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ORIGINAL ARTICLE

Ameliorative Effect of Fructo-Oligosaccharide Rich Extract of *Orchis latifolia* Linn. on Sexual Dysfunction in Hyperglycemic Male Rats

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Abstract Diabetes mellitus (DM) is one of the most prevalent modern degenerative disease that has been implicated for deleterious effects on male reproductive function possibly due to an increased oxidative stress. Fructans and fructooligosaccharides (FOS's) which are also considered as functional food components, have been reported to produce a benevolent effect against streptozotocin induced oxidative stress. The aqueous extract of *Orchis latifolia* rich in FOS's as well as phytosterols were evaluated for their efficacy against streptozotocin and alloxan induced sexual dysfunction. The behavioral analysis of rats was undertaken to observe the effect on mount, ejaculation and intromission latencies as well as frequencies, hesitation time and copulatory rate. It was observed that hyperglycemia has an adverse effect on overall sexual behavior. The deleterious effect was significantly reduced in animals treated with polysaccharide rich fraction of *O. latifolia*. The study suggests that the diabetes induced sexual disability may be ameliorated by proper usage of herbal drugs.

Keywords Aphrodisiac · *Orchis latifolia* · Fructooligosaccharide · Diabetes · Sexual dysfunction

Introduction

Sexuality is an integral part of being human and optimum sexual performance has been a matter of concern for a long time [1]. Sexual dysfunction has affected present society which has its origin in modern life style. Various substances of animal and plant origin have been used in folk medicines of different cultures for enhancing the overall sexual performance. Although, some have been identified pharmacologically, allowing for understanding of their mechanisms of action, most of these herbs remain scientifically

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unexplored. Diabetes, hypertension, myocardial ischemia, ischemic heart disease, atherosclerosis, Peyronies's disease, testicular failure, hypogonadism, hyper-prolactinaemia, klinefelter's syndrome, cryosurgical ablation of the prostate, vascular injury, and hypercholesteremia remain major causes of sexual dysfunction [2].

Diabetes and associated stress are major contributors to sexual dysfunction and impotence in the modern world. About 90% of people with diabetes have disturbances in sexual function, including a decrease in libido, impotence and infertility, in the latter case due to testicular dysfunction associated with sustained hyperglycemia [3]. Diabetes mellitus (DM) is a heterogeneous metabolic disorder characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both. Growing evidence indicates that oxidative stress is increased in diabetes resulting in production of free radicals and reactive oxygen species (ROS's) causing impaired sexual function [4]. Although a variety of ailments can be a cause of sexual disability, diabetes is one of the contributors of sexual dysfunction.

Orchis latifolia Linn. is popularly known as 'Salam Mishri'. The plant holds the reputation of being a 'Vajikaran Rasayana' the ayurvedic category of drugs having sexual stimulation properties [5]. In our previous publication we have reported on the effectiveness of herb in stimulating the sexual performance as well as pendiculation activity and preserving sperm count [6]. In another study conducted by the authors, FOS rich aqueous fraction of *C. borivilianum* was found to be effective in preventing the streptozotocin induced oxidative stress [7]. In connection to these findings it was envisaged to evaluate the effectiveness of the FOS's rich aqueous extract of *O. latifolia* in restoring the sexual functions in hyperglycemic rats.

Materials and Methods

Animal Stock

Wistar strain albino rats of either sex weighing 220–250 g were fed on standard diet and water *ad libitum*. The animals were housed at room temperature (24 ± 2°C) on a reversed day-night cycle (06:00–18:00 h.).

Preparation of Extracts

Dried roots of *Orchis latifoila* Linn. were procured from the local market at Sagar, M.P. India and identified at the Department of Pharmaceutical Sciences Dr. H.S. Gour Vishwavidyalaya Sagar M.P. (India). The powdered roots were subjected to aqueous extraction as shown in schematic (Fig. 1). The lyophilized extract was then suspended in 2% PVP solution and administered orally using metal canula. The drugs alloxan as well as streptozotocin were administered as aqueous solution i.p. Hyperglycemia was induced in experimental animals by intra peritoneal administration of either streptozotocin or alloxan in all the groups as mentioned below except untreated control group animal (group I).

Male animals in-group of six each were taken for the studies and dosing protocol for different groups were as follows.

Group I served as control and was administered vehicle only.
Group II: 50 mg/Kg b.w. streptozotocin intraperitoneally once.
Group III: 50 mg/Kg b.w. streptozotocin intraperitoneally once and 200 mg/Kg body weight of *O. latifolia* extract orally

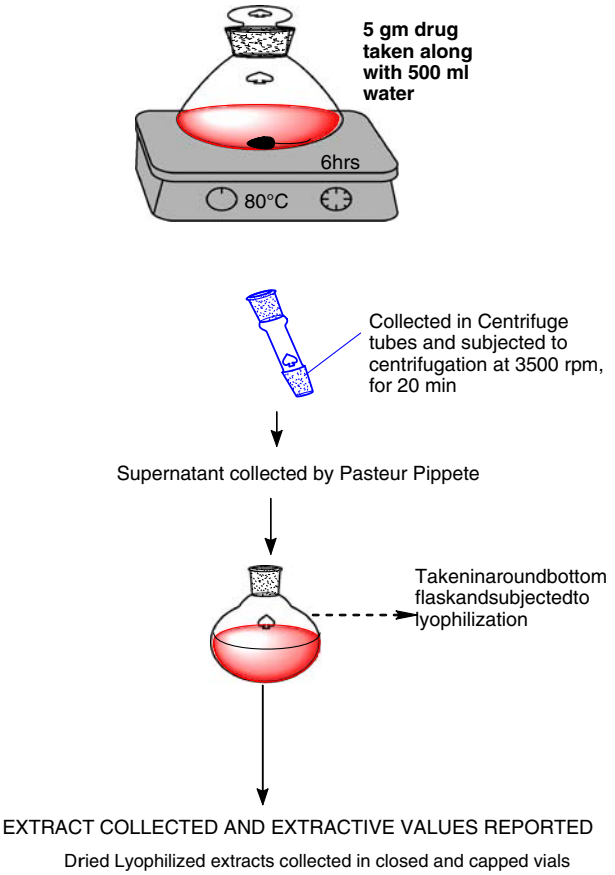

Fig. 1 Extraction Methodology for aqueous extract of *O. latifolia*

Group IV a single dose of 100 mg/Kg b.w. of alloxan intraperitoneally.
Group V a single dose of 100 mg/Kg b.w. of alloxan i.p. and 200 mg/Kg b.w. of aqueous extract of *O. latifolia*.

Blood glucose levels of animals of different groups was monitored 96 h after administration of streptozotocin or alloxan. Animals of group III and group V were given a daily oral dose of 200 mg/Kg b.w. of *O. latifolia* extract for 28 days.

The sexual behavior of animals was assessed on day 14 and day 28 of experimentation. The experiment was carried under dim red light and the behavioral aspects were video recorded using a digital camera (Olympus, EX120). Observational and behavioral analysis were performed in a wooden chamber with a glass wall (70 × 40 × 60 cm) under diffused red light in the dark phase of the light dark cycle. The chamber had a special small opening at the side for introducing the female as stimulus. The video recorded data was subjected to analysis using freeware version of Etholog v 2.2.5© E.B. Ottoni, (Sau Pauolo) [8, 9] run on Windows Xp. All the animal experimentation were carried out after prior permission from the institutional ethical committee of Dr. H.S. Gour University, Sagar (M.P.), India. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India were adhered to during the whole experimentation.

Chemical Characterization of Aqueous Extract

The aqueous extract of *O. latifolia* was characterized by HPTLC, GC, HPAEC, SEC and enzymatic analysis the results of which have been reported elsewhere. The extract was found to be rich in fructooligosaccharide of 2➔1, 2➔6 (neokestose type).

Studies Performed

Effect on Body Weights

The body and organ weights of all the groups were determined 28 days after administration of extracts and recordings were taken as described by Saxena and Dixit [10].

Orientation Behavior Analysis

Effect of extracts on behavioral aspects were gauged by evaluation of three different parameters viz. self-exploratory behavior which involved rearing, self-licking, anogenital sniffing. Environmental exploration comprising exploration, roaming, climbing and non-self-exploratory behavior which included mounting over female, licking, anogenital sniffing.

The methodology for scoring was the same as followed by Malmnas and Meyerson [11]. The scoring was made giving a value of 0 (no sexual activity), 1 (no interaction, rears and climbs on chamber), 2 (sniffs other rat), 3 self-exploratory behavior i.e., grooming and sniffing of genitals, 4 (grooms female rat anywhere), 5 (rears and climbs sexually), 6 (pursues and sniffs other rat, 7 (tries to mount but easily discouraged), 8 (mounts with an integrated deliberate manner, not easily discouraged), 9 reflex and almost involuntary mount.

Recordings were done on day 14 and day 28 after treatment (Table 2).

Attraction Towards Female or Heterosexual Attraction

Determination of attraction towards sexually receptive female was done using the methods reported by [12] modified by Thakur and Dixit [6]. On 7, 14, 21 and 28th day of treatment female rat was placed in a cage which had a wooden barrier of 15 cm separating male and female compartments which could be passed by a motivated male rat.

The hesitation time was recorded as the time (in seconds) required by the male rat before making an attempt to cross the barrier. In the same way, a scoring for attraction towards female was recorded by a score between 0 and 5 during an observation period of 15 min. A complete cross of the partition by the male rat each time was given a score of 5 while an attempt to climb was given a score of 2 and disinterest to climb was rated as 0. The readings were recorded on 14 and 28th day of treatment. This test is useful in determining the willingness of a male rat to cross an aversive position, thus indicating the intent of sexual attraction [12]. Cumulative observations of the group are presented in Table 2.

The sexual behavior in male rats was observed in the presence of sexually receptive female rat which was introduced silently from one side of the chamber as stimulus. The

whole pattern was digitally recorded and observations for various parameters were made as follows: *Mount latency* (*ML*) was calculated as the time from the introduction of female to the occurrence of first mount. *Intromission latency* (*IL*) was considered as the time for first intromission after introduction of female in the cage. *Intromission frequency* started with a mount and results in vaginal penetration, usually the male starts licking the erect genital past intromission. *Intromission ratio* was determined by dividing the number of intromissions by the sum of number of mounts and number of intromissions [13]. *Post-ejaculatory interval* (*PEL*) was calculated as time from ejaculation until next intromission. *Mount Frequency* (*MF*) was considered as total number of mounts within 30 min.

Penile Erection (*PE*) was determined using method reported by Benassi-Benelli et al. [14]. The rats of all the groups were given the treatment 30 min prior to experimentation. The rats of each group were placed in observation cages (6 at a time) and continuously observed for a period of 30 min. The penile erection was recorded when the rats bent down to lick their erect penis, ejaculation ensuing. *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 [15].

$$\text{Penile Erection Index} = \%\,\text{rats exhibiting erection} \times \text{Mean number of erections}$$

Coapulatory rate was calculated by determining the number of mounts plus number of intromissions divided by the time from the first mount until ejaculation.

$$\text{Coapulatory rate} = \frac{\text{Number of mounts} + \text{Number of intromissions}}{\text{Time from first mount till ejaculation}}$$

Statistical Analysis

Results are reported as mean ± SE. The treated groups were compared to control by ANOVA following Dunnet's test. Significance level was set at $p < 0.5$ and confidence level at 95%. Statistical analysis was carried out using Instat v 2.1 software residing in a Pentium IV processor run on Windows Xp©.

Results

Effect of Treatment on Body Weight and Blood Glucose Levels

All the male rats in streptozotocin and alloxan treated groups showed an increase in blood glucose level 96 h after injection. The blood glucose level was <250 mg/dl in all the groups. There was a moderate decrease in the blood sugar level of diabetic rats upon administration of *O. latifolia* aqueous extract on the 28th day. In streptozotocin and *O. latifolia* treated group it was (112.4 ± 2.01 mg/dl) as compared to the streptozotocin control (233.15 ± 2.01 mg/dl). Similarly, in alloxan and *O. latifolia* treated group it was 117.63 ± 1.01 mg/dl while in animals injected alloxan alone the blood glucose level was found to be 265 ± 3.11 mg/dl (Table 1).

After 28 days of treatment a decrease in the body weights of the male rats in streptozotocin and alloxan treated groups was observed. No significant weight loss was observed in the group III or group V animals which were administered aqueous extract of *O. latifolia* after administration of streptozotocin or alloxan. Similarly, a loss of up to 20% was noted

Table 1 Effect of various treatments on body weights and blood glucose level

Group	Weight of animal (g)		Weight of testes (g)		Weight of prostate(mg)		Blood glucose level in mg/dl	
	0 days	28 days	0 days	28 days	0 days	28 days	0 days	28 days
Group I	221.5 ± 0.84	224.1 ± 0.84	0.86 ± 0.01	0.88 ± 0.01	95.1 ± 1.5	96.6 ± 1.4	105 ± 2.1	106.2 ± 1.3
Group II	220.1 ± 0.61	192.6 ± 0.82	0.84 ± 0.1	0.62 ± 0.08	96.5 ± 1.2	88.1 ± 2.1	229.1 ± 1.2	233.15 ± 2.01
Group III	221.2 ± 0.5	216.1 ± 1.1**	0.87 ± 0.01	0.79 ± 0.01**	93.1 ± 1.6	90.2 ± 1.8*	231 ± 1.12	112.4 ± 2.01**
Group IV	221.3 ± 0.8	198.4 ± 0.13**	0.86 ± 0.01	0.60 ± 0.02*	94.2 ± 1.6	85.2 ± 2.18*	234 ± 0.98	265 ± 3.11
Group V	221.6 ± 0.81	216.7 ± 0.94**	0.85 ± 0.02	0.86 ± 0.03**	97.1 ± 1.1	88.1 ± 1.1**	236 ± 1.2	117.63 ± 1.01**

Group I: (no treatment) served as control and was administered vehicle only

Group II: 50 mg/Kg b.w. streptozotocin i.p. once

Group III: 50 mg/Kg b.w. streptozotocin i.p. once and 200 mg/Kg body weight of *O. latifolia* extract orally

Group IV: a single dose of 100 mg/Kg b.w. of alloxan i.p.

Group V: a single dose of 100 mg/Kg b.w. of alloxan i.p. and 200 mg/Kg b.w. of aqueous extract of *O. latifolia*

* $P < 0.05$ considered significant, ** $P < 0.01$ considered extremely significant

in testes weight in the animals of alloxan or streptozotocin treatment. This loss was almost normalized and even improved by administration of extract to group III and V animals (Table 1).

Effect on Orientation and Sexual Behavior

A progressively sluggish orientation behavior was observable in the rats of diabetic control groups (alloxan and streptozotocin alone treated). Whereas, in case of diabetic animals fed with the extracts a reduced orientation activity was observed after 14 days of treatment which was completely restored by the 28th day of observation and the behavioral parameters were even better than control group animals at the completion of experiment.

A similar effectiveness was also observable in case of sexual behavior analysis. The hesitation time was increased to 520 ± 7 s in case of diabetic rats. The hesitation time for extract treated diabetic animals was $\sim 17\%$ lesser ($P < 0.5$). Similarly, a significantly reduced score for attraction towards female was observed in diabetic animals. These scores were normal and even better than control group animals in *O. latifolia* treated groups. The values for the cumulative score of attraction were slightly superior to those observed for control group animals.

The frequencies (mount, intromission and ejaculatory) were significantly reduced in streptozotocin and alloxan induced diabetic rats ($P < 0.01$). The most interesting feature observed was that almost 70% of the animals did not indulge in any kind of sexual behavior in diabetic groups. While in diabetic animals treated with *O. latifolia* the normal sexual behavior was restored, but it was moderately higher than the vehicle treated control group animals.

The data for the latencies and frequencies is shown in Table 2. A clearly demarcated effectiveness of *O. latifolia* aqueous extract in restoring the vandalized sexual functions by streptozotocin as well as alloxan induced damage.

Discussion

The overall purpose of our study was to ascertain whether *O. latifoila* could be useful in restoring the sexual dysfunctions caused by hyperglycemia. *O. latifoila* is very rich in fructooligosaccharides (FOS's) which help in protecting the oxidative damage caused by streptozotocin. The tubers of *O. latifolia* are also considered as anti-diabetic in traditional literature. So the role of herb on sexual function in diabetic rats was investigated.

More than 90% of the patients suffering from diabetes have been reported to have one form of sexual dysfunction or the other ranging from loss of libido to decreased performance and vigor. One of the mechanisms thought to be underlying this is the degeneration of testicular function caused as a result of oxidative damage by ROS (reactive oxygen species). Although, there are reports suggesting the improvement of sexual function in diabetic individuals after treatment with anti-diabetic agents like glibenclamide or glipizide due to better management of diabetes. Still the improvement was only marginal with none of the persons with diabetes showing improvements better than the control group [16, 17].

Fructans and fructoligosaccharides have been shown to possess effectiveness in overcoming this damage. Also, the herb *O. latifolia* has stimulatory activity has been recommended in Ayruveda for sexual dysfunction [18]. Our results showing that fewer

Table 2 Effect of various treatments on sexual behavior in male rats after 28 days of treatment

Parameters	Treated groups				
	Group I	Group II	Group III	Group IV	Group V
Mount Latency ML (mean time ± SE in seconds)	169.9 ± 12.6	271.2 ± 11.2**	154.2 ± 3.4*	293.2 ± 8.2**	134.2 ± 2.1
Intromission latency IL (mean time ± SE in seconds)	313.2 ± 10.8	412.6 ± 7.4**	290.3 ± 1.2*	416.2 ± 7.1**	271.6 ± 1.4*
Post-ejaculatory latency PEL (mean time ± SE in seconds)	496.7 ± 5.8	761 ± 2.3**	482.1 ± 1.3*	772 ± 2.1**	416.2 ± 7.1
Hesitation time (mean time ± SE in seconds)	330 ± 8	520 ± 2**	184 ± 1.1*	531 ± 2**	142 ± 6*
Intromission Frequency IF	6.2 ± 0.1	2.1 ± 0.4**	6.8 ± 1.1	1.7 ± 0.3**	6.8 ± 0.8
Ejaculation Frequency EF	3.1 ± 0.9	0.2 ± 0.01**	3.8 ± 1.2*	0.1 ± 0.02**	3.5 ± 0.7*
Mount Frequency MF	15.6 ± 3.9	4.8 ± 2.9**	14.9 ± 0.89*	5.3 ± 1.4**	11.9 ± 1.4*
Number of bouts	0.6 ± 0.3	0.1 ± 0.02**	1.01 ± 0.1*	0. 1 ± 0.07**	1.63 ± 0.1*
Percent ejaculating animals	68.6 ± 0.3	21.2 ± 0.5**	81.2 ± 1.4*	28.2 ± 0.5**	80 ± 0.9*
Cumulative score for attraction towards female	22 ± 2	6 ± 1.2**	65 ± 2.1*	8 ± 1.1**	67 ± 2**
Penile Erection Index (PEI)	21.2 ± 1.1	6.2 ± 0.6**	38.6 ± 1.1**	7.1 ± 0.4**	40.2 ± 1.7**

Group I: (no treatment) served as control and was administered vehicle only

Group II: 50 mg/Kg b.w. streptozotocin i.p. once

Group III: 50 mg/Kg b.w. streptozotocin i.p. once and 200 mg/Kg body weight of *O. latifolia* extract orally

Group IV: a single dose of 100 mg/Kg b.w. of alloxan i.p.

Group V: a single dose of 100 mg/Kg b.w. of alloxan i.p. and 200 mg/Kg b.w. of aqueous extract of *O. latifolia*

* $P < 0.05$ considered significant, ** $P < 0.01$ considered extremely significant

One-way ANOVA followed by Dunnet's test comparing all versus control

diabetic rats reached ejaculation than control rats agree with the earlier reports that diabetes is associated with reduction in male copulatory behavior. There are a cascade of mechanisms responsible for diabetes induced sexual dysfunction but eventually better management of diabetes along with improved steroidogenesis and tonification of the system can be a major contributor in restoring the failing sexual functions in diabetic subjects [17].

The ability of *O. latifolia* extracts to restore the sexual function therefore, illustrates that the drug not only helps in steroidogenesis (probably due to the presence of steroidal precursors in the extract) but is also effective in ensuring the better availability of hormone to gonads. Secondly, previous reports on the ability of FOS's in reducing glucose levels could provide some insight into the possible mechanism behind the restoration of normal copulatory rate in diabetic animals treated with aqueous *O. latifolia* extract. Therefore, the multiple mechanism of herb can be a major reason for a significant improvement in all the sexual parameters. The improved activity which is even better than untreated control group clearly presents the herb as a potential potent stimulant in overcoming the sexual disability related to diabetes.

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