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EVALUATION OF APHRODISIAC ACTIVITY AND SPERMATOGENIC EFFECT OF SHILAJIT

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Abstract: This study was designed to evaluate the aphrodisiac and spermatogenic potential of the aqueous extract of Shilajit in rats. Male Wistar albino rats were divided into four groups. Rats were orally treated with (1) Control group: distilled water (2) Viagra group: 4 mg/kg/day sildenafil citrate (3) Shilajit 50 mg/kg/day and (4) Shilajit 100mg/kg/day and their sexual behaviour was monitored 1h later using a receptive female. Their sexual behaviour was evaluated on days 0, 7, 14, 21, 28, 35 and 42days of treatment by pairing with a oestrous phase female rat. For sperm count the treatment was continued further in all groups except the sildenafil citrate group for 42days. At 50 mg/kg, and 100mg/Kg/day dose of Shilajit had a marked aphrodisiac action, Mount frequency (MF) Intromission frequency (IF) marked increased, Similarly, Mount latency (ML): marked decrease. On day 43 day the sperm count increased significantly in both the Shilajit groups, 50 mg/kg and 100g/kg, in a dose dependent manner. Thus, Shilajit can be useful in the treatment of certain forms of sexual inadequacies, such as premature ejaculation and oligospermia.

Keywords: Aphrodisiac, Spermatogenic, Premature ejaculation, Mount frequency, Shilajit

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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. However, unfortunately, there has been a lot of ignorance, wrong information, fear and negative attitude as for as sex is concerned. Myths and misconceptions are rampant and are passed on from generation to generation. These sexual myths can result in sexual dysfunctions, misery, silent suffering, disturbed interpersonal relationships and even divorce. Sexual ignorance is a social disease and can only be resolved through comprehensive sex education, which can increase awareness and improve the environment. [2] Infertility is also a worldwide medical and social problem. It affects above 10-15% of married couples. WHO estimates that there are 60-80 million infertile couples worldwide. Infertility in itself may not threaten physical health but it can certainly have a serious impact on the mental and social wellbeing of infertile couple. In many countries the stigma of infertility often leads to marital disharmony, divorce or Ostracism. [3-4]

Research during the past two decades has an unfolded focus on impotence (erectile failure), premature ejaculation and male infertility. There are a number of prescription drugs which may act as sex stimulant and enhancing the sexual desire and activity in both men and women. Although the use of allopathic medicines have shown significant improvement in treating sexual disorders, but at the same time there are large number of side effects. These include irregularities of the rhythm of the heart, suicidal tendencies, mental disorders and tremors. The use of synthetic aphrodisiacs results in the dilation of blood vessels in other parts of the body causing

headache and fainting. Other side effects include facial flushing, stomach upset, blurred vision and sensitivity to light which usually occur at higher doses. [5]

Shilajit -is considered one of the wonder medicines of Ayurveda. Neither a plant nor animal substance, it is a mineral pitch that oozes from the rocks of the Himalayas, as they become warm in the summer months. There are four different varieties of shilajit which have been described in charka Samhita, namely Savrana, Rajat, Tamra and Lauha shilajit. Savrana shilajit is gold shilajit and is red in colour. Tamra is a copper shilajit and is blue in color. Rajat is a silver shilajit and is white in color while the Lauha shilajit is an iron-containing shilajit and is brownish-black in colour. Tamra and savrana shilajit are not found commonly but the last variety, i.e. lauha shilajit is commonly found in Himalayan ranges and is supposed to be most effective according to the therapeutic point of view. [6]

It is said to carry the healing power of these great mountains. Shilajit is an important drug of the ancient Hindu materia medica and is to this day used extensively by the Hindu physicians for a variety of diseases. Early ayurvedic writings from the Charaka Samhita and Susruta Samhita describe shilajit as a cure for all disease as well as a rasayana (rejuvenative) able to increasing longevity from 100 to 1000 years of age. It is composed of humus and organic plant material that has been compressed by layers of rock mixed with microbial metabolites. Traditional uses primarily focus on diabetes and diseases of the urinary tract, but also include edema, tumors, wasting, epilepsy and even insanity. Modern indications extend to all system of the human body with a significant number of additions in the reproductive and nervous system. The Ayurvedic energenics vary depending on the base rock that the shilajit comes from but it is generally thought to be tridoshic and only aggravating to Pitta (Fire) when used in excess. Clinical research confirms many of the properties that shilajit is used for. However, further investigations are required before many of shilajit's actions can be affirmed. Shilajit is generally considered safe in moderate doses and is readily available in the United States both as a stand-alone product and in the traditional Ayurvedic formula Chandraprabha. It has also compounded in many patent medicines from India. Shilajit is truly a remarkable substance with a long history of human usage for healing and should be subjected to further investigations. [7][8]

Procurement of Shilajit

The *Shilajit shuddh* was purchased from Patanjali Arogya Kendra. Shilajit was dispersed in purified water and used without any further purification. The final concentrations were prepared 50mg/ml and 100mg/ml.

MATERIAL AND METHODS:

Animals

Healthy adult albino rats of wistar strain, weighing about 150-200 g were obtained from the Arya College Animal house, Jaipur. The rats of either sex were isolated and housed in separate cages during the course of experimental period and kept them at room temperature (24±2°C) with a 12 h: 12 h light / dark cycle. The animals were fed with standard pellet diet and provided water *ad libitum.* All the procedures in this study were performed in accordance with the NIH guidelines for the care and use of laboratory animals, after getting the approval from the Arya College Animal Ethics Committee. (Approval No.1013/PO/c/06/CPCSEA)

Preparation of male rats

The male rats were trained for sexual behavior, two times a day for a period of minimum of 10 days. The male rat which did not show any sexual interest during the test period was

considered as an inactive male. The sexually active male rats were selected for testing aphrodisiac activity of the extracts.

Preparation of female rats

Female rats were housed in separate cages with food and water *ad libitum*. The female rats were brought in oestrous phase by treating them with Estradiol valerate (10 microgram/kg S.C. and Hydroxy progesterone 1.5mg/kg S.C., for 48 hours and 5 hours prior to experimentation, respectively, to make them sexually acceptable and were selected for the study.[10]

Experimental details

The sexually active male rate separated and divided into 6 groups; each group consisting of 6 animals. The animals in the divided groups received the treatment orally. Different groups of animals which received the Shilajit extract and the control are as follows (Table 1):

The sexual behavior of the experimental rats was observed weekly in a dim light at 10 a.m. in a specially designed cage that has glasses and wood as shown in photograph. The male experimental rat was first placed in the cage and then one female rat in estrous phase was introduced. An initial period of 10 minutes was considered as acclimatization period. After 10 minutes activity of male rat in each group was recorded individually for 30 minutes.

To determine the aphrodisiac activity of the extracts, several parameters were observed. These include measuring and observing the mount frequency, mount latency, intromission frequency:

(I) Mount latency (ML): Time taken for the first mount following the introduction of females.

(II) Mount frequency (MF): No. of mounts observed in 30 min;

(III) Intromission frequency (IF): No. of intromission observed in 30 min;

To determine the spermatogenic potential of the extracts, several parameters were observed.

(I) Weight of seminal vesicle.

(II) Weight of prostate gland.

(III) Weight of epididymis.

(VI) Effects on epididymis sperm count**.**

For evaluating the effect on sperm count the methods by Kempinas and Lamano-Carvalho were followed with minor modifications. The drug treatment of the drug groups I, II and the control group were continued up to 42 days. All the animals in the above three groups were

killed by decapitation on day 43. The left and right epididymis were from were isolated and freed of adjoining fat. The cauda portion was cut off so as to separate it from the caput epididymis portion and 5 ml of phosphate buffered saline (PBS) was aspirated into each cauda epididymis and the aspirate from both the cauda was collected together to give sperm suspension of 10 mL. This suspension was vortexed and diluted 10 times with PBS. This diluted suspension was then used for the sperm count on a Neubauer haemocytometer. The suspension was well mixed and charged into Neubauer's counting chamber. The total sperm count in four squares (except the centralerythrocyte area) of 1 mm2 each was determined and multiplied by 1.25 × 105 to express the number of spermatozoa/cauda epididymis.[11],[12]

Calculation for sperm count.

Total no. of sperms in 4 squares (each of 0.1 mm3) =*N.*

Total no. of sperms in 4 squares (each of 0.1 mm3) =*N.*

N/0.4 mm3= no. of sperms/mm3 of diluted suspension

*N**1000/0.4mm3 = no. of sperms/cc of diluted suspension

N *2500 = no. of sperms/cc of diluted suspension

N *2500 = dilution factor = no. of sperms/cc of undiluted suspension

Dilution factor= 10

N *2500*10 = no. of sperms/cc of undiluted suspension
N *25 000 = no. of sperms/cc of undiluted suspension
Total no. of sperms in undiluted 10 mL PBS suspension= *N* *25_000**10 = *N* *2.5 *105
Sperm count in 2 cauda epididymis= *N* *2.5 *105
Sperm count/cauda epididymis= *N* = 1.25 = 105
Statical Analysis: Data are expressed as Mean± SEM Statistical analysis was performed using one-way ANOVA followed by Dunnett's test. The $p \leq 0.05$ were considered statistically significant

RESULTS:

Aphrodisiac activity and Spermatogenic potential:

The aphrodisiac activity of aqueous extracts of Shilajit was studied on male Wistar albino rats at two dosages. The parameters observed during the study were mount frequency, mount latency, intromission frequency, weight of seminal vesicle, weight of testies, weight of prostate gland, weight of epididymis and total sperm count.

Mount frequency:

Administration of Sildenafil citrate 4 mg/kg showed significant ($p<0.01$ and $P<0.001$) increase in Mount frequency on 7, 14 and 21, 28, 35, 42 day of observational period respectively as compared with control. Administration of Shilajit (50 mg/kg) showed significant ($p<0.05$ and $p<0.01$) increase in Mount frequency on 14, 21, 28, 35 and 42 day of observational period as compared with control. Shilajit (100 mg/kg, p.o.) showed significant ($p<0.05$ and $p<0.01$) increase in Mount frequency on 14, 21 and 28, 35, 42 day of observational period as compared with control.

Mount latency :

Administration of (Sildenafil citrate 4 mg/kg p.o.) showed significant ($p<0.01$ and $P<0.001$) decrease in Mount latency on 07, 14 and 21, 28, 35, 42 day of observational period respectively as compared with control. Administration of Shilajit (50mg/kg) showed significant ($p<0.05$and $p<0.01$) decrease in Mount latency on 14, 28, 35 and 42 day of observational period respectively as compared with control. Shilajit (100 mg/kg, p.o.) showed significant ($p<0.05$, $P<0.01$ and $P<0.001$) decrease in Mount latency on 21, 28 and 35 and 42 day of observational period respectively as compared with control.

Intromission frequency:

Administration of (Sildenafil citrate 4 mg/kg p.o.) showed significant ($P<0.01$and $P<0.001$) increase intromission frequency on 7, 14 and 21, 28, 35, 42 day of observational period respectively as compared with control. Administration of Shilajit (50mg/kg) showed significant ($p<0.05$) increase in Intromission frequency on 28, 35, 42 day of observational period as compared with control. Shilajit (100 mg/kg, p.o.) showed significant ($p<0.05$ and $P<0.01$) increase in Intromission frequency on 21, 28 and 35, 42 day of observational period respectively as compared with control.

Sperm count: Administration of Shilajit (50mg/kg) showed significant ($p<0.05$) increase in sperm count as compared with control. Shilajit (100 mg/kg, p.o.) showed significant ($p<0.01$) increase sperm count as compared with control.

Weight of organ: Administration of Shilajit (50mg/kg) and (100 mg/kg, p.o.) show increase in weight of Testies, Seminal vesicle and prostate gland as compared with control but does not show significant increase in weight.

DISCUSSION:

The data presented here provides evidence about the ability of extracts of Shilajit to enhance male sexual behavior expression in sexually active rats. The data obtained reveal that an oral administration of different doses of shilajit extracts effectively facilitate several aspects of copulatory behavior. In the experimental analysis of male sexual activity, the concept of the existence of two different physiological mechanisms responsible for sexual behavior expression was introduced in the early 50s by Frank Beach. This notion holds that one of these mechanisms is responsible for sexual arousal and the other for sexual performance. This concept has been central for the neurobiology of sexual behavior. [13]

The Shilajit extracts were subjected for preliminary photochemical studies and aphrodisiac activity. The reports of photochemical studies showed the presence of moisture, gums, albuminoids, calcium, potassium, nitrogen, silica, resin, vegetable matter, magnesium, sulfur, iron, chloride, phosphorous, iodine, glycosides, tannic acid, benzoic acid and a number of vitamins and enzymes (US Patent No. 5,405,613). The Shilajit composition of biologically active components disclosed in the US Patent (No. 6,440,436) contains 0.3% by weight of oxygenated dibenzo-pyrone and 60% by weight of low molecular weight of fulvic acid. Amount these compounds; some of the compounds definitely possess aphrodisiac activity. It was found that an increased copulatory sexual behavior and mounting were observed.

Finally, based on this preliminary data, it can be concluded that the Shilajit is a safe drug without any known adverse effects and can be very useful in enhancing the male sexual activity and treating various sexual disorders like erectile failure, premature ejaculation, lack of sexual desire and ejaculatory incompetence. However, further detailed studies are needed to confirm the usefulness this extract in treating sexual disorders. This includes separation, purification, and characterization of different chemical constituents of these extracts and testing the aphrodisiac activity of purified compounds.

The results of the present study suggested that shilajit have a beneficial effect on male reproductive functions in rats. These data are confirmed by our observation on the increased sperm counts, motility. The increase in the absolute weight of the testis and epididymis could

therefore be due to increased androgen biosynthesis as evidenced by a significant increase in serum testosterone levels in the experimental rats. Androgens have been shown to be necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands and studies have shown that the level is positively correlated with the weight of testis, epididymis, seminal vesicle and prostate glands. The increased sperm count and motility thereby shows that treatment with shilajit improves and enhances the fertilizing capacity of the Semen. These qualities were often used as a measure of sperm production, testicular function and/ or male fertility.

CONCLUSION:

Hence, the results of present study revealed that, Shilajit extract improved sexual performance as well as sperm count. The effectiveness of the shilajit in multiple preclinical models with desire mechanism of action might be due to the presence of fulvic acid and minerals or both synergistic actions of these constituents. However, the exact role of chemicals and their mechanism of action need future investigation.

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Table : 1 Dose distribution schedule in experimentation

Group	Drug	Dose & Time
I	Control (Normal saline)	2ml/kg per day at 9 a.m.
II	Positive control (Sildenafil citrate)	4mg/kg per day.[10]
III	Aqueous extract of Shilajit	50 mg/kg per day at 9 a.m. [9]
IV	Aques extract Shilajit	100mg/kg per day at 9 a.m.[9]

Table: 2 Mount Latency

Groups	0 day	7 day	14 day	21 day	28 day	35day	42 day
Control	244± 24.87	271.3± 55.89	240.5± 16.81	232± 18.09	253.5± 22.89	215.2± 10.83	221± 7.78
Shilajit 50 mg/kg	276.2± 18.86	240± 5.02	203.5± 11.52*	185.7± 10.25	155.2± 15.11*	146.5± 9.65*	120.3± 10.11**
Shilajit 100 mg/kg	219.5± 30.33	187.2± 17.93	191.7± 10.3	157± 14.03*	157.3± 14.96*	139.3± 16.39**	92± 9.352***
Sildenafil citrate 4 mg/kg	227.7± 20.86	121.8± 10.14**	99.33± 6.596**	79.33± 11.3***	71.83± 6.61***	57.5± 3.334***	64.17± 10.02***

Table : 3 Mount Frequency

Groups	0 day	7 day	14 day	21 day	28 day	35day	42 day
Control	6.833± 0.4773	7± 0.5164	6.667± 0.6667	9± 1.033	6.833± 0.8724	7.667± 0.6146	7± 0.5774
Shilajit 50 mg/kg	7.333± 0.4944	10.67± 0.4944	14.5± 1.839*	16.67± 1.563*	17± 1.653*	18.17± 1.537*	20.67± 1.726**
Shilajit 100 mg/kg	6.167± 0.4773	13.33± 0.8433	16.17± 0.8333*	16.83± 0.6009*	20.17± 1.537**	23.83± 1.249**	24± 1.033**
Sildenafil citrate 4 mg/kg	7± 0.5774	25.17± 1.579**	26.83± 1.515**	31.33± 1.874***	29.83± 2.167***	32.17± 2.725***	36.83± 1.99***

Table : 4 Inter Mission Frequency

Groups	0 day	7 day	14 day	21 day	28 day	35day	42 day
Control	0.6667± 0.2108	0.6667± 0.2108	0.6667± 0.2108	0.5± 0.2236	0.6667± 0.2108	0.6667± 0.2108	0.8333± 0.1667
Shilajit 50 mg/kg	0.6667± 0.2108	0.8333± 0.1667	1.5± 0.2236	1.833± 0.1667	2.5± 0.2236*	3.333± 0.2108*	3.833± 0.3073*
Shilajit 100 mg/kg	0.5± 0.2236	1± 0	1.833± 0.3073	3± 0.2582*	4± 0.6831*	5.333± 0.5578**	7.5± 0.4282**
Sildenafil citrate 4 mg/kg	0.8333± 0.1667	6.5± 1.118**	8.833± 1.078**	11± 0.5164***	12.67± 0.9189***	11.67± 0.3333***	14± 0.3651***

Table : 5 Epididymal sperm count

Sperm count Group	Million/cauda	Increase in sperm count Compared with control (%)
Control	9.1	0
Shilajit 50 mg/kg	10.4*	14.2
Shilajit 100 mg/kg	10.8**	18.6

Table : 6 Weight of seminal vesicle

Groups	Wt. of seminal vesicle at 43days
Control	288± 3.992
Shilajit 50 mg/kg	308.8 ± 3.049
Shilajit 100mg/kg	315.7± 3.19

Table : 7 Weight of prostate gland

Groups	Wt. of Prostate gland at 43 days
Control	228.7± 4.485
Shilajit 50 mg/kg	247± 3.406
Shilajit 100mg/kg	256.8± 3.156

Table : 8 Weight of epididymis

Groups	Wt. of epididymis at 43 days
Control	547± 1.238
Shilajit 50 mg/kg	570.5±4.342
Shilajit 100mg/kg	595.2± 3.664

Fig.1 Photograph of Shilajit

Fig.2 Photograph of Shilajit

Fig. 3 Cage for aphrodisiac activity

Fig. 4 Rats during sexual act.

Fig. 5 Cauda part of Epididymis

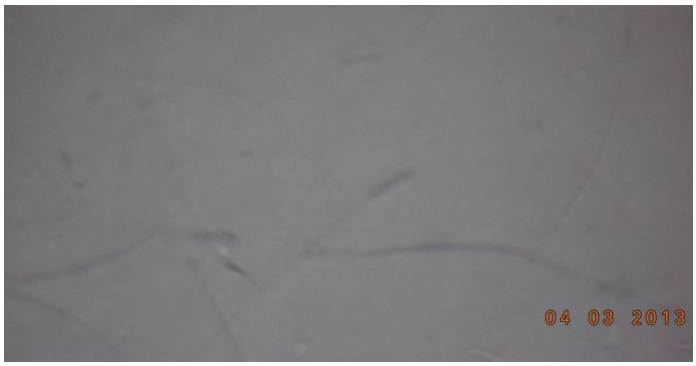

Fig. 6 Sperm Counting

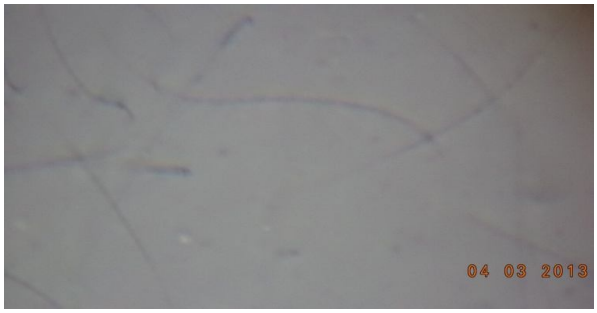

Fig. 7 Sperm Counting

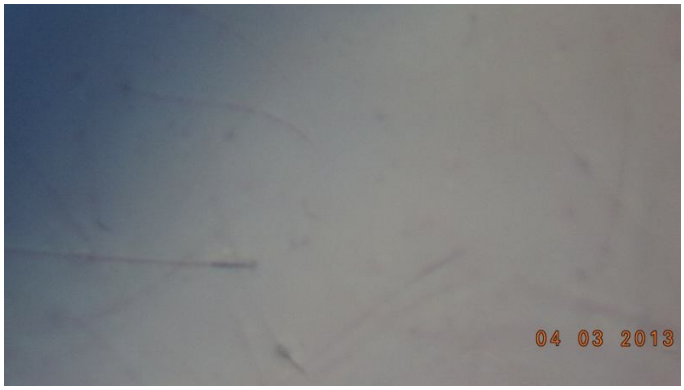

Fig. 8 Sperm Counting

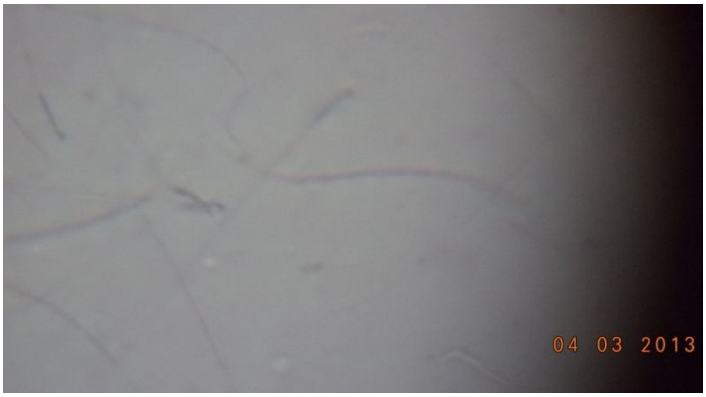

Fig. 9 Sperm Counting

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