



## A Comparative Analytical and Experimental Study on Wistar albino Rats using *Apamarga Ksharasutra* Prepared by Different Methodologies in Fistula in ANO

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### ABSTRACT:-

The *Ksharasutra* is a medicated thread prepared from alkalies substance. It is prepared by repeated coating of alkali substance on Linen Barbour thread no- 20. *Sushruta* has mentioned its reference in *Nadivarna Chikitsa*. *Ksharasutra* is a choice of treatment in the management of Fistula-in-ano. The *Apamarga Ksharasutra* is considered as standard. A study has been put forth to access the various Physico-chemical parameters of *Apamarga Ksharasutra* made with different methodologies and also to access its UCT in animal based model.

**Keywords:-** *Ksharasutra*, Fistula-in-ano, Animal model, Physico-chemical analysis.

### Introduction:-

The word *Kshara* is derived from the root *Kshara* which means to melt away. *Ksharasutra* is a thread made up of alkalie material which causes *Kshanana* of tissue.

*Acharya Sushruta* was 1<sup>st</sup> to describe *Ksharasutra*. He mentioned it in the treatment of *Nadivrana* but no description regarding the preparation was mentioned. Later *Chakrapani Dutta* was 1<sup>st</sup> to mentioned the preparation of *Ksharasutra* and indicated in *Bhagandara* managment. *Rasatarangini* also mentioned the method for preparation of *Ksharasutra*. But due to its intricate preparation and method it lost its popularity. Later Prof P J Deshpande developed the process of preparation and standardization of the *Ksharasutra*.

### Aim:-

To study the efficacy of *Apamarga Ksharasutra* made with different methodologies.

### OBJECTIVES

1. To prepare *Apamarga Ksharasutra* made with different methodologies.
2. To study the Physico-chemical aspect of *Apamarga Ksharasutra* made with different methodologies.
3. To Evaluate the Unit cutting time of *Apamarga Ksharasutra* made with different methodology using Wistar albino rat.

### Materials and Method:-

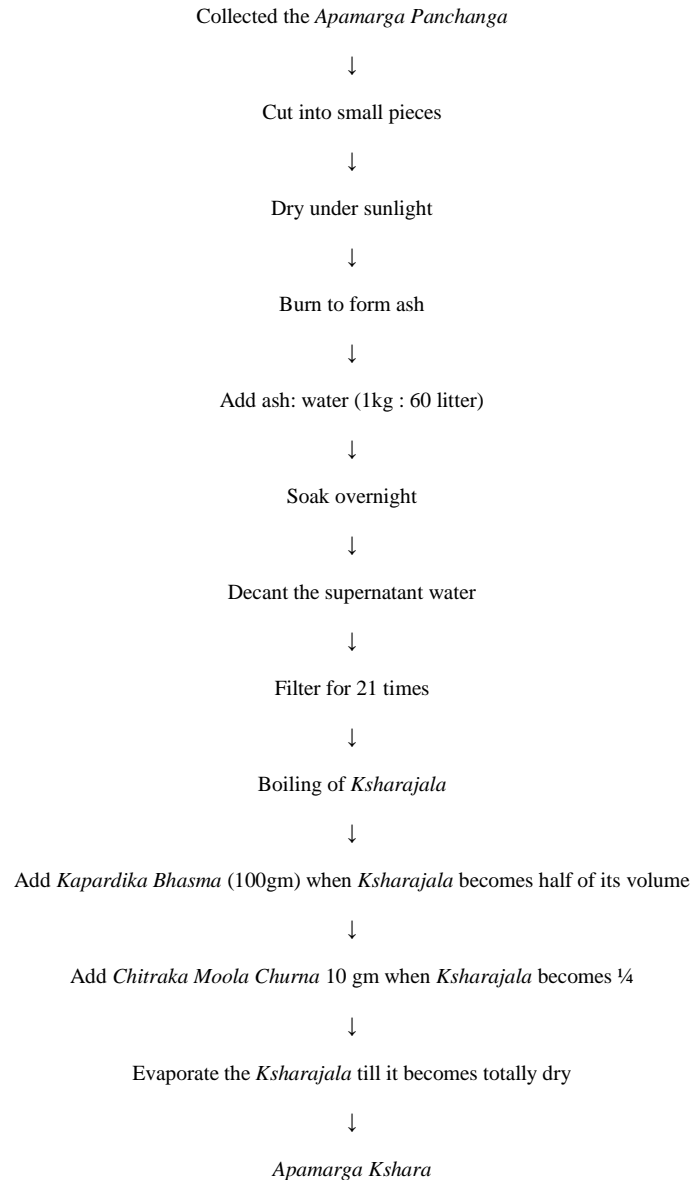
- **Selection of thread-** The selection of thread plays an important role in preparation of *Ksharasutra*, as the thread should have the appropriate thickness, quality and tensile strength to bare the whole process of the *Ksharasutra* preparation, hence the Linen Barbour thread no 20 is standard with tensile strength of 5 kg.
- **Collection of *Snuhi Ksheera*-** To yield large quantity of *Ksheera* it should be collected in the morning at 6<sup>o</sup>clock. It should not be stored for prolonged hours due to its tendency to separate into watery and gelatinous substance. Every time fresh *Ksheera* should be collected. The latex should be collected by incising the *Snuhi* plants stem with stainless steel blade and the *Ksheera* coming out through the incision is collected.
- ***Ksharasutra* cabinet:-** It is used for drying the *Ksharasutra* and for sterilization of the *Ksharasutra*.

## Preparation of Apamaraga Ksharasutra

- *Purvakarma*:- it includes preparation of *Apamarga Kshara*

S.N	Ingredients	Quantity
1	<i>Apamarga panchanga</i>	10 kgs
2	<i>Kapardika</i>	100gm
3	<i>Chitrak Mool Churna</i>	10gm

### Procedure-



- **Pradhana karma-**

It includes preparation of *Apamarga Ksharasutra*

### Group -1 Group 1- *Asamapramana Apamarga Ksharasutra* of 21 coatings

**Materials:** - Linen Barbour thread no 20, *Ksharasutra* cabinet, *Ksharasutra* hangers, bowl, blade

**Ingredients:** -

S.N	Ingredients	Quantity
1	<i>Snuhi Ksheera</i>	As per required
2	<i>Apamarga Kshara</i>	As per required
3	<i>Haridra Churna</i>	As per required

Number of coatings are as follow

- a) 11 coatings of *Snuhi Ksheera*
- b) 07 coatings of *Snuhi Ksheera + Apamarga Kshara*
- c) 03 coatings of *Snuhi Ksheera + Haridra Churna*.

**Group -2** Number of coatings are as follow

*Haridra Churna + Apamarga Kshara + Snuhi Ksheera* was taken one part each and was applied evenly over the thread under aseptic measures daily for 21 days. *Ksharasutra* was Dried in cabinet. After drying *Ksharasutra* was cut into 10-11 inches and sealed in glass tube.

**Group 3 – Asamapramana Apamarga Ksharasutra of 07 coatings**

**Materials:** - Linen Barbour thread no 20, *Ksharasutra* cabinet, *Ksharasutra* hangers, bowl, blade

**Ingredients:** -

S.N	Ingredients	Quantity
1	<i>Snuhi Ksheera</i>	As per required
2	<i>Apamarga Kshara</i>	As per required
3	<i>Haridra Churna</i>	As per required

**Procedure:** - The no of coatings done are as follows,

- a) 4 coatings of *Snuhi Ksheera*
- b) 2 coatings of *Snuhi Ksheera + Apamarga Kshara*
- c) 1 coating of *Snuhi Ksheera + Haridra Churna*

**Group 4- Samapramana Apamarga Ksharasutra of 07 coating**

**Materials:** - Linen Barbour thread no 20, *Ksharasutra* cabinet, *Ksharasutra* hangers, bowl, blade

**Ingredients:** -

S.N	Ingredients	Quantity
1	<i>Snuhi Ksheera</i>	1 part
2	<i>Apamarga Kshara</i>	1 part
3	<i>Haridra Churna</i>	1 part

**Procedure:** -

*Haridra Churna + Apamarga Kshara + Snuhi Ksheera* was taken one part each and was applied evenly over the thread under aseptic measures daily for 07 days. *Ksharasutra* was Dried in cabinet. After drying *Ksharasutra* was cut into 10-11 inches and sealed in glass tube.

**Experimental study of Apamarga Ksharasutra**

**Study design:**

30 Male Wistar albino rats were randomly selected and divided into five groups consisting of 6 rats in each group weighing approximately 250 - 350 gms were selected for the present study.

**Inclusive Criteria:**

1. Wistar Albino Rats weighing approximately 250 - 350 gms was selected.

2. Male Wister albino Rats were selected.
3. Active and healthy rats were selected.

#### Exclusion Criteria:

1. Rats used for other studies previously.
2. Diseased Rats.
3. Rats with anal infection

#### Grouping of Animals:

**Table. - Grouping of animals and type of Ksharasutra ligated in different groups.**

Sl. No	Group Name	Type of <i>Apamarga Ksharasutra</i>
Group 1	Control group	Plain linen Barbour thread no 20.
Group 2	Standard group	<i>Asamapramana Apamarga Ksharasutra</i> of 21 coatings.
Group 3	Test group	<i>Samapramana Apamarga Ksharasutra</i> of 21 coatings.
Group 4	Test group	<i>Asamapramana Apamarga Ksharasutra</i> of 07 coatings.
Group 5	Test group	<i>Samapramana Apamarga Ksharasutra</i> of 07 coatings.

#### Animal Study:-

The animal study was conducted to access the UCT of each thread using animal based model for Fistula-in-ano.

The experiment was conducted on Wistar albino rats of male sex. The animals were maintained under controlled condition of temperature (22+\_20), humidity (50+\_50), 12 hours of light and dark cycles, with food, water and healthy atmosphere in animal house. The rats were kept according to all required ethical standards.

**Animals** Wistar albino rats

**Sex** Male

**Body weight** 300 gms

**Drugs used** ketamine

**Tract length** 1cm

#### Pre-operative:-

- Anaesthesia given to rat through intraperitoneal route of administration
- Position of rat- Supine position with 4 limbs in abduction
- Part preparation

#### Operative:-

A threaded steel wire number 05 suture was passed through the anal sphincter musculature approximately 1cm lateral to the left. After perforation of anal canal by needle, the steel wire was cut and tied loosely through rotating movement around the anal sphincter muscle. The rats were maintained in individual cage during the study period with food and water ad libitum. The rats were checked daily, water and food were changed daily.

On 31<sup>th</sup> day the steel wire was removed. Thus, complete Fistula tract was created probing of the tract was done. *Apamarga Ksharasutra* was passed through the external opening and taken out through internal opening. Two ends of the *Apamarga Ksharasutra* were tied. The procedure was carried under aseptic measures. On the 37<sup>th</sup> day *Apamarga Ksharasutra* was changed in all rats and the length of the tract was measured.

**Result:-** On 31<sup>st</sup> day all the animals had steel wire properly tied around the anal sphincter with no signs of corrosion or destruction of the seton by the animal.

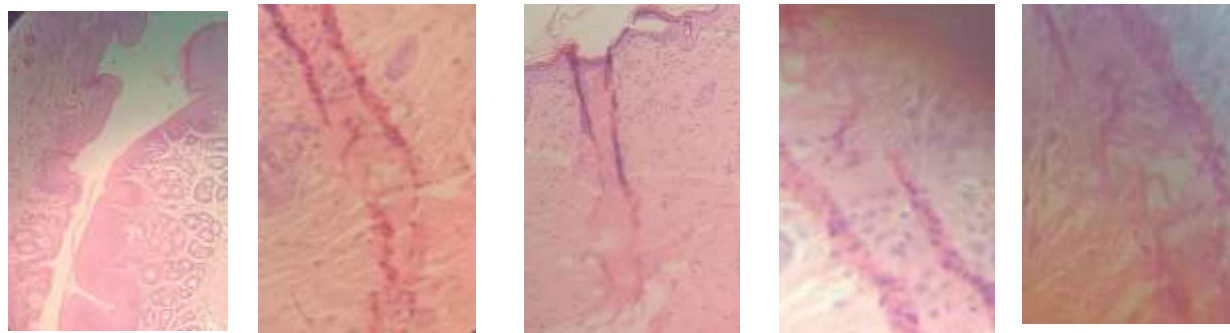
**The length of fistula tract on 1<sup>st</sup>,7<sup>th</sup>, and 14<sup>th</sup> day of treatment**

Group	Rats	Initial length of the tract on 1 <sup>st</sup> day	Length of the tract on 7 <sup>th</sup> day	Length of the tract on 14 <sup>th</sup> day
Control group	1	1 cm	1 cm	1 cm
	2	1 cm	1 cm	1 cm
	3	1 cm	1 cm	1 cm
	4	1 cm	1 cm	1 cm
	5	1 cm	1 cm	1 cm
	6	1 cm	1 cm	1 cm
Standard group	1	1cm	4 mm	0 mm
	2	1cm	4 mm	0 mm
	3	1cm	3 mm	0 mm
	4	1cm	5 mm	1 mm
	5	1cm	4 mm	0 mm
	6	1cm	3 mm	0 mm
Trail group I	1	1cm	5 mm	1 mm
	2	1cm	5 mm	0 mm
	3	1cm	3 mm	0 mm
	4	1cm	4 mm	0 mm
	5	1cm	5 mm	1 mm
	6	1cm	4 mm	0 mm
Trail group II	1	1cm	5 mm	2 mm
	2	1cm	3 mm	0 mm
	3	1cm	5 mm	1 mm
	4	1cm	4 mm	0 mm
	5	1cm	5 mm	2 mm
	6	1cm	4 mm	0 mm
Trail group III	1	1cm	5 mm	0 mm
	2	1cm	5 mm	2 mm
	3	1cm	3 mm	0 mm
	4	1cm	3 mm	0 mm
	5	1cm	5 mm	1 mm
	6	1cm	4 mm	0 mm

### Histological evaluation of the Fistula tract

After euthanasia a circumferential incision around the anus was performed using a cold scalpel, including the perineum and a margin lateral to the steel wire. Tract dissection was performed with removal of the piece in block extending to rectum.

The resected material was sectioned and submitted to staining method for histological analysis. Histologically all 12 specimens analysed which showed complete healing of the fistula tract with less inflammatory symptoms.



Control group

Standard group

Trail group- 1

Trail group- 2

Trail group 4

#### Analytical study:-

Sample Name	Diameter	Tensile strength	pH	Sodium	Potassium	Total alkalis	Curcumin
<i>Asamapramana Apamarga Ksharasutra</i> of 21 coatings	1.90mm	5 ½ kg	10	8%	45%	33%	0.6%
<i>Samapramana Apamarga Ksharasutra</i> of 21 coatings	1.95mm	5 ½ kg	10	7%	48%	40%	0.8%
<i>Asamapramana Apamarga Ksharasutra</i> of 07 coatings	0.85mm	6 kg	10	4 %	35%	30%	0.2%
<i>Samapramana Apamarga Ksharasutra</i> of 07coatings	0.84mm	6 kg	10	4%	30%	28%	0.1%

Sr.No	Sample Name	Length	Weight	Loss On Drying	Water Extractive Value	n-Hexane extractive Value
1	<i>Asamapramana Apamarga Ksharasutra</i> of 21 coatings	30cm	0.34g	1.2%	73%	8%
2	<i>Samapramana Apamarga Ksharasutra</i> of 21 coatings	30cm	0.31g	1.4%	53%	34.33%

3	<i>Asamapramana</i> <i>Apamarga Ksharasutra</i> of 07 coatings	30cm	0.16g	1.9%	43 %	2.3%
4	<i>Samapramana</i> <i>Apamarga Ksharasutra</i> of 07 coatings	30cm	0.16g	1.8%	56%	34%

### Discussion:-

The anal fistula is the common problem with incidence rate of 50,000-1,00,000 people per year and surgical treatment can bring some complication such as incontinence.

In Ayurveda a unique thread known as Ksharasutra is used to treat the Fistula-in-ano. It help in cutting, curetting, draining and healing of the fistula tract.

It destroys and removes unhealthy tissue and promote healing of the fistula tract due to caustic action, separate the debris and cleans the wound, controls infection and facilitates drainage of pus.

The experimental model of artificial anal fistula most accepted in the world was developed in pigs. Although the model has been successful, as demonstrated by MRI, presence of lumen and granulation tissue at histological analysis and the fact that it has been used for the study of new techniques, we believe that a similar model in rats would be easier to reproduce, with further contribution to advance in the study of technique for treatment of fistulas.

The use of rats in the experiment was justified by the fact that the surgical procedure in rats was easier, due to the small size as well as availability of the animals, and the fact that there was no description in the literature of the use of these animals as experimental model, with reports found only in dogs and pigs. Rats have the structure of the internal and external sphincters similar to humans. It is known that, unlike humans mucous anal glands, rats have the simplest type of sebaceous gland involving the anal canal and located mainly in the submucosal region.

The histological evaluation of this study showed complete healing of the tract with less inflammatory changes.

### Conclusion:-

1. The present study showed that Physico- chemical parameters varies with respect to coatings of *Apamarga Ksharasutra*.
2. It may be concluded that in this study *Apamarga Ksharasutra* made with different methodologies had a significant effect in treating Fistula in ano induced in Wistar albino rat as compared to control group.
3. When compared between the groups very minimal difference was seen in UCT among the groups hence *Ksharasutra* prepared with 07(Samapramana and Asamapramana) coatings is having similar effect that of 21 coatings.
4. On considering above facts the *Apamarga Ksharasutra* with 7 coatings showed more effect in treating fistula-in-ano induced in Wistar Albino rats when compared with *Apamarga Ksharasutra* of 21 coatings.
5. Hence it can be said that *Apamarga Ksharasutra* with 07 coatings is less time consuming in preparation but shows similar effect to that of 21 coatings.



Steel wire ligation

**CONTROL GROUP**



Day 01



Day 07



Day 14

**ASAMAPRAMANA APAMARGA KSHARASUTRA OF 21 COATINGS**



Day 01



Day 07



Day 14

**SAMAPRAMANA APAMARGA KSHARASUTRA OF 21 COATINGS**



Day 01



Day 07



Day 14



**ASAMAPRAMANA APAMARGA KSHARASUTRA OF 07 COATINGS**



Day 01



Day 07



Day 14

**SAMAPRAMANA APAMARGA KSHARASUTRA OF 07 COATINGS**



Day 01



Day 07



Day 14

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