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Spermatogenic Activity of Rajatachandrodaya Rasa

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***Abstract*— Rajatachandrodaya Rasa (RC) is a Sagandha, Sagni, Parada Moorchana which is Kanthastha and Bahirdhooma Kupipakwa Rasayana prepared as per the guidelines of Bheshaja Samhita. RC was prepared with 1:8:16 proportions of Shodhita Rajata, Shodhita Parada and Shodhita Gandhaka by Kupipaka method. RC is having therapeutic potential ranging in diseases such as *Hridroga*, Madhumeha, *and Jwara* and also possesses R*asayana* and *Vajeekarana* properties. Till date, no reported studies are available pertaining to vajikarana effect of Rajatachandrodaya Rasa. Hence an attempt has been made to evaluate spermatogenic activity of RC experimentally on wistar strained male Albino rats and compared to Siddha Makaradhwaja.Animal experimental study of RC done on Albino Male rats under all necessary precautions. RC was taken in two different doses (0.45 mg/0.2kg &0.54 mg/0.2kg.b.wt) and compared with Control (distilled water & food) and Standard (Siddha Makaradhwaja) groups. Study shows increase in sperm count (up to 95-99 million) which shows effect of RC on spermatogenesis. It showed statistically significant ($p<0.05$) Spermatogenic activity compared to control group ($p<0.05$) & standard group ($p<0.05$). Histopathological study also showed increase in size of seminiferous tubules. Basement membrane was tightly bound with germinal epithelium. The lumen of seminiferous tubule was filled with bundles of Spermatozoa suggestive of significant Spermatogenic activity.**

***Index Terms*— Rajatachandrodaya Rasa, Siddha Makaradhwaja, Spermatogenic activity.**

I. INTRODUCTION

Rasashastra, an advanced pharmaceutical science describes the conversion of metals and minerals into therapeutically safe and potent forms. Rasouhadhies are herbo-mineral compound formulations, known for their quick curative attributes with small dose. Kupipakwa Rasayana is one among the important formulations described under the Parada Murchana and is considered best in terms of its wide spectrum in therapeutics. Pharmaceutical preparation by Kupipakwa method enhances the property of the drug, to form a stable, safe, efficacious compound, which is evident by various studies on the same.

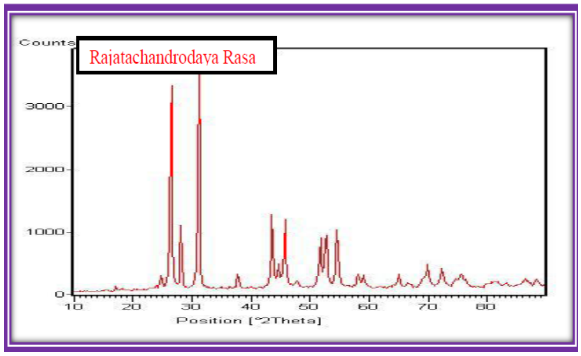
The word Rajatachandrodya is formed by the combination of two words i.e. Rajata & Chandrodaya. Chandrodaya is a Synonym of Kamadeva, the symbol of beauty. Person using RC will become handsome like Kamadeva, as quoted in Bheshaja Samhita. The other similar meaning of Chandrodaya is that, the users of it may gain brilliant shining of the body and look like rising Moon.RC was prepared in PG DEPT of Rasashasra & B.K, Ayurveda MahaVidyalaya, and Hubballi as per guidelines of Bheshaja Samhita. It is prepared by method of Kupipakwa in Valuka yantra with 19 hours of Mridwagni, 24 hours of Madhyamagni and 18 hours of Teevragni. All the standard parameters and Precautions were observed and documented during the procedure.RC Rasa was prepared with 1:8:16 proportions of Shodhita Rajata, Shodhita Parada and Shodhita Gandhaka by Kupipaka method in 61 hours. Out of 200gms of Kajjali, the yield of final product was 95gms (47%).Oranoleptic characters, physicochemical analysis, SEM; XRD & EDAX tests were performed to assess the quality of RC.Organoleptic Characters- Prepared sample of RC was reddish brown in colour, odourless, tasteless and in fine powder form. Physical Tests- pH-5,Tot al ash value-0.39%,Acid insoluble ash-0.22%,Water soluble ash-0.15%,Los on drying-0.14%.

Chemical tests - Silver-0.10%, Total Mercury-74.07%, Free Mercury-Nil, Total Sulphur-12.28%.Particle size by Lazer Diffraction Method-Mean value of total percentage of particles size of RC is 9.59µm.XRD -Report of RC shows d-space value as 3.37401, 3.1735, 2.87326, 2.38013, 2.08021, 1.77134, 1.73641, 1.68271, 1.43631, 1.30721, 1.25858 and 1.10645.

Many strong peaks were observed along with week peaks, after selecting certain strong peaks it was concluded that the RC has cubic crystal system with primitive brave lattice. SEM-RC morphology is just like cotton balls. The particles size ranges from 120 nm to 200 nm.

EDAX -Shows the chemical composition consists of mercury, sulphur and small particle was in Nano meter size. Three elements were observed in major concentration in the sample tested. They are S, Ag and Hg with weight % of 15.97, 10.13 and 73.90 and atomic % of 44.22, 20.18 and 35.60 respectively .

II. GRAPH SHOWING XRD RESULT OF RAJATACHANDRODAYA RASA



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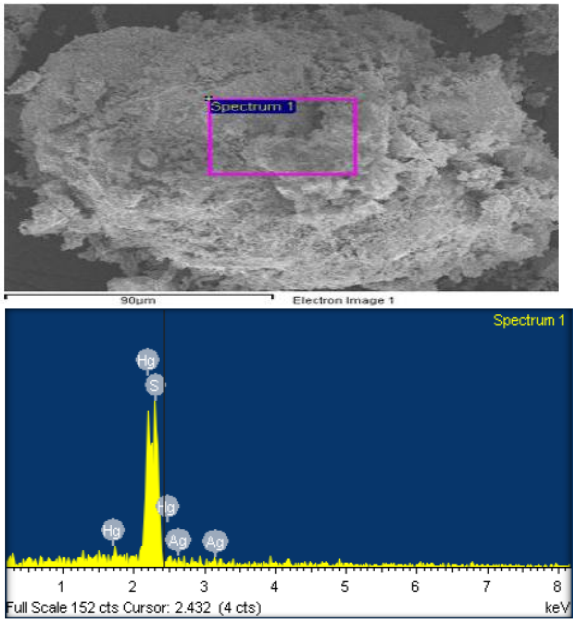

III. EXPERIMENTAL STUDY

RC was subjected for Spermatogenic activity in Albino rats and compared with standard group and control group. Animal were divided into four groups.

Group I is Control group received Normal food and distilled water,

Group II is Standard group received Siddha Makaradhwaja Rasa,

Group III is Trial group A1 received Rajatachandrodaya Rasa (dose 2.25 mg/kg.b.wt).

Group IV is Trial group A2 received Rajatachandrodaya Rasa (dose 2.7 mg/kg.b.wt).

IV. MATERIALS

Drugs	Test drug:Rajatachandrodaya Rasa. Standard drug:Siddha Makaradhwaja
Animals Used	Wister strain Albino Rats.
Equipments	Animal Cage, Day night cycle chamber, Microscope. Weight
Glass wares	Glass Beakers, Test tubes, Stirrer, Measuring jar, 18" needle & Disposable Syringe.
Chemicals and Reagents	Normal Saline, 10% Formalin, Bouin Holland solution, Paraffin wax, Chloroform

Healthy Albino rats of male sex weighing 150 -200 gm were used for the experimental study. The animals were purchased from Sri. Venkatesh Enterprises, Bangalore, India. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET'S College of Pharmacy, Dharwad, India (REG.No.112/1999/CPCSEA) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India.

The study of male and ovarectomized female Sprague Dawley rat are housed in sex- separated rooms at 21±1 0C in a reversed light-dark cycle with free access to food and water.The mating tests are performed between 12:30 and 17:00 during the dark phase of the lighting cycle.Drug was administered orally 1 hour before test to the male rat. The animal was allowed to adapt to the test area (60 cm diameter, 50 cm high) illuminated with a dim red light.

A. Sexual behaviour in male rat:

Male rat was placed in the observation glass chambers in order to acclimatize it with the cage environment. Sexually receptive female rat was then allowed to enter the test cage silently from a side door inside the cage. The behavioural observations were carried out taking into account the following parameters.

B. Mounting Behaviour:

It was determined and characterized by following parameters

(1) Mount frequency: Average number of mount during 30 min observation.

(2) Mount latency: The leg time from the introduction of female in the cage to first mount.

Intromission Behaviour: it was evaluated as follows.

Intromission latency: intromission latency (IL) was considered as the time for first intromission after introduction of female in the cage.

Parameters for sexual behaviour analysis.	**Group**	**0 day**	**15 day**
Mount Latency (Time in sec.)	Control	60±2.5	90±4.12
	Standard	90±6.2	210±2.4
	Test A1	60±3.2	210±1.8
	Test A 2	90±3.1	240±4.7
Mount Frequency	Control	6±0.7	6 ±0.9
	Standard	7±0.2	10±0.1
	Test A1	6±0.3	11±0.2
	Test A2	5±0.5	12±0.7
Intromission latency (in sec.)	Control	300±1.6	300±3.2
	Standard	330±4.5	270±1.8
	Test A1	270±2.4	300±5.4
	Test A2	300±2.2	330±4.6

V. Experimental Protocol

Selection of Animals.

Inclusive criteria:

- Healthy Albino rats of male sex will be considered.
- Albino rats weighting 150 -200 gms.
- Exclusive criteria:
- Less than 150 gm. and more than 200 gms.
- Rats which are under trial of other experiments.
- Sample Size.

24 Wister strain Albino rats divided into 4 groups (6 rats in each group)

VI. Method

The Rat dose was calculated by standard conversion factor 0.018 from human to the rat, according to body weight. The medicine was administered mixing with 0.5ml Distilled water.

Table1: Table showing days and dosage of Drug induced

Sl. No	Group	No. of Rats	Drug	Dose
1.	Control	6	Normal food and Distilled water.	1ml/kg.
2.	Standard	6	Siddha Makaradhwaja Rasa.	0.45mg/ 0.2kg, p.o.)
3.	Trial Sample A1(L)	6	Rajatachandrodaya Rasa.	0.45mg/ 0.2kg, p.o.)
4.	Trial Sample A2(H)	6	Rajatachandrodaya Rasa.	0.54mg/ 0.2kg, p.o.).

Table 2: Table Showing Dose, Duration and Mode of Administration:

Group	Dose	Duration	Mode of Administration
Control	**1ml**	**6 weeks**	**Oral**
Standard	**0.45mg/ 0.2kg, p.o.).**	**6 weeks**	**Oral**
Trial Sample A1(L)	**0.45mg/ 0.2kg, p.o.).**	**6 weeks**	**Oral**
Trial Sample A2(H)	**0.54mg/ 0.2kg, p.o.).**	**6 weeks**	**Oral**

After completion of 6 weeks, for histopathology of animals were studied by sacrificing by Ether Anesthesia. Then the abdomen was opened by taking incision over it & testis, epididymis was excised. Epididymis excised for purpose of semen analysis. Both the organs fixed in 10% formalin.

Then these were studied by compound microscope for the confirmation of Spermatogenetic activity. Spermatozoa were counted using Hematocytometer.

VII. RESULT

Table 3: Showing the comparative average calculated Sperm count in all Groups

Groups	Average sperm count (In million / ml)
Control	**86.8333 ± 14.905**
Standard	**98.333 ± 2.582**
Trial Sample A1(L)	**99.167 ± 6.401**
Trial Sample A2(H)	**99.333 ± 5.502**

Table 4: Showing the effect of the drugs on Spermatogenic Activity comparison between Groups:

Semen Analysis ANVOA Test followed by Tukey process

Activities	Groups	Test values one way Analysis (ANVOA Test)
Semen Analysis	Control Vs. Standard	N= 6, H=6.270, Q=3.510, P=0.012(P<0.05)
	Control Vs. Trial A_1	N=6, H=4.379, Q= 2.944, P=0.04(P<0.05)
	Control Vs. Trial A_2	N=6, H=4.078, Q=2.831, P=0.041(P<0.05)
	Standard Vs. Trial A_1	N=6, F=0.0143, P=0.907.
	Standard Vs. Trial A_2	N=6, F=0.0187, P=0.894.

All values expressed as mean ±S.D.P<0.05 and P<0.01 considered Significant as compared to control group.

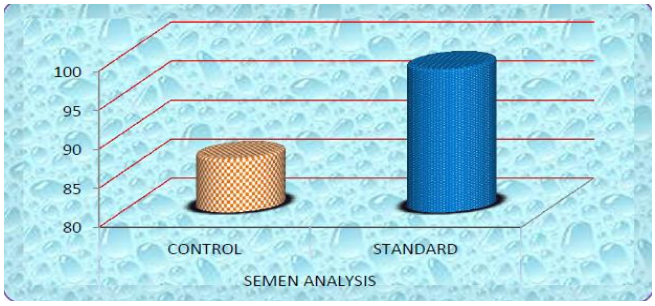

Fig 1: Graph Showing the Semen Analysis Control & Standard group.

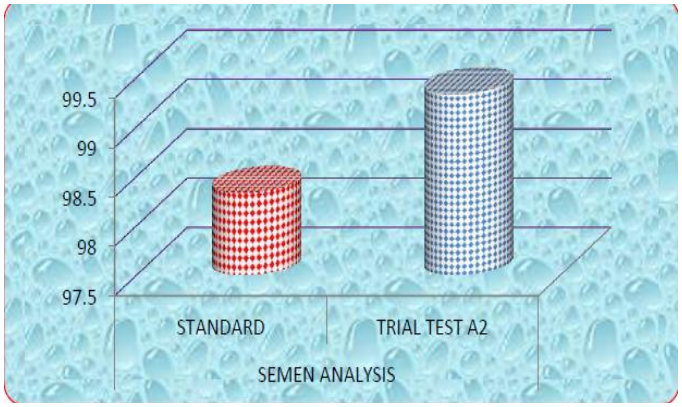

Fig 2: Graph Showing the Semen Analysis Standard & Trial test A2 group

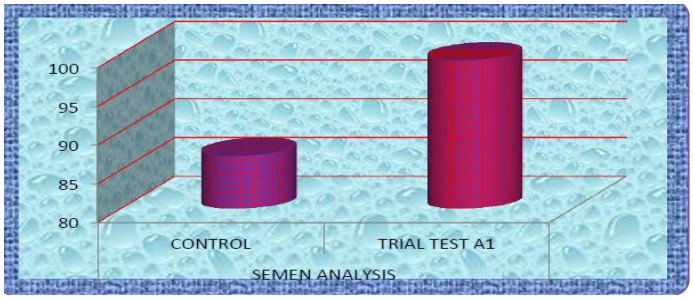

Fig 3:Graph Showing the Semen Analysis control & Trial testA1group

Table 4: Table showing the testes weight in gm. all groups

Control group	Standard group	Trial group A_1	Trial group A_2
2.5	1.8	3	2.5
2.4	3	2.2	2.9
2	2.1	2.5	2.8
2.9	3.2	2.3	2.4
3	3.1	3.3	2.3
2.1	2.8	3.5	3.1
Average	**Average**	**Average**	**Average**
2.48	2.7	2.8	2.7
±	±	±	±
0.41	0.6	0.5	0.3

Table 5:Table showing the epididymis weights in gm. all groups

Control group	Standard group	Trial group A_1	Trial group A_2
0.564	0.66	0.541	0.841
0.571	0.647	0.611	0.654
0.54	0.87	0.63	0.6
0.541	0.851	0.521	0.511
0.281	0.711	0.842	0.521
0.6	0.411	0.7	0.612
Average	**Average**	**Average**	**Average**
0.516	0.692	0.641	0.623
±	±	±	±
0.117	0.167	0.118	0.120

Table 6: Table showing the body weight in gm. all groups:

Control group	Standard group	Trial group A_1	Trial group A_2
285	252	341	344
265	271	301	321
271	284	311	311
280	241	285	281
275	235	248	254
280	257	298	211
Average	**Average**	**Average**	**Average**
276	256.7	297.3	287.0
±	±	±	±
7.21	18.4	30.6	48.7

Control group treated with distil water did not showed increase in the Spermatogenic activity as observed in first day of sperm count to that of final day. Standard group treated with siddha Makaradhwaja Rasa showed increase in the Spermatogenic activity as observed in first day of sperm count to that of final day.

Trial test A1 group treated with Rajatachandrodaya Rasa showed increase in the Spermatogenic activity as observed in first day of sperm count to that of final day.

Trial test A2 group treated with Rajatachandrodaya Rasa showed increase in the Spermatogenic activity as observed in first day of sperm count to that of final day.

VIII. HISTOPATHPLOGY REPORT

Source of Specimen: RAT TESTIS
Gross Examination: Received 2 testicles larger (m) 1.6x1x1 cm. Cut section gray white.
Microscopy: Sections show testis with normal spermatogenesis.
Impression: Features are Suggestive of Normal Spermatogenesis.

The section of the testis, of the Control group when compared with treated group albino male rats showed observable differences in various stages of Spermatogenesis. In Control group albino male rats, all stages of Spermatogenesis viz. Spermatogonia, primary

spermatocytes, secondary Spermatocytes, spermatid and Spermatozoa and connective tissue, blood vessels, lymph ducts and leydig's cell were observable and distinct. The proliferation was evidently more perceptible in case of treated group animals as compared to Control group. 2.25 mg/kg.b.wt. And 2.7 mg/kg.b.wt treated groups (RC) showed increase in size of seminiferous tubules. Basement membrane was tightly bound with germinal epithelium. The lumen of seminiferous tubule was filled with bundles of Spermatozoa. There was also increase in number of laydig's cell as cytoplasm was highly stained with eosin. Under normal condition the sertoli cells lie down near the basement membrane and are spaced at quite regular intervals whereby they perform their functions of supporting the developing Spermatogenic cells, in general the nucleus is at right angles to the wall and the cell is pyramidal in shape. In case of RC treated groups the primary Spermatogenic cells, the Spermatogonia are at the first state of repetitive cell division.

IX. Discussion

RC was obtained as Reddish brown shiny conical blocks. Nishchandratva indicate absence of mercury in elemental form and Varitaratva confirmed the fineness of the product. All the physico-chemical analytical parameters are on par with Pharmacopeial standards

In Rajatachandrodaya Rasa particle size is 9.59µm which is slightly larger because, it largely depends on trituration of the product. Here RC is a condensed and recrystallized product under high temperature and pressure, so segregation of particle may be responsible for increase in particle size. Also considerable point is Rajatachandrodaya Rasa. Possesses least ash and acid insoluble value indicating purity of product and its dissolution is more in acid media.

SEM analysis of RC shows surface is ultra-smooth and porous as shown by SEM. Some small particles of nanometer size can be seen on the surface. The porous structure may be by the virtue of unique pharmaceutical procedure. The particles size ranges from 120 nm to 200 nm.

EDAX refers to energy dispersion x ray spectroscopy and is employed here as an analysis tool to determine the elemental composition of RC. The EDS shows the chemical composition consisting of mercury, sulphur and small particles were seen in Nano meter size, so absorption of the drugs will be more with quicker action. SEM images captured randomly at 6 different areas were connected to the EDAX spectroscopy to generate elemental composition in both mass % and atomic %. S, Ag and Hg elements were observed in major concentration in the Rajatachandrodaya Rasa. With weight % of 15.97, 10.13 and 73.90 and atomic % of 44.22, 20.18 and 35.60 respectively.

Administration of aqueous extract of test drug in albino male rats showed increase in weight of Testes and epididymis significantly. The increase in body and organ weights was noticed and compared with Siddha Makaradhwaja Rasa. A significant anabolic effect of RC was observed as compared to the control group, which was comparable to that of Siddha Makaradhwaja Rasa.

The experiment clearly suggests enhancement of sexual activity which can be correlated to the enhancement of sexual pleasure. The aqueous extract of RC. increased the penile erection in male rats, similar to the increase in penile erection observed in Siddha Makaradhwaja Rasa indicating the involvement of nitrous oxide based intervention.

The effect of RC.treated group (2.7 mg/kg .b. wt) was much more pronounced when compared to Siddha Makaradhwaja Rasa. RC. (2.25 and 2.7/kg.b.wt.) modified both the orientation as well as sexual behaviour, conclusively suggesting a better sexual performance.

This activity was also validated by the Histopathological study on testis section. The results thus confirm that, the drug extracts can be useful in enhancement of overall sexual performance of male rats. This study also showed increased Spermatogenic activity. This may be due to the change in neurotransmitter level or their action in the cell could also change sexual behaviour. Spermatogenesis involves a complex interplay between the structural element of testis and the endocrine system. FSH (Follicle Stimulating Hormone) stimulates Spermatogenesis, also Testosterone cause direct stimulation of Spermatogenesis. Our results also show that there is increase in Spermatogenesis and increase in weight of sexual organ in comparison to control group. The improvement of sperm count suggests an improved Spermatogenic activity of the test extract. Similarly, Rajatachandrodaya Rasa increased the quality of sperm which eventually assist in better reproductive potential.

X. CONCLUSION

Rajatachandrodaya is a Sagandha, Sagni, Kupipakwa Rasayana which is obtained as Kanthastha product and prepared by adopting Bahirdhooma method using Valuka Yantra as per guidelines of Bheshaja Samhita. RC.was prepared with 1:8:16 proportions of Shodhita Rajata, Shodhita Parada and Shodhita Gandhaka by Kupipaka method in 61 hours with 19 hours of Mridwa agni, 24 hours of Madhyama agni and 18 hours of Teevra agni.

SEM reports the particles size of RC. was 120 nm to 200 nm which indicates fineness of the product.EDS reports of RC. reveals 40% particles were spherical and nano metric in size.XRD study showed – vermillion colour in cubic crystal structure.

Experimental Study shows increase in sperm count (up to 95-99 million) which shows effect of Rajatchandrodaya Rasa on spermatogenesis. It showed statistically significant ($p<0.05$) Spermatogenic activity compared to control group ($p<0.05$) & standard group ($p<0.05$).

Histopathological study also showed increase in size of seminiferous tubules. The lumen of seminiferous tubule was filled with bundles of Spermatozoa suggestive of significant Spermatogenic activity. Based on these observations it can be concluded that Rajatachandrodaya Rasa is competent with Standard drug Siddha Makardhwaja Rasa in Spermatogenic Activity.

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