RESEARCH PAPER

Fertility effects of phytochemical influences of Selaginella bryopteris (Sanjivani) in Swiss albino mice

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ABSTRACT

This Study investigate the potential of Selaginella bryopteris (Sanjivani) to improve reproductive levels in Swiss albino mice. Twenty-four male mice (n=24) were selected and put into four distinct groups (n=6 each). Group-1 (G1): control; Group-2 (G2): estradiol-treated; Group-3 (G3): pre-treated with oestradiol and Selaginella bryopteris (150 mg/kg body weight); Group-4 (G4): Oestradiol pre-treatment combined with Selaginella bryopteris at a dosage of 200 mg/kg body weight. G2, G3, and G4 mice received oestradiol treatment at a dosage of 25µg/kg body weight for a duration of forty-five days. Upon completion of the dose period, G2 was halted for examination, but G3 and G4 continued with the administration of Selaginella bryopteris at two distinct doses for a length of 35 days. Sperm quality, immunoreactive luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (T) were examined. The results of this investigation demonstrated that, in comparison to G1, the estradiol-treated G2 exhibited substantial alterations ($p<0.001$) in sperm count, sperm motility, sperm morphology, and blood levels of LH, FSH and testosterone. Rebound effects were noted in G3 and G4 following the injection of S. bryopteris. In comparison to G3, G4 yielded superior results. The histological examination of the testis demonstrated disorganisation of the cytoarchitecture in the seminiferous tubules, characterised by vacuolations, absence of lumen, and compartmentalisation of spermatogenesis. In comparison to LH and sperm density, oestradiol markedly inhibited FSH and sperm motility. The direct influence of oestradiol on the testis is mostly accountable for the fall in testosterone levels. However, the administration of S. bryopteris at 200 mg/kg body weight resolved the change more effectively than at 150 mg/kg body weight.

Keywords: Selaginella bryopteris, Estradiol, LH, FSH, Testosterone, and Mice.
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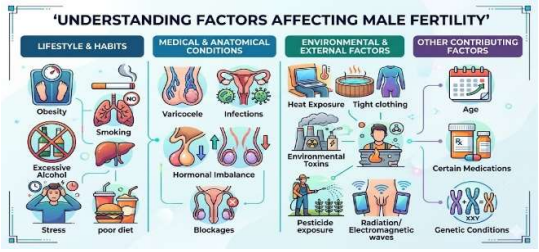
Introduction

In recent decade fertility achievement is involving as a biggest challenge, it is believed that over 15% of adults are facing fertility issues. Male infertility refers to a man's inability to cause pregnancy in a fertile female after a minimum of one year of regular, unprotected intercourse. World Health Organisation reports that over 50-60 million people around the globe affecting with such issues, in which around half of the cases related to male factor (Chu. et., al., 2010). These can be caused by a variety of factors, including hereditary issues, hormonal abnormalities, and medical conditions such as diabetes or infections. Lifestyle factors such as smoking, alcohol consumption, and exposure to environmental toxins may have an unfavourable effect on male fertility (Fainberg, J., & Kashanian, J. A. 2019). Low sperm production, low sperm motility, and abnormal sperm function are all typical reasons why men are unable to bear children. Globally, insufficient sperm production is thought to be he leading cause of male factor infertility.



The use of alternative medicine to treat male infertility has grown in popularity in recent years. Potential remedies for infertility in men have included a number of dietary changes, medicinal herbs, and antioxidants (Roozbeh & Mortazavian 2017). Throughout history, people all across the world have tried to regulate male fertility by using

medicinal herbs that either increase or decrease fertility. The fertility-related characteristics of these plants have also piqued the curiosity of modern scientists.

Supplemental methods of treating infertility have grown in popularity alongside the increasing number of males seeking for herbal cures for the condition (Yao & Mills 2016). According to a recent statement by the European Association of Urology, a multi-faceted integrative approach to treating male infertility involves adopting supplemental treatments based on traditional medicine. The World Health Organization (WHO) has urged researchers to discover rational uses for medicinal plants in the hopes of discovering novel treatments for infertility. Scientists are making efforts to clarify the effects of medicinal herbs on male fertility due to the growing interest in these substances. There is evidence that some of these plants can alter the release of hormones by the testicles, while others can increase sperm motility and count. Flavonoids and phenolic compounds found in certain plants are potent antioxidants that neutralise oxygen-containing free radicals. In fact, they do protect sperm from free radicals, which improves sperm quality and fertility prospects. The exact use of herbal remedies and our current scientific knowledge of their molecular mechanisms of action are, however, severely lacking. Thus, it is critical to identify natural chemicals with oestrogenic and anti-estrogenic actions and to study the impact of physiologically active herbal substances on male fertility.

Our study aimed to determine the phytochemical effects of *Selaginella bryopteris* (Sanjivani). which is lithophytes pteridophyte plant with extraordinary recovery powers, Selaginella bryopteris (Family: Silaginaceae) is better known as Sanjivani (Paswan, et al., 2017). According to Vashistha et al. (2021) and Gautam et al. (2023), the first known life-giving herb in India was *Selaginella bryopteris*, which is referred to in the famous epic of the Hindi poet-Swami Tulsi Das. According to scientific reports, this plant possesses pharmacological effects such as anti-bacterial, anti-protozoal, growth-promoting, anti-stress cell death properties. In clinical settings, the shown metabolites of this plant reduce levels of reactive oxygen species (ROS) by acting as antioxidants. Male infertility may be treatable using their metabolites (Chandrakant et al., 2015; Paswan et al., 2017). *Selaginella bryopteris* (Sanjivani) contains the following Phyto-compounds: amentoflavone, bilobate, Hevea flavone, amentoflavone, tetrahydro amentoflavone, (2S)-2,3- dihydroamentoflovone, Hing flavone, naringenin (4'", O, 3). kaempferol and robusta flavone have been studied by Paswan et al. (2020).

Material and Methods

Chemicals Used

The estradiol Valerate pills, acquired under the brand name Estraheal 2, from Healing Pharma India Pvt. Ltd. India.

Selaginella bryopteris (Sanjivani)

Selaginella bryopteris (Sanjivani) verification done by Systematics and Herbarium Division, CSIR-National Botanical Research Institute (NBRI), Lucknow, India

Preparation of *Selaginella bryopteris* whole plant Ethanolic extract

Selaginella bryopteris was collected, washed with distilled water, and dried thoroughly at room temperature before being ground into powder after soaking in ethanol as a solvent for 48 hours and drying with a rotatory evaporator. The doses for the study of plant extract were 150 mg/kg and 200 mg/kg body weight, respectively

Animals and Animal Ethics

The animal house of Mahavir Cancer Sansthan and Research Centre in Patna, India (IAEC. No. 1129/PO/ReBi/S/07/CPCSEA) supplied the twenty-four (n=24) male, healthy, albino mice, which ranged in age from 8 to 12 weeks and weighed 30-35g. Institutional Animal Ethics Committee (IAEC) No. 2021/1A-06/10/21 gave their approval to all of the experiment plans. The gathered mice were maintained in an environment with a consistent temperature (22 ± 2 ºC), a regular pattern of light and dark periods, and a food and water supply.

Animal Grouping:

Group-1 (G1): control,

Group-2 (G2): Estradiol treated (25µg/kg body weight)

Group-3 (G3): Pre-treated Estradiol + *Selaginella bryopteris* (150mg/kg body weight)

Group-4 (G4): Pre-treated Estradiol + *Selaginella bryopteris* (200mg/kg body weight).

Experimental Design:

Experimentally, G2, G3 and G4 mice were treated with estradiol at the dose of 25 µg/kg body weight for 45 days. Post dosage period G2 were sacrificed for analysis while rest G3 and G4 were continued for *Selaginella bryopteris* administration at two different dose for 35 days. After treatment of Selaginella bryopteris to G3 and G4 mice, they were also sacrificed for analysis.

Sample collection:

The animals in each group were sacrificed using the decapitation process. After drawing blood from the trunk and letting it coagulate at 40°C, it was centrifuged at 3000 g for 15 minutes. Sort the serum into its respective components (LH, FSH, and testosterone) and store it in an Eppendorf tube at -20ºC for later use.

Sperm count:

In the dissection of each group, the cauda epididymis of the mice was excised and

meticulously rinsed in normal saline (0.85%). The trimmed and purified cauda epididymis was punctured at many sites within a watch glass containing 1 cc of distilled water to promote sperm release. Subsequently, two drops of eosin Y were amalgamated gently. We transferred the sample into Neubauer's chamber and examined them at 450X magnification.

Sperm motility:
The cauda epididymis of each experimental mouse was dissected, ruptured, and prepared for microscopic examination on a slide. Subsequently, a cover slip was delicately positioned over the specimen, and the spermatozoa were examined.

Sperm morphology:
A precise evaluation of sperm morphology necessitates meticulous smear preparation, fixation, and staining. This experiment utilises Haematoxylin and Eosin (H&E) stain to prepare a sperm smear. Subsequent to staining, the slides were allowed to dry and subsequently examined under a light microscope.

Hormonal assay
The concentrations of the hormones LH (Luteinizing hormone), FSH (Follicle Stimulating Hormone), and Testosterone were quantified utilising the ELISA method. ELISA kit obtained from "Immuno Tag," the subsidiary brand of "Geno Technology" Inc., USA.

Scanning Electron Microscopy (SEM) Preparation
After being rinsed with PBS buffer, 1-2 mm thick samples are dipped into Krasnoyarsk's fixative, a combination of 4% para formaldehyde and 1% glutaraldehyde, and let to soak for 12 hours at 40°C.After the tissues were dehydrated with graded ethanol and dried, a mounting and coating procedure utilising a thin layer of gold was employed. Scanning Electron Microscopy (SEM) procedure perform at AIIMS (All India of Medical Science, Instrument facility system, New Delhi, India)

Statistical analysis
ANOVA analysis was applied for hormonal levels, sperm morphology, count and motility. The results were compared using Tukey's test for multiple comparisons. Statistical significance is considered at p values ($p<0.05$). All calculations were performed using GraphPad Prism software (GraphPad Software, Inc., San Diego, USA).

Result
This study examines the phytochemical effects of Selaginella bryopteris (Sanjivani) at two distinct doses against oestradiol in a group of mice, demonstrating significant impacts on many parameters, as detailed in Table-1.

Parameters	G1	G2	G3	G4
Serum Count (10^6/ml)	6.21±0.03	1.193±0.04	4.057±0.04	4.408±0.12
Sperm Motility (%)	71.68±0.02	30.18±0.03	52.03±0.18	64.33±0.17
Sperm Morphology (%)	91.05±0.09	71.62±0.3	76.55±0.19	75.14±0.11
Testosterone (pg/ml)	2.884±0.01	0.733±0.05	1.796±0.06	1.23±0.09
FSH (pg/ml)	2.792±0.01	1.183±0.01	1.050±0.06	1.843±0.03
LH (pg/ml)	2.128±0.001	1.899±0.15	1.655±0.01	1.938±0.17

Table-1: Comparative measurement of Sperm Motility, Sperm Morphology, and hormones

Effect on Sperm Count

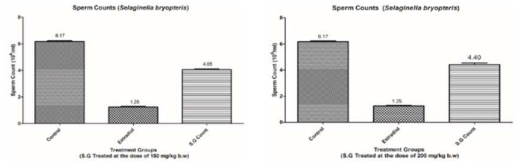


Figure-1: Comparative sperm count levels across different groups of mice (n=6; values presented as mean ± SD), significance at $p<0.001$.

The sperm count of the estradiol-treated mice was significantly lower than that of the control group. Nevertheless, after taking S. bryopteris, a notable improvement in testicular function was observed, leading to an increase in sperm count ($p<0.001$). G4 (S. bryopteris administered at 200 mg/kg body weight) was more effective and dependable than G3 (S. bryopteris administered at 150 mg/kg body weight) (Figure -3).

Effect on sperm motility

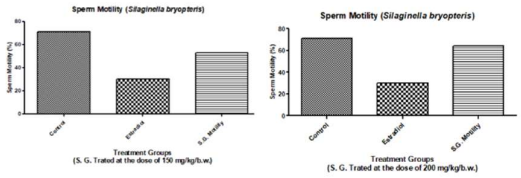
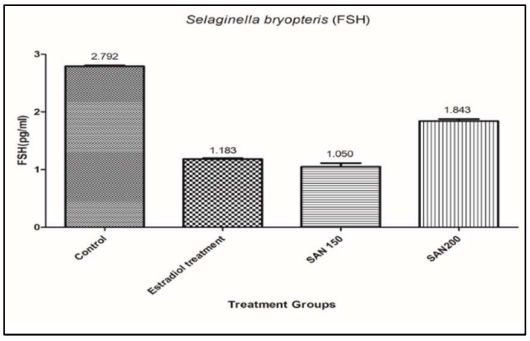


Figure-2: Comparative sperm motility levels across different groups of mice (n=6; values presented as mean ± SD), significance at $p<0.001$.

As seen in Figure 2, the sperm motility of the estradiol-treated mice was significantly lower than that of the control group. There was a noticeable improvement in sperm motility levels after administering S. bryopteris, indicating good

movement efficiency ($p<0.001$). A more dependable and effective method was G4, which involved administering S. bryopteris at a dose of 200 mg/kg body weight, as opposed to G3, which involved administering 150 mg/kg body weight.

Effect on sperm morphology



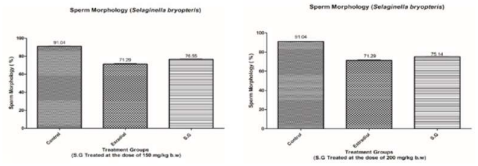


Figure-3: Comparative sperm morphology levels across different groups of mice (n=6; values presented as mean ± SD), significance at $p<0.001$.

Sperm morphology was significantly reduced in the estradiol-treatment compared to the control group (Figure -3). Significant sperm abnormalities were noted. There was a noticeable loss of sperm tails and intermediate pieces. Nevertheless, a notable normalisation was observed following the administration of S. bryopteris ($p<0.001$). There was little difference in efficacy between Groups 3 and 4, when S. bryopteris was administered at 150 mg/kg body weight and 200 mg/kg body weight, respectively.

Effect on Reproductive Hormones

Testosterone (T)

Figure-4: Comparative Testosterone (T) levels across different groups of mice (n=6; values presented as mean ± SD), significance at $p<0.001$.

A significant decrease ($p<0.001$) in testosterone (T) was seen in the estradiol-treated mice group as compared to the control group. Figure -6 shows that the testosterone level was significantly ($p<0.001$) raised following the injection of S. bryopteris. G4 was much more effective than G3 when it came to administering S. bryopteris at a dosage of 200 mg/kg body weight, as compared to 150 mg/kg body weight in G3.

Luteinizing Hormone (LH)

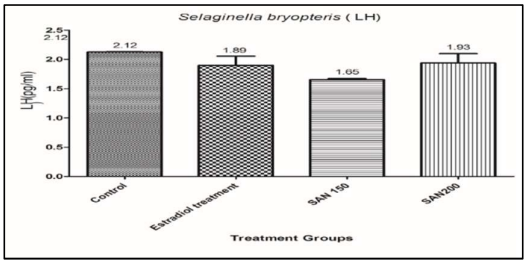


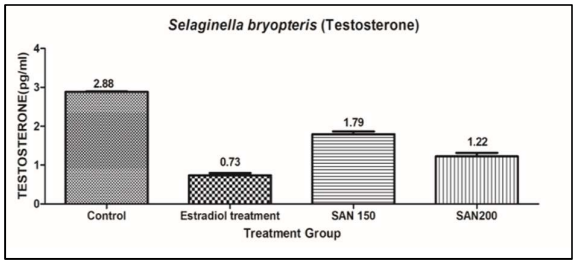
Figure-5: Comparative Luteinizing Hormone (LH) levels in different group of mice (n=6; values are expressed as mean ± SD, significance at $p<0.001$).

The group of mice treated with estradiol showed a little decrease in Luteinizing Hormone (LH) levels compared to the control group, with a significant difference ($p<0.001$). The administration of S. bryopteris (Sanjivani) extract at a dosage of 200 mg per kg of body weight raised the level of luteinizing hormone (Figure -5), with a substantial rise ($p<0.001$). When comparing the groups of mice treated with S. bryopteris (G3) and those treated with 200 mg/kg body weight (G4), the difference between the two groups was statistically significant, with G3 showing a marked decrease compared to the estradiol-treated group.

Follicle Stimulating Hormone (FSH)

Figure-6: Comparative Follicle Stimulating Hormone (FSH) levels in different group of mice (n=6; values are expressed as mean ± SD, significance at $p<0.001$).

There was a substantial decrease ($p<0.001$) in the amount of Follicle Stimulating Hormone (FSH) when compared to the control group. The level of FSH Hormone was significantly ($p<0.001$) raised after administering *S. bryopteris* (Sanjivani) extract (at 200 mg/kg body weight) (Figure7). Group 3 (S. bryopteris at 150 mg/kg body weight) showed less improvement as compared to Group 4 (S. bryopteris at 200 mg/kg body weight).



Scanning Electron Microscopy (SEM)

In this procedure, a high-quality image of the sperm was captured from every experimental group. The results showed that the oestradiol treatment caused

the neck disruption, which can alter or damage DNA, to disappear. In contrast, the sperm images from the groups treated with S. bryopteris at doses of 150 mg/kg body weight and 200 mg/kg body weight were normal, without any damage to the head, neck, or tail. There is evidence that S. bryopteris treatment is effective, as all sperm parameters are restored after oestradiol treatment, even at high doses.

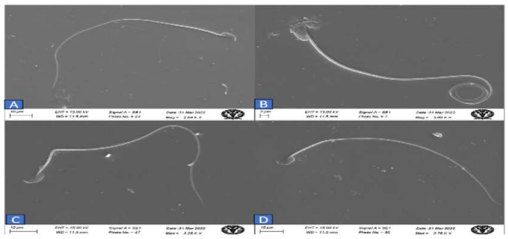


Figure-7, In Scanning electron microscopy control shows [A], normal sperm head, Neck and tail, while [B] belongs to estradiol treatment shown head disruption, Neck region also disappear. [C] and [D] show treatment of *S. bryopteris* at dose of 150mg/Kg b.w and 200mg/Kg b.w respectively shown normal sperm parameter. All picture taken at 3-5k X magnification.

Discussion

The male reproductive system relies on Estrogens to stay healthy and functional. The most effective oestrogen for men is oestradiol. The number of oestrogen receptors in the testis is high, and they include enzymes such as cytochrome P450 aromatase, the membrane-associated G-protein-coupled functional ER (GPER), and oestrogen receptors β and β. The conversion of testosterone to oestrogen is carried out by ERβ and ERβ (Bendis et al., 2024; Fietz et al., 2014; Oliveira et al., 2014; Bernardino et al., 2016). It has actions that are both stimulating and inhibiting. Puts a stop to the growth of Leydig cells and their ability to produce testosterone (Hess et al., 1997). It is well-known that oestradiol can control the proliferation of germ cells and other processes involved in spermatogenesis in testicular cells. Processes including differentiation, proliferation, survival, and apoptosis have been studied extensively in several fields (Fujikura & Fujinoki 2024; Chimento et al., 2010 & 2014; Royer et al., 2014; Pentikainen et al., 2000; MacCalman et al., 2017). Spermatozoa, immature germ cells, Leydig cells, Sertoli cells, the efferent ductile epithelium, and the proximal duct of the epididymis are among the epithelial cell types that synthesise it. The present investigation found that nearly exclusively complete Azoospermia resulted from severely impaired spermatogenesis in testicular seminiferous tubules.

The release of testosterone, luteinizing hormone (LH), and growth hormone (GH) is controlled by the hypothalamic-pituitary-testosterone axis. Both gonadal and non-gonadal tissues receive FSH and LH from the anterior pituitary gland, which are attached to receptors in. In order to prevent the pituitary gland and the central nervous system from producing FSH and LH, respectively, the androgens produced by the testes' Leydig cells stifle their production.

Phosphorylation of the cAMP response element-binding protein is one mechanism by which testosterone controls spermatogenesis. Compared to the control group, the treated animals showed a marked drop in testosterone levels, and then there was a trend toward lower levels of FSH and LH, according to our findings. According to Chandrakant et al. (2018), a drop in testosterone levels may have affected Leydig cell activity (Figure 6), but this disturbance did not cause a drop in FSH and LH as a result of a negative feedback process.

Recently conducted research has linked estradiol's direct action on the testicles to a lack of testosterone. Yet every one of the evaluated Treatment with *S. bryopteris* extract significantly improved the parameters. The reproductive ability was positively affected by a dosage of 150 mg/kg body weight of the same plant's root, stem, and leaves, as opposed to a dose of 200 mg/kg body weight. *S. bryopteris* may have a medical utility in boosting reproduction, according to the data.

The phytoconstituents found in *S. bryopteris*, including as tannins, steroids, alkaloids, and flavonoid derivatives, have a crucial role in increasing fertility in the estradiol-induced model (Girase and Talele 2015). There are a number of flavonoids and steroids in the *S. bryopteris* extract. that suggests flavonoids may have amplified the effects of steroids by increasing male sex hormones. All things considered, the flavonoids in the extract may have enhanced sperm parameters through their antioxidant actions.

Conclusion

Results showed that S. bryopteris extract significantly improved all measured variables. When comparing the effects on reproductive processes, the dosage of 200 mg/kg body weight was superior to that of 150 mg/kg body weight. Therefore, S. bryopteris can be recommended to those who are experiencing infertility.

Acknowledgement

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Conflict of Interest

There is no conflict of interest amongst the authors of this piece, as they have all stated.

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