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Research Article

EVALUATION OF POTENTIAL APHRODISIAC ACTIVITY OF *MORINGA OLEIFERA* SEED IN MALE ALBINO RATS

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ABSTRACT

Objective: Evaluation of the effect of the aqueous, alcohol and chloroform extract of *Moringa oliefera* on sexual behaviour of male albino rats.

Methods: Plant extracts (aqueous, alcohol and chloroform) at doses of 100, 200 and 500 mg/kg were administrated for 21 days. The female rats involved in mating were made receptive by hormonal treatment. The general mating behaviour, libido along with orientation behaviour was studied. The effect of the extract on body weight, reproductive and vital organ weight were determined. The most effective aqueous extract was further studied for its effect on hormonal assay and compared with the standard reference drug sildenafil citrate. Similarly adverse effects and acute toxicity of the extract were also evaluated.

Results: Oral administration of aqueous, alcohol and chloroform extract at doses of 100, 200 and 400 mg/kg significantly increased the Mounting Frequency, Intromission Frequency and Ejaculation latency with reduction in Mounting Latency, Intromission Latency and Post Ejaculatory Interval. It also significantly increased the libido. The extract was also observed to be devoid of any adverse effects and showed negative results for acute toxicity.

Conclusion: The results of the present study demonstrate that aqueous, alcohol and chloroform extract of *M. oliefera* seed enhance sexual behaviour in male rats. It also thus provides a rationale for the traditional use of *M. oliefera* as acclaimed aphrodisiac and for the management of male sexual disorders.

Keywords: Aphrodisiac, Herbal medicine, Male sexual behaviour, Male rat, *Moringa oliefera,* Seed

INTRODUCTION

An aphrodisiac is defined as an agent that arouses sexual desire. Many natural substances have historically been known as aphrodisiac [1]. Sexual dysfunction is a repeated inability to achieve normal sexual intercourse, which includes various forms like premature ejaculation, retrograded, or retarded ejaculation, erectile dysfunction, arousal difficulties, etc. Several management options employed are associated with some serious side effects and are not readily available and expensive. The search for natural supplement from medicinal plants is being intensified, probably because of reduced side effect, its ready availability and reduced cost. Therefore, the increasing used for search and screening of medicinal plants with aphrodisiac potential in male has been necessitated [2].

Moringa oleifera (Linn) is a medicinally important plant, belonging to family *Moringaceae*. The plant is also well recognized in India, Pakistan, Bangladesh and Afghanistan as a folkloric medicine [3]. *Moringa oleifera* is a small or medium sized tree up to 10 m tall, with thick, soft, corky, deeply fissured bark, growing mainly in semiarid, tropical and subtropical areas. Different parts of the tree have been used in the traditional system of medicine. 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 *Moringa oleifera* seeds is being used traditionally as an aphrodisiac [4]. The seeds have been used in indigenous medicine for over many decades as traditional medicine. The seeds are also known to exert its protective effect by decreasing liver lipid peroxides and, as an antimicrobial agent [5]. The leaves of *Moringa oleifera* are used as purgative, are applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and cataract; leaf juice is also believed to control glucose levels and applied to reduce glandular swelling [6, 7, 8]. The stem bark is used as an abortifacient and as an antioxidant activity [9, 10]. The root of *Moringa oleifera* were shown to possess antihelmithic, rubefacient, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, in treatment of rheumatism, inflammations, articular pains, lower back or kidney pain and constipation [11, 7].

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 *Moringa oleifera* seeds in the folklore medicine. Hence then, the present work was undertaken to validate scientifically the aphrodisiac role of *Moringa oleifera* seeds as acclaimed by the traditional tribal user of Melghat region of Amravati district, Maharashtra.

MATERIALS AND METHODS

Collection of Plant Material

The seeds *Moringa oleifera* plant were collected from Melghat region of Amravati district during the flowering period of September to February, identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. VZ- 1).

Procurement and Rearing of Experimental Animal

Healthy wistar strain male albino rats, two months old and weighing 200- 300 g 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 hours 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/1/2012)].

Preparation of Extract

The seeds of *Moringa oleifera* 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 presences of various constituents in the seed extract of M. oleifera were determined by preliminary phytochemical screening as per Thimmaiah [12].

Acute Toxicity Study

Healthy male albino rats were starved for 3- 4 hours and subjected to acute toxicity studies as per Organization of Economic Co-operation and Development (OECD) guidelines No: 423 [13]. They were divided into 4 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 different extract (aqueous, alcohol and chloroform) of *Moringa oleifera* seed, orally at the doses of 1000, 2000 and 5000 mg/kg daily for 7 days respectively. The rats were observed continuously for 2 hours for behavioural, neurological and autonomic profile, and for next 24 and 72 hours 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 g) 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 different extract (aqueous, alcohol and chloroform) of *Moringa oleifera* seed orally at the doses of 100, 200 and 500 mg/kg, respectively, daily for 21 days at 18:00 hour. Group 5 served as standard and was given suspension of sildenafil citrate (Vigora tablets, German Remedies) orally at the dose of 5 mg/kg, 1 hour prior to the commencement of the experiment. Since the male animals should not be tested in unfamiliar circumstances hence 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 [15] method (as the female rats allow mating only during the estrus phase) They were administered suspension of ethinyl oestradiol (Lynoral tablets, Organon Pharma) orally at the dose of 100 µg/animal, 48 hour prior to the pairing plus progesterone (Dubaget tablets, Glenmark Pharma) injected subcutaneously, at the dose of 1 mg/animal, 6 hour before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, experimental 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 hour 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 ratio. 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 recorded on audio video-cassette (Sony Handycam) as soon as they appeared. Their disappearance was also recorded. Later, the frequencies and sexual behaviour 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 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) and time from ejaculation and the first intromission of the following series or Post-ejaculatory interval.

Using the above parameters of sexual behaviour, the following computed parameters were calculated: % index libido= (number mated/ number paired)×100; % Mounted= (number mounted/ number paired)× 100; % Intromitted= (number of rats that intromitted/ number paired)× 100, Intromission ratio= (number of intromission/ number of mount + number of intromission), % Ejaculated= (number of rats that ejaculated/ number paired) × 100; Copulatory Efficiency= (number of intromission/ number of mounts)× 100; Intercopulatory Efficiency= (average time between intromissions) [18].

Test for Libido

The test was carried out by the method of Davidson [19] modified by Amin et al [16]. Healthy and sexually experienced male albino rats (200– 300 g) that were showing brisk sexual activity were selected for the study. They were divided into 11 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 different extract (aqueous, alcohol and chloroform) of *Moringa oleifera* seed orally at the doses of 100, 200 and 500 mg/kg, respectively, daily for 21 days at 18:00 hour. Group 5 served as standard and was given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hour 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 Mounting Frequency (MF) on the evening of 21st day at 20:00 hour. The penis was exposed by retracting the sheath and 5% xylocaine ointment (Lidocaine ointment, AstraZeneca Pharma) was applied 30, 15 and 5 min before starting the observations. Each animal male was placed individually in a cage and the receptive female rat was introduced in the same cage. The number of mountings, intromission and ejaculation were noted.

Orientation Activity

The test was carried out by the method of Sharma et al [20], modified by Islam et al [21]. Healthy and sexually experienced male albino rats (200– 300 g) 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 different extract (aqueous, alcohol and chloroform) of *Moringa oleifera* seed orally at the doses of 100, 200 and 500 mg/kg, respectively, daily for 21 days at 18:00 hour. Group 5 served as standard and was given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hour prior to the commencement of the experiment. The orientation activity was carried out on the 21st day of treatment and was analyzed in three segments with little modification [21].

Orientation behaviour of male rats was determined using following method of scoring:

Orientation towards female – (1 for every sniffing and 2 for every licking)

Orientation towards self – (1 for every non-genital grooming and 2 for every genital grooming)

Orientation towards environment – (1 for every exploration, 2 for every rearing and 3 for every climbing)

The cumulative score for each orientation behaviour noted in the half hour observation period was later calculated.

Effect on Sexual and Vital Organ Weight

After the mating behaviour analysis, the next morning (Day 22), all the control, standard and experimental groups of male rats were evaluated for their body weight. The animals were completely anaesthetized with anesthetic ether (Narsons Pharma), sacrificed by cervical decapacitation and then testis, seminal vesicles, epididymis, vas-deference, penis and prostate glands along with vital organ like liver, kidney, adrenal gland, and spleen were carefully removed and weighed using digital electronic balance [22, 23, 24].

Statistical Methods

All the data are expressed as mean ± S.E. Statistical analysis was done by Student's t-test and one way ANOVA [25].

RESULTS

Phytochemical Screening

Preliminary phytochemical screening of the seed extract of *Moringa oleifera* revealed the presence of alkaloids, flavonoids, steroids, phenolics, tannins and saponines whereas anthraquinones were not detected.

Acute Toxicity 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. 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 5000 mg/kg body weight. Hence one tenth of treated dose (500 mg/kg b. w.) was selected for present investigation.

Effect of the Extract on Mating Behaviour

The administration of *Moringa oleifera* 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 sexual behaviour parameters studied. The results of mating behaviour test show that the seed extract of *Moringa oleifera* (aqueous, alcohol and chloroform) at the dose of 100, 200 and 500 mg/kg body weight significantly increased the Mounting Frequency (MF) (P<0.001), Intromission Frequency (IF) (P<0.001) and Ejaculatory Latency (EL) (P<0.001). Similarly it also causes significant reduction in the Mounting Latency (ML) (P<0.001) and Intromission Latency (IL) (P<0.001) in experimental animals as compared to control group. Similarly, the standard drug also increased the MF, IF and EL as well as decreased the ML (P<0.001) and IL (P<0.001) in a highly significant manner as compared to control animals. The most appreciable effect was observed in the aqueous extract of *M. oleifara* at the dose of 100, 200 and 500 mg/kg body weight (Table- 1). The alteration in these parameters was statistically significant.

The computed male sexual behaviour parameters which include percentage index of libido, % mounted, % intromitted, % ejaculated and copulatory efficiency were found to be higher in the extract treated animals compared to the distilled water treated control animals (Table- 2). In contrast, the aqueous, alcohol and chloroform seed extract of *Moringa oleifera* reduced the intercopulatory interval of the animals in dose related manner compared to the distilled water administered control animals. Similarly the standard drug (Sildenafil citrate) treated group of animals also exhibited a decrease in the intercopulatory interval when compared to control animals (P<0.001). The decrease observed was statistically significant (P < 0.001, P < 0.01 and P < 0.05).

Table 1: Effect of aqueous, alcohol and chloroform extract of *Moringa oleifera* seed on mating behaviour in male rats

Treatment groups	Parameters						
	Doses (mg/kg Body wt)	Mount Frequency(MF)	Mount Latency (in Sec)	Intromission Frequency (IF)	Intromission Latency (in Sec)	Ejaculation Frequency (EF)	Ejaculation Latency (in Sec)
Group- I Control	Vehicle	4.5±0.66	248.6±11.7	4.33±0.68	341.4±1.76	1±0.25	262.8±5.73
Group- II	100	8.5±1.77**	246.8±6.5 ns	9.83±1.89*	228.4±10.5*	1.83±0.30 ns	407.4±16.8**
Aqueous extract	200	16±1.75***	180± 11.1***	19.16±3.14***	186.4±17.2*	2±0.36**	484.2±109.2*
	500	25.66± 4.98***	129.2±16.1***	21.5±3.33***	156±10.9**	2.5±0.30***	846.6±58.8***
Group- III	100	4.16±0.47*	230.4±10.8*	8.66±1.05*	353.8±46.8 ns	Absent	Absent
Alcoholic extract	200	4.66±0.61 ns	202.2±10.8*	16±0.57**	281.1±25.8**	1.66±0.40**	256.8±64.8 ns
	500	5.5±0.92*	176.6±15.4***	18±2.75**	189.3±22.2***	2.33±0.33***	320.4±100.2***
Group- IV	100	3.83±0.47*	393.6±33**	5.83±0.47 ns	661.2±173.4***	Absent	Absent
Chloroform	200	5.16±0.77*	243.6±16.8 ns	14±1.06***	390.6±25.8**	Absent	Absent
extract	500	7.16±0.60***	163.2±49.8***	20.66±1.26***	206.5±36.6***	1.33±0.21 ns	300.6±6**
Group- V Sildenafil citrate	5	6.5±0.36***	138.6±7.2*	5.66±0.33***	142.19±6.6***	2.5±0.42***	454.3±15.6***

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant.

Table 2: Effect of aqueous, alcohol and chloroform extract of *Moringa oleifera* seed on computed male rat sexual behaviour parameters

Treatment groups	Parameters							
	Doses (mg/kg Body wt)	% Index of libido	% Mounted	% Intromitted	Intromission ratio	%Ejaculated	% Copularory Efficiency	Intercopulatory interval (in sec)
Group- I Control	Vehicle	66.66	66.66	83.66	0.49	67	100	721±10.8
Group- II	100	100	100	83.33	0.53	100	100	154.8±21.8***
Aqueous	200	100	100	100	0.54	100	100	153.6±14.23**
extract	500	100	100	100	0.45	100	83.78	130±23.9***
Group- III	100	66.66	50	50	0.67	Absent	100	254.4±6**
Alcoholic	200	83.66	100	100	0.77	100	100	250.8±9.6*
extract	500	100	100	100	0.76	100	100	141±11.2***
Group- IV	100	50	33.33	50	0.60	Absent	100	425.4±3.26**
Chloroform	200	66.66	66.66	83.33	0.73	Absent	100	377.6±9.4***
extract	500	100	100	100	0.74	100	100	366±3.66**
Group- V Sildenafil citrate	5	83.33	100	100	0.50	100	100	306.6±21.7***

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant.

Effect of the Extract on Libido

The results obtained in the test for libido shows that the aqueous, alcohol and chloroform seed extract of *Moringa oleifera* at the dose of 100, 200 and 500 mg/kg, significantly increased the Mounting Frequency (MF) ($P < 0.001$, $P < 0.01$ and $P < 0.05$) as compared to control group. The standard drug also significantly increased the MF ($P < 0.001$) as compared to control animals. The intromission frequency increases in a significant manner in all the extract treated group in a dose dependent manner, however ejaculation was found to be absent in100 mg/kg b. w. alcohol extract and in 100 and 200 mg/kg b. w. chloroform extract treated groups. However a strikingly increased libido activity was observed in the 500 mg/kg body weight treated animals of all the extract treated groups with a marked increase in aqueous extract treated group (Table- 3).

Table 3: Effect of aqueous, alcohol and chloroform extract of *Moringa oleifera* seed on mounting frequency (test for libido) in male rats

Treatment Groups	Parameters			
	Doses (mg/kg body wt.)	Mounting Frequency (MF)	Intromission Frequency (IF)	Ejaculation (EJ)
Group-I Control	Vehicle	4.8±0.47	4±0.36	Absent
	100	8.5±0.88*	8.33±1.05 ns	Present
Group-III	200	8.83±1.70**	12±1.75**	Present
Aqueous extract	500	28.33±5.4***	24.16±2.54***	Present
	100	7.5±0.42 *	14.5±0.45***	Absent
Group-IV	200	13.66±0.93**	17.16±0.91***	Present
Alcoholic extract	500	17±1.79***	19.83±0.45***	Present
	100	6±0.57 ns	6.83±0.60 ns	Absent
Group-V	200	8.66±1.11***	8±1.06 **	Absent
Chloroform extract	500	10±1.46***	12.66±2.10***	Present
Group-II	5	17.83±0.70***	9.5±0.56***	Present
Sildenafil citrate				

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant

Effect of the Extract on Orientation Behaviour

The aqueous, alcohol and chloroform extracts *Moringa oleifera* seed at the dose level of 100, 200 and 500 mg/kg body weight markedly influenced the orientation behaviour of the treated animals, which showed more attraction towards female rats. The studies revealed significant increase in number of licking ($P< 0.001$, $P< 0.01$) and in the anogenital smelling ($P < 0.001$, $P < 0.01$ and $P < 0.05$) of treated male rats towards receptive female comparable to the standard drug treated group of animals. The behavioural assessment of rats towards environment (exploration, raring and climbing) was significantly decreased in experimental animals and moderately decreased in standard group. The studies on the genital grooming of male rats revealed that there was significant increase in genital grooming ($P < 0.001$, $P < 0.01$ and $P < 0.05$) in all extract treated groups, while moderate decrease in non-genital grooming was observed as compared with the control group. The standard drug also shows significant increase in genital grooming and decrease non-genital grooming of male rats as compared to control group (Table- 4).

Table 4: Effect of aqueous, alcohol and chloroform extract of *Moringa oleifera* seed on orientation activity in male rats

Treatment group	Doses (mg /kg b. wt.)	Mean activity score towards Female		Mean activity score towards Environment			Mean activity Score towards Self	
		Licking	Anogenital smelling	Exploration	Rarring	Climbing	Nongenital grooming	Genital grooming
Group-I Control	Vehicle	17±0.85	11±0.73	23.33±1.66	27.33±1.42	05±1.24	22.16±0.98	28±0.33
Group- II	100	14±0.38**	13.83±0.54ns	11.5±0.33***	16.66±0.33**	Nil	33.5±0.11**	30.5±0.66*
Aqueous	200	19.33±0.87*	20±0.13**	15.33±0.24**	22.16±0.28**	Nil	35±0.98***	34.14±2.31***
extract	500	31.66±1.21***	32±0.28***	21.4±0.43*	26.16±0.30 ns	Nil	43.33±1.22***	39±0.20***
Group-III	100	19.33±1.35*	15.8±0.11**	19.33±0.55***	23.33±0.86 ns	Nil	28±0.52**	30.5±0.68 ns
Alcoholic	200	25±0.90**	16.8±0.68**	20.66±1.08**	24.5 ±0.44*	Nil	28.5±0.22**	33.3±0.38**
extract	500	29.33±0.16***	23.5±1.09***	22.8±0.90 ns	29.8±1.05**	Nil	34±0.47***	40±0.24***
Group- IV	100	17±0.54 ns	13± 0.33 ns	22.16±0.45 ns	30.5±2.05***	3.5±0.55**	27.8±0.77*	28±1.64 ns
Chloroform	200	24±0.29***	17.8±0.40**	26.16±0.79**	33±1.41***	3±0.38**	29.66±0.86***	33.8±0.66*
extract	500	26.5±1.06***	24.5±1.20***	40.5±2.30***	33.66±1.06***	4±0.16*	39.5±1.89***	36.5±1.06**
Group- V	5	26.5±1.57***	23±0.70***	19±0.78	13±0.48**	03±0.23***	17±1.34***	36±2.42***
Sildenafil citrate								

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant.

Effect of Extract on Sexual and Vital Organ Weight

The intragastric (i. g.) administration of aqueous, alcohol and chloroform seed extract of *Moringa oleifera* at the dose of 100, 200 and 500 mg/kg, caused an increase in body weight, significantly, when initial and final body weight were compared. The relative weight of the reproductive organ like testes, caput segment of the epididymis, ventral prostate, seminal vesicle, penis and vas- deferens increased significantly when compared to control ($P < 0.001$, $P < 0.01$ and $P < 0.05$). Similarly, there was significant increase in the relative weight of the vital organs like liver, kidney, adrenal gland and spleen ($P < 0.001$, $P < 0.01$ and $P < 0.05$), when compared with control animal group (Table- 5). The significant increase in the weight of reproductive and vital organs was also observed in standard group when compared to control.

Table 5: Effect of aqueous, alcohol and chloroform extract of *Moringa oleifera* seed on body weight, male reproductive organ and vital organ weights of male rats

Treatment Groups	Doses (mg/kg body wt.)	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)
Group-I Control	Vehicle	200.16±3.28	212.83± 2.11	2.25± 0.08	0.408±0.09	0.809± 0.02	0.286± 0.01	0.338± 0.01	0.258± 0.11	7.198 ± 0.13	1.505 ± 0.01	0.035± 0.03	0.537 ± 0.03
Group-III Aqueous extract	100	236.33± 6.81	248.33± 5.51*	2.371 ± 0.11**	0.509±0.01**	1.549± 0.09***	0.303± 0.01*	0.301± 0.008*	0.310± 0.008*	7.957 ± 0.18***	1.526 ± 0.07 ns	0.044± 0.06**	0.423 ± 0.04***
	200	210± 1.38	221.8 ± 2.56**	2.928 ± 0.20*	0.396±0.02 ns	1.039± 0.07***	0.314± 0.04*	0.371± 0.01 ns	0.314± 0.02**	8.325 ± 1.45***	1.645 ± 0.06*	0.040± 0.002*	0.482 ± 0.01 ns
	500	215.45± 3.14	234.33± 2.33***	3.154 ± 0.31 ns	0.400±0.01 ns	1.086± 0.09***	0.581± 0.04***	0.273± 0.03**	0.301± 0.03**	7.642 ± 0.20**	1.837 ± 0.29***	0.046± 0.04**	0.477 ± 0.02**
Group-IV Alcoholic extract	100	219.16± 2.72	239.32± 4.92**	2.769 ± 0.08**	0.434±0.01*	1.013± 0.06***	0.173± 0.01***	0.225± 0.01***	0.243± 0.01 ns	7.095 ± 0.21*	1.454 ± 0.06**	0.030± 0.002 ns	0.447 ± 0.03**
	200	220± 1.69	241± 3.78**	2.289 ± 0.53 ns	0.298±0.01 ns	0.479± 0.03**	0.136± 0.01***	0.197± 0.01***	0.207± 0.01*	7.264 ± 0.33 ns	1.512 ± 0.08 ns	0.034± 0.001*	0.425 ± 0.04***
	500	219.33± 1.82	239.5 ± 2.12***	2.565 ± 0.15**	0.441±0.06**	0.781± 0.07 ns	0.239± 0.14 ns	0.219± 0.01**	0.320± 0.04**	7.512 ± 0.10*	1.814 ± 0.07***	0.037±0.001*	0.469 ± 0.02*
Group-V Chloroform extract	100	205.83± 3.76	226.66± 5.74*	2.669 ± 0.08**	0.437±0.05**	0.450± 0.01*	0.223± 0.02**	0.262± 0.03**	0.259± 0.02 ns	7.403 ± 0.26*	1.779 ± 0.08***	0.034± 0.006 ns	0.448 ± 0.01**
	200	210± 2.32	221.6 ± 3.61**	2.839 ± 0.08***	0.475±0.02***	1.060± 0.07***	0.300± 0.02*	0.316± 0.02 ns	0.304± 0.01**	8.958 ± 0.56***	1.735 ± 0.04**	0.032± 0.02*	0.475 ± 0.01***
	500	210.5± 2.64	223.33± 1.03	2.466 ± 0.28**	0.344±0.02**	1.189± 0.06***	0.324± 0.03*	0.266± 0.01**	0.294± 0.02*	8.413 ± 1.09***	1.488 ± 0.03**	0.035± 0.001 ns	0.429 ± 0.01**
Group-II Sildenafil citrate	5	230.16±1.18	251± 1.83**	3.874 ± 0.84***	0.468±0.21**	0.799± 0.07**	0.312± 0..07*	0.782± 0.04***	0.283± 0.03*	6.582 ± 0.68**	2.038 ± 0.32***	0.062± 0.02***	0.498 ± 0.01**

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant.

DISCUSSION

The seed of *Moringa oleifera* is been used traditionally 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.

The phytochemical screening helps to reveal the chemical constituent of the plant extract and the one that predominates over the other. It may also be used to search for bioactive agents as starting products used in the partial synthesis of some useful drugs [26]. The preliminary phytochemical screenings of the seed extract of *Moringa oleifera* revealed the presence of alkaloids, flavonoids, steroids, phenolics, tannins. It has been reported that steroids and saponin constituents found in many plants possess fertility potentiating properties, and are useful in the treatment of impotence [27]. Saponins found primarily in the leaf of *Tribulis terrestris* L. have been used as an aphrodisiac agent in both; Indian and Chinese traditional system of medicine [28]. The saponins may therefore boost the level of testosterone in the body as well as trigger libido enhancing effect [29] observed in this study. The presence of flavonoids in the *Moringa oleifera* extract which has been implicated to have a role in altering androgen levels [30] may also be responsible for the enhanced male sexual behaviour in this study. The alkaloid is also reported to cause facilitation of sexual behaviour and has effect on sexual behaviour [31]. Thus the improvement in sexual function demonstrated in the current study might thus be due to the presence of compounds such as flavonoids, saponins and alkaloids in *Moringa oleifera* seed extract. Further study is required to identify the active constituents responsible for the sexual function improvement activities.

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. Hence it can be suggested that short term use of *M. oleifera* seed extract for this purpose is apparently safe. Similar finding was also observed by Tajuddin, et al [32], while working on ethanolic extract of *Myristica fragrans.*

In male rats, latency for mount and intromission are considered as indicators of the sexual motivation, whereas intromission and ejaculation frequencies are considered as behavioural indication of sexual performance and facilitation [33]. After treatment with the various experimental doses of seed extract of *Moringa oleifera* there was a significant decrease in the latency for mount and intromission latencies indicating enhancing of sexual motivation, which was predominant at 21st day of observation. Similarly an increase in the number of ejaculations with an increase in the ejaculation latency indicated an increase in the sexual performance. The aqueous, alcohol and chloroform extracts have a pronounced effect on sexual behaviour shown by significant increase in Mounting frequency (MF) and Intromission frequency (IF) as compared to control. The MF and IF are considered the indices of both libido and potency. The significant increase in Ejaculation latency (EL) suggests that the all experimental extracts and standard drug prolonged the duration of coitus, which is an indicator of increase in sexual motivation [34]. The significant increase in computed male sexual behaviour parameters like % mounted , % intromitted, % ejaculated and the reduction in intercopulatory efficiency are indications of sustained increase in sexual activity and aphrodisiac property inherent in the plant extract [35]. The present findings show that the aqueous, alcohol and chloroform seed extract of *Moringa oleifera* produces a striking enhancement of over- all sexual performance of normal animals. Our finding are corroborated with the aphrodisiac effect of *Allium tuberosum* seeds extract, investigated in male rats at 500 mg/kg for 21 days, which significantly reduced ML and IL increased MF, IF and EL [36].

Mounting frequency 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 [19]. Therefore, in the present study all the extracts (aqueous, alcohol and chloroform) were also studied for their effect on these components of sexual behaviour. The effect of the aqueous, alcohol and chloroform seed extract of *Moringa oleifera* at the dose of 100, 200 and 500 mg/kg 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 extracts produced a significant increase in the MF of sexually normal male rats and in standard drug treated rats. Whereas, the MF was much reduced in control animal in which the penis had not been anaesthetized. However, the test for libido revealed that Intromission and Ejaculation were present in both 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 [37], while working on ethanolic extracts of *Myristica fragrans* and *Syzygium aromaticum* in male rats.

Administration of the aqueous, alcohol and chloroform extract of *Moringa oleifera* seed at the dose level of 100, 200 and 500 mg/kg body weight modified the rat orientation activities, which acts as a main determinant for measuring male sexual behaviour [38]. All the extracts of *Moringa oleifera* in the orientation activity study showed significantly more frequent and vigorous licking and anogenital sniffing of the receptive females sexually experience treated male rats and their increased genital grooming as compared to control animals. All these indices indicate into significant increase in sexual motivation and vigor [1].

In the present study, aqueous, alcohol and chloroform extract of *Moringa oleifera* seed at the dose level of 100, 200 and 500 mg/kg body weight of extract resulted in weight gain in treated animals. The weight of the reproductive organs likes testes, seminal vesicle, penis, epididymis, vas- deference and prostrate also increased significantly along with that of 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 [23]. Since androgenic effect is attributable to testosterone levels in blood [24], 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 [39], in addition to the intensity of orgasm and ejaculations which might also be expected to improve [40]. Similar conclusion was recorded by Watcho et al [41], while working on hexane extract of *Mondia whitei* on the reproductive organ of male rats.

CONCLUSION

This aphrodisiac activity study lends support to the claim for traditional usage of *Moringa oleifera* as a sexual function enhancing medicine. Thus, this study may prove to be an effective and safe alternative remedy in sexual disorders. Work is in progress on the isolation and characterization of the aphrodisiac principle in the plant extract, the actual mechanism of action of the crude extract and bioactive agents.

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