Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 15 [2] January 2026.47-53

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

A Comparative Study to Evaluate the Spermatogenic activity of Shalmali Gandhaka Yoga over Panchamruta Parpati - An Experimental study

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ABSTRACT

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. Shalmali Gandhaka Yoga is one among the Vajikarana Dravya, mentioned in Bhaishajya Ratnavali. Panchamruta Parpati is proved and established as spermatogenic activity. So, it is considered as standard drug. Panchamruta Parpati, Shalmali Gandhaka Yoga, Carboxy Methyl Cellulose and 18 Wister strain male Albino rats form the materials for the study. Three groups, in Standard group- Panchamruta Parpati (18mg/ 200gm body wt + 1ml CMC), in Test group- Shalmali Gandhaka Yoga (9mg/ 200gm body wt + 1ml CMC) and in Control group- Carboxy Methyl Cellulose (1ml/ 200gm body wt) was administered for 30 days period. Analysis of Body weight, Gravimetric and Micrometric, Biochemical estimation, Spermatogenic elements, Effect of drugs on accessory organs were followed. The test drug Shalmali Gandhaka Yoga has shown highly significant result compared to that of Standard drug Panchamruta Parpati in Spermatogenic activity. Shalmali Gandhaka Yoga has shown significant result than Panchamruta Parpati by promoting cell division, energy for motility, production of spermatogonia, maturation of spermatid to spermatozoa and significant in spermatocyte to spermatid. Shalmali Gandhaka Yoga nourishes Kapha Dosha which act as Brimhana, Tarpana, Balya, Shukrala, and Vrushya property. The Vrushya effect of Panchamruta Parpati due to Balya, Brumhana and Rasayana property.

Keywords: *Infertility, Spermatogenic activity, Panchamruta Parpati, Shalmali Gandhaka Yoga, Carboxy Methyl Cellulose.*

Received 22.11.2025 Revised 19.12.2025 Accepted 20.01.2026

INTRODUCTION

Ayurveda is one of the oldest medical sciences in the history of human beings. Rasashastra is one of the branches of ayurveda that deals with the Ayurvedic pharmaceutics, manufacturing and dispensing of the Rasaushadhis. These formulations are well known for treating the various acute as well as chronic diseases. Rasa dravyas are well known for its quick action and they are very effective in smaller doses [1]. Also, these preparations are palatable and have longer shelf life compared to the herbal formulations. These rasa dravyas exhibits various actions on the body and cures many diseases. Vrushya is the property or action (Karma) which enhances the production of healthy sperms, which leads to a healthy progeny.

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility affects millions of peoples and has an impact on their families and community. Estimates suggests that approximately one in every six couples of reproductive age worldwide experience infertility in their lifetime [2].

Globally, infertility affects approximately 13% to 15% of all couples, while 1 in 5 couples is unable to achieve pregnancy in the first year of trying [3] of all infertility cases, approximately 40-50% is due to "male factor" infertility and as many as 2% of all men will exhibit suboptimal parameters. There are numerous factors such as anatomical, physiological, environmental, lifestyle and genetic factors that cause male infertility. Male infertility can be diagnosed by different tools like physical examination, semen analysis, testicular biopsy, hormonal tests, immunobead test, sperm functional tests, sperm chromatin, abnormal deoxyribonucleic acid (DNA) assays, chromosome and genetic studies [4].

The healthy life of an individual is depending on Ahara (Diet), Nidra (Sleep), Bhramacharya (Celibacy) which are the three main pillars of humans Lifestyle [5]. The importance of the third pillar i.e.

Bhramhacharya lies in the fact that an entire discipline has been mentioned among the eight specialized branches of Ayurveda known as Vajikarana Tantra. It has been described as a branch of Ashtanga Ayurveda which deals with diagnosis and the management of defective semen and spermatogenesis along with sexual vigor [6].

Acharya Charaka in the context of Vajikarana mentioned that the person who doesn't have progeny will never get respect in community and he is treated like "Chitradeepa" [7]. Acharya Sushruta explained about the Shuddha Shukra Lakshana are Spatikabh, Drava, Snigdha, Madhura, Madhugandhi, Taila, Kshoudra, and Nibha [8]. Vajikarana is the Shrestha Chikitsa as it is included in Astanga Ayurveda. In Shukra kshaya, Vajikarana treatment will provide recipient with the virality of horse. Treatment tends to increase the aphrodisiac activity which increase the quality and quantity of sperm in semen.

Shalmali Gandhaka Yoga is a one among the Vajikarana Dravya as mentioned in Bhaishajya Ratnavali Vajikarana Rogadhikara [9]. Shalmali Gandhaka Yoga contains Shalmali (*Bombax ceiba* Linn.) and Shuddha Gandhaka (Sulphur) as main ingredients. Shalmali is an herbal drug widely available throughout India and have the properties like Madhura and Kashaya Rasa, Madhura Vipaka, Sheeta Virya, Laghu, Snigdha and Picchila Guna and does the Karmas like Purishavirajaniya, Vrushya, Balya, Shukravardhaka, Kapha Vardhaka etc [10]. Gandhaka is one among the Uparasa dravya. As Shuddha Gandhaka is mainly indicated in Kushta rogas, but it is also mentioned that it also has Vajikara therapeutic activity [11]. Hence, Shalmali Gandhaka Yoga is taken as test drug to be analyzed.

Panchamruta Parpati which has the ingredients like Shuddha Gandhaka, Shuddha Parada, Loha Bhasma, Abhraka Bhasma and Tamra Bhasma. It is indicated in Grahani roga but recent works proved that it possesses spermatogenesis activity [12]. Hence, it is taken as standard drug.

MATERIAL AND METHODS

Materials for Experimental Study:

1) **Drugs for Experimental Study:** Panchamruta Parpati, Shalmali Gandhaka Yoga, Carboxy methyl cellulose.
2) **Animals for Experimental Study:** 18 Wister strain Male Albino rats were taken for the study, 6 in each group and maintained under standard laboratory protocol.

Method:

Method of preparation of Shalmali Gandhaka Yoga:

Shalmali Gandhaka Yoga have Shalmali mula twak churna and Shuddha Gandhaka. Shalmali Gandhaka Yoga is prepared as per the reference Bhaishajya Ratnavali, Vajikaranadhikara Adhyaya, 21st Yoga. Shalmali Gandhaka Yoga was prepared in the Pharmacy, Department of PG and Ph.D. studies in Rasashastra & Bhaishajya Kalpana, BLDEA's AVS Ayurveda Mahavidyalaya, Hospital & Research Centre, Vijayapur.

Panchamruta Parpati:

Panchamruta Parpati were purchased from GMP certified Pharmacy "Shree Dhootapapeshwar". Batch No: P240300114.

Method of Experimental study:

IAEC approval was taken to conduct study on animals before to start the study with letter reference no. 616/A/2023-24 Dated 21/10/2023 from BLDEA's AVS Ayurveda Mahavidyalaya, Hospital and Research Centre, Vijayapura. CCSEA approved with registration number 533/CCSEA.

Selection of animals:

Inclusive Criteria: Healthy male albino rats, Age 90 to 120 days old, Weight ranges in between 150 to 200gms.

Exclusive Criteria: Female albino rats.

Experimental study protocol [13]:

Three groups were made for the experimental study. 6 Wister strain male albino rats in each group. For Group I rats 18 mg of Panchamruta Parpati in 1ml of Carboxy Methyl Cellulose suspension was prepared and fed to albino rats daily for 30 days. For Group II rats 9 mg of Shalmali Gandhaka Yoga in 1ml of Carboxy Methyl Cellulose suspension was prepared and fed to albino rats for 30 days daily. For Group III rats 1ml of Carboxy Methyl Cellulose suspension was prepared and fed to albino rats for 30 days daily.

On 30th day last dose of Panchamruta Parpati, Shalmali Gandhaka Yoga and Carboxy Methyl Cellulose was administered and later observation was done for 24 hours of fasting period. Body weight of each Wister strain male albino rats was measured and all animals from each group were sacrificed for analysis of the reproductive organs. The testis, epididymis, Vas deferens, seminal vesicles, prostate gland has been isolated and drained of surrounding fat and connective tissue, later weighed on electronic weighing machine. Blood sample was collected and sent for the hormonal assay.

Organs from one side of each animal were frozen in Formaldehyde for investigations on micrometrics, spermatogenic elements and drug effects on accessory organs. While the organs from other side of each

animal was fixed in Formaldehyde for biochemical assessment of the things like cholesterol, glycogen and protein.

BLOOD SAMPLING:

- Blood was collected after sacrificing the animals in a different Eppendorf tube.
- Plasma testosterone, follicle stimulating hormone and luteinizing hormone were assayed by the EIA kit.

Study Groups:

Table No.1 Showing Drug Schedule and Study groups.

Study design	Group I	Group II	Group III
Sample size	6 rats	6 rats	6 rats
Drug	Panchamruta Parpati	Shalmali Gandhaka Yoga	Carboxy methyl cellulose
Dose	18mg/200gm bd wt	9mg/200gm bd wt	1ml/ 200gm bd wt
Dosage Form	Suspension in 1ml of CMC	Suspension in 1ml of CMC	Suspension in 1ml of CMC
Route	Oral	Oral	Oral
Duration	30 days	30 days	30 days
Autopsy	31st day	31st day	31st day

Investigations:

- **Effect of drug on Accessory organs:** includes weight of Testis, weight of Epididymis, weight of Seminal vesicle, weight of Prostate and weight of Vas deferens.
- **Micrometric changes of testis:** includes Diameter of testis and Diameter of tubules.
- **Effect of drugs on spermatogenic elements:** includes Spermatogonia, Spermatocytes, Spermatids and Sperm count.
- **Biochemical Estimation:** includes Tissue Cholesterol, Tissue Glycogen and Tissue Protein.
- These investigations are evaluated by using Assay Kits at Dr. Karigoudar Diagnostic Laboratory, Vijayapur.

RESULTS

Table No.2: Showing the effect of yoga on body weight before and after yoga

Groups	Before Administration (gm) (Mean ±SD)	After Administration (gm) (Mean ±SD)	Before v/s After
Panchamruta Parpati (G1)	165 ± 4.47	195 ± 5.48	t= -10.37 & p < 0.0001 HS
Shalmali Gandhaka Yoga (G2)	164.17 ± 5.85	229.17 ± 18.82	t= -9.51 & p = 0.0001 HS
Carboxy Methyl Cellulose (G3)	163.33 ± 9.83	185.83 ± 22.23	t= -3.82 & p <0.01 HS

Table No.3: Showing the effect of yoga on body weight after treatment

	After treatment			G1 V/S G2	G1 V/S G3	G2 V/S G3
	G1 (Mean ±SD)	G2 (Mean ±SD)	G3 (Mean ±SD)			
Body weight	195 ± 5.48	229.17 ± 18.82	185.83 ± 22.23	t= -4.57 p=0.002 S	t=1.01 p=0.17 NS	t=2.99 p=0.015 S

Table No.4: Showing the effect of yoga on biochemical parameters. n=6

Biochemical Analysis	After the administration of Yoga			G1 V/S G2	G1 V/S G3	G2 V/S G3
	G1 (Mean ±SD)	G2 (Mean ±SD)	G3 (Mean ±SD)			
Cholesterol (mg /gm)	0.06 ± 0.00	0.060 ±0.000	0.04 ±0.000	t=-0.55 p=0.30 (NS)	t=8.78 p < 0.0001 (HS)	t=12.41 p=0.000 (HS)
Glycogen (mg /gm)	1.02 ±0.05	1.08 ±0.01	0.87 ±0.07	t=-2.46 p<0.02 (S)	t=6.02 p < 0.0000 (HS)	t=6.74 p=0.000 (HS)
Protein (mg /gm)	0.21 ±0.03	0.27 ±0.01	0.15 ±0.03	t=-5.41 p=0.001 (HS)	t=4.34 p<0.003 (HS)	t=6.89 p=0.000 (HS)

Table No.5: Showing the effect of the yoga on spermatogenetic elements

Spermatogenetic elements	After the administration of Yoga			G1 V/S G2	G1 V/S G3	G2 V/S G3
	G1 (Mean ±SD)	G2 (Mean ±SD)	G3 (Mean ±SD)			
Spermatogonia	117.83 ±8.68	119.17 ±6.01	99.17 ±6.55	t=-0.31 p=0.38 (NS)	t=3.73 p < 0.006 (HS)	t=4.17 p<0.004 (HS)
Spermatocytes	204.50 ±45.36	192.83 ±30.39	122.50 ±18.64	t=0.518 p=0.31 (NS)	t=3.71 p < 0.006 (HS)	t=3.67 p <0.007 (HS)
Spermatids	190.83 ±23.33	226.33 ±11.60	150.67 ±50.15	t=-3.37 p=0.009 (HS)	t=1.50 p=0.09 (NS)	t=3.14 p<0.02 (S)
Sperm count (millions/ cauda)	47.65 ±2.67	52.92 ±2.69	40.47 ±3.03	t=-3.30 p=0.01 (S)	t=4.68 p < 0.002 (HS)	t=5.83 p < 0.001 (HS)

Table No.6: Showing the effect of the Yoga on accessory organs

Accessory organs	After the administration of Yoga			G1 V/S G2	G1 V/S G3	G2 V/S G3
	G1 (Mean ±SD)	G2 (Mean ±SD)	G3 (Mean ±SD)			
Weight of testis (mg)	2.91 ±0.13	3.15 ±0.06	2.46 ±0.03	t=-5.93 p=0.0009 (HS)	t=7.40 p < 0.0003 (HS)	t=20.11 p=0.0000 (HS)
Epididymis (mg)	0.45 ±0.01	0.50 ±0.02	0.33 ±0.02	t=-5.59 p=0.001 (HS)	t=10.75 p < 0.0001 (HS)	t=10.51 p <0.0001 (HS)
Seminal vesicle (mg)	0.32 ±0.01	0.37 ±0.01	0.26 ±0.03	t=-9.77 p=0.000 (HS)	t=4.15 p=0.004 (HS)	t=7.73 p = 0.0002 (HS)
Prostate (mg)	0.06 ±0.01	0.08 ±0.01	0.04 ±0.010	t=-7.92 p=0.0002 (HS)	t=5.53 p < 0.001 (HS)	t=10.95 p < 0.0001 (HS)
Vas deferens (mg)	0.06 ±0.01	0.07 ±0.01	0.041 ±0.01	t= -5.96 p=0.0009 (HS)	t=2.15 p =0.041 (S)	t=4.74 p =0.002 (HS)

Table No.7: Showing the effect of the yoga on micrometric changes of Diameter testis & tubules

Micrometric changes of testis	After the administration of yoga			G1 V/S G2	G1 V/S G3	G2 V/S G3
	G1 (Mean ±SD)	G2 (Mean ±SD)	G3 (Mean ±SD)			
Diameter of testis (µm)	8933.33 ±186.19	9400.00 ±141.42	8133.33 ±175.12	t=-4.18 p =0.004 (HS)	t=6.76 p< 0.0005 (HS)	t=13.27 p< 0.0001 (HS)
Diameter of tubules (µm)	189.17 ±6.85	198.83 ±3.71	181.00 ±12.95	t=-2.45 p =0.023 (S)	t=1.27 p= 0.129 (NS)	t=3.14 p< 0.012 (S)

Table no.8: Showing the effect of yoga on hormonal parameter

Hormonal Test	After the administration of yoga			G1 V/S G2	G1 V/S G3	G2 V/S G3
	G1 (Mean ±SD)	G2 (Mean ±SD)	G3 (Mean ±SD)			
FSH (mIU/mL)	0.07 ±0.06	0.06 ±0.05	0.03 ±0.04	t=0.12 p=0.54 (NS)	t=1.73 p =0.07 (NS)	t=1.54 p=0.09 (NS)
LH (mIU/mL)	0.12 ±0.07	0.13 ±0.02	0.05 ±0.007	t=-0.25 p=0.406 (NS)	t=2.53 p =0.03 (S)	t=2.62 p =0.02 (S)
Testosterone (ng/mL)	1.70 ±2.43	2.01 ±2.53	0.38 ±0.34	t=-1.49 p=0.09 (NS)	t=1.25 p=0.13 (NS)	t=1.47 p = 0.09 (NS)

Fig No.1 Group-I (Panchamruta Parpati) Rats showing the Testis, Prostate, Epididymis, Seminal vesicle

Testis **Prostate** **Epididymis** **Seminal Vesicle**

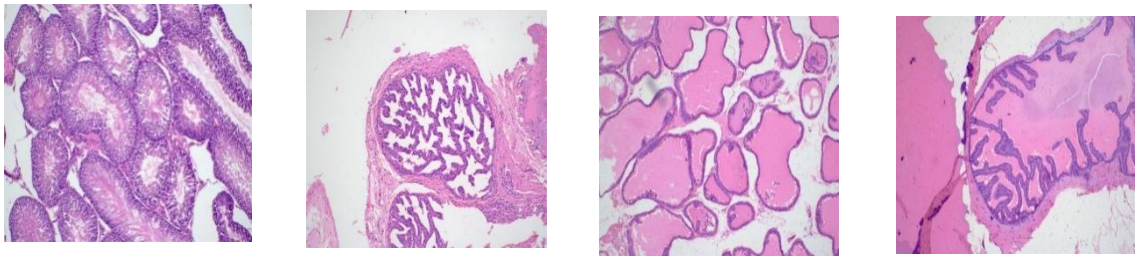
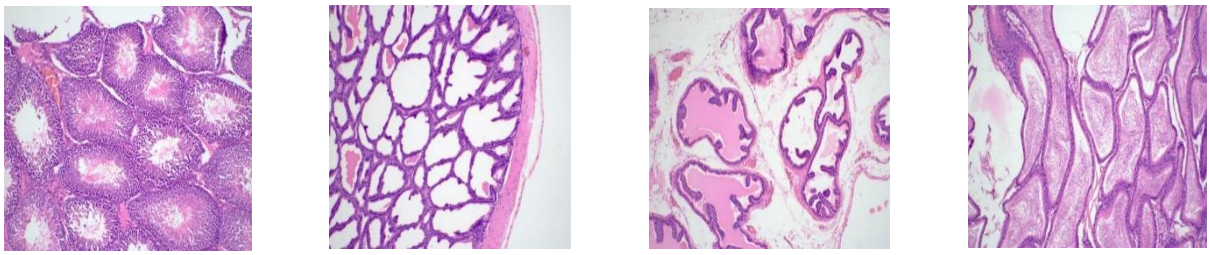


Fig No.2 Group-II (Shalmali Gandhaka Yoga) Rats showing the Testis, Prostate, Epididymis, Seminal vesicle, Vas deferens.

Testis **Prostate** **Epididymis** **Seminal Vesicle**

Fig No.3 Group-III (Carboxy Methyl Cellulose) Rats showing the Testis, Prostate, Epididymis, Seminal vesicle.



Testis **Prostate** **Seminal Vesicle** **Epididymis**

DISCUSSION

Shalmali Gandhaka Yoga is a Herbo mineral formulation mentioned in Bhaishajya Ratnavali Vajikarana Rogadhikara 74/ 49-51. Shalmali Gandhaka Yoga is indicated in conditions of Shukra kshaya, Vandya (Male infertility) as it possesses Vajikaraka and Vrushya property. Panchamruta Parpati is a mineral formulation mentioned in Bhaishajya Ratnavali Grahanirogadhikara 8/458-460. Panchamruta Parpati is proved and established as spermatogenic activity.

In this spermatogenic activity the following parameters like Body weight, Biochemical parameters like tissue Cholesterol, tissue Glycogen, tissue Protein, Spermatogenic elements like number of spermatogonia, Spermatocytes, Spermatids, spermatozoa, Micrometric study like diameter of Testis, diameter of Tubules & Hormonal assay like FSH, LH, Testosterone, along with Reproductive accessory organ weights like weight of testis, Epididymis, Seminal vesicle, Prostate and Vas deferens, were assessed for the spermatogenic activity study & the results compared with standard drug Panchamruta parpati and control drug CMC.

GI and GIII

GI (Panchamruta Parpati) has shown highly significant ($P<0.0001$) in tissue cholesterol and tissue glycogen, weight of Epididymis, ($P<0.0003$) in weight of Testis, ($P< 0.0005$) in diameter of testis, ($P<0.006$) in spermatogonia and spermatocytes, ($P<0.003$) in tissue protein, ($P<0.002$) in sperm count, ($P<0.001$) in weight of prostate, ($p=0.004$) in weight of seminal vesicles than GIII (Carboxy Methyl Cellulose).

GI (Panchamruta Parpati) has shown significant ($p=0.041$) in weight of vas deferens, ($p=0.03$) in LH than GIII (Carboxy Methyl Cellulose).

GI (Panchamruta Parpati) has shown not significant (p=0.17) in effect of yoga on body weight, (p=0.09) in spermatids, (p= 0.129) in diameter of tubules, (p=0.07) in FSH and (p=0.13) in Testosterone than GIII (Carboxy Methyl Cellulose).

GII and GIII

GII (Shalmali Gandhaka Yoga) has shown highly significant (p=0.000) in tissue cholesterol, tissue glycogen and tissue protein, weight of testis, (p<0.0001) in weight of epididymis and weight of prostate, diameter of testis, (p=0.0002) in weight of seminal vesicles, diameter of testis, (p<0.001) in sperm count, (p=0.002) in weight of vas deferens, (p<0.004) in spermatogonia, (p<0.007) in spermatocytes than GIII (Carboxy Methyl Cellulose).

GII (Shalmali Gandhaka Yoga) has shown significant (p<0.02) in spermatids, (p=0.015) in effect of yoga on body weight after treatment, (p<0.012) in diameter of tubules, (p=0.02) in LH than GIII (Carboxy Methyl Cellulose).

GII (Shalmali Gandhaka Yoga) has shown not significant (p=0.09) in FSH and testosterone than GIII (Carboxy Methyl Cellulose).

GII and GI

GII (Shalmali Gandhaka Yoga) has shown highly significant (p=0.000) in weight of seminal vesicles, (p=0.0009) in weight of testis and weight of vas deferens, (p=0.0002) in weight of prostate, (p=0.001) in weight of epididymis, (p< 0.006) in spermatogonia and spermatocytes, (p< 0.002) in sperm count, (p=0.004) in diameter of testis than GI (Panchamruta Parpati).

GII (Shalmali Gandhaka Yoga) has shown significant (p=0.002) in effect of yoga on body weight, (p<0.02) in tissue glycogen, (p=0.01) in sperm count, (p=0.023) in diameter of tubules than GI (Panchamruta Parpati).

GII (Shalmali Gandhaka Yoga) has shown not significant (p=0.30) in cholesterol, (p=0.38) in spermatogonia, (p=0.31) spermatocytes, (p=0.54) in FSH, (p=0.406) in LH, (p=0.09) in testosterone than GI (Panchamruta Parpati).

The result suggests that Shalmali Gandhaka Yoga and Panchamruta Parpati have highly significant effect in the increment of Body weight because of Brimhana, Balya, Tarpana properties. Shalmali Gandhaka Yoga has shown significant effect in testicular weight than Panchamruta Parpati. Shalmali Gandhaka Yoga has shown highly significant effect in diameter of tubule than significant increment in Panchamruta Parpati due to the thickening of proliferative layer of tubule, which suggest that Shalmali Gandhaka Yoga is highly proliferative in nature compared to Panchamruta Parpati.

Result convey that Shalmali Gandhaka Yoga has shown highly significant effect in the production of sex hormones, cell division, maturation of spermatogonia to spermatozoa, providing energy for the motility. It has significant effect in accessory sex gland secretions, increasing the life span of spermatozoa, morphological maturation, viability, storage, volume of seminal fluid.

CONCLUSION

Shalmali Gandhaka Yoga has shown highly significant effect in cell division, energy for motility, production of spermatogonia, maturation of spermatid to spermatozoa and significant in spermatocyte to spermatid than Panchamruta Parpati.

The test drug Shalmali Gandhaka Yoga has shown a significant result compared to that of Standard drug Panchamruta Parpati and Control group Carboxy Methyl Cellulose in Spermatogenic activity.

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CITATION OF THIS ARTICLE

Rahul K P, K A Patil, Kasinath H. A Comparative Study to Evaluate the Spermatogenic activity of Shalmali Gandhaka Yoga over Panchamruta Parpati - An Experimental study. Bull. Env. Pharmacol. Life Sci., Vol 15 [2] January 2026. 47-53