

Research article

Open Access

Evaluation of the Anabolic, Aphrodisiac and Reproductive Activity of *Anacyclus Pyrethrum* DC in Male Rats

Vikas SHARMA, Mayank THAKUR,
Nagendra Singh CHAUHAN, Vinod Kumar DIXIT *

Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar M. P., India

* Corresponding author. E-mail: vkdixit2011@rediffmail.com (V. K. Dixit)

Sci Pharm. 2009; 77: 97–110

doi:10.3797/scipharm.0808-14

Published: December 23rd 2008

Received: August 21st 2008

Accepted: December 23rd 2008

This article is available from: <http://dx.doi.org/10.3797/scipharm.0808-14>

© Sharma *et al.*; licensee Österreichische Apotheker-Verlagsgesellschaft m. b. H., Vienna, Austria.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Anacyclus pyrethrum DC (Compositae), commonly referred to as 'Akarkara', is widely recognized in Ayurvedic system of Indian medicine as tonic and rejuvenator. The roots are also considered aphrodisiac and sexual stimulant. Aqueous extract of the roots was studied for its effect on sexual behavior, spermatogenesis, and sperm count. Fructose levels in seminal vesicles of albino rats were also recorded. Two doses i.e. 50 and 100 mg/kg of aqueous extract on administration in albino rats showed pronounced anabolic and spermatogenic effect in animals of respective groups. The sperm count and fructose levels in seminal vesicle were markedly increased. Improvement in sexual behavior of male rats was characterized by increased mount and intromission frequency and reduced mount and intromission latency. The extract had a dose dependent influence on sperm count and seminal fructose concentration which increased significantly.

Keywords

Anacyclus pyrethrum • Akarkara • Aphrodisiac • Rasayana, Spermatogenesis • Vaajikaran

Introduction

Sexual dysfunction, impotence and other related problems is eluding scientific community and medical practitioners since time immemorial. Sexual dysfunction may have psychosocial implication affecting men in many ways. There has been a constant exploration for newer herbal and chemical agents to overcome these age-old problems of sexual dysfunction [1].

A variety of plants have been used as sex stimulants in traditional medicines of various countries. *Punica granatum* was a symbol of immortality and love in oriental regions [2]. Similarly a number of plants (like *A. racemosus*, *C. borivilianum*, *D. hatagirea*, *C. orchiodies*, *O. latifolia*) have been traditionally employed among different cultures in order to improve sexual performances [3–6]. Administration of *Trichopus zeylanicus* leaf to male mice stimulated their sexual behavior as evidenced by an increase in number of mounts and mating performance [7]. Herbal drugs have also been reported for enhancing the sperm count and eliciting androgenic effects [8].

Anacyclus pyrethrum DC roots which are commonly known as pellitory root (Akarkara in Indian trade), is one such herb which has been widely acclaimed in Ayurvedic system of medicine for its rejuvenative properties [9]. It is generally designated as a Vajikaran Rasayana herb and is known to improve sexual function especially in male [10]. Powder of this herb when consumed, has been known to arouse sexual desire and improve ejaculatory time [11].

Apart from being used as an aphrodisiac the herb *A. pyrethrum*, is widely used in folk remedies for stimulating salivary glands, curing chronic catarrh of the head or nostrils and when applied to the skin, it acts as a rubefacient. As a masticatory it has been found useful in toothache, apnea, paralysis of the tongue and muscles of the throat and neuralgic affections of the teeth. The plant is native to Asia and Africa and is mainly found in the northern region of India. Pellitory root has been frequently analyzed, and its activity proved to reside in an acrid matter, known as pyrethrin. But this so-called pyrethrin is, in reality, a mixed substance, consisting of a brown acrid resin. The root also contains a little volatile oil, gum, and traces of tannic acid [12]. Phytoconstituents mainly reported from the plant are, N-isobutyldienedynamide [13] and hot water soluble polysaccharides [14].

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

Materials and methods

Animal Stock

The protocol for experimentation was approved by Institutional Animal Ethics Committee of Dr H. S. Gour University, Sagar, India. Wistar strain albino rats of either sex weighing 120–150 g were fed on standard diet and water *ad libitum*. The animals were housed at room temperature ($24 \pm 2^\circ\text{C}$) on a reversed day-night cycle (06:00 h to 18:00 h). The guidelines

of CPCSEA, India the governing body for animal experimentations in India, were strictly adhered to during the whole animal experimentation protocol. The number for approval of ethical committee is 379/01/ab/CPCSEA and the proposal was approved by the meeting which was held on 23-10-07.

Preparation of extracts

Dried roots *Anacyclus pyrethrum* DC. (Akarkara) were purchased from local market, and were authenticated at Agharkar Research institute Pune, India (Authentication no. Auth. 07-86). The dried roots were crushed to moderately coarse powder (60–80 mesh size) and defatted with petroleum ether (60–80°C). Defatted drug was then extracted with water and the extract was dried using lyophilization technique as reported previously by Thakur and Dixit 2008 [5]. Lyophilized aqueous extract showed positive test for ketose sugar. The major constitute of lyophilized aqueous extract was fructans which were isolated and studied separately. The yield of polymeric ketoses was ~55% in aqueous extract.

Preparation of Test Samples

Lyophilized aqueous extract was suspended in 1% sodium CMC solution and administered orally. Testosterone was procured from Sun Pharma, Baroda, India as a generous gift. It was suspended in arachis oil and administered intramuscularly.

Treatment

Thirty two male rats were randomized into 4 groups comprising of 8 animals each. The animals were treated with respective extracts or drug for 28 days and various test parameters were evaluated.

Group I (vehicle only) served as control.

Group II aqueous extract 50 mg/Kg b. w. (p.o.) daily (Aq 50)

Group III was administered with daily dose of aqueous extract 100 mg/Kg b. w. (p.o.) daily (Aq 100)

Group IV was given 0.5 mg/Kg b. w. of testosterone suspension in arachis oil, twice a week intramuscularly.

The dose of extracts was decided after careful evaluation of doses of *A. pyrethrum* in various traditional literatures. The reported dose of dry root powder is 2-3 gm for an adult male. Based on the extractive values and traditional dosage the treatment doses were determined.

Studies Performed

Effect on sexual organ weight

After 28 days of treatment the body weight of animals was recorded. The animals were then sacrificed by cervical decapitation and testis, seminal vesicles, epididymis and prostate glands were carefully removed and weight of each organ was determined [3].

Histological studies

After 28 days of treatments to animals of all respective groups, testis of animals from each group were dissected out and testicular section of nearly 5 μ m thickness were fixed in Bouin's fixative, dehydrated by varying percentage of ethanol and stained with hematoxylin and eosin. Microscopic evaluation of the thin section was undertaken and variations in histoarchitecture were recorded [15].

Orientation behavior analysis

The analysis of orientation activity was carried out and analyzed in three segments with little modification [2].

Orientation behavior of male rats was determined using following method of scoring:

- Orientation towards female – (1 for every sniffing and 2 for every licking)
- Orientation towards self – (1 for non-genital grooming and 2 for genital grooming)
- Orientation towards environment – (1 for exploration, 2 for *rearing* and 3 for climbing)

Rats were observed daily for their orientation activity. The cumulative score after 0, 15, 28 days of the treatment as well as after 7 and 15 days past withdrawal of experimentation i.e. after withdrawal of extracts, were recorded (Table 2).

Sexual behavior analysis

Male rat was placed in the observation glass chambers in order to acclimatize it with the cage environment. Sexually receptive female rat was then allowed to enter the test cage silently from a side door inside the cage. The behavioral observations were carried out taking into account the following parameters[3, 6].

Mounting Behavior – It was determined and characterized by following parameters.

- (A) Mount frequency – average number of mount during 30 min observation.
- (B) Mount latency- The leg time from the introduction of female in the cage to first mount.

Intromission Behavior – It was evaluated as follows.

- (A) Intromission frequency – average number of Intromission during 30 min observation.
- (B) Intromission latency- Intromission latency (IL) was considered as the time for first intromission after introduction of female in the cage.

Penile Erection Index

Penile Erection (*PE*) was determined when the rats bent down to lick their erect penis during the observation period. *Penile erection index (PI)* was determined by multiplying the

percentage of rats exhibiting at least one episode of penile erection during 30-min observation period with the mean number of penile erections [3, 6].

PI = % of rats exhibiting penile erection X Mean number of erections

Sperm Count

(a) In-vivo sperm count

For counting spermatozoa left and right epididymes of four rats of each group were homogenized and taken into 5 ml of 1% sodium citrate solution, squashed thoroughly with the help of needle and forceps until a milky suspension was obtained. The suspension was filtered through 80 μ mesh and the final volume made up to 10 ml. The made up volume was inclusive of washings of the filter. The suspension was thoroughly shaken and the spermatozoa were counted using a hemacytometer. The average numbers of sperms determined in every group are reported [8].

(b) In-vitro sperm count preservation

For the determination of *in-vitro* sperm count preservation, the method of Thakur and Dixit (2007) was used. In brief, a total of six healthy male rats weighing between 110–130 g were taken and sacrificed by cervical decapitation. Left and right epididymes of all the rats were taken into 5 ml of 1% sodium citrate solution and squashed thoroughly, with the help of a needle and forceps until a milky suspension was obtained. The solution was filtered through 80 μ mesh and the volume was made up to 10 ml inclusive of washings of the filter. A 1 mg/ml solution of aqueous extract was prepared and added into the sperm specimens in the test sample in a ratio of 0.1:1 (100 μ l sperm solution: 1 ml extract solution). The spermatozoa were counted using hemacytometer at 0 and 30 min after incubation at room temperature (25 ± 1 °C) [4].

Determination of fructose concentration in seminal vesicle

For the determination of seminal fructose concentration the method reported by Gonzales, 1991 was used. The seminal vesicles were homogenized, and the homogenate was then deproteinized, by shaking 0.1 ml of seminal vesicle homogenate with 1.9 ml of distilled water, thereafter, 1 ml each of 1.8% Barium hydroxide and 2% Zinc Sulphate were added and the mixture shaken each time, after the addition of each of the reagents. This was followed by centrifugation at 2000 rpm for 30 min. The supernatant was used for the fructose estimations following the method described by Keleti and Lederer, 2003 [16,17].

Statistical Analysis

Results are expressed as mean \pm SEM. The treated groups were compared to control by ANOVA following Dunnet's test. All the statistical analysis was carried out using Instat version 2.1 software.

Results

Anabolic effect

The effect of the aqueous extract on sexual organ and body weight is summarized in Table 1. Treatment with Aq 50 showed 10.07 % increase in body weight, 6.5 % increase in

weight of testis, 6.6 % increase in weight of prostate gland, 6.8 % increase in seminal vesicles and 7.6 % in case of epididymis, while Aq 100 extract treatment showed an increment of ~ 14.2 % in body weights, ~ 8.6 % increase in weight of testis, ~ 10.5 increase in weight of prostate, 9.2 % increase in the weight of seminal vesicles and 9.2% in case of epididymis after 28 days of treatment. Testosterone administration produced a 9.08 % increase in body ~ 6.3% increase in testis, ~ 24.09% in case of prostate weights, 6.3% increase in the weight of seminal vesicles and ~ 4.20 % in case of epididymal weights (Table 1).

Histological examination

The sections of the testis of the control group when compared with treated group animals showed observable differences in various stages of spermatogenesis observed in different treated groups. In control group's animals, all stages of spermatogenesis were clearly observed *viz.* spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid and spermatozoa, beside connective tissue, blood vessels, lymph ducts and Leydig's cells were observable and distinct. The proliferation was evidently more perceptible in case of treated group animals as compared to control.

Tab. 1. Effect of aqueous extracts of *Anacyclus pyrethrum* on body/organ weights of albino rats

Group	Body weight (gm)		Weight of testes	Weight of prostate
	0 day	28 days	28 days	28 days
Control	131.6 ± 3.32	140.5±1.4	841±3.4	206±3.36
AE 50	135.0± 1.82	148.6±1.9*	896.1±3.9**	220.8±3.2*
AE 100	134.16±1.86	155.3±2.26**	914.8±2.4**	228.7±2.7**
TG	137.6±3.63	150.1±1.408*	894.1±2.5**	256.5±3.7**

Group	Weight of seminal vesicles	Weight of epididymides	Average number of sperms	Fructose content (mg/g)
	28 days	28 days	28 days	28 days
Control	291.5±7.04	547.2±2.9	120.2 ± 1.8	1.50±0.058
AE 50	311.4±5.4**	589.5±7.1**	131.5 ± 2.5**	2.04±0.068**
AE 100	328.8±3.3**	598.7±4.47**	146.7 ± 3.09**	2.48±0.062**
TG	310.1±3.16**	570±3.49**	135.7 ± 1.8**	1.81±0.041**

Weight of testes, prostate, seminal vesicles are expressed as mg/100g body weight.

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; AE 50: Aqueous extract (50 mg/Kg b.w.) p.o.; AE 100: Aqueous extract (100 mg/ Kg b.w.) p.o.; TG: Testosterone group: Testosterone (0.5 mg/KG b.wt.) i.m.

Aqueous extract 50 and 100 mg treated groups showed increase in the size of seminiferous tubules. Basement membrane was tightly bound with germinal epithelium. The lumen of seminiferous tubule was filled with bundles of spermatozoa. There was also

increase in number of leydig's cells as cytoplasm was highly stained with eosin. Under normal condition the sertoli cells lie down near the basement membrane and are spaced at quite regular intervals whereby they perform their functions of supporting the developing spermatogenic cells, in general the nucleus is at right angles to the wall and the cell is pyramidal in shape. In case of aqueous extract treated groups, the primary spermatogenic cells, the spermatogonia are at the first state of repetitive cell division. As can be seen in figure 2, 3 and 4 lumen size is decreased and an increase in vascularization is observable in extract treated group, the effect is slightly restrained in the testosterone treated group and is very meager in control. Similarly, a very clear view of leydig cells can be seen in photomicrograph for different extract treated groups, further confirming the efficacy of extracts of *A. pyrethrum* analogous to that of testosterone in spermatogenic activity. Comparison of paleness between stained control slide and the vascularized mounts of the test group testis suggests a proper differentiation and vascularization of the spermatids and spermatogonia. The figure also discloses a better and non-striated vesicle as compared to the control signifying for a better spermatogenetic activity of the extracts, which are comparable to those found in the figure of testosterone group. Histopathological studies revealed that the spermatid separation was enhanced by the administration of extracts and was comparable to the effect of testosterone. The tubules are widened by seminiferous epithelium resting on a basement membrane, which in turn is bordered by thin layer of fibrous connective tissues. The interstitial stroma is full of blood and lymph vessels and contains small group of interstitial cells of leydig. Histoarchitecture of testosterone treated group also show a profile similar to aqueous treated group. High number of spermatozoa in seminiferous tubules confirmed the increased spermatogenesis, this is also proved by an increase in spermatogenic elements as compared to control. In case of testosterone treated group, a distinct hypertrophy compared to control group animals was observed in case of leydig cells and interstitial cells. The solid packing also suggests a supposed role of testosterone in increasing the vascularization of testicular tissue (Figure 4).

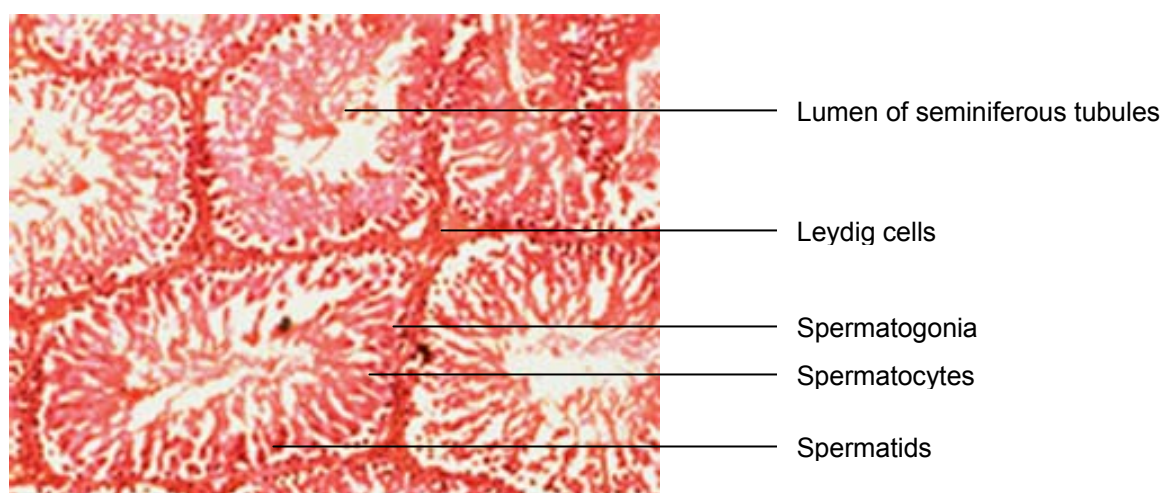


Fig. 1. Histoarchitecture of testis of control group

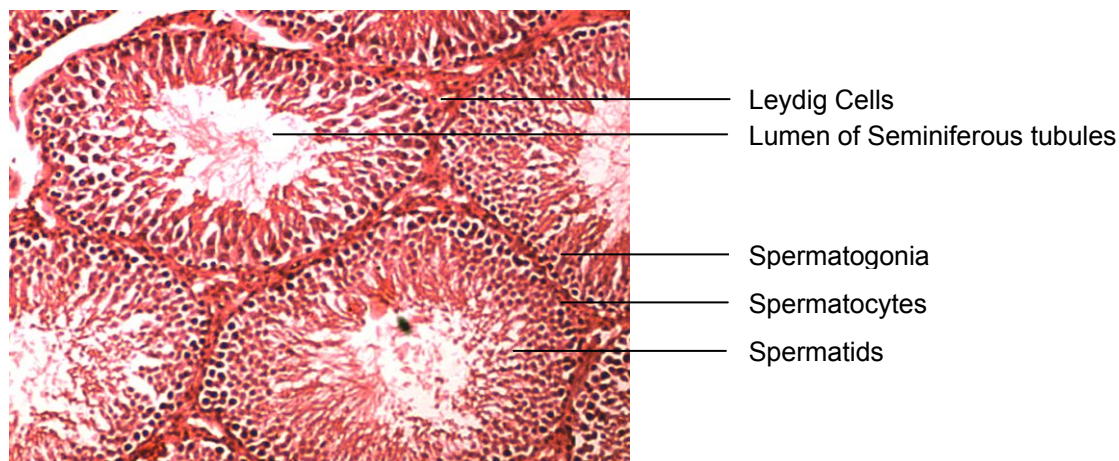


Fig. 2. Histoarchitecture of testis of aqueous extract 50 mg treated group

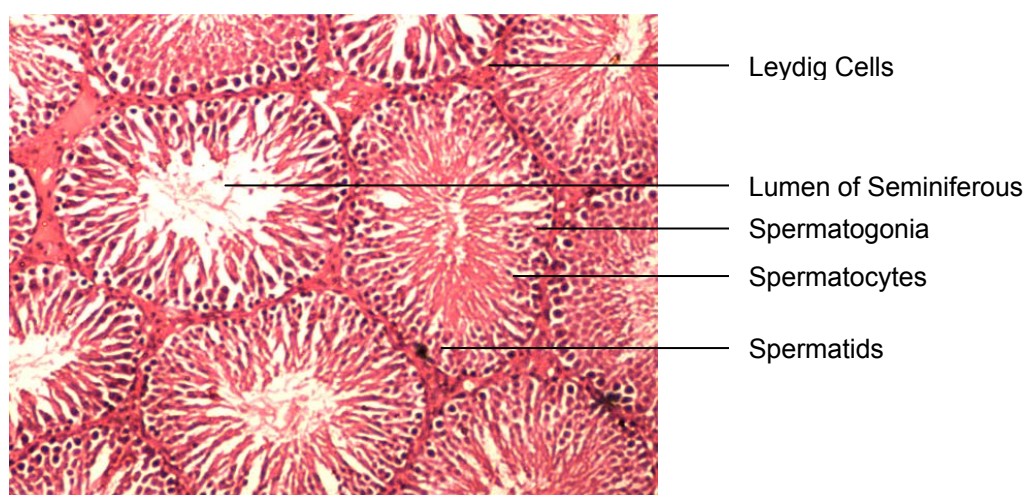


Fig. 3. Histoarchitecture of testis of aqueous extract 100 mg treated group

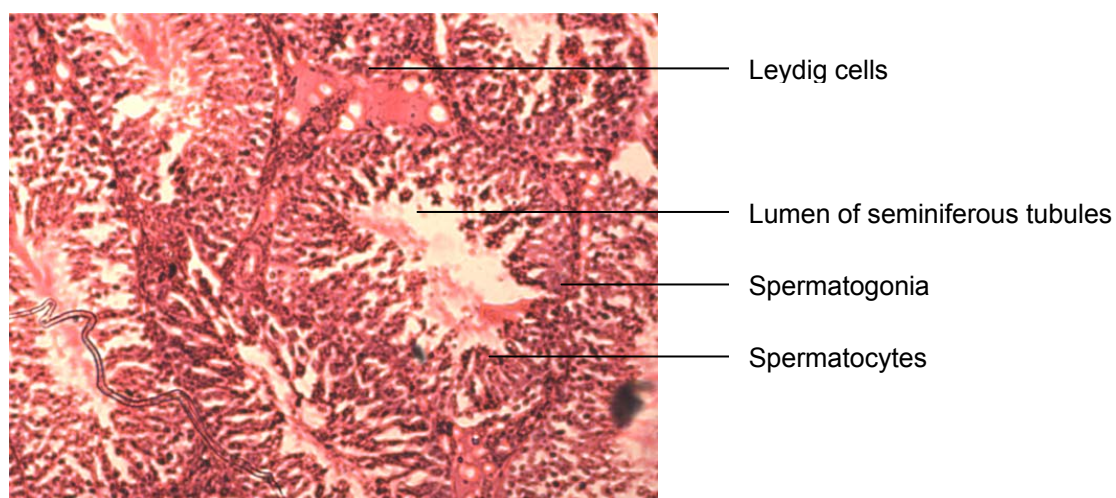


Fig. 4. Histoarchitecture of testis of testosterone treated group

Orientation behavior analysis

The aqueous extracts markedly influenced the behavior of the treated animals, which showed more attraction towards female rats. A more than two fold enhancement in attraction towards female was noticed in Aq 100, compared to nearly two times increase in Aq 50 and only one and half fold increase in testosterone treated group. The behavioral assessment of rats towards environment as well as self was also more pronounced as compared to control group animals (Table 2).

Sexual behavior

Penile Erection Index (PI) an indicator of enhanced vascular function in penile tissue, was also increased in treated groups, it was 3 folds better in case of Aq 100 treated group, compared to nearly 2.5 fold better in case of Aq 50 and testosterone treated groups as compared to control group values which were considered as normal benchmark (Table 2).

Sexual behavior of the animals was also improved upon treatment with aqueous extract. Administration of extracts had a pronounced effect on overall sexual performance, as evidenced by different parameters studied. The mount latency and intromission latency were significantly reduced in aqueous extract treated groups. Mount latency time was reduced by 35.5% in Aq 100 and 24.5% in Aq 50 treated group, whereas only 14.8% reduction was observed in case of testosterone treated group. Similarly, intromission time was reduced by 17% in Aq 100 and 11.8% in Aq 50 treated group whereas only 9.8% reduction was observed in case of testosterone treated group. The observation for mount frequency revealed an increase in mount frequency 12.83 ± 0.94 in Aq100, 10.66 ± 0.80 in Aq 50 and 9.36 ± 0.94 in testosterone treated group compared to 4.83 ± 0.40 in control group animals. The intromission frequency was also increased and was found to be 6.64 ± 0.23 in Aq100, 4.16 ± 0.30 in Aq50 and 3.80 ± 0.56 in testosterone treated group compared to 2.12 ± 0.42 in control group animals (Table 3). The results of behavioral studies conducted after 7 and 15 days of withdrawal of treatment illustrated that the improvements in case of mount and intromission frequencies and reduction in latencies was significantly improved, when compared with that of the untreated control group animals, this was most pronounced in case of Aq100 treated group animals. In case of testosterone treatment, the improvements were subsided and no effectiveness was observable after 15 days of withdrawal.

Sperm count

The sperm count in control and extract treated groups, at 0 min there is no significant difference (119 ± 2.0 in control group and 118 ± 1.23 in Aq group) in the average sperm counts in both groups, but a significant difference was clearly evident after 30 min. There was a significant reduction in average sperm number by 47 % (63 ± 1.26) in control group which was only reduced by 23 % (91.3 ± 1.4) in Aq treated group, when compared to their sperm count at 0 min.

In-vivo sperm count was also determined after 28 days of treatment of the all group animal, the epididymal sperm counts significantly increased in treated group to 9.4, 22 and 17 % in Aq50, Aq100 and testosterone group respectively, than those in the vehicle-treated group (Table 1).

Tab. 2. Effect of aqueous extracts of *A. pyrethrum* on orientation activities in male rats

Parameters for sexual behavior analysis:	Group	0 days	15 days of treatment	28 days of treatment	After 7 days Withdrawal of treatment	After 15 days Withdrawal of treatment
Mean activity Score Towards Female (Licking & Anogenital smelling)	<i>Control</i>	7.5 ±0.84	7.5 ±0.76	7.83 ±0.87	7.6±0.76	7.80±0.94
	<i>AE 50</i>	7.16 ±0.91	10.5±0.67	13.5±1.43*	11.16±1.19*	10.16±1.30
	<i>AE 100</i>	7.5 ±0.56	12.5±1.25**	17.5±1.2**	15.5±0.88**	13.83±0.83*
	<i>TG</i>	7.83 ±0.71	11.83±0.94**	12.0±0.94*	10.83±0.60	9.50±0.47
Mean activity Score Towards Environment (Exploration, Rearing and Climbing)	<i>Control</i>	13.3±0.88	13.5±1.4	14.16±1.07	14.5±0.99	14.83±1.01
	<i>AE 50</i>	13.5±1.38	16.52±1.05*	23.33±1.14**	18.83±1.07	17.0±0.89
	<i>AE 100</i>	13.0±0.85	18.66±1.2**	24.0±0.59**	23.33±1.3**	18.16±1.04**
	<i>TG</i>	12.14±1.08	14.1±0.83*	20.0±1.71*	16.16±1.1	15.66±1.01
Mean activity Score Towards Self (Nongenital grooming and Genital grooming)	<i>Control</i>	8.16±0.47	8.0±0.36	8.10±0.42	8.16±0.60	7.80±0.94
	<i>AE 50</i>	7.80±1.04	12.83±1.07*	13.50±1.47**	11.60±0.80*	10.30±0.33
	<i>AE 100</i>	8.60±0.91	13.50±0.99**	16.0±1.35**	13.0±0.76**	11.0±0.85**
	<i>TG</i>	7.50±0.42	11.83±1.55*	11.5±1.04*	11.16±1.3*	8.57±0.94
Penile Erection Index (PI, Mean ± SE)	<i>Control</i>	23.0±2.2	22.1±2.4	27.6±4.9	24.9±2.7	23.9±3.1
	<i>AE 50</i>	21.1±0.9	33.2±7.0	63.8±6.7**	47.15±6.6*	41.6±5.6
	<i>AE 100</i>	23.0±2.2	44.4±5.5*	69.5±7.9**	61.1±7.6**	47.2±5.1*
	<i>TG</i>	23.9±1.1	47.2±5.1*	63.1±3.3**	44.5±5.0*	27.9±6.6

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; AE 50: Aqueous extract (50 mg/Kg b.w.) p.o.; AE 100: Aqueous extract (100 mg/ Kg b.w.) p.o.; TG : Testosterone group: Testosterone (0.5 mg/KG b.wt.) i.m.

Fructose content

Seminal fructose content is an important parameter for evaluating the normal sexual functioning in male, as fructose is consumed during fructolysis for providing energy to immotile spermatozoa. Treatments with lyophilized aqueous extract improved seminal fructose content in a dose dependent manner. In case of Aq 50 treated group, the seminal fructose content was found to be 2.04 ± 0.06 mg/g, while in case of Aq 100 it was 2.48 ± 0.62 mg/g. In case of testosterone treated the seminal fructose level was found to be 1.81 ± 0.41 mg/g. (Table 1).

Tab. 3. Effect of aqueous extracts of *A. pyrethrum* on sexual performance parameters in male rats

Parameters for sexual behavior analysis:	Group	0 days	15 days of treatment	28 days of treatment	After 7 days Withdrawal of treatment	After 15 days Withdrawal of treatment
Mount latency (time in seconds)	Control	194.7 ± 5.6	196.6 ± 5.1	194.0 ± 4.5	195.0±5.6	193.3±6.1
	AE 50	193 ± 5.8	178.8±1.8*	146.3±4.4**	166.0±3.98**	178.3±3.29
	AE 100	195.8 ± 1.6	160.6±0.98**	125.1±2.0**	138.5 ± 6.2**	171.1±3.8**
	TG	193.7 ± 6.6	175.5 ± 3.6*	165.1±2.5**	170.5±3.77**	187.0±2.0
Mount frequency	Control	3.33±0.42	4.33±0.21	4.83±0.40	4.05±0.25	5.01±0.44
	AE 50	3.6±0.33	7.10±0.60**	10.66±0.80**	8.34±0.60**	7.50±0.42**
	AE 100	3.5±4.2	11.20±0.77**	12.83±0.94**	11.52±0.76**	10.16±0.60**
	TG	4.0±0.36	8.61±0.61**	9.36±0.94**	7.69±0.55*	4.80±0.60
Intromission latency (time in seconds)	Control	305.3±4.5	304.3±2.3	302.5±12.5	318.6±4.50	303.1±2.6
	AE 50	304.1±4.9	292.6±3.3	272.8±3.0**	277.5±4.04**	290.3±4.7*
	AE 100	305.5±9.6	278.3±3.4**	251.0±4.7**	265.0±5.20**	276.6±3.8**
	TG	306.4±12.9	295.6±3.9	267.3±2.1**	280.0±2.69*	297.1±2.9
Intromission frequency	Control	1.60±0.33	1.62±0.36	2.12±0.42	1.83±0.16	1.66±0.21
	AE 50	1.52±0.29	3.13±0.49	4.16±0.30*	3.60±0.33*	2.83±0.30
	AE 100	1.37±0.32	4.52±0.75**	6.64±0.23**	4.32±0.55**	3.51±0.42**
	TG	1.16±0.30	3.11±0.60	3.80±0.56*	3.11±0.78	2.27±0.25

All values expressed as mean ±S.E.M, n=6; P* $<$ 0.05 and P** $<$ 0.01 considered significant as compared to control; Control: No drug; AE 50: Aqueous extract (50 mg/Kg b.w.) p.o.; AE 100: Aqueous extract (100 mg/Kg b.w.) p.o.; TG: Testosterone group: Testosterone (0.5 mg/KG b.wt.) i.m.

Discussion

Administration of extract resulted in weight gain in treated animals. Testes and epididymal weights were also increased significantly, while a moderate but insignificant increase in prostate weight was also observed. The increase in body and organ weights was comparable with testosterone (Table 1). Since androgenic effect is attributable to testosterone levels in the blood, it is likely that the plant extracts may have a role in testosterone secretion allowing better availability of hormone to gonads [3]. A significant anabolic effect upon administration of extracts was observable as compared to the control group, which was comparable to that of the administration of testosterone suggesting a testosterone type action of the extracts. The experiment clearly suggests anabolic steroidal effect of *A. pyrethrum*. The enhancement of sexual activity has been directly correlated to the enhancement of sexual pleasure. Penile erection index is important for evaluating the effect of administration of extract on erectile function [18]. The aqueous extract of *A. pyrethrum* increased the penile erection of male rats, similar to the increase in penile erection index observed by administration of testosterone. An increase in PEI was observed in treated groups, indicating the involvement of nitrous oxide (NO) based intervention [19]. The effect in *A. pyrethrum* aqueous extract treated group (100 mg/Kg) is much more pronounced when compared to the administration of testosterone. Administration of extract (50 and 100 mg/Kg b. w.) modified both the orientation as well as sexual behavior, conclusively suggesting a better sexual performance after administration

of the extracts. This activity was also validated by the histopathological studies on testis section of various groups. The results thus confirm that, the drug extracts can be useful in enhancement of overall sexual performance of male rats. Drug also shows prolonged and sustained effect in overall sexual performance conducted during this study. Sexual desire may be affected directly by increasing serum testosterone level or by having testosterone like effect. This may be due to the change in neurotransmitter level or their action in the cell could also changed sexual behavior [7, 20]. Spermatogenesis involves a complex interplay between the structural element of testis and the endocrine system [15].

FSH stimulates spermatogenesis and LH stimulates synthesis and release of testosterone. Testosterone causes an increased blood flow and stimulates the growth of the target tissues. Testosterone cause direct stimulation of spermatogenesis. Our results also show that there is increase in spermatogenesis and increase in weight of sexual organ in extracted treated group as comparison to control group [21]. The improvement of *in-vivo* sperm count suggests an improved spermatogenic activity of the test extract. Similarly, the seminal fructose count improvement also indicates that the aqueous extract of *A. pyrethrum* enhances the quality of seminal parameters which eventually assist in better reproductive potential. Preservation of *in-vitro* sperm count is an indicative that the herb contains water soluble nutritive substances that help sperms in avoiding death due to lack of energy. One the other hand the fructose content is also regulated by androgens and fructose content establishes a narrow relationship among the levels of testosterone and fructose production by the seminal vesicle [16]. Therefore, the results corroborate the hype that the plant is capable of being nominated as herbal alternative for improving sexual function. Since, the herb had a beneficial effect on various physiological conditions responsible for exhibiting a better sexual performance i.e. improved anabolic activity, better orientation activities, improvement in seminal fructose level and an overall improvement in various sexual behavior parameters. Hence, these findings provide a scientific basis for acclaiming *A. pyrethrum* as 'Vajikarana Rasayana'.

Acknowledgement

One of author Vikas Sharma is thankful to UGC for providing the financial assistance.

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

References

- [1] Adimoleilija A.
Clinical application of Asian medicine in erectile dysfunction (ED).
Int J Impot Res. 1997; 9: 1–4.
doi:10.1038/sj.ijir.3900265

- [2] Islam MW, Tariq M, Ageel AM, Al-Said MS and Al-Yhya AM. Effect of *Salvia hematodes* on sexual behavior of male rats. *J Ethnopharmacol.* 1991; 33: 67–72. doi:10.1016/0378-8741(91)90163-8
- [3] Thakur M, Dixit VK. Effect of *Chlorophytum borivilianum* on androgenic & sexual behavior of male rats. *Indian Drugs.* 2006; 43: 300–306.
- [4] Thakur M, Dixit VK. Effect of some vajikaran herbs on peniculation activities and In-vitro sperm count in male. *Sex Disabil.* 2007; 25: 203–207. doi:10.1007/s11195-007-9051-y
- [5] Thakur M, Dixit VK. Ameliorative Effect of Fructo-Oligosaccharide Rich Extract of *Orchis latifolia* Linn. on Sexual Dysfunction in Hyperglycemic Male Rats. *Sex Disabil.* 2008; 26: 37–46. doi:10.1007/s11195-007-9063-7
- [6] Chauhan NS, Rao CV, Dixit VK. Effect of *Curculigo orchioides* rhizomes on sexual behaviour of male rats. *Fitoterapia.* 2007; 78: 530–534. doi:10.1016/j.fitote.2007.06.005
- [7] Subramoniam A, Madhavachandran V, Rajasekharan S, Pushpangadan P. Aphrodisiac property of *Trichopus zeylanicus* extract in male mice. *J Ethnopharmacol.* 1997;57: 21–27. doi:10.1016/S0378-8741(97)00040-8
- [8] Saxena S, Dixit VK. Role of total alkaloids of *Mucuna pruriens* Baker in spermatogenesis in male rats. *Indian J Nat Prod.* 1987; 3: 3–7.
- [9] The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy, Part I, Vol. II, 1st Edition, New Delhi. 2004.
- [10] Puri HS, 'Rasayana'—Ayurvedic herbs for longevity and rejuvenation. Taylor and Francis, London. 2003.
- [11] Singh G and Mukherjee T. Herbal aphrodisiacs: A review. *Indian Drugs.* 1998; 34: 175–182.
- [12] Bentley R, Trimen H. Medicinal Plants. Third vol., Asiatic Publishing House, Delhi. 2004.
- [13] Crombie L. Isolation and Structure of an N-isoButyldienedynamide from Pellitory (*Anacyclus pyrethrum* DC.). *Nature.* 1954; 174: 832–833. doi:10.1038/174832a0
- [14] Bendjeddou D, Lalaoui K, Satta D. Immunostimulating activity of the hot water- soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galangal* and *Citrullus colocynthis*. *J Ethnopharmacol.* 2003; 88: 155–160. doi:10.1016/S0378-8741(03)00226-5

- [15] Chauhan NS, Dixit VK.
Spermatogenic activity of rhizomes of *Curculigo orchioides* Gaertn on male rats.
Int J Appl Res Nat Prod. 2008; 1(2): 26–31.
- [16] Gonzales GF.
Functional structure and ultrastructure of seminal vesicles.
Arch Androl. 1989; 22: 1–13.
doi:10.3109/01485018908986745
- [17] Keleti G, Lederer WH.
Handbook of micromethods for the biological sciences.
Van Nostrand Reinhold Company, New York. 1974.
- [18] Benassi-Benelli A, Ferrari F, Pellegrini-Quarantotti B.
Penile erection induced by apomorphine and N-n-apopolymorphine in rats.
Arch Int Pharmacodyn Ther. 1979; 242: 241–247.
PMid:44457
- [19] Thakur M, Dixit VK.
Aphrodisiac activity of *Dactylorhiza hatagirea* (D.Don) Soo in Male Albino Rats.
Evid Based Complement Alternat Med. 2007; 4(Suppl 1): 29–31.
doi:10.1093/ecam/nem111
- [20] Gonzales GF, Córdova A, Vega K, Chung A, Villena A.
Effect of *Lepidium meyenii* (Maca), a root with aphrodisiac and fertility enhancing properties, on serum reproductive hormone levels in adult healthy men.
J Endocrinol. 2003; 176: 163–168.
doi:10.1677/joe.0.1760163
- [21] Zarrow MX, Yochim JM, McCarthy JL.
Experimental endocrinology, A sourcebook of basic technique.
Academic Press, New York and London. 1964.