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EVALUATION OF POTENTIAL APHRODISIAC ACTIVITY OF *HIBISCUS CANNABINUS* (LINN) SEEDS IN MALE ALBINO RAT

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ABSTRACT

The *Hibiscus cannabinus* (*H. cannabinus*) seed is used for curing male infertility and impotency in traditional medicine. However, the validity has not been scientifically evaluated. Therefore, the present study was undertaken to evaluate the effect of *H. cannabinus* seed on the sexual behaviour of male rats. Sixty six rats were randomized into five groups and were treated as one control group, three experimental and one standard group. All the extract doses resulted in significant increase in mount frequency, intromission frequency and reduction in mount and intromission latency in experimental animals. The animal group treated with *H. cannabinus* seed at dose of 400 mg/kg shows a maximum increase in the orientation activity toward female rats. This group also showed significant increase in serum testosterone concentrations. Results of this study led to a conclusion that the aqueous seed extract of *H. cannabinus* increased the blood testosterone concentrations and this may be the mechanism responsible for its aphrodisiac effects and various masculine behaviour. It may be used to modify impaired sexual functions in animals. Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, tannins and saponines.

KEYWORDS: ***Hibiscus cannabinus*, Aphrodisiac activity, Albino rat, Libido**

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INTRODUCTION

An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces veneral desire and increases pleasure and performance. This word is derived from 'Aphrodite' the Greek goddess of love and these substances are derived from plants, animals or minerals and since time immemorial they have been the passion of man[1]. There are two main types of aphrodisiacs, psycho physiological stimuli (visual, tactile, olfactory and aural) preparations and internal preparations (food, alcoholic drinks and love potion) [2]. Erectile dysfunction (ED) or (male) impotence is a sexual dysfunction characterized by the inability to develop or maintain an erection of the penis[3]**.** There are various underlying causes, such as cardiovascular leakage and diabetes, many of which are medically treatable. The causes of erectile dysfunction may be physiological or psychological[4]. Folk remedies have long been advocated, with some being advertised widely since the 1930s[5]. The introduction of the first pharmacologically approved remedy for impotence, sildenafil (trade name Viagra), in the 1990s caused a wave of public attention, propelled in part by heavy advertising[6]. There are many herbal drugs that have been used by men with ED with varying degrees of success. Most potent herbal aphrodisiacs are available and have little or very little side effects[7].

Survey in the tribal belt of Melghat region (20° 51′ to 21° 46′ N and to 76° 38′ to 77° 33′ E) of Amravati district of Maharashtra state of India revealed that the *Hibiscus cannabinus* (*H. cannabinus*) seeds is being used traditionally as an aphrodisiac. *H. cannabinus* L. (Malvaceae) is a woody to herbaceous annual plant producing large cream coloured flowers characterized by a reddish purple or scarlet throat, popular in the western world as "Kenaf" and widely grown as a fibre crop. It is native to China and grown widely as an ornamental plant throughout India. The plant is a hermaphrodite mostly unbranched, fast-growing with prickly stems[8]. For centuries, plant was used, as an antidote to chemicals (acid, alkali, pesticides) poisoning and venomous mushrooms[9], to treat bruises, bilious conditions, fever and puerperium. The stem peelings were applicable in treating dysentery and blood and throat disorders. The seeds were used externally to treat aches and bruises. In addition, this plant has been reported to be an anodyne, aperitif, aphrodisiac, as well as fattening, purgative and stomachic[10]. The flowers are considered emollient, and an infusion of the petals is used as a demulcent. Its decoction is given in bronchial disorder in India. Previous studies show that the plant possesses anticomplimentary, antidiarrhetic and antiphologistic activities[11]. The leaves and flowers have been found to be effective in the treatment of heart disorders. The present work was undertaken to validate scientifically the aphrodisiac role of *H. canabinus* seeds as acclaimed by the traditional tribal user of Melghat region. But to the best of our knowledge, there is no information in the open scientific literature that has substantiated or refuted the aphrodisiac claims of *H. canabinus* seeds in the folklore medicine.

MATERIALS AND METHODS

Collection of Plant Material

The seeds of *H. canabinus* plant was collected during the flowering period of October to January from Melghat region, identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. DIDACHICA13).

Procurement and Rearing of Experimental Animal

Healthy wistar strain male albino rats of about two months old and weighing 120- 200 gm were procured from Sudhakarrao Naik Institute of Pharmacy, Pusad (Maharashtra). The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hr light and dark

cycle approximately at 25 °C. They were fed on pellets (Trimurti Lab Feeds, Nagpur) and tap water *ad libitum.* The rats were allowed to acclimatize to laboratory environment for 15 days before experimentation.
All experimental protocols were subjected to the scrutinization and approval of Institutional Animal Ethics Committee [registration number 1060/ac/07/ CPCSEA (IAEC/7/2009)].

Preparation of Extract
The seeds of *H. canabinus* were collected, shade dried, powdered and subjected to soxhlet extraction successively with distilled water, ethanol and chloroform. The extract was evaporated to near dryness on a water bath, weighed and kept at 4 °C in refrigerator until further use.

Phytochemical Screening
The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as per Thimmaiah[12].

Acute Toxicity Study
Healthy male albino rats were starved for 3- 4 hr and subjected to acute toxicity studies as per Organization of Economic Co-operation and Development (OECD) guidelines No: 423[13]and a highest dose was selected for treatment. The rats were observed continuously for 2 hrs for behavioural, neurological and autonomic profile, and for next 24 and 72 hrs for any lethality or death.

Mating Behaviour Test
The test was carried out by the methods of Dewsbury and Davis Jr[14] and Szechtman et al[15], modified by Amin et al[16]. Healthy and sexually experienced male albino rats (200–300 gm) that were showing brisk sexual activity were selected for the study. They were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Groups 2–4 received suspension of the extract of *H. canabinus* orally at the doses of 100, 200 and 400 mg/kg, respectively, daily for 21 days at 18:00 hrs. Group 5 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hr prior to the commencement of the experiment. Since the male animals should not be tested in unfamiliar circumstances the animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment. The female animals were artificially brought into oestrus (heat) [17] by the Szechtman et al method (as the female rats allow mating only during the estrus phase) They were administered suspension of ethinyl oestradiol orally at the dose of 100 µg/animal 48 hrs prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal 6 hrs before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test and standard animals. The most receptive females were selected for the study. The experiment was carried out on the 21st day after commencement of the treatment of the male animals. The experiment was conducted at 20:00 hrs in the same laboratory and under the light of same intensity. The receptive female animals were introduced into the cages of male animals with 1 female to 1 male. The observation for mating behaviour was immediately commenced and continued for first 2 mating series. The test was terminated if the male failed to evince sexual interest. If the female did not show receptivity she was replaced by another artificially warmed female. The occurrence of events and phases of mating were called out to be recorded on audio-cassette as soon as they appeared. Their disappearance was also called out and recorded. Later, the frequencies and phases were determined from cassette transcriptions: number of mounts before ejaculation or Mounting Frequency (MF), number of intromission before ejaculation or Intromission Frequency (IF), time from the introduction of female into the cage of the male upto the first mount or Mounting Latency (ML), time from the introduction of the female up to the first intromission by the male or Intromission

Latency (IL), time from the first intromission of a series up to the ejaculation or Ejaculatory Latency (EL).

Test for Libido

The aqueous extract at 400 mg/kg was found to be the most active amongst the three treatments in aphrodisiac testing. Hence it was subjected to a detailed investigation for the study of test for libido. The test was carried out by the method of Davidson[18], modified by Amin et al[16]. Sexually experienced male albino rats were divided into 3 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2 received suspension of the extract of *H. canabinus* orally at the dose 400 mg/kg, respectively, daily for 21 days at 18:00 hr. Group 3 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 h prior to the commencement of the experiment. The female rats were made receptive by hormonal treatment and all the animals were accustomed to the testing condition as previously mentioned in mating behaviour test. The animals were observed for the Mounting Frequency (MF) on the evening of 21st day at 20:00 h. The penis was exposed by retracting the sheath and 5% xylocaine ointment was applied 30, 15 and 5 min before starting observations. Each animal was placed individually in a cage and the receptive female rat was placed in the same cage. The numbers of mountings were noted. The animals were also observed for intromission and ejaculation.

Orientation Activity

The aqueous extract at 400 mg/kg was found to be the most active amongst the three treatments in aphrodisiac testing. Hence it was subjected to a detailed investigation for the study of orientation activity. Sexually experienced male albino rats were divided into 3 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2 received suspension of the extract of *H. canabinus* orally at the dose 400 mg/kg, daily for 21 days at 18:00 hr. Group 3 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hr prior to the commencement of the experiment. The orientation of male rats towards female (licking, anogenital sniffing), towards self (nongenital grooming, genital grooming) and towards environment (exploration, climbing, raring). The orientation behaviour was observed at 10, 20 and 30 min after treatment to all groups. The severity of response can be scored as 1= presence, 2= moderately severe and 3= intense and continuous action. The cumulative score after 21 days of the treatment for each activity in the half hour was calculated[19].

Effect on Sexual and Vital Organ Weight

The aqueous extract at 400 mg/kg was found to be the most active amongst the three treatments in aphrodisiac testing. Hence it was subjected to a detailed investigation for the study of effect of extract on sexual and vital organ weight. After 21 days of treatment, all the control, standard and experimental groups of male rats were evaluated for their body weight. The animals were then sacrificed by cervical decapacitation and testis, seminal vesicles, epididymis and prostate glands were carefully removed and weight of each organ was determined[20- 22].

Effect on Hormonal Level

The most active aqueous extract at 400 mg/kg was subjected to a detailed investigation of hormonal assay. After 21 days of treatment, all the control, standard and experimental groups of male rats sera were analyzed for Testosterone level by Chemiluminescence immunoassay (CLIA) method with semi automated Chemiluminescence analyzer and autoplex- A processor for CLIA[23. 24].

Statistical Analysis

The data are expressed as mean± SE. Statistical analysis was done by Student´s t-test[25].

RESULTS

Preliminary phytochemical screening of the seed extract of *H. canabinus* revealed the presence of alkaloids, flavonoids, steroids, tannins and saponines whereas anthraquinone were not detected. Clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Similarly no mortality and changes in the behavioural, neurological and autonomic profile were observed in treated groups of the rats up to highest dose of 4000 mg/kg body weight in acute toxicity study. No death was observed at highest dose (4000 mg/kg body weight) used. The administration of *H. canabinus* aqueous, chloroform and alcohol seed extract for 21 days to male rats resulted in remarkable increase in the sexual vigor of the male rats, as evidenced by the different parameters studied. The results of mating behaviour test show that the seed extract of *H. canabinus* (aqueous, alcohol and chloroform) at the dose of 100, 200 and 400 mg/kg body weight, significantly increased the Mounting Frequency (MF), Intromission Frequency (IF), and caused significant reduction in the Mounting Latency (ML), Intromission Latency and (IL) Ejaculatory Latency (EL) in experimental animal as compared to control group. Similarly, the standard drug also increased the MF, IF as well as decreased the ML, IL and EL in a highly significant manner as compared to control animals (Table 1). The results obtained in the test for libido show that the seed extract of *H. canabinus* at the dose of 100, 200 and 400 mg/kg, significantly increased the Mounting Frequency (MF) ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively) as compared to control group. The standard drug also significantly increased the MF ($P < 0.001$) as compared to control animals. Intromission was observed in control and experimental group while in standard groups it was absent, however ejaculation was noted only at the dose of 400 mg/kg body weight (Table 2).

The aqueous extracts markedly influenced the orientation behaviour of the treated animals, which showed more attraction towards female rats. A more than two fold enhancement in attraction towards female was noticed in aqueous seed extract of *H. canabinus* at the doses of 100, 200 and 400 mg/kg body weight compared to control whereas the increase was only half fold in sildenafil citrate treated group. The behavioural assessment of rats towards environment (exploration, raring and climbing) was significantly decreased in experimental animal and moderately in standard group. A moderate increase in female anogenital smelling was observed, however there was a significant increase in the licking of female animal treated with aqueous seed extract of *H. canabinus* at various doses. The studies on the genital grooming revealed that there was significant increase in genital grooming while moderate decrease in nongenital grooming was observed in animals treated with aqueous seed extract of *H. canabinus* at concentration of 100, 200 and 400 mg/kg body weight (Table 3). In the present investigation, the administration of aqueous seed extract of *H. canabinus* at the dose of 100, 200 and 400 mg/kg body weight resulted increase in the body weight, reproductive organ weight and vital organ weight in treated animals. The weight of the organs likes testes, seminal vesicle, penis, epididymis, vas- deference and prostrate increased significantly along with the weight of vital organ like liver, kidney, spleen and adrenal glands (Table 4). The effect of experimental extract on hormonal assay of male rats shows increase in serum testosterone level in extract treated rats as compared to control (Table 5).

Table 1

Effect of aqueous, chloroform and alcoholic seed extracts of Hibiscus cannabinus on sexual behaviour of rats on 15th day

Treatment groups	Drug dose (mg/kg body wt)	Mount latency (time in sec)	Mount frequency (No.)	Intromission latency (time in sec)	Intromission frequency (No.)	Ejaculatory latency (time in sec)
Group-I Control (vehicle)	-	204±0.01	3.33±0.20	543±0.18	2.33±0.33	739.2±0.80
Group-II Aqueous Extract	100	154±1.42*	7.83±0.02**	181.1±0.09**	15±0.93***	208.8±0.09^{ns}
	200	147.2±1.20**	12.5±0.05^{ns}	160.8±0.16	22.5±0.04^{ns}	144±0.74***
	400	125.4±0.16***	14±0.09***	140.4±0.12**	29.83±3.08**	186±0.19**
Group-III Chloroform extract	100	201±0.33**	4±0.33^{ns}	208±0.45**	2.83±0.33***	Nil
	200	180±0.67***	5.5±0.56**	196.8±0.33^{ns}	5.59±0.67**	Nil
	400	139.2±0.80*	11.83±1.14*	151.2±1.24^{ns}	6.42±0.11***	Nil
Group-IV Alcoholic extract	100	258±1.80^{ns}	2.33±0.03***	334.8±1.41***	3.40±0.58**	Nil
	200	247.8±1.34*	3.5±0.05**	312±0.98**	3.97±0.08^{ns}	Nil
	400	201.6±0.18**	6.16±0.18***	271±0.66*	4.21±0.02*	Nil
Group-V Sildenafil citrate	5	180±0.02***	10.33±0.20***	429.7±0.16**	4.66±0.80***	190.2±0.07

Values are mean± SE from 6 animals in each group, P values: *<0.05, **<0.01, ***<0.001, when compared with control group, ns=non significant

Table 2

Effect of aqueous seed extracts of Hibiscus cannabinus on libido in male rats

Treatment groups	Doses (mg/kg body wt)	Mounting frequency (MF)	Intromission frequency (IF)	Ejaculation (EJ)
Group-I Control	-	1.16±0.91	3.16±0.48	Absent
Group-II Aqueous extract	100	4.83±0.75**	8.16±0.16***	Present
	200	7±0.48**	13.83±1.20*	Present
	400	7.16±0.47***	33.5±2.96***	Present
Group- III Sildenafil citrate	5	2.33±0.26***	Nil	Absent

Values are mean± SE from 6 animals in each group, P values: * <0.05, **<0.01, ***<0.001, when compared with control, ns= non significant.

Table 3

Effect of aqueous seed extracts of Hibiscus cannabinus on orientation activities in male rats.

Orientation Towards		Group-I Control	Group- II Standard	Group-III Aqueous seed extract of *Hibiscus cannabinus*		
		Distilled water 10 ml/kg	Sildenafil citrate 5 mg/kg	100 mg/kg b. w	200 mg/kg b. w	400 mg/kg b. w
Female	Licking	17	27	19	26	34
	Anogenital smelling	11	23	05	13	14
Environment	Exploration	23	19	10	05	06
	Rarring	27	13	14	10	07
	Climbing	05	03	02	02	01
Self	Nongenital grooming	22	17	09	13	19
	Genital grooming	28	26	22	36	43

Scored Point 1=presence, 2=moderately severe and 3= intense and continuous action.

Table 4

***Effect of aqueous seed extracts of Hibiscus cannabinus on body weight, male reproductive organ and various organ weights of* male rat**

Treatment Groups	Doses	Body weight (gm)		Testes (gm)	Epididymis (gm)	Seminal vesicle (gm)	Ventral prostate (gm)	Vas-Deferens (gm)	Penis (gm)	Liver (gm)	Kidney (gm)	Adrenal Gland (gm)	Spleen (gm)
		Initial	Final										
Group-I Control	Distilled Water 10 ml/kg	189.16 ±3.28	191.83 ±2.11	3.25 ±0.08	2.08±0.09	0.809 ±0.02	0.286 ±0.01	0.638 ±0.01	0.258 ±0.01	7.198 ±0.13	2.505 ±0.01	0.055 ±0.03	0.537 ±0.03
Group-II Positive Control	Sildenafil citrate 5 mg/kg	196.66 ±2.11***	200.66 ±5.67**	3.202 ±0.06***	1.64±0.13*	0.691 ±0.05^{ns}	0.366 ±0.01***	0.659 ±0.01^{ns}	0.277 ±0.10**	7.93 ±0.17***	1.667 ±0.10*	0.053 ±0.03**	0.269 ±0.01*
Group-III Aqueous seed extract of *H. cannabinus*	100 mg/kg	181.35 ±0.49**	223 ±0.60**	3.537 ±0.02*	2.768±0.22*	0.832 ±0.009*	0.389 ±0.33**	0.636 ±0.32***	0.186 ±0.28**	7.240 ±0.58^{ns}	1.694 ±0.08**	0.048 ±0.04^{ns}	0.285 ±0.20*
	200 mg/kg	193 ±1.70***	232.66 ±1.80**	3.767 ±0.58***	2.673±0.23**	0.845 ±0.21***	0.390 ±0.05^{ns}	0.678 ±0.19^{ns}	0.179 ±0.02***	7.892 ±0.33**	2.375 ±0.27^{ns}	0.053 ±0.01*	0.269 ±0.67***
	400 mg/kg	212.5 ±3.24***	248.83 ±1.52***	3.936 ±0.46^{ns}	2.813±0.03**	0.895 ±0.10**	0.398 ±0.09***	0.673 ±0.02**	0.269 ±0.16**	7.942 ±0.70***	2.387 ±0.17***	0.063 ±0.02*	0.426 ±0.16**

Values are mean±SE from 6 animals in each group, P values: *<0.05, **<0.01, ***<0.001, when compared with control, ns=non significant

Table 5

Effect of aqueous seed extracts of H. Cannabinus on testosterone hormone in male rats

Treatment group (doses, mg/kg body wt)	Group-I Control	Group-II Sildenafil citrate	Group-III Aqueous seed extract of *H. Cannabinus*		
	-	5 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Testosterone (ng/ml)	1.41±0.79	2.15±0.68*	2.23±0.66***	2.49±0.33**	3.13±0.90***

Values are mean± SE from 6 animals in each group, P values: * <0.05, **<0.01, ***<0.001, when compared with control, ns= non significant.

DISCUSSION

The seed of *H. canabinus* has been in use by the tribals of Melghat region as a means of treating sexual inadequacy and stimulating sexual vigor even without recourse to the scientific validity of the claim. Hence this study was carried out to validate scientifically this tribal claim.This plant was traditionally prescribed in traditional folk medicine in Africa and India; reported to contain several active components as tannins, saponins, polyphenolics, alkaloids, lignans, essential oils and steroids[26- 27]. Phytochemical screening of *H. canabinus* plant revealed the presence of phenolics, tannin, saponin, alkaloids and steroids; our finding was also corroborating with Agbor et al[26]. This phytochemicals have been implicated as possible bioactive agents responsible for the aphrodisiac effect in *Tribulus terrestris* extract[28]. In the present study, clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment.Similar observation was revealed by Babu et al[29], while working on ethanol extract of *Cassia kleinii* leaf in streptozotocin - induced diabetic rats. In male rats latency for mount and intromission are considered as indicators of the sexual motivation, intromission and ejaculation are considered as a behavioural indication of sexual performance and facilitation[30]. After treatment with the experimental doses of seed extract of *H. canabinus* there was a significant decrease in the latency for mount and intromission indicating an enhancing of sexual motivation, which was predominant at 21st day of observation. Also an increase in the number of ejaculation with a decrease in the ejaculation latency indicated an increase in the sexual performance. The aqueous extract has a pronounced effect on sexual behaviour by shortening mount latency (ML) and ejaculation latency (EL), which are considered to be the indicators of an increase in sexual motivation[31]. Similar findings were reported by Subromoniam et al[32] and Gonzales et al[33] in their study on *Trichopus zeylanicus* and *lepedium meyenii* plants respectively.

The MF and IF are considered the indices of libido. Thus, the increase in the MF and IF, indicates that *H. canabinus*, increases libido. The significant decrease in the Ejaculatory Latency (EL) suggests that the extract and standard drug prolonged the duration of coitus. The test drug also caused a significant reduction in the Mounting Latency (ML) and Intromission Latency (IL) as compared to control animals, while a highly significant decrease was observed in the ML of animals treated with the referent drug. This also provides an evidence for aphrodisiac effect of the test drug. These findings show that the test drug produces a striking enhancement of over-all sexual performance of normal animals. MF after penile anesthetization of rats is a reliable index of 'pure' libido and the penile reflexes of the rats are a good model of pure potency[34].Therefore, in the present study the extract was also studied for effect on these components of sexual behaviour. The effect of the test drug on libido was studied by assessing the MF after genital anaesthetization which does away with the reinforcing effect of genital sensation thus affording the study of pure libido or intrinsic sexual desire. During the experiment the test drug produced a significant increase in the MF of sexually normal male rats. Whereas, the MF was much reduced in control and standard animals in comparison with the MF of corresponding groups in mating behaviour test where the penis had not been anaesthetized. However, the test for libido revealed that Intromission and Ejaculation were present in control and experimental groups of animals. Thus, it may be inferred that the test drug produced a striking increase in 'pure' libido. Similar finding was also recorded by Tajuddin et al[35], while working on ethanolic extracts of *Myristica fragrans* and *Syzygium aromaticum* in male mice.

In the present study it was observed that the behavioural assessment of rats towards

environment (exploration, raring and climbing), and self nongenital grooming was significantly decreased. The increase in female anogenital smelling, licking and genital grooming in animal treated with aqueous seed extract of *H. canabinus* at various doses was observed. This supports the aphrodisiac activity of *H. canabinus* seed in male rats. Our result also corroborates with the finding of Sharma et al[36], while working on anabolic, aphrodisiac and reproductive activity of *Anacyclus Pyrethrum* DC in male rats. Administration of extract resulted in weight gain in treated animals. The weight of the organs likes testes, seminal vesicle, penis, epididymis, vas- deference and prostrate also increased significantly along with vital organs like liver, kidney, spleen and adrenal glands. Genesis of steroids is one of the causes of increased body and sexual organ weight and an increase in these parameters could be regarded as a biological indicator for effectiveness of the plant extract in improving the genesis of steroidal hormones[21]. Since androgenic effect is attributable to testosterone levels in blood[20], it is likely that the plant extracts may have a role in testosterone secretion allowing better availability of hormone to gonads.Testosterone supplementation has previously been shown to improve sexual function and libido[37], in addition to the intensity of orgasm and ejaculations which might also be expected to improve[38]. Spermatogenesis involves a complex interplay between the structural element of testis and the endocrine system[19]. FSH stimulates spermatogenesis and LH stimulates synthesis and release of testosterone. Testosterone causes an an increased blood flow and stimulates the growth of the target tissues. Testosterone causes direct stimulation of spermatogenesis[39]. Our results also show that there is increase in spermatogenesis and increase in weight of sexual organ in extract treated group as compared to control group, thereby suggesting the possibly role of the seed extract of *H. canabinus* in inducing spermatogenesis.

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