Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

STUDY OF KAJJALIKODAYA MALAHARA FOR EVALUATION OF WOUND HEALING ACTIVITY

Jayalaxmi [1]*, MS Krishnamurthy [2]

[1] PhD Scholar, HOD and Associate Professor, Department of Rasa Shastra and Bhaishajya Kalpana, Shri Basaveshwara Ayurvedic Medical College Hospital and Research Centre, Kalaburagi, Karnataka, India

[2] HOD, Department of Rasa Shastra and Bhaishajya Kalpana, Alvas Ayurveda Medical College, Moodbidri, Mangalore, Karnataka, India

Received on: 25/1/24 Accepted on: 25/3/24

***Corresponding author**
E-mail: drjayalaxmim@gmail.com

DOI: 10.7897/2277-4343.15237

ABSTRACT

Introduction: Kajjalikodaya malahara, mentioned in the Rasatarangini text, is indicated in different types of wounds like chronic wounds and fistula in ano, which did not respond to other treatment modalities. So, this formulation was selected for the present study. Material and methods: The Kajjalikodaya malahara prepared as per reference -anagni (without fire) and also modified version- agni siddha (with fire) method and subjected for antimicrobial, acute dermal toxicity studies and also screened for wound healing efficacy in excision and incision wound healing models. Results: The trial drug prepared by both methods was found safe in an antimicrobial study and did not reveal any acute dermal toxic conditions. In excision and incision wound healing model showed better therapeutic efficacy. Conclusion: Both formulations were efficient in the wound healing model's study. Compared to a modified version of the agni siddha test drug, classically prepared anagni siddha was better in therapeutic efficacy.

Keywords: Malahara, Agni Siddha, Kajjali, Excision, Wound

INTRODUCTION

Malahara kalpana is the primary external application explained in Ayurvedic classics. These are generally prepared by siktha (bee wax) and tila taila in a specific ratio based on the season present. Like Bhallataka shothahara lepa[1], navaneeta is rarely used as a base. Many malahara kalpana are mentioned with herbal or herbo-mineral ingredients, but only few have minerals as main ingredients like Kajjalikodaya malahara.

Kajjalikodaya malahara is one such external application explained by Rasa taranginikara[2] for treating different types of wound healing. This is prepared with Kajjali[3], shodhita Mriddarashringa[4], Kampillaka[5], and Tuttha[6] with the base Siktha taila[7].

The test drug KJM (Kajjalikodaya malahara*)* was prepared by the anagni siddha method as mentioned in classics and modified by the agni siddha method. Both formulations were evaluated through experimental study in the excision and incision wound healing model. The drug was also screened for antimicrobial activity and acute dermal toxicity before subjecting to an experimental study. The results were found to be supportive of both models. The KJM prepared as per classical guidelines was found to have better therapeutic efficacy in both wound healing model studies than prepared by the modified method.

MATERIALS AND METHODS

The KJM prepared using both methods as per classical guidelines was subjected to an antimicrobial study at CRF, KLE Belagavi, and Karnataka. The experimental study was conducted at SDM Udupi, Karnataka.

Experimental study: Antimicrobial study

Total bacterial count: Plate count for bacteria: Mixture of 1 ml of pre-treated extract preparation and about 15 ml of liquefied casein soybean digest agar (using Petri dishes 9 to 10 cm in dm) added to each dish. Alternatively, the pre-treated extract preparation spread on the surface of the solidified medium in a Petri dish of the same dm. The pre-treated extract preparation was diluted, so a colony count of not more than 300 may be expected. At least two such petri dishes were prepared using the same dilution and incubated at 30 ^{0}C to 35 ^{0}C for 5 days unless a more reliable obtained count lies in a shorter time. The number of colonies were counted that are form. The results were calculated using plates with the greatest number of collies but taking 300 colonies per plate as the maximum consistent with good evaluation. [8]

Fungi plate count: In this, the same procedure was followed as in the case of fungi, but sabouraud dextrose agar with antibiotics in place of casein soyabean digest agar and incubated the plates at 20 ^{0}C to 25 ^{0}C for 5 days, unless a more reliable count is obtained in a shorter time. The results were calculated using plates with not more than 100 colonies.

Acute dermal toxicity[9]

An acute dermal toxicity study was done to assess any local irritation or systemic toxicity with repeated doses associated with topical administration of the test drug. A total of 24 Wistar albino rats of either sex of body weight 200-260g were selected according to AOT software and divided into control, standard, test 1, and test 2 groups containing 6 rats each. And the results were encouraging to predict the response needed for further analysis. Hence, the test drug Kajjalikodaya malahara(anagni siddha and agni siddha) was subjected to the acute dermal toxicity

study. IEC NO: MC/UCMS/IAEC/AAMC/CPCSEA/IAEC/ 2018-1019-AL-09

Test conditions (applies for both agni siddha kjm and anagni siddha kjm)

1. Animal species: Rats
2. Strain: Wistar albino
3. Source: Animal house attached to SDM Research Centre, SDM Ayurveda College Udyavara.
4. Selection: A total of 6 healthy either sex of body weight 200-260 g. Rats were selected according to AOT software.
5. Acclimatization period: All the selected animals were kept under acclimatization For 7 days before dosing.
6. Numbering and identification: The animal was marked with saturated Picric acid solution in water.

Table 1: Marking within the cages

Animal Number	Marking
1	Head
2	Neck
3	Middle of the back
4	The base of the tail
5	For limb
6	No mark

Table 2: The group number, animal number, and sex of the animal were identified with the help of cage cards

Identification of Animals	Desired dose (according to AOT)	Body weight (grams)
Head	1000 mg/kg	215
Neck	1000 mg/kg	200
Back	2000 mg/kg	253
The base of the tail	2000 mg/kg	237
For limb	5000 mg/kg	200
No mark	5000 mg/kg	210

Husbandry condition

Housing: Rats were housed in each polypropylene cage with a stainless steel top grill. The dry husk was used as bedding material and was changed every morning.

Environment: The animals were exposed to 12 hours of light and 12 hours of dark cycle with a relative humidity of 50 to 70 % and an ambient temperature of 22 ± 03®c.

Diet: rat pellet feed supplied by Sai Durga Feed Bangalore was provided throughout the study period except on the previous night of dosing, i.e. (overnight) fasting before dosing. The drinking water was given *ad / libitum* in polypropylene bottles with stainless steel sipper tubes.

Preparation of test formulation for administration

1. Test drug: Agni siddha and anagni siddha
2. Dose preparation: All the animals were dosed with constant dose volume1000mg/kg, 2000mg/kg, 5000mg/kg
3. Schedule: Single dose per animal.

a) Administration: The test formulation was applied uniformly over the exposed area of dorsal/flank skin (i.e., at least 10 % of the total body surface area).
b) Dose fixation: According to the AOT Software.
c) Route: Topical
d) Dose: 1000 mg/kg, 2000 mg/kg, 5000 mg/kg test substance
e) Dose-volume: As per requirement

Excision Wound Healing Study

A total of 24 Wistar albino rats of either sex of body weight 200-260 g were selected. These were grouped into control, standard, test 1 (KJM agni siddha), and test 2 (KJM anagni siddha) groups containing 6 rats in each group. The excision wound was made as per standard guidelines in all groups, and then the test drugs (KJM agni siddha-test 1 and anagni siddha- test 2) were applied topically. The standard drug(betadine) was also applied in the standard group by following the same parameters. Then, a change in contraction was observed in all the groups. [10]

Incision wound healing study

The grouping and selection of rats were the same as in the excisional wound healing study. Two parallel six cm paravertebral incisions were made through the full thickness of the skin after giving anaesthesia, and 1 cm lateral to the midline of the vertebral column after giving anaesthesia were closed with interrupted sutures. Then, the wound was applied with standard and test drugs. The sutures were removed on the 7th post-wounding day. Wound-breaking strength was measured on the 10th post-wounding day in anaesthetized rats. [9]

RESULTS

Table 3: Results of bacteria and fungi count

	Normal Limits	Anagni Siddha	Agni-siddha
Tot bacterial count	30-300 cfu/ml	09 cfu/ml	07cfu/ml
Tot fungal count	10-100cfu/ml	07 cfu/ml	02 cfu/ml

Table 4: Test for specified microorganisms (qualitative): *Escherichia coli* and others

	Limits (as per IP)	Anagni siddha	Agni- siddha
Escherichia coli.	Absent/100 ml	Absent	Absent
Staphylococcus aureus.	Absent/100 ml	Absent	Absent
Pseudomona. aerginosa	Absent/100 ml	Absent	Absent
Salmonella abony.	Absent/100 ml	Absent	Absent

Acute dermal toxicity There were no physical and behavioural changes (except a mild increase in motor activity, irritability and cyanosis seen in 4 rats in the group 2000 mg/kg and 5000 mg/kg) in all the treated animals on day one at ½, 1, 2, 3, 4 hours intervals, after dosing and after that once daily for 14 consecutive days. Thus, the data obtained from the study on single dose topical administration of anagni siddha and agni siddha KJM. Up to 14 days of observation does not result in physical and behavioural changes. All the animals belonging to the treated group survived throughout the 14-day observation period after dosing.

Excision wound healing model

The percentage of change in wound contraction was observed in all groups from day 3 to day 24. 11%,17%,30%,8% of contraction was found on day 3, which reached 47%, 74%, 69%, 53% on day 9, and 80%, 93%, 95%, 103% on day 18, and 89%, 99%, 98%, 79% on day 24 in control standard, test1, test2 groups respectively.

Incision wound healing model

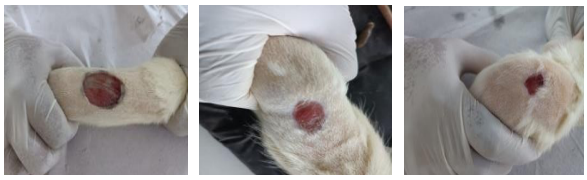
Table 5: As and when the wound just opened up, the weight was stopped and noted

Time taken in seconds(wound breaking)			
Control	**Standard**	**Test 1 (*agni siddha*)**	**Test 2 (*anagni siddha*)**
480	450	490	480
420	470	500	510
410	500	500	520
410	500	510	510
390	490	550	540
400	490	520	530

Preparation of excision wound

KJM Agni siddha

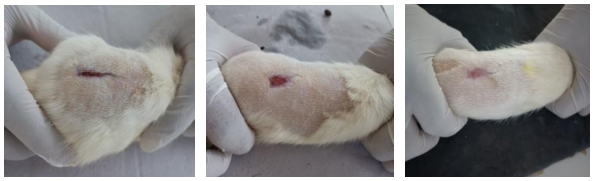
KJM Anagni siddha



Preparation of Incision Wound

KJM Agni Siddha

KJM Anagni Siddha



DISCUSSION

The test drug Kajjalikodaya malahara was screened for antimicrobial study and acute dermal toxicity study, and then the experimental study proceeded.

Antimicrobial Study: Microorganisms like *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella abony* were absent in the test drug prepared by both methods. Hence, this also reconfirms that the test drugs are of standard quality. The screening of the formulations prepared by both the anagni siddha and agni siddha methods for bacterial and fungal count results falls below the normal limits. It indicates that the formulations prepared by both methods are of standard quality and genuine. An antimicrobial study confirms that KJM prepared using both methods is safe for further evaluation.

Acute dermal toxicity: The test drugs did not produce any mortality up to the dose of 5000 mg/kg on topical application. At the dose level studied, the drug also did not produce any observable toxic effect except for a mild increase in motor activity, irritation and cyanosis in animals receiving the doses 2000 mg/kg and 5000 mg/kg, and thus, it could be concluded that the test drugs do not pose any toxic potential even at the dose of 5000 mg/kg in animals. Therefore, KJM was found to be safe. Then, it is screened for excision and incision wound healing model.

Excision wound healing model: After observing the data from day 3 to day 24, one can say that the change in the percentage of contraction was quite evident in the test 2 group from most negligible value 8 % to more than 50 % on day 9 and retains higher rate 89% on day 18, 95% on day 24. On the other hand, group test 1 reached 30% to 98 % on days 3 and 24. Standard from 16% to 99% on day 24. So, by these values, it can be said that the lowest percentage to a higher value is better observed in test 2 groups, compared to test 1 and standard groups. Test 1 and standard drug run parallel values from day 3 to day 24.

Incision wound healing model: The wound-breaking strength was assessed in all groups. The duration taken in the control group varies from 390 to 480 sec. On average, the duration was 418 sec in the control group. The duration taken in the standard group ranges from 450 to 500 sec. On average, the duration was 483 sec in the standard group. The duration taken in the test drug 1 (*agni siddha*) group varies from 490 to 550 sec. On average, the duration was 506 sec in the test drug 1 (*agni siddha*) group. The duration taken in the test drug 2 (*anagni siddha*) group varies from 480 to 540 sec. On average, the duration was 515 sec in the test drug 2 (*anagni siddha*) group.

The more resistance in wound breaking, the better the strength of the drug. This was seen in test 1 and test 2 groups, with an average time of 511 and 515 seconds, respectively. Whereas it was 416 1nd 483 sec in control and standard, respectively. Test 2 group holds better therapeutic efficacy compared to all other groups.

CONCLUSION

Kajjalikodaya malahara mentioned in the the classical text Rasa tarangini when subjected to evaluation for wound healing efficacy, showed better results than the control and standard group in the excision and incision wound healing model. From the values obtained from the above wound healing study, one can conclude that the test groups showed better efficacy in the excision and incision wound healing models than others. Among test 1 and test 2 groups, test 2 retains better wound healing efficacy than test 1. So, classically formulated Kajjalikodaya malahara anagni siddha is better compared to all other groups in the present excision and incision wound healing model

ACKNOWLEDGEMENT

Authors thank the CRF, KLE Ayurveda Medical College Belagavi, Karnataka, SDM Ayurveda Medical College Udupi, Karnataka and Alvas Ayurveda Medical College Moodabidri, Karnataka, India.

REFERENCES

1. Tripathi B, Sharangdhar Samhita Uttarkhand 11/7, Edition 2007, Chaukhamba Surbharati Prakashan, Varanasi, p. 392
2. Sadananda Sharma, Rasa Tarangini 6 /129-134, Edition 2012 Motilal Banarasi Das, Varanasi, p. 129.
3. Sadananda Sharma, Rasa Tarangini 2/27, Edition 2012, Motilal Banarasi Das, Varanasi, p. 16.
4. Sadananda Sharma, Rasa Tarangini 21/167-169, Edition 2012 , Motilal Banarasi Das, Varanasi, p. 551.
5. PHC. Murthy, Rasashastra (The mercurial system), Edition 2008, Chaukhamba Sanskrit Series, Varanasi, p. 285.
6. Sadananda Sharma, Rasa Tarangini 21/108, Edition 2012 Motilal Banarasi Das, Varanasi, p. 540.
7. Sadananda Sharma, Rasa Tarangini 6/59-61, Edition 2012 Motilal Banarasi Das, Varanasi, p. 114.
8. Ayurvedic pharmacopeia of India, Part II, Vol II. Appendix 2.4-2.4.2, p. 184-195.
9. OECD guidelines 402.
10. S Murthy, MK Gautam *et al.,* Evaluation of *in vivo* wound healing activity of *Bacopa monniera* on different wound model in rats, Biomed Research International. DOI: http://dx.doi.org/10.1155/2013/972028

Cite this article as:

Jayalaxmi and MS Krishnamurthy. Study of Kajjalikodaya malahara for evaluation of wound healing activity. Int. J. Res. Ayurveda Pharm. 2024;15(2):47-50
DOI: http://dx.doi.org/10.7897/2277-4343.15237

Source of support: Nil, Conflict of interest: None Declared