IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 3, January 2026
Impact Factor: 7.67

Musli Pak Supplementation Improves Male Reproductive Health in Adult Parkes Strain Mice

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Abstract: *Aim: To study the effect of Musli Pak supplementation on male reproductive physiology in Adult Parkes strain mice*

Study design: Twenty-four adult Parkes strain male mice of twelve to fourteen weeks age were placed in 4 different groups (6 mice /group). Standard optimum conditions were maintained; mice were treated with the respective dose (50 mg/kg BW,100 mg/kg BW, and 200 mg/kg BW) of Musli Pak along with control group for 35 days.

Place and Duration of Study: Ethical clearance was approved by the IAEC, Department of Zoology, Banaras Hindu University with a validity of two years.

Methodology: Following autopsy, reproductive organ indices, sperm quality, testicular daily sperm production (TDSP), steroidogenic enzyme activities(3β-HSD,17β-HSD), serum hormones, epididymal sialic acid, seminal vesicle fructose, and histoarchitecture of testis, seminal vesicle and epididymis of adult Parkes strain mice were evaluated.

Results: In Musli Pak treated mice testis weight increased at all doses however relative testis and epididymis weight increased in mice treated with doses 100 & 200 mg/kg, body weight. Seminal vesicle weight was significantly higher in all treated groups. Musli Pak treatment appeared to enhance sperm count, motility, viability, TDSP and protected against abnormal morphology. Serum testosterone, estradiol,3β-HSD and 17β-HSD expression were elevated in mice particularly at 100 & 200 mg/kg dose of Musli Pak. PCNA expression was found highest at 50 and 200 mg/kg. Histology of testis, epididymis and seminal vesicle showed normal architecture with germinal epithelium thickening and sperm-rich lumina without degenerative changes. Biochemical analysis showed increased sialic acid and fructose concentration indicated improved epididymal and seminal vesicle function.

Conclusion: Musli Pak supplementation enhances spermatogenesis, steroidogenesis, and sperm maturation in a dose-dependent manner, with 200 mg/kg showing maximal benefits and 50 mg/kg triggering strong proliferative activity. The findings support its traditional fertility-boosting use of Musli Pak without histological or biochemical toxicity..

Keywords: Musli Pak, steroidogenic enzymes, testosterone, estradiol, daily sperm production, sperm quality, fructose, sialic acid

I. INTRODUCTION

In the 21st century, reproductive health in adult men has become an important global concern. Worldwide, around 15–20% of couples were experienced infertility, with male factors accounting for approximately half of these cases(Agarwal et al., 2015;Mishra et al., 2018).Over the past five decades, a notable decline in both sperm quantity and quality has been observed (Joffe, 2003). Contributing factors such as endocrine, genetic, psychological, lifestyle-related, and environmental influences have further raised reproductive issues in men (Yadav et al., 2022). In the Indian subcontinent, infertility imposes not only medical but also social and psychological burdens, as childlessness is often associated with stigma and feelings of incompleteness(Seshagiri, 2001). Despite significant technological advances in

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reproductive medicine, high costs and limited accessibility remain major concerns (Kamel, 2010). Consequently, herbal medicines are often considered safer alternatives to synthetic pharmaceuticals (Mishra et al., 2012).

As per estimated data from the World Health Organization (WHO, 2002), between 70 and 80 percent of people worldwide use traditional medicine, with up to 80 percent of medical care in underdeveloped nations coming from herbal therapies (Chan, 2003;Singh & Singh, 2009; Mishra & Singh, 2016) Ayurveda, the traditional Indian system of medicine, emphasizes *Vajikarana Chikitsa* (aphrodisiac therapy) under *Rasayana* formulations, which aim to enhance vitality, fertility, and longevity (Yadav et al., 2012; Yadav & Mishra, 2025). Traditional herbal formulations such as Musli Pak, Vanari Gutika, Makardhwaja, Shilajit Rasayana, Shatavari Kalpa, and AshwagandhadiLehyam are described in Ayurveda under Vajikarana and Rasayana therapies and are supported by scientific literature for their fertility-promoting, aphrodisiac, and rejuvenating properties(Dalal et al., 2013;Khanam et al., 2013;Rajpoot et al., 2025; Gurav et al., 2023).Among these, Musli Pak is a classical polyherbal Ayurvedic preparation, recognized for its aphrodisiac (*vrishya*), strength-promoting (*balya*), and rejuvenating (*rasayana*) properties (*Charaka Samhita*, 100 BC).

Musli Pak is a traditional Ayurvedic formulation whose principal ingredient, *Chlorophytum borivilianum* (Safed Musli), is rich in steroidal saponins and glycosides that are known to stimulate spermatogenesis, increase testosterone levels, and improve libido (Sen et al., 2008). Additionally, it has been reported that using this herb as a dry powder or an ethanolic or aqueous extract can enhance sexual function and boost sexual performance in experimental animals(Kenjale et al., 2008;Thakur et al., 2009). Musli Pak additionally contains *Curculigoorchioides* (Krishna Musli), *Mucuna pruriens (konch)*, *Asparagus racemosus* (Shatavari),*Tribulus terrestris* (Gokshura), and *Withania somnifera* (Ashwagandha), along with spices and nutritive ingredients such as clove, almond, long pepper, cinnamon, black pepper, ginger, ghee, milk, and sugar. Collectively, these bioactive components provide glycosides, alkaloids, flavonoids, saponins, vitamins, and minerals that exert androgenic, spermatogenic, anabolic, and restorative effects.

Scientific validation of Ayurvedic aphrodisiac formulations is expanding. For example, treatment with *Vanari Gutika* has been shown to improve testicular histomorphology, daily sperm production, and sperm motility (Rajpoot et al., 2025) while Makardhwaja treatment enhanced testosterone levels, steroidogenic enzyme activity, and germ cell proliferation(Tiwari et al., 2024). However, the comprehensive effects of the standardized Musli Pak formulation on male reproductive physiology and the mechanisms underlying its biological activities remain unclear.Therefore, the present study was undertaken to assess the impact of Musli Pak supplementation on reproductive physiology in adultParkes strain male mice. The study focused on sperm characteristics, health of the reproductive organ, serum hormone levels, testicular cell proliferation, and β-hydroxysteroid dehydrogenase (β-HSD) enzymes expression, to elucidate the mechanisms underlying its potential role in spermatogenesis and male fertility.

II. MATERIAL AND METHODS

2.1 Chemicals & reagents

The chemical used were of analytical-grade, utilized in this investigation came from SRL Pvt. Ltd., and E. Merck India Ltd. Mumbai in India.

2.2 Animal care and maintenance

Ethical clearance (Approval No.: BHU/DoZ/IAEC/2021-2022/010) for the present study was obtained from the IAEC, Zoology Department, Institute of Science, Banaras Hindu University, Varanasi. Total 24 adult male albino mice (Parkes strain), aged 12-14 weeks and weighing 30-40g, were obtained from the animal house facility, Zoology Department, Banaras Hindu University. The animals were kept in conventional (430 x 270 x 150 mm) polypropylene cages with dry husk bedding and grill tops made of stainless steel. Mice were kept in a controlled environment with a 12:12-hour light-dark cycle and a temperature of 24 ± 2°C and a humidity of 50 ± 10%.Throughout the research, water and a standard pellet diet were freely accessible to the animals(Mishra & Singh, 2005).

2.3 Experiment design and dose preparation

Musli Pak drug (Batch No. ADF2100008) was procured from a licensed distributor under the trade name Patanjali India Ltd., Varanasi, Uttar Pradesh, India. Musli Pak was ground into a fine powder and then dissolved in cow's milk for oral

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use. Ayurvedic principles state that people have historically taken herbal medicines with milk, ghee, honey, etc.(Nitin & A.K, 2022). Milk was then chosen to administer Musli Pak and fed to the control group as well. Additionally, three doses of Musli Pak50, 100, and 200 mg/kg BWwere selected as the desired dose. The 24 male mice were randomly assigned to four groups of six mice each, and they received oral treatment for 35 days (a mouse spermatogenic cycle lasts 35 days), as indicated below.

1. Group I (Control): Received vehicle (cow milk) orally for 35 days.
2. Group II: Received 50 mg/kg body weight of Musli Pak orally for 35 days.
3. Group III: Received 100 mg/kg body weight of Musli Pak orally for 35 days.
4. Group IV: Received 200 mg/kg body weight of Musli Pakorally for35 days

2.4 Autopsy and sample collection

After the treatment period, animals were anesthetized and also euthanized by decapitation (Mishra & Singh, 2008).During autopsy the body weight of all mice (control and treated) was recorded for biometric study and the trunk blood was obtained for hormonal analysis.Cauda epididymis was excised immediately for sperm parameters analysis. The seminal vesicle, testes, and epididymiswere excised, cleared of blood, and assessed. The testiswas used to assess testicular daily sperm production (TDSP) and immuno-blot of steroidogenic enzymes (3β HSD and 17β HSD) as well as proliferative markers (PCNA), while the epididymis and seminal vesicles were used for biochemical estimations. Histological analyses were carried out using testis, seminal vesicle and epididymis (Mishra & Singh, 2005).

2.5 Biometric analysis

At the time of dissection, body weights of control and Musli Pak-treated mice were measured. The testis, seminal vesicle, and epididymiswere carefully excised, and their absolute and relative weights were calculated (Mishra & Singh, 2016).

2.6 Sperm analysis (count, motility, viability and morphology)

Sperm analysis was performed by excising the caudal epididymis from mice of both control and treated groups and incubating it in a 35-mm glass Petri dish containing 0.5 mL of normal saline at 37 °C. The tissue was carefully dissociated using fine forceps and a needle to obtain a sperm suspension. This suspension was utilized for the evaluation of sperm count, sperm viability, sperm motility,and sperm morphological characteristics, following the methods described by (Mishra & Singh, 2009) and (Patel, Singh, Singh, et al., 2017).

2.6.1 Sperm Count

Sperm count was tested according to international procedures described by the World Health Organization (1999;2010). A 10 µL sample was diluted 20-fold and placed on a Neubauer haemocytometer. Spermatozoa were counted using a WBC counting chamber under a bright-field microscope at 40X magnification, as described by (Mishra & Singh, 2016)with minor modification.

Concentration of spermatozoa =Average number of spermatozoa counted (N) X multiplication factor (10,000) X dilution factor (20)

= N X 10,000 X 20

= N X 0.2 X 10^6 spermatozoa

2.6.2 Sperm Motility

The sperm motility was assessed soon after the animal were sacrificed. A coverslip was placed over a tiny drop of sperm suspension on a spotless glass slide. The slideswere observed after 1 minute of incubation at 37°C under light microscope at 40X magnification.Sperm that moved in any way were classified as motile, whilst those did not move were classified as non-motile. The quantity of motile spermatozoa relative to the total number of spermatozoa detected was used to compute the percentage of sperm motility (Mishra & Singh, 2016;Tiwari et al., 2024).

Motility (%) = (Number of motile spermatozoa / Total number of spermatozoa) × 100

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2.6.3 Sperm Viability

Sperm viability was evaluated using the Eosin–Nigrosin staining protocol in accordance with World Health Organization guidelines (1999; 2010), with minor modifications (Mishra & Singh, 2016). The cauda epididymis was finely minced in 0.5 mL of pre-warmed normal saline (0.9%) at 37 °C to obtain a sperm suspension. Equal volumes of the suspension and staining solution were combined, and uniform smears were made on glass slides. The slides were examined under a bright-field microscope at 40X magnification. The unstained spermswere categorized viable, whilestained sperms as non- viable.

2.6.4 Sperm Morphology

Evaluation of sperm morphology involved the preparation of sperm smears on clean glass slides, which were subsequently analyzed under a bright-field microscope at 40X magnification. morphological abnormalities in spermatozoa were assessed using the criteria ofWyrobek & Bruce, (1975) and Zaneveld and Polakoski (1977)

2.7 Testicular Daily Sperm production (TDSP)

Evaluation of testicular daily sperm production (TDSP) was assessed to evaluate the effect of Musli Pak supplementation in control and treated mice. Sonication-resistant elongated spermatids representing developmental steps 14–16 were counted following the method described by (Meistrich&van Beek, 1993) with minor methodological changes (Mishra & Singh, 2008;Rajpoot et al., 2025).To extract spermatid heads, one testis was homogenized in 1 mL of ice-cold, cooled distilled water and sonicated for 1.5 minutes for spermatid head. Neubauer hemocytometer was used to count the heads at 40xmagnification using a phase-contrast microscope. Since spermatids at stages 14–16 stay in the testis for 4.84 days in mice, the total number of spermatids was divided by 4.84 to determine TDSP.

2.8 Testosterone and Estradiol Assay

Determination of serum testosterone (DKO002) and estradiol (DKO003) concentrations the commercially available enzyme-linked immunosorbent assay (ELISA) kits were from DiaMetra (Italy) were used following the manufacturer's instructions.

2.9 Western Blot Analysis for Testicular Steroidogenic Marker

To investigate steroidogenic activity, the expression profiles of3β-HSD,17β-HSD and PCNA were assessed using western blotting, (Patel et al.,2017).The conventional Lowry method (Lowry et al., 1951) was used to measure protein concentration. The Chemi-Doc detection system (Analytik Jena) was used to visualize the protein bands. Densitometric analysis was used to measure band intensity using AlphaEaseFC software (AlphaImager 2200).While β-actin served as the reference loading control. Comprehensive details of the primary antibodies employed and their working dilutions are presented in Table.

Antibody(Ab)	Host species	Dilutions	Catalog no.	Source
Primary Ab				
3β-HSD	Mouse monoclonal	WB (1:500)	sc-515120	Santa Cruz Biotechnology
17β-HSD	Rabbit polyclonal	WB (1:250)	sc-32872	Santa Cruz Biotechnology
PCNA	Mouse monoclonal	WB (1:2000)	sc-56	Santa Cruz Biotechnology
Secondary Ab				
Rabbit IgG	Goat	WB (1:2000)	114038001A	GeNei
Mouse IgG	Goat	WB (1:1500)	114068001A	GeNei

2.10 Histological analysis

Histological evaluation was performed by carefully dissecting the testis, epididymis, and seminal vesicles, followed by thorough cleaning and fixation in freshly prepared Bouin's solution. The protocol was adapted from the established methodology (Mishra & Singh, 2009), with slight modifications (Yadav et al., 2022). To assess the effects of Musli Pak

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treatment, seminiferous tubule sections were analyzed from six testis of six mice in each group. The identification and staging of seminiferous tubules were performed in accordance with the spermatogenic stage classification described by Russell et al. (1990).

2.11 Biochemical analysis

The fructose content of seminal vesicles was quantitatively assessed using the (Lindner & Mann, 1960) method. Estimation of epididymal sialic acid content was determined using the procedure described by (Aminoff, 1961), with slight modifications as reported by Mishra and Singh (2016).

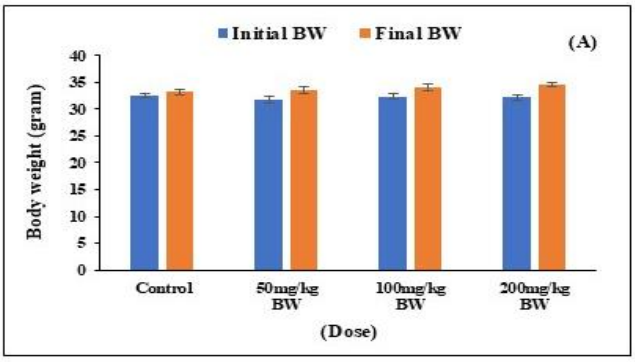
2.12 Statistical analysis

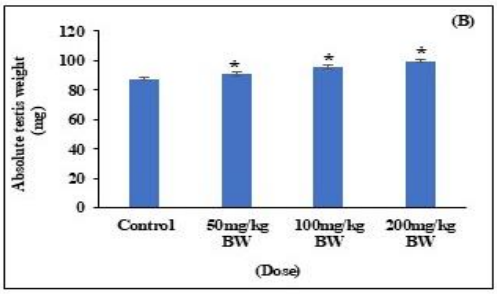
The mean ± SEM was used to express all of the experimental results. The Student's t-test was used to statistically evaluate body weight, while one-way analysis of variance (ANOVA) and the Newman-Keuls post hoc test were used to evaluate the remaining data. At $p < 0.05$, differences were regarded as statistically significant. The statistical study was conducted using IBM SPSS Statistics 16 for Windows.

III. RESULTS

3.1 Biometric analysis

Throughout the experimental period, all adult male mice appeared healthy. There were insignificant differences observed between initial and final body weight in control and treatment groups (Fig. 1A). As compared to control, the absolute weights of testis, epididymis, and seminal vesicles increased significantly in all treated groups (Fig. 1B, D, F). In contrast, the relative weight of testis showed a significant rise only at 200 mg/kg BW, whereas the relative weights of the epididymis and seminal vesicles showed no significant variation among the treatment groups (Fig. 1C, E, G).





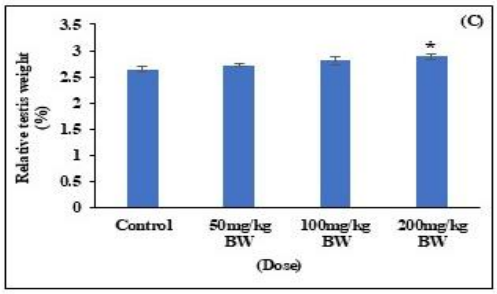


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Fig. 1: Effect of Musli Pak supplementation on body weight and reproductive organ weights in adult male mice.(A) Initial and final body weight,(B) absolute testis weight, (C) relativeweight of testis, (D) absolute epididymis weight, (E) relative weight of epididymis , (F) absolute seminal vesicle weight, and (G) relative weight of seminal vesicle as compared to control .Statistically different from control (*p < 0.05).

3.2 Sperm Analysis

As compared to control group, Musli Paktreated groups showed that the number of sperm had significantly increased (Fig. 2A). Sperm motility considerably increased at 100 and 200 mg/kg BW (Fig. 2B). while sperm viability increased significantly at all doses (Fig. 2C). Caudal epididymal sperm abnormal morphology decreased in all treated groups, with reductions in number of the headless and tailless sperm at higher doses (Fig. 2F–I).

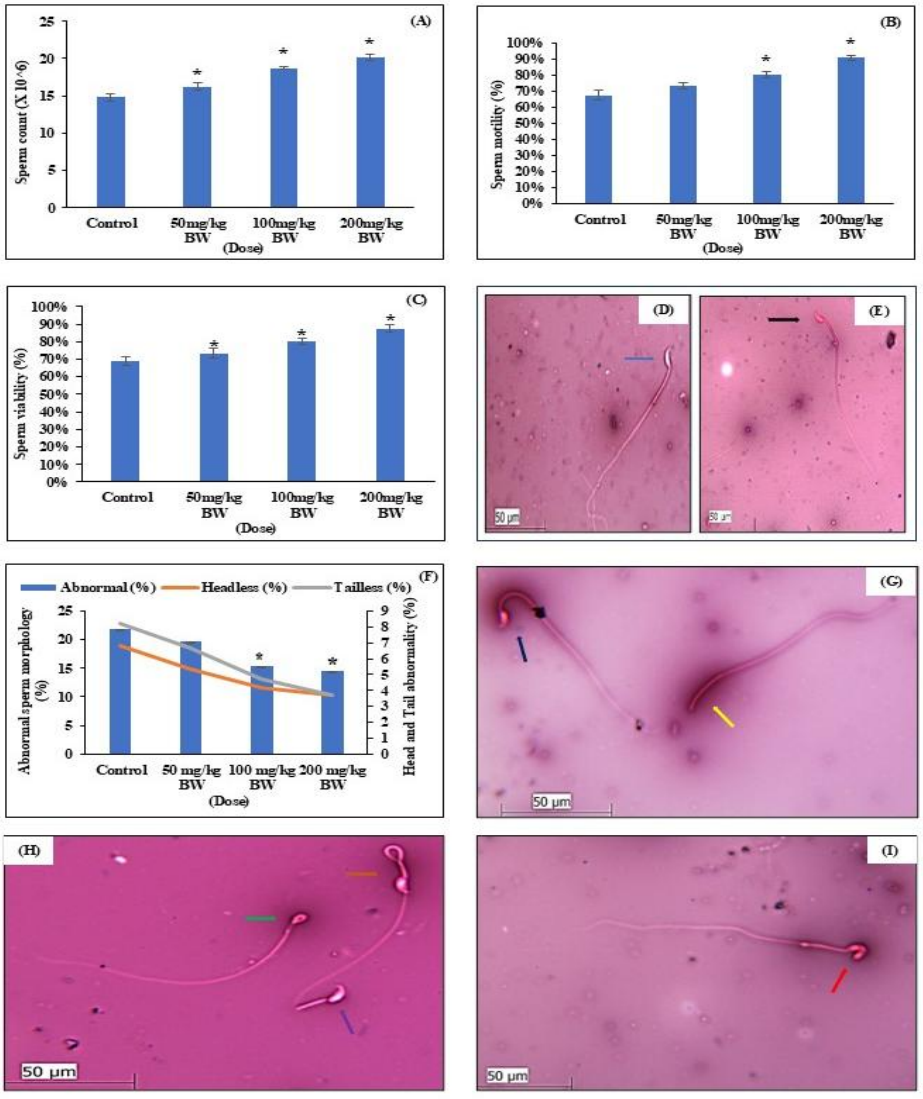

Fig. 2: Effect of Musli Pak supplementation on sperm parameters in adult male mice.(A) Sperm count, (B) sperm motility, (C) sperm viability, and (F) abnormal sperm morphology including headless and tailless spermatozoa. Representative images show (D) viable sperm (blue arrow), (E) non-viable sperm (black arrow), (G) bent head,

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headless sperm (dark blue and yellow arrow), (H) sperm with round head ,coiled/bent tails, isolated/detached sperm head abnormality(green, orange and purple arrows), and (I) hook head abnormality (red arrow). Data are mean ± SEM; *p < 0.05 vs. control. Scale bar = 50 µm (applies to all micrographs).

3.3 Testicular Daily Sperm Production (TDSP)

Testicular daily sperm production (TDSP) was significantly increased in all Musli Pak–treated groups compared to the control, with the maximum rise observed at 200 mg/kg BW (Fig. 3A).

3.4 Serum Hormones level (testosterone and estradiol)

Male mice treated with Musli Pak showed a statistically significant increase in serum testosterone levels at doses of 100 and 200 mg/kg body weight, as well as elevated in serum estradiol levels at both 100 and 200 mg/kg, compared to the control group. (Fig. 3B and C).

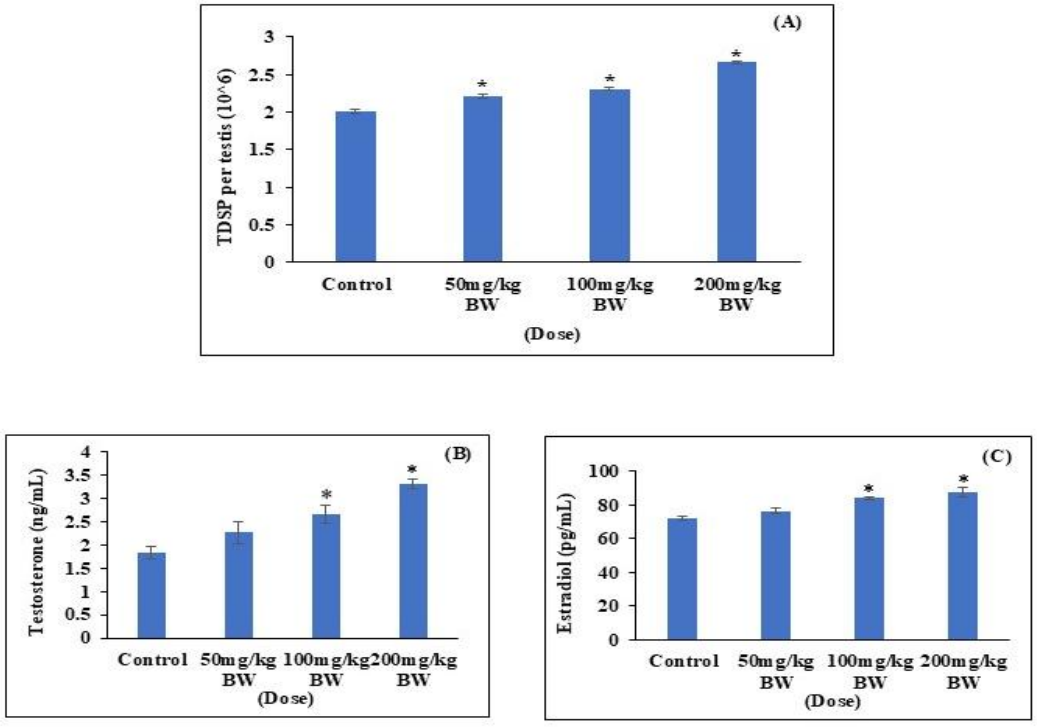

Fig. 3: Effect of Musli Pak supplementation on daily testicular sperm production (TDSP) and serum hormone levels in adult male mice. (A) TDSP per testis ($\times 10^6$), (B) serum testosterone levels, and (C) serum estradiol levels measured following Musli Pak administration at different doses. Data are expressed as mean ± SEM. $p < 0.05$ indicates a statistically significant difference compared with the control group.

3.5 Testicular steroidogenic markers (3β-HSD and 17β-HSD) and Proliferation marker (PCNA)

Densitometric analysis of Western blot data revealed a statistically significant increase in 3β-HSD and 17β-HSD protein expression in Musli Pak–treated groups compared with the control group (Fig. 4B and C). In addition, proliferating cell nuclear antigen (PCNA) expression showed a significant increase at doses of 50 and 200 mg/kg body weight when compared to the control group (Fig. 4D).

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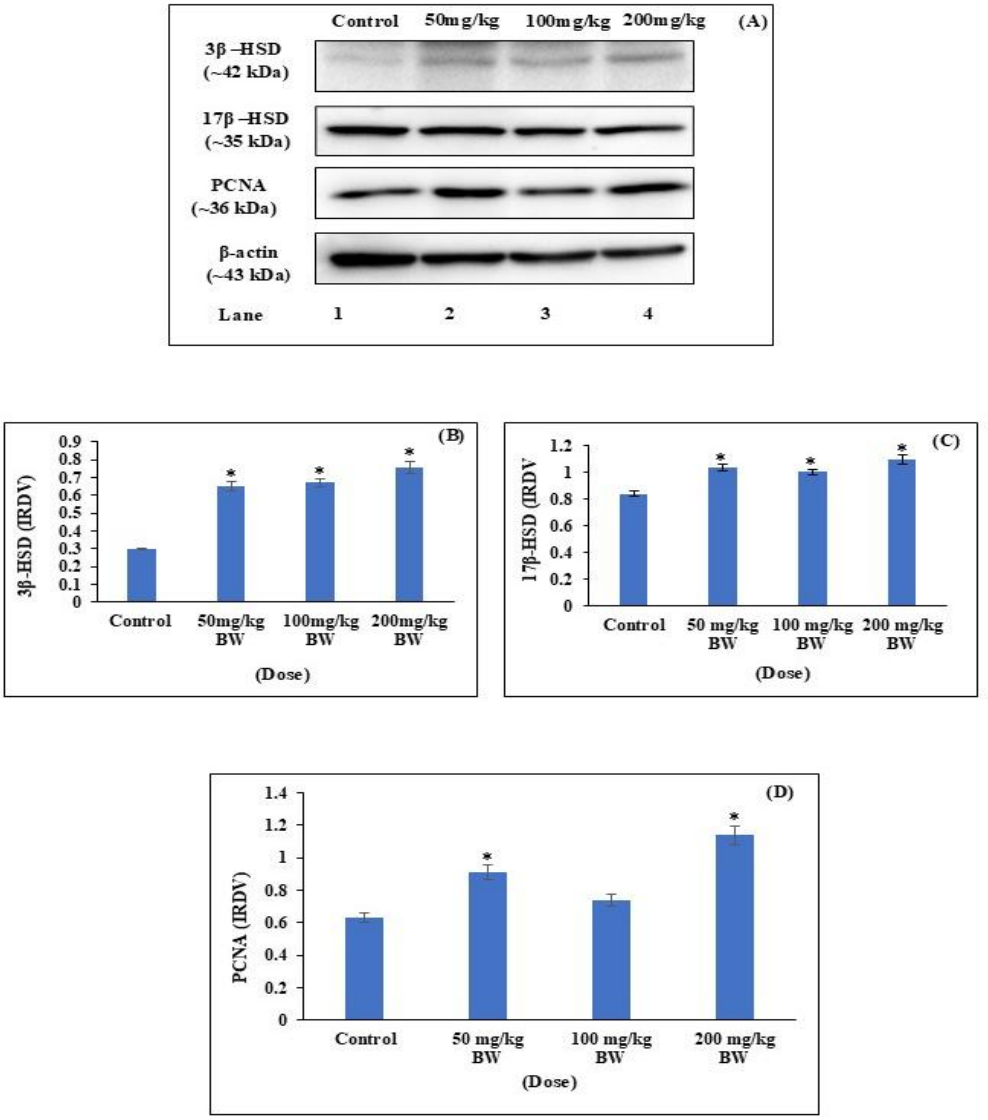

Fig. 4: Effect of Musli Pak supplementation on testicular steroidogenic and proliferative protein expression in adult male mice. (A) Representative Western blot images showing the expression of 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), and proliferating cell nuclear antigen (PCNA), with β-actin used as a loading control. (B–D) Densitometric analysis of 3β-HSD (B), 17β-HSD (C), and PCNA (D) protein expression, expressed as integrated relative density values (IRDV). Data represent the mean ± SEM of triplicate experiments. $p < 0.05$ indicates a statistically significant difference compared with the control group.

3.6 Histological examination of testis

On histological examination of testis, control, 50 mg/kg and 100 mg/kg Musli Pak–treated mice revealed normal histoarchitecture in the seminiferous tubules (Figs. 5A–F). Substantial histological changes, such as increased cellular density, thickened germinal epithelium, and abundant spermatozoa filling the lumen, were observed in the testis of mice treated with 200 mg/kg BW (Fig. 5G–H).

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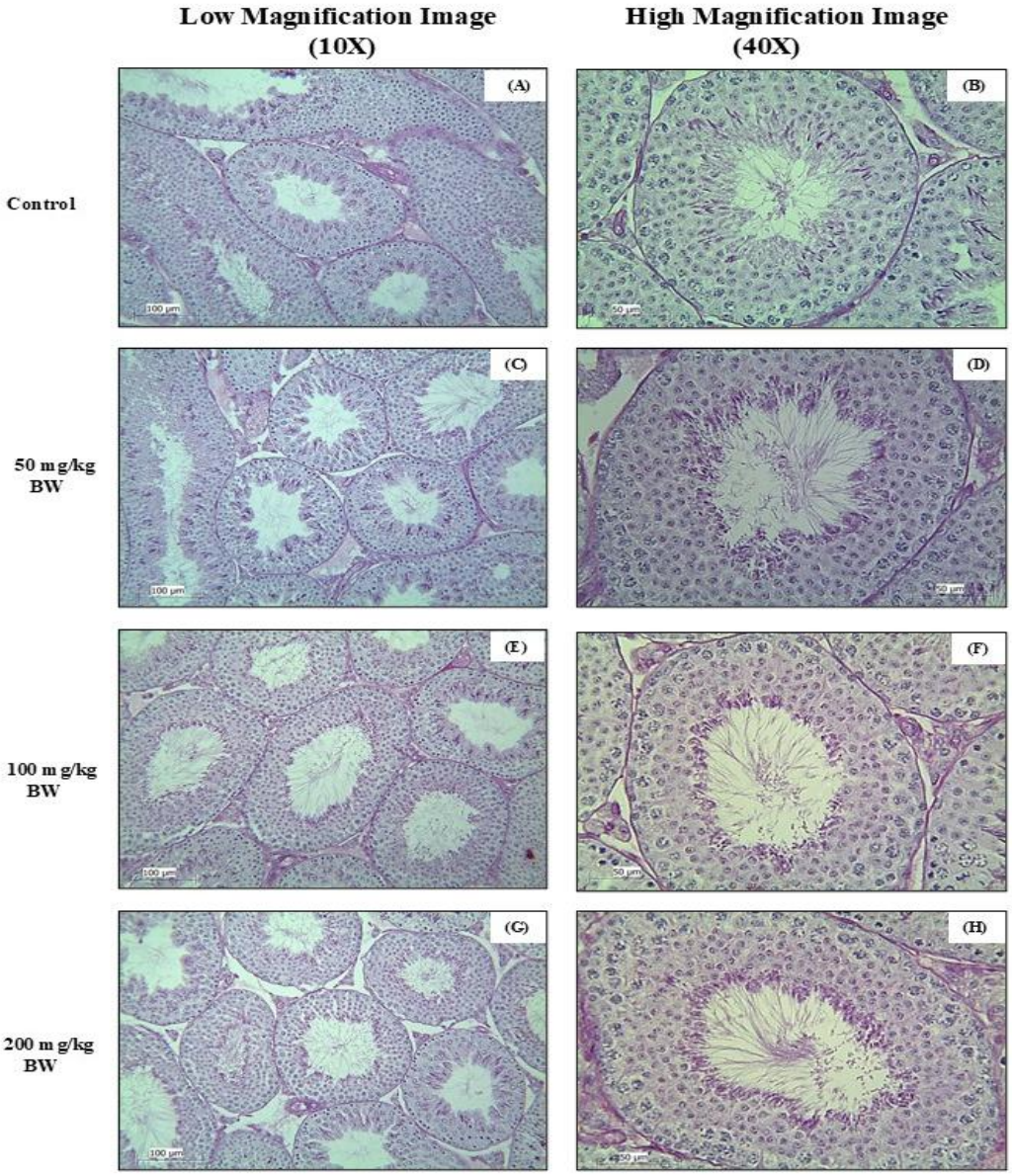

Fig. 5: Effect of Musli Pak supplementation on testicular histology in adult male mice. Representative Periodic Acid Schiff – hematoxylin stained of the testis at low magnification (A, C, E, G) and high magnification (B, D, F, H). (A–B) Control testis showing normal seminiferous tubules. (C–H) Musli Pak–treated groups (50, 100, and 200 mg/kg) showing progressively enhanced germinal epithelium organization, increased spermatogenic layers, and higher sperm density in the lumen. Scale bars: 100 µm (A, C, E, G; low magnification) and 50 µm (B, D, F, H; high magnification).

3.7 Histological examination of epididymis

Histological analysis of the epididymis of control mice and Musli Pak–treated groups at 50, 100, and 200 mg/kg BW revealed a normal epithelium with intact epithelial lining and lumina filled with abundant spermatozoa (Fig. 6A–L). No degenerative changes were observed at any tested dose.While quantitative parameters are described separately in Section 3.9.

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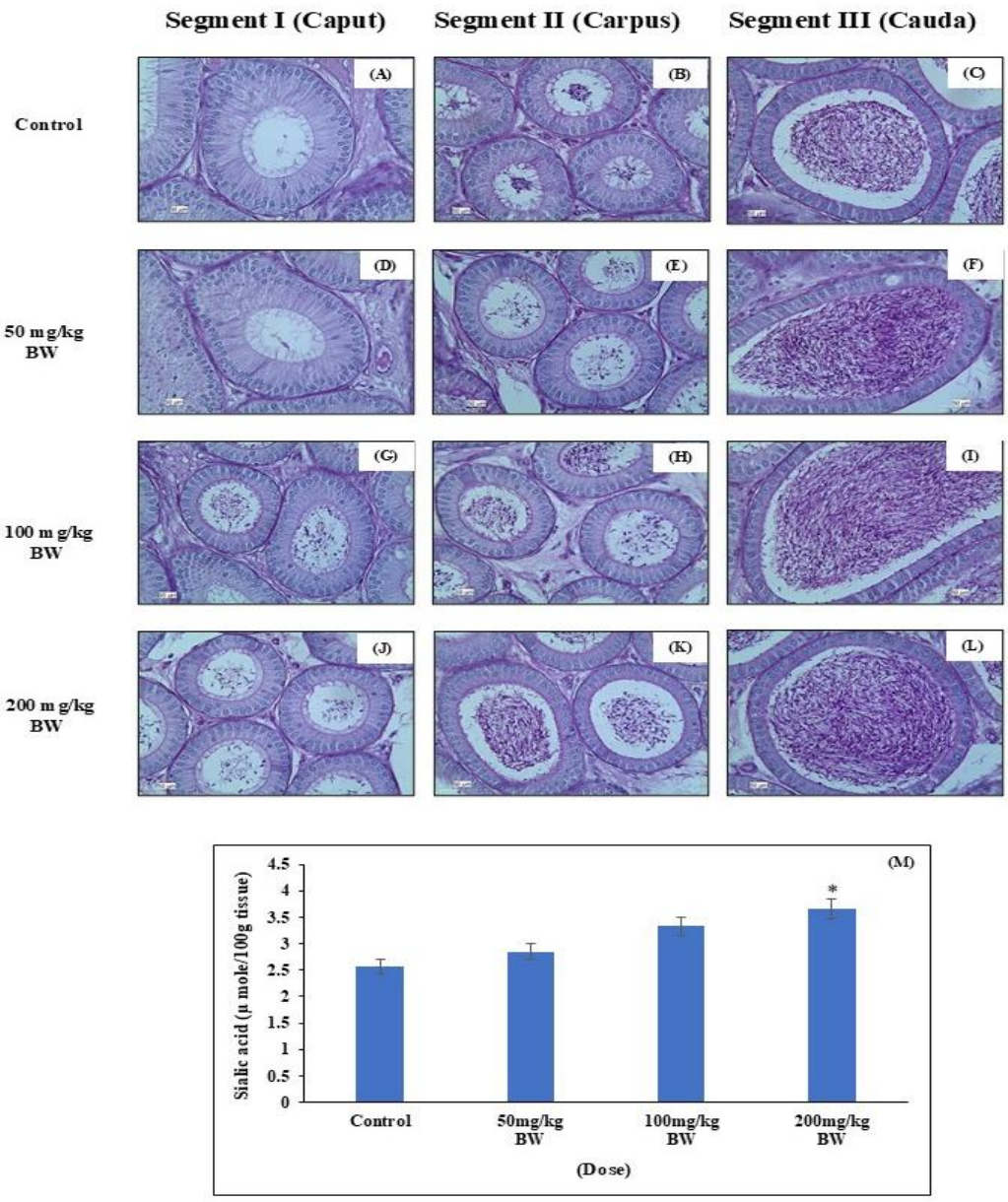

Fig. 6: Effect of Musli Pak supplementation on epididymal histology and sialic acid concentration in adult male mice. Representative PAS–Hematoxylin stained sections of the epididymis (caput, corpus, and cauda) at 50 µm scale. (A–C) Control group showing normal epithelium and lumen with fewer spermatozoa. (D–F) 50 mg/kg Musli Pak group showing increased sperm density within the lumen. (G–I) 100 mg/kg Musli Pak group showing higher accumulation of spermatozoa and improved epithelial structure. (J–L) 200 mg/kg Musli Pak group showing maximum sperm density and enhanced epithelial activity. (M) Quantitative analysis of epididymal sialic acid concentration. Values are expressed as mean ± SEM. (*$p < 0.05$), significantly different from control. Scale bar = 50 µm.

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3.8 Histological examination of seminal vesicle

Seminal vesicle histological inspection of control and Musli Pak–treated mice revealed a normal appearance with intact mucosal folds lined by columnar epithelium and lumina filled with secretory material (Figs. 7A–H). No vacuolation, atrophy, or structural disruption was observed in any treated group, and the architecture remained comparable to that of the control. indicating that Musli Pak preserves the structural integrity and functional activity of the seminal vesicles.While quantitative parameters are described separately in Section 3.9.

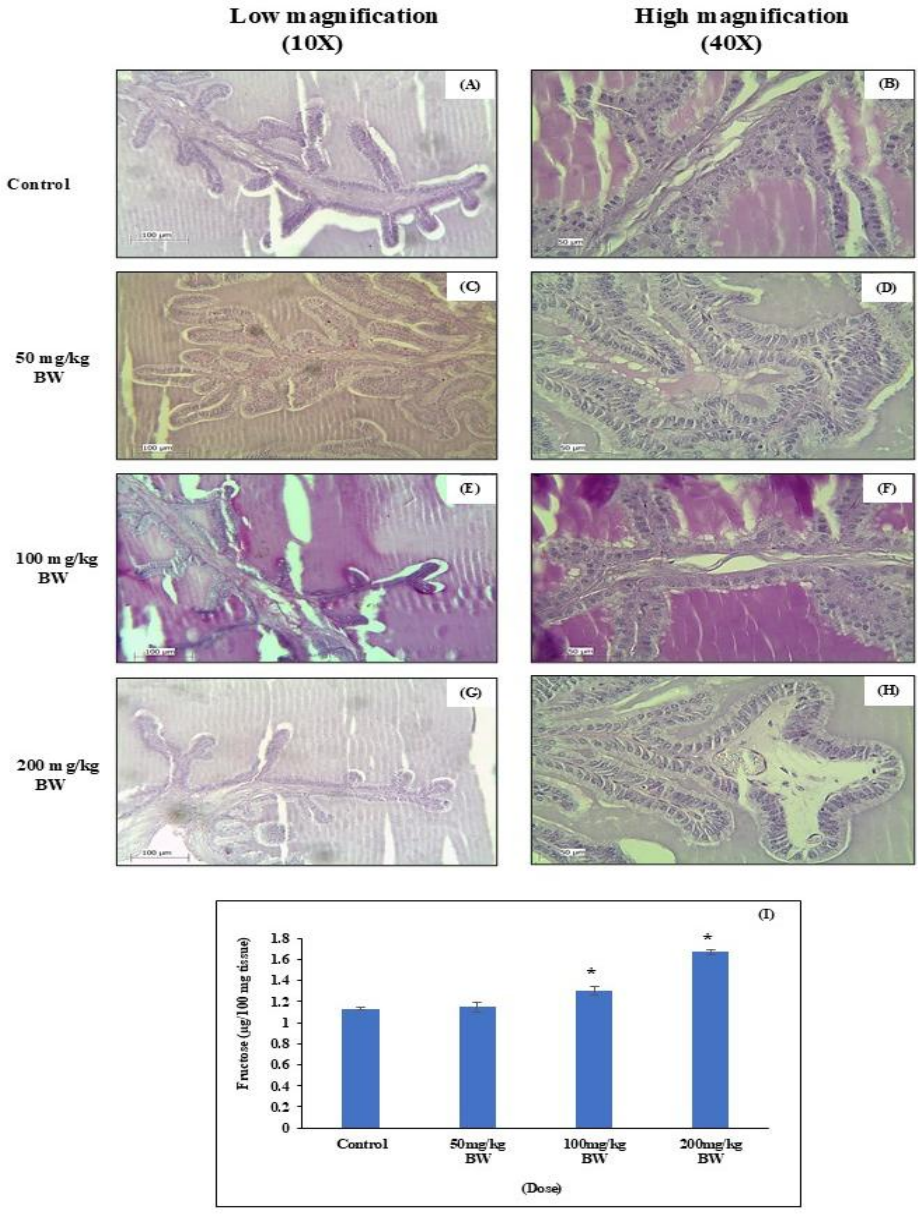

Fig. 7: Effect of Musli Pak supplementation on seminal vesicle histology and fructose concentration in adult male mice. Representative PAS-Hematoxylin stained sections of the seminal vesicle. (A–B) Control group showing normal glandular epithelium with moderate secretion. (C–D) 50 mg/kg Musli Pak group showing mild epithelial folding and

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increased secretory activity. (E–F) 100 mg/kg Musli Pak group showing highly folded epithelium with dense secretory granules. (G–H) 200 mg/kg Musli Pak group showing maximum epithelial activity, prominent vacuoles, and lumen filled with secretion.(I) Quantitative analysis of seminal vesicle fructose concentration. Values are expressed as mean ± SEM. (*$p < 0.05$), significantly different from control. Scale bars: 100 µm (A, C, E, G; low magnification) and 50 µm (B, D, F, H; high magnification).

3.9 Biochemical assay of sialic acid and fructose

Musli Pak supplementation at 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW produced a dose-dependent elevation in sialic acid concentration, in all treated groups, showing significantly higher values compared to the control (Fig. 6M). A similar trend was observed for seminal fructose concentration, which also increased progressively across the Musli Paktreated groups relative to the control, reaching the maximum level at 200 mg/kg BW (Fig.7I).

IV. DISCUSSION

In Ayurveda, Musli Pak has been traditionally used to manage male sexual and reproductive disorders. It is a classical Vajikarana formulation prepared with 24–25 ingredients, with Musli (*Chlorophytum borivilianum*) as its principal component. Musli is rich in glycosides and saponins, known for its aphrodisiac, adaptogenic, and spermatogenic properties (Charaka Samhita, 100 BC; Sharma, 2001; (Chauhan et al., 2007). Traditionally, it has been employed to treat sexual weakness, oligospermia, and male infertility. However, the effects of Musli Pak on the functional physiology of male reproductive organs and the mechanisms underlying its activity remain poorly understood.

Previous studies indicate that *Chlorophytum borivilianum* is non-toxic at these doses and does not affect body or reproductive organ weights (Thakur, Bhargava, et al., 2009). In the present study, animals were orally administered graded doses of Musli Pak (50, 100, and 200 mg/kg BW) for 35 days, equivalent to one spermatogenic cycle, to assess its role in modulating spermatogenesis. As expected, all adult male mice were in a healthy condition throughout experimental period and exhibited normal appearance. Furthermore, all the Musli Pak treated groups demonstrated negligible differences between their initial and final body weights. Our results are consistent with established reports that Musli Pak formulation is generally safe(Mishra et al., 2018).

Following Musli Pak treatment in mice, the absolute and relative weights of the testis significantly increased in a dose-dependent manner (absolute weights about 14% and relative weights 9.5% at 200 mg/kg BW). Similar outcomes have been reported for Shilajit in cadmium-induced infertile mice (Mishra et al., 2018). It was postulated that, the increased testicular weight could be due to increased sperm production. Furthermore when measured TDSP in the Musli Pak treated mice, a positive correlation was observed between TDSP level and the dose of MusliPak. The observation supported that, the increased testicular weight could be due to increased sperm production in the testis. Similar reports are available from the studies involving treatment of mice with aphrodisiacs such as Shilajit and VanariGutika(Mishra et al., 2012;2018). Overall, the data indicate that a similar biological mechanism may regulate TDSP and the observed increase in testicular weight induced by different aphrodisiac agents.It is established that sperm production is an outcome of germ cell proliferation in mouse testes. Proliferative Cell Nuclear Antigen (PCNA) a cell cycle regulatory protein that is strongly linked to cellular DNA synthesis. PCNA is a useful indicator of spermatogonia and spermatocyte proliferation. These results, which demonstrated that Musli Pak had a beneficial effect on proliferative activity, were compatible with the current study's considerable rise in PCNA expression in the group treated with Musli Pak(Mishra & Singh, 2013).

Furthermore, it is established that germ cell proliferation is androgen dependent, which in turn is synthesized through a series of enzymatic activities (Smith & Walker, 2014)Pregnenolone is converted to progesterone and dehydroepiandrosterone (DHEA) to androstenedione by the enzyme 3β-HSD, whereas androstenedione is converted to testosterone by 17β-HSD (Stucco and McPhaul, 2006). A significant rise in the level of both these steroidogenic enzymeswas observed in the in the Muli Paktreated mice when compared to the control group, suggesting that Musli Pak positively influences the activities of 3β-HSD and 17β-HSD. Consistent with these enzymatic changes, the treated groups showed a clear increase in testosterone levels, with a 45.64% rise at 100 mg/kg and an 81.27% rise at 200 mg/kg compared to the control. This dose-dependent pattern indicated enhanced steroidogenic activity in treated mice.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 3, January 2026
Impact Factor: 7.67

Conversion of testosterone to estradiol occurs through aromatase activity in Leydig and Sertoli cells (Carreau et al., 2010). Correspondingly, estradiol levels increased significantly, showing a 16.71% rise at 100 mg/kg and a 21.63% rise at 200 mg/kg. Therefore, it can be deduced that Musli Pak treatment in mice promotes androgen synthesis and germ-cell proliferation, which ultimately leads to increased sperm production and higher testicular weight.

Histological examination of the testis revealed that Musli Pak treated groups (50, 100, and 200 mg/kg) preserved the normal architecture of seminiferous tubules without any signs of vacuolation or degenerative alterations. These findings are consistent with reports describing protective effect of *Chlorophytum borivilianum,* a principal constituent of Musli Pak (Sharma & Kumar, 2012). Similarly, Ray reported that *C. borivilianum* restored spermatogenic activity in a cyproterone acetate–induced subfertility model (Ray et al., 2014). Recently, Manani et al. (2024) confirmed the positive spermatogenic potential of C. borivilianum through detailed histological analyses in rat testis. Collectively, these findings substantiate that Musli Pak preserves testicular structure and sustains spermatogenesis against potential histopathological damage.

In comparison to the control group, all Musli Pak–treated mice's, absolute and relative weights of the seminal vesicles increased by 5.10–14.23% and 6.00–9.74%, respectively, reflecting enhanced structural growth and secretory activity. The epithelial height and secretory activity of the seminal vesicles were also increased, indicating the androgenic influence of Musli Pak. Seminal fructose concentration increased dose-dependently: 1.5% at 50 mg/kg, 15% at 100 mg/kg, and 47% at 200 mg/kg, demonstrating progressive enhancement of fructose-producing secretory capacity. These quantitative changes are supported by previous studies, where *Chlorophytum borivilianum* (Safed Musli) treatment similarly increased seminal fructose levels in male rats (Giribabu et al., 2014; Mishra & Singh, 2009; Kumari & Singh, 2012), consistent with androgendependent regulation of seminal vesicle function (Price, 1936; Maggi et al., 1970; Lung & Cunha, 1981.). Histological observations revealed preserved epithelial morphology and active secretory vesicles, confirming the structural and functional improvements. Collectively, Musli Pak appears to enhance seminal vesicle structure, secretory function, and androgen-dependent physiology, similar to the effects of other Rasayana aphrodisiac formulations such as Shilajit and VanariGutika(Mishra et al., 2012; Rajpoot et al., 2025). Thus, our results suggest a common underlying mechanism by which Musli Pak and other Rasayana formulations improve androgen-dependent glandular activity and contribute to enhanced male reproductive efficiency.

Musli Pak treatment improved the histological structure of the seminal vesicles. All treated groups showed higher epithelial cells, more mucosal folds, and increased secretory material, indicating activation of androgen-dependent functions. Similar androgen-driven recovery of epithelial height and secretion has been reported earlier in the Hershberger assay(1953), where testosterone restored normal seminal vesicle structure after androgen deprivation(Nishino et al., 2004).In present study, these improvements were seen in all treated groups, with the 200 mg/kg dose showing the highest epithelial activity. Our results align with previous research, where androgenic or Rasayana treatments produced the same pattern of increased epithelial height, secretory activity and role of androgens in maintaining seminal vesicle morphology and function (Welsh et al., 2010).

Musli Pak at doses of 100 mg/kg BW and 200 mg/kg BW in mice significantly increased the absolute epididymal weight by 19.79% and 27.59%, respectively, while relative weight showed no significant change. At the same doses, sialic acid concentration in the epididymis increased by 43%, indicating enhanced epididymal secretory activity (Setty & Jehan, 1977), consistent with findings from high-dose Shilajit treatment (Mishra et al., 2018), indicating towards shared mechanism of action between Musli Pak and Shilajit treatment improved sperm parameters in a dose-dependent manner. Sperm count increased by 9.45%, 26.45%, and 36.18% at 50, 100, and 200 mg/kg BW, respectively; sperm motility improved by 17.65% and 33.82%; sperm viability increased by 5.80%, 15.94%, and 27.54%; and abnormal sperm morphology decreased by 31.82% and 36.36% at 100 and 200 mg/kg BW. These findings align with previous reports wherein *Chlorophytum borivilianum* (Safed Musli) root extract (Giribabu et al., 2014),has been used. Collectively it indicates that Musli Pak enhances epididymal function and overall semen quality.

These findings demonstrate that Musli Pak exerts a dose-dependent enhancement of epididymal function and sperm quality, supporting its efficacy in improving male reproductive health through both biochemical (sialic acid) and functional (sperm parameters) mechanisms.

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ISSN: 2581-9429 Volume 6, Issue 3, January 2026 Impact Factor: 7.67

Histological examination of the epididymis showed that Musli Pak treatment at all doses (50, 100, and 200 mg/kg) maintained a normal structural pattern similar to the control group. The epithelial lining remained intact and uniform, and the lumina were filled with healthy spermatozoa without any signs of degeneration or vacuolation. This suggests that Musli Pak does not cause harmful changes in epididymal tissue. Similar observations were reported by (Vyas et al., 2023) wherein it has been reported that *Chlorophytum borivilianum* extract preserved epididymal sperm structure without causing damage.

V. CONCLUSION

In summary, Musli Pak improves sperm production, testis weight, sperm quality, and hormone levels in a dose-dependent way while keeping reproductive organs healthy. These results support its traditional use for male fertility. Future study may seek to examine the mechanism of action at the molecular level, check long-term safety, and test its effectiveness on infertility in humans.

ACKNOWLEDGEMENTS

Male Reproductive Physiology lab, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221005, India for an opportunity for experimentation and availing the various facilities of the Department. Moreover, our sincere gratitude towards the Department of Zoology, Smt. CHM College for allowing me to work and complete my Research work.

AUTHORS' CONTRIBUTIONS

Raghav Mishradesigned the study, prepared the protocol and collaborated in materials and lab required for the work.
Raj Kumar Yadav performed the literature search and animal experimentation, and drafted the manuscript.
Vind Kumar Tiwari and Arti Rajpoot managed the statistical analysis and prepared the illustrations.
Shashibhal M. Pandey supervised the work, contributed to data interpretation, finalized and communicated the manuscript.
All authors read and approved the final manuscript.

ETHICAL APPROVAL

All animal experiments were conducted in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985) and CPCSEA guidelines. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Department of Zoology, Institute of Science, Banaras Hindu University (BHU), Varanasi, India (IAEC Approval Reference No. BHU/DoZ/IAEC/2021-2022/010). All authors read and approved the ethical compliance of the study.

COMPETING INTERESTS

The authors have stated that there are no conflicting interests.

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International Journal of Advanced Research in Science, Communication and Technology
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Impact Factor: 7.67

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