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EVALUATION OF APHRODISIAC ACTIVITY OF METHANOLIC EXTRACT OF *Cicer arietinum* SEEDS IN SEXUALLY SLUGGISH MALE ALBINO RATS

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ABSTRACT

The present study was designed to evaluate the potential aphrodisiac effect of seeds of methanolic extract of *Cicer airetinum* (MECA) in sexually sluggish male albino rats. Sexual behavioral parameters like mount frequency (MF), intromission frequency (IF),ejaculation frequency (EF), ejaculation latency (EL), mount latency(ML) and intromission latencies (IL) were observed in male rats. The male serum cholesterol and testosterone concentrations were also recorded. Oral administration of MECA at 200 and 400 mg/kg body weight significantly increased the MF, IF, EF and EL ($P < 0.05$)in comparison to control groups. ML and IL significantly decreased ($p<0.05$). The extract also significantly ($p<0.05$) increased the serum cholesterol and testosterone levels. From these effects the MECA posses significant increase in the sexual activity in male rats.The augmented sexual behavior in male rats might be due to the presence of alkaloids, saponins and flavonoids found in MECA.

Keywords: *Cicer arietinum*, Aphrodisiac, Cholesterol, Testosterone, Sexual behavior

INTRODUCTION

One of the main aims of marriage is the procreation (reproduction) and more importantly for sexual fulfillment of both partners. For life to continue, an organism must reproduce itself before it dies. In Homo sapiens, reproduction is initiated by the mating of a male with a female in sexual intercourse which facilitates the coming together of sperm and egg for the purpose of fertilization[1]. For there to be a normal sexual intercourse and sexual fulfillment in males, the male sexual organs (the copulatory organ, the penis) and factors relating to erection must function normally. Inability to perform this function effectively is a major problem facing the reproductive process. This is known as sexual dysfunction [2]. This condition which is of various types can be managed by the use of aphrodisiacs.

The word 'Aphrodisiac' is derived from 'Aphrodite, the Greek goddess of love. An aphrodisiac can therefore be described as any substance that enhances sex drive and or sexual pleasure. Aphrodisiac can also be viewed as any food, drug, scent or device that can arouse or increase sexual drive or libido [3]. Several reports have suggested a stress related decline in semen quality, sperm concentration, morphology and percentage of motility. This is leading to a close correlation between the sperm concentration and fertility potential of male[4].

Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year. About 25% of couples do not achieve pregnancy within 1 year, 15% of the couples seek medical treatment for infertility and ultimately less than 5% remain childless. Infertility affects both men and women. Male causes for infertility are found in 50% of these couples[5]. A variety of disorders ranging from hormonal disturbances to physical problems, to psychological problems can cause male infertility. Allopathic drugs for sexual dysfunctions were usually associated with many deleterious side effects. So, in view of reducing these unwanted side effects extensive research is being done on plants to find out the effectiveness of natural drugs.

The plant used for the present study is *Cicer arietinum* belongs to family Fabaceae commonly called as chick peas which is widely distributed throughout India and frequently used in traditional medicines. It is commonly known as Sanagalu in Telugu and its juice is used for application in cuts, wounds, dropsy, antidiabetic & antihyperglycaemic[6], Leaves juice is stomachic, laxative, chronic diarrhoea, skin disease, weight control & obesity, antibacterial, antifungal, antipyretic and rheumatic affections[6]. However till date there is no systemic and scientific study were carried out on seeds of *Cicer arietinum* to assess the aphrodisiac activity in rats. Hence, the present study was designed for screening of aphrodisiac activity on extract of *C.Arietinum* seeds. The seeds of *Cicer arietinum* contains carbohydrates(55-62%), proteins (25-29%),saponins[27], alkaloids[27], tannins[26], steroids[26], flavonoids[26], phenolic compounds[26].

MATERIALS AND METHODS

Plant material: The dried seeds of *Cicer arietinum* were procured from the local market of Hyderabad in telangana state. The seeds were authenticated by Dr. S. Madhav chetty, Associate Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India.

Preparation of Extrac*t:* The dried seeds of *Cicer arietinum* was powdered and subjected to soxhlet extraction with methanol. The extract was concentrated by vacuum distillation. The extract was subjected to phytochemical screening.

Experimental animals: Wistar Albino rats of both the sex (150-200 g) were procured from Albino Research Center. They were randomly housed in standard polypropylene cages and maintained under the standard conditions: room temperature was (25±3)ºC with humidity 45%-55%. Animals were housed in a reversed 12/12 h light/dark cycle (light on from 20:00 to 08:00) for 10days before the start of experiment[7,8]. They were fed with commercially available pellet diet obtained from Amruth foods, Pranav Agro Industries, Sangli, India and water was allowed *ad libitum.* The animals were acclimatized to laboratory conditions minimum one week prior to the behavioral experiments. Behavioral studies were carried out during the reversed dark phase (between 11:00 and 16:00 using a dim red light for illumination). Animals used in this study were treated and cared in accordance with the guidelines recommended by CPCSEA (Reg No:1662/PO/a/CPCSEA,2013). The study was performed as per the protocols and recommendation of the Institutional Animal Ethics Committee of Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad.

Sexual behavior study: Female rats were ovariectomized under ether anesthesia, and after full recovery, they were brought into the estrous state by the subcutaneous administration of 10µg/kg body weight of estradiol benzoate and 500µg/kg body weight of progesterone at 48h and 4h prior to pairing[9,10]. They were checked for sexual receptivity[11] with sexually active adult males, and only those showing copulatory behavior in response to mounting, were used in the study. A preliminary screening was carried out to identify the sexually sluggish males. Briefly, the male rats were placed singly with sexually receptive females for 30min at seven different occasions, with a gap of 5days between each exposure. Male animals that failed to achieve ejaculation during any of the last three exposures were considered to be sexually sluggish[12] and used in the present study.

Evaluation of Aphrodisiac activity: Aphrodisiac activity was assessed by oral administration of methanolic extract of *Cicer arietinum* seeds. A total of 18 sexually sluggish male wistar rats were selected and housed separately. They were randomly divided into three groups of six animals each. Group I received 1ml distilled water and served as control, group II received methanolic extract low dose (200mg/kg) and group III received methanolic extract high dose (400mg/kg). The drug/vehicle treatment was continued for 13 days. Fifty minutes after the drug/vehicle administration on day 14, the animals were placed in a glass cage (40x50x40cm). After an adaptation period of 10min, a sexually receptive female was presented to the male by dropping into the cage[13]. The following sexual behavioral parameters were recorded.

- *Mount latency*: Time duration (in seconds) from the introduction of the female into the cage till the first mount.
- *Intromission latency*: Time duration (in seconds) from the introduction of the female into the cage till the first intromission (vaginal penetration).
- *Ejaculation latency*: Time duration (in seconds) from the first intromission till ejaculation.
- *Mount frequency*: Total number of mounts preceding ejaculation.
- *Intromission frequency*: Total number of intromission preceding ejaculation.
- *Ejaculation frequency*: The number of times there was expulsion semen by males after

vaginal penetration –characterized by rhythmic contraction of the posterior abdomen.

- *Copulatory Efficiency:* is the number of intromission divided by the number of mounting multiplies with hundred.

Copulatory Efficiency = Number of Intromissions x 100 / Number of Mounts

Determination of Serum cholesterol[14](Chod PAP method[15]) and Testosterone[16,17]: After recording of the sexual behavioral parameters, blood samples were collected from retro orbital plexus, centrifuged and serum was separated, samples stored at-20^{0}C which was used for the testosterone[17] level estimation by using ELISA kit. Serum cholesterol concentrations may be determined by the Chod-PAP method[15]. Briefly, 0.02cm^3 of the sample (serum) is mixed with 2.00cm^3 of working reagent and the absorbance of the resulting mixture read after 5min at 546nm wavelength. The blank and standard are composed in a similar way except that they are replaced with 0.02cm^3 each of distilled water and standard solution respectively. The biochemical estimations were done using respective kits.

Statistical analysis: The data obtained from this study were expressed as mean ± SEM, (n=6). Statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnett's test. P value less than <0.05 was considered to be statistically significant.

RESULTS

Oral administration of methanolic extract of *Cicer arietinum* (MECA) seed produced a dose-dependent increase in sexual behavior, which was significant as compared with the control animals (Table1). On administration of MECA (200 and 400mg/kg), a significant decrease ($p<0.05$) in the mount latency, intromission latency and ejaculatory latency, whereas a significant increase in mount frequency, intromission frequency, ejaculatory frequency and copulatory efficiency was observed. A significant increase ($p<0.05$) in serum cholesterol and testosterone levels was also observed as compared with the control animals (Table 2).

DISCUSSION

In the present days the main problem for decrease in sexual potency and libido is due to physical and mental stress. This leads to changes in life and diet style resulting in infertility problems. Now –a-days a number of allopathic drugs are available for treating the infertility problems but because of their unwanted side effects folk remedies are gaining importance. Hence, the present study was aimed to evaluate the traditional claim of aphrodisiac and sexual stimulant activity of *C. aeritinum* in an experimental model of sexual sluggish rats.

In the present study the behavioural parameters were calculated to estimate the potency of the *C. aeritinum* extract. Mount latency and intrommision latency are indicators of sexual motivation. The decrease in the time period by the administration of the extract observed in this study might imply stimulation of sexual motivation and arousability.It may also be an indication of enhanced sexual appetitive behaviour in the male rats[10].

Mount frequency and intromission frequency are useful indices of vigorous libido and potency. Increase in these frequencies by the administration of the extract might be due to increase in the concentration of several anterior pituitary hormones and serum testosterone, which in turn stimulated dopamine receptor synthesis and sexual behavior[18]. This is attributed to the flavonoid and saponin constituents of the plant. since they have been reported to alter androgen levels i.e. increase in testosterone levels[13].

Many plants with medicinal properties acts as aphrodisiacs through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins. It is evident that sexual behavior and erection are dependent on levels of androgen that act through central and peripheral mechanisms[19].Treatments that alter the concentration of circulating sex hormones may also modify sexual behavior. Previous data on testosterone also suggest that a slight increase in the levels of the hormone in adult males results in a significant increase in sexual desire and libido[20]. In this study, by the administration of C.aeritinum extract the testosterone concentration was increased. Dehydroepiandrosterone (DHEA), a major circulating steroid in the plasma, and a common precursor for both androgens and estrogens, facilitate sexual function[21]. Although, the levels of DHEA was not measured in this study. The involvement of saponins in the biosynthesis of DHEA[22] may therefore increase the level of testosterone in the body as well as trigger libido enhancing effect observed in this study. In addition, the presence of flavonoids in the extract which has been implicated to have a role in altering androgen levels may also be responsible for the enhanced male sexual behaviour in this study[23]. In addition, the presence of alkaloidswhich have been reported to have ergogenic

properties either by inducing vasodilation of the blood vessels through the production of nitric oxide and thus allowing erection or stimulates steroidogenesis in the testes of the animals. Alkaloids may also act peripherally by relaxing *Corpus cavernosum* smooth muscle in the copulatory organ of the male rats.

In the present study there is a significant increase serum cholesterol concentration which may imply stimulation in the steroid genesis, that leads to increased testosterone concentration[24]. Such increase in testosterone concentration should normally reflect a corresponding increase in libido[25].Hence, from the results the aphrodisiac effect of the plant extract may be due to the presence of alkaloids, saponins and flavonoids through a multitude of central and peripheral mechanisms.

CONCLUSION

On the basis of our results the present study revealed that the methanolic extract of seeds of *C. aeritinum* posses significant dose dependent increase in sexual behavioral parameters in male rats. From this we conclude that the *C. aeritinum* seed extract has the potential to be used as a safe therapeutic alternative to current modalities for the management of sexual dysfunction in males.

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Table 1: Effect of methanolic seed extract of *C. arietinum* on sexual behavior of male rats

Treatment	ML	IL	EL	MF	IF	EF	CF
Control (distilled water)	441.17±16.30	530.67±17.00	166.50±5.807	5.17±0.40	2.17±0.31	0.83±0.31	31.67±2.36
MECA (200mg/kg)	221.33±8.12*	361.00±10.71*	149.33±4.99*	8.17±0.70*	4.33±0.42*	2.33±0.42*	53.40±3.84*
MECA (400mg/kg)	188.33±10.20*	314.33±9.66*	136.00±4.79*	9.17±0.70*	4.83±0.40*	3.17±0.31*	53.04±3.19*

Values are expressed as mean ± S.E.M.(n=6); * = P < 0.05 as compared to Control.
ML=Mount latency; IL= Intromission latency; EL= Ejaculatory latency; MF= Mount frequency; IF= Intromission frequency; EF= Ejaculatory frequency; CF= Copulatory efficiency; MECA= Methanolic extract of *Cicer arietinum*

Table 2: Effect of methanolic seed extract of *C. arietinum* on testosterone and cholesterol concentration in male rats

Treatment	Testosterone (ng/ml)	Cholesterol (mg/dL)
Control	2.99 ± 0.049	61.202 ± 2.9
MECA (200mg/kg)	3.17 ± 0.064*	73.224 ± 2.892*
MECA (400mg/kg)	3.56 ± 0.069*	83.060 ± 2.892*

Values are expressed as mean ± S.E.M.(n=6); * = P < 0.05 as compared to Control.
MECA= Methanolic extract of *Cicer arietinum*

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