



Research Article

THE SPERMATOGENIC ACTIVITY OF *SHUDDHA GANDHAKA* AND *KAMDEEPAKA RASA* - A COMPARATIVE STUDY IN ALBINO RATS

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KEYWORDS: *Shuddha Gandhaka, Kamdeepaka Rasa, Spermatogenic activity.*

ABSTRACT

In last 10 years significant decrease in human fertility has been observed. Male factor contributes to infertility as much as 51.2% according to WHO study of more than 8500 couples. *Shuddha Gandhaka* and *Kamdeepaka Rasa* have *Vrishya* property expected to contribute better in infertility. *Gandhaka Shodhana* was prepared as per *Rasatarangini* and *Kamdeepaka Rasa* as per Bhaishajya Ratnavali, Vajikarana Adhaya, subjected to Spermatogenic activity on albino rats

Method: *Kamdeepaka Rasa* prepared as per *Bhaishajya Ratnavali. Shuddha Gandhaka* (9mg), *Kamdeepaka Rasa* (9mg), milk (0.5ml), male albino rats formed the materials for the study parameter like body weight, biochemical Spermatogenic element, histometric studies and reproductive accessory organ weight were followed.

Results: In analytical study Si, S, Zn, were deducted in *Gandhaka*, Si, S, Cl, K, Ca, Iron, Mg, Zn, and Cu. *Kamdeepaka Rasa* has shown significant Spermatogenic activity compare to *Gandhaka* and milk.

Conclusion: The chemical and phytochemical component and *Balya, Brumhna, Rasayana*, and *Vrishya* properties have contributed for Spermatogenic activity thus *Kamdeepakarasa* may be used in different condition of Male Infertility.

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INTRODUCTION

Ayurveda is a system of medicine with historical roots in the Indian subcontinent Ayurveda therapies have varied and evolved over more than two millennia. Therapies are typically based on complex herbal compounds, minerals and metal substances (perhaps under the influence of early Indian alchemy or *Rasashastra*).

In Ayurvedic medicine, the traditional medical lore of Hinduism, *Rasashastra* is a process by which various metals, Minerals and other substances, including mercury, are purified and combined with herbs in an attempt to treat illnesses.

Vajikarana deals with (Oligospermia) (Qualitatively defective Sperm) (Azoospermia), of sperm quality along with treatment like *Prasadan, Upachaya* and *Sukrajanana* (Spermatogenesis) also

Praharshajanana (increase pleasure of orgasm. All these things are explain in *Vajikarana Tantra*.^[1]

Infertility is a disease of the reproductive system defined by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse.^[2] Reproduction is the biological process by which new individual organisms- "offspring"- are produce from their parents.^[3]

Number of therapies and formulation has been mentioned for rejuvenation and sexual vigor in Ayurvedic classics. In ancient time it was believed that without having child one cannot get *Dharma, Arth, Yasha* etc.^[4] This may reason it is included *Astanga Ayurveda* as *Vajikarana Chikista. Brihatrayees* have explain *Vajikarana Chikista* in separate chapter in *Samhita* and *Tantra*.^[5,6] *Gandhaka* is one among the *Uparasa*.^[7] Traditional uses of *Gandhak* primary focused on skin disorder

but it is also mention that *Shuddha Gandhak* has Spermatogenic property.^[8]

It is used in many ingredients in various formulation and also as individual drug. *Kamdeepaka Rasa (Chadalini Yoga)* contains *Gandhaka* and this is one of the *Vajikara Yog* mentioned in *Bhaishajya Ratnavali*.^[9]

Objective

1. To evaluate the Spermatogenic activity of *Shuddha Gandhaka* and *Kamdeepaka Rasa*
2. To compare Spermatogenetic activity between *Shuddha Gandhaka* and *Kamdeepaka Rasa*

Methods

Methodology of Pharmaceutical Study

***Kamdeepak Rasa* preparation**

- *Punarnava Moola Churna, Mocharasa Churna, Shalmali Moola Churna* was prepared.^[10]
- Thereafter *Shalmali Moola Kwatha* was also prepared.^[11]
- Seven times *Shalmali Moola Kwatha Bhavit Punarnava Churna* made.
- *Shuddha Gandhaka* made by *Goghrita* and *Godugdha* method. Raw *Gandhaka* was taken in *Khalva Yantra* and was powdered. Cow milk taken in another container and it's covered neatly by cotton cloth. *Goghrita* taken in Iron pan container and it is melted by heat. After melting of *Goghrita*, powdered *Gandhaka* was added in it and stirred well until *Gandhaka* completely melts in it. Then melted *Gandhaka* poured into the container which contains milk through cloth. Remove the cloth after pouring of *Gandhaka*. Solid slab form was obtained. The slab is again converted into multiple small pieces and

subjected to dry in open air for overnight. This procedure is repeated for three times.

- *Kamdeepak Rasa* prepared as per *Bhaishajya Ratnavali*.^[12] 50gm of *Punarnava Churna* (dried *Shalmali Moola Kwatha Bhavita*) and 50gm of *Mocharasa Churna* taken in *Khalva Yantra*. Then 100gm of above *Churna* mixed with 100gm of *Shuddha Gandhaka Churna* and subjected to trituration and mixed well.

Methodology of Experimental Study

Materials

1. *Gandhaka*
2. *Kamdeepaka Rasa*
3. *Godugdha*
4. Male Albino rats

Experimental Protocol

Sample Size: 18 male albino rats were taken for experimental study. 6 albino rats are taken in each group.

Inclusive Criteria

1. Healthy male albino rats.
2. Rat of 90-120 days old.
3. Weight ranges between 150-200gms.

Exclusive criteria

1. Unhealthy male albino rats
2. Weight below 150gm and above 200gm
3. Below 90 days old and above 120 days.

Study group

Group I: Administration of *Shuddha Gandhak*

Group II: Administration of *Kamdeepak Rasa*

Group III: Administration with milk.

Table 1: Showing Experimental Protocol (n=06)

Study design	Group I	Group II	Group III
Drug	<i>Shuddha Gandhak</i>	<i>Kamdeepak Rasa</i>	Milk
Dose	9mg S.G + 0.5ml Propylene Glycol	9mg K.D+0.5ml Propylene Glycol	0.5ml
Dosage form	Liquid	Liquid	Liquid
Route	Oral	Oral	Oral
Duration of study	30 days	30 days	30 days
Autopsy	31 st day	31 st day	31 st day

Data of body weights

Body wt of control and test group animal was recorded before and after the study period.

Organ weights

The organ weights of all experimental animals were recorded to the nearest gram after 30 days of drug administration. The experimental

animals were sacrificed on the next day after the last day of drug administration. The testis, epididymis, Vas deferens, seminal vesicles, prostate glands were dissected out and freed from surrounding fat and connective tissue and weighed up to the nearest milligram on the electronic

balance. The organ weights were calculated per 100gm body weight by using the formula as follows. Organ wt/100gm body wt = Organ wt/Body wt x 100. Then the organ from one side of each animal was fixed in bouin's fluid for histological studies.

Sperm Parameters

Sperm Count^[13]

A known amount of tissue was utilized for determining sperm count in cauda epididymis.

Sperm Count was done according to the method of Prasad *et. al* (1972) using a W.B.C Neobauer's counting chamber of haemocytometer. A known amount of tissue was taken (50mg/ml) in physiological saline. The tissue was teased gently in order to release the sperm. Thus prepared sperm suspension was sucked up to 0.5 mark in W.B.C pipette. It was diluted with 5% NaHCO₃ till the 11 mark. Here the sodium bicarbonate acts as a spermicidal for killing the sperms and facilitating their counting. Then a drop was placed and number of sperms in 64 small square were counted. The number of spermatozoa were expressed as million (10⁶/ml) of suspension.

Calculation

Volume of one square = $\frac{1}{4} \times \frac{1}{4} \times \frac{1}{10} = 0.1/16$ cu.mm.

64 small squares contain 0.4cu.mm.

Dilution=0.5 to 11=20

Total no Sperms = $N \times 20/0.4 = N \times 50$ cu.mm.

Number of spermatozoa = $N \times 50 \times 1000 = N \times 10^6$ /ml

Histology and Histometry^[14]

The histology of testis, epididymis was carried out by standard Haemotoxyline Eosin Staining technique (Gurr 1962). The tissues were blotted free of blood and fixed in the Bovines fixative. The tissue were then washed by placing in 30%, 50%, 70% grades of alcohol for minimum of 10 min in each grade. Frequent changes with 70% alcohol were given till the yellow colour disappeared. The tissue were dehydrated through

RESULTS

Shodhana process of Gandhak

Table 2: Quantity and time taken to complete Shodhana of Gandhaka

Shodhana of Gandhaka	Quantity of Gandhaka	Quantity of Godugdha	Quantity of Goghrita	Time taken to complete the process
I st	240gm	500ml	240gm	45 min
II nd	220gm	500ml	220gm	35 min
III rd	210gm	500ml	210gm	30 min

Preparation of Kamdeepak Rasa

Kamdeepak Rasa prepared by the mixture of Shalmali Moola Kwatha Bhavita Punarnava Churna (50gm), Mocharasa Churna (50gm), Shuddha Gandhaka (100gm) and total quantity of Kamdeepak Rasa obtained is 200gm.

80%, 90% and absolute alcohol by placing for 20-30min and giving 3-4 changes in each grades and are cleared by keeping in mixture of Xylene and absolute alcohol (1:1) for 15-20 min and Xylene only till they become completely transparent. Then tissues were transferred to molten paraffin wax in oven. The paraffin wax was changed 3 times with the duration of 15, 30, and 45-60min each. The paraffin blocks of the tissues were made using 'L' shaped metal bars. The tissues in wax blocks were micro toned at a thickness of 4.5µm and sections are affixed on slide using Mayer's albumin. The paraffin from the sections was removed by placing them in Xylene (10-15min). Then the slides are passed in 100%, 90%, and 70% alcohol (5min in each) grades and washed in distilled water. Then slides were stained with haemotoxylene. Washed in running tap water and placed in distilled water, before transferring to 70% and 90% alcohol. Then counter stained to eosin and up graded to 100% alcohol after placing in Xylene for 5min the sections were mounted in DPX.

Micrometry

Histometric measurement of the diameter of the testis, seminiferous tubules were made using ocular and stage micrometer from randomly chosen 10 round sections from each group.

Counting Spermatogenic Elements

Randomly chosen 10 good sections of testis from each group were observed under the microscope. Spermatogenic elements like spermatogonia, spermatocytes and spermatids were counted from 20 round seminiferous tubules of each section of each group and then average of each Spermatogenic elements was calculated.

Statistical analysis

Statistical analysis was done using statistical significance test i.e. Annova Test and paired and unpaired t test.

Table 3: Organoleptic Character of *Gandhaka* and *Kamdeepak Rasa*

S. No.	Organoleptic Character	Description of <i>Shuddha Gandhaka</i>	Description of <i>Kamdeepak Rasa</i>
1	Colour	Dark Yellow	Grey
2	Odour	Ghee Smell	Pungent odour
3	Taste	Tasteless	Pungent
4	Touch	Rough	Smooth / Soft
5	Appearance	Solid	Amorphous Powder

Analytical results

- *Kamdeepak Rasa* sparingly soluble in distilled water, Methanol, Chloroform and not soluble in Petroleum ether, Ethyle alcohol, Xylene.
- Specific Gravity of *Shuddha Gandhaka* and *Kamdeepaka Rasa* is 0.994 and 1.004 respectively
- pH Value of *Kamdeepaka Rasa* and *Gandhaka* is 7.6 & 7.7 respectively.
- Moisture content percentage of *Kamdeepak Rasa* and *Gandhaka* is 2.4% and 0.4% respectively
- Ash value of *Kamdeepaka Rasa* is 25.66%
- Analytical reports of *Shuddha Gandhaka* sample was obtained by using XRF contains Silicon <00.10%, Sulphur 99.17%, Zinc <1.0 ppm.
- Analytical reports of *Kamdeepak Rasa* sample was obtained by using XRF contains Silicon 00.13%, Sulphur 86.68%, Chloride 00.96%, Potassium 00.77%, Calcium 00.65%, Iron 00.20, Manganese 00.17ppm, Zinc 04.4ppm, Copper 235.58ppm.

Results of Experimental Study:**Table 4: Histopathological study of Rat Specimen (Test drug- *Shuddhha Gandhaka*)**

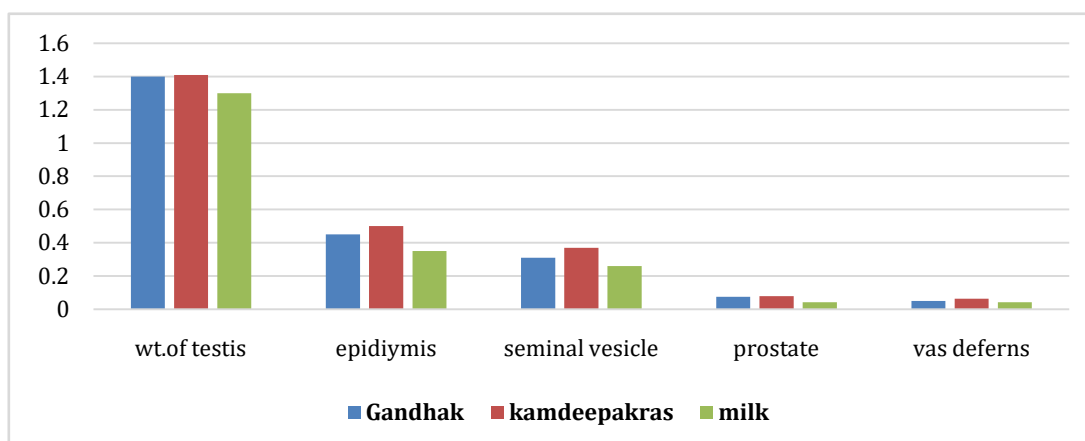
No	Parameters	Group I (<i>Shuddhha Gandhaka</i>)						MEAN
		A	B	C	D	E	F	
	Macrometers							
1	Diameter of Testis	9200	9300	9400	9400	9400	9300	9333.33
2	Diameter of Tubules	206	206	208	204	204	206	205.67
	Spermatogenic Elements							
1	Spermatogonia	98	102	102	98	100	100	100.00
2	Spermatocytes	174	172	174	172	176	176	174.00
3	Spermatids	218	220	216	215	220	216	217.50
4	Sperm Count (Millions/ Cauda)	48.5	48.5	45.5	47.5	46.5	44.5	46.833
	Organ Weights							
1	Wt of Testis (mg/100mg bd wt)	1.38	1.38	1.40	1.40	1.48	1.38	1.4033
2	Wt. of Epididymis	0.46	0.45	0.46	0.45	0.46	0.46	0.457
3	Wt. of Seminal Vesicle	0.30	0.32	0.30	0.32	0.30	0.34	0.3133
4	Wt. of Prostate	0.08	0.07	0.06	0.08	0.08	0.08	0.075
5	Wt. of Vas deferens	0.06	0.05	0.06	0.05	0.06	0.06	0.051

Table 5: Histopathological study of Rat specimen (Test drug- Kaamdeepak Rasa)

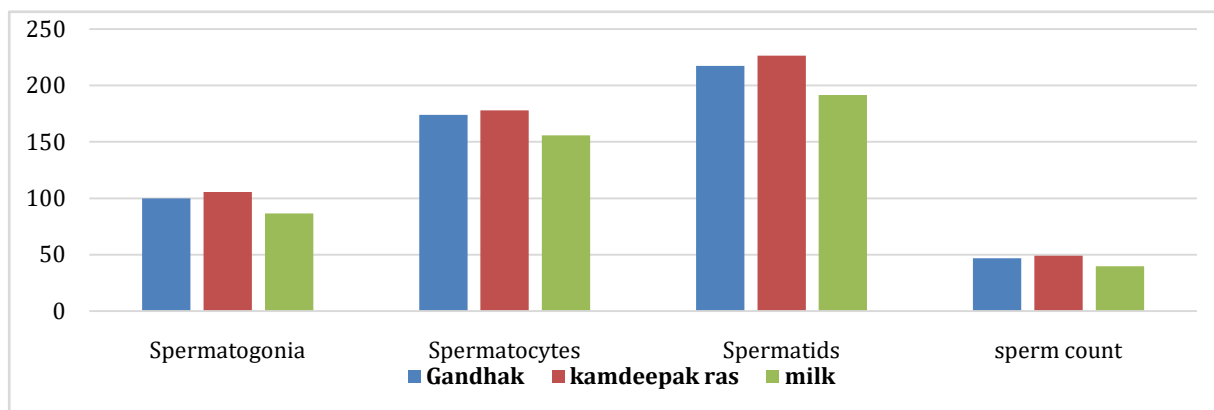
No	Parameters	Group II (Kaamdeepak Rasa)						MEAN
		G	H	I	J	K	L	
Macrometers								
1	Diameter of Testis	9600	9700	9700	9800	9400	10200	9733.33
2	Diameter of Tubules	208	208	210	209	207	207	208.17
Spermatogenic Elements								
1	Spermatogonia	109	102	108	105	106	104	105.67
2	Spermatocytes	170	181	190	172	178	177	178.00
3	Spermatids	220	230	219	218	230	242	226.50
4	Sperm Count (Millions/ Cauda)	49.5	49.0	50.4	48.4	48.0	50.0	49.217
Organ Weights								
1	Wt of Testis (mg/100mg bd wt)	1.38	1.37	1.42	1.40	1.42	1.47	1.410
2	Wt. of Epididymis	0.49	0.54	0.48	0.49	0.49	0.52	0.502
3	Wt. of Seminal Vesicle	0.38	0.40	0.37	0.36	0.32	0.40	0.3717
4	Wt. of Prostate	0.08	0.09	0.06	0.07	0.09	0.08	0.078
5	Wt. of Vas deferens	0.07	0.06	0.07	0.07	0.06	0.05	0.063

Table 6: Histopathological study of Rat Specimen (Test drug- Milk)

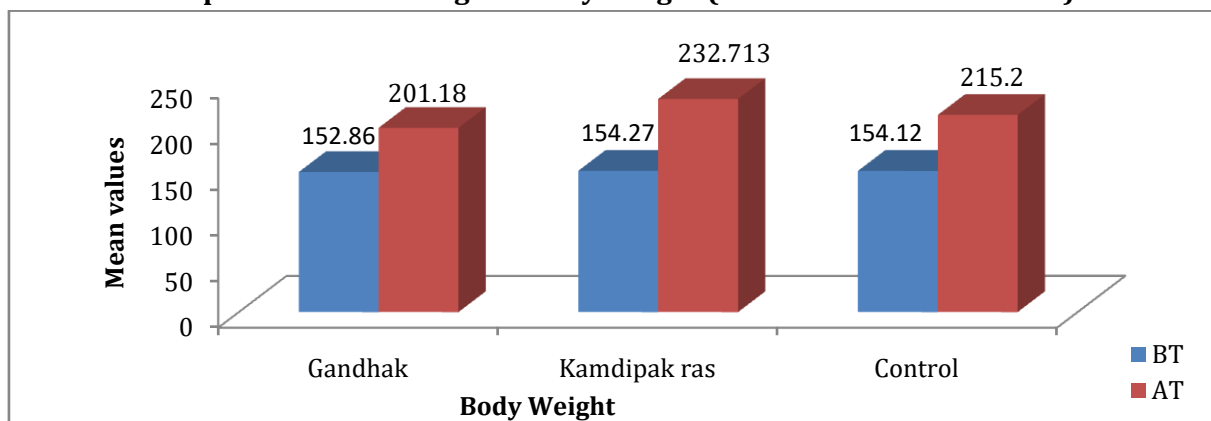
No.	Parameters	Control Group III (Milk)						MEAN
		M	N	O	P	Q	R	
Macrometers								
1	Diameter of Testis	7200	7900	7900	7800	8000	7900	7783.33
2	Diameter of Tubules	175	188	180	190	182	180	182.50
Spermatogenic Elements								
1	Spermatogonia	84	89	84	87	91	84	86.50
2	Spermatocytes	154	152	158	154	160	157	155.83
3	Spermatids	185	196	190	189	201	188	191.50
4	Sperm Count (Millions/ Cauda)	36.5	37.0	42.5	41.5	40.0	40.6	39.683
Organ Weights								
1	Wt of Testis (mg/100mg bd wt)	1.30	1.28	1.32	1.34	1.27	1.32	1.305
2	Wt. of Epididymis	0.35	0.33	0.36	0.38	0.32	0.38	0.353
3	Wt. of Seminal Vesicle	0.28	0.26	0.27	0.29	0.24	0.27	0.2683
4	Wt. of Prostate	0.03	0.06	0.04	0.05	0.04	0.04	0.043
5	Wt. of Vas deferens	0.05	0.04	0.03	0.04	0.05	0.04	0.042

Graph 1: Histopathological study of Rat Specimen

Graph 2: Histopathological study of Rat Specimen



Graph 3: Effects of drugs on body Weight (Before & After Treatment)



DISCUSSION

Gandhak Shodhana was done with reference of *Rasatarangini* by *Godugdha* method. The powdered *Gandhaka* taken in iron pan with *Goghrita*, then it melted by slow heat and poured into the container which contains milk covered by cloth. The total loss of after three *Shodhana* procedure is 40gm. *Kamdeepaka Rasa* prepared as per *Bhaishajya Ratnavali* which contains *Shalmali Moola Kwath Bhavita Punarnava Mool, Mocharasa* and *Shuddha Gandhaka*. Increase in weight of *Punarnava Churna* by 19.20gm due to *Bhavana* of *Shalmali Moola Kwath*. 1125ml of *Shalmali Kwatha* used for *Bhavana* procedure.

Shuddha Gandhaka shows more alkaline in nature than *Kamdeepaka Rasa*. Percentage of Moisture content of *Kamdeepaka Rasa* (2.4%) found more than *Gandhaka* (0.4%).

In solubility test *Shudhha Gandhak* not soluble in all solvent and whereas *Kamdeepak Rasa* Sparingly Soluble in Distilled water, Methanol, Chloroform and not soluble in Petroleum ether, Ethyle alcohol, Xylene. *Kamdeepakarasa* having more specific Gravity than *Shuddha Gandhak*. *Kamdeepaka Rasa* having 25.66% ash value and there is no ash value for *Gandhak*.

Analytical reports of *Shuddha Gandhaka* sample was obtained by using XRF contains

Silicon<00.10%, Sulphur 99.17%, Zinc<1.0 ppm. Analytical reports of *Kamdeepaka Rasa* sample was obtained by using XRF contains Silicon 00.13%, Sulphur 86.68%, Chloride 00.96%, Potassium 00.77%, Calcium 00.65%, Iron 00.20%, Manganese 00.17ppm and Zinc 04.45ppm, Copper 235.58ppm.

In experimental study there are three groups, G-I (*Shudhha Gandhak*), G-II (*Kamdeepaka Rasa*) and G-III (milk) were subjected for the evaluations and compare Spermatogenic activity in male albino Rats.

G-I shown increase in Spermatogenic elements [number of Spermatogonia (+13.5), Spermatocytes (+18.17), Spermatids (+26), Spermatozoa (+7.15 mili/susp)], Reproductive accessory organ [weights of testis (+0.1mg), epididymis (+0.10mg), seminal vesicle (+0.045mg), prostate (+0.032mg), vas deferens (+0.008mg)] and Histometric study [diameter of testis (+1550µm) & tubules (+23.70µm)] than G-III (control). Statistically G-I shown highly significance as compared with G-III.

G-II shown increase in Spermatogenic elements [number of Spermatogonia (+19.17), Spermatocytes (+22.17), Spermatids (+35), spermatozoa (+9.53 mili/susp)], Histometric study [diameter of testis (+1950µm) & tubules

(+25.67µm)], Reproductive accessory organ weights [weight of testis (+0.11mg), Epididymis (+0.148mg), Seminal vesicle (+0.103mg), Prostate (+0.035mg) and Vas deferens (+0.021mg)] than G-III (control). Statistically G-II showed highly significance as compared with G-III.

G-II shown increase in Spermatogenic elements [number of spermatogonia (+5.67), spermatocytes (+4), spermatids (+9), spermatozoa (+2.38mili/susp)], Histometric study [diameter of testis (+400µm) & tubules (+2.5µm)] and Reproductive accessory organ weights [weight of testis (+0.01mg), Epididymis (+0.045mg), Seminal vesicle (+0.058mg), Prostate (+0.003mg) and Vas deferens (+0.012mg)] than G-I (Gandhaka). Statistically G-II shown highly significance than G-I (Gandhaka) in parameter of diameter of testis, diameter of tubules, Spermatogonia, Spermatocytes, Spermatids, Epididymis and Seminal vesicle etc. and insignificance in sperm count, weight of testis, weight of prostate etc.

Kamdeepaka Rasa shown highly significant effect in production of sex hormones and accessory sex gland secretions, cells division and maturation of the Spermatogonia to spermatozoa and increasing the life span of spermatozoa. It has significant effect on morphological maturation, providing energy for the motility, viability, storage and ejaculation of sperm and volume of seminal fluid.

CONCLUSION

Shuddha Gandhak and *Kamdeepak Rasa* have *Vrishya* property. *Shuddha Gandhak* shows more alkaline nature than *kamdeepaka Rasa*. *Kamdeepaka Rasa* having more specific gravity than *Shuddha Gandhak*, due to other herbal ingredients in it. Experimental study reveals that *Shuddha Gandhak* shows more Spermatogenic activity than milk. *Kamdeepak Rasa* shows more Spermatogenic activity than milk. *Kamdeepaka Rasa* shows more Spermatogenic activity than *Shuddha Gandhak*.

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Pharmaceutical Study *Kamadeepakarasa*



Raw Gandhaka



Row Mocharas



Row Punarnavamul



Shalmalimul

Gandhaka Shodhana Procedure



Gandhaka Churna



Gandhaka Melted in Ghee

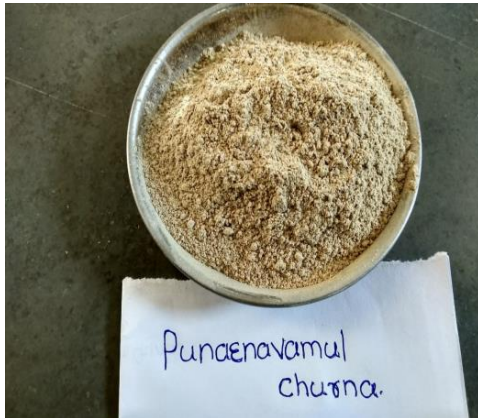


Melted Gandhaka Poured in Godhugdha



Shudha Gandhaka

Preparation of Kamadeepakarasa



Punarnavamul Churna



Shalmali Mool Kwatha Preparation



Shalmali Mool Kwatha



Bhavna with Shalmali Kwatha



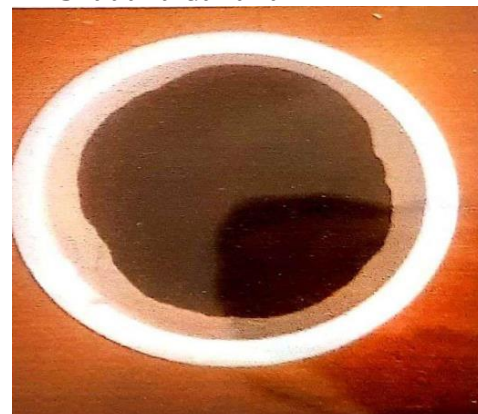
After 7 Bhavna



Shuddha Gandhak



Mochrasa Churna



Kamdeepakarasa

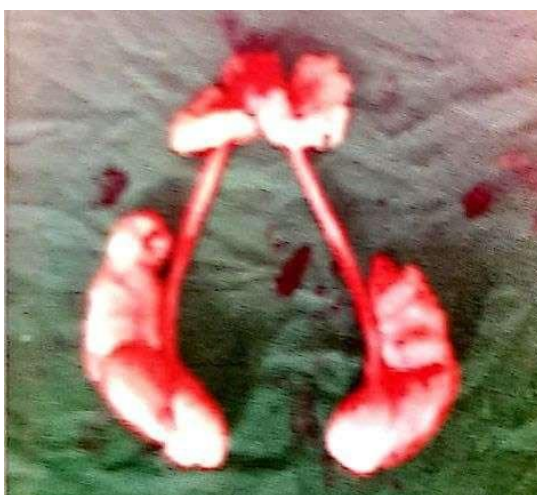
Experimental Study



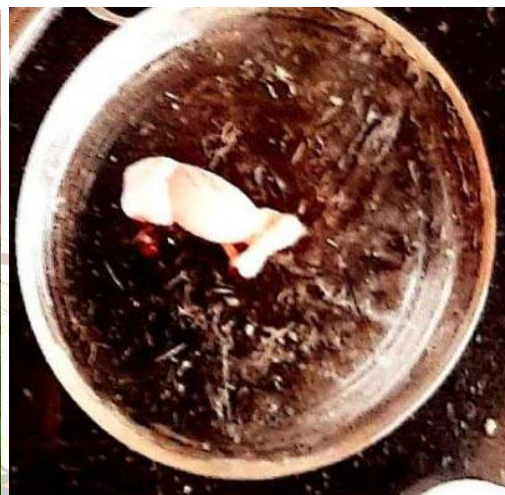
Administration of Drug



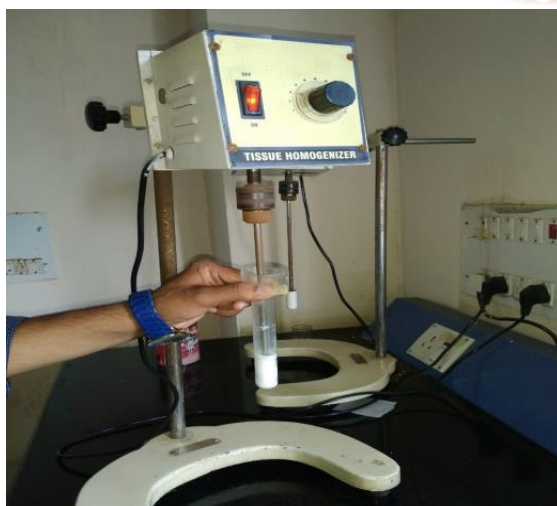
Rat Dissected After 30 Day



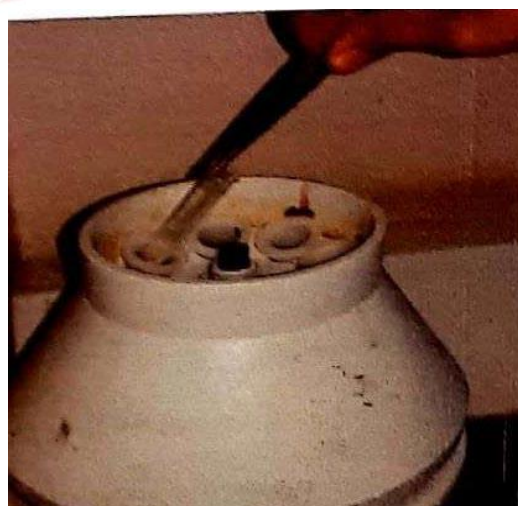
Collected Reproductive Organ



Tissue Sample for Crushing

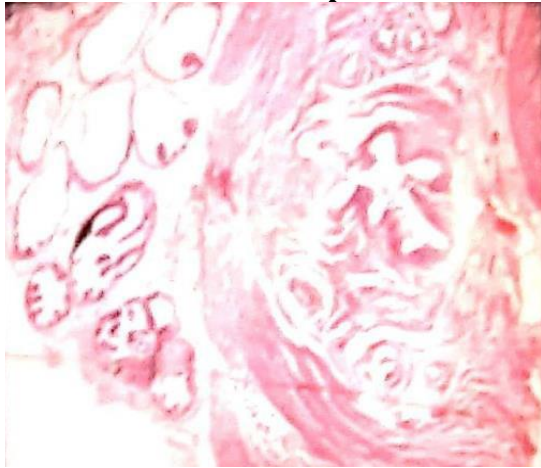


Tissue Homogeniser

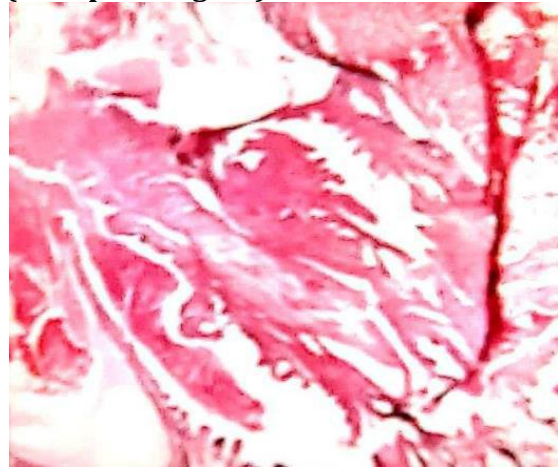


Centrifuging the Tissue

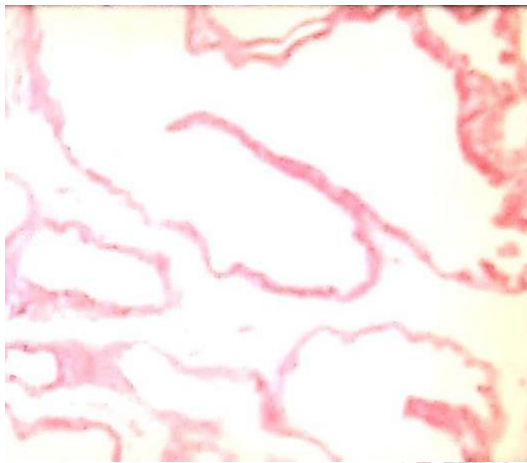
Experimental Study (Histopathological)



G1 Vas deferens



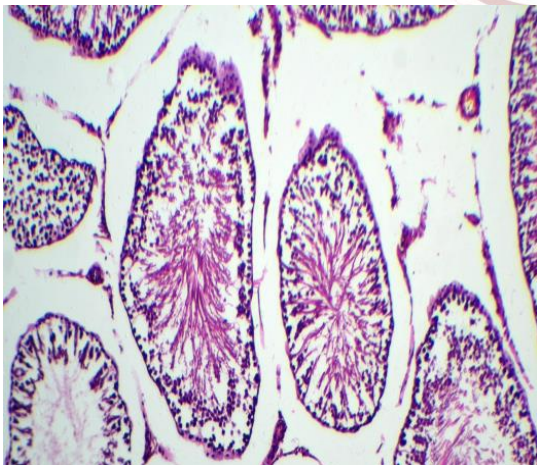
G2 Vas Deferens



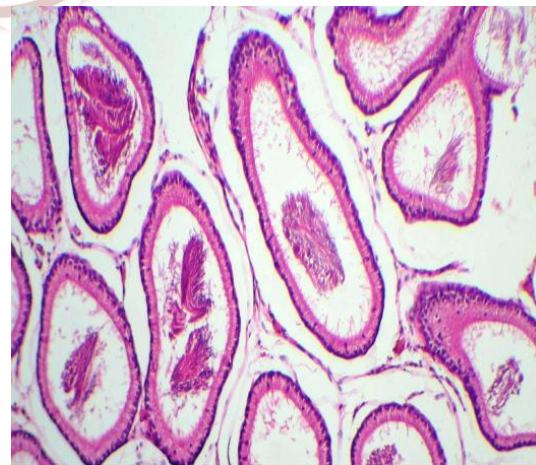
G 1 Seminal Vesicle



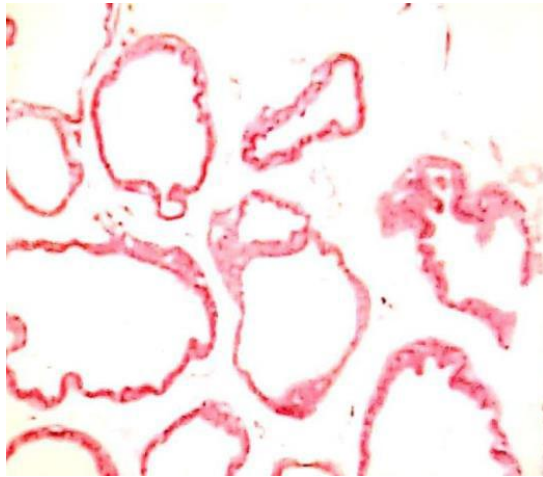
G 2 Seminal Vesicle



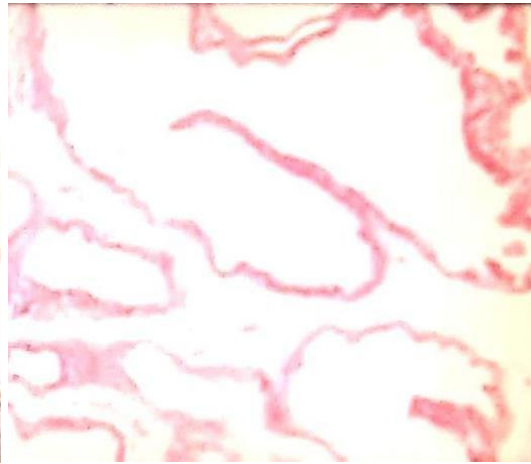
G1 Testis



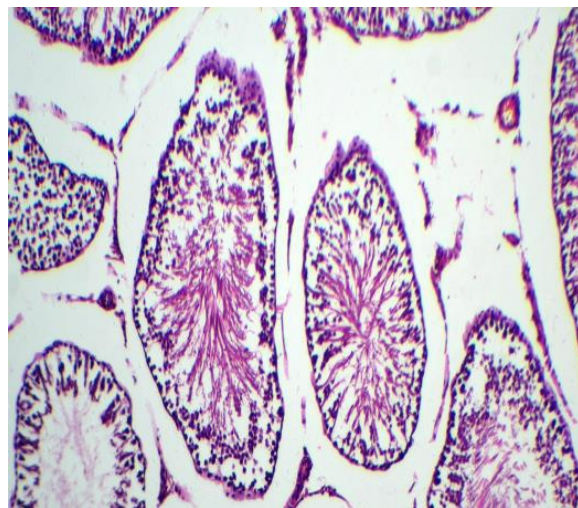
G2 Testis



G3 Vas deferens



G 3 Seminal Vesicle



G3 Testis