



## Research Article

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### AN EXPERIMENTAL EVALUATION OF VRANA ROPANA KARMA (WOUND HEALING ACTIVITY) OF ARKA (*CALOTROPIS PROCERA* AIT R. BR.) AND SNUHI (*EUPHORBIA NERIIFOLIA* LINN.) KSHEER (LATEX) OINTMENT

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#### ABSTRACT

The aim of this study is to evaluate the wound healing activity of Arka (*Calotropis procera* AitR.Br.) and Snuhi (*Euphorbia neriifolia* Linn.) latex ointment in excision and incision wound model of Wistar strain albino rats. Methods- Effect of test samples on excision wound: For the study, the animals were divided into 5 groups each group comprising 6 rats. Group A served as Negative control group and received Ointment base. Group B, C and D were test groups. Where the animals of Group B were treated with Arka Ointment. Group C with Snuhi Ointment and Group D treated with combination of Arka, Snuhi Ksheer ointment, Whereas the animals of the Group E received soframycin ointment which was standard. Result- Wound contraction property of test Group D had found more potent than standard Group E. Effect of test samples on incision wound: At Incision Wound Model wound breaking strength. Test Group - Group C, Group D and Standard Group E found to have statistically response. Wound breaking strength of Group D and Standard Group E had been found closely similar response.

**Keywords** -Ayurveda, Arka Ksheer, Snuhi Ksheer, Ointment, Vrana ropana

#### INTRODUCTION

Ayurvedic system of medicine has its own history since, origination of human civilization. Feasibly, after been exposed to injury, plants or its parts were used for the treatment mostly which are easily available in the surrounding. Treatments of Vrana (Wound) evolved from magical supplications, lotions, and ointments, to a systematic text of wound care in evaluation of Veda, Epics, Samhita, Chikitsa grantha with a fine example of Sandhana of injured leg of queen Bishala<sup>1</sup>. Laksha for Vranarohana<sup>2</sup> and Maanshrohini for Vrana ropana<sup>3</sup>. According to Epics-Ramayana, Lakshmana was treated by four Mahaushadhi (drugs)- Mritasanjivani, Vishalyakarani, Savarnakarani and Sandhanakarni after being injured in the battlefield Valmiki Ramayana<sup>4</sup>. Several drugs of plant, mineral and animal origin are described in the Ayurveda for their wound healing properties under the term Vranaropanaeg. Elaborated description of wounds and wound healing (Vrana and Vrana ropana) is available in the Sushruta Samhita written by Acharya Sushruta in 3<sup>rd</sup> century BC. In Sushruta Samhita, along with healing of these wounds, Acharya Sushruta also describes many medicinal plants for treatment of wounds both individually or groups mentioned in two different chapters. Sushruta has stated not only procedures of cleaning of wound (Vrana Shodana) followed by healing (Vrana Ropana) but also medicines to help treatment of Vranas.

Proper wound healing well should be done with minimal scar formation, with minimum pain, fast healing. This is the major slogan of every surgeon. The problematic of wound healing has been distributed at several levels since the beginning of humanity and uniform today there is a massive scope for researchers to work out and find a well clarification of wound healing. In Charak Samhita<sup>5</sup> and Astanga Hridayam<sup>6</sup> described the many types of

Vrana and its treatment. The most important role of Ayurveda is for prevention and treatment of disease. To treat a wound is to bring out the procedure of repair of the tissue in the most conductive manner for the present and future well-being of the patients. Such an important responsibility cannot be carried out without comprehensive and suitable knowledge of Vrana, shodhan and Ropana (wound healing) along with the modern medical doctrine. Wound healing is a basic and fundamental problem for surgeons as well as for biomedical researchers. For the understanding of biological process of wound healing, multidirectional approach is necessarily required. It is amazing to note that; in Indian Indigenous System of medicine, i.e. *Ayurveda*, which is one of the most ancient systems of medicine of the world, the subject of wound healing dealt with the spirit of multidimensional approach<sup>7</sup>. It is likely found that more effective wound healing agents would be developed from natural products Classics of Ayurveda especially Acharya Sushruta has elaborately elucidated the details of wound and its management.

“Vranagatravichuranevranatitivanah”<sup>8</sup>

In Ayurvedic Chikitsa granthas many oils and ghrita based formulation is used for Vrana shodhana and Vrana ropana like Arka<sup>9</sup> and Snuhi<sup>10</sup> are the constituents of Sanshodhana Ghrita<sup>11</sup>. Oil cooked with latex of Snuhi and Arka along with bee-wax heals chronic ulcers<sup>12</sup>.

#### Aims and objectives

To evaluate the wound healing activity of Arka (*Calotropis procera* Ait R. Br.) and Snuhi (*Euphorbia neriifolia* Linn.) Latex Ointment in excision and incision wound model of Wistar strain albino rats.

## MATERIAL AND METHODS

In present study, Ksheer of both (Arka and Snuhi) the plants were collected early morning during Sharad Ritu. Snuhi Ksheer is collected in Shishir Ritu, but in present study both the drug samples were collected in Sharad Ritu because both Ksheer had to be mixed together while preparation of drug<sup>13,14</sup>. Pharmacognostical and preliminary phytochemical evaluation of test of both drugs were carried out. Ointment preparation was done in National Institute of Ayurveda, laboratory. Study on animals were carried out after obtaining the approval from Institutional CPCSEA. Animal house was attached to Bilwal Medchem and Research Laboratory Pvt. Ltd. H-9 SKS Reengus Industrial Area, Reengus, Rajasthan. The experimental protocol was submitted to the animal ethics committee of the institute, and approval was obtained for conducting the experiment. Approval No: BMRL/AD/CPCSEA/IAEC/2018/2/I. Sixty wistar albino rats were selected; weighing 130-180 g. Animals were caged in polypropylene cages. Wistar albino rats have been divided in to 5 groups (A, B, C, D, and E); Control, standard and test group. Six in each and area of wound epithelization and wound breaking strength observed after treatment. Experimental procedure was done in two models Excision wound model and Incision wound model. Each group were designed as in Group A, 6 Wistar strain albino rats were applied ointment base twice a day. In Group B, 6 Wistar strain albino rats were applied ointment of Arka Ksheer locally twice a day. In Group C, 6 Wistar strain albino rats were applied ointment of Snuhi Ksheer locally twice a day. In Group D, 6 wistar strain albino rats were applied ointment of mixture of Arka, Snuhi Ksheer locally twice a day. In Group E, 6 wistar strain albino rats were applied soframycin ointment locally twice a day.

### Excision Wound Model

Under Ketamine Xylazine anesthesia, the animal was secured to the operation table in its natural position. An impression was made on the depilated dorsal thoracic surface 2 sq. CMS behind the ears, 1 cm away from the vertebral column. The full thickness of the impressed area was excised to obtain a wound area of 200 sq. mm. The physical attributes of wound healing namely, wound closure (contraction) and epithelization time were studied in this model. Contraction, which mainly contributes for wound closure in the first two weeks, was studied by tracing the raw wound area on tracing paper on the wounding day followed by 0, 7, 14, and 21

and subsequently on every alternate day, till complete wound contraction occurred. The criteria for complete wound contraction are being the fall of scab without any raw surface. Wound area was measured by retracing the wound on a millimeter scale graph paper. The degree of wound healing was calculated as percentage closure in wound area from original wound area. The mean and standard error values were calculated. The number of days for complete wound contraction was noted.

### Incision Wound Model

Under Ketamine Xylazine anesthesia, two paravertebral linear incisions of 6 CMS each were made through the entire thickness of skin on either side of the vertebral column with the help of a sharp blade as described by Ehrlich and Hunt. After complete homeostasis, the wounds were closed by means of interrupted sutures placed at equidistant points about 1 cm apart, using 4-zero silk thread and straight round body needles. Wounds were then mopped with cotton swabs soaked in 70% alcohol. The animals were caged individually. Removal of the sutures was done on the 8th post wounding day. Wound breaking strength was determined on the 10th post wounding day as described below. The anaesthetized animal was secured to the operation table. Two Allieps forceps were firmly applied on the lines facing each other; the forceps on one side was hooked to a metal rod fixed firmly to the operation table. The other forceps were tied with a string, which ran over a pulley. To the other end of the string, serial measuring weights in ascending order were added. The basal weight added to the string was 50 g and the weight was gradually increased. As soon as the wound gaping was observed, the weights were immediately removed, and the total weight was noted down. The wound breaking strength was expressed as the minimum weight at which the wound started to gape. Three such recordings were made for a given incision wound and the procedure was repeated on the other site.

### Statistical analysis


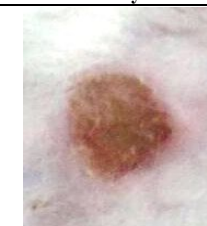



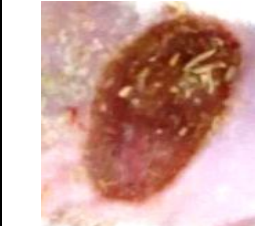


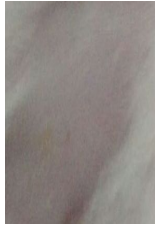
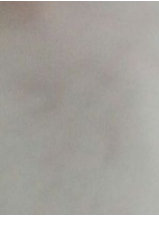
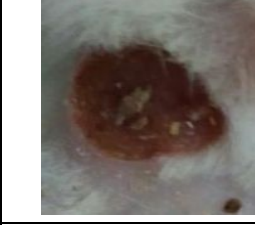

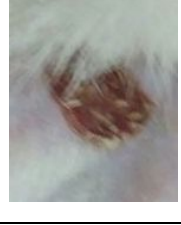

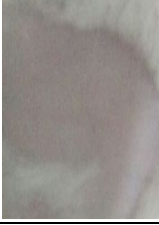



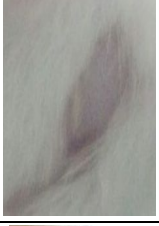
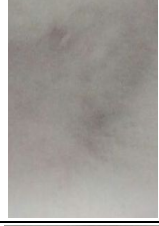
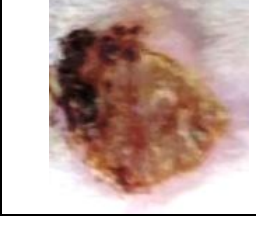

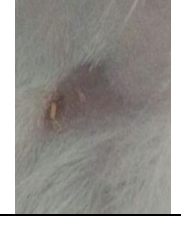
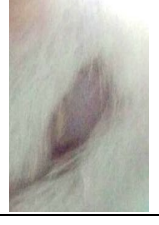
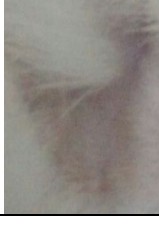
All the values were expressed as mean  $\pm$  standard error of the mean (S.E.M) of six animals each across the groups. Statistical analysis of data was carried out using one-way and two-way ANOVA analysis of variance (ANOVA) with help of Graph pad Prism software. Dunnett's multiple comparisons test. P value < 0.05 was considered to be statistically significant.

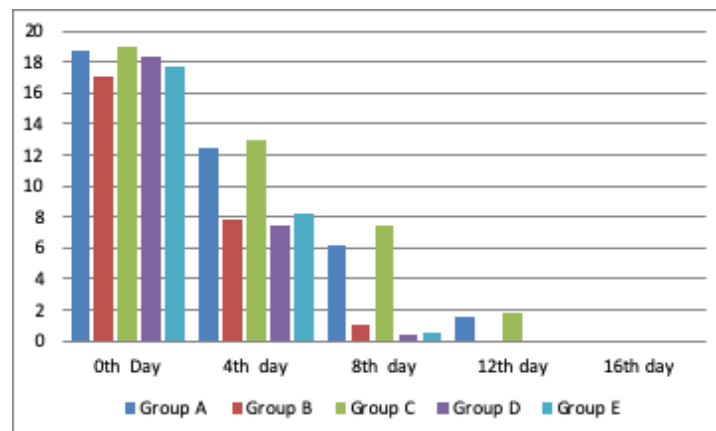
## RESULT

Table 1: Excision Wound Model (Diameter of wound)

| Groups  | Diameter of wound (mm)                |                                       |                                       |  |  |
|---------|---------------------------------------|---------------------------------------|---------------------------------------|--|--|
|         | 0 <sup>th</sup> Day<br>Mean $\pm$ SEM | 4 <sup>th</sup> day<br>Mean $\pm$ SEM | 8 <sup>th</sup> day<br>Mean $\pm$ SEM | 12 <sup>th</sup> day<br>Mean $\pm$ SEM | 16 <sup>th</sup> day<br>Mean $\pm$ SEM |
| Group A | 18.67 $\pm$ 0.919                     | 12.50 $\pm$ 0.719                     | 6.17 $\pm$ 0.477                      | 1.50 $\pm$ 0.428                       | 0.0 $\pm$ 0.0                          |
| Group B | 17.00 $\pm$ 0.856                     | 7.83 $\pm$ 0.872                      | 1.00 $\pm$ 0.365                      | 0.0 $\pm$ 0.0                          | 0.0 $\pm$ 0.0                          |
| Group C | 19.00 $\pm$ 1.211                     | 13.00 $\pm$ 1.065                     | 7.50 $\pm$ 0.885                      | 1.83 $\pm$ 0.307                       | 0.0 $\pm$ 0.0                          |
| Group D | 18.33 $\pm$ 1.282                     | 7.50 $\pm$ 0.671                      | 0.33 $\pm$ 0.333                      | 0.0 $\pm$ 0.0                          | 0.0 $\pm$ 0.0                          |
| Group E | 17.67 $\pm$ 1.085                     | 8.17 $\pm$ 0.307                      | 0.50 $\pm$ 0.342                      | 0.0 $\pm$ 0.0                          | 0.0 $\pm$ 0.0                          |

Table 2: Images of Excision Wound Model

| Groups  | 0 <sup>th</sup> Day   | 4 <sup>th</sup> day   | 8 <sup>th</sup> day  | 12 <sup>th</sup> day  | 16 <sup>th</sup> day  |
|---------|---|---|--|---|---|
| Group A |    |    |    |    |    |
| Group B |    |    |    |    |    |
| Group C |    |    |    |    |    |
| Group D |   |   |   |   |   |
| Group E |  |  |  |  |  |



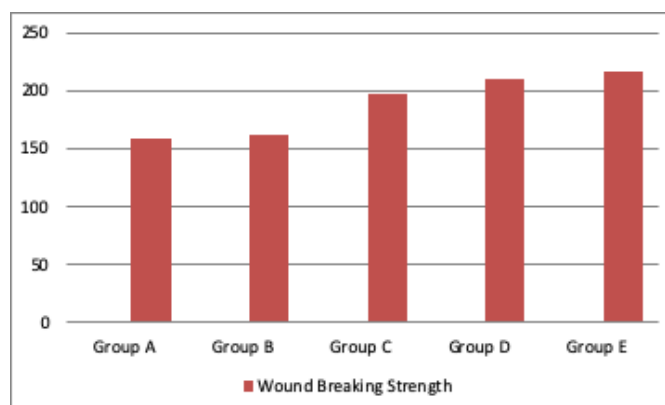
Graph 1: Excision Wound Model (Diameter of wound)

Table 3: Incision Wound Model (Wound Breaking Strength)

| Incision Wound Model    | Group A<br>Mean $\pm$ SEM | Group B<br>Mean $\pm$ SEM | Group C<br>Mean $\pm$ SEM | Group D<br>Mean $\pm$ SEM | Group F<br>Mean $\pm$ SEM |
|-------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Wound Breaking Strength | 158.17 $\pm$ 1.973        | 161.67 $\pm$ 3.989        | 198.00 $\pm$ 9.377        | 210.33 $\pm$ 7.486        | 215.83 $\pm$ 3.781        |

Table 4: Dunnett's multiple comparisons test

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|--------------|---------|------------------|
| Group A vs. Group B                 | -3.5       | -25.5 to 18.5      | No           | ns      | 0.9811           |
| Group A vs. Group C                 | -39.83     | -61.84 to -17.83   | Yes          | ***     | 0.0003           |
| Group A vs. Group D                 | -52.17     | -74.17 to -30.16   | Yes          | ****    | 0.0001           |
| Group A vs. Group E                 | -57.67     | -79.67 to -35.66   | Yes          | ****    | 0.0001           |



Graph 2: Incision Wound Model (Wound Breaking Strength)

## DISCUSSION

### Formulations: (Arka and Snuhi Ksheer Ointment)

According to review of Samhita and Nighantu Arka and Snuhi pacify Kapha and Vata dosha. In both the Samhita and Nighantu, most of the therapeutically uses of Arka ksheer are given in Kustha, Arsha, Vrana, Gulma. In the pathogenesis of these diseases twak, mansa, rakta involved as Dushta dhatu and imbalance of Tridosha. Arka and Snuhi ksheer is abundantly used in single form or formulation in Samhitas in Kustha as well as Vrana also. In this work study Arka ksheer and Snuhi Ksheer combined formulation which is mentioned in Sahasrayogam<sup>15</sup> was taken for study. This formulation is made in Tila tail and madhuchhishth which are them self well known for their Vranaropaka action and Til tail is mentioned for its Sansakaranuvartana property. It enhances the tridoshashamaka property of the formulation. After Sansakaranuvartana, the property of formulation is Sarvarogapaha or according to dosha and dushya. But when same drugs are used in single form they act by virtue of Rasa, Guna, Veerya, Vipaka, Dosha-karmta.

### In-vivo wound healing study

Wound healing is a natural process of regenerating dermal and epidermal tissues. Whenever there is a wound, a set of overlapping events takes place to repair the damage<sup>16</sup>. These processes had been categorized into phases which include the inflammatory, proliferative and the remodeling phases<sup>17</sup>. In the inflammatory phase, bacteria and debris are phagocytosed and removed and cytokines and mediators are released that cause the migration and division of cells involved in the proliferative phase. Angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction occurs in proliferative phase. During epithelialization, the epithelial cells crawl across the wound bed to cover it<sup>18</sup>

### Effect of test samples on excision wound

For the study, the animals were divided into 5 groups each group comprising 6 rats. Group A served as Negative control group and received Ointment base. Group B, C and D were test groups; where the animals of Group B were treated with Arka Ointment. Group C with Snuhi Ointment and Group D treated with combination of Arka, Snuhi Ksheer ointment; whereas the animals of the Group E received soframycin ointment which was standard.

Reduction of wound area of different groups till the 16<sup>th</sup> day for excision wound model was calculated.

The size of wound on at 0<sup>th</sup> day on different Groups (A, B, C, D and E) was found respectively 18.67, 17.00, 19.00, 18.33, 17.67 mm. after treatment of tests sample in different Groups. All test Group (B, C, D) and Standard Group (E) was found complete wound contraction on 8<sup>th</sup> day and contraction of wound was found statically significant. But the contraction of wound Group D at 8<sup>th</sup> day (0.33 mm) and contraction of Group E Standard Group 0.50 mm value of test Group D was lesser than standard group. Wound contraction property of test Group D had found more potent than standard Group E. (Table 1, 2)

### Effect of test samples on incision wound

At Incision Wound Model wound breaking strength at 7<sup>th</sup> day at all the groups was found respectively 158.17, 161.67, 198.00, 210.33 and 215.83 gm. Test Group -Group C, Group D and Standard Group E found to have statistically response. Wound breaking strength of Group D (210 gm) and Standard Group E (215 gm) had been found closely similar response (Table 3, 4).

It was concluded from excision wound model and incision wound model that wound healing property of test Group D (Arka and

Snuhi Ksheer Ointment) had found more potent than standard Group E (Soframycin).

**Probable Mode of Action**

Owing to the above said properties and the Pharmacodynamic properties of the drug ingredients as discussed in the drug review chapter they found Vrana Ropana Karma effectively and so the probable mode of action of Arka and Snuhi ointment on the wound was made as follows-Vrana Ropana involves Shodhana as well as Ropana, so for healing to occur two stages are to be gone through practically that is removal of local Dhatu Dushti and checking of the inflammatory features.

**Removal of Local Dhatu Dushti**

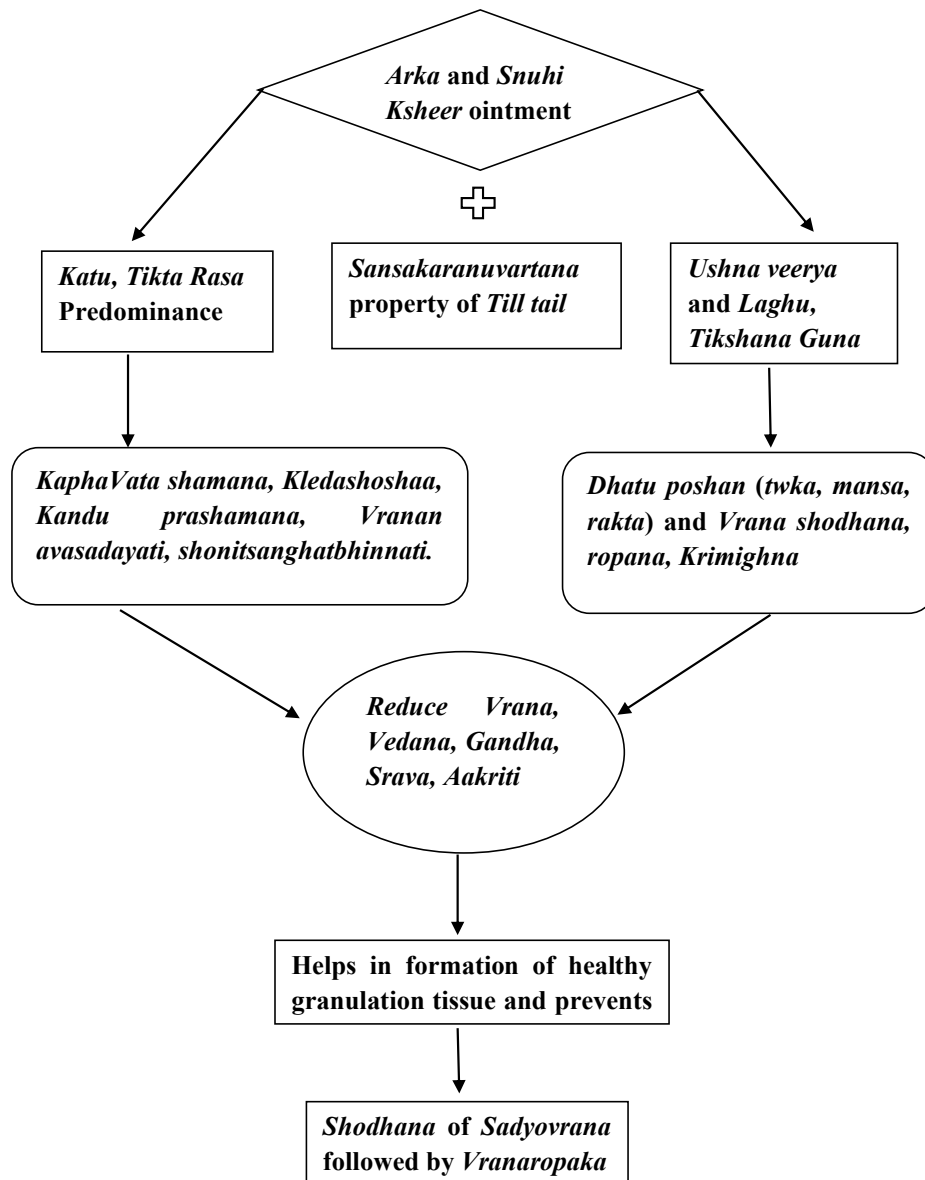
1. Severity of wounds depends on the local Dhatu Dushti with imbalance of local tissues Twaka and Mamsa Dhatu with involvement of Rakta Dhatu.

2. Both drugs having Lekhana, Shoshana, Shodhana, actions followed by the Samshodhana (detoxifying, cleansing) karma, which cleaned or removed vitiated Dhatu and carried out proper therapeutic debridement at the site of Vrana increasing to speed of wound healing.

**Examination of the Clinical features**

Due to Katu rasa, it removes the local discharge (Vranashodhana), irritation (Kandu prashamana), acts as an anti-infective (Krimighana), and discourages excessive granulation tissue formation (Vranan avasadayati), break blood clots and other obstruction (shonitsanghat bhinnati). As prior mentioned tikta rasa in Arka is having Kapha, Kleda, pooya, lasika upshoshana property. Due to Katu rasa, Ushna virya, Laghu and Tikshana guna, Drug penetrates in deep tissues, debride the wound and clarify the passages (Marga-vivrunoti). Actions of katu Rasa played an important role in scraping the debris and slough<sup>19</sup>.

**PROBABLE MODE OF ACTION OF DRUG:**



## CONCLUSION

The present study entitled “An Experimental Evaluation of Vrana Ropana Karma (Wound Healing Activity) of Arka (*Calotropis procera* Ait R.Br.) and Snuhi (*Euphorbia neriifolia* Linn) Ksheer (Latex) Ointment” based on the findings of the study, these conclusions can be put forth –Wound healing property of test drug (Group D) had been found more potent than standard drug (Group E) in excision wound models. After application of test drug (Group D) and standard drug (Group E) showed wound breaking strength was found closely similar. So, test sample had been showed closely similar response (statistically response) in incision wound model.

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