PRECLINICAL EVALUATION OF ETHANOLIC EXTRACT OF *DALBERGIA SISSOO* FOR FEMALE SEXUAL DYSFUNCTION IN EXPERIMENTAL RATS.

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Abstract: In this study, we tested the effect of hydroalcoholic extract of *Dalbergia sissoo* on female sexual dysfunction (FSD) in 30 days postsurgical bilateral ovariectomized female rats. 3-month old female Wistar rats were used and distributed in 5 groups, ovariectomized (Ovx) groups with 30 days, Ovx treated hydroalcoholic extract of *Dalbergia sissoo* (200 & 400 mg/kg), Ovx treated with standard β Estradiol (0.1mg/kg/s.c) and estrous control rats. All treatments were given for further 28 days after post-surgical period (30 days) in ovariectomized female rats. They were evaluated on the 14th day and on 28th day in the copulatory arena with the sexually experienced male rats and for serum estrogen levels. After 28 days of treatment, histopathology of uterus and vagina were quantified. The treatment of the hydroalcoholic extract of *Dalbergia sissoo* (200 and 400 mg/kg) in the Ovx rats shows significance increase in the sexual activity, lordosis, serum estrogen level and histology results. All the results of *Dalbergia sissoo* are comparable with standard β Estradiol. Results suggest that 28 days of treatment with hydroalcoholic extract of *Dalbergia sissoo* is able to increase the sexual function in estrogen deficient females, serum estrogen levels and decreased atrophy of vaginal epithelium and uterus.

Index Terms - Dalbergia sissoo, **Post menopause, Copulatory arena, Serum estrogen level, Histopathology, Atrophy.**

1.0 INTRODUCTION

Estradiol is a predominant female sex hormone in women that helps maintain the integrity of vaginal mucosal epithelium and promotes lubrication. Estrogen plays a major role in regulating sexual function and nitric oxide synthesis in the vagina and clitoris (Rupesh, 2007). It also has vasoprotective and vasodilator effects on the vagina. After menopause, vaginal lubrication and sexual desire and frequency decrease, which may result in vaginismus (Sarrel, 1998)[1]. Adjustment in estradiol levels results in vaginal wall smooth muscles atrophy and can increased vaginal canal acidity, eventually leading to discomfort and stress (Berman and Goldstein, 2001). Estrogen replacement therapy in postmenopausal women has been shown to improve vaginal lubrication and sexual desire. Female sexual dysfunction (FSD) is considered as a significant age-related, progressive and highly prevalent problem that affects a substantial number of women (Renata *et al*, 2010). Women are commonly more affected by sexual dysfunction than men, with one study reporting that 43% of women experienced sexual problems as compared to only 31% of men (Laumann *et al*, 1999).

Loss of interest in sexual activity may occur due to a medial or psychiatric condition, abrupt change in internal hormonal milieu such as major depressive disorder and the initiation of menopause (Lopez *et al*, 2007). A drop-in libido at menopause can be due in part to physical changes, including vaginal dryness or atrophy that can further lead to vaginal pain or irritation, fatigue, sleep disturbances, hot flashes, night sweats, and general health concerns (Rioux *et al*, 2000).

In Indian traditional system of medicine, many herbs have been claimed for its effect on libido in females. However, among these herbs, very few have been scientifically documented so far. Despite the increasing handiness of effective typical medical treatments, plant derived and seasoning remedies still give a preferred different for men and ladies seeking to enhance their sex life due to their effects which is associated with comparatively lesser side effects (Rowland and Tai, 2003).

Dalbergia sissoo (DS), popularly known as shisham in India, is an erect deciduous tree. *D. sissoo* is widely available throughout the Indian subcontinent. Various pharmacological properties of *D. sissoo*, including stimulation of new cell growth and tissue regeneration, have been reported. Phytopharmacological evaluation program aimed at finding an effective alternative therapy for postmenopausal osteoporosis, we recently reported that several phytoestrogens, particularly methoxy isoflavones, were present in the crude extract made from the leaves of *D. sissoo* and exhibited in vitro bone-forming activity (Bijauliya *et al*, 2017). Comprehensive investigation of *D. sissoo* reported to contain estrogenic flavonoids and some sterols with estrogenic activity. The reported results of phytochemical analysis indicated to the presence of flavonoids in *Dalbergia sissoo* (Dixit *et al*, 2012). Thus, the objective of the present study was to evaluate the effect of hydro alcoholic extract of *Dalbergia sissoo* on copulatory behaviors in bilateral ovariectomized induced post-menopausal female rats.

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material:

D. sissoo leaves were collected from the surrounding area of rural Pune during September 2018. The plant was identified and authenticated by M/s. Shamantak Enterprises, Dr. Gautam, Botanist, Pune, India.

2.2 Preparation of Plant Extract:

A weighed quantity (50g) of the air-dried powdered leaves of *D. sissoo* was drawn and then it was extracted with 90% ethanol in a Soxhlet extractor. The hydroalcoholic extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50˚ C to get a solid residue. Different concentration (200mg/kg, 500mg/kg and 1000 mg/kg p.o.) of hydroalcoholic extract of leaves of *D. sissoo* was given according to body weight of animals (Hajare *et al*, 2001).

2.3 Animals:

The study was undertaken at the AISSMS College of Pharmacy, Pune. The Institutional Animal Ethical Committee approved the protocol (CPCSEA/IAEC/PC-08/01-2K18) for the study. Wistar rats of both sexes (200-250g) were used. They were maintained at 25±2º C and relative humidity of 45 to 55% and under reversed light dark cycle (12 h light: 12 h dark cycle) (Johansen *et al*, 2008). The animals had free access to food and water ad libitum throughout study. All experiments were carried out between 9:00 – 16:00 hours.

All rats were free of any toxicity as per acceptable range given by the OECD guidelines up to the dose of 1000 mg/kg. From this data and study reports; two different doses 200 mg/kg and 400 mg/kg were selected for the study.

2.4 Surgical Procedure of bilateral Ovariectomy (OVX):

The acclimatized rats were ovariectomized using the dorsal midline skin incision. The rats underwent surgical procedure after being anesthetized with ketamine (80 mg/kg). Rat was put on its ventral surface and ovariectomy was preceded by a single 2 cm long longitudinal skin incision on the dorsal midline (the hump) and the base of tail. After deep incision the bilateral ovaries were found, surrounded by a variable amount of fat. Ligation of blood vessels was necessary. Both ovaries were identified and then silk thread was tightly tied around the oviduct, including the ovarian blood vessels. The oviduct was cut and ovary was removed, taking good care in leaving the knot intact. The uterine horn was returned into the abdominal cavity. The muscle incision was required suturing with 0 size chromic absorbable catguts. The skin was sutured with non-absorbable silk thread. Broad spectrum antibiotic Neosporin antibiotic powder was used topically after surgery for 15 consecutive days (Waynforth and Flecknell).

2.5 Animal Grouping and Treatment Protocol:

After 3 months, ovariectomized rats were categorized into 5 groups. Group 1 – Negative control (Ovariectomized rats), Group 2 – Estrous Control group (induction of behavioral estrous by subcutaneous administration of 10µg/100g estradiol benzoate 48 hours prior and 500 µg/100g of progesterone 05 hours prior before testing), Group 3 – Ovx treated with Estradiol (0.1 mg/kg/s.c), Group 4 and 5 – Ovx treated with hydroalcoholic extract of *Dalbergia sissoo* (200 and 400 mg/kg/p.o). Treatments i.e. standard Estradiol (0.1 mg/kg/s.c), *Dalbergia sissoo* (200 & 400 mg/kg/p.o), respectively were given for a period of 28 days.

2.6 Training of Male Rats for sexual Experience:

To make sexually experienced, male rats were given 04 training test sessions (twice a week for 2 weeks) with non-experimental receptive females. Only males displaying at least 02 ejaculations and active mounting behaviors during the 04 training test sessions were included in the final experiment (Rossler *et al*, 2006).

2.7 Estimation of Serum Estradiol Level:

Serum Estradiol level measured based on CLIA – Fully Automated Chemiluminescence system on 14th and 28th day of dosing (Yang *et al*, 2004).

2.8 Copulatory Test:

This behavioral test consists of 30 minutes session. Sexually experienced adult male rats were used as copulatory partners. These same partners were paired with each of the experimental female groups, thus controlling potential difference in male responsiveness (Lopez *et al*, 2007). Female rat copulatory signs are further divided into proceptivity and receptivity (Avitsur *et al*, 1999). Measures of proceptivity are Dart, Hop, Ear wiggling, Rejection and Solicitation (Avitsur *et al*, 1999; Agmo *et al*, 2010) while measures of receptivity are Lordosis and Lordosis Quotient (Matuszcyk *et al*, 1998). Male rat copulatory signs included Mount Latency (ML), Mount Frequency (MF), Intromission Latency (IL), Intromission Frequency (IF), Ejaculation Latency (EL), Ejaculation Frequency (EF) and Post Ejaculatory Interval (PEI) (Agmo *et al*, 2010; Tajuddin *et al*, 2004; Kenjale and Sathaye, 2008). All these female and male copulatory signs were evaluated on the 14th and 28th day of the experiment.

2.9 Histopathology of Vaginal tissue and Uterus:

Formalin fixed vagina and uterus tissues were trimmed and processed. Tissue processing was carried out to dehydrate in ascending grades of alcohol, clearing in xylene and fixed in paraffin wax. Paraffin wax embedded tissue blocks were sectioned at 3-5 µm thickness with the Rotary Microtome. Slides were stained with Hematoxylin & Eosin (H & E) stain. The prepared slides were examined under microscope by Pathologist to note histopathological lesions, if any. Distribution of the lesions was recorded as focal, multifocal and diffuse.

2.10 Statistical Analysis:

Statistical analysis was carried out using GraphPad Instat 3. All of the data is shown as the mean ± standard error of the mean (S.E.M) and were analyzed using one-way analysis of variance (ANOVA). Significant differences between the estrous control and experimental groups were determined using Tukey-Kramer test all comparison test, $P<0.001$ was considered significant.

3.0 RESULTS

3.1 Serum Estrogen level:

The result of serum estrogen level is decreased in Ovx group (negative control) as compared to standard group. While the treatment with DS (200 and 400 mg/kg) significantly ($p<0.001$) increased in serum estrogen levels as compared to Ovx group on

the 28th day of dosing as compared to 14th day. The results of the present study are comparable with standard Estradiol. The results are mentioned in the **Table 1**.

Table 1 Effect of DS extract on Serum Estrogen Levels

SERUM E2 LEVEL	ESTROUS CONTROL	NEG CONTROL	STANDARD	DS 200 MG/KG	DS 400 MG/KG
14th Day	51.64±4.794	12.49±1.468	82.45±13.214*	16.12±1.902*	23.15±2.017*
28th Day	65.06±7.519	12.87±1.227	77.085±6.511	22.09±2.883**	27.09±4.416**

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test. *p<0.05, **p<0.001.

3.2 Copulatory Test:

Female rat Copulatory Signs:

Proceptive Parameters: The 14 days pretreatment with DS 200m/kg showed significant equipotent increase in ear wiggling and solicitation (p<0.05 and p<0.001) and thereby exhibited proceptive behavior. Hops and darts of the 200 mg/kg were significantly increased than OVX group. While 28 days pretreatment with DS-200 and 400 mg/kg showed significant increase in hops, darts, ear wiggling and solicitation. DS- 200 mg/kg was found to be more significant than DS- 400 mg/kg. The results are mentioned in **Table 2 and Table 3**.

Table 2 Effect of Ds Extract (After 14 Days of Treatment) On Female Proceptive Parameters

PARAMETERS	ESTROUS CONTROL	NEG CONTROL	STANDARD	DS-200 MG/KG	DS-400 MG/KG
Hops	9±0.7303	3±0.2582	13.16±0.6009**	8.3±0.5578	8.3±0.4773
Darts	7.16±0.6540	2.66±0.3333	3.66±0.8433**	7.83±0.7923	7.66±0.7149
Ear wig.	8.33±0.4944	2.33±0.2108	10.50±0.5627*	5.50±0.2236**	4.833±0.3073**
Solicitation	4.83±0.6009	3.00±0.3651	11.33±0.4644**	6.50±0.5000	7.16±0.4773*
Rejection	1.33±0.3333	3.667±0.3333	0.66±0.3333	0.83±0.3073	0.50±0.3416

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test. *p<0.05, **p<0.001.

Table 3 Effect of DS Extract (After 28 Days of Treatment) On Female Proceptive Parameters

PARAMETERS	ESTROUS CONTROL	NEG CONTROL	STANDARD	DS-200	DS-400
Hops	9±0.5477	2.83±0.3742	14.3±0.400**	9.8±0.3742	8.8±0.3742
Darts	7±0.3162	2.6±0.4000	15.4±0.4000**	9.2±0.5831*	7.6±0.4000
Ear wig.	7.4±0.4000	1.8±0.2000	10.8±0.3742**	6.8±0.3742	4.4±0.2449*
Solicitation	4.33±0.5578	2±0.3651	12.16±0.3073**	7.5±0.4282*	7±0.4472*
Rejection	1.5±0.6191	3.6±0.5099	0.5±0.2236	0.66±0.33	0.66±0.2108

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test. *p<0.05, **p<0.001.

Receptive Parameters: The major receptive behavior i.e., lordosis and the lordosis quotient were significantly improved by DS- 400 mg/kg on the on the 14th and 28th days of treatment. The results are mentioned in the **Table 4 and Table 5**.

Table 4 Effect of Ds Extract (After 14 Days of Treatment) On Female Receptive Parameters

PARAMETERS	ESTROUS CONTROL	NEG CONTROL	STANDARD	DS-200 MG/KG	DS-400 MG/KG
Lordosis	4.66±0.3333	3.33±0.4944	13.16±0.7923**	5.66±0.4944	8.0±0.5164*
LQ (%)	52.91±5.940	35.35±5.279	84.75±1.513**	59.12±2.876	71.38±3.053*

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test. *p<0.05, **p<0.001.

Table 5 Effect of Ds Extract (After 28 Days of Treatment) On Female Receptive Parameters

PARAMETERS	ESTROUS CONTROL	NEG CONTROL	STANDARD	DS-200 MG/KG	DS-400 MG/KG
Lordosis	4.33±0.33	3±0.3651	13.66±0.6146**	8.5±0.7188**	8.16±0.3073**
LQ (%)	42.31±3.658	28.32±2.885	88.15±1.884**	67.64±4.832**	59.26±2.582*

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test. *p<0.05, **p<0.001.

Male rat Copulatory Signs:

Mount Latency (ML) and Mount Frequency (MF): The mount latency of male rats paired with DS treated female rats showed significant (p<0.01) reduction at the doses of 200 mg/kg and 400 mg/kg after 28 days of treatment. But DS 400 mg/kg did improve the number of mounts significantly (p<0.05) in the period of 30 minutes. **(Table 6 and 7)**

Intromission Latency (IL) and Intromission Frequency (IF): All the doses of DS did not show any significant difference in the intromission latency after 14 days and even after 28 days. **(Table 6 and 7)**

Ejaculation Latency (EL) and Ejaculation Frequency (EF): No change was observed in the ejaculation latency of the male rats after the DS treatment, irrespective of the treatment period. However, DS 400 was found to be significant (p<0.05) for ejaculation latency after 28 days. DS-400 mg/kg significantly increased (p<0.05) the ejaculation frequency after 28 days also. **(Table 6 and 7)**

Post Ejaculatory Interval (PEI): No significant changes in the post ejaculatory interval were seen with DS treated on 14the and 28th day of treatment. **(Table 6 and 7)**

Table 6 Effect of Ds Extract (After 14 Days of Treatment) On Male Copulatory Parameters

PARAMETERS	ESTROUS CONTROL	NEG CONTROL	STANDARD	DS-200 MG/KG	DS-400 MG/KG
ML (min)	3.2±0.1585	3.39±0.2013	2.27±41.963	2.91±0.2695	3.38±0.2902
IL (min)	4.48±0.2366	4.51±0.2557	3.37±0.2168*	4.18±0.1668	4.38±0.2719
EL (min)	1.51±0.02565	1.34±0.01856	1.20±0.02525**	1.38±0.01352	1.41±0.01291*
PEI (min)	4.88±0.1830	6.02±0.3539	4.65±0.1478	4.85±0.3541	4.86±0.2538
MF	8.66±0.5578	9.16±0.4773	15.5±0.7638**	9.5±0.4282	11.16±0.3073*
IF	14.33±0.7601	15.66±0.8819	14.66±0.4944	16±0.5774	16.33±0.6667
EF	8.16±0.7923	7±0.5164	8.33±0.4944	8±0.3651	7.5±0.7188

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test. *p<0.05, **p<0.001.

Table 7 Effect of Ds Extract (After 28 Days of Treatment) On Male Copulatory Parameters

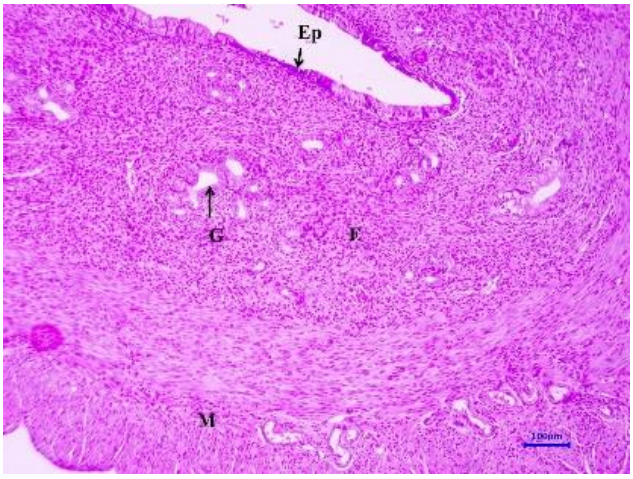
PARAMETERS	ESTROUS CONTROL	NEG CONTROL	STANDARD	DS-200 MG/KG	DS-400 MG/KG
ML (min)	2.765±0.1656	3.06±0.1715	1.94±0.2037	2.41±0.2620	2.82±0.2058
IL (min)	3.76±0.1674	3.85±0.2142	2.93±0.2613	3.29±0.2117	3.79±0.1784
EL (min)	1.31±0.03363	1.43±0.04609	1.05±0.1031*	1.2±0.05285	1.30±0.04022
PEI (min)	4.6±0.2163	5.92±0.2838	4.13±0.1934	4.59±0.3130	4.66±0.2071
MF	10.33±0.5578	12.5±0.4282	15.5±0.6191**	12.5±0.4282*	13.83±0.4014**
IF	13.33±0.8028	15.66±0.6146	12.83±0.4773	14.66±0.6146	15±0.4472
EF	7.5±0.5627	5.66±0.4944	9.16±0.7923	8.66±4944	8.33±0.6146

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test. *p<0.05, **p<0.001.

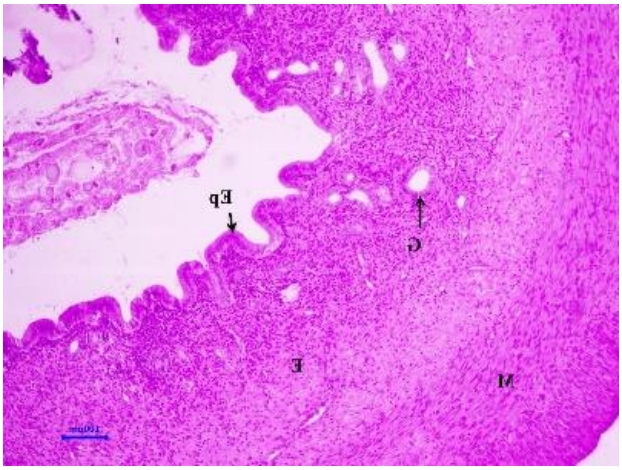
3.3 Histopathological Study:

Vagina and uterus of rats of control group did not reveal any lesion of pathological significance.

Rats treated with standard drug showed multifocal mild hyperkeratosis of vaginal epithelium, whereas no morphological variation is seen in uterus. Ovariectomized rats treated with test drug at 200 mg/kg showed multifocal moderate endometrial atrophy of uterus and atrophy of vaginal epithelium. Ovariectomized rats treated with test drug at 400 mg/kg showed multifocal mild endometrial atrophy of uterus and atrophy of vaginal epithelium. Severity of the atrophic changes is reduced effectively by treatment of test drug at 400 mg/kg by weight. All the Histopathology study images are mentioned **Figure 1 and Figure 2.**



Uterus Control



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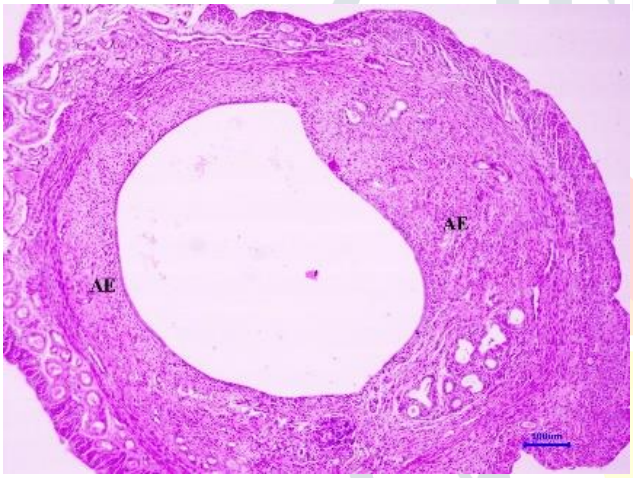
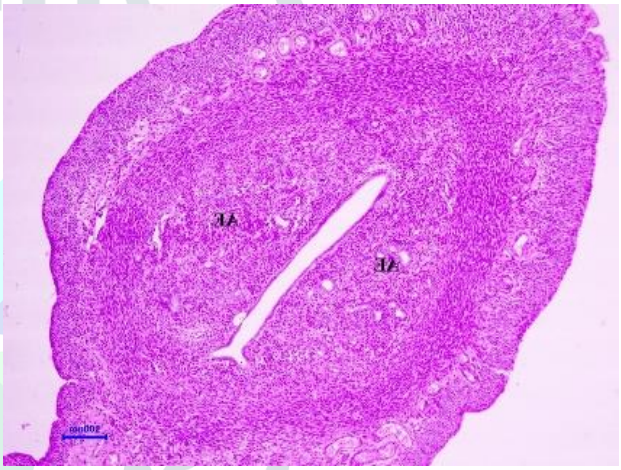
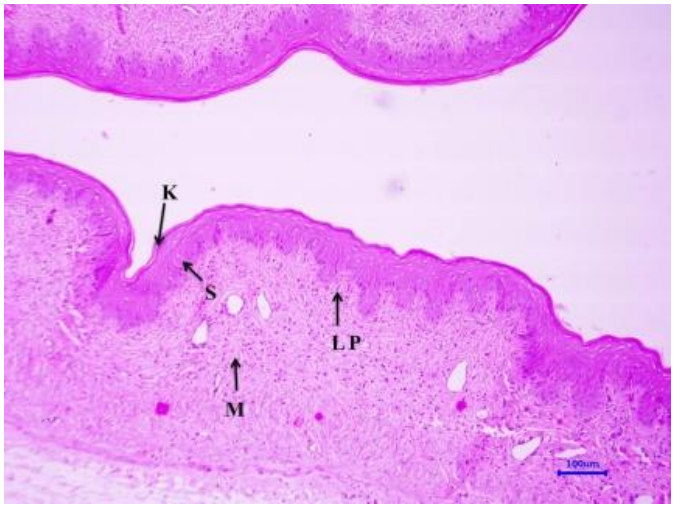


Uterus DS 200 MG/KG

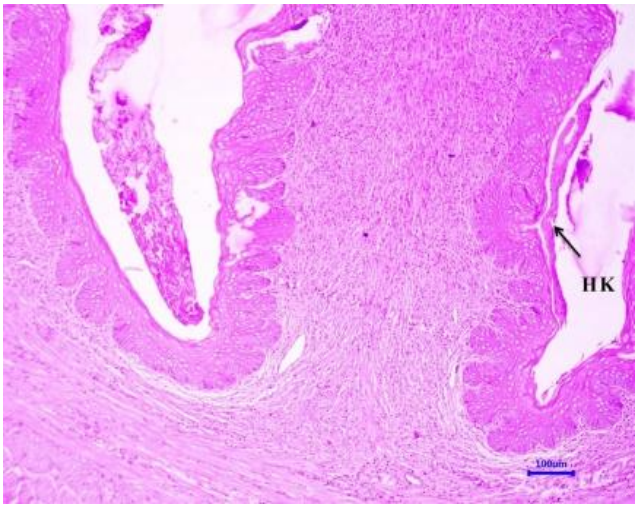


Uterus DS 400 MG/KG

Fig. 1 Histopathological uterus slide stained with Hematoxylin & Eosin (H & E) stain



Vaginal Tissue Control



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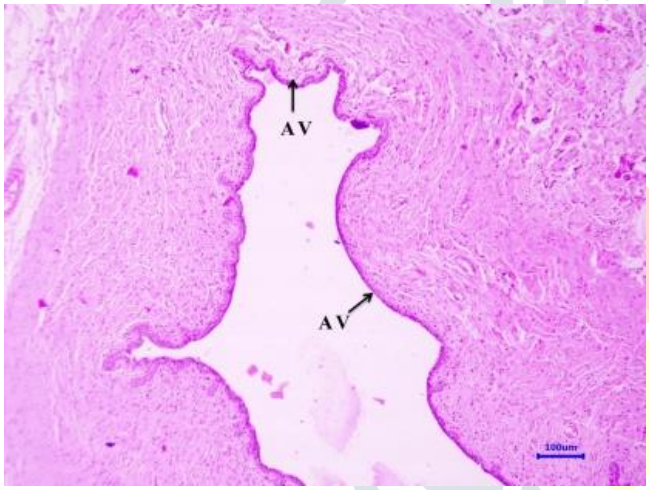
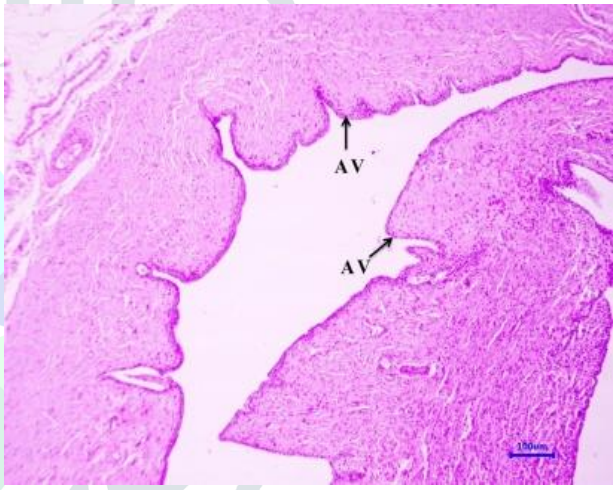


Vaginal Tissue DS 200 MG/KG



Vaginal Tissue DS 400 MG/KG

Fig 2 Histopathological vaginal tissue slide stained with Hematoxylin & Eosin (H & E) stain

4.0 DISCUSSION

The overall purpose of our study was to ascertain whether *D. sissoo* could be useful in correcting the sexual dysfunction caused by post menopause or estrogen deficiency. *D. sissoo* is rich in phytoestrogens (estrogenic flavonoids) and sterols (Khedgikar *et al*, 2012). The traditional systems of medicine like Ayurveda mention several plants claimed to be effective in sexual dysfunction (Al-Snafi, 2017). This has resulted in scientists taking a keen interest in exploring the potential of the plants using scientifically validated methodology (Khadabadi and Bhajipale, 2010). However, unlike in male sexual dysfunction, in female sexual dysfunction scientifically documented evidence is lacking.

In the present study, the efficacy of *D. sissoo* leaves extract was investigated in Female Sexual Dysfunction in rats. Pharmacological model viz. Copulatory test (Mating behavior) was used for screening of *D. sissoo* leaf extract. The rodent model

as selected for this study since, the basic neural and behavioral mechanisms controlling sexual desire and motivation are similar rodents and in humans (Agmo and Soria, 1976). Along with this investigation, supportive studies were also done viz. serum estrogen and histopathological study. These supportive studies were conducted to explore some of the possible mechanisms by which these plants may be effective in FSD.

Evaluating the effect of *D. sissoo* extract on mating behavior in Copulatory arena which is a specialized chamber that provides the rats an environment favorable to mating while also enabling observation by nonintrusive recording system. The two different aspects of sexual behavior displayed by estrous female rats in the presence of sexually active males are sexual receptivity and proceptivity (Beach, 1976). These sexual activities are similar to the arousal in women, which may be classified into genital arousal called as potency and psychological arousal called as libido or motivation. The psychological arousal in women could be very close to proceptivity and hence the study of proceptive behaviors are of great important to preclinically investigate potential of compounds affecting libido and to treat female sexual dysfunction (FSD) (Uphouse, 2014).

To study the aforementioned sexual behaviors in female rats, direct parameters (female rat copulatory behaviors) and indirect parameters (male rat copulatory behaviors) were recorded.

Proceptive behaviors signal readiness to mate and to govern timing of sexual stimulation received by the female (Uphouse, 2014) and receptive behavior is represented by lordosis behavior, which can be quantified by using lordosis quotient or lordosis score. The lordosis quotient is the percentage of time the female exhibited lordosis in response to a sexual contact with the male rat.

Hops, darts, ear wiggling and solicitations, as a measure of proceptive behaviors of female rats were significantly ($p<0.01$) increased by both doses of *D. sissoo* extract (200 and 400 mg/kg) in 28 days of treatment. Lordosis and lordosis quotient (LQ), as a measure of receptive behavior were also increased by both the doses of *D. sissoo*. Proceptivity represents an antecedent condition to the copulatory act. These behaviors have been shown to be dependent on the action of progesterone (Pazol *et al*, 2006; Tunnent *et al*, 1980). Receptive behaviors are those that facilitate the act of copulation of female rats (Uphouse, 2014). It is well known that sexual receptivity in the female rat is dependent on the presence of estrogen (Kow and Pfaff, 2004). Thus, a female rat needs both estrogen and progesterone to display the full complement of sexually motivated behavior (Acosta-Martinez *et al*, 2007).

Also, the female rat has to alert males that she is ready for mating (Lewis, 2018); olfactory signals during estrous phase are used to communicate receptivity (Achiraman *et al*, 2010). Male rat was more attracted by odours derived from clitoral gland during estrous than in other phases of the reproductive cycle (Zhang *et al*, 2008). Therefore, indirect parameters that is male rat parameters like mounting frequency (MF), intromission frequency (IF) and ejaculation frequency (EF) were also significantly increased while intromission latency (IL, ejaculation latency (EL) and post ejaculatory interval (PEI) were significantly reduced when they were placed in copulatory test (30 minutes) with females treated with different doses of DS extract for 28 days.

Both doses of *D. sissoo* extract (200 and 400 mg/kg) showed significant increase in serum estrogen level in female rats as compared to the serum estrogen level of Estrous control and standard group rats. Estrogen regulates eNOS by genomic mechanisms involving mRNA transcription and protein synthesis in vaginal and clitoral tissue. eNOS plays a key role in vascular

function, relaxation, blood flow, engorgement, lubrication, vasodilation homeostasis of vaginal tissue and clitoris of rat, rabbit and human being and it synthesize the nitric oxide (NO) from arginine (NO/ cGMP pathway) which is important for arousal.

Menopause may damage nerve fibers within the lamina propria and muscularis and, in humans, may damage the possible G-spot leading to difficulty in sexual arousal or the achievement of orgasm and thus contribute to dyspareunia and associated issues (Li *et al*, 2017). Thus, in the post-menopausal/ ovariectomized rats' endometrial atrophy of uterus and atrophy of vaginal tissue was seen. Therefore, severity of the atrophic changes is reduced effectively by treatment of test drug at 400 mg/kg.

In summary, the present study provides evidence that the ethanolic extract of *Dalbergia sissoo* is a potent stimulator of sexual behavior, particularly of sexual arousal in post-menopausal/ ovariectomized female rats. On this basis, this extract can be considered to possess libido enhancing properties.

5.0 ACKNOWLEDGEMENT:

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