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Fertility booster effect of *Selaginella bryopteris* (Sanjivani) against high dose of estradiol exposed Swiss Albino mice

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Abstract

The present investigation was aimed to examine the antifertility effects of estradiol and phytochemical influences of *Selaginella bryopteris* (Sanjivani) in *Swiss albino* mice. Male twenty- four (n=24) mice were selected which was divided into four different group (n=6; each). Group-1 (G1): Control, Group-2 (G2): Estradiol treated, Group-3 (G3): Pre-treated estradiol + *Selaginella bryopteris* (150mg/Kg body weight) and Group-4 (G4): Pre-treated estradiol + *Selaginella bryopteris* (200mg/Kg body weight). Forty-five days of estradiol treatment at 25µg/Kg body weight were given to G2, G3, and G4 mice. After completion of dose duration G2 were stopped for examination and the rest G3 and G4 were continued for *Selaginella bryopteris* administration at two different doses for 35 days. Sperm quality, immunoreactive Luteinizing hormone (LH), Folliclestimulating hormone (FSH), and Testosterone (T) were analysed. The outcomes of this study indicated that compared to G1 the estradiol treated G2 had significant ($p<0.001$) alteration in spermcounts, sperm motility, sperm morphology, serum level of LH, FSH, and testosterone. Significant ameliorative effects were observed in G3 and G4 after the administration of *S. bryopteris* extract. Compared to G3 vs. G4; the G4 had better results than G3. The testicular architecture was analysed through histological study of testis revealed disorganization of the cytoarchitecture in the seminiferous tubules, vacuolations, absence of lumen and compartmentalization of spermatogenesis in estradiol treated group. Moreover, compared with LH and sperm density, estradiol significantly suppressed FSH and sperm motility. It is evident that the direct action of estradiol on the testis is mostly responsible for the testosterone reduction. But, after the administration of *S. bryopteris* 150 mg/Kg body weight and 200 mg/Kg body weight there was significant amelioration in all the studied parameters. Among the two doses, the dose at 150 mg/Kg body weight showed best impact on reproductive functions than the dose of 200 mg/Kg body weight. However, the entire study depicts that *S.bryoteris* possess fertility booster activity which can be used for therapeutic purposes.

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

Introduction

Estrogen (estradiol) plays an important role in men's daily health. Level of estradiol and testosteroneare closely related to each other and essentials to balance rest of hormones (Gillies, et al., 2010). Men's bodies produce estradiol in form of estrogen but, its accuracy on male fertility is still unknown (Cooke, et al., 2017). However, estradiol - the predominant form of estrogen plays a precarious role in male sexual function. The estrogen receptor aromatase enzyme converts estrogen (estradiol) to testosterone. This aromatase enzyme found in estrogen producing tissue, such as adrenal glands, brain, fatty tissue, and testis (Rosati, et al., 2021). Estradiol is often referred to as E2, which refers one hormone in a class of hormones called "estrogens". Among some other hormones, this class also includes estradiol, estriol, and estrone (Levin, et al., 2011). Production of estrogen in men's body is completely normal and necessary process (Hess, R. A. 2003). It is essential to be healthy and healthy physiologic functions of brain, and other important organs including erectile function and sex interest, maintenance of bone health, regulation of fat mass vs. lean mass, lipid metabolism and skin metabolism (Hess, 2003; Cooke, et al., 2017; Rosati, et al., 2021).

To understand the estradiol's effect on testosterone, it is important to evaluate its effect on libido atlow and normal level of circulating testosterone (Davis, et al., 2008). The decreased level of testosterone is clearly associated with low libido in males. Many of the research shown that the administration of estradiol increases libido in males who agonised from low level of testosterone (Castello-Porcar, et al., 2016). Furthermore, in a unique case report of a males with aromatase deficiency and hypogonadism, both estrogen and testosterone were required to increase libido, whereas neither hormone could achieve the effect alone (Rochira and Carani, 2009; Kacker, et al., 2012; Tan, et al., 2014; Schulster, et al., 2016). Report suggesting that estrogen plays essential role in sexual desire in the setting of low testosterone. The ratio of testosterone/estradiol (TE) can be used as a proxy for hormonal balance between androgens and estrogens. This ratio has been associated with various health abnormalities including cardiovascular disease, and metabolicdisorders (Schulster, et al., 2016). The elevated level of estrogen increases the incidence of erectile dysfunction (ED). In testis, spermatogenesis is modulated at every level by estrogen, starting with the hypothalamus-pituitary-gonadal axis, followed by leydig, sertoli, and germ cells which finishing with the ductal epithelium, epididymis, and mature sperm (Makela and Toppari, 2017; Das, et al., 2023). The testicular cell regulation by estradiol shows both an inhibitory and a stimulatory influence, including an intricate symphony of dose-dependent and temporally sensitive modulation (Ulloa-Aguirre, et al., 2003; Schulster, et al., 2016).

Elevated level of estradiol in males is associated with metabolic disorder have been implicated in the development of common diseases, including cardiovascular disorders, mental sickness, and stress (Williams, G. 2012; Patel, et al., 2018). Predisposing causes of hyperestrogenism includes diseases of adrenal cortex, testicles, or medications affecting the hypothalamus-pituitary-gonadal axis. Estradiol is also very essential in males for modulating libido, erectile function, and spermatogenesis (Dick, et al., 2020). To control the complex process of spermatogenesis, testosterone and pituitary gonadotropins play a key role along with many other hormones (Singh, et al., 2017; Sengupta, et al., 2019; Santi, et al., 2020). The estrogen (E) hormone has significant contribution in spermatogenesis which is associated with the improvement in semen parameters (Peivandi, et al., 2019). After administration of aromatase inhibitor, it improves sperm concentration, motility and morphology by normalising the T/E ratio (Schulster, et al., 2016; Patel, et al., 2018; Yang, et al., 2022). There is dearth of antitoxic drug are available to combat the side effect of estradiol (Schulster, et al., 2016; Patel, et al., 2018; Dennerstein, et al., 2002; Russell, et al., 2017; Tan, et al., 2019; Nguyen, et al., 2017; Busetto, et al., 2020). Hence, medicinal plant canbe targeted to reduce the effect of estradiol and after the positive response can be recommended as antidote. From the last several decades world revolution started in the form of synthetic medicines,wherever several diseases have been treated well. These synthetic medicines have certain side effects that have been observed after crossing few generations (Abd-Elkareem, et al., 2021). So, there is the need of modern neuropathy i.e. use of those compounds which is extracted from naturalsources. From the mature, numerous plant extracts/metabolites are used throughout India for the improvement of male fertility but there is the need of scientific validation. Therefore, present study has been used to identify the phytochemical influences of *Selaginella bryopteris* (Sanjivani) against antifertility effects of estradiol.

Selaginella bryopteris (Family: Silaginaceae); commonly known as Sanjivani is a lithophytes pteridophytic plant renowned for its remarkable recovery capabilities (Paswan, et al., 2017). These wonder herb plant "Sanjeevani" is referenced in the well-known epic by the Hindi poet-Swami Tulsi Das, making *Selaginella bryopteris* the first known life-giving herb in India (Vashistha, et al., 2021; Gautam, et al., 2023).

Scientifically, it has been declared that this plant has pharmacological effect i.e.; anti-bacterial (Agarwal, et al., 1999), anti-protozoal (Miki, et al., 2008), growth promoter (Sah, 2008), anti-stress cell death (Paswan, et al., 2017), provide relief from heat stroke and burning sensation from urination, memory enhancement, anti-hyperglycemic activity, relief from stomach-ache and anti-depression activity (Kumari, et al., 2014; Rupa, et al., 2014). Clinically, the proven metabolites of this plant act as antioxidants which lowers down the reactive oxygen species (ROS) level significantly. Metabolites of these could be used as treatment of infertility in males (Chandrakant, et al., 2015; Paswan, et al., 2017). The available phytocompounds in *Selaginella bryopteris* (Sanjivani) are: Amentoflavone, (2S)-2,3-Dihydroamentoflovone, Tetrahydroamento flavone, Hinokiflavone, Bilobetin, Heveaflavone, Amentoflavone, Naringeninyl- (4’’’, O, 3) - Kaempferol, and Robustaflavone (Paswan, et al., 2020; Shivaraj, et al., 2021). Traditionally, this herbal medicine possesses great significance have rational background. Therefore, interest in herbal medicines in term of investigating the rationality of their use in modern scientificterms that has been increasing in recent years (Agarwal, et al., 1999; Rupa, et al., 2014; Paswan, etal., 2017). The present study thus aims to study the fertility booster effect of *Selaginella bryopteris* (Sanjivani) against high dose of estradiol exposed Swiss albino mice.

Materials and MethodsChemical Used

The estradiol tablets were purchased from Healing Pharma India Pvt. Ltd., India

Selaginella bryopteris (Sanjivani)

The Sanjivani plant were collected and identified by Dr. K. M. Prabhu Kumar, Senior Scientist and Herbarium Curator (LWG), Plant Division, Systematics and Herbarium Division, CSIR-National Botanical Research Institute (NBRI), Lucknow, India.

Preparation of Sanjivani (*Selaginella bryopteris*) whole plant ethanolic extract

In this present study, plant Sanjivani were collected, cleaned, and washed. Then after plants were grinded to make it in powder form. The next step was to soak the powdered part of the plant in alcohol for 48 hours. For the final ethanolic extract, the powdered leaves were put through to a vacuum evaporation run. The doses of plant were taken for the study as 150mg/Kg and 200mg/Kg body weight after the titration.

Animal Ethics approval

Twenty-four (n=24) male healthy Swiss albino mice weighing 30-35g and their age between 8-12 weeks, were obtained from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, India (IAEC. No. 1129/PO/ReBi/S/07/CPCSEA). Through Institutional Animal Ethics Committee this experimental work was approved by the proposal No. IAEC No. 2021/1A-06/10/21. The collected mice were kept at constant temperature (22 ± 2 ºC) with a regular light and dark cycle with providing nutritious diet and water. Above allotted mice were randomly divided into four groups, each consisting of six animals. Drug was administrated orally using a ball tripped stainless steel gavage attached to a syringe. An individual body weight was obtained for test animals’ prior administration daily.

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

Experimental Design

In line with experimental design; estradiol treatment at 25µg/Kg body weight were given to G2, G3, and G4 mice for 45 days. After completion of dose duration G2 were sacrificed for examination and the rest G3 and G4 were continued for *Selaginella bryopteris* administration at two different doses for 35 days. After completion of *Selaginella bryopteris* administration upon G3 and G4 mice; they were also sacrificed for examination.

Sample collection

The animals in all groups were sacrificed by decapitation method. The collection of blood from the trunk was allowed to clot at 4 ºC then after centrifuge at 3000g for 15min. Separated the serum in eppendorf and frozen at -20 ºC for ELISA test of LH, FSH, and testosterone.

Sperm counts

During dissection of each group; mice Cauda epididymis was trimmed out and washed thoroughly in normal saline (0.85%). The trimmed washed cauda epididymis was punctured in watch glass at several places in 1mL of distilled water as to allow the sperm to ooze out. Then after two drop of eosin Y was mixed gently. The sample were pored gently on Neubauer's chamber and the observation was taken at 450x magnification.

Sperm motility

Each of the experimented mice's cauda epididymis was dissected out and ruptured on microscopic slide. Then after cover slip were gently placed over it and the spermatozoa was examined.

Sperm morphology

Accuracy-based sperm morphology assessment was done by smear preparation, fixation, and staining. Under this experiment Haematoxylin and Eosin (H&E) stain were used for the preparation of sperm smear. After staining process slide were kept for dry and then observed under light microscope.

Hormonal assay

The hormone LH (Luteinizing hormone), FSH (Follicle Stimulating Hormone), and Testosterone levels was analysed by using ELISA method. The ELISA kit was purchased from "Immuno Tag" (trade mark for the ancillary brand of "Geno Technology" Inc. USA).

Histopathological study

Experimented all groups mice testis were fixed into 10% formalin for 24 hours. Then after the tissues were dehydrated through graded ethanol concentration and were embedded into paraffin. The tissues section was grossed at 5µm thickness through digital rotary microtome (Microm HM 340E, Thermo Scientific, USA) and stained with haematoxylin and Eosin (H&E) for investigation of histopathological changes under light microscope.

Statistical analysis

The hormonal concentrations, sperm morphology, count, and motility were subjected to ANOVA. Output was significantly determined by Tukey's test with multiple comparisons. P values ($p<0.05$) considered as statically significant. Calculations were performed through GraphPad Prism program (GraphPad Software, Inc. San Diego, USA).

Results

In this study, the phytochemical influences of *Selaginella bryopteris* (Sanjivani) at two different doses against estradiol upon mice group had significant effect on several parameters which is givenbelow in **Table-1**.

Parameters	G1	G2	G3	G4
Sperm Counts (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 levels of Sperm Motility, Sperm Morphology, and hormones

Effect on Sperm Counts

The estradiol-treated mice had marked reduction in their sperm count in comparison to control. However, the administration of *S.bryopteris*, there was seen significant ($p<0.001$) amelioration which increase the level of sperm count denoting normalisation in the testicular function. Comparedto G3 (administration of *S.bryopteris*, at 150 mg/Kg body weight) vs. G4 (administration of *S.bryopteris*, at 200 mg/Kg body weight); G4 was more reliable and effective than G3 (**Figure -3**).

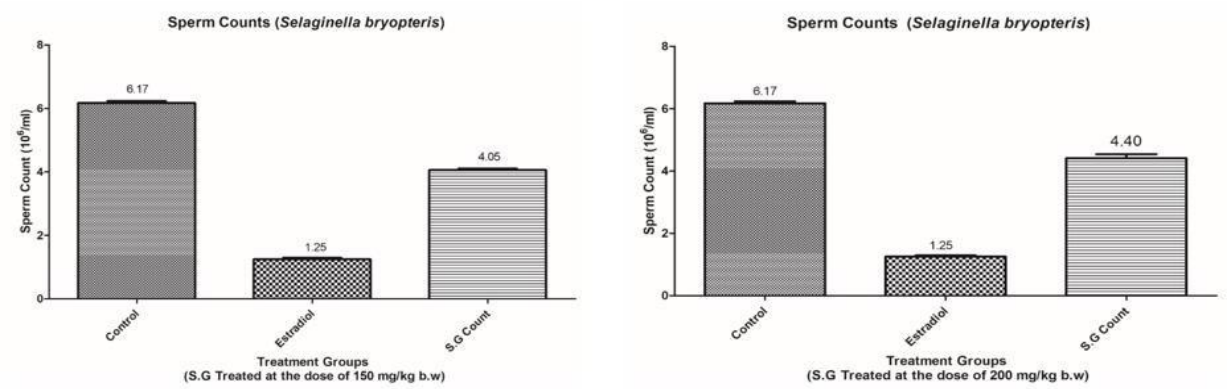

Figure-3: Comparative levels of sperm count in various group of mice (n=6; values are expressed as mean ± SD), significance at $p<0.001$.

Effect on sperm motility

The estradiol-treated mice had marked reduction in their sperm motility in comparison to control (**Figure -4**). The administration of *S.bryopteris*, there was seen significant ($p<0.001$) remediation which accelerate the level of sperm motility denoting good efficiency of movement. Compared to G3 (administration of *S.bryopteris*, at 150 mg/Kg body weight) vs. G4 (administration of *S.bryopteris*, at 200 mg/Kg body weight); G4 was more reliable and effective than G3.

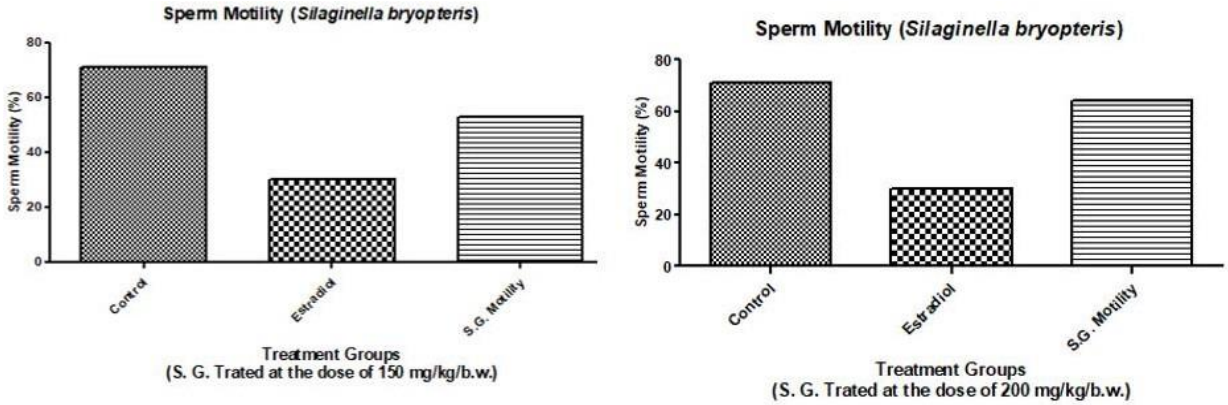

Figure-4: Comparative levels of sperm motility in various group of mice (n=6; values are expressedas mean ± SD, significance at $p<0.001$).

Effect on sperm morphology

The estradiol-treated mice had marked reduction in their sperm morphology in comparison to control (**Figure -5**). The major sperm abnormalities had been observed. Loss of sperm tails, coilingin sperm tails, etc. were seen. However, after the administration of *S.bryopteris* there was seen significant ($p<0.001$) normalisation. Compared to G3 (administration of *S.bryopteris*, at 150 mg/Kg body weight) vs. G4 (administration of *S.bryopteris*, at 200 mg/Kg body weight); G4 had little moreeffective than G3.

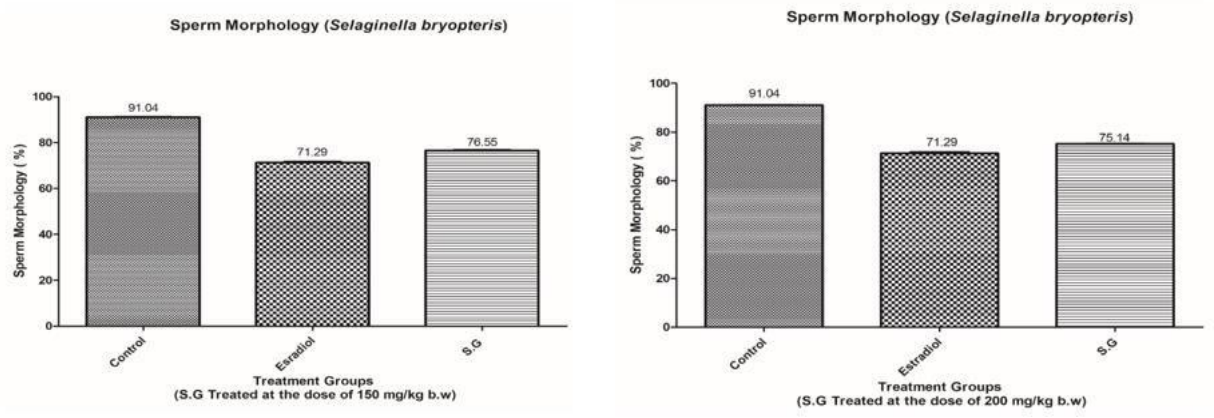

Figure-5: Comparative levels of sperm morphology in various group of mice (n=6; values are expressed as mean ± SD, significance at $p<0.001$).

Effect on Reproductive Hormones

Compared to control group the testosterone (T) tested estradiol-treated mice group had showed significant ($p<0.001$) reduction. After administration of *S.bryopteris* the level of testosterone was significantly ($p<0.001$) increased, denoting normalization in the endocrine function (**Figure -6**). Compared to G3 (administration of *S.bryopteris*, at 150 mg/Kg body weight) vs. G4 (administration of *S.bryopteris*, at 200 mg/Kg body weight); G3 was highly effective than G4.

Testosterone (T) Levels

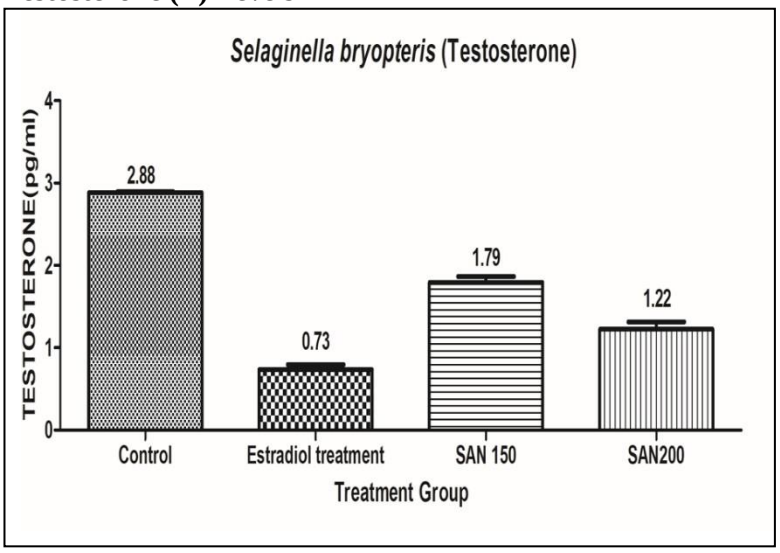

Figure-6: Comparative levels of Testosterone (T) in various group of mice (n=6; values are expressed as mean ± SD), significance at $p<0.001$.

Luteinizing Hormone (LH)

Compared to the control group the Luteinizing Hormone (LH) tested estradiol-treated mice group

significantly ($p<0.001$) had mild reduction. *S.bryopteris* (Sanjivani) extract administration (at the dose of 200 mg/Kg body weight) significantly ($p<0.001$) increased the level of Luteinizing Hormone (**Figure -7**). Compared to G3 (administration of *S.bryopteris*, at the dose of 150 mg/Kg body weight) vs. G4 (administration of *S.bryopteris*, at 200 mg/Kg body weight); G4 was effective where G3 had significant reduction in comparison to estradiol-treated mice group.

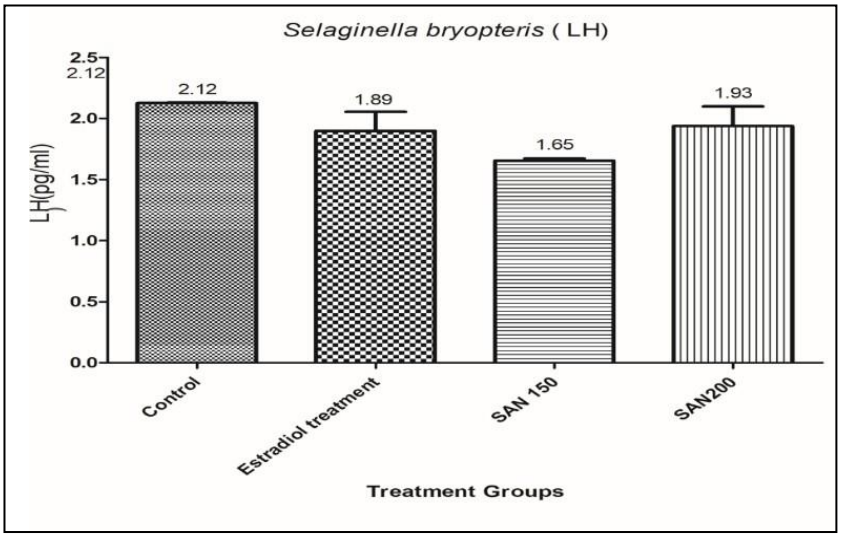

Figure-7: Comparative levels of Luteinizing Hormone (LH) in various group of mice (n=6; values are expressed as mean ± SD, significance at $p<0.001$).

Follicle Stimulating Hormone (FSH)

Compared to control group the level of Follicle Stimulating Hormone (FSH) was significantly ($p<0.001$) reduced. *S.bryopteris* (Sanjivani) extract administration (at 200 mg / Kg body weight) significantly ($p<0.001$) increased the level of FSH Hormone (**Figure -8**). Compared to G3 (administration of *S.bryopteris*, at 150 mg/Kg body weight) vs. G4 (administration of *S.bryopteris*, at 200 mg/Kg body weight); G3 was un-effective not shown any remediation.

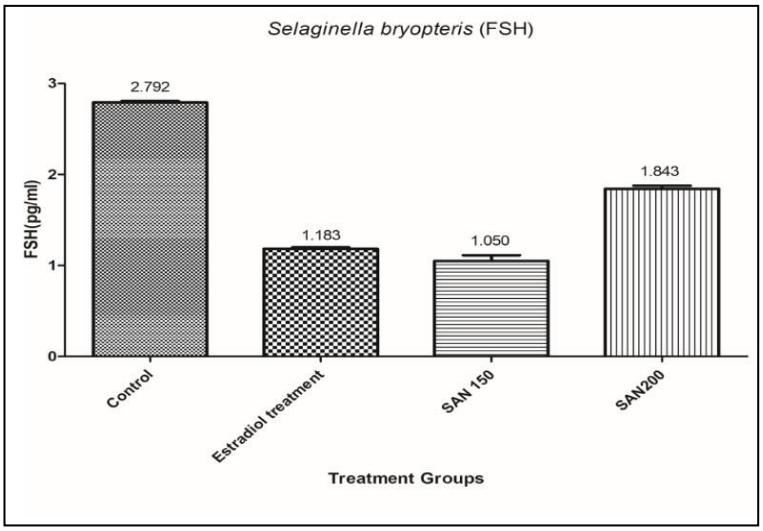

Figure-8: Comparative levels of Follicle Stimulating Hormone (FSH) in various group of mice (n=6; values are expressed as mean ± SD, significance at $p<0.001$).

Histopathological Study

In the Seminiferous Tubules (ST), major spermatocytes, spermatogonia, spermatids (SPM), and spermatozoa are all are well arranged in the control testis of mice testis. Fig. 9A shows that during normal spermatogenesis, the leydig cells that align the inter-seminiferous tubules are functioning precisely. But after estradiol treatment, in the testicular sections there is disrupted functions as onlyleast active spermatogenetic

phases are observed or just 35% of the normal activity Figure 9B. Moreover, there is hemorrhaging in the leydig cells, which indicates that they are likewise in a severely degenerative state. However, after the administration of *S.bryopteris* at the dose of 150mg/Kg b.w, there has been significant restoration is observed as the spermatogenetic stages are restored to their normal state - spermatozoa, spermatids spermatogonia, and the primary spermatocytes, it means that cellular activity has restored to normal Figure 9C. Moreover, the normal leydig cell function is also reinstated. However, after the administration of *S.bryopteris* at the dose of 200mg/Kg b.w, the recovery in the spermatogenesis is mild especially in the primary spermatocytes, but the spermatogonia, spermatid and spermatozoa are much restored Fig. 9D.

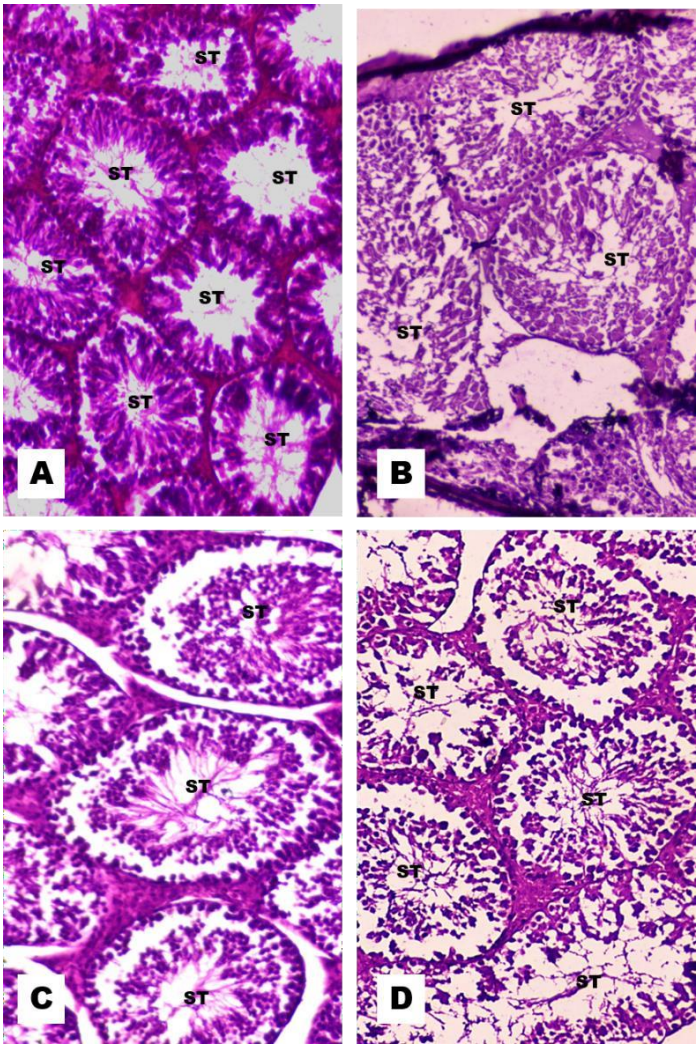

Figure 9: Sections stained with haematoxylin and eosin in a microphotograph of **[A]** A section of the testis of a control mice demonstrating the typical arrangement of the spermatogenetic stages (ST) 500x. **[B]** Testis of Estradiol-treated mice exhibiting significantly reduced spermatogenetic stages (ST) and aberrant architecture of seminiferous tubules 500x. **[C]** Testis of mice administered *S.bryopteris* at the dose of 150mg/Kg b.w restored in function during spermatogenetic stages 500x. **[D]** Testis of mice administered with *S.bryopteris* at the dose of 200mg/Kg body weight shows mild restoration 500x.

Discussion

Depending on the cell type on which it acts, estradiol treatment has been shown to have both inhibitory and stimulatory effects on testicular cells, according to the present research. Estradiol and the addition of other medications have the ability to regulate the magnitude of the effects (Estradiol 2024; 32. Hariri & Rehman 2023). Estrogens are crucial for maintaining the health and functionality of the male reproductive system. Estradiol is the primary biologically active estrogen in males. The aromatization of testosterone serves as the principal origin of this hormone. The testis contains a substantial number of estrogen receptors, including cytochrome P450 aromatase, ERα and ERβ, the membrane-associated G-protein-coupled functional ER (GPER), and ERβ and ERβ, respectively. ERβ and ERβ are responsible for the conversion of testosterone to

estrogen (Bendis et al., 2024; Fietz et al., 2014; Oliveira et al., 2014; Bernardino et al., 2016). Estradiol is synthesized in various epithelial cells, including spermatozoa, immature germ cells, Leydig cells, Sertoli cells, the epithelium of the efferent ductules and the proximal epididymal duct, and Turner et al. (2002); Foucault et al. (1994). Subsequently, estradiol levels in the testis and sperm may surpass those detected in the vasculature of females (Gurun et al., 2023; Bujan et al., 1993; Luboshitzky et al., 2002[a,b]; Naderi et al., 2003; Chieffi et al., 2003). Whether estradiol is produced in cells or not influences its function. Estradiol is recognized for its ability to control various facets of spermatogenesis in testicular cells, such as germ cell proliferation, differentiation, survival, and apoptosis (Fujikura & Fujinoki 2024; Chimento et al., 2010 & 2014; Royer et al., 2014; Pentikainen et al., 2000; MacCalman et al., 2017). Estradiol is implicated in the regulation of Leydig cell function and the modulation of cell communication via the tight junctions of Sertoli cells (Zhai et al., 1996; Abney et al., 1991; Yang et al., 2016). Previous studies have demonstrated that estradiol influences Sertoli cell proliferation, ion transport regulation, and apoptosis regulation (Bernardino et al., 2016; Simões et al., 2013; Chen et al., 2014). Furthermore, it has been established that estradiol impedes the regeneration of Leydig cells and suppresses the production of testosterone bythese cells (Hess et al., 1997). Estradiol is implicated in the reabsorption of fluids within the efferent ductules; consequently, it influences the concentration, motility, and morphology of sperm (Adamczewska et al., 2022). The fact that estradiol exerts both inhibitory and stimulatory effects on testicular cells indicates that its modulation is subtle, dose-dependent, and time-sensitive. Spermatogenesis in testicular seminiferous tubules was significantly impaired in the present study as well, resulting almost exclusively in complete azoospermia.

The hypothalamic-pituitary-testosterone axis controls the release of growth hormone (GH), luteinizing hormone (LH), and testosterone. Bound to receptors in both gonadal and non-gonadal organs, FSH and LH are released by the anterior pituitary. The androgens produced by the Leydig cells in the testes inhibit the pituitary gland and the central nervous system, respectively, to regulate the production of FSH and LH. Androgens' negative feedback impact lowers pituitary reactivity togonadotropin-releasing hormone, which in turn lowers plasma LH level and reduces the amplitudeof the LH pulse (Widyastuti et al., 2020). An increase in testosterone plays a crucial role in sperm quality and quantity. Testosterone controls spermatogenesis by phosphorylating the cAMP response element-binding protein. Our investigation found that compared to the control group, the treated animals' testosterone levels dropped dramatically, and then there was a trend toward lower FSH and LH levels. Although Figure 6 shows that a drop in testosterone may have affected Leydig cell activity, this disturbance did not cause the drop in FSH and LH via a negative feedback mechanism (Chandrakant et al., 2018).

In the present study, there was significant decline in the sperm counts, sperm motility and sperm mortality in arsenic treated group in comparison to the control group. However, there was significant amelioration in the sperm counts, sperm motility and sperm mortality after the treatment with the *S.bryopteris* extract at the two doses – 150mg/Kg b.w and 200mg/Kg b.w. In the hormonal study also there was significant decline in the serum testosterone, FSH and LH levels, but there wassignificant restoration in these levels after the treatment with the *S.bryopteris* extract at the two doses – 150mg/Kg b.w and 200mg/Kg b.w. At the histopathological study of testis, there was significant degeneration in the seminiferous tubules in the spermatogenesis phases – primary spermatocytes, spermatogonia and spermatozoa in comparison to the control testis. However, there was significant restoration in the testis of the *S.bryopteris* extract treated mice testis at the two doses – 150mg/Kg b.w and 200mg/Kg b.w, in the seminiferous tubules. In comparison to the 2 doses of *S.bryopteris* extract, at the dose of 150mg/Kg b.w., the amelioration was much more in comparison to the dose 200mg/Kg b.w.

In the present study, *S.bryopteris* was used as antidote against estradiol caused infertility in mice. Selaginella contains a substantial quantity of phenolic compounds known as "flavonoids" in the form of biflavonoids, which likely contributed to the amelioration caused by *S. bryopteris.* Antitumor, antimalarial, anti-allergic, anti-thrombotic, anti-inflammatory, hypotensive, antibacterial, antioxidant, anti-hepatotoxic, estrogenic, and antiviral properties are all exhibited by these secondary metabolites. These are low molecular weight polyphenolic compounds comprising flavones, flavonols, flavon-3-ols, flavonones, isoflavones, and anthocyanins. Flavonoids have been observed to enhance the effects of vitamin C, ultimately serving as antioxidants. Enzyme activities such as cyclooxygenase, lipoxygenase, and prostaglandin synthase can be inhibited by them. The flavonoid antioxidant activity is directly attributable to the arrangement of functional groups surrounding the nuclear structure (Harbone et al., 2000; Adnan 2001). This denotes that *S.bryopteris* contain these compounds which play vital role in controlling the testicular damage caused by estradiol at sperm morphological level, hormonal level and histological level.

Conclusion: The present thus depicts that the compounds present in *S. bryopteris* possess antifertility activities which can combat the damage caused by estradiol or infertility caused. Moreover, the of *S.bryopteris* extract, at the dose of 150mg/Kg b.w plays vital role in controlling the damage in comparison to the dose of 200mg/Kg b.w. The plant product contains therapeutic compounds which can be used for the novel drug development against infertility.

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