Effect of *Mucuna pruriens* (Linn.) on Sexual Behavior and Sperm Parameters in Streptozotocin-Induced Diabetic Male Rat

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ABSTRACT

Introduction. Sexual dysfunction is one of the major secondary complications in the diabetic. *Mucuna pruriens*, a leguminous plant identified for its antidiabetic, aphrodisiac, and improving fertility properties, has been the choice of Indian traditional medicine.

Aim. Objective of the present study was to analyze the efficacy of *M. pruriens* on male sexual behavior and sperm parameters in long-term hyperglycemic male rats.

Methods. Male albino rats were divided as group I control, group II diabetes induced (streptozotocin [STZ] 60 mg/kg of body weight (b.w.) in 0.1 M citrate buffer), group III diabetic rats administered with 200 mg/kg b.w. of ethanolic extract of *M. pruriens* seed, group IV diabetic rats administered with 5 mg/kg b.w. of sildenafil citrate (SC), group V administered with 200 mg/kg b.w. of extract, and group VI administered with 5 mg/kg b.w. of SC. *M. pruriens* and SC were administered in single oral dosage per day for a period of 60 days. The animals were subjected to mating behavior analyses, libido, test of potency, and epididymal sperms were analyzed.

Main Outcome Measure. The mating behavior, libido, test of potency, along with epididymal sperms were studied.

Results. The study showed significant reduction in sexual behavior and sperm parameters in group II. Daily sperm production (DSP) and levels of follicular stimulating hormone, luteinizing hormone, and testosterone were significantly reduced in group II, whereas the animals with diabetes administered with seed extract of *M. pruriens* (group III) showed significant improvement in sexual behavior, libido and potency, sperm parameters, DSP, and hormonal levels when compared to group II.

Conclusion. The present work reveals the potential efficacy of ethanolic seed extract of *M. pruriens* to improve male sexual behavior with androgenic and antidiabetic effects in the STZ-induced diabetic male rats. This study supports the usage of *M. pruriens* in the Indian system of medicine as sexual invigorator in diabetic condition and encourages performing similar study in men. **Suresh S, and Prakash S. Effect of *Mucuna pruriens* (Linn.) on sexual behavior and sperm parameters in streptozotocin-induced diabetic male rat. J Sex Med **;**:**–**.**

Key Words. *Mucuna pruriens*; Sexual Behavior of Diabetic Rat; Spermatogenesis; Antidiabetic; Androgenic

Introduction

Diabetes-associated reproductive dysfunction affects approximately 150 million men worldwide and is one of the most common clinical problems that worsen the quality of the life of patients with diabetes [1,2]. Sexual dysfunction is one of the major secondary complications in the diabetic animal and human [3,4] compared with the nondiabetic. Diabetes manifests decreased sexual libido, potency, erectile dysfunction, and ejaculation difficulties [4,5], decreased sperm count, motility and impairment of spermatogenesis [6], vascular complication [7], central and peripheral neuropathy [8,9], and derangement of pituitary testicular axis [10]. Natural herbal medicine with good therapeutic properties is preferred to various commercial drugs which would otherwise cause drastic side effects. Herbal plants are in ancient use in the Indian system of medicine for treating similar disorders, however without any scientific validations.

Mucuna pruriens, a leguminous plant, is identified as herbal medicine for improving fertility in the Indian traditional system of medicine. Its habitat includes India, Sri Lanka, South East Asia, and Malaysia [11]. The plant is rich in alkaloids such as prurienine, prurieninine, and prurienidine [12]. Triterpenes and sterols (β-sitosterol, ursolic acid, etc.) are discovered in its root and seeds. The seeds also contain proteins, amino acids such as Levo-3,4-dihydroxyphenylalanine (L-DOPA) [13,14], methionine, tyrosine, lysine, glycine, aspartic acid, glutamic acid, leucine, and serine along with globulins and albumins [15], fatty acids, carbohydrates, and related compounds such as oleic acid, linoleic acid, and palmitic acid [16–18].

Furthermore, it is reported to sustain the sexual and androgenic activities in adult male rats while improving the muscles mass [19,20]. Our previous studies proved that ethanolic seed extract of *M. pruriens* has a good sexual enhancer and increases sperm count and motility in normal rat [14]. It also improves viability and reduces structural and functional abnormalities under oxidative stress in aged rat sperm [13]. The seed extract is also reported to have hypoglycemic effects [21]. The bioavailability and natural source of essential bioactive compounds in the seed of *M. pruriens* has been the motivating force for the design of present work. Hence, the present work is aimed at evaluating the efficacy of the ethanolic seed extract on the male sexual behavior and sperm parameters in long-term diabetic rats.

Materials and Methods

Animals

Albino rats of Wistar strain were used for this study. Twelve-week-old female and male rats of body weight (b.w.) around 175–200 g and 225–250 g, respectively, were selected. They were housed individually in separate standard cages and maintained under standard laboratory conditions (temperature 24–28°C, relative humidity 60–70%, and 12-hour light–dark cycle) with free access to solid pellet diet and water ad libitum throughout the study. The study was approved by Institutional Ethical Committee (IAEC No. 01/044/07). The quarantine procedures and the animal maintenance were according to the recommendations of Canadian Council Guide to the Care and Use of Experimental Animals [22] and CPCSEA (India) Guidelines for laboratory animal facility [23]. Animals were randomly divided into six groups (N = 6). Group I represented control animals (received citrate buffer), group II received single dose of 60 mg/kg b.w. of the streptozotocin [STZ] in 0.1 M citrate buffer, group III (diabetes induced and treated with ethanolic seed extract of *M. pruriens* 200 mg/kg b.w.), group IV (diabetes induced and treated with sildenafil citrate [SC] 5 mg/kg b.w.), group V (received ethanolic seed extract of *M. pruriens* 200 mg/kg b.w.), and group VI rats were administered with SC (5 mg/kg b.w.) reference drug, that served as positive control. The extract and the SC were administrated once daily for 60 days by gavage; the dosage used in the present study was based on our previous study [13,14].

Chemicals

STZ and SC were purchased from Sigma (St. Louis, MO, USA). Other chemicals were purchased from SISCO Research Laboratory (SRL, Mumbai, India).

Drug Preparations and Phytochemical Screening

The ethanolic extract of *M. pruriens* seed was used in the present study. Collection of plants and extract preparation, percentage of yield, and phytochemical analysis were described elsewhere [13,14].

Body Weight, Food, and Water Intake

Every day before the drug administration, the body weight, food and water intake were estimated in all the experimental groups.

Blood Glucose Level

The serum glucose levels were estimated at every 30-day intervals. A drop of blood was collected from the tail vein and blood glucose level was determined by using commercially available glucometer (Accu Chek active, Roche, Germany).

Hormonal Analyses

On the 60th day, the blood was collected from retro-orbital venous plexus around 8:00 in the morning. The blood was left for 10 minutes at room temperature to clot. Serum was aspirated with Pasteur pipettes in to clean, dry sample bottles and was used for analysis of testosterone (T), follicular stimulating hormone (FSH), and luteinizing hormone (LH) by the radioimmunoassay.

Mating Behavior Test

The test was carried out according to the method of Agmo [24] and as described previously [14].

Animals from each group were allowed to mate with sexually receptive and estrus-phased females at 1:2 ratio. The occurrence and disappearance of events and phases of mating were recorded as soon as they appeared. Later, the frequencies and phases were determined by the recorded 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 up to 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), number of intromission in a single attempt or number of intromission (NI), number of mount in a single attempt or number of mount (NM), and time from the first ejaculation up to the next intromission by the male or post-ejaculatory interval (PEI), time between two adjacent intromission or inter-intromission interval (III). The precoital sexual behaviors such as chasing, nosing, anogenital sniffing and mounting were observed for up to 2 hours of pairing. The value of the observed parameters of control and experimental groups were taken. Along with the above parameter, the sexual behavior parameters computed include % of libido = (number of mated/number of paired) × 100; % of mounted = (number of mounted/number of paired) × 100; % of intromitted = (number of intromitted/number paired) × 100; % of ejaculated = (number of ejaculated/number of paired) × 100; copulatory efficiency = (number of intromission/number of mount) × 100; intercopulatory efficacy = average time between the intromission, intromission ratio, or copulatory rate = (number of intromissions/number of mount) + number of intromissions.

Test for Libido

The libido was assessed according to the method described earlier [14]. This test was done using the MF and IF of the mating behavior test on the 60th day. The numbers of mounting along with intromission until the ejaculation were analyzed.

Test for Potency

The effect of the *M. pruriens* on potency was studied according to the method described by Amin et al. [20], and as described previously modified by Suresh et al. [14]. On the 60th day, the test for penile reflexes was carried out by placing the animal on its back in a glass cylinder partial restraint. The preputial sheath was pushed behind the glands by means of thumb and index finger and held in this manner for a period of 15 minutes. Such stimulation elicits a cluster of genital reflexes. The following components were recorded: erections (E), quick flips (QF), long flips (LF), and total genital reflex (TGR). Along with the above parameters, the penile erection index (PEI) = % rats exhibiting erection × mean number of erections was also recorded.

Locomotors Test (Actophotometer)

The locomotor activity was monitored using actophotometer according to the methods of Reddy [25]. The animals were individually placed in activity meter for 3 minutes before counting actual locomotor activity for the next 5 minutes. The locomotor activity was expressed in terms of total photobeam counts for 5 minutes per animal.

Fertility Test

The fertility test was carried out according to the methods described by Nusier et al. [26]. After mating, when spermatozoa were found in the vaginal smear, it was considered as day 0 of gestation. Positive animals were allowed for the full gestation periods for normal delivery and the numbers of litters were counted and it was compared between the control and other experimental groups.

Daily Sperm Production

At the end of experimental period, animals were sacrificed, and immediately, the right side testes were dissected out and decapsulated. The testicular capsule and parenchyma were subjected to morphological analysis. The testicular parenchyma was placed in 0.25 M sucrose solution buffered to pH 7.5 with 0.02 M Tris–HCl (hydroxymethylamino methane) and homogenized in a fluid containing 150 mM NaCl, 0.05% (v/v), triton X-100, and 3.8 mM NaN_3 [27]. Round spermatids were counted using a hemocytometer and such evaluations were made in duplicate by two separate observations. The daily sperm production (DSP) was estimated by dividing the number of round spermatids by the products of weight of parenchyma and time divisor assigned to that particular species [28]. The time divisor for rats was taken 6.1 days [6]. DSP = (No. of round spermatid/Weight of testicular parenchyma × time divisor).

Sperm Analysis

Sperm Count, Viability, and Motility

Sperm analysis was performed according to the methods described earlier [13]. Briefly, spermatozoa were collected from caudal portion of the right epididymis by mincing caudal epididymis with anatomical scissors in 5 mL prewarmed physiological saline placed in a rocker for 10 minutes and incubated at room temperature for 2 minutes. The supernatant fluid was diluted 1:100 with a solution containing 5 g sodium bicarbonate, 1 mL formalin (35%), and 5 mg eosin/100 mL of H_2O. The sperm count was analyzed by using hemocytometer. Approximately, 10 μL of diluted sperm suspension was transferred to each counting chamber and made to stand for 5 minutes and counting was done under a light microscope at 400× magnification. About 20 μL of sperm suspension was mixed with an equal volume of 0.05% eosin-Y and nigrosin. After 2 minutes incubation at room temperature, slides were viewed by bright-field microscope with 400× magnification (Nikon Corporation, Tokyo Japan). Dead sperm appeared pink and live sperms were not stained. Two hundred sperms were counted for each sample and viability percentages were calculated. Percentage of motile sperms were assessed using graded semiquantitative scale of 0–5 and the spermatozoa was evaluated for the rate of forward movement and graded accordingly, i.e., 0 = No movement, 1 = Sluggish or tail movement alone, 2 = Intermittent sluggish movement, 3–4 = Fair and good movement, and 5 = Maximum movement in forward direction. Sperm collection procedure carried out around 9:00 in the morning.

Morphological Study

At the end of the experimental period, the animals were sacrificed by the overdose of anesthesia (thiopentone sodium 60 mg/kg b.w.), and transcardial perfusion was done by using the 4% paraformaldehyde in 0.01 M phosphate buffer (pH 7.4). Organs (testes, epididymis, vas deferens, prostate, seminal vesicle, and bulbourethral gland) were dissected out and postfixed in the same fixative, and organ weights were measured by using the electronic monobalance, the length, breadth and the width were measured by Vernier caliper, and the volume was measured by water displacement method.

Statistical Analysis

The data were statistically analyzed using analysis of variance (ANOVA). When the "F" ratio was statically significant, the data were subjected to the "one-way ANOVA test" [29]. Values were considered significant at $P < 0.05$.

Results

Body Weight, Food, and Water Intake

The observed data showed significant reduction in body weight and significant increase in the food and water intake in the group II and IV when compared to group I. In group III, the body weight was improved significantly and the food and water intake were also regularized when compared to group II. No significant changes were observed in the group V in body weight, food, and water intake compared to group I. In group VI, no changes were observed in the body weight and food intake; however, significant increase in water intake was observed when compared to group I (Table 1).

Blood Glucose Level

Blood glucose levels were found to be significantly increased in groups II and IV compared to group I. Administration of *M. pruriens* seed extract (group III) showed significant reduction within 30 days and it was maintained until the end of experimen-

Table 1 Animal body weight, food, and water intake

Groups	Body weight (g)		Food intake (g)	Water intake (mL)
	Initial	Final		
Group I	222 ± 11.22	263 ± 12.88	19.08 ± 1.76	60.33 ± 12.24
Group II	269 ± 33.97	91 ± 3.74 a***	47.05 ± 6.68 a***	151.33 ± 10.48 a***
Group III	222 ± 12.88	211 ± 22.90 b***	20.01 ± 1.45 b***	79.17 ± 9.02 b***
Group IV	225 ± 7.07	109.83 ± 6.73 a***	55.83 ± 12.41	167.83 ± 15.51 a***
Group V	225 ± 19.49	281 ± 37.34	20.91 ± 0.86	66 ± 7.29
Group VI	221 ± 27.64	260 ± 24.70	19.24 ± 2.04	97.83 ± 10.49 a***

Shows the body weight, food, and water intake of the control and experimental groups. Each value indicates the mean ± standard deviation (N = 6).
***$P < 0.001$.
a = group I; b = group II.

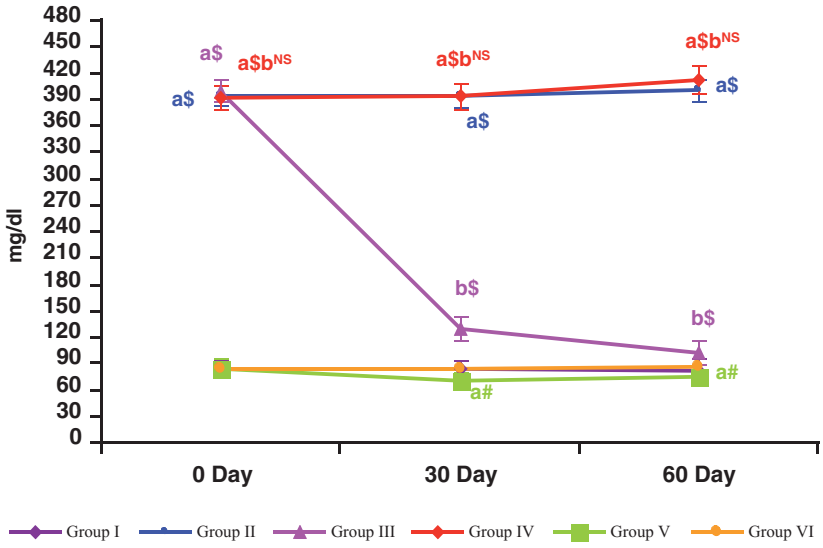

Figure 1 Shows the blood glucose levels in control and various experimental groups. Each points indicates mean ± standard error of the mean (N = 6) animals.

tal period. Administration of SC (group IV) did not show any effect on the blood glucose level. Group V showed significant hypoglycemic effect compared to group I. No significant effect was observed in the group VI (Figure 1).

Hormonal Analyses

The hormonal study showed significant reduction in serum T, LH, and FSH levels in groups II and group IV compared to group I. The supplementations of the *M. pruriens* seed extract in diabetic animals (group III) significantly increased the serum hormone levels. Group V showed significant increase in the serum hormone T and LH, but significant reduction was observed in FSH level when compared to group I. No significant changes were observed in the group VI (Figure 2).

Mating Behavior Test

The mating behavior study showed significant increase in the ML, IL, EL, PEI, and III in the group II when compared to group I. Supplementations of *M. pruriens* seed extract (group III) and SC (group IV) showed significant reduction in EL as well as ML, IL, PEI, and III when compared to group II. Groups V and VI showed significant effects in overall performance, but the net effect was less significant in group V compared to group VI. In Figure 3, NM and NI were found significantly decreased in group II when compared to group I. Supplementation of *M. pruriens* and SC (group III and group IV) significantly increased NM and NI when compared to group II. The group VI significantly increased NM and NI when compared to group I. Both the extract and the standard

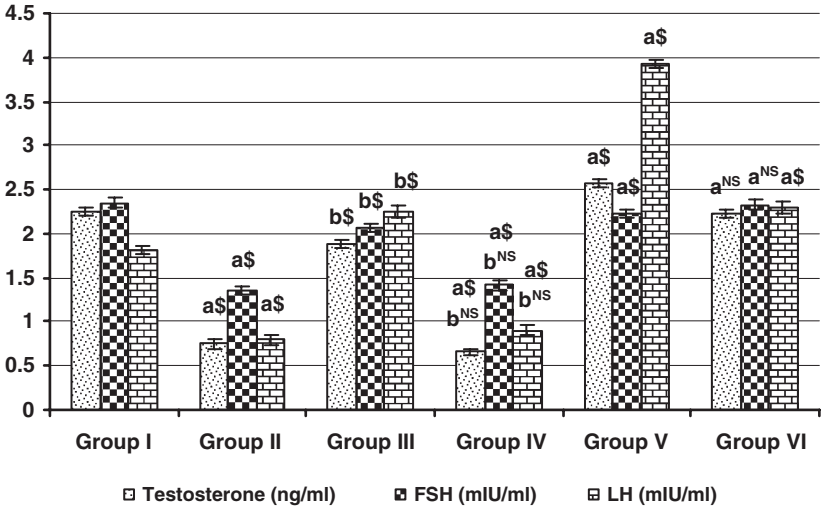

Figure 2 Shows the serum hormone levels in control and various experimental groups. Each bar indicates mean ± standard error of the mean (N = 6) animals. FSH = follicular stimulating hormone; LH = luteinizing hormone; a = group I; b = group II; NS = not significant; $ = $P < 0.001$.

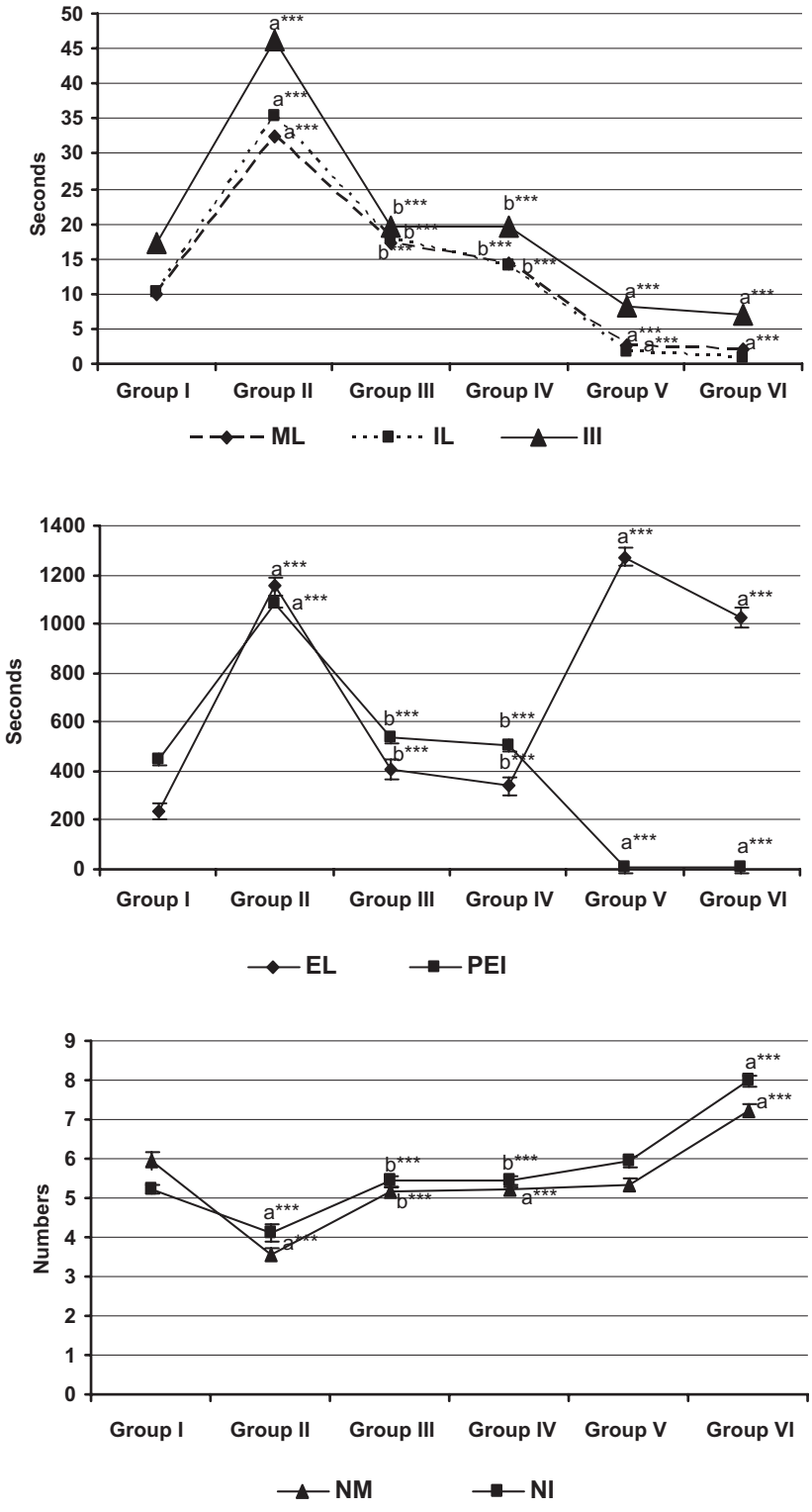

Figure 3 Shows the sexual behavior parameters of control and various experimental groups. Each point indicates mean ± standard error of the mean (N = 6) animals. ML = mounting latency; IL = intromission latency; III = inter-intromission interval; EL = ejaculatory latency; PEI = post-ejaculatory interval; NM = number of mount; NI = number of intromission; a = group I; b = group II; *** = $P < 0.001$.

drug-treated animals showed significant increases in all the computed male rat sexual behavior parameters investigated, except the intercopulatory efficiency that was significantly reduced (Table 2).

Test of Libido

Data showed significant reduction in MF and IF in group II animals when compared to the group I. The supplementation of the *M. pruriens* in group

Table 2 Computational data of sexual behavior

	Group I	Group II	Group III	Group IV	Group V	Group VI
% of libido	75 ± 5.12	25 ± 6.10 a*	75 ± 5.01 b*	75 ± 2.98 b*	100	100
% of mounted	75 ± 3.21	25 ± 3.24 a*	75 ± 4.13 b*	75 ± 6.31 b*	100	100
% of intermitted	80 ± 4.21	40 ± 4.10 a*	80 ± 2.89 b*	80 ± 5.13 b*	100	100
% of ejaculated	80 ± 3.85	20 ± 3.12 a*	80 ± 6.10 b*	55 ± 5.68 b*	100	100
Copulatory efficacy	113.58 ± 6.25	102.75 ± 3.12 a*	105.04 ± 4.05 b**	104.21 ± 4.26	111.26 ± 3.42	110.21 ± 3.71
Intromission ratio	0.53 ± 0.002	0.50 ± 0.003 a*	0.51 ± 0.002	0.51± 0.001	0.53 ± 0.002	0.52 ± 0.003
Inter copulatory efficacy	17.24 ± 1.08	46.1± 2.85 a*	19.68 ± 3.74 b*	19.65 ± 2.92 b*	8.2 ± 1.62 a*	6.98 ± 1.05 a*
Penile erection index	541.5± 11.05	83± 2.52 a*	530.25 ± 10.89 b*	539.25 ± 22.51 b*	1900 ± 31.85 a*	2232 ± 33.58 a*

Show the computed data of sexual behavior of the control and experimental groups. Each value indicates the mean ± standard deviation (N = 6).
*$P < 0.001$, **$P < 0.05$.
a = group I; b = group II.

III and the SC in group IV significantly reversed both the MF and IF when compared to group II. Groups V and VI showed significant increase in the overall performance when compared to group I (Figure 4).

Test for Potency

The data indicate significant reduction in the E, QF, LF, and TGR in group II when compared to group I. Supplementation of *M. pruriens* in group III and SC in group IV showed significant improvement in the E, QF, LF, and TGR when compared to group I. Groups V and group VI showed significant increase in all the parameters when compared to group I (Figure 5).

Fertility Test

The fertility test showed significant reduction in group II compared to group I. The administration of seed extract significantly increased the potency of the fertility in group III compared to group II. Group IV and group VI did not show any significant change when compared to group II and group I, respectively. In group V, the fertility was increased (but not significantly) when compared to group I (Figure 6).

Locomotors Test (Actophotometer)

The analysis of data showed significant reduction in group II compared to group I. Supplementation of the *M. pruriens* (group III) and SC (group IV) significantly improved the locomotor activity. Groups V and VI were found to be significantly increased in the spontaneous activity compared to group I (Figure 7).

DSP

The DSP was significantly reduced ien group II and group IV compared to group I. Supplementation of the *M. pruriens* (in group III) had significantly increased toward control (in group I). Groups V and VI showed significant increase in DSP compared to group I (Table 3).

Sperm Count, Viability, and Motility

Group II animals showed significant decrease in sperm count, viability, and motility when compared to group I. These defects were significantly reduced in group III administrated with ethanolic seed extract of *M. pruriens*. Group IV did not show any significant change when compared to group II. Groups V and VI showed significant increase in all the parameters compared to group I (Table 3).

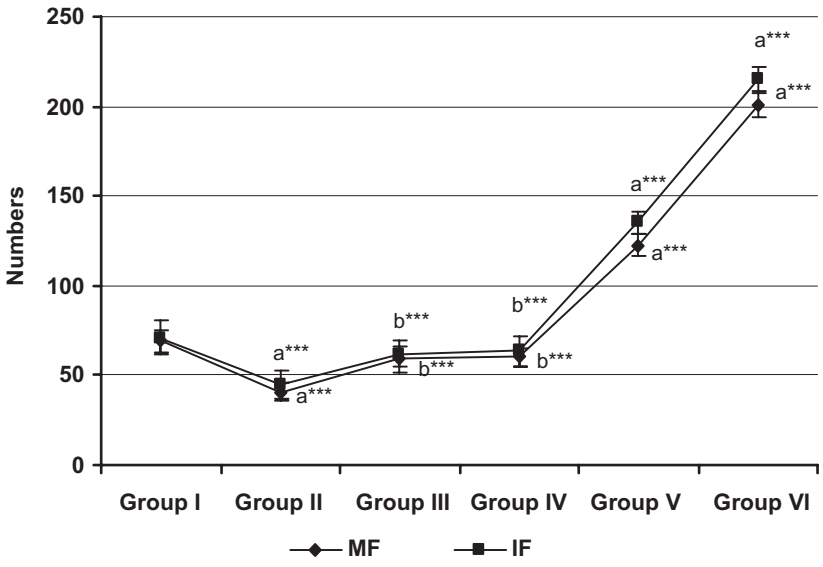

Figure 4 Shows the test of libido of control and various experimental groups. Each point indicates mean ± standard error of the mean (N = 6) animals. MF = mounting frequency; IF = intromission frequency; a = group I; b = group II; *** = $P < 0.001$.

Morphological Study

The weights of the organs were found to be significantly reduced in groups II and IV compared to group I. These changes were significantly reversed by the supplementation of *M. pruriens* seed extract in diabetic rats (group III) compared to group II. Group V and group VI also showed significant increase in the weight of epididymis, vas deferens, seminal vesicle, and bulbourethral gland; however, there was no change in the weight of the testis and prostate gland when compared to group I (Figure 8).

Discussion

The bioavailability of herbal plants and clinical significance of herbal-based drugs is a blooming tool in recent research worldwide. Furthermore, the contribution of herbal drugs has been massive in diabetic and cancer research, also equally progressive on male sexual dysfunctions. The present study reveals the potency of the ethanolic seed extract of *M. pruriens* as a sexual enhancer. The mating behavior tests showed significant increase in ML, IL, EL, PEI, and III in diabetic rats; the possible cause of which could be the androgen insufficiency [30]. But this is not the only factor for the copulatory dysfunction, because testosterone replacement therapy did not reverse the adverse effects of diabetes on sexual behavior [31]. However, in diabetes, sexual dysfunction may be due to the reduced testosterone responsiveness [6]. The supplementation of *M. pruriens* in diabetic animals (group III) showed significant recovery in

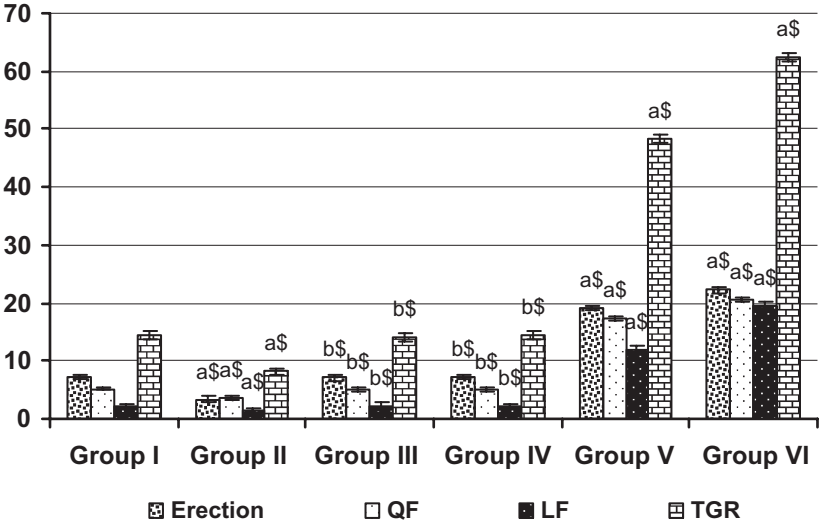

Figure 5 Shows the test of potency parameter of control and various experimental groups. Each point indicates mean ± standard error of the mean (N = 6) animals. QF = quick flip; LF = long flip; TGR = total genital reflex; a = group I; b = group II; $ = $P < 0.001$.

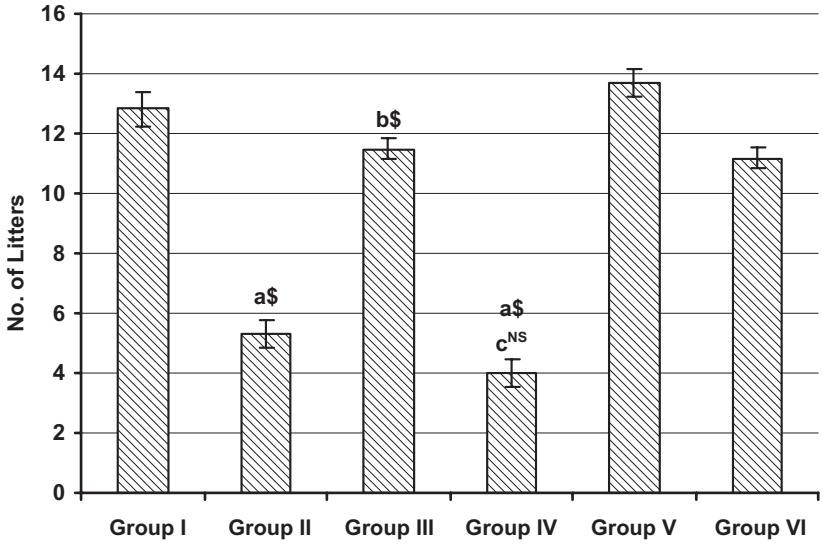

Figure 6 Shows the fertility status of control and various experimental groups. Each bar indicates mean ± standard error of the mean (N = 6) animals. a = group I; b = group II; c = group III; NS = not significant; $ = $P < 0.001$.

ML, IL, EL, PEI, and III. Hence, this suggests the efficacy of extract in promoting the EL, with significant reduction of the ML and IL compared to control. These observations indicate the aphrodisiac potential of *M. pruriens* not only in normal rats [14] but also in diabetic rats. A significant increase in PEI (test of potency and libido) observed in the diabetic animals clearly indicates that diabetes causes loss of potency and libido [6,32]. However, the extract treatment significantly decreased PEI in group III. The extract might have possibly recovered the animals from the sexual exhaust, thereby enhancing the potency and libido. The ML, IL, and PEI were considered inversely proportional to sexual arousal or motivational effects, while III was considered inversely proportional to the performance or potency [33]. The III was found to be increased in diabetic rats, but the animals with extract administration showed significant reduction in III. Similarly, the animals treated with extract alone copulated more vigorously than control as observed in our earlier study [14]. These results clearly demonstrate that *M. pruriens* has the potency to enhance the sexual performance in sexually impaired diabetic rats.

The effect on potency was evaluated by testing the frequency of penile reflexes such as E, QF, LF, and TGR. For the penile erection, a well-coordinated system of vascular, endocrine, and neural network is essential. The potency tests was significantly reduced in all the above parameters in diabetic animals; thus, diabetes induces reduction

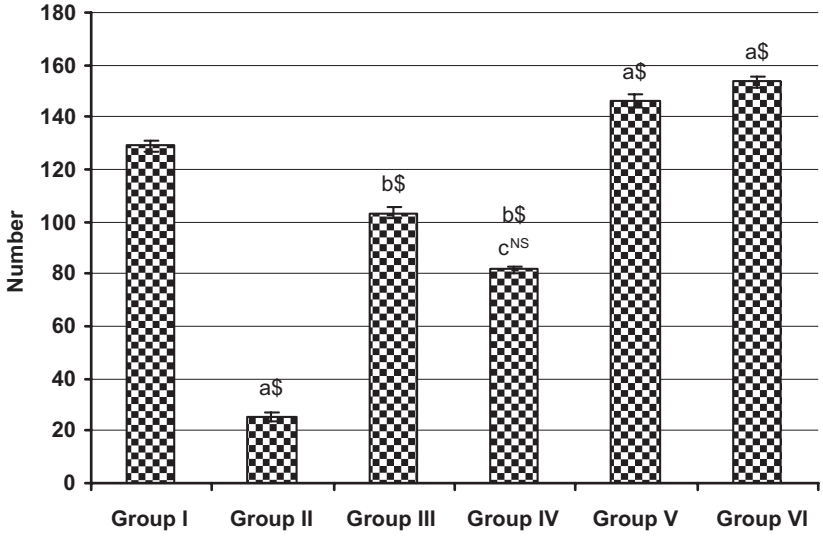

Figure 7 Shows the spontaneous activity of control and various experimental groups. Each bar indicates mean ± standard error of the mean (N = 6) animals. a = group I; b = group II; c = group III; NS = not significant; $ = $P < 0.001$.

Table 3 Sperm parameters and daily sperm production

	Group I	Group II	Group III	Group IV	Group V	Group VI
Sperm count	210.83 ± 13.58	139.17 ± 6.11 a*	197.67 ± 7.36 b*	141.5 ± 6.32 a*	248.5 ± 3.73 a*	270 ± 8.37 a*
Motility						
Motility in (%)	97.83 ± 0.75	49.17 ± 6.31 a*	87.67 ± 1.63 b*	50.83 ± 5.19 a*	98.33 ± 0.52	97.83 ± 0.75
Semiquantitative	4.8	2.3 a*	4.3 b*	2.6 a*	4.8	4.8
Viability (%)						
Viable	96.42 ± 2.02	50.5 ± 1.05 a*	92.17 ± 3.82 b*	58.5 ± 3.15 a*	98.67 ± 0.52	96 ± 1.41
Dead	3.58 ± 1.02	49.5 ± 2.05 a*	7.17 ± 2.71 b*	41.5 ± 1.98 a*	1.33 ± 0.52	4 ± 1.41
Weight of parenchyma (gm)	1.783 ± 0.12	1.142 ± 0.14 a*	1.558 ± 0.27 b*	1.09 ± 0.09 a*	1.77 ± 0.17	1.721 ± 0.13
Weight of capsule (gm)	0.075 ± 0.001	0.098 ± 0.004	0.079 ± 0.005	0.099 ± 0.006	0.072 ± 0.003	0.079 ± 0.004
Volume of parenchyma (mL)	1.685 ± 0.07	0.882 ± 0.07 a*	1.1 ± 0.14 b*	0.7 ± 0.09 a*	1.7 ± 0.19	1.47 ± 0.10
Volume of capsule (mL)	0.25 ± 0.05	0.36 ± 0.05	0.3 ± 0.07	0.42 ± 0.04	0.23 ± 0.05	0.28 ± 0.04
Number of spermatid ($\times 10^6$/testis)	331.66 ± 5.85	136.83 ± 2.62 a*	271.42 ± 4.36 b*	144.83 ± 6.83 a*	384.67 ± 2.72 a*	343.58 ± 5.71 a**
Daily sperm productions ($\times 10^6$/testis/days)	30.59 ± 2.09	19.74 ± 3.23 a*	28.58 ± 1.67 b*	21.85 ± 5.34 a*	35.99 ± 4.15 a**	32.71 ± 9.06 a**

Show the sperm parameters and the daily sperm production in control and various experimental groups. Each value indicates the mean ± standard deviation (N = 6).
*$P < 0.001$, **$P < 0.01$.
a = group I; b = group II.

in erection or sexual potency [4,6]. Administration of ethanolic seed extract of *M. pruriens* was found to significantly enhance the E, QF, LF, and TGR. These observations suggest the probable effect of the extract to induce changes in the neurotransmitter levels in local organs or in the neuronal centers that involve erectile response [34,35]. The high amount of L-DOPA is the predominant phytochemical constituent in the extract [14]; thus, the potency of the extract to enhance the erection and sexual behavior could be its influence on the nerve cells, especially dopaminergic neurons or/and dopaminergic pathway; however, this requires further study.

The actophotometer analysis exposed the depressive mood of the long-term diabetic animals. This may influence sexual function in diabetes and cause energy-mediated immobility [36], supported by the facts that body weight, food, and water intake, and blood glucose levels significantly altered in diabetic rats. This may play a crucial role in the diabetic-mediated sexual behavioral impairment. Supplementation of *M. pruriens* significantly increased the spontaneous activities, improved the food and water intake, and controlled the blood glucose level toward normal. Thus, the ability of the *M. pruriens* to keep the animals healthy by showing hypoglycemic property might influence sexual behavior. Furthermore, the control animals fed with *M. pruriens* showed significantly low glucose levels.

The sperm morphology was significantly affected under hyperglycemia. However, the epididymal sperm count, motility, as well as DSP were significantly reduced in diabetic rats, which clearly indicate impairment of spermatogenesis and the epididymal sperm maturation under hyperglycemic condition [6,37]. This may be due to the decreased androgen level or hyperglycemic-induced degeneration of seminiferous epithelium and epididymal function [38,39]. The poor quality sperm may be the cause for the decreased fertility of the diabetic rats [6]. Supplementation of *M. pruriens* significantly increased the epididymal sperm count, viability, motility, and testicular DSP, which clearly indicates the spermatogenic efficacy of the extract. These observed effects clearly demonstrate that the extract improves the fertility of the diabetic animals. This may be due to the improvement in the androgen biosynthesis by activating the hypothalamo-pituitary-testicular axis [13,14] and supplemented by the effect of lowering the blood glucose levels. *M. pruriens* directly or indirectly prevents hyperglycemic-induced degen-

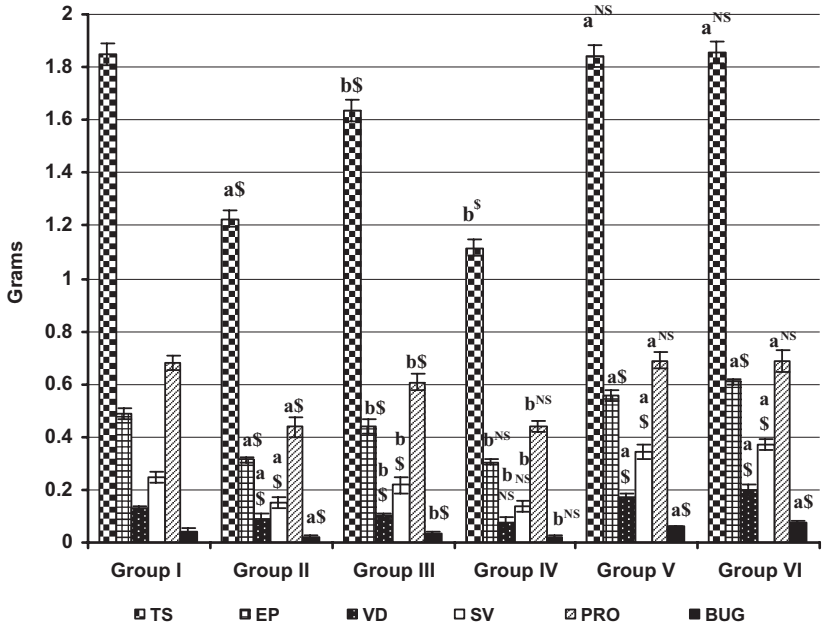

Figure 8 Shows the weight of the testis and other accessory reproductive organs of control and various experimental groups. Each bar indicates mean ± standard error of the mean (N = 6) animals. TS = testis; EP = epididymis; VD = as deferens; SV = seminal vesicle; PRO = prostate; BUG = bulbourethral gland; a = oup I; b = group II; NS = not significant; $ = $P < 0.001$.

erative changes in seminiferous epithelium and improves the epididymal sperm. Thus, the present study clearly demonstrates that the extract possesses potent androgenic and hypoglycemic effects.

Reduction in the weight of the accessory organs in diabetic animals might be androgen-mediated degenerative changes [6,40]. Administration of the *M. pruriens* significantly improve their weight; this may be due to the restoration of the androgen levels [14] or reduced hyperglycemic-induced degenerative changes. Thus, these organs may play a role in improving the sperm quality and fertility potential after the extract administration.

With regard to the SC, it was predominantly used for erectile dysfunction, sexual dysfunction of psychogenic nature, and reported to increase sperm number and function [41]. Similarly, in the present study, the sexual behavior, libido, and potency, and sperm parameter in normal rats were increased. However in diabetic rats, SC showed good effect on the sexual behavior, but failed to reverse the spermatogenic and androgenic impairment.

Hence, from the present study, we conclude that ethanolic seed extract of *M. pruriens* is not just discovered to be a potent sexual enhancer, but is also expressing spermatogenic, androgenic, and antidiabetic effects in STZ-induced diabetic rats, and thus, supporting the usage of *M. pruriens* in Indian system of medicine as sexual invigorator in diabetic condition. This study is encouraging to perform another study in men with diabetes-induced sexual dysfunction.

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