experiment, different preparation of Tridax had shown different pattern of activity in initial, intermediate and late phase of wound healing. Such as Ash and Kalka has shown rapid wound contraction in initial stage whereas oil failed to contract wound. On contrary Oil caused to dilate the wound

area in some cases in initial stage. Ethanolic extract of Tridax promoted wound contraction in intermediate stage of wound healing whereas it delayed the last stage of wound healing. This typical behavior may be due to the phytochemical contents of Tridax in that particular dosage form, may be due to physical nature of dosage forms such as Ash is in an absorbent powder form, Kalka itself contracts and along with contracts the wound as it dries, Oil prevents the wound from drying and contracting, etc. or it may be the combined action of phytochemicals, vehicles and physical form of the dosage form. A separate study of each dosage form may unwind the mystery^{6,7}.

Another interesting observation was that, the Tridax Kalka promoted hair growth after healing the wound. Whereas other dosage forms and control group showed absence of hairs on the scar area. The Kalka might have got some novel property that promotes healthy epithelialisation and formation of hair follicles. Generally excised wounds heal through collagen and fibrous tissue formation that lack hair follicles. Further histological investigation is necessary to find out exact pattern of tissue development⁸.

Finally the traditionally used form of Tridax that is Kalka is found to be most useful for wound healing purpose. This might be one of the reasons why other dosage forms of Tridax could not evolve in the course of time. Another most useful dosage form of Tridax is found as its Ash. Also other dosage forms may be purposefully used as they promote wound healing at different stages of wound healing. Further detailed study including histological and histochemical investigation will be beneficial in order to develop a good wound healing agent of future from Tridax⁹.

Conclusion

Tridax is traditionally being used for wound management in India. Ayurvedic text quotes no reference about different dosage forms of Tridax. The experimental trial of different dosage forms of Tridax on wound healing in Albino rats reveals that, Tridax has got highly significant improvement in wound healing including wound contraction and epithelialization, except the oil that is not significant in reducing time for epithelialisation. Kalka the most famous traditional dosage form of Tridax and Ash were found to be best in wound healing, followed by the Ghana. Different preparation of Tridax shows different pattern of activity in initial, intermediate and late phase of wound healing. Tridax Kalka also promoted hair growth on scar after healing the wound. Further detailed study including phytochemical, histological and histochemical investigation will be beneficial in order to develop a good wound healing agent of future from Tridax.

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References

1. Bapalalvaidya, NighantuAdarsh Part-I:769
2. Shodhal, ShodhalNighantuPart I:70,72,80,
3. Ambulkar P.Characterization, Pharmacological and Microbiological activity of various dosage forms of Tridaxprocumbens L., thesis submitted to Rajasthan Ayurveda University, Jodhpur (2011)
4. Morton J. Evaluation of vulneray activity by an open wound procedure in rats. Arch. Int. Pharmacodyn Ther. 196(1): 117-26.
5. Yaduvanshi B et al. Evaluation of Wound Healing Potential of Topical Formulation of Leaf Juice of TridaxProcumbens L. in Mice. Indian J Pharm Sci. 2011; 73(3): 303–306.
6. Diwan P et al. Influence of Tridaxprocumbens on wound healing. Indian J Med Res. 1982;75:460–4.
7. Diwan P et al. Steroid depressed wound healing and Tridaxprocumbens. Indian J Physiol Pharmacol. 1983;27:32–6.
8. Udupa S et al. Influence of Tridaxprocumbens on lysyl oxidase activity and wound healing. Planta Med. 1991;57:325–7.
9. Kasture A, Wadodka S. Preliminary phytochemical study of Tridaxprocumbens Linn. Indian J Pharmacol. 1971;33:96.