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An Experimental Study to Assess the *Vrana Sandhana* and *Rakta Stambhana* Effect of *Guduchi* on Excision Wound in Wistar Strain Albino Rats

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ABSTRACT

BACKGROUND: *Sandhaniya Mahakashaya Varga* is one among the *Panchashanmahakashaya* explained in *Charaka Samhita, Sutra Sthana* 4th Chapter. Chakrapanihas commented that *Sandhaniya* means *Samgrahana* (withholding) in general (that is explained under *Atisara*). This indicates that there are certain other meanings too for the word *Sandhaniya*. Few are healing (as in case of *Vrana Sandhana*) and few are *Stambhana* (as in case of stopping *Raktapravrtti*). This paves a way to explore the wider actions of the drugs mentioned under *Sandhaniya Gana* as *Stambhana* along with *Vranasandhana* effect which is the need of the hour. As there are different meanings for the word *Sandhana*, an effort was made to see the *Vranaropana* and *Stambhana* action of *Guduchi*, one drug among the *Sandhaniya Gana*.

AIM: To assess the *Vrana Sandhana* and *Rakta Stambhana* action of *Guduchi* on excision wound.

METHODOLOGY: An experimental study was carried out to assess haemostatic and wound healing activity of *Guduchi* by using excision wound model technique and bleeding induced experiment in Wistar Albino rats by taking wound contraction, histopathological study of wound tissue and bleeding time, clotting time as parameters respectively.

RESULT & INTERPRETATION: Observations after the study showed that there was non significant increase in the percentage of wound contraction in test group which shows the wound healing enhancing property of the drug *Guduchi* and histopathological study also supported the same. *Guduchi* showed statistically significant result with respect to reduction in bleeding time. There was statistically non-significant reduction in the clotting time.

CONCLUSION: The results of the study revealed that the test drug *Guduchi* has both wound healing enhancing property as well as haemostatic property. Thus it can be concluded that the word *Sandhana* means *Vrana Sandhana* in case of wound healing and it means *Rakta Stambhana* in case of *Raktatipravrtti*.

Key Words *Vrana Sandhana, Rakta Sthambhana, Guduchi, Sandhaniya Gana, Raktatipravrtti*

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INTRODUCTION

Wound healing is a complex phenomenon that results in the restoration of disrupted anatomical continuity and disturbed functional status of skin, accomplished by several processes which involve different phase including inflammation, granulation, fibro genesis, neo vascularization, wound contraction and epithelialization. Haemostasis is a process to prevent and stop bleeding, meaning to keep blood within a damaged blood vessel. It is the first stage of wound healing. *Sandhaniya Mahakashaya* explained by *Acharya Charaka* includes ten drugs which are *Madhuka*, *Madhuparni*, *Prishniparni*, *Ambashtaki*, *Samanga*, *Mocharasa*, *Dhataki*, *Lodhra*, *Priyangu*, *Katphala*¹. All these ten drugs are having this unique phenomenon of *Sandhana*.

The word *Sandhana* means binding or joining together². Thus we can say that these drugs have the property of *Vrana Sandhana*. *Sandhana* also means *Stambhana* in case of *Raktatipravrutti*³. Thus there is a need to explore the wider actions of the drugs which are mentioned in *Sandhaniya Dashemani*. In the recent past various herbal products have been used in the management of wounds and also for arresting of bleeding, but they lack enough scientific data to support the claims in ancient literature. *Guduchi* is one of such agent that has not been subjected for scientific evaluation on *Vrana Sandhana* and *Stambhana*.

Among the *Sandhaniya Dashemani*, *Madhuparni* or *Guduchi* is taken up for this study. In view of the paucity of information related to the effect of *Guduchi* on wound healing and haemostasis and since it is easily available and is of cost effective, the present study was undertaken with the aim of investigating the influence of topical application of *GuduchiChurna* on excision wound model and bleeding induced experiment in Wister Albino rats.

AIM

To assess the *Vrana Sandhana* and *Rakta Stambhana* action of *Guduchi* on excision wound.

REVIEW OF LITERATURE

Review on *Vrana Sandhana* and *Raktastambhana*:

Vrana Sandhana is one among the *Shashti Upakrama* of *Vranadescribed* in *Dvivraniya Chikitsa Adhaya* of *Sushruta Samhita*⁴. *Sandhana* means joining of the body parts which are separated due to any injury. *Sandhana* is also considered as one among the methods to prevent bleeding. Wounds which are not having any complications of ripening, localized in the muscles and having wide opening are indicated for *Sandhana Karma*⁵. *Sandhana Karma* is also indicated in *Sadhyovrana* which is extensive. It is explained that the drugs having *Kashaya Rasa* joints or unites the edges of the wound. The word *Stambhana* means that which prevents movement or *Gati*⁶. *Shonita Sthapana* is mentioned as one



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among the *Shashti Upakramas* of *Vrana* by *Acharya Sushruta* in *Chikitsa Sthana*⁷. When too much of blood flows out, then appropriate methods to control the bleeding should be adopted by the physician. *Acharya Sushruta* has described

the process of *Raktastambhanain Sutrasthanachapter 14* under the heading of *Shonitavarnaniya Adhyaya*. There are four measures for stopping the arresting the bleeding. They are: *Sandhanam*, *Skandanam Pachanam* and *Dahanam*, where *Sandhana* means to arrest the bleeding is arrested by the process of contracting the affected part or constriction of capillaries and blood vessels.⁸ The drugs with the astringent property possess such an action. eg. *Udumbara*, *Dhataki*, *Lodhra* etc. The *Dravyasha* having *Drava*, *Tanu*, *Sara*, *Sheeta Guna* and having *Swadu*, *Tikta*, *Kashaya Rasa* properties causes *Stambhana*⁹.

Drug review:

Botanical name: *Tinospora cordifolia*

Family: *Menispermaceae*

Properties:¹⁰

Rasa – Tikta, Kashaya Rasa

Guna – Guru, Snigdha

Vipaka – Madhura

Virya – Ushna

Karma – Sangrahana, Laghu, Balya, Agnidipana, Tridosahara, Cures Trit, Daha, Murchchha, Kasa, Pandu, Kamala, Kushta, Vatarakta, Jwara, Krimi, Arshas, Hridroga, Vatarogas.

All experiments were performed after prior permission from IAEC (Ref No. SDMCAU/ACA-49/AEC24/2018-19), SDM Centre for Research in Ayurveda and Allied Sciences, Udupi.

Experimental Design:

Rats were randomly divided into 3 groups of 6 rats each as mentioned in table no.1. Group I was taken as Control, Group-II was treated with 5% Povidone Iodine Powder (Standard drug), Group-III was treated with *Guduchi Churna*.

Drug Preparation:

About 500gms of dried sample of *Guduchi* stem was kept under sunlight for drying for 2 days. Then it was then grinded and fine powder was prepared.

Inclusion Criteria:

Healthy wister albino rats of either sex will be considered, weighing about 200 ± 50grams

Exclusion Criteria:

- Pregnant and diseased rats
- Rats which are under trial for other experiments

Route of Drug Administration:

- External application on the wound which is made on the dorsal thoracic central region of the rats with a diameter of 2.5 cm.

Dose Fixation:

- Quantity sufficient to cover the wound area.

Drug administration procedure and schedule:

Excision wound model:

MATERIALS AND METHODS

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➤ Quantity sufficient *Guduchi Churna* is dusted on the wound, till the area of wound is covered.

➤ Drug is applied daily at once in the morning.

Bleeding induced experiment:

➤ Quantity sufficient *Guduchi Churna* was dusted on wound after inducing bleeding.

➤ This procedure of inducing bleeding for assessing the haemostasis was done on every 5th day starting from the 1st day of wounding. Clotting time was assessed on every 10th day starting from 1st day of wounding using capillary method.

Duration of the Study:

Time line of 24 days

Animal Grouping :

Group	Intervention
Control Group	No Intervention
Standard Group	Povidone Powder
Test group	<i>Guduchi Churna</i>

Table 1: Animal Grouping and Intervention

The grouping of animals and intervention is shown in Table no.1. Each group having 6 rats were kept in separate metabolic cages.

EXPERIMENTAL METHODOLOGY

Procedure for Excision Wound Model

This was conducted according to the technique developed by Morton and Malone. The animals were anaesthetized using Pentobarbitone (45mg/kg) intra-peritoneal route. After the animals are sufficiently anesthetized, they are secured to the dissection plate in prone position. The hairs were removed using shaving blade

from the part to be operated and subsequently the area is cleaned. A round seal of 2.5 cm in diameter was impressed on the dorsal thoracic central region 5cms away from the ears of the anaesthetized rats. Full skin thickness from the marked area was excised in circular fashion with the help of forceps, surgical blade and scissors. After achieving full haemostasis, the animals are placed in individual cages.

External application of *Guduchi Churna* was started from the 1st day of post wounding. Control groups left without applying drug to observe the natural healing process and 5% Povidone Iodine Powder is applied for the rats in the standard group. Quantity sufficient powder is dusted on the wound, till the area of wound is covered. Drug is applied once a day for 24 days.

Procedure of inducing bleeding:

After making the wound in rats, on the 1st day of wounding bleeding was induced by pricking with surgical needles and scissors over the wound till proper bleeding occurs. After inducing bleeding, the test drug *Guduchi Churna* was applied to test group topically and standard drug povidone iodine dusting powder was applied to the standard group rats. Stopwatch was started and bleeding time was noted after every 30 seconds by using a blotting paper. Bleeding time was noted in all the 3 groups on every 5th day starting from the 1st day of making the wound till the 24th day after the wound excision.

Procedure to assess the clotting time:

Firstly the rats were given overdose of diethyl ether anesthesia. Blood samples were collected
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WOUND CONTRACTION		
Days	Standard Group	Test Group
3	NSI	NSI
6	NSD	NSD
9	NSD	NSD
12	NSD	NSD
15	NSD	NSI
18	NSI	NSI
20	NSI	NSI
21	NSI	NSI
24	NSI	NSI
BLEEDING TIME		
1	NSD	NSD
5	NSI	NSD
10	NSD	VSD
15	NSI	VSD
20	NSI	NSD
24	NSD	VSD
CLOTTING TIME		
1	NSI	NSD
10	NSI	NSI
20	NSD	NSD
24	NSD	NSD

from retro orbital plexus using a capillary tube and kept on a side to allow clotting. Stopwatch was started and a piece of capillary tube was broken every 30 seconds till thin fibrin thread appears which suggests a clot. Clotting time was assessed in all the 3 groups on the 10th and 20th day of wound excision.

Statistical Analysis:

- The data obtained was analysed by employing One way ANOVA with Dunnett's multiple t- test as post HOC test.
- Graph pad Instat software 3 was used for this purpose.

Assessment criteria:

The criteria to assess wound healing was wound contraction and histopathological study of wound tissue and to assess haemostasis criteria used are bleeding time and clotting time.

OBSERVATION AND RESULTS

Table 2 Consolidated statement of the results

Histopathological examination of the results:

Grading done for various features as follows

+slight; ++ moderate; +++ extensive; – absence.

Slide No	Epidermal regeneration	Granulation tissue	Inflammatory cell infiltration	Angiogenesis	Proliferation of fibroblast cells	Collagen deposit
C1	-	++	+++	+	+	+
C2	-	+	++	++	+	+
C3	-	+	+++	+	+	+
S1	+	++	+++	+	+	+
S2	+++	++	+	+	++	++
S3	+	++	+	++	+++	++
T1	++	++	+	+	++	++
T2	+	+	++	++	+	+
T3	++	+++	++	++	++	++

Table 3 Evaluation of Histological parameters for assessing wound healing

Table no. 3 shows the different gradings of evaluation of histopathological parameters for assessing wound healing

DISCUSSION

Effect of Guduchi on wound contraction:

The percentage of wound contraction was calculated on 3rd, 6th, 9th, 12th, 15th, 18th, 21st and November 10th 2021 Volume 15, Issue 3 Page 87

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24th post wounding days. On the 3rd day there was a slight increase in the percentage of wound contraction in test group compared to the control and standard group. On the 6th, 9th and 12th day there was an increase in the wound contraction percentage in test group as well as standard group. On the 15th, 18th, 21st and 24th days there was a marked increase in the percentage of wound contraction in the test group compared to the standard and control groups which was found to be statistically non-significant. The result of histopathological examination of wound tissue showed that the formation of granulation tissue, **epidermal** regeneration, collagen deposits, angiogenesis was more in the test group compared to the control group. Thus it can be interpreted that *Guduchi Churna* showed good effect on the wound since day 1. There was non-significant increase in the wound contraction percentage when compared with standard and control group, which shows that it has wound healing enhancing property in the early stages of wound healing.

Effect of Guduchi on bleeding time:

Bleeding time was calculated on 1st, 5th, 10th, 15th, 20th and 24th post wounding days. Clotting time was calculated on 1st, 10th, 20th and 24th post wounding days.

On day 1 there was no any observable changes noted in the bleeding time in test group. In standard group there was slight reduction in bleeding time. On day 5, there was a marked decrease in test group whereas there was increase

in bleeding time in standard group. On day 10 and 15, there was statistically significant decrease in the bleeding time in test group when compared to the standard group. On day 20 there was very much reduction in bleeding time in test group in comparison to other groups. On the day 24 there was statistically very significant decrease in bleeding time compared to the other groups. Bleeding time in standard remained almost constant throughout study. Thus it can be interpreted there was significant changes observed on bleeding time factor. Statistically the test group showed very significant reduction in the bleeding time when compared with standard and control group. This shows that *Guduchi Churna* acts locally at the site of injury and reduces the bleeding. Reduction in bleeding time was markedly observed after 5th day.

Effect of Guduchi on clotting time:

The clotting time was ranging between 30-90sec in all the 3 groups. There was slight reduction in clotting time in test group in comparison to other groups on first day. On 10th day there was slight reduction in clotting time in both standard and test group, which remained constant on day 20 and 24. There was reduction in the clotting time when compared with standard and control group. The reduction was statistically non-significant suggesting no considerable effect of *Guduchi* on clotting time. It shows the systemic effect of *Guduchi* is very limited as clotting time is

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calculated after drawing blood from the retro-orbital plexus.

Probable mode of action of the drug:

Vrana&Raktatipravrutti



Guduchi Churna application



Drug enters the tissue through *Siramukha*

Drug acts according to *Prabhava and Guna*



Vranasandhana&Raktastambhana

CONCLUSION

Acharya Charaka has described *Sandhaniya Dashemani* in *Charaka Samhita Sutrasthana Shadvirechana Shatashritiya Adhyaya*. Since there is different meanings for the word *Sandhana* in different contexts there is a need to explore the wider actions of the drugs included in *Sandhaniya Dashemani*. Hence an attempt has been made to evaluate the *Vranasandhana* and *Raktastambhana* effect of *Guduchi* which is among the *Sandhaniya Dashemani*.

In the experimental study, in case of period of epithelialization or the number of days taken for complete wound healing was less in the test group compared with the standard and control groups. Histopathological examination of wound bed revealed better wound maturation profile in test group compared to control group. In the *Guduchi Churna* applied group, more of epithelial regeneration, mature collagen fibre deposition and good granulation tissue formation was observed. In

the bleeding induced experiment, there was a very significant decrease in the bleeding time of test group on 10th, 15th and 24th days of the experiment. In the clotting time there was non-significant decrease observed in the test group compared to the control group.

The study clearly shows that the quality of wound healing process is enhanced by *Guduchi Churna* application. It can be suggested that the test drug has wound contraction enhancing property in the early stages of wound maturation process.

By analyzing the results of bleeding time and clotting time it can be suggested that the drug possess haemostatic effect. From the present study it could be inferred that the test drug has got both *Vranasandhana* and *Rakta Stambhana* effect.

Thus it can be concluded that the word *Sandhana* means *Vranasandhana* in case of wound healing and it means *Raktastambhana* in case of *Raktatipravrutti*.

Hence the study has helped to give scientific validation for the textual reference of *Sandhana*.

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