Research Article

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VAJEEKARANA EFFECT OF VIDARI CHURNA WITH AND WITHOUT BHAVANA WITH REFERENCE TO SPERMATOGENIC ACTIVITY: AN EXPERIMENTAL EVALUATION

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ABSTRACT

Vajeekarana or Vrushya Chikitsa (aphrodisiac therapy) is a specialized branch of Ayurveda aimed at enhancing reproductive capacity, sexual vitality and improving the qualitative and quantitative status of Shukra dhatu (reproductive tissue). Classical texts emphasize that therapeutic efficacy of a drug is optimized only after appropriate Samskara (pharmaceutical processing). Vidarikanda (*Pueraria tuberosa*) is a classical well-documented Vrushya (spermatogenic), Balya (strength-promoting), and Brimhana (nourishing). In the present study, Vidarikanda Churna was prepared with and without Bhavana Samskara using Vidari Kwatha and evaluated for its Vajeekarana efficacy through pharmaceutical, analytical and experimental parameters. Cyclophosphamide-induced spermatogenic suppression in Wistar albino rats was employed as the experimental model. Analytical findings demonstrated enhanced extractive values, ash content, and alkalinity in the Bhavita sample, suggesting improved bioavailability. Experimental evaluation showed that Bhavita Vidari Churna produced greater improvement in sperm count, motility, morphology, and histopathological restoration of testicular architecture compared to the non-Bhavita sample. The study substantiates the classical concept that Bhavana Samskara significantly potentiates the spermatogenic activity of Vidarikanda Churna validating classical ayurveda principles.

Keywords: Vidarikanda churna, *Pueraria tuberosa,* Bhavana, Vajeekarana, Spermatogenic activity, Pharmaceutico- analytical study.

INTRODUCTION

Ayurveda recognizes Aushadha (medicine) as one of the four fundamental pillars of treatment[1]. Classical texts emphasize that raw drugs attain therapeutic suitability only after undergoing appropriate Samskara, which enhances efficacy, safety, Sukshmata (subtlety) and bioavailability. Vajeekarana therapy aims to promote sexual vigor, fertility and optimal functioning of Shukra Dhatu. (reproductive tissue), particularly in conditions associated with Shukra kshaya. According to Acharya Charakapani[2], Vajeekarana improves libido, facilitates timely ejaculation, and supports reproductive competence.

Vidarikanda (*Pueraria tuberosa*) is described in Brihattrayi as Brimhana (nourishing) balya (strength-promoting), Shukravardhaka (spermatogenic) and Vatapittahara. Its Madhura rasa, guru guna, Sheeta virya and Madhura vipaka makes it especially suitable for nourishing Shukra dhatu. Acharya Dalhana differentiates Vrushya (sukra-janana) and Vajeekarana (Sukra-pravartana), while acknowledging that Vrushya drugs generally contribute to both actions[3]. From a disease perspective, Shukra Kshaya and related spermatogenic dysfunctions are described as conditions characterized by reduced semen parameters and impaired reproductive potential. These conditions may manifest due to Dhatukshaya, Agni Vaishamya (metabolic imbalance) and lifestyle-related factors. Experimental models employing cytotoxic agents such as cyclophosphamide closely resemble the pathological features of Shukra Kshaya by inducing testicular damage, impaired spermatogenesis, and reduced sperm quality, thereby serving as suitable platforms for evaluating Vajeekarana drugs.

Phytochemical studies of *Pueraria tuberosa* reveal the presence of bioactive isoflavonoids such as puerarin, daidzein, genistein, quercetin, irisolidone, biochanin-A, biochanin-B, isoorientin, mangiferin[4], which exhibit antioxidant, androgen-mimetic and testis- protective effects. These constituents are reported to improve spermatogenic parameters and protect against chemically induced testicular toxicity.

Bhavana samskara, a key pharmaceutical process in Rasashastra, involves repeated levigation of powdered drugs with a liquid medium[5] to enhance absorption, tissue penetration, improve stability, shelf-life and targeted action particularly towards Sukhravaha srotas. It enhances Sukshmata and imparts Vyavayi and Yogavahi properties by reducing particle size and improving bioavailability. The tuber of this plant is sweet and is widely used in the treatment of fever, menorrhagia, skin diseases, wounds, infertility, bronchial asthma, and jaundice, etc. In Raj Nigantu – it is explained under Mulakadi varga[6] and in Dhanvantri nigantu and Bhava Prakasha – explained under Guduchyadi varga[7]. Although Vidarikanda is well studied for its Vajeekarana activity, comparative experimental evaluation of Vidarikanda Churna with and without Bhavana Samskara is scarcely documented. This

lacuna necessitated the present study to scientifically evaluate the role of Bhavana in augmenting spermatogenic activity.

Aims and Objectives

To prepare Vidarikanda Churna as per Ashtanga Hridaya and Sharangadhara Samhita

To prepare Vidarikanda Churna pharmaceutically without and with bhavana using Vidari kwatha.

To evaluate analytical parameters of both preparations.

To assess spermatogenic activity using a cyclophosphamide-induced rat model.

MATERIALS AND METHODS

Drug Authentication and Preparation

Raw vidarikanda tubers were procured from a local vendor and authenticated, Churna was prepared in the Rasashastra and Bhaishajya Kalpana laboratory following standard procedures. The prepared churna was divided into two samples: T1 (without Bhavana) and T2 (with Bhavana). Seven Bhavana cycles were administered to T2 using Vidari kwatha.

Dose Fixation: The classical human dose of Vidarikanda Churna (1 karsha ≈ 12g) was converted to the rat dose using body surface area conversion, yielding 1.08g/kg body weight. The test drugs were suspended/ dissolved in a vehicle comprising ghee (15ml) and honey (5ml) as Anupana (Adjuvant)[8].

Experimental animals: The study was carried out after receiving approval from the Institutional Animal Ethical Committee (SDMCRA/ IAEC/ KA-D-10). 24 Male Wistar Albino rats (6 in each group) weighing between 140-250 grams were used in the study, The animals were obtained from the animal house attached to the Pharmacology Laboratory of SDM Centre for Research in Ayurveda and Allied sciences. Animals were maintained under standard laboratory conditions in a room with a temperature of 22 ± 30°C, relative humidity of 50–60%, and an illumination cycle of 12 hours dark and 12 hours light. And was provided with pellet diet and purified water ad libitum.

Experimental design: The animals were randomly allocated into four experimental groups and treated as shown in Table 1.

Table 1

Group	Name	Treatment	Number of rats
I	Normal Control	Normal diet and water	06
II	Cyclophosphamide control	Cyclophosphamide treatment	06
III	Trial drug 1 (T1)	Vidari Churna without bhavana	06
III	Trial drug 2 (T2)	Vidari Churna with bhavana	06

The normal control drug received distilled water and normal diet for 65 days. T1 and T2 groups were administered with their respective test drugs orally. To model spermatogenic impairment, Cyclophosphamide (25mg/kg) was administered every 7th day to induce spermatogenic suppression.

Experimental procedure: After 65days of drug administration, animals of all groups were weighed and given anesthesia. All the animals for the study were acclimatized under normal conditions and Spermatogenic assessment for - Epididymal sperm concentration, motility, morphology and histopathological evaluation of testis, prostate and seminal vesicles were assessed following standard methods.

Epididymal sperm concentration: As described by Saalu *et al*. (2011), spermatozoa in the right epididymis were counted by a modified method of Yokoi and Mayi (2004). The right cauda epididymis was minced in 5 mL of physiological saline and placed on a rocker for 10 minutes, followed by 2 minutes of incubation at room temperature. The supernatant was then diluted 1:100 using a diluent containing 5 g sodium bicarbonate and 1 mL of 35% formalin. Ten microliters of the diluted suspension were loaded into a Neubauer improved hemocytometer and allowed to settle for 5 minutes. Spermatozoa were counted in five 16-squared grids under a binocular microscope. Sperm concentration was calculated as: sperm count = X x 10^6 mL^{-1} where X represents the average number of spermatozoa counted per 16-celled square.

Sperm count: The count was done with a haemocytometer (Neubauer- improved counting chamber). After proper cleaning of the chamber, the cover slip was placed in position and the thoroughly mixed semen solution was charged in between, smaller squares (400 smallest) were used for counting i.e. RBC chamber. Calculations were made to make the count in million/caudal epididymal tissue suspension. Live sperms appeared bright and colorless, whereas dead sperms showed blue heads.

Sperm motility: Before doing the counting, the sperm motility was observed by viewing the sperm under microscope and counting the number of live cells in percentage under high power microscope. They were categorized into sperms showing rapid linear progressive, slow linear progressive and immotile. This was evaluated by earlier method Sonmez *et al.* (2005). The fluid obtained from the left cauda epididymis with a pipette was diluted to 0.5 mL with tris buffer solution. A slide was placed on light microscope with heater table, an aliquot of this solution was kept on the slide, and percentage motility was evaluated visually at a magnification of x400. Motility estimates were performed from three different fields in each sample. The mean of the three estimations was used as a final motility score. Samples for motility evaluation were stored at 35^{o} C.

Sperm morphology: The sperm morphology was evaluated with the aid of light microscope at x400 magnification. Caudal sperm was taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin. Five hundred sperms from the sample were scored for morphological abnormalities. Briefly, in wet preparations using phase contrast optics, spermatozoa were categorized.

Histopathological Assessment: Prostate gland, testis and seminal vesicle were carefully excised at the end of the study cleaned and transferred to 10% formalin for pre-fixation before being processed for histopathological examination by taking their section employing standard laboratory procedures. The organs were exposed under a light microscope after being cut into thin slices using a microtome and stained with haematoxylin–eosin and seen under microscopy (200X) for abnormalities, after which photo-micrographs were taken.

Calculation and Statistical Analysis: Automated method: the results in the IFU was calculated automatically using a 4PL curve fit. 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 and values obtained.

OBSERVATION AND RESULTS

Table 2: Analytical Results

Parameter	Results n = 3 %w/w (Avg ± SD)	
	Vidari churna with bhavana	Vidari churna without bhavana
Loss on drying	2.32±0.03	2.02±0.03
Total Ash	3.93±1.36	2.85±1.45
Acid Insoluble Ash	1.94±0.02	0.00±0.00
Water soluble Ash	0.97±0.03	2.90±0.00
Alcohol soluble extractive value	6.77±0.02	2.14±0.02
Water soluble extractive value	22.01±0.02	4.99±0.01
pH	8.02	5.77

Table 3: Effect of drugs T1 and T2 on Sperm Count, Sperm Morphology, And Ponderal Changes

Parameters (Million/ml) MEAN ± SEM	Normal Control	Cyclophosphamide Control	T1	T2
Sperm Count	4.06 ± 0.54	4.53 ± 0.37	3.85 ± 0.96	6.08 ± 0.88
Sluggish Motile	20.16 ± 4.54	26.16 ± 2.72	30 ± 7.05	37.33 ± 2.56
Non – Motile	79.83 ± 4.54	75.5 ± 1.52	70 ± 7.05	62.66 ± 2.56
Sperm Morphology				
Normal	86.16 ± 0.83	82.16 ± 0.60 *	82 ± 0.93	82.6 ± 1.25
Amorphous head	0	0.16 ± 0.16	0.33 ± 0.21	0.83 ± 0.30
Hook less	13.33 ± 0.66	17.16 ± 0.79*	16.5 ± 0.80	16 ± 1.31
Curled tail	0.33 ± 0.21	0.5 ± 0.22	1.16 ± 0.16	0.16 ± 0.16
Ponderal Changes				
Weight of Testis	2.69 ± 0.073	2.76 ± 0.099	2.47 ± 0.066	2.59 ± 0.085
Weight of Prostate	0.66 ± 0.024	0.60 ± 0.061	0.58 ± 0.086	0.72 ± 0.010
Weight of Seminal vesicle	0.55 ± 0.034	0.53 ± 0.030	0.33 ± 0.035**	0.54 ± 0.024

Data: MEAN ± SEM -- *P < 0.05; **P < 0.01

Table 4: % change results of the parameters assessed

Parameters (Million/ml) MEAN ± SEM	Cyclophosphamide Control	T1	T2
Sperm Count	11↑	15↓	34↑
Sluggish Motile	29↑	14↑	42↑
Non – Motile	5.4↓	7.2↓	17↓
SPERM MORPHOLOGY			
Normal	4.64↓	0.19↑	0.53↑
Amorphous head	0	106↑	418↑
Hook less	28↑	3.8↓	6.7↓
Curled tail	51↑	132↑	68↓
PONDERAL CHANGES			
Weight of Testis	2.6↑	10.5↓	6.15↓
Weight of Prostate	9.0↓	3.33↓	20↑
Weight of Seminal vesicle	11.7 ↓	16.6↑	20↓

↑- Increase ↓- Decrease

Table 5: Histopathological Report of testis

Groups	Degenerated spermatids and spermatocytes	Depletion and degeneration of elongated spermatids	Tubular degeneration/ atrophy of tubules	Spermatid retention
NC 1	-	-	-	-
NC 2	-	-	-	-
NC 3	-	-	-	-
NC 4	-	-	-	-
PC1	+	+	-	-
PC2	+	++	-	-
PC3	+	+	-	-
PC4	-	+	-	-
GA-1	-	-	-	-
GA -2	-	-	-	-
GA -3	+	+	-	-
GA -4	-	-	-	-
GB-1	-	+	-	-
GB-2	-	-	-	-
GB-3	-	-	-	-
GB-4	-	-	+	-

Table 6: Histopathological Report of Prostate & Seminal Vesicle

Groups	Decrease of Secretion compared with NC group -- Prostate	Decrease of Secretion compared with NC group -- Seminal vesicle
NC 1	-	-
NC 2	-	-
NC 3	-	-
NC 4	-	-
PC1	-	-
PC2	-	-
PC3	-	-
PC4	-	-
GA-1	-	-
GA -2	-	-
GA -3	-	-
GA -4	-	-
GB-1	-	-
GB-2	-	-
GB-3	-	-
GB-4	-	-

NC – Normal control; PC- Positive control; GA – T1 without bhavana; GB – T2 with Bhavana. Nil; + Mild; ++ Moderate; +++ Severe.

Figure 1: Experimental Study

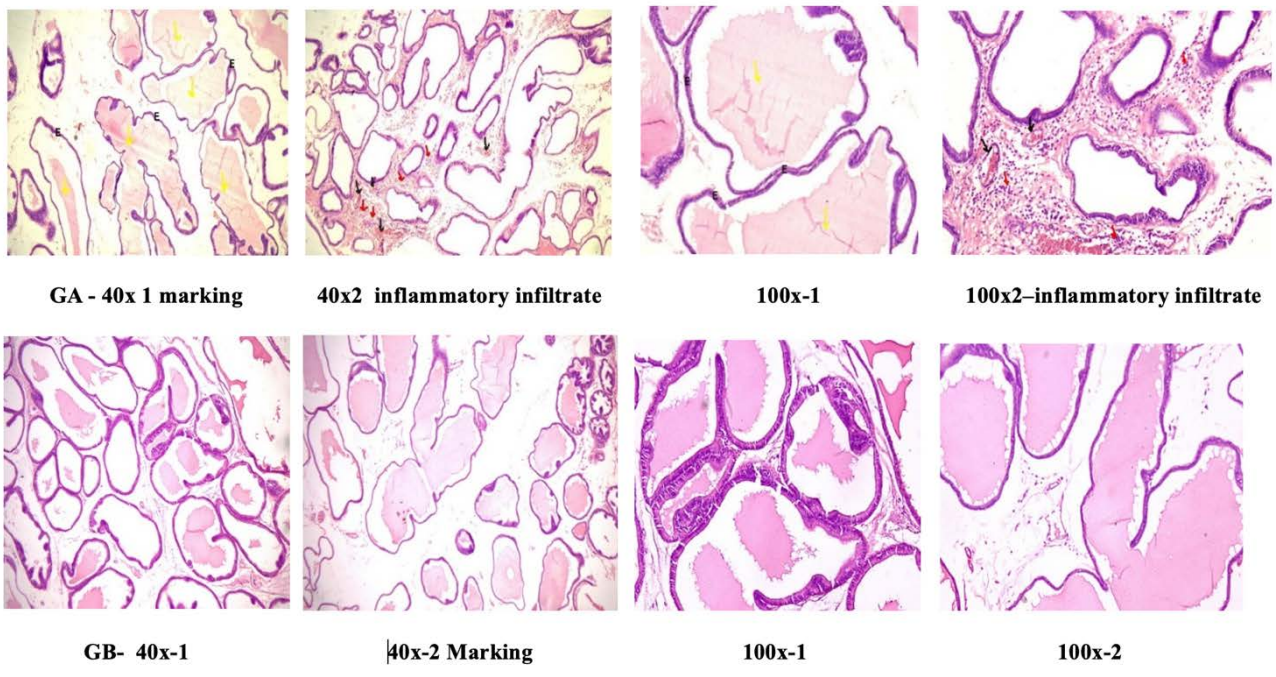

Figure 2: Histopathological image results of Prostate

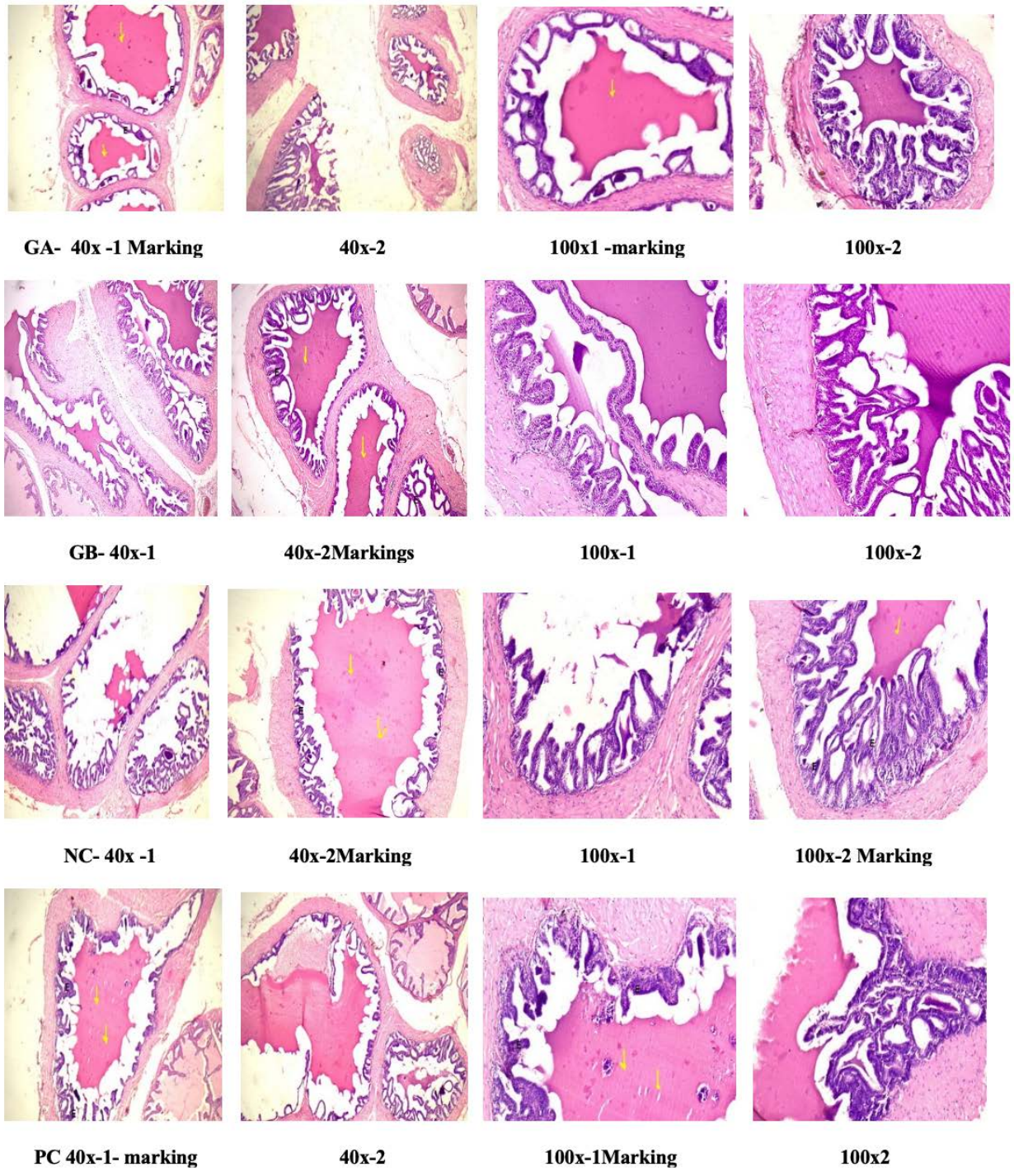

Figure 3: Histopathological Image Results of Seminal Vesicle

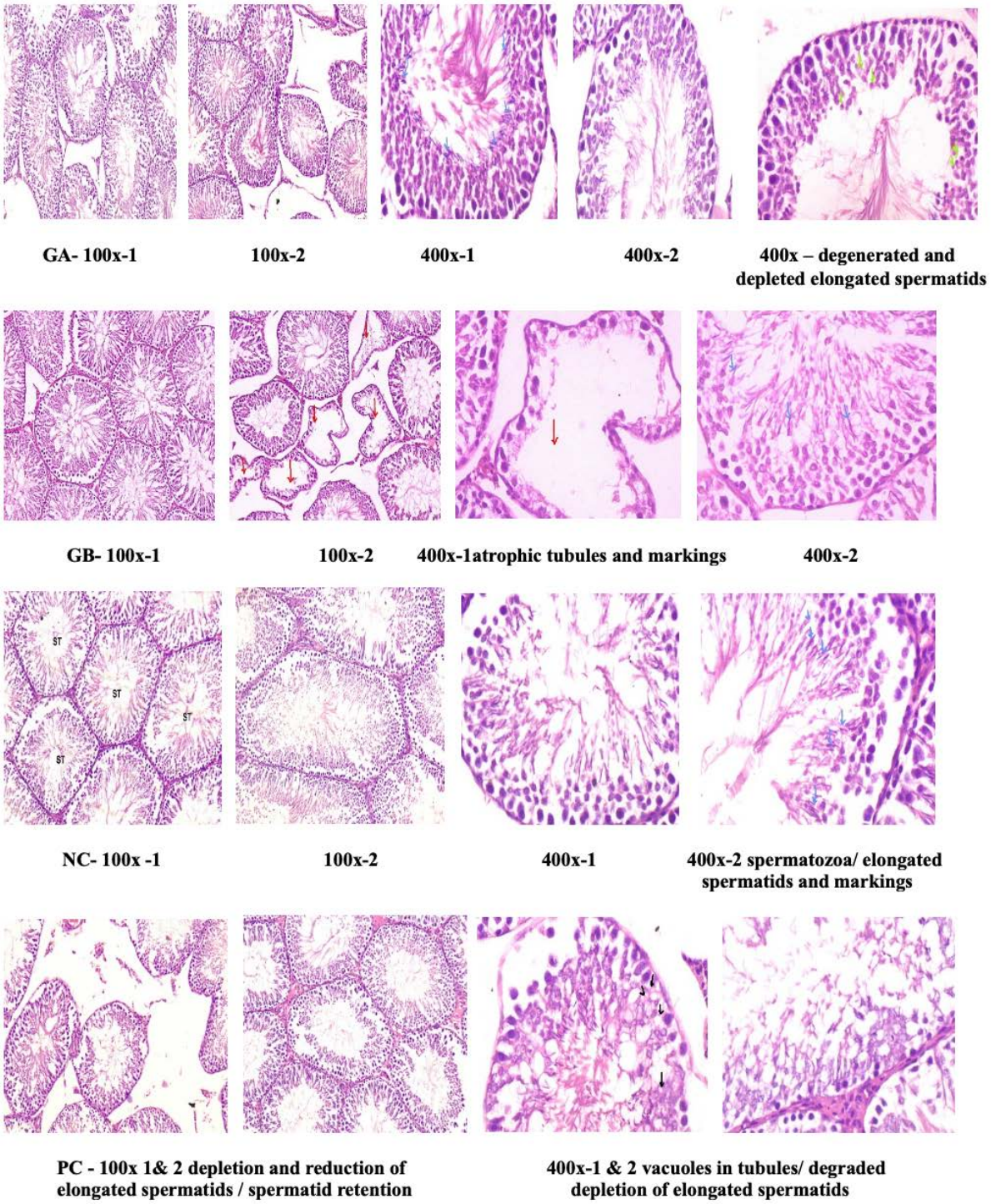

Figure 4: Histopathological Image Results of Testis

The data in Tables 3 and 4 shows the MEAN ± SEM and decrease or increase % changes of parameters assessed in T1 and T2 groups when compared to normal control and cyclophosphamide control in the spermatogenesis model. Based on which the observed decrease or increases was found to be statistically significant or non-significant.

DISCUSSION

The classical Ayurvedic drug formulation Vidarikhanda Churna is prominently described in authoritative texts particularly under the Vajeekarana (aphrodisiac) category. The churna synergistically act to improve nourishment, tissue regeneration, and hormonal balance, which are essential in the process of spermatogenesis. The selection of this formulation is therefore rooted in both traditional wisdom and pharmacological rationale, making it an ideal drug for evaluating Vajeekarana efficacy.

Pueraria tuberosa phytochemical profiling reveals that it contains large amounts of isoflavonoids (including puerarin, daidzein, genistein), flavonoids, saponins, steroids, chalcones, etc. Puerarin is a principal isoflavone, with a glycoside bond linking a glucose moiety and multiple phenolic hydroxyl groups,

enabling antioxidant activity, potential interaction with hormonal pathways, and capacity for modulating membrane interfaces.
Animal studies show that *P. tuberosa* ethanolic extract increases sperm count, improves testis weight, enhances sexual behaviour, and elevates FSH, LH, testosterone; in separate studies puerarin alone improves chemically-induced spermatogenic toxicity (e.g., busulfan) by reducing oxidative stress, restoring BTB (blood-testis barrier), Sertoli cell function, and moderating MAPK (Mitogen-Activated Protein Kinase) pathway activation. *Puerarin* can improve spermatogenic parameters, promote sexual behaviours, increase hormone levels, and reduces damage to testes.

Puerarin is not absorbed by the stomach but is absorbed very quickly in the intestine. After absorption, the product is excreted rapidly in urine and feces as glucuronic acid metabolites.[9] For another sample Vidarikanda Churna II – Vidarikanda Churna is given Bhavana with Vidarikanda Kwath. The main marker isoflavone in *Pueraria*. *Puerarin*, was found to be a C-glycoside of daidzein and to be relatively thermally stable when the tubers of Vidari were coarsely pounded and subjected to mandagni for kwatha preparation. This indicates that *puerarin* is frequently retained in kwatha made from Vidarikanda[9].

Both Vidarikhanda Churna I and II were subjected to qualitative and quantitative changes of sperm (spermatogenetic activity) variation. The following results can be found in both invitro and invivo experimental modules.

In *in-vitro* observation in a 65days test module were noted. The sperm analysis revealed significant variations among different experimental groups. The cyclophosphamide control group exhibited a marked reduction in sperm count, sluggish motility and an increased percentage of non-motile sperms. Morphologically increased abnormal sperms indicating spermatogenic suppression due to cytotoxic effect. In contrast both trial drug groups demonstrated improvement in sperm parameters, suggesting restoration of spermatogenic function. Among the two trial drugs, the Bhavita group (T2) showed higher sperm counts, improved motility and greater proportion of morphologically normal sperms compared to the trial group without bhavana (T1). The absence of necrotic or degenerated spermatogenic cells and the presence of actively dividing germ cells in the Bhavita group further confirm its superior efficacy.

In vivo experimentation after sacrificing the animal there was marked increase in the structural ramification of testis, prostate and seminal vesicles. The HPE of testis, prostate and seminal vesicle revealed significant differences among the groups. The Normal Control (NC) group exhibited normal architecture with well-organized seminiferous tubules, active spermatogenesis and intact germinal epithelium. In contrast, the cyclophosphamide – treated positive control (PC) group showed marked degeneration and depletion of spermatogenic cells, tubular atrophy and necrotic changes, indicating severe testicular damage due to cytotoxic insult. The groups treated with trial drugs demonstrated a remarkable protective and restorative effect. In Group-A (without bhavana) – moderate regeneration of spermatogenic cells and improvement in seminiferous tubular structure were observed, with no signs of necrosis or degeneration. The Group-B (with Bhavana) showed near- normal histoarchitecture with well-developed germ cells and active spermatogenesis, suggesting enhanced therapeutic efficacy and better tissue recovery compared to the Group-A group. The absence of necrosis, inflammation or vascular congestion in both Group-A and Group-B groups indicates the safety of the formulations on testicular tissue.

Similarly, in prostate and seminal vesicles, normal glandular architecture and secretory activity were preserved in the drug-treated groups, whereas the cyclophosphamide group showed a decrease in secretion and mild degenerative changes. These findings suggest that the trial drugs, particularly, the Bhavita group (Group-B) exert a protective and rejuvenating effect on male reproductive organs, improving spermatogenic activity and maintaining semen health. Thus, the drug processed with Bhavana T2/Group-B exhibits a more pronounced Vajeekarana activity compared to the non Bhavita drug and the cyclophosphamide control.

CONCLUSION

In the present study, pre-clinical evidence is generated for the formulation Vidarikanda churna with and without Bhavana for possessing substantial spermatogenic efficacy. According to the results, the drugs T1 and T2 protect testis tissues damage and maintains normal testis functions in a rat model. Therefore, it is plausible to suggest that compared to non-bhavita churna, bhavita churna sample can prevent testicular degeneration Therefore, Vidarikhanda Churna, especially when fortified with Bhavana samskara, holds significant promise as a safe, natural, and effective formulation in promoting spermatogenesis, reproductive health, and Vajeekarana therapy in Ayurveda. Both forms of the preparation contribute valuable therapeutic outcomes, but the Bhavita sample may be preferred where stronger and sustained Vajeekarana action is required.

Scope of Future Study: The advanced pharmaco – therapeutics selected androgen receptor modulation theory of anabolic steroids and testosterone has to be applied and seen in the case of Bhavana modulation of Vidari Churna.

Other Karmas of Shukra like Dhairya, Preeti, Dehabala need to be analysed. Further studies should be done on Human subjects through clinical trial to assess other attributes of Shukra.

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