International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2020; 8(3): 130-133
Received: 25-01-2020
Accepted: 28-02-2020

BGSM Sumanasinghe
PG Scholar, Department of Dravyagunavignana, IPGT and RA, GAU, Jamnagar, Gujarat, India

RN Acharya
Professor and HOD, Department of Dravyagunavignana, IPGT and RA, GAU, Jamnagar, Gujarat, India

Mukesh Nariya
Head, Pharmacology Laboratory, IPGT and RA, GAU, Jamnagar, Gujarat, India

K Nishteswar
Professor, Department of Dravyagunavignana, IPGT and RA, GAU, Jamnagar, Gujarat, India

Corresponding Author:
BGSM Sumanasinghe
PG Scholar, Department of Dravyagunavignana, IPGT and RA, GAU, Jamnagar, Gujarat, India

Pharmacological evaluation of *vrishyakarma* (Aphrodisiac activity) of leaf of *Clitoria ternatea* Linn. (*Aparajita*- Blue variety)

BGSM Sumanasinghe, RN Acharya, Mukesh Nariya and K Nishteswar

Abstract
Aphrodisiacs are the substances which are used to increase sexual activity and help in fertility. The basic and fundamental purpose of sex and sexuality is the "continuation of progeny" and the survival of human race. *Clitoria ternatea* Linn. is a herbal drug belongs to family Fabaceae found in classical texts as *Aparajita*. Pharmacologically it is attributed for antioxidant, hypolipidemic, anticancer, anti-inflammatory, analgesic, antipyretic, antidiabetic, CNS, antimicrobial, gastro-intestinal antiparasitic etc. This study is to evaluate its aphrodisiac activity (*Vrishya*) of leaf of *Aparajita*. The sexually active male rats were chosen separately and divided into 4 groups; each group consisting of 6 animals and the study was done using the suitable animal experimental model. The data generated during the study will be analyzed by employing Unpaired 't' test and one-way ANOVA test as applicable to determine significant difference between groups at $P<0.05$. Milk treated group showed significant increase in body weight, sperm count, motility and sexual behavior parameters comparing to control group. Drug treated group also showed significant increase in body weight and non-significant increase in sperm motility. In this study milk showed anabolic, sparmatogenic and aphrodisiac activity.

Keywords: *Clitoria ternatea* Linn, *Aparajita*, Aphrodisiac, *Vrishya*, Sparmatogenic

Introduction
Aphrodisiacs are the substances which are used to increase sexual activity and help in fertility. Sexual feelings are an inevitable part of life. The basic and fundamental purpose of sex and sexuality is the "continuation of progeny" and the survival of human race [1]. The sex is the most intimate, indispensable and an integral part of every individual and can be a source of pleasure and fulfillment.

Clitoria ternatea Linn. is a herbal drug belongs to family Fabaceae found in classical texts as *Aparajita*. It is a perennial vine abundantly found all over India having two varieties according to the colour of the flower i.e. blue and white. *Acharya Charaka* mentioned in *shirovirechana* [2] and *vayahsthapana* [3] *dashemani* in *sutrasthana* fourth chapter and *Sushrutasamhita* mentioned *Aparajita* in *arkadi gana* [4] and *samshodhana varg* [5]. *Ashtangahridaya* mentioned under *arkadi* [6] and *asanadi gana*. [7] Bhava Mishra mentioned the indications and properties of *Aparajita* in *guduchyadi varga* as *medhya* (good for intelligence), *khanthya* (good for throat), bestows good vision, *kushta* (cures leprosy), *mutra roga* (disease of urine), *visha* (poison), *amashotha* (unriped swelling) etc [8].

In preliminary study, the plant leaves showed the presence of flavonoids and glycosides [9]. The plant showed many pharmacological effects including antioxidant, hypolipidemic, anticancer, anti-inflammatory, analgesic, antipyretic, antidiabetic, CNS, antimicrobial, gastro-intestinal antiparasitic, insecticidal and many other pharmacological effects [10].

Ethnomedicinally, *Aparajita* is an important herb, more than 16 tribal groups all over India are using this herb for their disease conditions such as diabetes mellitus, tuberculosis, pneumonia, whooping cough, cough, goiter, leprosy, as an antidote, as a medico sexual drug, syphilis, leucorrhoea etc. The drug is attributed with laxative, purgative, diuretic, aphrodisiac and antidote activities [11].

Ethno botanical claims have been reported for *Clitoria ternatea* Linn. leaves as an aphrodisiac [12]. Many experimental studies have been carried out on *Clitoria ternatea* Linn. But no studies have been conducted to evaluate its aphrodisiac activity till date. Keeping this in view study has been planned to evaluate the folklore claim.

2. Material and Methods
2.1 Plant material
Leaf of *Aparajita* was collected from university premises of I.P.G.T & R.A, Jamnagar and the

same were botanically identified, confirmed and authenticated by the pharmacognosist, pharmacognostical laboratory, I.P.G.T & R.A, Jamnagar. The leaves were cleaned properly and make into fine powder sending through sieve number 120.

2.2 Animals
Healthy Charles Foster albino rats of either sex weighing between 150-200 g were used for experimental study. The animals were obtained from the animal house attached to the pharmacology laboratory of I.P.G.T & R.A. Animals were exposed to the natural day and night cycles with ideal laboratory condition in terms of ambient temperature (22 +/- 2OC) and humidity (50- 60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro industries and water given ad libitum. All the experiments were carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC). (IAEC/18/2015/23)

2.3 Selection of male rats
The selected active male rats (weight between 150- 200g) were kept separately for 10 days prior to study. They were trained for sexual experience with normal estrus female rats in estrus cycle. Male rats were again allowed to acclimatize to the new atmosphere in the transparent cage, which was used as test arena. It was of square structure made of fiberglass with opening at both upper and lower ends.

2.4 Selection of female rats
Adult female rats of the weight between 150- 210g were selected for the study from animal house. They kept in groups in separate cages after marking. To select the rats for the copulatory study, their vaginal smears were taken prior to experimentation and only estrus female were used for the study.

2.5 Experimental details
The sexually active male rats were chosen separately and divided into 4 groups; each group consisting of 6 animals. The animals in the divided groups received the treatment orally.

Table 1

Group	Treatment	Dose
I	Control group- Water control	-
II	Vehicle control- Received milk	22ml/Kg
III	Leaf powder of Clitoria ternatea L.	900mg/Kg
IV	Standard drug- Sildenafil citrate	9mg/Kg

2.6 Rout of administration
The test drug, vehicle and standard drug were administered according to the body weight of the animals by oral route with the help of oral cannula.

2.7 Dose fixation schedule
Dose of the drug was fixed by extrapolating the human dose to laboratory animals on the basis of body surface area ratio as per the table of Paget and Barnes (1964) [13].
Aparajita leaf powder- 900mg/kg
Milk - 22ml/kg (Amul taaza)
Standard drug (Sildenafil citrate) - 9mg/kg

The test drug and vehicle were administered to respective groups for 30 consecutive days. On 30th day, one hour after test drug administration, sexual behavior of each male rat was observed visually in the transparent area with selected female estrus rats. First, vaginal smear of female rats were taken to assess the estrus stage. Only estrus female rats were selected for the experimentation. The estrus female rat was placed into the transparent cage with individual male rat 30 minutes under dim light with green curtains on the window and complete silent. Presence of, if present, duration of sexual activities were recorded (time in seconds) with aid of stop watch. The following parameters were noted for aphrodisiac activity of test drugs:

1. Initiation of latency of sexual behavior i.e. grooming of genitals, sniffing, licking etc.
2. Mounting latency, mounting frequency.
3. Average duration of sexual activity.

All the treated and control group animals were observed individually with fresh receptive female rats. Any jerking movement of the mating area was avoided. Sufficient space for the animals in the mating area was provided to be enabling them to chase each other. Cleaning of the mating area was done after each trial.
On the 31st day the animals were weighed and sacrificed by cervical dislocation. Immediately about 100mg of cauda epididymal tissue was excised out carefully and transferred to physiological saline (5ml) and teased gently with forceps to liberate the spermatozoa; incision was made in the inguinal region and suspension in saline was studied for sperm count, motility and morphology assessment. The blood sample was also collected; serum was separated and used for estimation of serum testosterone [14]. The other organs like testis, prostate and seminal vesicle were dissected out, weighed and transferred to 10% formalin solution for histopathology studies.

2.8 Statistical analysis
The data generated during the study will be analyzed by employing Unpaired ‘t’ test and one-way ANOVA test as applicable to determine significant difference between groups at $P<0.05$.

3. Results and Discussion
3.1 Spermatogenesis
During experimental study, only milk group showed significant increase in sperm count and motility respectively. Milk is having madhura rasa, guru snigdha guna, sheeta virya and jivana, brimhana and mentioned as having vrishya properties [15]. These properties are similar to the vrishya properties mentioned by Acharya Charaka [16]. So we can assume that vrishya properties of milk acts on albino rats to increase their sperm parameters. Studies showed that, intake of full-fat dairy were inversely related to sperm motility and morphology [17]. But in this experimental study, low fat milk was used, may be the reason to increase sperm parameters of rats. (Amul taaza)
Drug treated group also showed non-significant increase of sperm parameters, comparatively it was higher than standard group. Katu tikta rasa, laghu guna, katu vipaka of Aparajita [18] is dissimilar to the properties of vrishya mentioned by Acharya Charaka. So we can assume that, these properties might cause reduction the vrishya action of drug treated group, might have negative action on spermatogenesis in comparison to milk control group but, on other hand drug till have effect along with milk in comparison to control group. (Table 2)

Table 2: Effect of Aparajita leaf powder on sperm count & motility of albino rats

Groups	Dose (per kg)	Sperm count (million/ml)	% Change	Sperm motility	% Change
Control	-	302.00±14.46	-	60.00±5.16	-
Milk	22ml	350.83±12.27*	16.17↑	85.83±2.01**	43.05↑
C. ternatea	900mg	316.00±8.18	4.63↑	73.33±4.01	22.21↑
Sildenafil citrate	9mg	329.17±10.68	8.99↑	70.83±3.74	18.05↑

Data: Mean ±SEM, ↑- Increase
*P<0.05, **P<0.01 when compared to control group (Annova followed by Dunnett's multiple 't' test)

All the groups couldn't show significant results on serum testosterone level. Vehicle control group showed non-significant increase of testosterone level comparing to control group while drug treated group and standard group showed non-significant decrease in serum testosterone level. (Table 3)

Table 3: Effect of Aparajita leaf powder on serum testosterone of albino rats

Groups	Dose (per kg)	Sr. testosterone (mg/dl)	% change
Control	-	181.89±64.63	-
Milk	22ml	265.51±63.68	45.97↑
C. ternatea	900mg	95.00±22.91	67.18↓
Sildenafil citrate	9mg	117.68±45.27	59.34↓

Data: Mean ±SEM, ↑- Increase, ↓- Decrease

3.2 Aphrodisiac activity

Only milk control group showed significant increase on mounting latency and mounting frequency. All the other groups failed to show any significant results on sexual parameters. Standard drug showed non-significant increase of mounting frequency and mounting latency comparing to control group. Drug treated group didn't show any significant effect on sexual behaviour parameters. (Table 4)

Table 4: Effect of Aparajita leaf powder on sexual behavior activity of albino rats

Groups	Dose (per kg)	Sexual behavior in rats- 30th day		
		ML (sec.)	MF (no.)	L (no.)
Control	-	0±0	0±0	8.00±1.95
Milk	22ml	552.00±204.19*	1.67±0.48*	11.67±1.73
C. ternatea	900mg	0±0	0±0	3.83±1.35
Sildenafil citrate	9mg	276.67±176.05	0.33±0.21	7.66±1.20

Data: Mean ±SEM, ↑- Increase, ML: Mounting Latency, MF: Mounting frequency, L: Licking
*P<0.05 when compared to control group (Annova followed by Dunnett's multiple 't'test)

Libido refers to increased sexual desire (vaginal licking, sniffing etc. of rats) and is influenced by an array of factors like- visual, olfactory, tactile, auditory and hormonal stimuli. Penile tumescence leading to erection depends on the increased flow of blood in to the lacunar network after complete relaxation of the arteries and corporal smooth muscles. The corpora at micro level are made up of a mass of smooth muscles supplied by endothelium lined vessels. Compression of the trabecular smooth muscles against the fibro-elastic tunica albuginea causes passive closure of the emissary veins and blood accumulation in the corpora- this leads to erection. CNS plays important role in modulating this activity. The erectile response is mediated by combination of central and peripheral innervations. Nitric oxide which acts as a gaseous neurotransmitter plays important role in the maintenance of erection and this activity is opposed by another set of neurotransmitters known as endothelin-1 [19]. Thus, the observed significant increase in the male rat libido (vaginal licking, sniffing etc.) can attribute to either or both of the above mechanisms. The milk may appear to be the testosterone like effect and nitric oxide based intervention [20] or may be decreasing the formation of endothelin-1 or may have an androgenic effect, contribute to increase sexual activity of rats.

3.3 Effect on ponderal changes

Increase of the weight of a specific organ, hypertrophy of an organ may be due to stimulation of its activity. Decrease of the weight, atrophy of an organ may be due to the degenerative changes or loss of tissue of that particular organ. In the present study all the three groups didn't show significant results on weight of testis and seminal vesicle. Standard drug group showed significant decrease in relative weight of prostate. (Table 5, 6, 7)

Table 5: Effect of Aparajita leaf powder on weight of testis of albino rats

Groups	Dose (per kg)	Absolute Weight (g)	% change	Relative weight (g/100g)	% change
Control	-	2.474±0.127	-	1.180±0.038	-
Milk	22ml	2.580±0.119	4.28↑	1.077±0.049	8.72↓
C. ternatea	900mg	2.478±0.034	0.16↑	1.050±0.027	11.01↓
Sildenafil citrate	9mg	2.478±0.083	0.16↑	1.320±0.193	11.86↑

Data: Mean ±SEM, ↑- Increase, ↓- Decrease

Table 6: Effect of Aparajita leaf powder on weight of prostate of albino rats

Groups	Dose (per kg)	Absolute weight (g)	% change	Relative weight (g/100g)	% change
Control	-	0.365±0.048	-	0.175±0.023	-
Milk	22ml	0.427±0.03	16.98↑	0.177±0.008	1.14↑
C. ternatea	900mg	0.257±0.022	29.58↓	0.151±0.009	13.71↓
Sildenafil citrate	9mg	0.253±0.017	30.68↓	0.117±0.006*	33.14↓

Data: Mean ±SEM, ↑- Increase, ↓- Decrease
*P<0.05 when compared to control group (Annova followed by Dunnett's multiple 't' test)

Table 7: Effect of Aparajita leaf powder on weight of seminal vesicles on albino rats

Groups	Dose (per kg)	Absolute Weight (g)	% change	Relative weight (g/100g)	% change
Control	-	0.755±0.078	-	0.363±0.039	-
Milk	22ml	0.860±0.101	13.90↑	0.357±0.040	1.65↓
C. ternatea	900mg	0.754±0.058	0.13↓	0.317±0.019	12.67↓
Sildenafil citrate	9mg	0.550±0.059	27.15↓	0.253±0.021	30.30↓

Data: Mean± SEM, ↑- Increase, ↓- Decrease

Body weight is an indicator of a living being. Gain in body weight indicates the normal progressive health status of animals and if decreases, considered to indicate interference with body functions and also possibility of degenerative changes. According to the above table, group which received milk as vehicle showed significant results in both actual body weight change and percentage increase of body weight. The test drug group also showed significant increase of actual body weight and percentage increase of body weight comparing to control group. Milk is having brimhana (bulk increasing) properties and we can assume that may cause to increase the body weight of rats. Milk is reported to be having anabolic activity which may be the reason for significant

increase the weight of rats [21]. Comparing to vehicle control group and drug treated group, vehicle control group showed more results. That may be due to katu. tikta rasa, katu vipaka and laghu guna may act on reduce brimhana properties of milk. (Table 8)

Table 8: Effect of Aparajita leaf powder on body weight of albino rats

Group	Dose (per kg)	Body weight (g)			
		Initial	Final	Actual change (g)	% Increase
Control	-	173.33±8.82	194.16±8.10	20.83±2.38	12.31±1.60
Milk	22ml	180.00±6.83	240.00±7.30	60.00±10.0**	34.31±6.57**
C. ternatea	900mg	186.66±7.14	236.66±5.72	50.00±5.00**	27.32±3.43*
Sildenafil citrate	9mg	183.33±6.66	201.66±4.21	18.33±2.79	10.34±1.96

Data: Mean ±SEM
*P<0.05, **P<0.01 when compared to control group (Annova followed by Dunnett's multiple 't' test)

Histopathological studies of testis, prostate and seminal vesicle suggest, the milk acts better in reproductive organs which increased spermatogenesis of testis, stimulation of secretion and increased size of alveoli and epithelium of prostate and stimulation of secretion and increased size of epithelium of seminal vesical. Testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate [22]. So action of the milk can be correlated with that of action of testosterone.

The drug treated group showed normal moderate stimulation of secretion and increased size of epithelium of seminal vesicle. Standard drug group showed mild improvement of spermatogenesis in testis and stimulation of secretion and increased size of alveoli in prostate. But studies showed, sildenafil had no statistically significant effect on sperm motility, count or density; the percentage of abnormal sperm forms; or the percentage of living sperm. It also did not affect ejaculate volume or viscosity [23].

Thus the data generated during this study milk showed presence of moderate androgenic activity in rats. Aparajita powder didn't increase libido, shows absent of androgenic activity on rats. As a whole the study indicates that there is some basis for the usage of milk for enhancing male sexuality and tribal claim of Aparajita leaf powder with milk couldn't show enhancing of sexuality of rats.

Conclusion

Aparajita leaf powder showed significant increase in body weight, non-significant increase in sperm motility but, failed to produce any significant effect on sperm count or sexual behavior parameters in experimental animals.

Milk showed significant increase in sexual behavioral parameters like mounting frequency and latency and sperm count, motility and body weight.

The standard drug, Sildenafil citrate also showed non-significant aphrodisiac activity when compared to milk.

References

1. Kothari P. common sexual problems. 3rd ed. UBS publishers, New Delhi. 2001; 1-6(155):203-204, 221.
2. Acharya Charaka, Ram Karan Sharma, Vaidya Bhagawan Dash. Charakasamhita. Part-1, Sutrastana 4/8/27, Chaukhambha Sanskrit Series, Varanasi, 2013, 63.
3. Ibidem, Ram Karan Sharma and Vaidya Bhagawan Dash. Charakasamhita. Part-1, Sutrastana 4/8/50, Chaukhambha Sanskrit Series, Varanasi, 2013, 66.
4. Acharya Susruta, GD Singhal. Susrutasamhita. Part-I, Sutrasthana 38/16-17, Chaukhmbha Sankrit Pratishthan, Delhi, 2007, 311.
5. Ibidem, G.D. Singhal. Susrutasamhita. Part-I, Sutrasthana 39/3, 39/6, Chaukhmbha Sankrit Pratishthan, Delhi. 2007; 321:322.
6. Acharya Vagbhata, Prof KR. Srikantha Murthy. Ashtangahridaya. Part- I, Sutrasthana 15/19, Chaukhambha Krishnadas Acadamy, Varanasi, 2009, 204.
7. Ibidem KR, Srikantha Murthy. Ashtangahridaya. Part- I, Sutrasthana 15/28,: Chaukhambha Krishnadas Acadamy, Varanasi, 2009, 203.
8. Bhavamisra KR. Srikantha Murthy. Bhavaprakasha. Dugdhavarga, Chowkhamba Krishnadas Academy, Varanasi. 2011; 1:245.
9. Anonymous, Ayurveda Pharmacopeia of India. Part- 1, Govt of India, Ministry of Health and Family welfare, Dept of ISM & H. 1999; 1:8-9.
10. Ali Esmail Al-Snafi. Pharmacological importance of Clitoria ternatea – A review. IOSR Journal of Pharmacy. 2016; 6(3):68-83.
11. BGSM Sumanasinghe K, Nishteswar Sushama B. Bhuvad. Ethnomedicinal claims and pharmacological studies of Clitoria ternatea Linn. A Review. Punarna V. 2015; 2(6):10-22.
12. Koppula Hemadri. A treatise on tribal medicine. 1st edn. Koppula Hemadri's House of tribal medicine, Vijayawada, 2011, 12.
13. Paget GE, Barns JM. Evaluation of drug activities. In: Lawrence, D.R. and Bacharach A.L. Pharmacometrics. Academic press, New York. 1964; 1:161.
14. Morales A. Androgen supplementation in practice: the treatment of erectile dysfunction associated with hypotestosteronemia. In: Oddens BJ, Vermeulen A. (editors). Androgens and aging male. Parthenon Publishing Group, London, 1996, 233-245.
15. Bhavamisra KR, Srikantha Murthy. Bhavaprakasha. Dugdhavarga, Chowkhamba Krishnadas Academy, Varanasi. 2011; 1:454.
16. Ibidem, Ram Karan Sharma, Vaidya Bhagawan Dash. Charakasamhita. Part- III, Chikitsa 2/4/36, Chaukhambha Sanskrit Series, Varanasi, 2013, 101.
17. Afeiche M *et al*. Dairy food intake in relation to semen quality and reproductive hormone levels among physically active young men. Hum Reprod. 2013; 28(8):2265-2275.
18. Ibidem KR, Srikantha Murthy. Bhavaprakasha. Guduchyadi varga, Chowkhamba Krishnadas Academy, Varanasi. 2011; 1:245.
19. McVary KT. Erectile dysfunction and lower urinary tract symptoms secondary to BPH. Eur Urol. 2005; 47(6):838-45.
20. Rajesh Garg Gupta VB. Comparative aphrodisiac activity profile of milk and aqueous decoction of Asparagus racemosus willd. in male albino rats. journal of global pharma technology. 2010; 2:7.
21. Sabikhi L. Compendium on advances in formulated foods. Karnel. National Dairy Research institute, 2000, 278-80.https://en.wikipedia.org/wiki/Testosterone.
22. Ken Purvis, Gary Muirhead J, Jane Harness A. The effects of sildenafil on human sperm function in healthy volunteers. Br J Clin Pharmacol. 2002; 53(1):53-60.