



Aphrodisiac Activity of *Cocos nucifera* Attenuates Ceric Sulphate Induced Infertility in Male Rats

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ABSTRACT

Objective: In the present study, we examined the effect of hydroalcoholic extract of apical bud of *cocos nucifera* (HACN) in ceric sulphate induced infertility in male rats. **Methods:** Rats weighing 180 to 200 gm were randomized into 4 groups of 6 animals each that included saline treated rats (normal) were orally administered once daily with 0.5ml of distilled water, Ceric sulphate treated (1 mm/100 gm., s.c.) and HACN treated (200 and 400 mg/kg., p.o.). Ceric sulphate was given to the rats for 7 days for all the groups except saline treated rats then followed by administration of the HACN extract for 21 days. **Parameters Evaluated:** Sexual behavioural parameters including mount and intromission frequency (MF and IF) and mount, intromission and ejaculation latency (ML, IL, EL) as well as post ejaculatory interval (PEI) and body weight (BW) were recorded in male rats one hour after injection of extract by mating with a receptive female (1:1). Biochemical evaluation such as serum and cauda epididymal testosterone and dihydrotestosterone (DHT) and histopathological analysis of testis were also assessed. **Results:** Ceric sulphate treated rats significantly decreased sexual behaviour and also reduced the serum and cauda epididymal tissue testosterone and DHT when compared to control rats. All these changes were significantly attenuated by ceric sulphate rats (with HACN extract). Ceric sulphate was found to be inducing sterility in male rats which has been confirmed after analyzing all the parameters of the study. **Conclusion:** From the results it may be concluded that hydroalcoholic extract of *cocos nucifera* may be used as a good aphrodisiac activity which can promote the fertility rate.

Key words: Aphrodisiac, Ceric sulphate, *Cocos nucifera*, Infertility, Testosterone.

INTRODUCTION

Abnormalities in male reproductive system like impotency, erectile dysfunction and vice versa are one of the main problems that lead to infertility. Erectile dysfunction (ED) is considered as one of the most important public health problem, since it affects higher percentage of men. It had been estimated that more than 152 million men worldwide subjected to sexual dysfunction and this number might increase to approximately 322 million by the year 2025.¹ In the last 50 years, a significant decrease in human fertility has been observed. This condition produces great impacts not only on the patient but also on their marital life. Reports in recent years have shown that incidence of male infertility has increased as a result of various factors such as

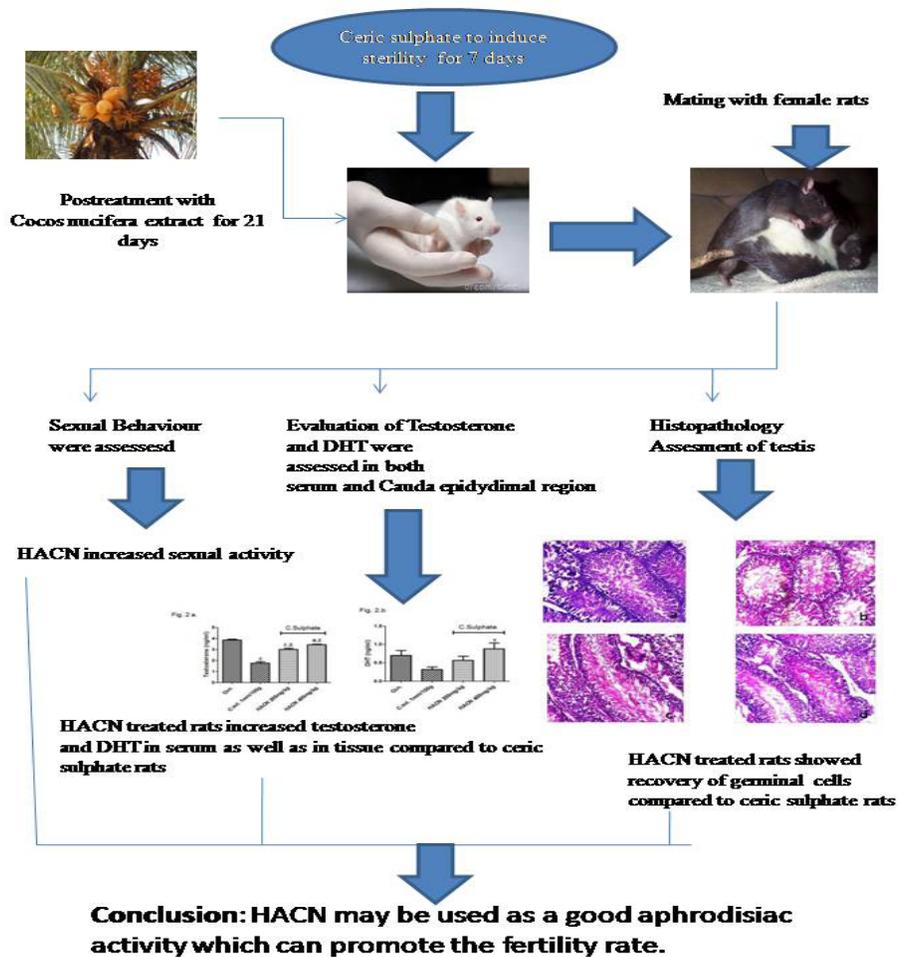
environmental pollution, stress, lifestyle and chemical industries.² In modern world, the growth of the chemical industry a number of new compounds is essential for development of country. But some of them have been found to have deleterious effects on the reproductive organs.³ Cerium is attaining wide application in ceramic and glass industries, medicine, as catalytic agent and in nuclear technology. 1 ml of 1 mm/100 gm body weight dose of ceric sulphate causes sterility in albino rats.⁴ Traditional medicine still plays a significant role in the lives of many people for sexual problems. Aphrodisiac substances or foods have been long term used for treating sexual dysfunction and enhancing the sex lives in traditional folklore.

Cocos nucifera is an unarmed, erect, tall palm belongs to arecaceae family contains fixed oil, volatile oil, wax, triglycerides of fatty acids such as lauric acid and myristic acid, vitamins A, B, C, protein, saccharose, oxidase, catalase fiber and antioxidants. The edible part of the coconut fruit is the endosperm tissue.⁵ It has been reported that *Cocos nucifera* acts as anthelmintic, antidotal, antiseptic, astringent, bactericidal, diuretic, purgative, vermifuge.⁶ *Cocos nucifera* have been used as traditional medicine for various medicinal purposes including aphrodisiac. However the advocated sexual stimulant activities of the *Cocos nucifera* are not scientifically tested and validated, with

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Graphical Abstract

this background the main aim of the present study was undertaken to investigate the effects of *Cocos nucifera* on the male sexual behavior of rats.

MATERIALS AND METHODS

Plant Material

Fresh apical bud of *Cocos nucifera* was collected in the month of Oct 2012 from Vijayawada, Andhra Pradesh. Plant material was identified (Ref.no. PARC/2013/2021) and authenticated by Dr. P. Jayaraman, Director of plant Anatomy Research Institute (PARC) medicinal plant research unit, West Tambaram, Chennai, India.

Preparation of hydro alcoholic extract of apical bud of *Cocos nucifera*

About 1000 g of apical bud of *Cocos nucifera* was cut into small pieces were immersed in hydroalcoholic solution (80% ethanol) in a 5000 ml flat bottom flask and was cold extracted for 7 days with occasional shaking and warming. On seventh day the clear filtrate was obtained by filtering through a Buchner funnel. The filtrate was further concentrated by vacuum distillation, cooled, transferred into a Petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the hydro-alcoholic extract was kept in a desiccator for 15 days to remove the excessive moisture and was used for further studies.

Phytochemical analysis

HACN was subjected to phytochemical screening through qualitative chemical analysis for confirmation of the phytoconstituents.⁷

Procurement and Maintenance of Experimental Animals

Adult Wistar rats, weighing 150-200 g obtained from C. L. Baid Metha College of Pharmacy, Chennai were used. Animals were housed in steel cages and maintained under standard conditions (12 h light cycle/ 12 h dark cycle; 25 ± 3°C; 35-60% relative humidity). Rats feed (Hindustan Lever Ltd.) and tap water was provided ad libitum. The research work has cleared the ethical clearance from IAEC No. IAEC/II/01/CLBMCP/2012/19/12/12.

Preparation of male rats

The male rats were trained, for sexual behavior, two times a day for a period of minimum of 10 days initially before administering the drugs.⁸

Preparation of Female rats

Adult healthy young female rats of 8 weeks old weighing about 130-140 gm were selected and administered benzoate oestradiol 10 µg/100 g body weight to bring the female rats for oestrous phase 48 hrs before copulatory study and progesterone 500 µg/100 g body weight was administered through subcutaneous route 4 hours before the copulatory studies.⁹

Experimental Design

To observe the effect of *Cocos nucifera* on ceric sulphate induced infertility in male rats, 30 rats (6 in each group) were randomly divided into five groups.

Groups	Treatment
I	Normal saline for 28 days (0.5 ml/day, p.o.)
II	Ceric sulphate 1 m.mol/100 gm.,s.c. for 7 days + Normal saline for 21 days
III	Ceric sulphate 1 m.mol/100 gm.,s.c. for 7 days + HACN 200 mg/kg.,p.o for 21 days
IV	Ceric sulphate-1 m.mol/100 gm.,s.c. for 7 days + HACN 400 mg/kg.,p.o for 21 days

Body weight

On the test day 1, the body weight (BW) of each animal was measured immediately before drug treatment as well as on day 28 after injection of either saline or extract. The changes in body weight was calculated using this formula

$$\% \text{ Decrease in Body weight} = \frac{\text{after treatment} - \text{before treatment}}{\text{Before treatment}} \times 100$$

Sexual behavior studies

Each male rat was placed in the observation chamber for 5 min to acclimatize with the cage environment. The primed female was then introduced into the chamber and the following sexual behavior parameters were recorded such as *Mount frequency* (MF): the number of mounts in a series, *Intromission frequency* (IF): was total number of intromissions observed during the observation period, *Mount latency* (ML): was calculated as the time from the introduction of the female to the occurrence of the first mount, *Intromission latency* (IL): was considered as the time for first intromission after introduction of the female in the cage; *Ejaculation latency* (EL): time from the first intromission until ejaculation and *Post ejaculatory interval* (PEI): was calculated as time from ejaculation until next intromission. If male rat couldn't have an intromission within the first 15 minutes, that rat was removed and replaced with a new one.¹⁰

Separation of Cauda Epididymal Tissue

Epididymal spermatozoa were separated as per the method of Brooks¹¹ by cutting the epididymal segments approximately into 1.0-mm³ pieces, with a sharp razor blade in Krebs's Ringer's phosphate (KRP) buffer (pH 7.4), after separated cauda regions as per the guidelines of Hamilton.¹² Spermatozoa from the epididymal pieces were completely removed by vortexing gently in KRP buffer, and the suspension was allowed to settle for 5 min. Spermatozoa released in the buffer were aspirated, centrifuged at 8003 g for 15 min and used for biochemical estimations. All these manipulations were done at 4°C.

Serum and Epididymal Tissue Hormones

Diluted serum/homogenized epididymal tissue sample (in distilled water) was vortexed with diethyl ether (twice). Ether extract was collected and allowed to evaporate completely. Residue was dissolved in Trisbuffer and used for the estimation of testosterone and dihydro testosterone (DHT), as described in the procedure enclosed with the kit purchased from Amersham International Plc. (UK).

Histopathological evaluation of testis

The Gendre's fluid fixed testes were processed in different percentage of alcohol, xylene and embedded in paraffin wax. Eight sections (5 µm thick) were taken at different levels in each half of testis using rotary microtome and stained by hematoxylin and eosin dye. The stained slides were mounted and carefully observed for histological changes and morphometric analysis was done.

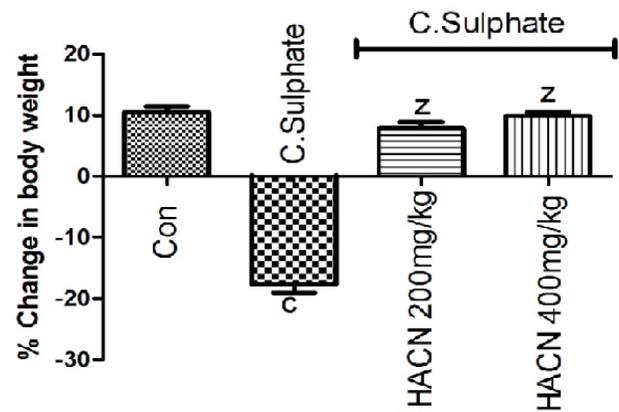


Figure 1: Effect of HACN on Body weight

c p<0.001 when compared with control group z p<0.001 when compared with ceric sulphate treated rats.

Statistical analysis

Data was expressed as mean ± SEM. Mean difference were analysed by one way ANOVA followed by Tukey multiple comparison test. Statistical analysis was performed using Graph Pad Prism, 5.01 (San Diego, US). P<0.05 was fixed as the statistical significance criterion.

RESULTS

Phytochemical screening

HACN subjected to preliminary phytochemical screening revealed the presence of steroids, sterols, flavanoids, carbohydrates, proteins, gums and mucilage, saponins and terpenes.

Effect of HACN on body weight

Ceric sulphate treated rats significantly decreased the body weight when compared with control rats (p<0.001). HACN treated rats (200 and 400 mg/kg) did not show any significant changes in body weight when compared to control rats. HACN treated rats (200 mg/kg and 400 mg/kg) significantly (p<0.001 and p<0.001) increased the body weight when compared to ceric sulphate treated rats (Figure 1).

Effect of HACN on copulatory study of the rats

Ceric sulphate treated rats significantly decreased the copulatory behaviour such as mount and intromission frequency (p<0.001 and p<0.01) and significantly (p<0.001) increased in mount latency, intromission latency, ejaculatory latency and post ejaculatory interval when compared with control rats. The effects of HACN (200 and 400mg/kg) treated rats produced significantly (p<0.001) increased the copulatory behaviour such as MF and IF and significantly (p<0.001) decreased the sexual behavior such as ML, IL, EL and PEI when compared with ceric sulphate treated rats. (Table 1)

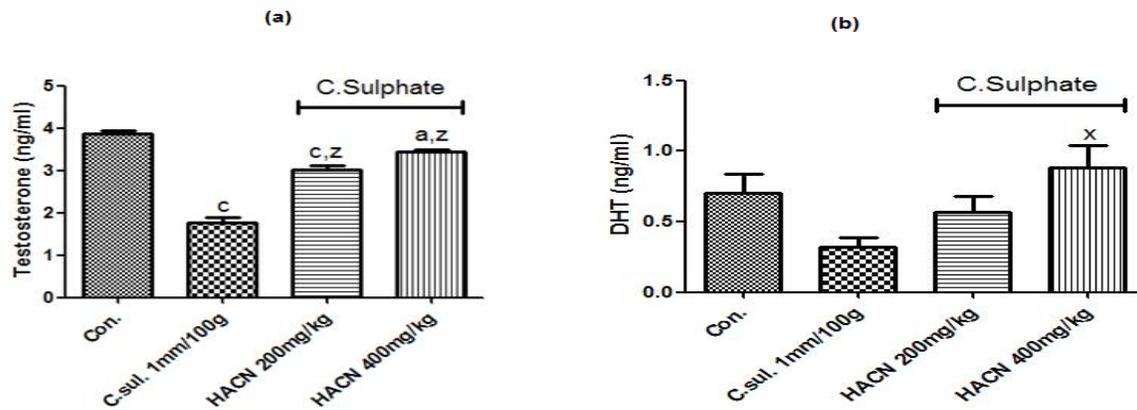
Effect of HACN of serum and cauda epididymal tissue testosterone and DHT

Ceric sulphate treated rats significantly decreased in serum testosterone and cauda epididymal DHT in tissue concentration level when compared with control rats (p<0.001 and p<0.001) but did not significantly altered the testosterone level in cauda epididymal tissue and dihydro testosterone concentration in serum when compared to control rats. HACN (200 and 400 mg/kg) treated rats significantly increased in both serum (p<0.001, p<0.001, p<0.05) and cauda epididymal tissue (p<0.001, p<0.05) testosterone as well as DHT concentration when compared to ceric sulphate treated rats (Figure 2. a,b and 3. a,b).

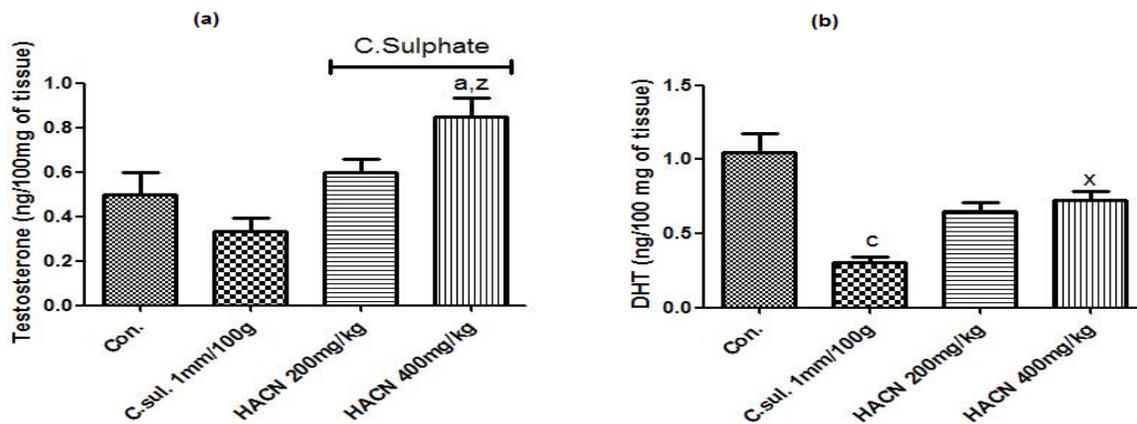
Table 1: Effect of hydroalcoholic extract of *Cocos nucifera* (HACN) on sexual behavior in male rats

Groups	MF	IF	ML (sec)	IL (sec)	EL (min)	PEI (min)
Con	3.12±1.04	1.35± 0.12	280.15±7.26	650.15±216.16	2.13±0.68	5.32±0.89
C. Sulphate	0.86±0.12 ^c	0.39±0.24 ^b	380.16±8.85 ^c	980.16±338.82 ^c	5.29±0.71 ^c	12.24±1.27 ^c
C. Sulphate + HACN 200 mg/kg	2.98±1.40 ^y	0.58±0.46	200.26±5.54 ^z	450.16±202.34 ^z	4.27±0.69 ^z	8.87±0.86 ^z
C. Sulphate +HACN 400 mg/kg	4.96±0.86 ^z	1.32±0.40 ^y	170.16±6.65 ^z	316.72±180.56 ^z	2.56±0.89 ^z	7.07±0.94 ^z

^a p<0.05, ^b p<0.01 and ^c p<0.001 when compared with control group * p<0.05, ^y p<0.01 and ^z p<0.001 when compared with ceric sulphate treated rats. (Tukey's multiple comparison test).

**Figure 2 a,b: Effects of HACN treatment on serum testosterone and DHT concentrations**

Values are expressed as mean \pm SEM. Significance with Tukey's multiple comparison test following one way ANOVA is indicated symbol denote the significance level ap<0.05 cp<0.001 when compared with control group.xp<0.05 xp<0.001 when compared with ceric sulphate treated rats.

**Figure 3 a,b: Effects of HACN treatment on on the cauda epididymal testosterone and DHT concentrations**

Values are expressed as mean \pm SEM. Significance with Tukey's multiple comparison test following one way ANOVA is indicated symbol denote the significance level ap<0.05 cp<0.001 when compared with control group.xp<0.05 xp<0.001 when compared with ceric sulphate treated rats.

Histomorphometry of testis

The section of testis of normal saline treated rats showed normal architecture of tubules with different types of germinal cells and lumen filled with spermatozoa. Ceric sulphate rats testis showed detachment and loss of germinal cells and presence of vacuoles in the tubular lumen when compared to control testis. The section of the testis of rats treated with HACN 200 mg/kg for 21 days showing partial loss of germinal cells and decrease in number of vacuoles in the tubular lumen when compared with ceric sulphate treated rats. The section of the testis of rats treated with HACN 400 mg/kg for 21 days showing recovery of germinal cells and luminal spermatozoa

when compared with ceric sulphate treated rats, and shown almost same that of control rats histogram (Figure 4. a,b,c,d)

DISCUSSION

Herbal medicine and plants even though their modes of action are unknown but they still comprise of a huge number of medicinal properties and use by medical practitioner and some local peoples of different areas of the country. In nature various plants were known to have aphrodisiac properties that modulate the sexual desire in men.¹³ In phytochemical evaluation on HACN extract has found it contain higher concentrations of steroids and sterols and minimal

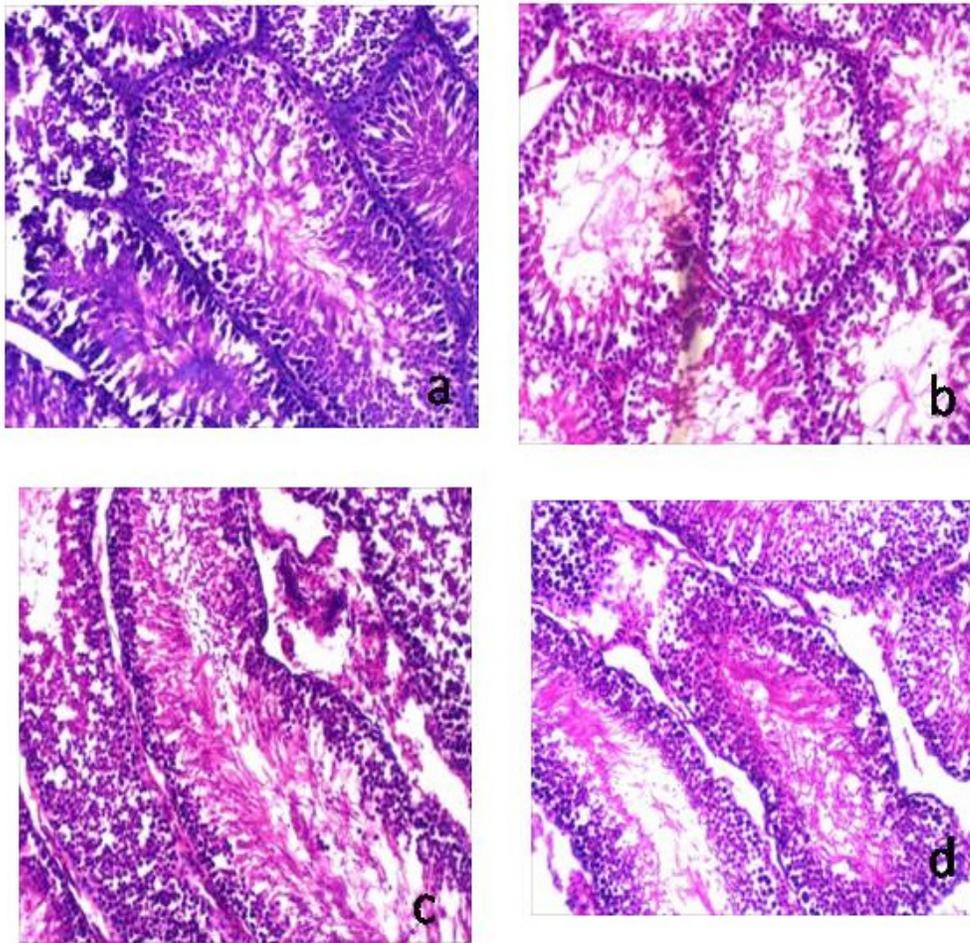


Figure 4: Effects of HACN on histomorphometry on testis

a. Normal saline treated rats showed normal architecture of tubules. b. Ceric Sulphate rats testis showed detachment and loss of germinal cells and presence of vacuoles in the tubular lumen when compared to control testis. c. HACN 200 mg/kg partial loss of germinal cells and decrease in number of vacuoles. d. HACN 400 mg/kg showing recovery of germinal cells and luminal spermatozoa when compared with ceric sulphate treated rats.

concentrations of flavanoids, phenols, carbohydrates, gums, mucilage and lipids. Many research findings can support our study it can reveal that steroidal constituents found in the plants possess fertility potentiating properties, and they have been found to be useful in the treatment of impotency.¹⁴

The present study provides evidence that the apical bud of hydroalcoholic extract of *Cocos nucifera* having aphrodisiac activity enhances the expression of male sexual behavior attenuates the ceric sulphate induced sterility. Ceric sulphate was infused at a dosage of 1 ml of 1 millimole/100 gram body weight for 7 days to induce sterility.¹⁵ In present study ceric sulphate was administered for seven days to all the groups except saline treated rats followed by either with saline or HACN 200 and 400 mg/kg.

The decreases in body weight in ceric sulphate treated rats compared to saline treated rats were observed. Previous reports support our present study treatment with ceric sulphate can cause reduction in body weight due to lack of sex pleasure effect.¹⁶ In both doses of HACN (200 and 400 mg/kg) treated rats increase the body weight compared to ceric sulphate treated rats. The observed anabolic effect evidenced by increased in body weight is attributed to steroid saponins in the extract is suggestive of testosterone intervention of the drug extracts.¹⁷

Ceric sulphate treated rats produced significantly decrease in MF and increase in both ML and IL that implies reduction in the desire component of sexuality. Mount Frequency and Intromission Fre-

quency are useful indices of vigour, libido and potency. While the number of mount (MF) reflects sexual motivation, increase in the number of intromission (IF) shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated.

Increased MF and IF in HACN (200 and 400 mg/kg) treated rats indicating the sexual motivation and efficiency of erection and penile orientation. Both of the tested doses exhibited a higher increment of mating performance. The main phytochemical constituents of HACN are steroids, flavonoids and lipids. These constituents can increase the sexual behavior. They can stimulate endogenous testosterone level probably by raising the level of luteinizing hormones (LH).¹⁸

Etiology of erectile dysfunction has also been correlated with low serum testosterone levels. In present study ceric sulphate treated rats significantly decrease in serum and tissue testosterone and DHT when compared to saline treated rats. Epididymis derives testosterone from circulation and testicular fluid.¹⁹ The low epididymal testosterone could be due to the decreased serum and testicular testosterone concentration.²⁰ The decrease in serum and epididymal DHT may be due to low testosterone available for 5α -reduction and increased conversion of DHT to 5α -androstane- 3α , 17α -diol and 5α -androstane 3α , 17α -diol, as reported in Leydig cells.²¹ Increase in testosterone level has been associated with a moderate but significant increase in sexual desire as well. Increased level of testosterone and DHT level in serum and tissue in HACN (200 and 400 mg/kg) treated rats were observed compared to ceric sulphate treated rats.

The testes produce spermatozoa and testosterone is the vital gonadotrophic hormone in the male. Ceric sulphate rats testis showed detachment and loss of germinal cells when compared to normal rats. HACN 200 mg/kg testis showed partial loss of germinal cells and decrease in number of vacuoles in the tubular lumen when compared with ceric sulphate treated rats tissue. HACN HACN 400 mg/kg for 21 days showing recovery of germinal cells and luminal spermatozoa when compared with control rats, and shown almost same that of control rats histogram.

CONCLUSION

Finally, our present investigation showed that HACN posses facilitatory effects that increase state of sexual desire during sexual interaction with female rats. The aphrodisiac effects of HACN may be due to phytochemical constituents such as steroids, flavonoids, alkaloids, proteins through central and mechanism pathway.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Highlights of Paper

- *Cocos nucifera* is an unarmed, erect, tall palm belongs to arecaceae.
- *Cocos nucifera* used as traditional medicine for various medicinal purposes including aphrodisiac.
- Ceric sulphate was infused at 1 ml of 1 mm/100 gram body weight for induce sterility.
- Phytochemical screening of hydroalcoholic extract of *cocos nucifera* (HACN) revealed the presence of steroids, sterols, flavanoids, carbohydrates, proteins, gums and mucilage, saponins and terpenes.
- HACN (200 and 400 mg/kg) treated rats produced significantly increased the copulatory behaviour such as MF and IF and significantly decreased the sexual behavior such as ML, IL, EL and PEI when compared with ceric sulphate treated rats.
- HACN (200 and 400 mg/kg) treated rats significantly increased in both serum and cauda epididymial tissue testosterone as well as DHT concentration when compared to ceric sulphate treated rats.
- The testis of rats treated with HACN 400 mg/kg showing recovery of germinal cells and luminal spermatozoa when compared with ceric sulphate treated rats.

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ABBREVIATIONS

- HACN**: Hydroalcoholic extract of *cocos nucifera*
BW: Body weight
DHT: Dihydrotestosterone
ED: Erectile dysfunction
MF: Mount frequency
IF: Intromission frequency
ML: Mount Latency
IL: Intromission Latency
EL: Ejaculation latency
PEI: Post ejaculatory interval

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