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Evaluation of Wound Healing Activity of Siddha Herbo-Mineral Formulation Rathinagara Rasa Mezhugu in Experimental Rats

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Abstract: The aim of this study was to investigate the wound healing potential of Herbo-mineral formulation Rathinagara rasa mezhugu (RNM) using excision wound model in Wistar albino rats. The animals were randomly selected and divided into five groups (I, II, III, IV and V) of six rats (n=6) each. Group I animals received only palm jaggery as vehicle control. Group II received dexamethasone (0.17 mg/kg/IM) as standard drug. Groups III,IV and V were treated with 20 mg/kg, 45 mg/kg, and 90 mg/kg of RNM for 14 days. Healing potential was assessed by parameters like rate of wound contraction and period of epithelialization. Oral administration of Rathinagara rasa mezhugu at two dose levels (dose II and dose III) significantly decreased ($P<0.01$) the wound area, percentage of wound contraction and period of epithelization when compared to control in the observed time point of 4 to 16 days. While comparing other doses of RNM, dose level at 90 mg/kg was found to be extremely significant ($P<0.001$) and decreases the wound area and all other parameters. The study strongly suggests that the Rathinagara rasa mezhugu exhibits good wound healing potential in the excision wound model in experimental rats. Clinical trials must be done on the drug to support the traditional use claimed in Siddha literature. So , in future this formulation can be used as a wound healing agent for treating various skin conditions by Siddha physicians.

Keywords: Siddha medicine, Wound healing activity, Rathinagara rasa mezhugu, Excision wound model, Period of epithelization

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Introduction

Wound healing remains a difficult clinical challenge despite scientific advances in the field. A proper and efficient wound management is very important. A focus on innovative and alternative therapeutic techniques, as well as the development of technologies for wound management is necessary. Wounds are physical injuries that result in a breaking or opening of the skin. A suitable wound healing approach is needed for the restoration of disrupted anatomical

continuity and impaired functional status of the skin (Ayyanar and Ignacimuthu *et al*., 2009). It may also results from a hematoma, contusion, laceration, or an abrasion (Enoch and John Leaper, 2005). Ulcer can range from a simple break in epithelial integrity of skin or deep into subcutaneous tissues with damage to structures like muscles, vessels, nerves, tendon, and bones (Robson *et al*., 1996). Wounds are classified as acute, Chronic, and complicated depending upon the time of repair. Wound healing involves several processes including inflammation, granulation, fibrogenesis, neovascularization, wound contraction, and epithelialization. The basic principle of wound healing is to minimize tissue damage and provide proper nutrition, adequate tissue perfusion, oxygenation, and moist wound-healing environment to restore the damaged part's anatomical continuity and function. Wound healing is determined by interaction of various epithelial and mesenchymal cells with cytokines, chemokines, and growth factors (Dehkordi *et al*., 2019).When there is a tissue injury, the coagulation pathway is activated, which results in the development of a temporary fibrin matrix that allows cells to migrate to the wounded area. At the same time, Platelet-derived factors attract leukocytes, and activating the inflammatory response. Growth factors and cytokines are produced by platelets and immune cells, which promote wound re-epithelialization, extracellular matrix (ECM) deposition, and angiogenesis. When the wound healing process is disrupted, a chronic wound forms that is far more difficult to heal (Qing, 2017).

Wound healing is a complex phenomenon which depends on the immunity and status of the wound bearing person. Synthetic drugs may lead to adverse effects, drug resistance with high cost of treatment. In recent times, Herbal research has been receiving much attention and high surge for herbal medicines. In traditional medicine, many medicinal plants are effective for wound healing properties. There are few plants/formulations in Siddha system which has been preclinically evaluated for wound healing activity. Siddha medicines are popular for its easy availability and affordability with less or no side effects and improving the overall quality of life of patients. But for getting wide recognition and acceptance, employing modern, up to date scientific techniques and to validate evidence-based approaches to alternative therapies like Siddha for wound healing to promote these treatments are safe and efficacious. Rathinagara rasa mezhugu (RNM) is a classical Siddha Herbo-mineral formulation that is mentioned in Anuboga vaidhaya naveneetham Part 5 (Hakeem. Pa. Mu. Abdullasayub, 2014). It has been used for the management of Pun (Ulcer), Karunkuttam, Senkuttam (leprosy), Pilavai (carbuncles), Kandamalai (Cervical adenitis), Puraiyodina punkal (deep ulcers/sinus). The present study aimed to evaluate the wound healing activity of Siddha Herbo-mineral formulation Rathinagara rasa mezhugu (RNM) using excision wound model in Wistar albino rats.

Materials and Methods

Ingredients of Rathinagara rasa mezhugu: Table 1 illustrates various ingredients of Rathinagara rasa mezhugu.

Table 1: Various ingredients of Rathinagara rasa mezhugu

S. No.	Name of the drug	Scientific name/Common name	Quantity
1	Sitramanakkennai	*Ricinus communis L./Castor oil*	¼ palam (8.75 g)
2	Vaalai Rasam	Hydrargyrum/ Absolute mercury	1 palam (35 g)
3	Gandhakam	Purified Sulphur	1 palam (35 g)
4	Serankottai	*Semecarpus anacardium* Linn./ Marking nut	30 nos.
5	Pasu nei	Cow's ghee	1 palam (35 g)
6	Panai vellam	Palm Jaggery	2 palam (70 g)

Raw Drug collection: Lingam (Cinnabar), Ganthagam (Sulphur), and Serankottai

(*Semecarpus anacardium*) were procured from a reputed country shop in Parrys Corner, Chennai, India. The other ingredients *Sitramanakku ennai* (castor oil), Pasu nei (ghee), and Panai vellam (palm jaggery) were purchased locally from the Tambaram market, Chennai, India.

Identification and Authentication of the drug:

The herbal ingredients were identified and authenticated by the Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Tambaram sanatorium (Certificate No: NISMB4922021). The metal drugs were identified by Pharmacologist, Department of Gunapadam, National Institute of Siddha, Tambaram Sanatorium, Chennai (Certificate no: Gun/Aut/026/21).

Preparation of trial drug Rathinagara rasa mezhugu:

The Castor oil is taken into a vessel and heated. Then Purified sulphur is powdered and added with the heated castor oil. When the sulphur melts, Semecarpus seeds were cut into two pieces and put into the oil. Then thailam is taken when Semecarpus seeds turn red and float. The thailam is called Rathi nagara thailam. Mercury and Rathi nagara thailam were mixed and ground. When mercury is ground well, Ghee and palm jaggery are added, ground to get Rathinagara rasa mezhugu.

Evaluation of wound healing activity of RNM by Excision wound model in Wistar Rats:

Procurement and rearing of experimental animal:

Healthy adult Wistar albino rats weighing between 200- 320 g were used for the study. The animals were housed in poly propylene cages and were kept in well-ventilated with 100% fresh air by air handling unit. A 12h light/12h dark cycle were maintained. Room temperature was maintained between 22 + 2 ℃ and relative humidity 50–65%. They were provided with food and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol (Table 2) was approved by The Institutional Animal Ethics Committee of National Institute of Siddha (NIS/IAEC-22/R02/16112021/E4) Chennai, Tamil Nadu, India.

Protocol of experiment and Excision wound model:

Rats were fasted for 12 h before the experiment. Animals were shaved on the dorsum portion using depilatory cream (Nair) and anesthetized with Ketamine (70 mg/kg/IP), Xylazine (6 mg/kg/IP) and for relieving pain Melonex (5 mg/kg/IP) were given intraperitoneally in pre- and post-surgical procedure. An impression was made on shaved dorsal region and area of the wound to be created and marked. A full-thickness excision wound with a circular area of ≈500 mm^2 -700 mm^2 was created along the marking using toothed forceps, a surgical blade, and pointed scissors by a Standard ring. Rats were left undressed to the open aseptic environment. In this model, wound contraction and epithelialization were evaluated.

Measurement of wound contraction:

In the excision model, wound areas were measured by tracing wound with the help of Vernier caliper on the day of wounding and Wound contraction was measured every 4^{th} day until complete wound healing and represented as percentage healed of wound area. Wound healing potential was determined by wound contraction and wound closure time (Meena Kumari *et al*., 2010). The wound healing potential of the drug was calculated accordingly to percentage of wound contraction (Dev *et al.*, 2019).

$$\text{Percentage Wound contraction} = \frac{\text{Initial Wound Size} - \text{Wound size on the day}}{\text{Initial Wound Size}} \times 100$$

Statistical analysis:

Results obtained *in vivo* excision model were expressed as Mean ± SD and were compared with the corresponding control group by one-way ANOVA test for assessing statistical significance.

Results

It was observed that the wound area of control group rats for the observed time point of 4 to 16 days varied from 424.61 ± 60.95 mm^2 to 147.27 ±

Table 2: Wound healing activity group with description

S. No.	Groups	Treatment	No. of Animals
1	Group I	Vehicle Control (Palm jaggery)	06
2	Group II	Standard drug Dexamethasone (0.17 mg/kg/IM)	06
3	Group III	Treated with RNM (20 mg/kg)	06
4	Group IV	Treated with RNM (45 mg/kg)	06
5	Group V	Treated with RNM (90 mg/kg)	06

Table 3: Effect of RNM on Wound area

Treatment	Wound Area (mm^2)				
	0 day	4^{th} day	8^{th} day	12^{th} day	16^{th}day
Control	483.74± 56.68	424.61± 60.95	339.51± 56.86	254.86± 49.65	147.27± 38.94
Standard	459.45± 61.11	306.02± 46.80*	210.51± 31.54*	123.37± 28.64**	32.47± 13.13***
Test Dose I	532.06± 64.65	437.25± 64.31	340.04± 54.69	253.33± 41.47	158.96± 42.01
Test Dose II	472.97± 52.29	357.64± 63.25	249.01± 61.29	174.36± 77.28*	93.27± 43.05*
Test Dose III	446.88± 20.82	290.06± 19.71*	178.72± 17.87**	111.08± 37.32***	37.68± 8.92***

Values are Mean±SEM (n=6); *P<0.05, **P<0.01 and ***P<0.001 Vs control

38.94 mm^2. Treatment with trial drug RNM at dose level 20 mg/kg, 45 mg/kg, and 90 mg/kg have shown a significant decrease in wound area in the observed time point to the maximum of 437.25± 64.31 mm^2 to 158.96± 42.01 mm^2 for test dose I (20 mg/kg), 357.64± 63.25 mm^2 to 93.27± 43.05 mm^2 for test dose II (45 mg/kg) and 290.06± 19.71 mm^2 to 37.68± 8.92 mm^2 for test dose III (90 mg/kg) drug treatment when compared to that of the standard drug treated group with the wound area of 306.02± 46.80 mm^2 to 32.47± 13.13 mm^2 (Table 3, Fig. 1). The wound healing effect of the drug is ascertained based on the percentage of wound contraction index. It was observed that the maximum wound contraction rate of RNM (dose I) treatment was found to be 51.66±4.21 mm^2. Further significant percentage contraction was observed in the dose II and dose III treatment group 61.60 ±4.69mm^2, 73.17 ± 3.32 mm^2, respectively when compared to that of the standard drug treated group with the maximum contraction index of about 80.86±3.60 when compared to the control group with a contraction index of about 41.78±2.82 (Table 4, Fig. 2).

Discussion

Wound is a major health problem in terms of morbidity and mortality. The wound healing process consists of various phases such as granulation, collagenation, collagen maturation,

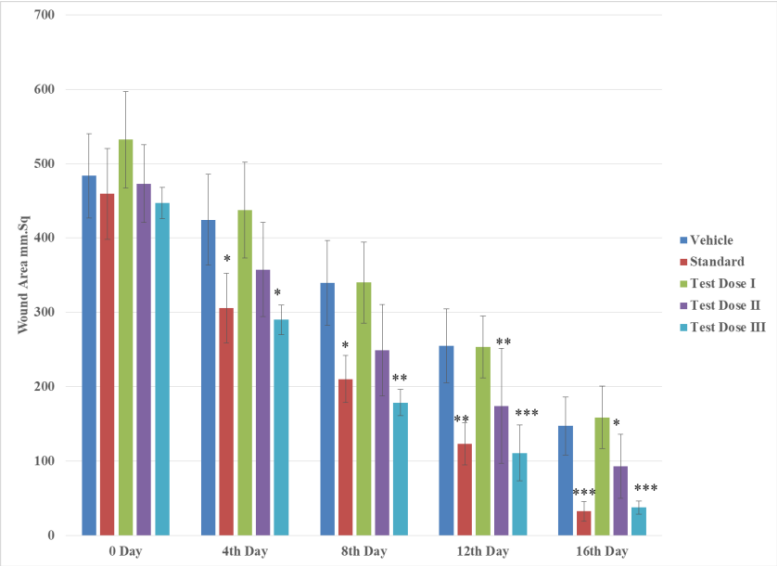


Fig. 1: Effect of RNM on Wound area.

Table 4: Effect of RNM on % Contraction

Treatment	4th day	8th day	12th day	16th day
Control	5.93± 1.14	18.36± 2.44	30.52± 3.21	41.78± 2.82
Standard	20.71± 3.47***	32.56± 2.62***	53.72± 2.73***	80.86± 3.60***
Test Dose I	10.37± 1.12***	27.12± 3.63**	38.25± 5.67*	51.66± 4.21*
Test Dose II	17.81± 3.44***	34.92± 6.63***	53.08± 5.59***	61.60± 4.69**
Test Dose III	19.55± 2.53***	39.84± 2.27***	59.53± 3.88***	73.17± 3.32***

Values are Mean±SEM (n=6); *P<0.05, **P<0.01 and ***P<0.001 Vs control

and scar maturation which are concurrent but independent to each other (Agarwal *et al*., 2009). The duration of healing predominantly depends upon the rate of wound contraction which enhances and facilitates the wound closure via rapid re-epithelization by shortening the migratory distance travel by keratinocytes (Tang *et al*., 2007)It also reduces the extracellular matrix required to recover the damaged epithelium (Strodtbeck, 2001). In this study wound healing potentials of Siddha Herbo-mineral formulation Rathinagara rasa mezhugu (RNM) were evaluated by excision wound model in Wistar albino rats with dexamethasone as the standard drug. Dexamethasone is a corticosteroid that decreases inflammation and increases vasculogenesis. Long-term treatment with steroid drugs has many side effects such as increased risk of infection, high blood pressure, osteoporosis, and development of diabetes (Liu, 2014). It has been suggested that

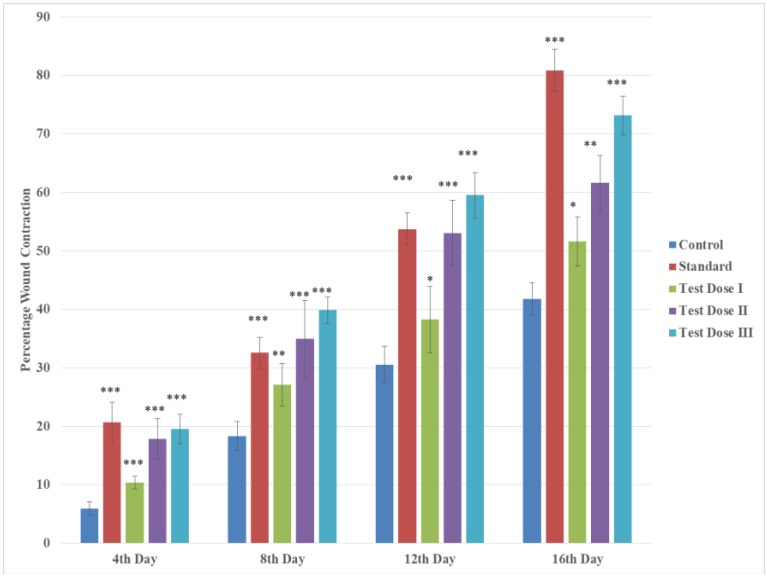
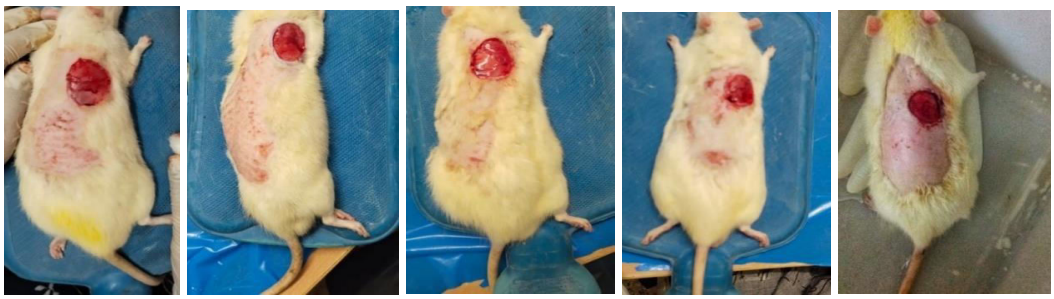


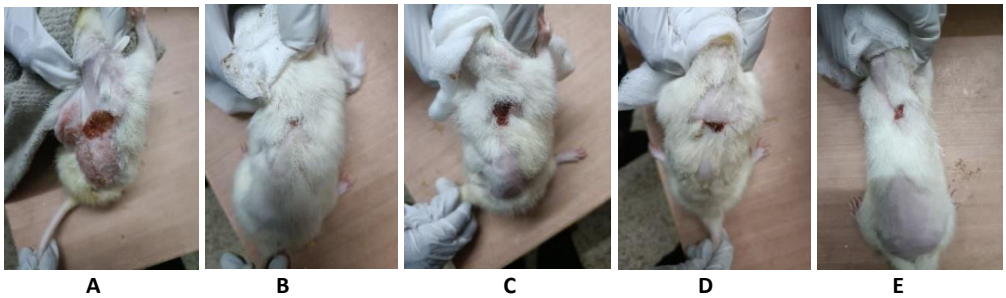
Fig. 2: Effect of RNM on % Wound Contraction. The data are expressed in mean ± SEM. Results were analyzed using one-way ANOVA followed by Dunnett's test. Differences were considered statistically significant if p< 0.05. when compared with the control. p value ***P<0.001 is considered to be extremely significant, **P<0.01 and *P<0.05 were considered as significant and P>0.05 indicates non-significant.

Day of Excision before treatment



A B C D E

16th Day of Wound area post treatment with test and Standard drug



A B C D E

Fig. 3: Wound Area before and After treatment of RNM and Standard drug. A-Group 1 (Vehicle control); B- Group 2 (Standard); C- Group 3 (Test dose I); D- Group 4 (Test dose II); E-Group 5 (Test dose III).

the anti-inflammatory and immunosuppressant activities may have a negative effect on wound healing and might increase the risk of infection with long-term treatment (Busti, 2005).

In the excision wound model, animals were treated with the RNM at three dose levels (20 mg/kg,45 mg/kg, and 90 mg/kg). The test drug and standard drug showed considerably minimum duration for complete recovery against the untreated group. Treatment with RNM at dose II and dose III ($P<0.01$) showed a significant decrease in wound area compared to control. RNM at these dose levels exhibited a significant decrease in the epithelization period, as evidenced by the shorter period for the fall of eschar when compared to control in the observed time point of 4 to 16 days. Percentage of wound contraction index determines the wound healing effect of a drug. The drug also facilitated the rate of wound contraction significantly which increases at these dose levels. The faster wound contraction by RNM may be due to stimulation of interleukin-8, an inflammatory α-chemokine that affects the function of various inflammatory cells, fibroblasts, and keratinocytes, and may increase the gap junctional intracellular communication in fibroblasts, and induces a more rapid maturation of granulation tissue (Moyer *et al*., 2002).While comparing these three doses of RNM, the dose at 90 mg/kg was found to be significant ($P<0.001$) and more effective. There was an overall decrease in the wound area, significant percentage of wound contraction parameters in the RNM at 90 mg/kg treatment group of rats.

Among the key ingredients, Sittramanakku ennai (*Ricinus communis*), Serankottai (*Semecarpus anacardium*), Valai Rasam (Elemental mercury), and Gandhagam (Purified sulphur) have already proven the wound healing potential in preclinical studies. These ingredients containing medicines like Rasagandhi mezhugu, Serankottai nei, Gandhaga Mezhugu, Idivallathi mezhugu, Neeradimuthu vallathi, Parangipattai pathangam, Sivanar amritham are widely used in clinical practices for treating skin diseases. *R. communis* promotes the wound healing process by enhancing wound contraction and increases rate of epithelialization (Sonali and Shonkor, 2015). The wound healing potential of the *R. communis* aqueous extract might be related to the presence of flavonoids, rutin, quercetin, epicatechin and polyphenols (Gallic and ellagic acid) and gentesic acid, these compounds which are potent anti-oxidant and anti-inflammatory agents (Saini *et al*., 2010). Inflammation is the primary wound-related characteristics caused by the release of prostaglandins, leukotriene, eicosanoids, and reactive oxygen species. The *Ricinus communis* exhibits wound healing potential due to the active principle of castor oil which produce antioxidant activity and inhibit lipid peroxidation. Due to the astringent and antimicrobial activity the tannins, flavonoids, triterpenoids and sesquiterpenes promotes the wound healing process, which are responsible for wound contraction and increased rate of epithelialization. Castor oil helps to reduce the scar area, epithelization time in excision wound model and promoting the wound healing potential (Prasad *et al*., 2011).Methanolic leaves extract of *R.communis* shows a protective effect in the prevention of cellular events during edema formation and in all the stages of acute inflammation (Ilavarasan, 2006). The wound healing activity of stem bark methanol extract of *Semecarpus anacardium* was evaluated by incision, excision, and dead space wound models using wistar albino rats. Methanol extract at 100 mg /kg exhibited significant wound healing activity but was lesser than standard. The study supported the ethnomedicinal claims of *S. anacardium* (Lingaraju *et al*., 2012). Various phytoconstituents such as tannins, flavonoids, glycosides, alkaloids, saponins, and terpenoids act as a therapeutic agent for the treatment of wounds and skin regeneration (Qu *et al*.,2018). So, considering the all parameters, our study has created scientific preclinical evidence for using Rathinagara rasa mezhugu (RNM) for the management of wounds or ulcers. However, further studies on the phytochemicals responsible for wound healing effect, their molecular mechanisms and clinical trials are needed.

Conclusion

Based on the data obtained from the present investigation it is concluded that the drug RNM exhibited significant wound-healing activity in the excision wound model in Wistar albino rats. The Wound healing activity of the drug may be due to the possible synergistic action of key ingredients used in the formulation. More specific experiments are required to understand the exact molecular mechanisms of action. This finding suggested that the drug RNM to be implemented as a wound-healing agent and uphold the traditional use of the drug claimed in Siddha literature for the treatment of ulcers or wounds. This study throws the limelight for conducting extensive clinical trials to prove the efficacy of this formulation in humans also.

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