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Pharmacological and toxicological evaluation of herbal extracts in rat model of erectile dysfunction

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Abstract
Erectile dysfunction is a global medical condition, can affect any individual irrespective of background, causing psychological imbalances in his life. Market today is loaded with plethora of medical therapies which include oral medications, intracavernosal injection of the vasoactive agents, penile implants etc. Popping a pill, usually 5-phosphodiestrase inhibitor tops the list of medical therapy used by a patient for resort. These medications come with a payback price of associated side effects which cannot be overruled. Therefore, world today is foreseeing use of herbal medications which treat the root cause of ailment and improve overall bodily functions without causing side effects. In order to contribute to the herbal medical therapy, we analyzed the potential of Withania somnifera, Asphaltum, Tribulus terrestris, Mucuna pruriens, Asparagus racemosus and Sida cordifolia individually and synergistically in hypercholestrolemic rat model of erectile dysfunction. These herbs showed comparable pharmacological activity to sildenafil in treatment of erectile dysfunction.

Keywords: witheringia, traditional herb, physalins, biological activities

1. Introduction
Sexual dysfunction mainly erectile dysfunction, impotence and other related problems are eluding scientific community and medical practitioners since time immemorial. Erectile dysfunction (ED) is the inability to obtain or sustain erection of the penis to permit satisfactory intercourse [1]. ED also has psychosocial implications which affect men in many ways. Of men aged 40-70 years, an estimated 34.8% have ED [2]. It has been recently estimated that more than 152 million men world-wide experienced ED in 1995 and this number may rise by 170 to approximately 322 million by the year 2025 [3]. Approximately 18 million men in the US are estimated to have ED [4].
Sildenafil (PDE-5 inhibitor), a prototype drug for the treatment of erectile dysfunction was approved by FDA in 1998. This class of inhibitor facilitates cGMP and subsequent corpus cavernosal smooth muscle relaxation. However, these drugs have their fair share of side effect which include headache (16%), facial flushing (10%), dyspepsia (7%), nasal congestion (4%), vision disturbances (3%), serious cardiovascular and cerebrovascular events including myocardial infarction, sudden cardiac death, ventricular arrhythmia, cerebrovascular hemorrhage, pulmonary hemorrhage, hypertension, confusion, dizziness, anxiety, agitation, nervousness, attention disturbance and irritability, emotional or psychological disturbances such as abnormal thinking, depression, abnormal dreams, delirium, amnesia, and aggressive behavior (including rape, suicide, or murder) have also been reported [5].
Although synthetic drugs such as PDE-5 inhibitors are effective in improving erectile function, their use is also associated with a plethora of adverse effects. There has been a constant increase in exploration for newer herbal and chemical agents for the management of sexual dysfunction [6]. Recent years have seen a shift form modern medicine towards traditional medicine for the search of newer chemical entities that are effective in the management of erectile dysfunction. In this study, we have explored the effectiveness of *Withania somnifera* (Ashwagandha), *Asphaltum* (Shilajit), *Tribulus terrestris* (Gokhru), *Mucuna pruriens* (klwanch or konch), *Asparagus racemosus* (Shatavri) and Sida cordifolia (Bala Extract).

2. Materials and Methods
2.1 Plant extracts
The standardized extracts of Withania somnifera, Asphaltum, Mucuna puriens, Tribulus terrestris, Asparagus recemosus and Sida cordifolia were provided by Sanat Products Ltd., Delhi. The test doses of the extracts were determined after evaluation of doses mentioned in traditional literature.

2.2 Experimental Animals

Wistar rats (120-150 g) of both sex were procured from in-house Animal Facility of AIIMS, maintained at 23 ± 2 °C, 55 ± 10% humidity under 12 h light/dark cycle with free access to standard pellet diet and water. The animals were acclimatized to the laboratory environment for the duration of 7 days before experimentation. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), AIIMS, New Delhi. The guidelines of CPCSEA, India, were strictly adhered during the whole procedure.

2.3 Experimentation conducted on normal animals

2.3.1 Estimation of efficacy of each test extract

A total of 30 animals were randomly divided into five groups (n = 6): Group I (vehicle only) served as control, Group II received sildenafil citrate (10 mg/kg) and served as standard, Group III to V received escalating doses of the test drug (doses of 25, 50 and 100mg/kg respectively for all the drugs) over a duration of 28 days.

2.3.2 Safety Studies

After evaluation of the efficacy, toxicity studies were carried out in rodents to evaluate the safety of the extracts. Adult male Wistar albino rats were used for the study. They were acclimatized to the laboratory conditions for a period of 7 days at 25±1°C with 12:12 h dark light cycle. They had free access to standard pellet diet and water during this period.

2.3.3 Limit test

A limit test was performed in 5 rats (per drug) in order to determine whether the compound was toxic to rats at a dose of 2000mg/kg body weight according to the method described in OECD guidelines for testing of chemicals[7].

2.3.4 Sub-acute toxicity study

Compound that demonstrated a LD50 value >2000mg/kg were further subjected to rigorous 28 days oral toxicity study. It was done according to the OECD guidelines for testing of chemicals [8]. 42 Wistar albino rats (150-200g) were divided into 7 groups (n=6). One group served as the normal control for biochemical and histopathological parameters. The remaining six groups received the test compounds in a dose equal to that of twice the highest dose used in each drug group for a period of 28 days. The body weight of the animals was monitored daily. On Day 28, the animals were sacrificed by an overdose of anaesthetic ether. Blood samples were collected for biochemical (SGOT, SGPT, and ALP) and hematological (Clotting time, WBC count, RBC count, and Hb) estimations. The liver, kidneys and testes were removed and subjected to examination for histopathological abnormalities [9].

2.4 In Vivo Animal Model to Study Mating Behavior

The study was conducted in a plexiglass test chamber in a dimly lit room so as to imitate the natural set. Single male rat was placed in test chamber and was allowed to acclimatize for 20-30 minutes. Then a sexually receptive female (brought into estrous by a single subcutaneous injection of estradiol benzoate and progesterone and confirmed by vaginal smears) rat was presented to the male by dropping gently into the chamber [10].

2.5 Parameters Evaluated

2.5.1 Sexual behaviours exhibited by male before introducing the female

- Pelvic thrusts followed by an upright stance
- Penile grooming
- Licking of the paws, head, body, tail, penis and pelvic area
- Emergence of the engorged glans penis and distal penile shaft

2.5.2 Sexual behaviours exhibited by male after introducing the female

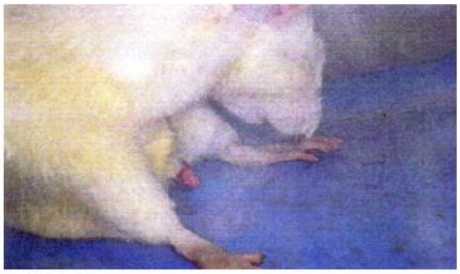
Mounting Behavior

- **Mount frequency**: average number of mount during 30 min observation.
- **Mount latency**: The lag time from the introduction of female in the cage to first mount.

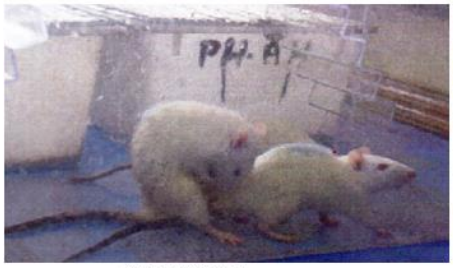
Intromission Behavior

- **Intromission frequency:** average number of Intromission during 30 min observation.
- **Intromission latency**: Lag time for first intromission after introduction of female in the cage.

Genital Licking



Penile Erection



Genital Licking

Mounting

Mounting Post ejaculatory penile grooming

Fig 1: Sexual behavior demonstrated by male rat

2.5.3 Penile Erection Index

Penile Erection was determined when the rats bent down to lick their erect penis during the observation period. Penile erection index (PEI) was determined by multiplying the percentage of rats exhibiting at least one episode of penile erection during 30 min observation period with the mean number of penile erections [8].

PEI = % of rats exhibiting penile erection x Mean number of erections

2.6 In-vitro Sperm Count Preservation

For the determination of in-vitro sperm count preservation, a total of six healthy male rats weighing between 110-130 g were taken and sacrificed by cervical decapacitation. Left and right epididymes of all the rats were taken into 5 ml of 1% sodium citrate solution and squashed thoroughly, with the help of a needle and forceps until a milky suspension was obtained. The solution was filtered through 80 µ mesh and the volume was made up to 10 ml inclusive of washings of the filter. A 1 mg/ml solution of aqueous extract was prepared and added to the sperm suspension in a ratio of 0.1:1 (100 µl sperm solution: 1 ml extract solution). Normal saline was added to control. The spermatozoa were counted using hematocytometer at 0 and 30 min after incubation at room temperature (25 ± 1 °C) [8].

2.7 Sperm Count and Serum Testosterone Level Estimation

For the determination of sperm count, only the rats from the most efficacious dose were selected. The blood was collected for estimation of testosterone. After blood collection, the male rats were sacrificed by cervical dislocation. Left and right epididymes of all the rats were taken into 5 ml of 1% sodium citrate solution and squashed thoroughly, with the help of a needle and forceps until a milky suspension was obtained. The solution was then filtered through 80 µ mesh and the volume was made up to 10 ml inclusive of washings of the filter. The spermatozoa were then counted by using a hemaocytometer [8]. Serum was separated from the blood by centrifugation at 3000 rpm (room temperature). Serum testosterone levels were estimated according to the manufacturer's instructions (Testosterone EIA kit — 582701: Cayman Chemicals, Michigan, USA).

2.8 Hypercholestrolemic rat model for erectile dysfunction

After selection of the most effective doses in the above experiments, a compound formulation of the test plants was evaluated in the hypercholestrolemic model of erectile dysfunction. The drugs were mixed in a ratio of 1:1:1:1:1:1. Four groups of male Wistar rats were used in this study. Group I received standard diet and served as normal. Groups II-IV received a 1% cholesterol diet for 4 months. After two months, Group II received normal saline and served as the control, group III received the standard drug sildenafil and Group IV received the test formulation (CF: 100mg/kg). Serum Cholesterol levels were measured at baseline i.e. 2 months and 4 months after initiation of 1% cholesterol diet to confirm hypercholesterolemia. At 4 months, sexual behaviour and erectile function was evaluated as described above. Blood was collected for estimation of serum testosterone levels. The seminal vesicles were harvested for estimation of sperm count [11].

2.9 Statistical analysis

Results have been expressed as mean ± SE. Comparison between groups was done by one-way ANOVA followed by Dunnett's multiple comparison.

3. Results

3.1 Effect of herbal drugs treatment in normal rats

Drug treatment produced a dose dependent increase in male sexual behaviour in the entire drug treated groups as compared to control rats as depicted by the decrease in mount latency, intromission latency, ejaculation latency and an increase in mount frequency, intromission frequency and penile erection index Tribulus terristeris (Table 1), Asphaltum (Table 3), Withania somnifera (Table 5), Sida cordifolia (Table 7), Asparagus racemosus (Table 9), and Mucuna puriens (Table 11). The therapeutic effect produced by the entire treatment groups at the dose of 100mg/kg treated in increasing the sexual behaviour was comparable to the standard drug sildenafil in experimental animals. Among all the test drugs that were evaluated, asphaltum was the most efficacious in increasing sexual behaviour in the tested animals. A significant increase in sperm count and serum testosterone level was also observed in all the treatment groups as compared to control Tribulus terristeris (Table 2), Asphaltum (Table 4), Withania somnifera (Table 6), Sida cordifolia (Table 8), Asparagus racemosus (Table 10), and Mucuna puriens (Table 12).

Table 1: Influence of T. terrestris administration on copulatory behavior in rats

Treatment	ML(s)	MF(no.)	IL(s)	IF(no.)	EL(s)	PEI(s)
Control	164.2±11.30	4.2±0.60	87.6±8.20	3.7±0.80	380.1±35.20	412.0±43.2
Sildenafil Citrate	24.2±2.11**	12.7±1.01**	21.6±1.04**	13.6±1.07**	211.5±23.20**	1790.4±92.7**
T. terristeris (25mg/kg)	159.7±8.9	5.9±0.75	78.9±6.9	5.8±0.50	338.5±37.9	570.5±47.8

T. terristeris (50mg/kg)	105.5±2.3*	9.4±0.85*	54.2±2.9**	10.9±1.01**	300.8±21.9**	890.4±52.4*
T. terristeris (100mg/kg)	26.4±2.00**	11.3±0.60**	24.4±1.00**	12.7±0.90*	217.4±18.70*	1640.2±89.2**

ML=mount latency; MF=mount frequency; IL=intromission latency; IF=intromission frequency; EL=ejaculatory latency; PEI= penile erection index. Values are mean ± SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

Table 2: Effect of T. terrestris administration on sperm count and testosterone level

Treatment	Sperm count (million/ml)	Testosterone level (ng/ml)
Control	56.20±2.21	2.0±0.03
Sildenafil Citrate	69.10±2.08**	4.36±0.06**
T. terristeris (100mg/kg)	88.13±1.84**	5.12±0.06**

Values are mean±SEM (n=6). *P<0.05, **P<0.01 'compared with control rats (ANOVA followed by Dunnett's test).

Table 3: Influence of Asphaltum administration on copulatory behavior in rats

Treatment	ML(s)	MF(no.)	IL(s)	IF(no.)	EL(s)	PEI(s)
Control	164.2±11.30	4.2±0.70	87.6±8.20	3.7±0.80	380.1±35.20	412.0±43.2
Sildenafil Citrate	24.2±2.11**	12.7±1.01**	21.6±1.04**	13.6±1.07**	231.5±23.20**	1790.4±92.7**
Asphaltum (25mg/kg)	142.8±6.2	6.9±1.05	61.5±2.9*	7.4±1.05*	310.5±27.3	815.4±59.72*
Asphaltum (50mg/kg)	95.5±4.3*	12.7±0.9*	43.6±1.19**	13.2±0.95**	249.4±19.7**	1495.2±91.5*
Asphaltum (100mg/kg)	31.4±1.80**	13.3±0.50**	22.4±1.05**	13.8±0.90*	228.4±17.40*	1690.2±85.2**

ML=mount latency; MF=mount frequency; IL=intromission latency; IF=intromission frequency; EL=ejaculatory latency; PEI= penile erection index. Values are mean±SEM (n=6). *P<0.05, "P<0.01 compared with control rats (ANOVA 'followed by Dunnett's test).

Table 4: Effect of Asphaltum administration on sperm count and testosterone level

Treatment	Sperm count (million/ml)	Testosterone level (ng/ml)
Control	56.20±2.21	2.00±0.03
Sildenafil Citrate	69.10±2.08**	4.36±0.06**
Asphaltum (100mg/kg)	84.12±3.84**	5.32±0.08**

Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Othirittt's test).

Table 5: Influence of W. somnifera administration on copulatory behavior in rats

Treatment	ML(s)	MF(no.)	IL(s)	IF(no.)	EL(s)	PEI(s)
Control	164.2±11.30	4.2±0.70	87.6±8.20	3.7±0.80	380.1±35.20	412.0±43.2
Sildenafil Citrate	24.2±2.11**	12.7±1.01**	21.6±1.04**	13.6±1.07**	231.5±23.20**	1790.4±92.7**
W. somnifera (25mg/kg)	151.5±12.25	5.9±0.50	71.9±5.80	6.3±0.50	370.2±28.50	590.2±43.9
W. somnifera (50mg/kg)	86.7±8.9**	9.7±0.60*	56.4±5.05*	9.7±0.85*	250.4±22.50**	1185.5±73.2**
W. somnifera (100mg/kg)	36.6±2.01**	10.3±0.65**	24.4±1.05**	11.7±0.84*	222.4±19.70*	1550.5±91.2**

ML=mount latency; MF=mount frequency; IL=intromission latency; IF=intromission frequency; EL=ejaculatory latency; PEI= penile erection index. Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's. test).

Table 6: Effect of W. somnifera administration on sperm count and testosterone level

Treatment	Sperm count (million/ml)	Testosterone level (ng/ml)
Control	56.20±2.21	2.0±0.03
Sildenafil Citrate	69.10±2.08**	4.36±0.06**
W. somnifera (100mg/kg)	72.13±1.50**	4.92±0.06**

Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

Table 7: Influence of S. cordifolia administration on copulatory behavior in rats

Treatment	ML(s)	MF(no.)	IL(s)	IF(no.)	EL(s)	PEI(s)
Control	164.2±11.30	4.2±0.60	87.6±8.20	3.7±0.80	380.1±35.20	412.0±43.2
Sildenafil Citrate	24.2±2.11**	12.7±1.01**	21.6±1.04**	13.6±1.07**	211.5±23.20*	1790.4±92.7**
S. cordifolia (25mg/kg)	143.5±12.95	5.3±0.50	69.5±5.75	5.9±0.20	320.4±21.05	650.5±43.06
S. cordifolia (50mg/kg)	92.5±5.65*	7.5±0.85	45.6±4.79*	9.75±0.50*	276.5±20.5	815.8±59.75*
S. cordifolia (100mg/kg)	37.4±2.10**	10.3±0.70*	26.4±0.90**	11.7±0.90*	243.4±19.50**	1320.2±62.2**

ML=mount latency; MF=mount frequency; IL=intromission latency; 1F=intromission frequency; EL= ejaculatory latency; PEI= penile erection index. Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

Table 8: Effect of S. cordifolia administration on sperm count and testosterone level

Treatment	Sperm count (million/ml)	Testosterone level (ng/ml)
Control	56.20±2.12	2.00±0.03
Sildenafil Citrate	69.10±2.08**	4.36±0.06**
S. cordifolia (100mg/kg)	73.13±2.14**	5.01±0.09**

Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

Table 9: Influence of A. racemosus administration on copulatory behavior in rats

Treatment	ML(s)	MF(no.)	IL(s)	IF(no.)	EL(s)	PEI(s)
Control	164.2±11.30	4.2±0.60	87.6±8.20	3.7±0.80	380.1±35.20	412.0±43.2
Sildenafil Citrate	24.2±2.11**	12.7±1.01**	21.6±1.04**	13.6±1.07**	231.5±23.20**	1790.4±92.7**
A. racemosus (25mg/kg)	150.5±12.90	6.5±0.50	70.5±6.01	6.5±0.75	310.5±24.6*	580.2±49.7
A. racemosus (50mg/kg)	108.4±8.60*	9.33±0.95*	56.2±4.05*	8.5±0.50*	285.4±19.7*	784.6±49.5*
A. racemosus (100mg/kg)	43.4±1.90**	10.3±0.60*	29.4±2.06**	10.7±0.85**	245.4±16.80**	1140.2±79.3**

ML=mount latency; MF=mount frequency; IL=intromission latency; IF=intromission frequency; EL=ejaculatory latency; PEI= penile erection index. Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

Table 10: Effect of A. racemosus administration on sperm count and testosterone level

Treatment	Sperm count (million/ml)	Testosterone level (ng/ml)
Control	56.20±2.21	2.00±0.03
Sildenafil Citrate	69.10±2.08**	4.36±0.06**
A. racemosus (100mg/kg)	75.13±2.50**	4.52±0.06**

Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

Table 11: Influence of M. puriens administration on copulatory behavior in rats

Treatment	ML(s)	MF(no.)	IL(s)	IF(no.)	EL(s)	PEI(s)
Control	164.2±11.30	4.2±0.60	87.6±8.20	3.7±0.80	380.1±35.20	412.0±43.2
Sildenafil Citrate	24.2±2.11**	12.7±1.01**	21.6±1.04**	13.6±1.07**	211.5±23.20*	1790.4±92.7**
M. puriens (25mg/kg)	145±12.40	4.5±0.65	71.3±4.90	4.5±0.33	350.5±26.10	510.2±82.7
M. puriens (50mg/kg)	89.5±5.7*	6.9±0.9	58.5±4.33*	9.36±0.75*	292.4±19.71*	880.35±75.3*
M. puriens (100mg/kg)	47.4±3.10**	9.3±0.60*	34.4±2.09**	11.7±0.70**	250.4±14.30**	1140.2±70.2**

ML=mount latency; MF=mount frequency; IL=intromission latency; IF=intromission frequency; EL=ejaculatory latency; PEI= penile erection index. Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

Table 12: Effect of M. puriens administration on sperm count and testosterone level

Treatment	Sperm count (million/ml)	Testosterone level (ng/ml)
Control	56.20±2.21	2.00±0.03
Sildenafil Citrate	69.10±2.08**	4.36±0.06**
M. puriens (100mg/kg)	81.13±3.04**	5.52±0.06**

Values are mean±SEM (n=6). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

3.2 *In-vitro* sperm count preservation

There was no statistical difference between the sperm counts in the control or drug treated groups at 0 min (before addition of the vehicle/extract suspension). However, at 30 minutes post incubation, there was a significant decrease in the sperm count in the control animals as compared to the extract treated groups (Table 13).

Table 13: Effect of test drugs on in-vitro sperm count preservation

Treatment	Sperm count (million /ml) 0 minutes	Sperm count (million/ ml) 30 minutes
Control	59.20±2.12	31.90±2.79
Tribulus terristeris	62.10±3.09	49.20±2.05**
Asphaltum	61.55±2.45	52.60±3.15**
Withania somnifera	58.90±2.08	49.30±1.90**
Asparagus racemosus	63.85±3.86	51.90±2.75**
Sida cordifolia	62.90±2.58	48.50±2.40**
Macuna puriens	60.85±2.75	49.66±2.54**

Values are mean±SEM (4sets/group). *P<0.05, **P<0.01 compared with control rats (ANOVA followed by Dunnett's test).

3.3 Toxicity studies on the test drugs

Administration of the test drugs at a dose of 2000mg/kg (limit test) did not produce any mortality in any of the animals throughout the observation period of 14 days. As the test drugs were found to be non-lethal at a dose of 2000mg/kg, LD50 determinations were not carried out. Since the highest dose (100mg/kg) of all the test extracts produced the maximum efficacy in the behavioural study, a dose of 200mg/kg was used for evaluation of 28 day oral toxicity of the drugs. Although there was an increase in body weight in all drug treated groups, this increase was not statistically significant as compared to normal animals (Table 14). However, there was a statistically significant increase in the organ weights of the testis, cauda epidymis and seminous vesicles in the extract treated groups as compared to normal. Histopathology of the visceral organs also did not demonstrate any toxic effects after test extract of administration (Fig. 2-4). Histopathological pictures of liver as in Fig. 2 demonstrated good structural integrity with normal hepatocytes. Patency of central vein and portal triad was also maintained in all drug treated groups. As visible in Fig. 3, H & E of rat kidney demonstrated good structural integrity with no disruption of nephrons, glomerulus was observed to be intact and tubular structures appeared to be normal. The structural integrity of testes (Fig. 4) was also preserved by all the drug treated groups with no indication of inhibition of spermatogenesis.

Table 14: Effect of 28 day test drug administration on experimental animals

Treatment	Increase in body weight (g)	Testis (g)	Cauda epididymes (g)	Seminal vesicle (gm)
Normal	23.6±1.45	1.24±0.02	0.20±0.01	0.23±0.01
Tribulus terristeris (200mg/kg)	29.5±1.55	1.76±0.01*	0.41±0.01*	0.38±0.01*
Asphaltum (200mg/kg)	26.3±1.39	1.81±0.02*	0.42±0.01*	0.40±0.01*

Withania somnifera (200mg/kg)	24.9±1.50	1.73±0.01*	0.39±0.01*	0.37±0.01*
Asparagus racemosus (200mg/kg)	28.3±2.13	1.69±0.01*	0.37±0.02*	0.38±0.01*
Sida cordifolia (200mg/kg)	26.7±1.76	1.70±0.01*	0.40±0.01*	0.37±0.01*
Macuna puriens (200mg/kg)	28.3±1.78	1.72±0.01*	0.36±0.01*	0.37±0.01*

Values are mean ± SEM. (n=6). *P<0.05, **P<0.01 as compared with vehicle-treated rats (ANOVA followed by Dunnett's test).

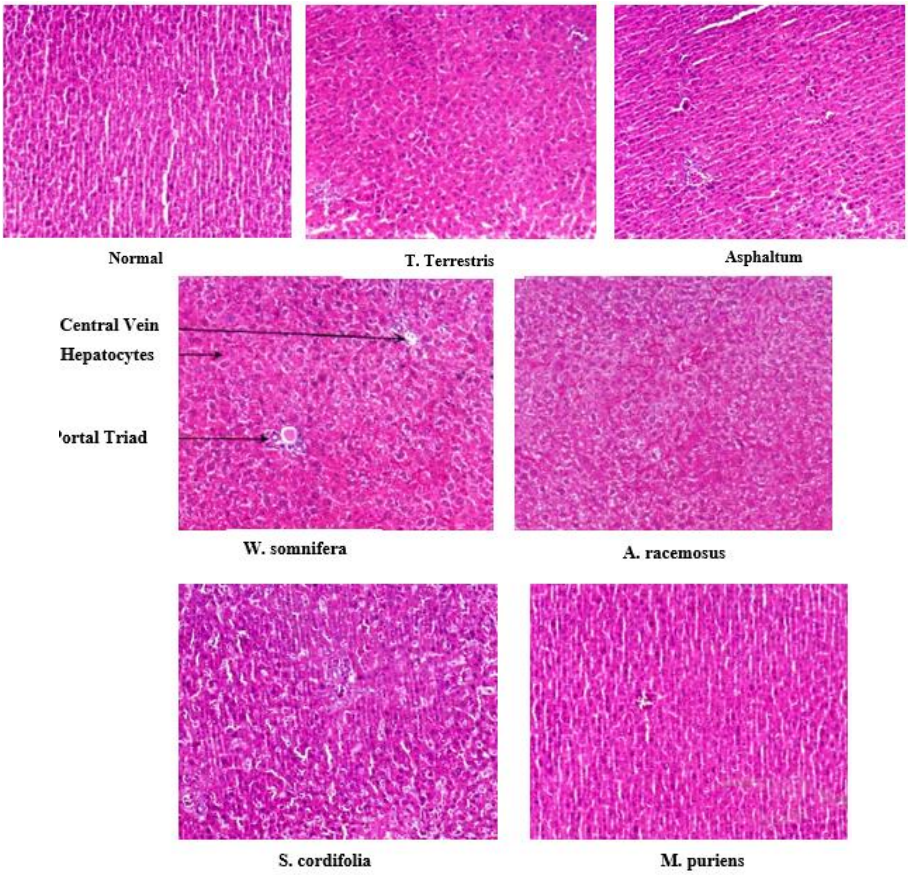


Fig 2: Representative photomicrographs of histopathologic sections of liver with different drug treatments using H & E Staining. The picture shows well defined hepatocytes and central vein in the entire drug treated groups suggesting the use of the drugs to be safe (Magnification, ×20).

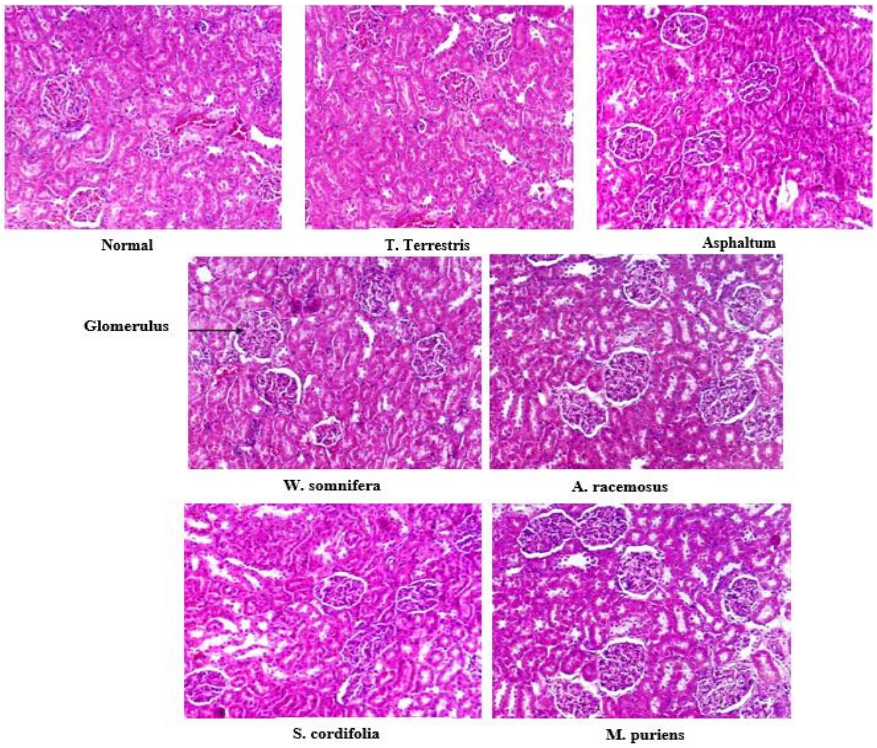


Fig 3: Representative H & E images of rat kidney from all the drug treated groups. Light microscopical examination of H & E stained tissue did not indicate any histopathological changes in the sectioned tissue. Nephrons demonstrate good structural integrity with no signs of disruption. Glomerulus and tubular structures appear to be intact and normal (Magnification, ×20).

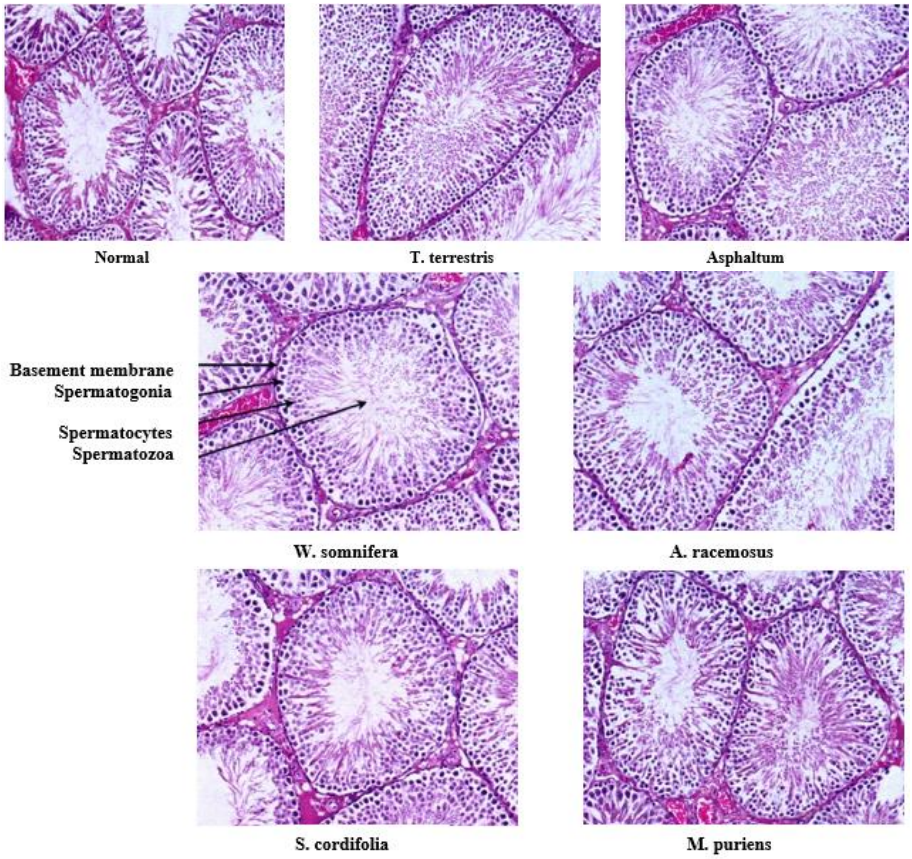


Fig 4: Pictorial representation of histological analysis of rat testes. The testes from entire drug treated groups were sectioned and stained in H & E. The sections demonstrated good structural integrity with no indication of inhibition of spermatogenesis (Magnification, ×20).

3.4 Effect of compound formulation (CF) on sexual behaviour of hypercholesterolemic rats

High cholesterol diet induced sluggishness in sexual behaviour of the control rats as can be seen by an increase in mount latency, intromission latency, ejaculation latency, and a decrease in mount frequency, intromission frequency and penile erection index as compared to normal (Table 15). A similar effect was also observed in the serum testosterone levels and sperm count (Table 16).

CF administration produced a significant decrease in mount latency, intromission latency, ejaculatory latency and a significant increase in mount frequency, intromission frequency and penile erection index. A similar ameliorative effect was also observed in sperm count and serum testosterone levels and both of these values demonstrated a significant increase for disease control and were almost similar to normal.

Table 15: Effect of Compound Formulation (CF) administration on copulatory behavior in rats

Treatment	ML(s)	MF(no.)	IL(s)	IF(no.)	EL(s)	PEI(s)
Normal	160.2±10.30	4.1±0.65	85.6±7.20	3.5±0.60	378.1±31.21	428.0±31.5
Control	210.2±17.30	2.1±0.30	107.6±8.60	1.7±0.30	480.1±40.50	305.0±23.5
Sildenafil Citrate (10mg/kg)	29.2±2.11**	10.7±1.01**	28.6±1.04**	10.6±1.07**	271.5±20.20**	1490.4±42.7**
CF (100mg/kg)	32.4±1.50**	11.3±0.60**	34.4±1.00**	9.2±0.80*	274.4±14.70*	1310.2±23.2**

Values are mean±SEM. (n=6). *P<0.05, **P<0.01 .as compared with control rats (ANOVA followed by Dunnett's test)

Table 16: Effect of Compound Formulation (CF) administration on sperm count and testosterone level

Treatment	Sperm count (million/ml)	Testosterone level (ng/ml)
Normal	59.20±2.35	2.09±0.04
Control (vehicle)	35.50±2.42	1.25±0.03
Sildenafil (10mg/kg)	45.13±2.84**	1.37±0.07
CF (100mg/kg)	53.10±2.18**	2.08±0.04**

Values are mean±SEM. (n=6). *P<0.05, **P<0.01 as compared with control rats (ANOVA followed by Dunnett's test)

4. Discussion

The present study investigates the efficacy of six herbal drugs viz, Tribulus terrestris, Asphaltum, Withania somnifera, Asparagus racemosus, Sida cordifolia and Macuna puriens in animal models of sexual behaviour and erectile dysfunction.

The efficacy of the test drugs was first evaluated as its ability to increase sexual behaviour in normal animals. All the tested drugs produced dose dependent and significant increase in sexual behaviour as demonstrated by a decrease in mounting latency, intromission latency, ejaculatory latency, and an increase in parameters like mounting frequency, intromission frequency and penile erection index during the observation period. These findings are in accordance with the reports published by other workers where an increase in sexual behaviour was observed in T terrestris [12], A. racemosus [8], Macuna puriens [13], Withania somnifera [14], Asphaltum [15, 16] and Sida cordifolia [16] treated animals as compared to experimental controls in different models. In our study, these drugs exhibited equivalent therapeutic efficacy to sildenafil (10mg/kg) at the highest dose of the extracts tested.

In the complex mechanism that regulates copulatory behavior,

testosterone is considered to contribute towards penile erection by acting both centrally and peripherally in concert with other determinants. The increased levels of the hormone have been involved in the ability of different medicinal plants to improve sexual function [12]. It has been demonstrated that, besides central effects, testosterone peripherally mediates nitrergic neurotransmission, accentuates nitric oxide synthase activity and nitric oxide release [17, 18] and all these factors in turn contribute towards penile tumescence.

In our study, all the tested herbal drugs produced an increase in serum testosterone levels in all the tested animals as compared to control. However, the feedback inhibition of sperm production due to the high circulating testosterone levels [19] was not seen in any of the drug treated groups. All test drugs demonstrated an increase in sperm count as compared to control animals, thus demonstrating that the abovementioned drugs do not decrease FSH and LH levels.

Safety studies of the test drugs also demonstrate their safety after 28 day oral administration in rats. There was no significant increase in overall body weight or the weights of organs like heart, liver and kidneys as compared to control. No adverse effects were observed in any hematological parameters in the drug treated groups. However, there was a significant increase in the weight of sex organs in the drug treated groups as compared to normal animals. This could have been the result of increased circulating testosterone levels in the treated groups as observed during efficacy studies. Although the test drugs were found to be safe on 28 day oral administration in rats, these results cannot be directly extrapolated to higher mammals as the hormonal and humoral milieu differs across species. Therefore further studies are required to establish the efficacy and safety of these individual herbal drugs in humans.

After establishing the efficacy of the test drugs in increasing sexual behaviour in normal animals, we evaluated the efficacy of a combination of these six agents (in a ration of 1:1:1:1:1:1) in enhancing sexual function in hypercholesterolemia induced sexually sluggish animals. Administration of a high cholesterol diet produced a decrease in sexual behaviour in the control rats as compared to normal (normal diet fed animals). As seen with individual drugs, the compound formulation also produced a significant increase in sexual behaviour of the tested animals as compared to control. The therapeutic efficacy shown by the compound formulation was comparable to sildenafil citrate (10mg/kg) in the behaviour parameters; mount latency, intromission frequency, ejaculation latency and penile erection index. Moreover, compound formulation produced a grater increase in mount frequency as compared to the standard drug sildenafil. Also, the compound formulation produced a greater increase in sperm count and testosterone levels as compared to sildenafil.

5. Conclusion

The compound formulation showed significant increase in sexual activity in the hypercholestrolemic rats establishing the efficacy of the composition. The study approves of aphrodisiac potential of the test drugs. The drugs appear to be a potential substitute to the allopathic drugs that carry a hefty baggage of unavoidable side effects. Hence, the study suggests further analysis of these drugs to strengthen their positive effect in treatment of sexual dysfunction with least possible side effects.

6. References

1. McVary KT. Erectile Dysfunction. New Eng J Med, 2007; 357:2472-2481.
2. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA. 1999; 281:537-544.
3. Kandeel FR, Koussa VKT, Swerdlo RS. Male sexual function and its disorders: physiology, pathophysiology, clinical investigation and treatment. Endocrine Rev. 2001; 22:342-388.
4. Selyin E, Burnett AL, Platz EA. Prevalence and Risk factors for Erectile Dysfunction in the US. Am J Med. 2007; 120:151-157.
5. US Food and Drug Administration (FDA), Adverse Event Reporting System (ASRS), Freedom of Information Report, Adverse Reactions Reports of Viagra, for the period January 4, 1998, through Washington, DC: Department of Health and Human Services, 2001.
6. Padma NH, Giuliano F. Oral Drug therapy for Erectile Dysfunction.' Urologic Clinics of North America. 2001; 28:321-334.
7. OECD. OECD Guidelines for the testing of chemicals. (No. 425), 2001.
8. OECD. OECD Guidelines for the testing of chemicals. (No. 407), 2008.
9. Thakur M, Bhargava S, Dixit VK. Effect of Asparagus racemosus on sexual dysfunction in hyperglycemic male rats. Pharmaceutical Biol. 2009; 47:390-395.
10. Thakur M, Dixit VK. Effect of Chlorophytum borivilianum on androgenic and sexual behavior in male rats. Indian Drugs. 2006; 43:300-306.
11. Kang KK, Yu JY, Yoo M, Kwon JW. The effect of DA-8159, a novel PDE5 inhibitor, on erectile function in the rat model of hypercholesterolemic erectile dysfunction. International Journal of Impotence Research. 2005; 17:409-416.
12. Gauthaman K, Adaikan PG, Prasad RN. Aphrodisiac properties of Tribulus Terrestris extract (Protodioscin) in normal and castrated rats. Life Sci. 2002; 71(12):1385-96.
13. Ahmed KA, Venkataraman BV. Assessment of a polyherbal Ayurvedic medicine for sexual activity in rats. Indian Drugs. 1999; 36:576-582.
14. Grandhi A, Mujumdar AM, Patwardhan B. A comparative pharmacological investigation of Ashwagandha and Ginseng. Journal of Ethnopharmacology. 1994; 44:131-135.
15. Mitra SK, Muralidhar TS, Rao DRB. Experimental Assessment of Relative Efficacy of Drugs of Herbal Origins on Sexual Performance and Hormone Levels in Alcohol Exposed and Normal Rats. Phytother Res. 1996; 10:296-299.
16. Mitra SK. Assessment of a polyherbal Ayurvedic medicine for sexual activity in rats. Indian Drugs. 1999; 36:576-582.
17. Aversa A, Mazzilli F, Rossi T, Delfino M, Isidori AM, Fabbri A. Effects of sildenafil (Viagra) administration on seminal parameters and postejaculatory refractory time in normal males. Hum Reprod. 2000; 15:131-134.
18. Lefievre L, De Lamirande E, Gagnon C. The cyclic GMP-specific phosphodiesterase inhibitor, sildenafil, stimulates human sperm motility and capacitation but not acrosome reaction. J Androl. 2000; 21:929-937.
19. Handelsman DJ, Conway AJ, Boylan LM. Suppression of human spermatogenesis by testosterone implants. J Clin Endocrin Metab. 1992; 75:1326-1332.