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TO STUDY AN APHRODISIAC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *WITHANIA SOMNIFERA* DRIED ROOTS IN FEMALE WISTAR RATS

D. Kaspate [*1], A.R. Ziyaurrahman [2], T. Saldanha[1], P. More[1] S. Toraskar [1], K. Darak [1], S. Rohankhedkar [1] and S. Narkhede [1]

AISSMS College of Pharmacy[1], Near R.T.O., Kennedy Road, Pune, Maharashtra- 411001, India
M.C.E. Society's Allana College of Pharmacy [2], Pune Maharashtra- 411001, India

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Correspondence to Author:
Dipti Kaspate

S.NO.229/2, "Sopandev" Buildg., Opp. '"KESAR Sonigara" Buildg., Chhatrapati Chowk, Kaspate wasti, Wakad, Pune-411057, India

E-mail: dkaspate@gmail.com

ABSTRACT: Female sexual dysfunction is a complicated disorder and mostly ignored. Worldwide, reduced sexual function occur in all ages but largest reduction is seen in menopausal women. Now, in the market various conventional medicines are available but they have various side effects and are less efficacious. Therefore the scientists diverted towards the medicinal plants claimed in the traditional systems of medicines. *Withania somnifera*, one of the plants from Ayurvedic medicinal system has been traditionally claimed to enhance the sexual activity in male and female. In females, it has not been scientifically documented like study on male sexual dysfunction. So, in the present study hydroalcoholic extract of dried roots of *Withania somnifera* (100, 200 and 300mg/kg/day, p.o. for 21 days) was investigated for increase in sexual activity in tubal ligated female rats (150-200g). Readings for sexual behaviours were taken on 11th and 21th day by using automated runway and Copulatory arena apparatus. At the end of the study female rats were decapitated, blood samples were collected for estimation of serum estradiol level and different organs like vagina, uterine horn and ovary were removed for histopathology. The results showed increase in sexual behaviours, hormonal level and normal histology of genital organs of female rats.

INTRODUCTION: Libido refers to a fluctuating state of sexual motivation in all organisms wherein there exist constant fluctuations in sexual arousal, desire, reward and inhibition [1, 2]. Sexual motivation is altered by internal, factors like circulating steroidal hormones and feedback from sexual stimulation; external factors, such as the presence of sexually relevant incentives; and by the cognitive processing of these factors that provides variations in sexual arousability and expectation of sexual reward [2]. Female sexual dysfunction is defined as disturbance in sexual functioning involving one or multiple phases of the sexual response cycle or pain associated with sexual activity [3].

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The National Health and social Life Survey found that sexual dysfunction is reported in 43% of women as compared to 31% men signifying that sexual dysfunction is more common in women than men [4].

The past several studies have shown that there are many reasons for female sexual dysfunction and these are multifactorial and combines, psychological, biological factors, interpersonal, and contextual factors [5, 6]. Female sexual dysfunction has strong association with psychological health issues [4, 5, 7, 8] such as depression, anxiety, stress, low self-esteem issues, body image perception disorders, fear of rejection, sexual performance anxiety, traumatic sexual experience in the past, and history of abuse [5, 9]. A second major factor is the quality of the relationship [5, 7]. Biological risk factors including medical conditions like urogenital, neurological, and endocrine disorders, pelvic floor disorders, menopause, pregnancy,

obesity, diabetes, hypertension [5,10] as well as pharmacological and other therapies like antineoplastic agents, antipsychotic, antidepressant, antianxiolytic, antihypertensive agents and major surgical operations, radiation therapies [11,12]. Other reasons are limits social reasons, financial difficulties, religious beliefs, educational backgrounds, employment status and lack of exercise [5, 13].

Withania somnifera has chosen for female sexual dysfunction because along with aphrodisiac activity it has many other activities to cure some psychological and biological risk factors of female sexual dysfunction. That is *Withania somnifera* also provides other health benefits beyond those specifically related to sexual performance and therefore there is a need to use *Withania somnifera* for female sexual dysfunction.

Withania somnifera (Solanaceae) is a semiwoody or perennial shrub growing in several tropical, subtropical countries. It has been used as an aphrodisiac, appetizer activity, anti-oxidant, anxiolytic, anti-aging, antibiotic activity, anticonvulsant activity, anti-depressant, anti-diabetic, anti-inflammatory, anti-hypertensive, antimicrobial, antiparkinsonian, anti-stress, cardioprotective, chondroprotective activity, hypolipidemic, hypothyroidism, immune modulating agent, nootrpic activity, rejuvenator, testicular development, thyroid dysfunction, etc. [14-18].

Phytochemically *Withania somnifera* has different alkaloids like Withaninine, Somniferine, Somniferinine, Somninine, Withasomine, and steroidal lactones like Withaferin-A, Withanone, Withanolide E-M and steroids like cholesterol, β-sitosterol, Sitoinosides VII-X and flavanoids [15,19].

MATERIALS AND METHODS:

Plant material:

The hydroalcoholic extract of dried roots of *Withania somnifera* was obtained from M/S. Shamantak Enterprises, Pune. Certificate of Authentification number of *Withania somnifera* is APCP/30/2013-14.

Preparation of Drug Solution:

Stock solution was freshly prepared daily by using the hydroalchoholic extracts of *Withania somnifera* in distilled water before dosing from which the different doses were administered by selecting the appropriate concentration**.**

Chemicals and drugs:

Ketamine Hydrochloride Injection (Aneket, Neon Laboratories Limited, Mumbai), Xylazine Hydrochloride Injection (Xylazine, Indian Immunologicals Ltd., Hyderabad), Framycetin cream (Soframycin, Safoni India Ltd., Goa), Estradiol benzoate AR (Estradiol Benzoate E.P. Analab Fine Chemicals, Mumbai) and Progesterone AR (Progesterone For Biochemistry, HiMedia Laboratories Pvt. Ltd., Mumbai), Sigma-Aldrich, USA), Propylene glycol (1,2-Propanediol Purified, Merck Specialities Private Limited, Mumbai), Sesame Oil (Research-lab fine chem. Industries, Mumbai), surgical cotton thread, surgical cotton, were purchased from New Neeta Chemicals, Pune,

Animals:

Healthy female Swiss albino mice (18-22g) were used for acute oral toxicity study and healthy female wistar rats were used for sexual behavioural study. They were maintained at 25±2 ºC, relative humidity of 45 to 55% and under standard environmental conditions (12 hrs light and 12 hrs dark cycle). The animals had free access to food (Nutrivet life sciences, Pune) and water ad libitum. Institutional Animal Ethical Committee approved the protocol. All the experiments were carried out between 9:00am-16:00pm.

Acute Oral Toxicity Study:

Acute toxicity study was performed in healthy female Swiss albino mice (150-200g) as per guidelines (AOT 425) suggested by the Organization for Economical Cooperation and Development (OECD). The rats were observed continuously for 4 hr for behavioural and autonomic profiles and for any other sign of toxicity or mortality up to a period of seven days.

Surgery:

The oviduct of female rats were ligated 14days prior to starting of the experiment using standard aseptic surgical techniques under deep anaesthesia. Female rats were anaesthetized with ketamine

(90mg/kg, i.p.) and Xylazine (10mg/kg, i.p.). In tubal ligation, a knot was tied up in between ovary and uterine horn by surgical cotton thread. Reason for ovary ligation in female rats is to avoid unwanted pregnancies during the course of experiment, so that they can be used for desired time period of study without any interruption in their hormonal levels, which occurs during their pregnancy period and it make possible to study the ovarian follicles after treatment.

Induction of behavioural estrous:

For induction of behavioural estrus, tubal ligated female rats were subcutaneously (SC) administered with 10µg/100g body weight estradiol benzoate (estradiol benzoate; in 0.1ml sesame oil) 48 hrs and 500µg/100g body weight of progesterone (progesterone; in 0.1ml propylene glycol) 05 hrs before testing for 11th day and 21th day [9, 20, 21].

Equipments used:

Automated Runway Apparatus (VJ Instrumnets, India) and Copulatory Arena (VJ Instrumnets, India).

Experimental Procedure:

Automated Run-Way Apparatus:

Motivation testing took place within two straight-arm runway consisting of a startbox (25×25×20 cm), an alley (160×10×20cm), and a cylindrical Plexiglass goalbox (50cm diameter×30cm height). **Fig. 1** [9] and **Fig. 2** show the schematic diagram and photograph of the runway apparatus respectively. Removable Plexiglass doors were located between the starbox, and alley and between the alley and goalbox. Within the goalbox, a removable Plexiglass partition divided the arena into two semicircular halves. Thirty-five diameter holes of 1 cm drilled into the partition allowed air to pass between the two sides of the goalbox. This partition prevented tactile, although visual, auditory, and olfactory cues were accessible.

Three infrared photocell emitter-detector sensor pairs were placed within the apparatus to detect rat motion. Sensor #1 was located just outside the startbox and was triggered when the rat entered the alley. Sensor #2 was located within the goalbox (15cm from the entry) and was triggered when the rat' entire body was within the goalbox. These two sensor pairs were linked to an electronic timer that recorded "run time." This timer started when the rat triggered sensor #1 and stopped when the animal triggered sensor #2. Sensor #3, located within the alley (25cm from the goalbox entry), became active only after an initial goalbox entry. Sensor #2 and #3 allowed for measurement of rat's "proximity time."

A second electronic timer started when the rat first entered the goalbox and triggered sensor #2. If the rat left the goalbox and triggered sensor #3, the timer stopped. If the rat re-entered the goalbox and triggered sensor #2, the time would start again. This continued for a period of 3 minutes, following the initial entry of the rat into the goalbox [9]. In addition to proximity time, retreat also counted. Retreat defines as a complete return to the startbox after the rat had entered the goalbox. Every time the rat made a circuit between sensor #2 (goalbox) and sensor #1 (startbox), an electronic counter increased by one [22].

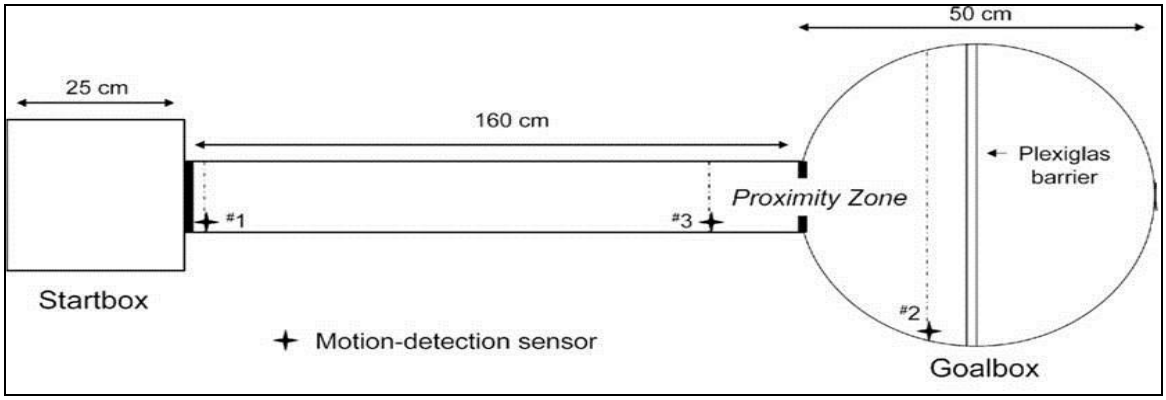


FIG.1: SCHEMATIC DIAGRAM OF AUTOMATED RUNWAY APPARATUS TO ASSESS SEXUAL MOTIVATION IN RATS. SENSORS #1 AND #2 ALLOWED FOR THE MEASUREMENT OF RUN TIME, WHILE SENSORS #2 AND #3 ALLOWED FOR THE MEASUREMENT OF PROXIMITY TIME [9].

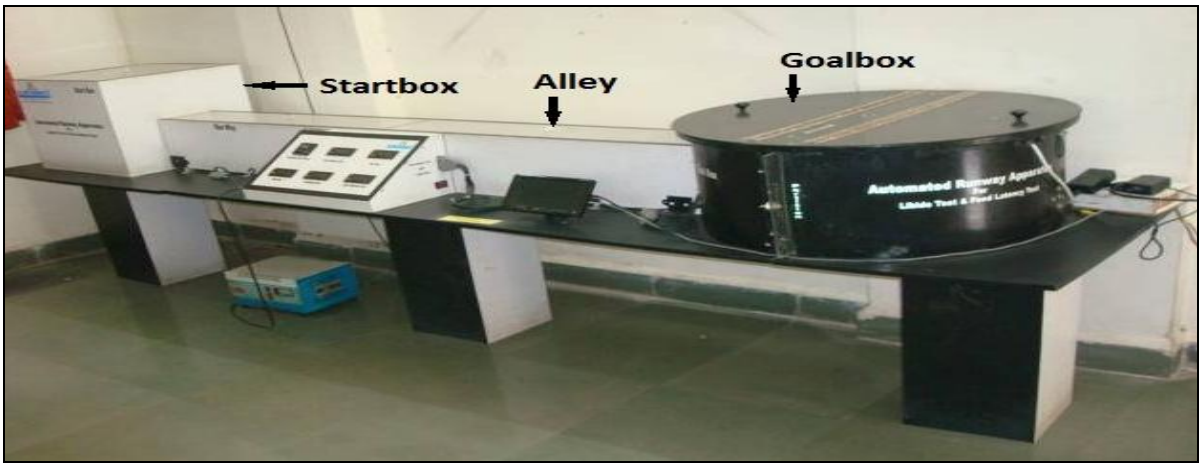


FIG. 2: AUTOMATED RUNWAY APPARATUS TO ASSESS SEXUAL MOTIVATION IN RATS.

Performance in the automated run-way apparatus:

Female rats were habituated to the empty run way apparatus for 10 minutes on two consecutive days. Baseline socio-sexual motivation of 24 female rats was measured over the next 6 days. Each rat was tested in a nonestrous state for their motivation to maintain close proximity to one of three different goalbox: an adult sexually activated male rat, nonestrous female rat, or an empty goalbox. Rats did not receive hormone or drug treatment. On any given day, all rats ran for the same target in the goalbox; only one trial per day per rat was conducted. Thus, rats run for each goalbox target twice during the baseline phase. The order of goalbox targets was randomly determined.

Following completion of the baseline phase, rats were divided into required experimental groups such that mean baseline run times and proximity times were approximately equal between groups. Rats were orally treated as per given **Table 1** and then re-tested in the runway for their motivation to approach the same three goalbox targets (empty, female, male) under estrous phase and their run time, proximity time, and retreats were measured for the period of 03 minutes on 11th and 21st. After 03 minutes period expired, the rat was removed from the runway and returned to their environment. The entire runway apparatus was cleaned with a 10% ethanol solution at the end of each trial [9].

TABLE 1: DRUG TREATMENT SCHEDULE FOR ESTROUS FEMALE RATS BY AUTOMATED RUN WAY APPARATUS.

Group. no.	Treatment for 21 days	Readings on 11th and 21st day against 3 Goalbox targets		
		Empty	Male	Female
1.	Estrous Control (distilled water as a vehicle 10 ml/kg, p.o.)		n=6	
2.	WS (100mg/kg, p.o.)		n=6	
3.	WS (200mg/kg, p.o.)		n=6	
4.	WS (300mg/kg, p.o.)		n=6	

WS-Withania somnifera, n-showing that number of female rats

Copulatory Arena (Mating Behavior):

Copulatory tests took place within cylindrical Plexiglass arena.

Selection of male rats for inclusion in the study:

To make sexually experienced, male rats were given 4 training sessions (twice a week for 2 weeks) with receptive females for the period of 30 minutes. Only males displaying at least 2 ejaculations during the 4 training test sessions were included in the study [23]. Female rats were divided into 04 groups with 06 rats in each group. They were orally treated as shown in the **Table 2** for the period of 14 days. On 11th and 21st day, 01 hr after the respective treatments, the sexually-experienced, adult male rat was placed in a rectangular plexiglass chamber, 10 minutes before the introduction of treated female rat, for it to get acclimatized to the chamber. Ten sexually-experienced, adult male rats were used as copulatory partners for all gropus. These same 10

partners were paired with each of the experimental female groups, thus controlling for potential differences in male responsiveness. The treated, estrous, tubal ligated female rat was then introduced into the chamber. During 30 minutes of test period, following female proceptive (i.e. hops, darts, ear wigglings and solicitations) and receptive (i.e. lordosis and lordosis quotient) behaviours along with male rat behaviour were observed. Copulatory arena were cleaned with a 10% ethanol solution after completion of test [9].

TABLE 2: DRUG TREATMENT SCHEDULE FOR COPULATORY TEST.

Group. No.	Group (n=6)	Treatment 21 days
1.	Estrous Control	Estrous Control-vehicle (distilled water) 10 ml/kg, p.o.
2.	WS1	WS-100mg/kg, p.o.
3.	WS2	WS-200mg/kg, p.o.
4.	WS3	WS-300mg/kg, p.o.

WS-*Withania somnifera*, n-number of female rats

The following parameters were observed in mating behaviour for treated female rats

Measures of proceptivity:

Proceptivity represents an antecedent condition to the copulatory act [24].

1) **Hop:** It is the short lip or jump with stiff legs followed by immobility in which female is landing on all four paws, followed by assumption of crouching posture [25, 26]. The distance covered by one hop by the hind feet is approximately equivalent to the whole length of the extended body [27].

2) **Dart:** It corresponds to a run of several steps, typically 3-5 steps, abruptly terminated by assumption of crouching posture [26].

3) **Ear wiggling:** Rapid lateral shaking of the head causing the appearance of distinctive anteroposterior ear vibrations [24, 26, 28].

4) **Solicitation:** It corresponds to head wise orientation of female towards the male followed by abrupt run away [2].

Measures of receptivity:

Receptive behaviours are those that facilitate the act of copulation [24].

1) **Lordosis:** Lordosis characterized by a crouching, sway-backed posture, with the tail flicked to the side to facilitate the male mounting and intermitting [24, 29].

2) **Lordosis Quotient:** A lordosis quotient can be computed by dividing the number of lordosis (dorsiflexion of back in response to mounting) with total number of mounts and multiplying this ratio with 100 [29].

The following parameters were observed in mating behaviour for sexually active male rats:

1) Mounting Frequency (MF): The number of mounts without intromission from the time of introduction of the female until ejaculation [20].

2) Intromission Frequency (IF): The number of intromissions from the time of introduction of the female rat until ejaculation [20].

3) Ejaculation Frequency (EF): The number of ejaculations in a given period of time [30].

4) Mounting Latency (ML): The time interval between the introduction of the female rat and the first mount by the male rat [20, 30].

5) Intromission Latency (IL): The interval from the time of introduction of the female rat to the first intromission by the male rat [20].

6) Ejaculation Latency (EL): The time interval between the first intromission and ejaculation [20].

7) Post-Ejaculatory Interval: The time interval between first ejaculation and the first intromission of the following series [20].

Serum Estradiol Levels Assay: After evaluation of socio-sexual motivation and mating behaviours

female rats were scarified by decapitation. Then, their blood was collected by cardiac puncture method. Serum for determine estradiol level was separated by centrifugation at 1000 rpm for 10 minutes at room temperature and kept at -80 ºC until analysis. Serum estradiol levels were measured by using Chemiluminescence Immunoassay (CLIA) with Advia Centaur at Dr. Lal Path Labs, Pune.

Histological Analysis:

After decapitation and collection of blood, genital organs like vagina, uterine horn and ovary were removed. Then fixed organs in 10% buffered formalin, processed routinely and stained with hematoxylin and eosin. Histological sections were prepared and examined with Image Microscope (Magnus, Pune) under 4X, 10X, 40X magnification values for identification of any effects caused by *Withania somnifera* (WS).

Statistical Analysis:

The results were expressed as mean±SEM (n=6). The data was analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. Significance set at *P<0.05, **P<0.01.

RESULT:

Acute Oral Toxicity Test:

All mice were free of any toxicity as per acceptable range given by the OECD guideline up to the dose 2000mg/kg. From this data and pilot study reports; three different doses 100, 200 and 300mg/kg were selected for further study.

Automated Run Way Methodology Performance:

Baseline phase: After completion of the baseline phase **Table 3**, rats were divided into groups such that mean baseline runtimes and proximity times were approximately equal between groups.

TABLE 3: BASELINE READINGS OF FEMALE RATS.

Parameters	Baseline readings of female rats with no hormone and no treatment against 3 goalbox targets		
	Empty goalbox	Male goalbox	Nonestrous female Goalbox
Run Time	39.18±2.13	26.22±1.14	27.26±1.13
Proximity Time	62.97±2.80	79.12±2.59	81.53±3.17
Retreats	12.41±0.73	6.83±0.38	8±0.35

Values are expressed in Mean±SEM (n=6),
n-number of animals

Effect of *Withania somnifera* (WS) extract on automated run way methodology performance of female rats estrous state:

For study of all parameters, 21 days of treatment was done. Dosing was performed 1 hr prior to the 30 minutes test.

Run Time:

Withania somnifera (WS) at all doses significant decreased the run time (P<0.05, P<0.01) of estrous female rats as compared to Estrous Control female rats for male goalbox. **Table 4, Fig.3**.

TABLE 4: EFFECT OF *WITHANIA SOMNIFERA* (WS) EXTRACT ON RUN TIME OF ESTROUS FEMALE RATS FOR THREE GOALBOX TARGETS.

Treatment (mg/kg)		Run Time (seconds)		
		Empty target	Male Target	Female Target
Estrous Control	Vehicle (10ml/kg)	23.56±0.37	16.37±0.36	17.55±0.34
After 11 days Trestment	WS-100mg/kg	22.75±0.540	14.55±0.51*	16.48±0.31
	WS-200mg/kg	22.53±0.75	13.06±0.43**	16.23±0.33
	WS-300mg/kg	22.66±0.29	12.15±0.46**	16.2±0.21
After 21 days treatment	WS-100mg/kg	22.35±0.35	13.71±0.50**	16.68±0.28
	WS-200mg/kg	21.9±0.58	12.61±0.41**	16.41±0.26
	WS-300mg/kg	22.05±0.59	11.21±0.34**	16.31±0.19

Values are expressed a mean±SEM (n=6).*P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

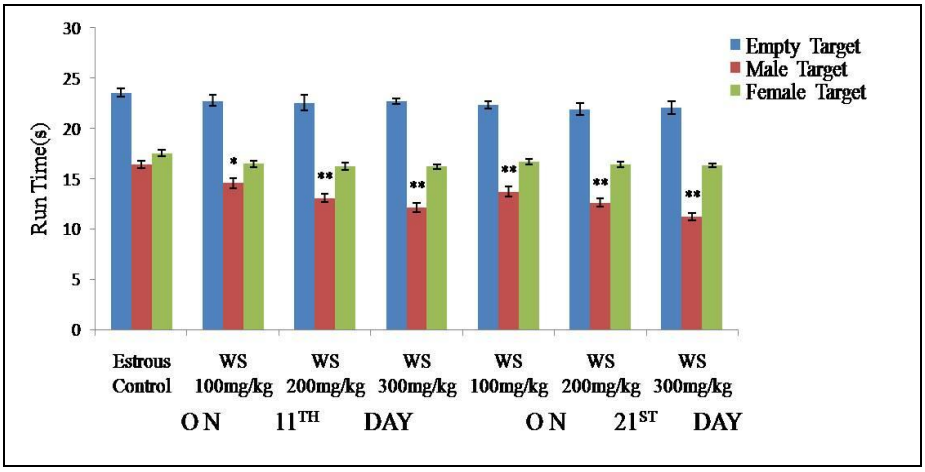


FIG. 3: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON RUN TIME OF ESTROUS FEMALE RATS FOR THREE GOALBOX TARGETS.

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

Proximity Time:

After 11 days of treatment, *Withania somnifera* (WS) at doses of 200 and 300mg/kg significant improved the proximity time (P<0.05, P<0.01) and after 21 days of treatment all doses of *Withania somnifera* (WS) improves the proximity time (P<0.05, P<0.01) of estrous female rats as compared to Esrous Control female rats for male goalbox target. See **Table 5, Fig.4**.

TABLE 5: EFFECT OF *WITHANIA SOMNIFERA* (WS) EXTRACT ON PROXIMITY TIME OF ESTROUS FEMALE RATS FOR THREE GOALBOX TARGETS.

Treatment (mg/kg)		Proximity Time (seconds)		
		Empty target	Male Target	Female Target
Estrous Control	Vehicle (10ml/kg)	67.01±1.41	97.03±1.88	93.52±1.79
After 11 days treatment	WS-100mg/kg	65.95±1.14	103.4±0.85	93.81±0.61
	WS-200mg/kg	67.83±0.84	112.96±1.61**	94.95±0.72
	WS-300mg/kg	67.56±1.57	123.76±1.80**	95.53±1.63
After 21 days treatment	WS-100mg/kg	66.1±1.82	115.51±1.34**	95.15±1.58
	WS-200mg/kg	67.66±1.59	126.21±1.35**	96.25±0.45
	WS-300mg/kg	68.6±0.78	131.21±0.96**	97.88±0.65

Values are expressed a mean±SEM (n=6).*P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test).WS-*Withania somnifera*, n-number of animals

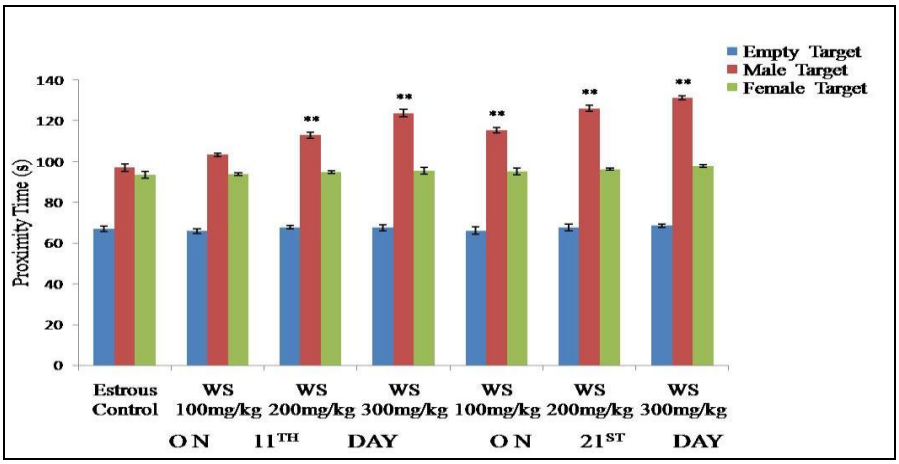


FIG. 4: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON PROXIMITY TIME OF ESTROUS FEMALE RATS FOR THREE GOALBOX TARGETS.

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test).WS-*Withania somnifera*, n-number of animals

Retreat: After 21 days of treatment, *Withanis somnifera* (WS) at all doses significant decreased the retreats (P<0.05, P<0.01) of estrous female rats as compared to Estrous Control female rats for male goalbox target. See **Table 6, Fig. 5**.

TABLE 6: EFFECT OF *WITHANIA SOMNIFERA* (WS) EXTRACT ON RETREAT OF ESTROUS FEMALE RATS FOR THREE GOALBOX TARGETS.

Treatment (mg/kg)		Retreat (number)		
		Empty Target	Male Target	Female Target
Estrous Control	Vehicle (10ml/kg)	11.22±0.43	7.22±0.31	7.38±0.42
After 11 days treatment	WS-100mg/kg	10.5±0.49	7±0.57	7.16±0.60
	WS-200mg/kg	10.5±0.42	6.5±0.42	6.66±0.49
	WS-300mg/kg	10.16±0.49	6.16±0.22	6.33±0.42
After 21 days treatment	WS-100mg/kg	10±0.44	5.83±0.40*	6.83±0.60
	WS-200mg/kg	9.66±0.66	5.83±0.30*	6.16±0.47
	WS-300mg/kg	9.5±0.42	5.16±0.47**	6±0.36

Values are expressed a mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

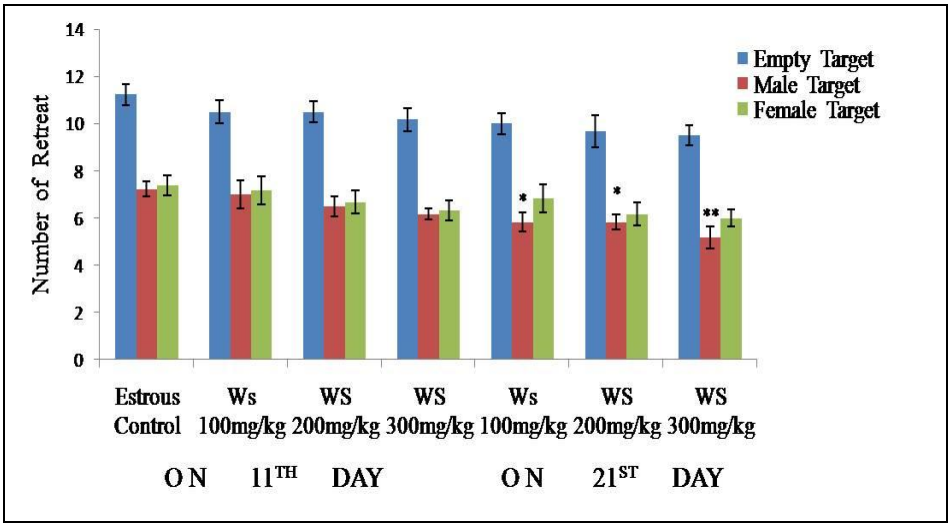


FIG.5: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON RETREAT OF ESTROUS FEMALE RATS FOR THREE GOALBOX TARGETS.

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

Mating Behaviour in Copulatory Arena:

Female rat Copulatory signs:

Proceptive Behaviours: *Withania somnifera* (WS) at all doses significant improved (p<0.05, p<0.01) hops, darts, ear wiggling and solicitation of estrous female rats for male partner. See **Table 7, Fig. 6.**

TABLE 7: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON FEMALE PROCEPTIVE PARAMETERS RATS.

Treatment (mg/kg)		Hops	Darts	Ear wiggling	Solicitation
Estrous Control	Vehicle (10ml/kg)	19.5±0.42	17.5±0.88	18.33±0.42	4.66±0.33
After 11 days treatment	WS-100mg/kg	25.33±0.33**	22.33±0.42**	23.16±0.40**	9±0.51**
	WS-200 mg/kg	25.83±0.40**	23.83±0.47**	24.16±0.74**	10.16±0.30**
	WS-300 mg/kg	26.66±0.33**	26±0.51**	26.83±0.47**	11.66±0.49**
After 21 days treatment	WS-100 mg/kg	29.66±0.21**	26.16±0.54**	27±0.51**	10.5±0.42**
	WS-200 mg/kg	10.5±0.42**	28±0.51**	27.33±0.71**	11.66±11.66**
	WS-300 mg/kg	30.5±0.56**	30.66±0.42**	31±0.44**	13±0.57**

Values are expressed a mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

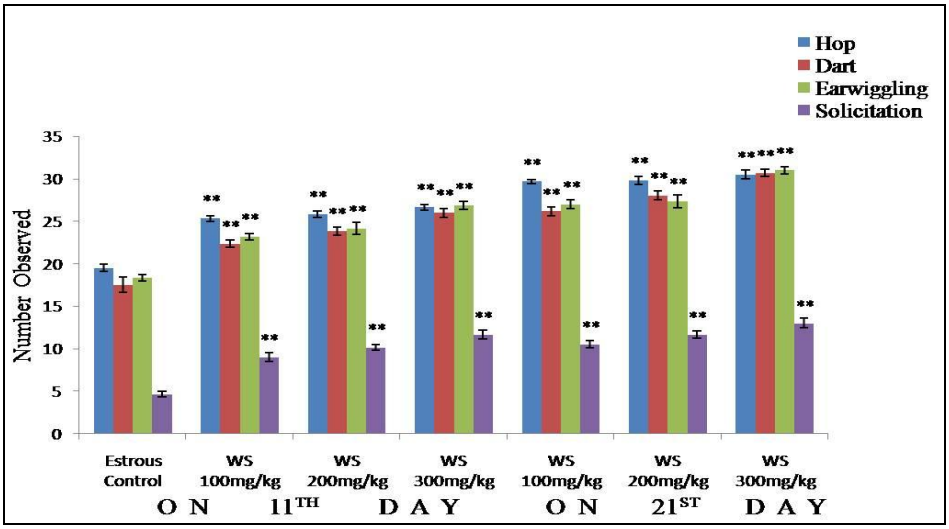


FIGURE 6: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON HOPS, DARTS AND EAR WIGGLING OF ESTROUS FEMALE RATS.

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rat (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

RECEPTIVE BEHAVIOURS: LORDOSIS: No significant effect was observed by any of doses of *Withania somnifera* (WS) on Lordosis. See **Table 8, Fig.7.**

TABLE 8: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON LORDOSIS OF ESTROUS FEMALE RAT.

Treatment		Lordosis
Estrous Control	Vehicle (10ml/kg)	19.5±0.5
After 11 days treatment	WS-100mg/kg	20.83±0.30
	WS-200mg/kg	20.66±0.49
	WS-300mg/kg	20.83±0.30
After 21 days treatment	WS-100mg/kg	20.66±0.33
	WS-200mg/kg	20.66±0.49
	WS-300mg/kg	20.5±0.22

Values are expressed a mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

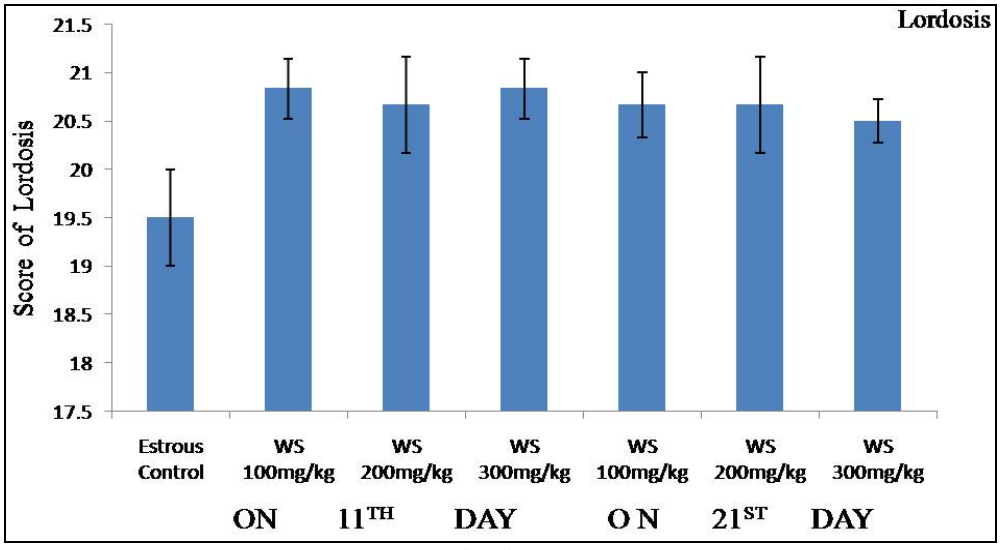


FIG.7: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON LORDOSIS OF ESTROUS FEMALE RATS.

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

Lordosis Quotient: After 11 and 21days of treatment only 300mg/kg dose of *Withania somnifera* (WS) treated estrous female rats showed increase in Lordosis Quotient. See **Table 9, Fig. 8.**

TABLE 9: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON LORDOSIS QUOTIENT OF ESTROUS FEMALE RAT.

Treatment		Lordosis Quotient (%)
Estrous Control	Vehicle (10ml/kg)	84.79±0.83
After 11 days treatment	WS-100mg/kg	86.24±1.32
	WS-200mg/kg	88.64±1.25
	WS-300mg/kg	89.27±0.96*
After 21 days treatment	WS-100mg/kg	87.30±0.17
	WS-200mg/kg	87.93±1.26
	WS-300mg/kg	89.15±0.90**

Values are expressed a mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera* n-number of animals

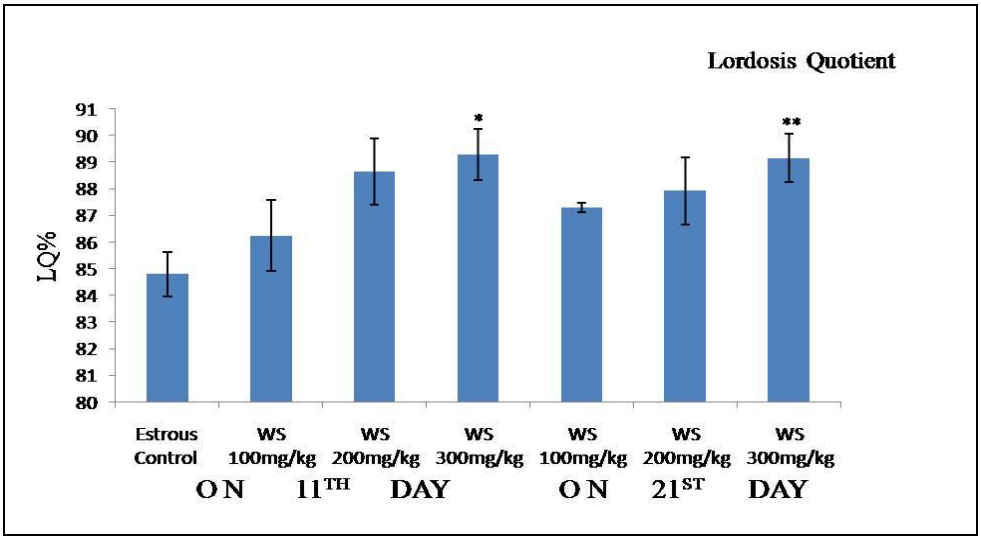


FIGURE 8: EFFECT OF *WITHANIA SOMNIFERA* (WS) ON LORDOSIS QUOTIENT OF ESTROUS FEMALE RATS.

Values are expressed a mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test).WS-*Withania somnifera*, n-number of animals

Male Rat Copulatory Sign:

Mounting Frequency (MF): Male rats paired with *Withania somnifera* (WS) treated female rats did not show any improvement in mounts on 11th and 21st day of treatment in the period of 30 minutes. See **Table 10, Fig.9.**

Intromission Frequency (IF) and Ejaculation Frequency (EF): Male rats paired with treated female rats with all doses showed significant improved (P<0.01) Intromission and Ejaculation Frequency on 11th and 21st day of treatment in the period of 30 min. See **Table 10, Fig. 9.**

TABLE 10: MOUNTING FREQUENCY (MF), INTROMISSION FREQUENCY (IF) AND EJACULATION FREQUNECY (EF) OF MALE RATS FOR ESTROUS FEMALE RATS TREATED WITH *WITHANIA SOMNIFERA* (WS).

Treatment (mg/kg)		MF	IF	EF
Estrous Control	Vehicle (10ml/kg)	23±0.57	15.33±0.21**	11.16±0.30
After 11 days treatment	WS-100mg/kg	24.16±0.30	17.83±0.30**	12.66±0.33**
	WS-200mg/kg	23.33±0.61	18.16±0.47**	13±0.51*
	WS-300mg/kg	23.33±0.21	18.5±0.34**	13.66±0.33**
After 21 days treatment	WS-100mg/kg	23.66±0.33	18.33±0.33**	13.16±0.16**
	WS-200mg/kg	23.5±0.42	18.66±0.49**	13.5±0.42**
	WS-300mg/kg	23±0.25	19±0.44**	14.16±0.30**

Values are expressed a mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

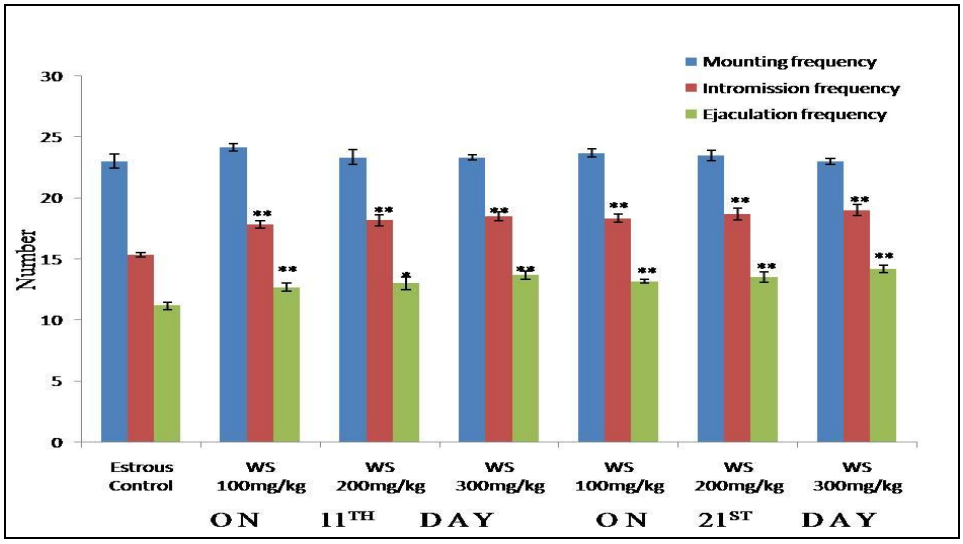


FIG. 9: MOUNTING FREQUENCY (MF), INTROMISSION FREQUENCY (IF), AND EJACULATION FREQUENCY (EF) OF MALE RATS FOR ESTROUS FEMALE RATS TREATED WITH *WITHANIA SOMNIFERA* (WS).
Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

Mounting Latency (ML):
Male rats paired with treated female rats did not show any improvement in Mount Latency on 11th and 21st day of treatment in the period of 30 minutes. See **Table 11, Fig. 10.**

Intromission Latency (Il), Ejaculation Latency (El) And Post-Ejaculatory Interval (PEI):
Male rats paired with treated female rats showed significantly reduction (P<0.05, P<0.01) in Intromission Latency, Ejaculation Latency and Post-Ejaculatory Interval on 11th and 21st day of treatment in the period of 30 minutes. See **Table 11, Fig.10.**

TABLE 11: MOUNTING LATENCY (ML), INTROMISSION LATENCY (IL), EJACULATION LATENCY (EL) AND POST-EJACULATORY INTERVAL (PEI) OF MALE RATS FOR ESTROUS FEMALE RATS TREATED WITH *WITHANIA SOMNIFERA* (WS).

Treatment (mg/kg)		ML	IL	EL	PEI
Estrous Control	Vehicle (10ml/kg)	0.94±0.09	1.87±0.24	6.69±0.31	4.67±0.34
After 11 days treatment	WS-100mg/kg	0.75±0.11	1.08±0.16*	6.25±0.16	3.09±0.29**
	WS-200mg/kg	0.66±0.07	0.98±0.12**	5.70±0.34	2.67±0.20**
	WS-300mg/kg	0.66±0.06	1.05±0.17*	4.39±0.22**	2.72±0.21**
After 21 days treatment	WS-100mg/kg	0.62±0.07	0.80±0.13**	5.95±0.29**	2.35±0.33**
	WS-200mg/kg	0.81±0.15	0.75±0.04**	5.34±0.19**	2.05±0.18*
	WS-300mg/kg	0.61±0.04	0.98±0.15**	4.18±0.15**	1.87±0.16**

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

Effect of *Withania somnifera* (WS) Extract on Serum Estradiol Levels: After 21 days of treatment, 100mg/kg dose of *Withania somnifera* (WS) increased (not significantly) serum estradiol level as compared to Estrous Control female rats while doses 200mg/kg and 300mg/kg significant increased (P<0.05, P<0.01) serum estradiol levels as compared to Estrous Control female rats, see **Fig. 11.**

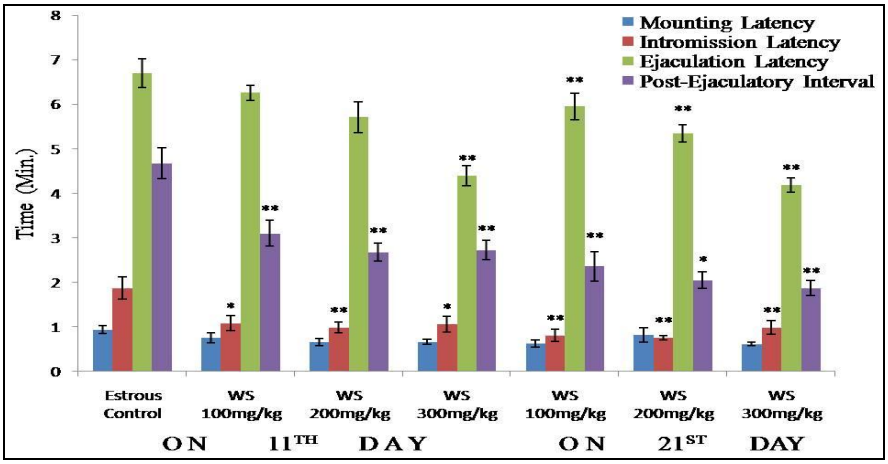


FIG. 10: MOUNTING FREQUENCY (ML), INTROMISSION FREQUNECY (IL), EJACULATION FREQUENCY (EL), AND POST-EJACULATORY INTERVAL (PEI) OF MALE RATS FOR ESTROUS FEMALE RATS TREATED WITH *WITHANIA SOMNIFERA* (WS).

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

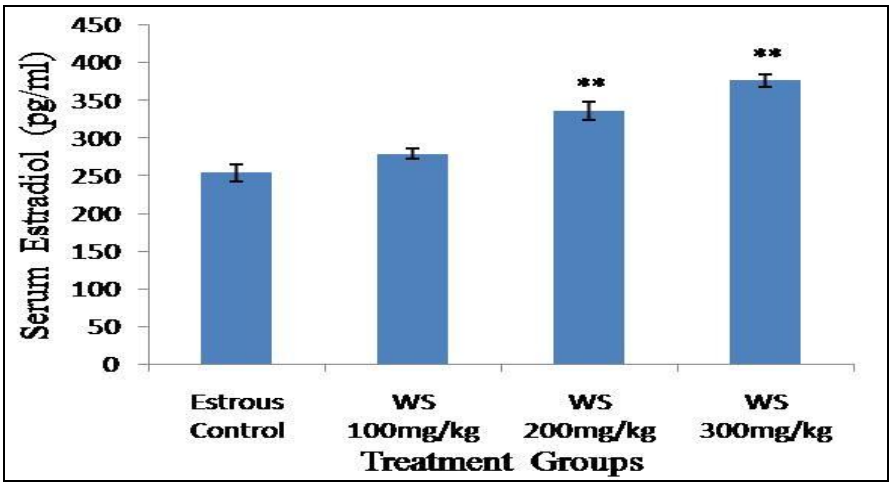
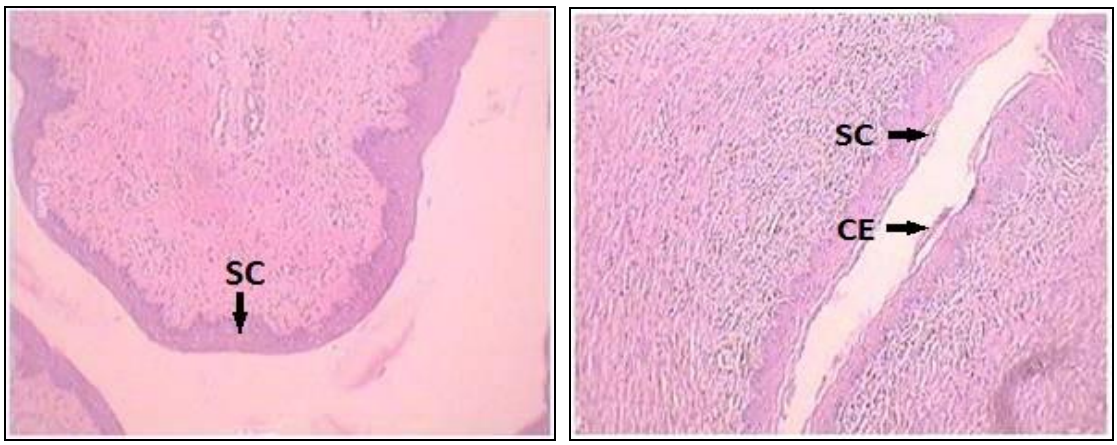


FIG.11: EFFECT OF *WITHANIA SOMNIFERA* (WS) EXTRACTON SERUM ESTRADIOL LEVELS

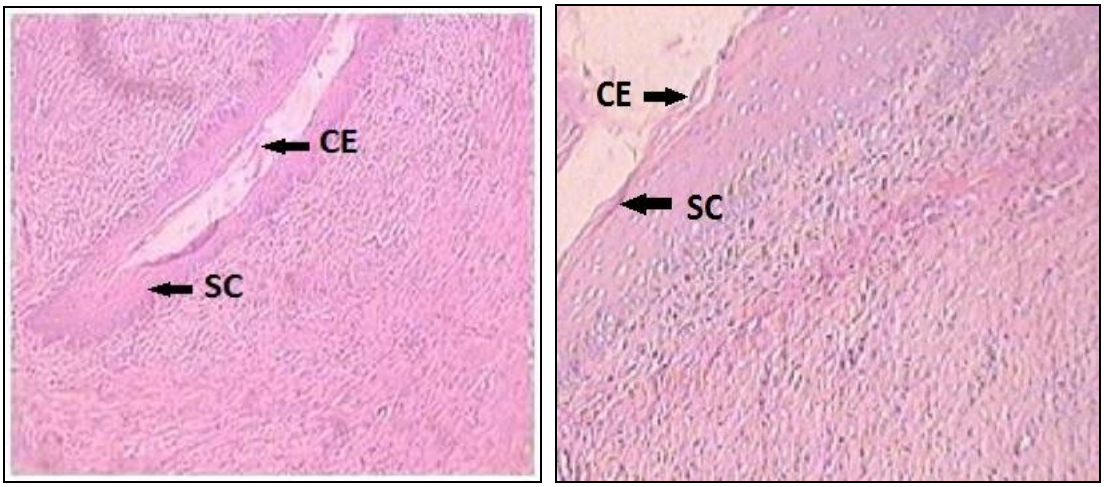
Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01 compared with vehicle-treated Estrous Control female rats (ANOVA followed by Dunnett's test). WS-*Withania somnifera*, n-number of animals

Histological Analysis:

At the end of the histological study of vagina, uterine horn and ovary, showed normal histoarchitecture. There was no any sign of toxicity like lesions or degeneration of organs. See **Fig. 12, 13, 14.**



ESTROUS CONTROL **WS-100mg/kg**

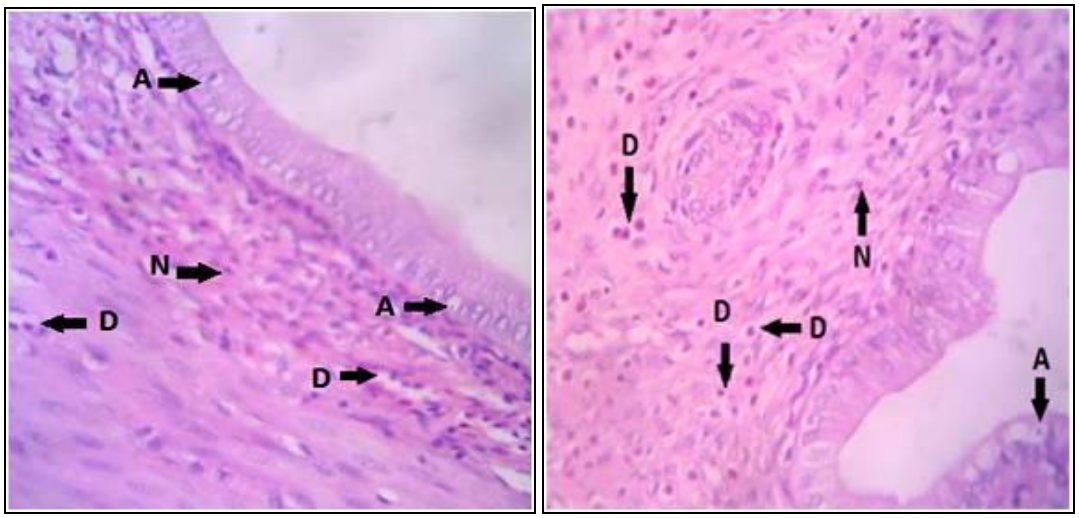


WS-200mg/kg **WS-300mg/kg**

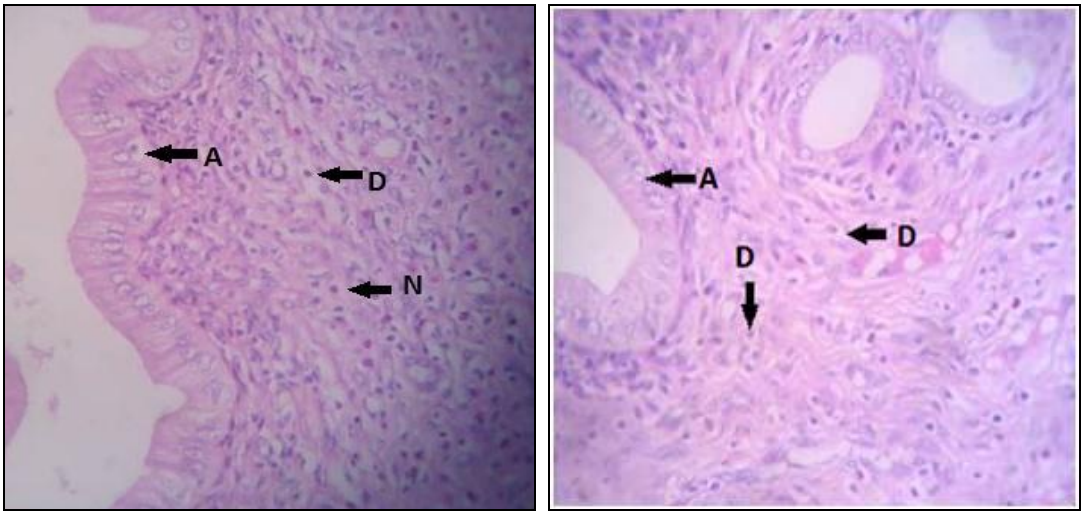
FIG. 12: HEMATOXYLIN AND EOSIN STAINING OF VAGINA OF FEMALE RATS.

SC-Stratum corneum, CE-Cornified epithelial cell

Observation: Stratum mucification absent, well developed Stratum Corneum, Cornified Epithelial Cells typically present in lumen.



ESTROUS CONTROL **WS-100mg/kg**

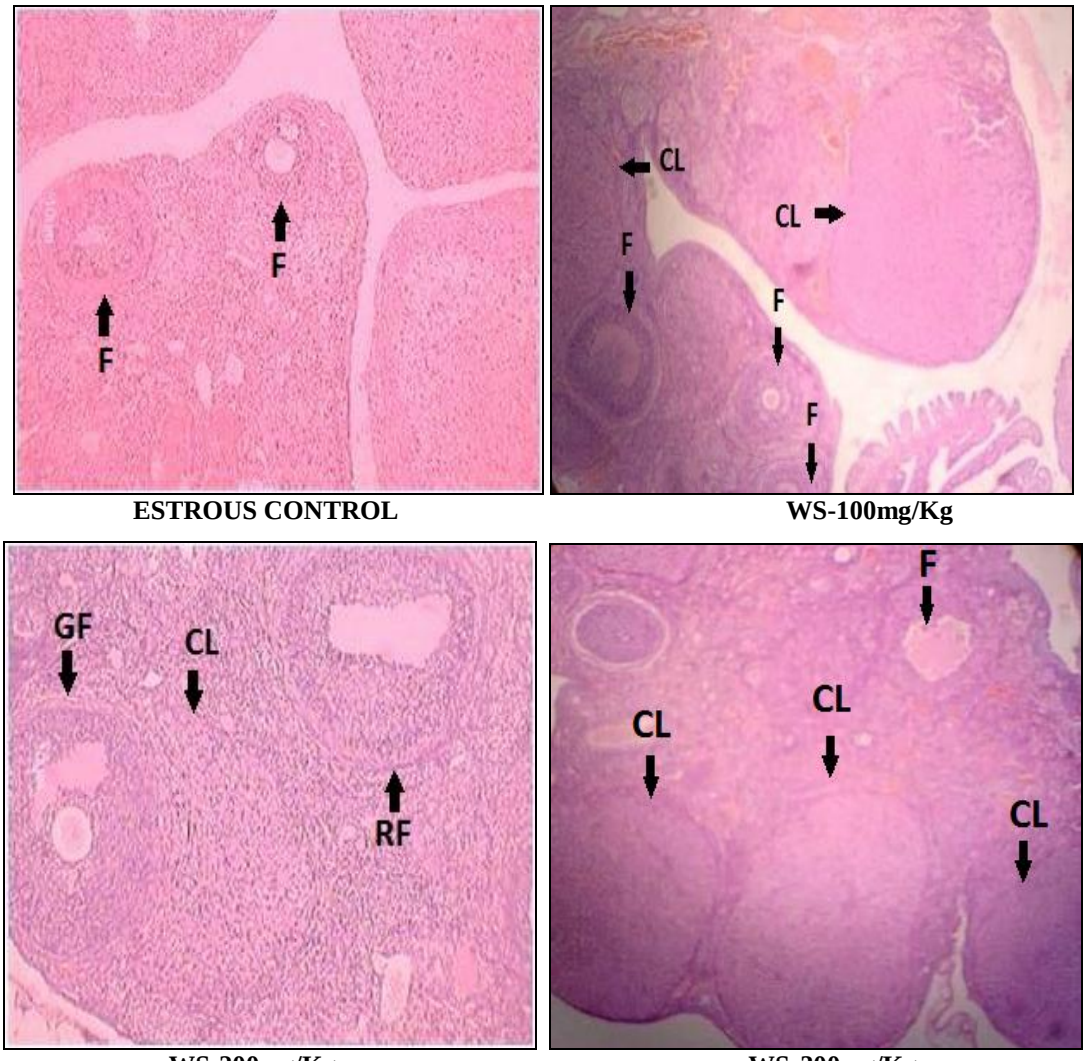


WS-200mg/kg **WS-300mg/kg**

FIG. 13: HEMATOXYLIN AND EOSIN STAINING OF UTERINE HORN OF FEMALE RATS.

D-Vacuolar degeneration, A-Apoptosis, N-Polymorphonuclear cells infiltrate the lamina propria and endometrial glands

Observation: Marked epithelial degeneration, apoptosis, accompanied by a loss of mitotic activity.



ESTROUS CONTROL **WS-100mg/Kg**

WS-200mg/Kg **WS-300mg/Kg**

FIG.14: HEMATOXYLIN AND EOSIN STAINING OF OVARY OF FEMALE RATS.
CL-Immature corpus luteum, RF-Ruptured follicles, F-Follicle, GF-Graffian follicle

Observation: Immature corpora lutea, Degenerate ovarian corpora lutea are often present at estrous phase, but others are small, with cells showing a basophilic cytoplasm and occasionally a central fluid-filled cavity retained from the follicular stage. Central fibrous tissue is generally not present.

DISCUSSION: The goal of this experiment was to assess the effect of *Withania somnifera* (WS) administration on various aspects of female rodent sexuality: sexual motivation, proceptivity and receptivity. The first model successfully demonstrated the predictive validity of the runway methodology in assessing experimentally-induced changes in female sexual motivation. Herbal extract treatment, significantly increased female interest in male targets. This enhancement of sexual motivation was manifested in shorter latencies to approach males (run time), as well as greater amounts of time spent in close physical proximity to male target located on the other side of a Plexiglas barrier (proximity time). That these two variables are behaviourally independent and yet are affected similarly by action of herbal drug, increases our confidence that they reflect underlying incentive-motivational processes. Notably, herbal treatment did not show motivation to approach an empty goalbox, and female target goalbox.

It is already proven that, ovarian hormones increase female sexual motivation (male seeking behaviour)

through generation of a central motive state that serves to energize behaviour and sensitize the individual to sexual-incentives which signal potential mating opportunities [5, 31, 32]. In one study it is already stated that when the chemosensory signals of sexually active male rat are received in the sensory neurons of the olfactory epithelium and the vomero nasal of female rats these signals are processed and relayed in the Main and Accessory Olfactory Bulb. They are then further integrated in the medial and cortical nuclei of the amygdale and preoptic area. Main and Accessory Olfactory Area include a large number of neurons with sex steroid receptors, suggesting an involvement of the Main and accessory Olfactory Area in regulating sex specific and sex steroid dependent olfactory preference for the odour of conspecific individuals. That is motivated estrous female rats are attracted by the odor of sexually active male rats rather than by that of female rats or male rats [33-35]. At the end of this study, serum estradiol was also estimated in blood sample of female.

It showed significantly improvement as compared to Estrous Control group, this may be due to steroidal saponins of *Withania somnifera* extract [19]; Because it is already proven that *Withania somnifera* increases steroidal hormones [36,37] and the regulation of female sexual motivation in rats by ovarian hormones has been demonstrated previously in numerous experiments [5,3 8-40].

In mating behaviour by copulatory arena, direct parameters (female rat copulatory behaviours) and indirect parameters (male rat copulatory behaviour) were studied. The two different aspects of copulatory behaviour displayed by estrous female rats in the presence of sexually active male rat are sexual receptivity and proceptivity [41]. 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 [26]. According to Uphouse et al., 1993, the psychological arousal in women could be very close to proceptivity and hence the study of proceptive behaviours are of great importance to preclinically investigate potential of compounds affecting libido and to treat female sexual dysfunction (FSD) [42].

All the female parameters showed improvement in sexual behaviours like Hops, Darts, Ear Wiggling, Lordosis Quotient, etc. and improvement in these parameters are due to the chemosensory signals of male rats and its processing in female rat's brain is mentioned in the above paragraph. Male rats also showed improvement in their sexual behaviours. Also the female rat has to alert males that she is ready for mating [43]; olfactory signals during estrous phase are used to communicate receptivity [44, 45]. Male rat were more attracted by odours derived from clitoral gland during estrous than those derive from this gland in other phases of the reproductive cycle [46].

Therefore indirect parameters that is male rat parameters like Mounting Frequency (MF), Intromission Frequency (IF), and Ejaculation Frequency (EF) were also significantly increased and Intromission Latency (IL), Ejaculation Latency (EL), and Post-Ejaculatory Interval (PEI) were significantly reduced when they were placed in copulatory test with treated female rats with different doses of *Withania somnifera* (100, 200 and 300mg/kg) for 21 days.

After performance in Automated runway apparatus and Copulatory arena, histology study of vagina, uterus horn and, ovary also done. All the histoarchitecture showed all characteristics like estrous phase without any toxic effect, degeneration or legions due to *Withania somnifera* (WS) treatment [46, 47].

CONCLUSION: This study on the basis of result and published scientific evidence of *Withania somnifera* (WS), it is proven that *Withania somnifera* (WS) increases sexual stimulation which was tested by automated runway apparatus and Copulatory arena and also increases serum estradiol hormone. Histology of genital organs like vagina, uterine horn and ovary was also normal; that's mean there were no any lesions or degeneration of tissues. More studies are in progress for evaluating its significance in Nω-Nitro-L arginine methyl ester (L-NAME) induced female sexual dysfunction with other plant formulations which are cited in traditional literature for their possible synergistic action.

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