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Aphrodisiac properties of *Polygonatum verticillatum* leaf extractImran Kazmi<sup>1</sup>, Muhammad Afzal<sup>1</sup>, Mahfoozur Rahman<sup>2</sup>, Gaurav Gupta<sup>1\*</sup>, Firoz Anwar<sup>1\*\*</sup><sup>1</sup>Siddhartha Institute of Pharmacy, Dehra Dun, Uttarakhand, India<sup>2</sup>Dreamz College of Pharmacy, Mandi, Himachal Pradesh, India

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## ABSTRACT

**Objective:** *Polygonatum verticillatum* is the main ingredient of Astavarga. Astavarga is the important ingredient of various classical Ayurvedic formulations like Chavyanprash. Astavarga has been assigned various medicinal properties by ancient Materia Medica dealing with Ayurveda. As per Ayurveda the main property of this plant is in the treatment of vata, pitta, general weakness, aphrodisiac etc. **Aim of the study:** In the present study, we examined the effect of *Polygonatum verticillatum* leaf aqueous extract upon the expression of male rat sexual behavior, in order to know whether *Polygonatum verticillatum* leaf aqueous extract possess aphrodisiac property. **Methods:** The aphrodisiac activity of *Polygonatum verticillatum* leaf aqueous extract was investigated in male rats. The extract (500 mg/kg body weight/day) and L-dopa (100 mg/kg body weight/day) were administered orally by gavages for 28 days. Mount latency (ML), intromission latency (IL), ejaculation latency (EL), mounting frequency (MF), intromission frequency (IF), ejaculation frequency (EF) and postejaculatory interval (PEI) were the parameters observed before and during the sexual behavior study at day 0, 7, 14, 21 and 28. **Results:** The *Polygonatum verticillatum* leaf aqueous extract reduced significantly ML, IL, EL and PEI ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ ). The extract also increased significantly MF, IF and EF ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ ). These effects were observed in sexually active and inactive male rats. **Conclusions:** Present findings provide experimental evidence that the *Polygonatum verticillatum* leaf aqueous extract possesses aphrodisiac property.

## 1. Introduction

*Polygonatum verticillatum* All. (Nooreallam) is a member of the genus *Polygonatum* (King Solomon's-seal, Solomon's seal) of about 57 species belonging to the family Liliaceae or Convallariaceae [1, 2]. *Polygonatum verticillatum* are considered members of Ashta-verga and are referred to as Meda and Mahameda, respectively, in the Ayurvedic system of medicine. In the traditional system of treatment, It has been used for thousands of years. Ethnobotanical uses of the plant include as emollient, aphrodisiac, vitiated condition of pitta and vata, appetizer and tonic, galactagogue (increases milk release), weakness [3]. In the area of Northwest Himalaya, tubers/leaves are baked with ghee, dried and powder and taken with milk for increase sexual potency [4]. In Western Himalayan, roots locally known as 'Salam mishri' used traditionally among the tribal communities in treatment of spermatorrhoea and piles [5]. *Polygonatum verticillatum* is one of the eight medicinal

plants of Astavarga. These plants are considered as a very good Rasayana with rejuvenating and health-promoting properties, and are known to strengthen the immune system and have immense cell regeneration capacity. Astavarga is useful in promoting body fat, healing fractures, seminal weakness, fever, abnormal thirst, diabetic conditions and as a cure for vata, pitta, rakta doshas. Due to high medicinal value, Astavarga plants are used in different forms, e.g. Taila (oil), Ghritam (medicated clarified butter), Churana (powder) and formulations in the traditional medical system (TMS) including Chyavanprasha, a health-promotive and disease-preventive tonic. Some of these formulations are available in Indian markets as pharmaceutical products, e.g. Chyavanprasha and the commonly used medicine Sudarshana Churna are available in almost every primary healthcare unit in rural areas of India. It is the most economically important medicinal plant of Indian Himalayan region [4].

Although, *Polygonatum verticillatum* is a constituent of number of herbal formulations that are known for improving sexual performance, there is no scientific report on *Polygonatum verticillatum* substantiating its usage as sexual

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tonic or stimulant. Keeping in view the growing popularity and market interest for the drug, present studies were undertaken to provide scientific support for its purported folkloric usage.

## 2. Materials and methods

### Plant material

Leaves of *Polygonatum verticillatum* All were collected from Garhwal region, Uttarakhand in the month of October 2010, and were authenticated by Dr. S.B.Singh, Scientist NISCAIR, NewDelhi. A voucher specimen no. NISCAIR/RHMD/consult/-26-02-11/1450/263 is deposited in the herbarium of NISCAIR, India.

### Preparation of extracts

The dried leaves were grinded into small particles and soaked in distilled water (dH<sub>2</sub>O) in the ratio of 1:20 (w/v) and left for 24 h. Then, the mixtures were filtered using cloth filter, cotton wool and Whatman No. 1 filter paper to obtain the supernatant, which was subjected to freeze drying process. The extraction processes was repeated three times up to 72 h using the same residue. This method was carried out according to Zakaria et al. with slight modifications [6].

### Drugs

L-dopa, Progesterone and Oestradiol benzoate were purchased from Sigma chemical Co. (St. Louis, MO, USA).

### Animal

Albino wistar rats (age-90 days) of either sex weighing between 150 to 200 gms were procured from registered breeders (LAR, CPCSEA No-196, IVRI, Bareilly). The animals were housed under standard conditions of temperature (25-20°C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water ad libitum. All the studies conducted were approved by the Institutional Animal Ethical Committee (1435/PO/a/11/CPCSEA) of Siddhartha institute of Pharmacy, Dehradun, India.

Male rats ( $n = 5/\text{group}$ ) were trained for sexual experience. To provide sexual experience, each male rat was allowed 30min exposure to a female rat in behavioral estrous, several days before testing for copulatory performance in a transparent arena. The animals were tested three times over a 10-day period for copulatory behavior and divided into active and inactive groups [7]. Sexually active animals were divided into control, L-dopa-treated (100 mg/kg body weight) and PVAE -treated (500mg/kg body weight) groups. The animals that did not show any sexual interest during training were considered the inactive group that was administered PVAE (500mg/kg body weight). Female rats

were ovariectomised using a standard procedure [8]. They were allowed to recover from the surgery for 10 days. They were brought into estrous by the administration of a single subcutaneous dose of 2 $\mu$ g/kg body wt. of estrogen benzoate and 500 $\mu$ g/kg body wt. progesterone 48 and 6 h before the copulatory study.

### Toxicity studies

The toxicity study of *Polygonatum verticillatum* leaf aqueous extract (PVAE) was performed in a single dose administration of 2000 mg/kg (p.o.). Rats were fasted for 24 h before the administration of PVAE. A group that received dH<sub>2</sub>O represented the control group. The toxicity signs and symptoms or any abnormalities were observed at 0, 30, 60, 120, 180 and 240 min after PVAE administration. The observation was continued once a day for the next 14 days. The number of rats that survived was recorded at the end of the study period.

### Sexual behavior study

The following guidelines were followed in the study [9, 10]:

- a) males were kept individually but females were kept in groups (3);
- b) training of each male for 15 min at a time was performed until sexual behavior was elicited and when the behavior was noticed, males were exposed to receptive females (1 male with 5 females);
- c) repeated training to overcome the lack of sexual response in the presence of observers;
- d) the study was conducted in a silent room under dim red light;
- e) any jerking movement of the mating area was avoided to enable the rats to chase each other; and
- g) cleaning of the mating area was performed after each trial, since the urine trails left by one rat might alter the sexual behavior of the next rat.

PVAE (500mg/kg body weight) and L-dopa (100 mg/kg body weight) in distilled water were administered for 28 days orally by gavage. L-dopa served as standard [11-14]. The control group received 1 ml saline. Each group consisted of six animals (1 male and 5 female). The following parameters of the copulatory behavior were recorded

- (1)Mount latency (ML): the time from introduction of the female until the first mount;
- (2)Intromission latency (IL): the time from introduction of the female until the first intromission (vaginal penetration);
- (3)Ejaculation latency (EL): the time from the first intromission to ejaculation;
- (4)Mount frequency (MF): the number of mounts preceding ejaculation;
- (5)Intromission frequency (IF): the number of intromission preceding ejaculation; and

(6)Post-ejaculatory interval (PEI): the time between the occurrence of ejaculation and the resumption of sexual activity, as indicated by next intromission.

*Statistical analysis*

Results were expressed as mean±SEM, (n=6). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey’s test using Prism 5 Graphpad Software. Mean differences were considered statistically significant if P<0.05.

**3. Results**

*Toxicity studies*

No mortality and changes in the behavior were observed in all the treated and control groups of mice up to a dose of 2000mg/kg.

*Sexual behavior study*

The observation of the sexual behavior study is presented in Table 1. PVAE reduced ML, IL, EL and PEI significantly in both active and inactive male rats (Figure–1, 2, 3 and 4). PVAE also increased MF, IF and EF significantly in both active and inactive male rats (Figure–5, 6and7). All these effects were observed on the 21st and 28th day but some effects were also observed on 14th day of the study. The MF was significantly altered in PVAE–treated active and inactive rats (14th day), whereas L–dopa did not this parameter on the 14th day. The EF was significantly altered in PVAE–treated inactive rats (14th day), whereas L–dopa and PVAE–treated active rats did not alter this parameter on 14th day. The EL and IF were significantly altered in PVAE–treated and L–dopa treated active rats (14th day), whereas PVAE–treated inactive rats did not alter these parameters on 14th day. The ML was significantly altered in PVAE–treated active rats (14th day), whereas the PVAE–treated inactive rats and L–dopa treated active rats did not alter this parameter on 14th day. PVAE for both active and inactive male rats and L–dopa did not alter any of all these parameters on the 7th day.

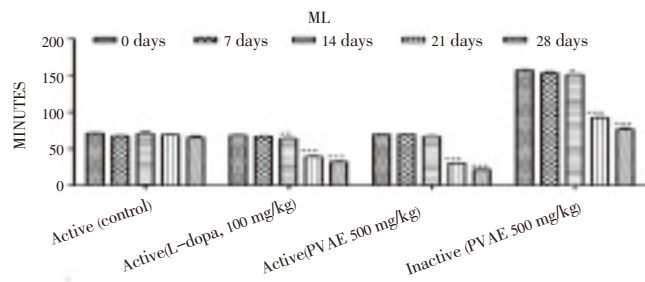
**Table 1**

Sexual behavior study of *Polygonatum verticillatum* leaf aqueous extract

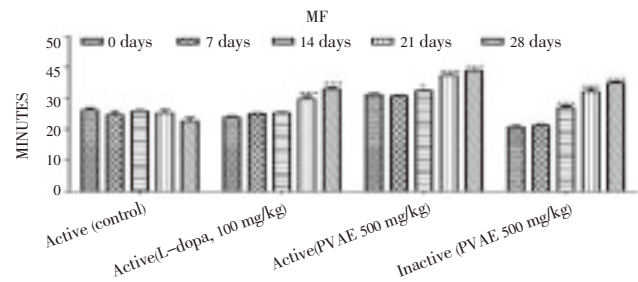
Group	Sexual BehaviorParameters	Mean±SEM				
		0 day	7th day	14th day	21st day	28th day
Active (control)	ML	70.99±0.64	67.36±0.86	70.36±2.61	69.41±0.67	65.10±2.32
	IL	167.6±1.28	172.5±2.02	170.0±0.90	162.5±1.28	164.2±1.03
	EL	7.47±0.18	7.94±0.04	6.73±0.20	6.92±0.31	7.52±0.11
	MF	26.23±0.63	24.71±1.25	25.76±0.52	25.23±1.12	22.65±1.16
	IF	6.31±0.18	6.33±0.27	6.67±0.33	6.38±0.41	6.78±0.06
	EF	2.19±0.09	2.04±0.24	1.94±0.02	2.06±0.02	2.04±0.06
	PEI	9.34±0.25	9.04±0.12	10.41±0.46	8.71±0.23	8.55±0.28
Active (L–dopa 100mg/kg Body weight)	ML	68.41±0.39	66.75±0.49	63.44±0.97b	39.52±1.10c	32.37±0.68c
	IL	192.6±1.93	186.9±3.20	173.4±0.97c	136.0±2.58c	100.5±0.59c
	EL	10.41±0.21	10.02±0.42	9.09±0.10b	6.23±0.15c	6.07±0.10c
	MF	23.96±0.32	25.00±0.19	25.26±0.27	29.80±0.89c	32.94±0.53c
	IF	7.31±0.15	7.59±0.35	8.45±0.13b	10.48±0.10c	12.25±0.15c
	EF	1.69±0.10	1.74±0.08	1.70±0.11	2.43±0.16b	2.72±0.15c
	PEI	7.53±0.24	7.07±0.06	5.15±0.17c	3.52±0.14c	3.31±0.08c
Active (PVAE 500mg/kg body weight)	ML	69.82±0.51	69.78±0.27	66.72±1.26	30.36±0.11c	21.43±0.86c
	IL	182.1±0.71	179.8±0.25	164.2±1.69c	99.90±0.51c	89.01±2.75c
	EL	8.79±0.09	8.29±0.15	7.98±0.02b	5.37±0.15c	4.59±0.20c
	MF	31.15±0.46	30.76±0.35	32.44±0.19a	37.42±0.20c	38.87±0.18c
	IF	12.42±0.18	11.93±0.05	9.66±0.18c	8.53±0.12c	7.89±0.02c
	EF	1.54±0.10	1.75±0.10	1.62±0.01	2.41±0.16c	2.97±0.02c
	PEI	8.89±0.30	8.15±0.19	5.46±0.15c	3.61±0.19c	3.05±0.04c
Inactive (PVAE 500mg/kg body weight)	ML	157.1±1.22	153.2±1.52	150.9±0.58a	92.28±0.86c	76.65±1.70c
	IL	297.9±1.21	290.3±1.21	224.7±1.68c	187.1±2.30c	156.6±2.79c
	EL	5.43±0.19	5.62±0.36	5.40±0.68	3.39±0.24b	2.81±0.19c
	MF	20.72±0.65	21.40±0.32	27.00±0.57c	32.21±0.76c	35.02±0.33c
	IF	7.79±0.32	8.14±0.19	8.16±0.07	11.61±0.44c	13.51±0.11c
	EF	1.10±0.04	1.19±0.02	1.65±0.16a	2.41±1.16c	2.96±0.02c
	PEI	7.43±0.11	7.27±0.12	6.59±0.22b	4.36±0.14c	3.34±0.13c

ML,mount latency; IL, intromission latency; EL, ejaculation latency;MF, mount frequency; IF, intromission frequency; EF, ejaculation frequency; PEI, post-ejaculatory interval.

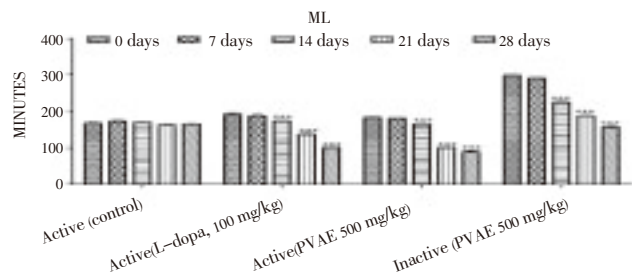
\* Significance level: \*P < 0.05, \*\*P<0.01 and \*\*\*P<0.001



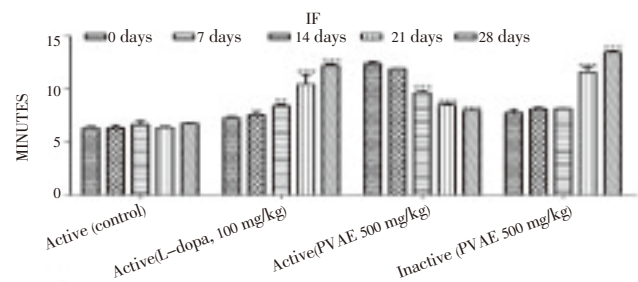
**Figure 1:** Effect of administration of *Polygonatum verticillatum* leaf aqueous extract on Mount Latency (ML) of male rats. Values are effects of various groups on 0, 7, 14, 21 and 28th day. Whereas \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$



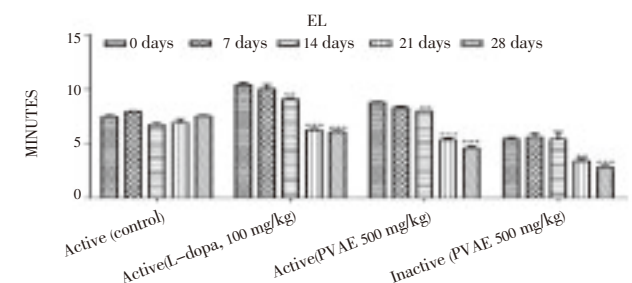
**Figure 5:** Effect of administration of *Polygonatum verticillatum* leaf aqueous extract on Mount Frequency (MF) of male rats. Values are effects of various groups on 0, 7, 14, 21 and 28th day. Whereas \* $P < 0.05$  and \*\*\* $P < 0.001$



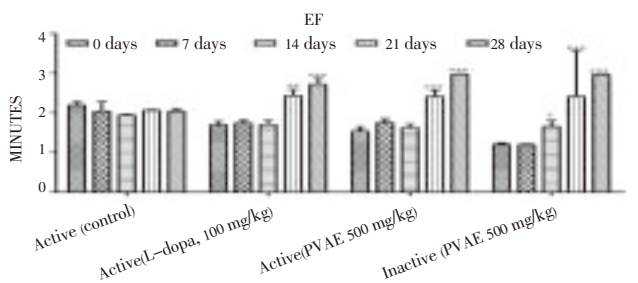
**Figure 2:** Effect of administration of *Polygonatum verticillatum* leaf aqueous extract on Intromission Latency (IL) of male rats. Values are effects of various groups on 0, 7, 14, 21 and 28th day. Whereas \*\*\* $P < 0.001$



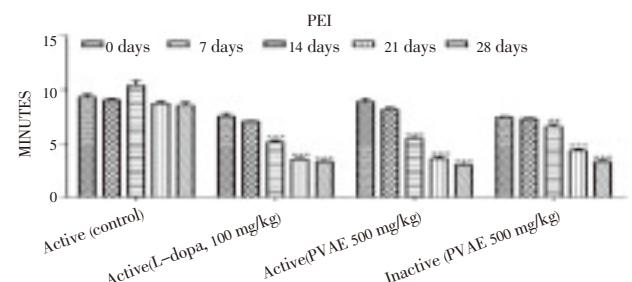
**Figure 6:** Effect of administration of *Polygonatum verticillatum* leaf aqueous extract on Intromission Frequency (IF) of male rats. Values are effects of various groups on 0, 7, 14, 21 and 28th day. Whereas \*\* $P < 0.01$  and \*\*\* $P < 0.001$



**Figure 3:** Effect of administration of *Polygonatum verticillatum* leaf aqueous extract on Ejaculation Latency (EL) of male rats. Values are effects of various groups on 0, 7, 14, 21 and 28th day. Whereas \*\* $P < 0.01$  and \*\*\* $P < 0.001$



**Figure 7:** Effect of administration of *Polygonatum verticillatum* leaf aqueous extract on Ejaculation Frequency (EF) of male rats. Values are effects of various groups on 0, 7, 14, 21 and 28th day. Whereas \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$



**Figure 4:** Effect of administration of *Polygonatum verticillatum* leaf aqueous extract on Post-Ejaculatory Interval (PEI) of male rats. Values are effects of various groups on 0, 7, 14, 21 and 28th day. Whereas \*\* $P < 0.01$  and \*\*\* $P < 0.001$

#### 4. Discussion

The present study provides evidence for the ability of the crude extract of *Polygonatum verticillatum* leaf, to enhance male sexual behavior expression in sexually active rats and to promote sexual activity in sexually inactive male animals. The data obtained reveal that when orally administered the aqueous extract of *Polygonatum verticillatum* leaf (500mg/kg, body weight), effectively facilitates several aspects of copulatory behavior. The results of present investigations show that the PVAE significantly increased the Mounting Frequency (MF) and Intromission Frequency (IF) in both



sexually active and inactive male rats as compared to control. The MF and IF are considered the indices of both libido and potency. Thus, the increase in the MF and IF, indicates the PVAE, along with increasing libido, probably also increases the potency. The significant increase in the Ejaculatory Latency (EL) suggests that the extract prolonged the duration of coitus. The significant decrease in the Post Ejaculatory Interval (PEI), suggests that the PVAE intensified sexual activity in sustained manner. The PVAE also caused a significant reduction in the Mounting Latency (ML) and Intromission Latency (IL) as compared to control animals. This also provides an evidence for aphrodisiac effect of the PVAE. These findings provide experimental support to the traditional use of *Polygonatum verticillatum* leaf as a sexual stimulant for the treatment of erectile dysfunction. Generally sexual behavior is enhanced by elevated testosterone levels. Drug-induced changes in neurotransmitter levels or their action in the cells could also change sexual behavior. The brain area most associated with sexual behavior is the limbic system. Research with various animal and human models indicates a relationship between brain dopamine, 5HT and sexual behavior [8, 15]. Both dopamine and 5HT are implicated in depression. The relationship of dopamine to human sexual behavior is supported by reports of persexuality behavior induced by L-dopa in parkinsonian patients. Stimulants and antidepressants are known to affect libido, erection, ejaculation and orgasm. In this connection the PVAE was also subjected to a toxicity testing and it was tested upto a high concentration of 2000mg/kg, orally (four times more than the aphrodisiac dose, evaluated in the present study). Even at this dose the extract did not produce signs of toxicity or treatment related adverse effects in the tests for aphrodisiac activity. This study clearly suggests that *Polygonatum verticillatum* leaf is a potent sex stimulant in rats. The chemical constituents and mechanism of action responsible for this activity are not known. However, this plant is rich in alkaloids, phenols and steroidal saponins [16–18].

Further studies are suggested to isolate the active principle of *Polygonatum verticillatum* leaf and to determine whether the alkaloids or steroidal saponins or phenols contribute towards the sex stimulant activity of this plant.

Sexual behavior was studied in sexually active and inactive rats to further understand the role of *Polygonatum verticillatum* leaf as an aphrodisiac. There was an overall increase in the sexual behavior parameters in the *Polygonatum verticillatum* leaf aqueous extract treated groups of rats as reflected in MF, IF and EF, and reduction in ML, IL, EL and PEI. These results were statistically significant. It is concluded that *Polygonatum verticillatum* leaf aqueous extract appears to possess aphrodisiac activity.

### Conflict of interest statement

We declare that we have no conflict of interest.

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