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Effect of Vajikaran Rasayana herbs in sexual behavior of old age rats

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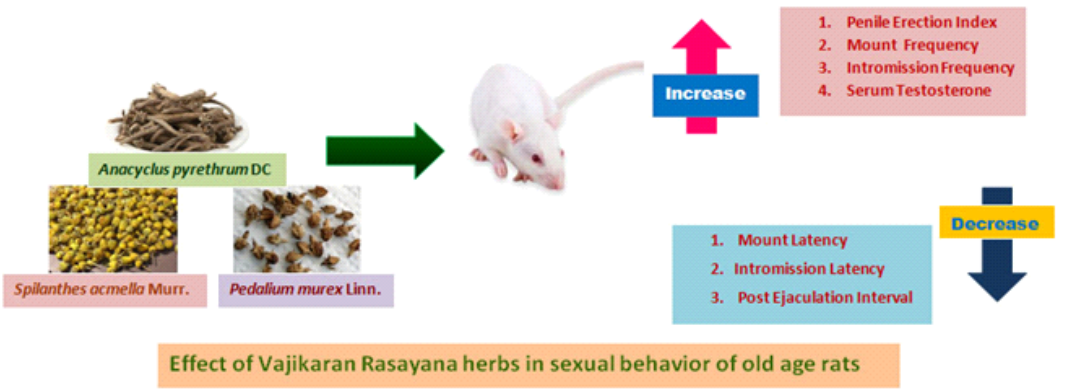
Graphical Abstract



Abstract

Background: According to Indian Systems of Medicine, *Spilanthes acmella* (L.)Murr.*Anacyclus pyrethrum* DC, *Pedalium murex* Linn.is considered effective in the treatment of sexual deficiencies especially due to aging.

*Purpose:*To assess the effect of ethanolic extracts of the*Anacyclus pyrethrum* roots*, Spilanthes acmella* flowers and *Pedalium murex* fruits in sexual behavior of old age rats

*Method:*In the present study ethanolic extracts of the*Anacyclus pyrethrum* roots*, Spilanthes acmella* flowers and *Pedalium murex* fruits were investigatedon general mating pattern, penile erection and serum hormone levels of old age male Wistar albino rats and compared with sildenafil citrate and Testosterone. For assessment of sexual behavior, animals were divided into six groups of six male rats. All extracts (150 mg/kg body weight/day orally) and sildenafil citrate(5mg/kg body weight/day orally) and Testosterone (0.5mg /Kg b. w. i.m. in arachis oil twice weekly) (positive control) were administered for 28 days. The behavioral and sexual parameters were observed at day 0, 15 and 28 of drug treatment.

Results: The orally administered extract had a positive effect on mounting frequency, intromission frequency and ejaculation frequency and the most significant effects ($p < 0.05$) were observed *Anacyclus pyrethrum* treatment. A positive effect was also observed on the testosterone serum levels. Sildenafil citrate exhibited also a significant effect on penile erection, but no effect on hormone levels of rats was observed.

Conclusion: The aphrodisiac potential of ethanolic extract of *Anacyclus pyrethrum, Spilanthes acmella* and *Pedalium murex* was demonstrated *in-vivo*. Study lends support to the traditional utilization Vajikaran Rasayana herbs as a sexual stimulating agent in old age.

Keywords: Aphrodisiac; Male sexual behavior; *Anacyclus pyrethrum* DC; *Spilanthes acmella*; *Pedalium murex* Linn.

Introduction

Sexual dysfunctions increase with ageing and etiological factors, including degenerative diseases, increase in injuries and stress associated with industrialized lifestyles. However, plant-derived and herbal remedies continue to be a popular alternative (Sharma et. al., 2011).

For several hundred years, people around the world have used locally grown plants as supplements to energize, vitalize, and eventually to improve male sexual functions.

Scientific investigation has supported the long-held notion that sexual function in men declines with age. A number of studies have found a small but reliable age-related decline in testosterone, a hormone of vital importance to sexual response in men (Chauhan et al., 2014).

A special class of Rasayana drugs is known as Vrishya or Vajikarana. They are associated with an improvement of male sexual potency and thereby ensure a *supraja,* or better progeny. Traditionally, the main aim of using Vajikaran was to achieve successful copulation for healthy reproduction, along with an

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improvement in sexual pleasure as an additional benefit. Vajikaran drugs are specially recommended to people suffering from sexual insufficiency and people in advanced age losing interest in sexual act or failing in sexual performance (Sharma et al., 2010). Besides having many specific drugs for enhancing sexual functions, the most commonly used are Akarkara and Gokharu.

In our previous studied *Anacyclus pyrethrum* (*A. pyrethrum*),*Spilanthes acmella*(*S.acmella*)and *Pedalium murex* (*P. murex*) extracts show the maximum effects in dose of 150mg/kg b.w after 28 days treatment (Sharma et al., 2010, 2011, 2012, 2013). This maximum effective dose (150mg/kg) was thus used for further experiments.

Materials and methods

Animal stock

The protocol for experimentation was approved by Institutional Animal Ethics Committee of Dr. Hari Singh Gour University, Sagar, India (Animal Eths Comm/IE/98/Reg No379/01/ab/CPCSEA) and was in accordance with international standard on the care and use of experimental animals. Inbred, 36 male rats 10-12 months old and weighed 250-300 g was used. Female from the same strain rats, used as stimulus for evaluation of sexual behavior, were prepared for experimentation, using the method reported by Agmo (Agmo, 2003). In brief, before all testing sessions, female estrus was induced by administration of estradiol benzoate (25 µg/rat), followed by progesterone (250 µg/rat), 48h later. Females were used between 4 and 8 h after the progesterone administration. Both steroids were purchased from Sigma (St.Louis, MO, USA). They were dissolved in arachis oil (Kriti, India) and injected subcutaneously in a volume of 0.1 ml/rat. The rats were housed at room temperature (24 ± 2°C)on a reversed day-night cycle (dark from 06:00 to 18:00) and relative humidity of 50–55%. They were fed with a standard pellet diet and water *ad libitum.*

Preparation of extracts

The flowers of the *Spilanthes acmella* and fruits of *Pedalium murex*plant were collected from the area in vicinity of the campus and were dried at room temperature (25-35°C). The plant *Spilanthes acmella* (L.)Murr.and*Pedalium murex* Linn.wasidentified and authenticated at Department of Botany, Dr. H. S. Gour University Sagar (M.P.) The roots of *Anacyclus pyrethrum* DC. were procured from the market and authenticated by Agharkar Research Institute, Pune (Authentication no. Auth. 07-86). The plant materials were reduced to powder and passed through a sieve (60 mesh), fed in a soxhlet extractor and extracted with ethanol (95%) till complete exhaustion. The extract was collected and dried under vacuum by using a rota vapor (Heidolph, Germany). *A. pyrethrum* ethanolic extract was dark brown colored and semisolid in consistency (yield 7.2%w/w). Ethanolic extract of *S. acmella* was greenish brown and semisolid with a yield of 6.1% w/w. *P.murex* ethanolic extract was brown colored, semisolid with a characteristic odor (yield 5.2% w/w). Before oral administration, allextractswas suspended in 1% sodium CMC.

Treatment

The male rats 10-12 months old and weighed 250-300 g was used for present study. The animals were divided into six groups of six rats each. Group I (Control) animals served as the control and received only vehicle 1 ml of 1% sodium CMC solution. Groups II (AP 150), III (SP 150) and IV (PM 150) were given ethanolic extracts of *A. pyrethrum, S. acmella and P. murex* respectively, with dose of 150mg/kg b.w. for 28 days. Group V (SC) was treated with sildenafil citrate (5 mg/kg body weight) in 1% sodium CMC solution orally, for 28 days, whereas Group VI (TG) was subcutaneously given 0.5 mg/kg of testosterone suspended in arachis oil twice weekly and served as the positive control for studies.

Functional studies

Penile erection

Penile erection was determined by using the method reported by Thakur et al. (2009). In brief, each male rat was placed in a transparent plexi glass cabin (60×40×40 cm) that was divided in half by 2 sheets of plastic fiber mesh, preventing contact but allowing auditory, visual, and olfactory stimuli. Ventral as well as lateral viewing and recording of the whole experimentation was facilitated by appropriately placing a mirror. After a 5-minute adaptation period, the test was started by placing an estrus female on the other side of the cage. Cages were cleaned before shifting the animals of different groups. The number of erections were recorded and tabulated. Erection in rats was marked by the visibility of the penis out of its sheath or by grooming of the penis, which is another indicator of penile erection in rats. Penile erection index (PEI) was calculated as per the methodology reported by Islam et al. (1991), *i.e.* by multiplying the percentage of rats exhibiting at least one episode of penile erection during 30-minute observation period with the mean number of penile erections: PEI= percentage of rats exhibiting penile erection × mean number of erections.

Parameters for sexual behavior analysis

The experimentation began by switching off the light at 08:00 A.M. After an interval of 20 minutes past turning off the light, the experimentation room was lit with a dim red illumination. The male rat was placed in a rectangular plexiglass chamber. After about 10 minutes, when the rat was acclimatized to the chamber condition, a sexually receptive female rat was dropped silently from one side of the chamber as stimulus. The observations in the 30 minute for sexual behavior were recorded for the following parameters (Thakuret al., 2009; Chauhan et al., 2009).

Mounting behaviour

Mount frequency (MF)wasdetermined by counting the number of pre-ejaculatory mounts with and without intromission in given period of observation.Mount latency (ML) was calculated as the time lapse from the introduction of female to the occurrence of first mount.

Intromission behavior

Intromission latency (IL) was calculated as the time when first intromission was observed after introduction of female in the cage. Intromission frequency (IF) was considered as total number of (pre-ejaculatory) intromission within 30 minutes.

Ejaculation behavior

Ejaculation frequency (EF) was calculated as the number of ejaculations observed in the 30 minute period and Post-ejaculatory Interval (PEjI) was considered as the time interval between ejaculation and the first mount of the 30 minutes period.

Serum total testosterone measurement

On the 28th day, blood was collected to measure serum testosterone level. Blood samples were spun at 2500 *g* for 10 minutes in a table top centrifuge. The serum samples obtained were analyzed to determine the concentration of testosterone. Serum concentration of total testosterone was measured by using a double antibody ELISA kit (Eiagen Testosterone kit, Italy). The protocol used for the testosterone determination was according to the method described for the kit. Serum concentrations of hormones were determined in triplicate samples (Chauhan and Dixit, 2010).

Statistical analysis

Results are expressed as mean ± standard error mean (SEM). The groups were compared by ANOVA, followed by Dunnet's test. All the statistical analysis was carried out using Instat version, 2.1 software. Etholog 2.1 was used for computation of the behavioral parameters.

Results

Penile erection Index

Penile erection index was improved in all the extract treated groups. It was marginally increased in testosterone treated group and maximum in sildenafil citrate group signifying the involvement of androgens and nitric oxide in this process. It also shows that erection is under the control of central as well as peripheral action. Compared to a PEI of 19.3±2.2 in control groups the values observed for *A. pyrethrum*group was 39.71±3.51, for *S. acmella* treated group it was found to 36.7± 4.40, for *P. murex* treated group it was 29.4±3.50.

In case of sildenafil citrate treated group it was 47.15±4.61 and it was found 27.56±3.32 in testosterone treated group (Table 1 and Fig. 1).

Sexual behavior

The observations of the sexual behavior study are illustrated in Fig. 2,3,4,5,6, and 7 while the numerical data are given in Table 1.

Mount latency was reduced significantly in all the treated groups after 28 days of treatment. In control group animals, the mount latency period was 365.5 ± 28.31, in *A. pyrethrum* treated group it was 206.3±16.9, for *S. acmella* treated group it was 219.6± 19.3 and for *P. murex* treated group 229.3±21.5. In case of sildenafil citrate and testosterone treated group the mount latency period was found to be 257.6±22.7 and 239.1±15.1 respectively.

Similar to mount latency a reduction in intromission latency time was also observed in different treated groups. In control group animals, the intromission latency period was 439.8±32.91, while in *A. pyrethrum* treated group it was reduced to 296.3±29.53, for *S. acmella* treated group it was found to be 298.9±23.9 and for *P. murex* treated group it was 310±22.8 .

In case of sildenafil citrate and testosterone treated group the intromission latency was found to be 346.6±18.71 and 316.6±22.51 respectively.

A reduction in Post ejaculatory latency time was also observed after treatment of animals with ethanolic extracts. In control group animals, the PEjL was 854.5±37.3, while in *A. pyrethrum* treated group it was reduced to 445.0±18.93, for *S. acmella* treated group it was found to be 455.3±28.54 and for *P. murex* treated group it was 468.66±25.5. In case of sildenafil citrate and testosterone treated group the post ejaculatory latency was found to be 682.0±32.69 and 752.0±22.19 respectively.

The reduction in latency time for all the parameters evaluated place the overall superiority of the extracts in following order.

A. pyrethrum>S. acmella> P. murex

Apart from the latency time an evaluation of mount and intromission frequency was also performed. The results exhibited the fact that the drug extracts under test were effective in this part as well.

The evaluation of mount frequency resulted in following observation. In control group animals, the mount frequency during 30 minute observation period was 4.6±0.32; in *A. pyrethrum* treated group it was 14.3±1.02, for *S. acmella* treated group it was found 12. 6±0.94, for *P. murex* treated group it was 11.3±0.89. In case of sildenafil citrate treated group the mount frequency was 7.0±0.45 and it was found 7.9±0.71 in testosterone treated group.

For evaluation of intromission frequency the following results were obtained. In control group animals, the intromission frequency during 30 minute observation period was 2.10±0.14, in *A. pyrethrum* treated group it was 7.16±0.23, for *S. acmella* treated group it was found to 7.11±0.39, for *P. murex* treated group it was 6.80±0.37. In case of sildenafil citrate treated group it was 4.01±0.41 and it was found 4.61±0.32 in testosterone treated group. Ejaculation frequency was also observed after 28 days of treatment. In control group animals, the ejaculation frequency was 1.4±0.2, while in *A. pyrethrum*

Table 1: Effects of ethanolic extracts of *A. pyrethrum, S. acmella and P. murex*onsexual behavior andpenile erection index in old agedmale rats

Groups	Sexual behavior parameter after 28 days of treatment						
	Mount latency (ML)	Mount frequency (MF)	Intromission latency (IL)	Intromission frequency (IF)	Ejaculation frequency (EF)	Post-ejaculatory Interval (PEjI)	Penile erection index (PEI)
Control	[illegible]	4.6±0.32	439.8±32.91	2.10±0.14	1.4±0.2	854.5 ± 37.3	19.3 ± 2.2
AP 150	206.3±16.9**	14.3±1.02**	296.3±29.53**	7.16±0.23**	3.2±0.16**	445.0±18.93**	39.71±3.51**
SP 150	219.6±19. 3**	12. 6±0.94**	298.9±23.9**	7.11±0.39**	2.8±0.12**	455.3±28.54**	36.7±4.40*
PM 150	229.3±21.5**	11.3±0.89**	310±22 .8**	6.80±0.37**	2.3±0.27	468.66±25.5**	29.4±3.50*
SC	257.6±22.7**	7.0±0.45*	346.6±18.71**	4.01±0.41*	1.9±0.25*	682.0±32.69*	47.15±4.61**
TG	239.1±15.1**	7.9±0.71*	316.6±22.51**	4.61±0.32*	1.8±0.20	752.0 ± 22.69*	27.56 ±3.32*

All values are expressed as mean ±S.E.M, n=6; P*<0.05 and P**<0.01 Considered significant as compared to control.
Control: No drug; AP 150: Ethanolic extract *A. pyrethrum* (150 mg/Kg b.w.) p.o.; SA 150: Ethanolic extract *S. acmella* (150 mg/ Kg b.w.) p.o.; PM 150: Ethanolic extract *P. murex* (150 mg/ Kg b.w.) p.o.; SC: Sildenafil citrate (5 mg/ Kg b.w.) p.o.;TG: Testosterone group: Testosterone (0.5 mg/KG b.wt.) i.m.

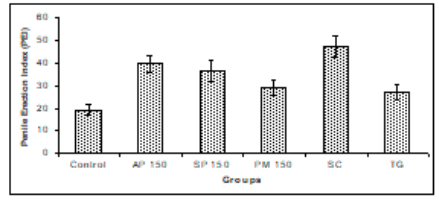

Fig.1. Effects of ethanolic extracts of *A pyrethrum, S. acmella and P. murex* on penile erection index in old aged male rats.

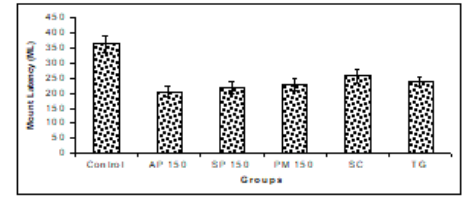

Fig.2. Effects of ethanolic extracts of A. pyrethrum, S. acmella and P. murex on mount latency in old aged male rats.

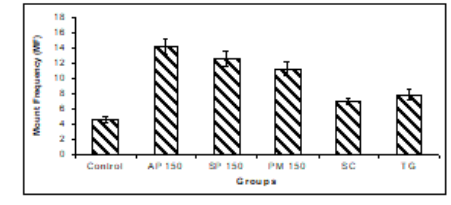

Fig.3. Effects of ethanolic extracts of *A pyrethrum, S. acmella and P. murex* on mount frequency in old aged male rats.

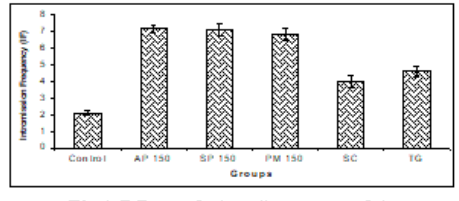

Fig.4. Effects of ethanolic extracts of *A. pyrethrum, S. acmella and P. murex* on intromission latency in old aged male rats.

All values are expressed as mean ± S.E.M, n=6; P*<0.05 and P<0.01 Considered significant as compared to control.**

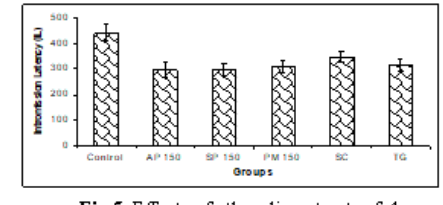

Fig.5. Effects of ethanolic extracts of *A pyrethrum, S. acmella and P. murex* on intromission frequency in old aged male rats

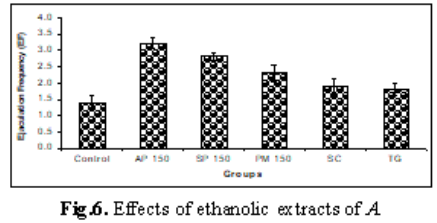

Fig.6. Effects of ethanolic extracts of *A pyrethrum, S. acmella and P. murex* on ejaculation frequency in old aged male rats.

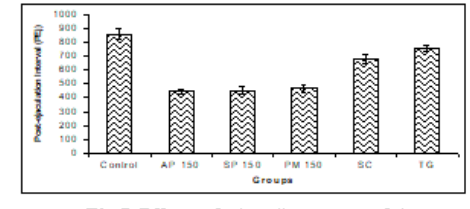

Fig.7. Effects of ethanolic extracts of *A pyrethrum, S. acmella and P. murex* on post-ejaculation interval in old aged male rats.

and *S. acmella* treated group it was increased to 3.2±0.16 and 2.8±0.12 respectively. In case of *P. murex* treated group ejaculation frequency was found 2.3±0.27. In case of sildenafil citrate and testosterone treated group the ejaculation frequency was found to be 1.9±0.25 and 1.8±0.20 respectively.

Serum testosterone levels

The testosterone level of untreated control group male rats was 1.13±0.11 ng/ml. In extract treated rats, serum testosterone level increased significantly. The mean levels of testosterone to 2.47±0.17 in *A. pyrethrum* group, 2.29±0.21 in case *S. acmella* treated group and 2.17±0.26 in case of *P. murex* treated group. In case of sildenafil citrate treated group testosterone level was 1.1±0.20 and level was found 1.92±0.21 in testosterone treated group. The observation therefore clearly suggest that administration of testosterone as such does not result in very high testosterone levels in serum while in case of extracts treatment improves the level of testosterone to a greater level. No effect of sildenafil citrate was observed on the hormone levels in rat sera (Table 2).

Discussion

Androgens have long been known to have a major stimulatory influence on several aspects of male sexual behavior, including penile erection.Sexual behavior is dependent on normal functioning of the hypothalamo-pituitary-gonadal axis (Thakuret al., 2011). In most mammalian species studied, castration has been found to decrease substantially the erectile responses to a variety of stimuli, whereas androgen replacement reversed these effects (Andersson, 2001).

Testosterone (T) is the main male gonadal hormone produced by the interstitial cells of the Leydig in the testis. It also helps in maintaining body shape, and increasing muscle mass and strength. The increase in testosterone should enhance androgen-dependent parameters such as mating behaviour and maintenance of spermatogenesis (Chauhan and Dixit, 2010). Testosterone may also facilitate male sexual behaviour by increasing dopamine release in the medial preoptic area and potentiating nitrergic neurotransmission (Hull et al., 1999; Putnam et al., 2001).

Table 2: Effect of administration of ethanolic extract of *A. pyrethrum, S. acmella and P. murex* on old aged male rat serum Testosterone level

Group and treatment	Serum Testosterone level (ng/ml)
Control	
AP150	2.47±0.17*
SA150	2.29±0.21**
PM150	2.17±0.26**
SC	1.1±0.20
TG	1.92±0.21

All values are expressed as mean ±S.E.M, n=6; P*<0.05 and P**<0.01 Considered significant as compared to control. Control: No drug; AP 150: Ethanolic extract *A. pyrethrum* (150 mg/Kg b.w.) p.o.; SA 150: Ethanolic extract *S. acmella* (150 mg/ Kg b.w.) p.o.; PM 150: Ethanolic extract *P. murex* (150 mg/ Kg b.w.) p.o.;

Present findings provide experimental evidence that the ethanolicextract of *A.pyrethrum, S. acmella* and *P. murex* used as a traditional medicine, possesses aphrodisiac properties. We demonstrated that the oral administration of its ethanolic extract was able to improve sexual performance of old age rats, particularly of sexual arousal in old age male rats and that it promotes the expression of male sexual behaviour. Compared with the controls, the percentages of mounting and intromission in rats were significantly increased in animals by administration of the extract. The reduction of ML, IL and PEI is generally suggested to be indicative of an improved copulatory behaviour, particularly when observed together with an increase in mounting and ejaculating animals (Bitran and Hull, 1987).

The ability of different medicinal plants to improve sexual function as illustrated in the case of *Tribulus terrestris, Panax ginseng, Ferula hermonis* was ascribed to increased levels of testosterone in the serum (Zanoli et al., 2009).

There is a possibility of developing the ethanolicextract of *A. pyrethrum, S.acmella* and *P. murex*as a therapeutic principle for stimulating male sexual activity, especially in cases where there are old age sexual deficiencies. The presence of alkylamides in the extract may be suspected as possible contributor to the observed effect of improved sexual function. The basis for such a premise is drawn from the libido enhancing properties of alkylamides isolated from the roots of *Lepidium sativum*.Oral administration of a purified lipidic extracts from *Lepidium meyenii* and *Anacyclus pyrethrum* increased intromissions and mounts frequency in normal mice (Zheng et al., 2000 and Sharma et al.,2010). In our study, ethanolic extract treated rats also showed increased intromissions and mount frequency.It is likely that alkylamide may mimic the action like testosterone or

stimulate secretion of testosterone that improves sexual behaviour.

In conclusion, the present study provides evidence that the ethanolic extract of *A. pyrethrum, S. acmella* and *P. murex* are a potent stimulator of sexual behaviour in old age male rats. There was an overall increase in the sexual behaviour parameters for the treated groups, as reflected in the increasement of MF, IF and EF, and reduction in ML, IL and PEI. These results were statistically significant even after discontinuing the treatment. The effect persisted, suggesting encapacitation of the body for improved sexual activity. The observed effects of the plant extracts in sexual behavior and performance suggest its efficacy in diminishing sexual energy or function specially in advancing age or debilities due to many reasons.

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Conflict of interest

No conflict to disclose

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