

AN EXPERIMENTAL EVALUATION OF *PURNACHANDRA RASON* SPERMATOGENIC EFFECT

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ABSTRACT:

Purnachandra Rasa is an *Ayurvedic* formulation containing herbo mineral compounds. It is claimed that it possess *Vrushya* (Aphrodisiac) property on reproductive organs. Antecedently, there were no studies reported on spermatogenic effect of *Purnachandra Rasa*. In this study, *Purnachandra Rasa* was prepared accordance with description of *Bhaishajya ratnavali*. Spermatogenic activity of *Purnachandra Rasa* was evaluated experimentally on albino rats. 30 male albino rats were selected & divided in 5 groups: Normal control, Negative control (Cyclophosphamide administered), Positive control (Testosterone administered), Test group 1 (*Purnachandra Rasa* 250 mg) & Test group 2 (*Purnachandra Rasa* 500 mg). *Purnachandra Rasa* was co-administered with Cyclophosphamide in both the Test groups for 60 days. The findings were compared with Negative control & Positive control group. *Purnachandra Rasa* was produced significant increase in weight of reproductive organs as compared to Normal group, Negative group & Standard group ($P<0.001$). The sperm counts in Cauda epididymis were significantly increased ($P<0.01$) in Test Groups. Histopathological sections of Seminal vesicle & Testis also showed normal architecture, normal interstitial connective tissue, uninjured germ cells and presence of plenty of sperms in Test groups. It is concluded that administration of *Purnachandra Rasa* suggested increased Spermatogenic activity in male Albino rats.

KEYWORDS: *Purnachandra Rasa*, *Rasoushadhi* (Herbo mineral medicine) *Vrushya* (Aphrodisiac), *Rasayana* (Rejuvenative property), Spermatogenesis, Cyclophosphamide, Testosterone.

INTRODUCTION:

The use of cytotoxic agents in the treatment of several types of diseases affects the fertility and other aspects of reproductive functions. For example, Cyclophosphamide is used clinically in the treatment of cancer patients & this drug has been associated with Oligospermia, Azoospermia and Seminiferous tubular atrophy. This drug is considered strong immune depressive¹.

In *Rasashastra* science, it has been stated that mineral medicine serves as therapeutic alternatives as well as effective treatment. These minerals have to be converted in to *Bhasma* form with help of classical purification and incineration process. In *Rasashastra* sciences, many *Rasayana* - *Vajjakrana* formulations have been mentioned & have better action on reproductive organs. Out of which *Purnachandra Rasa* is considerable herbo mineral formulation mentioned in the text *Bhaishajya Ratnavali*. It is Herbo-mineral preparation which contains *Abhraka Bhasma*, *Swarnamakshika Bhasma*, *Shodhita Shilajatu*, *Loha Bhasma*, *Rasa Sindura* *Vidanga Choorna*².

It has been mentioned that *Rasoushadhi* produces their therapeutic effects immediately after administration³. Considering this fact, *Purnachandra Rasa* has been selected to evaluate the Spermatogenic effect when combined administration with Cyclophosphamide. Consequently the Experimental study is required to decide the therapeutically effective & safety in the human beings. In the literature, properties of the ingredients have been claimed that they possess *Vrushya* property. Even though *Purnachandra Rasa* was time tested, we need to give a scientific base to understand its spermatogenic activity through in vivo study. Hence the present experimental study was undertaken to evaluate the *Vrushya* activity of *Purnachandra Rasa* in the animal models using OECD guidelines.

MATERIALS & METHODS:

Test drug: *Purnachandra Rasa*.

Source of drug: the raw materials were procured from local market & authenticated as per the *Ayurvedic* criteria in *Rasashastra & Bhaishajya kalpana* department of Shri J.G.C.H.S.Ayurvedic Medical College, Ghataprabha.

Mineral ingredients were prepared according to the classical texts mentioned and collected from Teaching Pharmacy of Shri J.G.C.H.S. *Ayurvedic* Medical College, Ghataprabha.

Materials used:

Table no 1: Ingredients of *Purnachandra Rasa*

SI no	Ingredients	Quantity
1	<i>Rasa sindhur</i>	100 gm
2	<i>Abhrak bhasma</i>	100 gm
3	<i>Loha bhasma</i>	100 gm
4	<i>Shodhita Shilajatu</i>	100 gm
5	<i>Vidang churna</i> (Embilia Ribes Burm.F.)	100 gm
6	<i>Suvarna makshik bhasma</i>	100 gm

Procedure: All drugs were taken in *khalwa yantra* (mortar) & mixed uniformly with Pestle. Obtained mixture was *Purnachandra Rasa*. 2 *Gunja* (500mg) size of tablet was prepared⁴.

EXPERIMENTAL STUDY:

Conducting laboratory & location:

Experimental protocol was permitted by the institutional Ethical Committee (IAEC) under CPCSEA, approved no: Reg.No. 112 /1999/CPCSEA, dated 01-04-2016 of Pharmacology Laboratory of SET’S Pharmacy College, Dharwad, Karnataka.

The study was carried out under prevailing husbandry conditions in the animal house as per OECD Guideline of CPASEA⁵.

Experimental Animals:

30 adult albino rats of male sex, weighing 150-200grams were selected. Rats were fed with rat pellet and tap water ad libitum and they were exposed to natural day and night cycles. The standard laboratory condition was maintained with temperature of 25 ± 2 ° C, humidity 55 ± 5%.

Chemicals & Reagents: Normal saline 10%, Formalin, Bouin Holland solution, Paraffin wax, Chloroform.

Sample size: The Albino rats were divided in to 5 groups of 6 animals each.

Dose calculation for experimental animals (Standard formula):

Test drug: The Dose of *Purnachandra Rasa* was calculated by standard conversion formula

Human dose x Body surface area constant of the rats, i.e. 0.0185 x 5/kg body wt.

$$= 125 \text{ mg} \times 0.0185 \times 5 / \text{Kg body wt.}$$

$$= 11.56 \text{ mg} / \text{Kg body wt.}$$

$$= 0.0115 \text{ mg} / \text{g body wt.}$$

Cyclophosphamide: = 6.1mg/kg/day for 6 weeks.

$$= 0.0061$$

mg/g/day for 6 weeks.

The medicine was administered with 0.5 ml distilled water.

Duration: 60 days.

Route of administration: The drugs were administered in a suspension form in distilled water orally with the help of feeding needle. The testosterone is administered subcutaneously.

Water: 50 ml / day.

Food: 10gm – 15 gm / day.

Experimental design:

Table no 2: Experimental Groups

Groups n = 6	Drug administered	Groups Served as
Group 1	Distilled water	Normal control
Group 2	Cyclophosphamide (producing organ toxicity)	Negative control
Group 3	Testosterone propionate	Positive control
Group 4	<i>Purnachandra Rasa</i> 250mg with Cyclophosphamide	Test group 1 (Protective)
Group 5	<i>Purnachandra Rasa</i> 500mg with Cyclophosphamide	Test group 2 (Protective)

Statistical Analysis:

The data generated during the study have been presented as Mean ± SEM. The difference between different groups was determined by one way ANOVA followed by Dunnett multiple t test.

After 60th day, animals were sacrificed by Ether anesthesia, abdomen was

dissected. Then Testis & Epididymis was excised for semen analysis. Both organs were fixed in 10 % formalin. Sections were stained with Haematoxylin & Eosin. These were examined by compound microscope & spermatozoa were counted by Haematocytometer (Neubauer counting chamber).

RESULTS:

Table No.3: Effect of *Purnachandra Rasa* on Mortality Rate in Albino rats.

Group	Dose	Alive/Total	Percentage (%)
1	100mg/kg	6/6	100%
2	40mg/kg	6/6	100%
3	4mg/kg	6/6	100%
4	250mg/kg	6/6	100%
5	500mg/kg	6/6	100%

Table No.4: Effect of *Purnachandra Rasa* on Body Weight in albino rats.

Group	Dose	Initial Body weight(g)	Final Body weight(g)	Percentage (%)
1	100mg/kg	181.2 ± 2.9	183.8 ± 3.1	1.40↑
2	40mg/kg	185.4 ± 3.3	185.6 ± 3.1	0.10↑
3	4mg/kg	186.0 ± 2.7	187.4 ± 2.6	0.75↑
4	250mg/kg	178.6 ± 4.3	180.6 ± 4.2	1.10↑
5	500mg/kg	180.8 ± 1.6	183.2 ± 1.7	1.30↑

Table no 5: Effect of *Purnchandra Rasa* on weight of Reproductive organs

Group	Dose	Weight of Testis (mg)	Weight of Epididymis (mg)	Weight of Vas deference (mg)	Weight of Seminal vesicle (mg)	Weight of Prostate gland (mg)
1	100mg/kg	567.4±8.276	304.1±1.8	353.4±2.8	285.9±0.41	196.8±0.55
2	40mg/kg	411.2±3.544	236.6±6.1	283.3±2.4	230.1±3.10	156.0±1.07
3	4mg/kg	858.4±5.121	345.1±1.3	369.3±1.5	313.8±0.96	216.7±1.25
4	250mg/kg	917.7±1.729* **	322.1±2.5**	366.0±1.9**	304.3±1.16* **	218.4±1.61** *
5	500mg/kg	1082±4.631** *	330.7±2.2** *	393.6±1.2** *	324.9±1.10* **	274.1±1.43** *

Data: MEAN ± SEM,*P<0.05; **P<0.01; ***P<0.001

Table no 6: Effect of *Purnchandra Rasa* on Spermatogenesis

Group	Dose	Spermatogonia	Spermatocytes	Spermatids	Sperm counts in Cauda epididymis millions/mg
1	100mg/kg	79.53±0.44	126.9±0.37	207.5±1.78	79.47±2.75
2	40mg/kg	96.36±0.47	213.6±1.40	306.9±1.91	69.64±2.97
3	4 mg/kg	85.29±0.57	156.0±0.75	224.9±1.10	92.98±0.91
4	250mg/kg	83.08±0.95**	147.6±0.89***	274.6±1.90***	85.82±0.53
5	500mg/kg	87.87±0.41***	164.8±1.14***	236.0±1.66***	87.76±0.41**

Data: MEAN ± SEM, *P<0.05; **P<0.01; ***P<0.001

All animals were alive in *Purnachandra Rasa* treated group as well as other groups (Table no 3). Body weight was maintained during the administration period in Test groups (Table no 4). The weight of testis, seminal, prostate, vas deference were significantly increased (P<0.001) in Group 5 (treated group) compared to Normal control group & Negative control group (Table no 5). The weight of Testis, Seminal Vesicle, Prostate, Vas deference was significantly decreased in Group 2 when compared to normal control group (Table no 5). Group 2 shows that the administration of Cyclophosphamide caused a significant increased in Spermatogonia, Spermatocytes & Spermatids. But when observed the sperm count, it was decreased after 60th day (Table no 6). In Group 4 & 5, Co-administration

of *Purnachandra Rasa* & Cyclophosphamide causes significant increase (P<0.01) in the sperm count (Table no 6). When compared to Positive control group, there was significant increase in Testosterone treated animals (Table no 6).

HISTOPATHOLOGICAL

OBSERVATIONS:

Histopathology of Seminal vesicle and Testis was done on albino rats which were selected for evaluate the spermatogenic activity of *Purnachandra Rasa*.

Figure 1.: Normal control section of Seminal vesicle showing Normal architecture of tubules, absence of Epithelial desquamation & any congestion. Have plenty of sperms.

Figure 2: Cyclophosphamide treated Seminal vesicle section showing Disruption of tubule, Mild congestion,

presence of Epithelial desquamation.
Absence of sperms.

Figure 3: Treatment section received *Purnachandra Rasa* 250mg+ Cyclophosphamide: showing Normal architecture of tubules. Mild congestion, absence of Epithelial desquamation. Plenty of sperms.

Figure 4: Treatment section received *Purnachandra Rasa* 500mg/kg + Cyclophosphamide: showing Normal architecture of tubules. Mild congestion, absence of Epithelial desquamation, presence of many sperms.

Figure 5: Testosterone treated section: showing Normal architecture of tubules. Mild congestion, absence of Epithelial desquamation. Presence of many sperms.

Figure 6: Normal Control section of Testis: showing Normal architecture of testis, normal interstitial connective tissue, germ cells are intact, absence of Epithelial desquamation & congestion.

Figure 7: Cyclophosphamide treated Testis: showing mild congestion, Fibrosis of interstitial connective tissue, moderate detachment of germ cells, moderate Epithelial desquamation.

Figure 8: Treatment section of *Purnachandra Rasa* 250mg/kg + Cyclophosphamide: showing Normal architecture of testis, Mild congestion, normal interstitial connective tissue, germ cells are intact, absence of Epithelial desquamation.

Figure 9: Treatment section (*Purnachandra Rasa* 500mg/kg + Cyclophosphamide: showing Normal architecture of testis. normal interstitial connective tissue, germ cells are intact, Absence of congestion & Epithelial desquamation.

Figure 10: Testosterone treated section: showing Normal architecture of testis, normal interstitial connective tissue, germ cells are intact, Absence of Epithelial desquamation & congestion.

DISCUSSION:

Mortality rate reveals that there is no any effect of toxicity of *purnachandra rasa* on albino rats (Table no 2). This indicated the safer drug for administration in human being for the purpose of *Vajikarana* treatment. When *Purnachandra rasa* was co-administered with Cyclophosphamide, there were no any significant changes in body weight of albino rats (Table no 3); indicated that *Purnachandra rasa*

may help to maintain the *Meda dhatu* (Adipose tissue) by the *Lekhana* (act of scraping) and *Ruksha* (Dryness) property of *Loha bhasma*⁶. The administered Cyclophosphamide (Group 2) reduces the weight of Testis, Epididymis, Vas deference, Seminal vesicle, prostate gland, when compared to the Normal control group (Table no 5); indicated the toxicity sign & by this make the animal infertile.

Statistically significant increase ($P < 0.001$) in weight of Testis, Epididymis, Vas deference, Seminal Vesicle, Prostate in the Group 5 when compared to Negative control & Positive control Group (Table no 5). This indicated the protective effect of *Purnachandra rasa* was co-administered with Cyclophosphamide. The increase in the weights might be action of *Rasayana* property of *Loha bhasma*, *Suvarna makshika bhasma*, *Rasa sindhura* & *Vidanga churna*^{7,8,9}. Due to the presence of *Rasayana* (Rejuvenative) property, the immune system was protected against Cyclophosphamide. The Group 2 showed significant increase in spermatogonia, spermatocytes & spermatids compared to Positive

control, Test group 1 & Test group 2 (Table no 6); suggested that there was accumulation of undifferentiated, immature germ cells. Whereas the Group 2 showed significant reduction in sperm count in Cauda Epididymis compared to Positive control, Test group 1 & Test group 2 (Table no 6); suggested Cyclophosphamide induces the damage to germinative cells resulted in decrease in mature spermatozooids. Sperm counts in Cauda Epididymis were significantly increased in Group 3 when compared to Negative control, Test group 1 & Test group 2 (Table no 6). Here Group 3 showed significant increase in sperm counts (Table no 5); indicated the Testosterone, influences the Hypothalamus & Pituitary gland which are involved in spermatogenic activity in Testis¹⁰. However, Testosterone has certain Side effects & claimed that administration in longer duration causes lowering of HDL & rise in LDL levels, Hepatic carcinoma etc¹¹. In Group 4 & Group 5, the sperm counts were significantly increased ($P < 0.01$) compared to Negative control group; indicated the activity of *Vrushya* (Aphrodisiac) property of *Abhrak bhasma*¹² (Table no 6).

Histopathological study:

Histological sections of testis & Seminal vesicle from the group 4 & 5 revealed the spermatogenic effect of *Purnachandra rasa*, co administered with Cyclophosphamide. These sections showed Normal architecture of tubules. Mild congestion, No epithelial desquamation & presence of many sperms (Figure 4, 5, 9 & 10); indicated confirmation of spermatogenic activity; Here the tissues and cells were not affected by Cyclophosphamide; this might be due to the presence of *Balya* (Strengthening) property in *Shilajatu*, that which raises the functional capacity of the cells & tissues¹³. Administration of Cyclophosphamide (Group 2) could cause Disruption of tubules. Mild congestion, epithelial desquamation & absence of sperms in Seminal vesicle & Testis (Figure 2 & 7); indicated inhibition of the spermatogenesis. The normal features also observed in Testosterone treated group (Figure 5 & 10).

CONCLUSION:

In conclusion, these results & Histopathological report confirms:

- Ingredients of *Purnachandra Rasa* play a major role in the

regulation of spermatogenesis and sperm production.

- Ingredients contain *Rasayana* activity protects cells from being damaged by Cyclophosphamide.
- Combined effect can overcome the Immuno-depressive and infertility by the *Rasayana-Vajikarana* property of *Purnachandra Rasa*.
- The findings indicated there was no synergetic action observed in co-administration of *Purnachandra Rasa* & Cyclophosphamide.
- It was observed that, *Purnachandra Rasa* has similar mechanism of Testosterone. On the other hand, it is claimed that, for long term administration of Testosterone causes side effects.
- Hence, the *Purnachandra Rasa* can be taken for pre-clinical study.

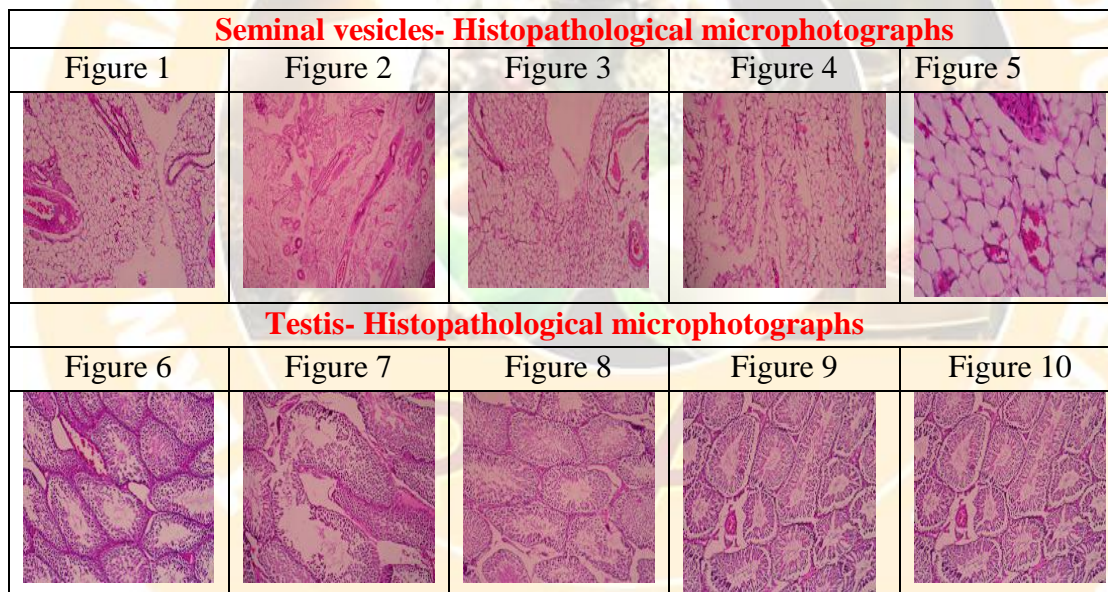
ACKNOWLEDGEMENT

Author is thankful to Dr Prasana V. Habbu. Head of Department. Dept of Pharmacognosy. SET'S College of Pharmacy. S.R.Nagar, Dharwad, Karnataka.

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Source of Support: NIL
Conflict of Interest : None declared