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ORIGINAL ARTICLE

Effect of *Curculigo orchioides* on hyperglycemia-induced oligospermia and sexual dysfunction in male rats

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Sustained hyperglycemia is considered as a major cause of sexual and erectile dysfunction in human population. *Curculigo orchioides* (CO) is considered as a sexual tonic in Ayurvedic system of medicine with potent antioxidant and adaptogenic properties. The aqueous extract of the herb was evaluated for its effectiveness against streptozotocin-induced hyperglycemic stress and subsequent sexual dysfunction due to hyperglycemia in male rats. Six groups with eight male rats in each group were used for this study and the study was carried out for 28 days. The body and organ weights of the animals were recorded. Behavioral analysis of rats was undertaken to observe the effect on mount, ejaculation and intromission (latencies and frequencies) and hesitation time. Blood glucose and serum testosterone levels were determined 28 days past treatment with CO at 100 and 200 mg kg^{-1} doses. Glibenclamide and sildenafil citrate were used as positive controls. This deleterious effect of sustained hyperglycemia and associated stress was prominently ameliorated in animals treated with aqueous extract of CO. CO treatment was helpful in ameliorating the damage caused by sustained hyperglycemia evidenced in the principle parameters *viz.* male sexual behavior, sperm count, penile erection index and seminal fructose content Antioxidant and anabolic activities of the extract under investigation could be a major attribute in preserving the sexual functions in hyperglycemic male rats. The study validates the use of CO in traditional medicine for curing diabetes-induced sexual dysfunction and compromised sexual potency.
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Introduction

Sexual dysfunction including erectile dysfunction (ED) is common in men with diabetes. Diabetic men are three times as likely to develop ED as non-diabetic men.[1,2] The causes of diabetes-induced sexual dysfunction are multi-factoral, but most commonly reflect endothelial dysfunction and autonomic neuropathy. Increased oxidative stress is also considered as an important factor causing diabetes-induced sexual dysfunction.[3]

Ayurveda the traditional system of medicine in India is endowed with numerous plants having the ability to cure sexual dysfunction in males, and the plants having this kind of activity are defined as rasayana. A rasayan may be defined as smooth pro-stressor, which reduces reactivity of host defense systems and decreases damaging effects of various stressors due to increased basal level of mediators involved in the stress response.[4,5]

Curculigo orchioides (CO) Gaertn. (family Amaryllidaceae) is a well-known herb in traditional Indian system of medicine and has been designated as a Vajikaran Rasayan.[6,7] It is claimed to be useful in treatment of piles, asthma, jaundice, diarrhea, colic, gonorrhea and sexual dysfunction.[8] It is also a constituent of 'Chywanprash' an age-old Ayurvedic formulation reported in ancient texts for invigorating and rejuvenating the physiological functions. CO has been used in several metabolism enhancing and aphrodisiac formulation in the Indian system of medicine. Rhizomes possess immunostimulant[7] aphrodisiac,[5,6] antidiabetic[9] and hepatoprotective activity.[10]

Diabetes and sustained hyperglycemia results in reduced libido.[11] Ayurveda and traditional Chinese

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medicine recognizes CO as an important aphrodisiac herb. Ayurveda the traditional system of medicine in Indian subcontinent also recommends the use of this herb as a 'Virya Rasayan'. Virya Rasayanas are herbs useful in improving the sperm count. Sustained hyperglycemia is reported to be major cause of ED, lower sperm count and low serum testosterone (T) levels, thus this study was designed to evaluate the role of this herb on erectile function, and low values for seminal parameters (that is, seminal fructose content, sperm count) in male rats.[12]

Materials and methods

Animal stock

A total of 66 Wistar strain albino rats (48 males and 18 females) weighing 220–250 g were fed on standard diet and water *ad libitum*. The animals were housed at room temperature ($24 \pm 2\,^{\circ}C$) on a reversed light–dark cycle (06:00 hours to 18:00 hours). Animal experimentation was carried out after prior permission from the Institutional Ethical Committee of Dr HS Gour University, Sagar (MP), India. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, India were adhered to during the whole experimentation.

Male animals in group of eight each were taken for the studies and dosing protocol for different groups were as follows. The experiment started 2 weeks after the confirmation of diabetes in all the groups, the experiment lasted 28 days thereafter.

Group I: was administered vehicle only (normoglycemic control).

Group II: $50\,mg\,kg^{-1}$ body weight (b.w.) streptozotocin (STZ) intraperitoneally (i.p.) once (hyperglycemic control).

Group III: $50\,mg\,kg^{-1}$ b.w. STZ, i.p. once and $100\,mg\,kg^{-1}$ b.w. of CO extract p.o., daily (CO100).

Group IV: $50\,mg\,kg^{-1}$ b.w. STZ, i.p. once and $200\,mg\,kg^{-1}$ b.w. of p.o., daily (CO200).

Group V: $50\,mg\,kg^{-1}$ b.w. STZ i.p. once and $600\,\mu g\,kg^{-1}$ b.w. of glibenclamide i.p. (positive control for hyperglycemia) daily ($STZ50 + Glib_{600}$).

Group VI: $50\,mg\,kg^{-1}$ b.w. STZ i.p. once and $5\,mg\,kg^{-1}$ b.w. of sildenafil citrate i.p. (positive control) daily ($STZ50 + Sil_5$).

Glibenclamide is an orally administered antidiabetic agent and was used as a positive control for evaluating the role of better management of glucose metabolism on sexual behavior and erectile function if any. Similarly, sildenafil citrate that is a phosphodiesterase inhibitor is useful in treatment of ED. Because sustained hyperglycemia may cause ED therefore, to evaluate whether the treatment with phosphodiesterase inhibitor may be useful in diabetes-associated ED, $STZ50 + Sil_5$ group was included in the study as a positive control.[1,3]

Female rats were also divided in three groups comprising of six animals in each group. Receptive female rats were prepared for the experimentation as per the methodology reported by Agmo.[13] After 15 days of preparation the female rats were used for experimentation.

Blood samples were collected by retro-orbital puncture from rats fasted overnight. First sample was collected 96 h after STZ injection. On day 28 the blood samples were collected by retro-orbital puncture 3 h after pellet diet and administration of extract. Blood glucose levels were determined using the glucose oxidase kit as per instructions (Invitrogen, Darmstadt, Germany).

Sexual behavior of male rat was assessed on day 14 to make the rats sexually experienced (data not shown) and day 28 of experimentation. The experiment was carried under dim-red light and the behavioral aspects were video recorded for the duration of 60 min for each rat using a digital camera (Olympus, Hamburg, Germany, EX120). Observational and behavioral analysis was performed in a wooden chamber with a glass wall ($70 \times 40 \times 60\,cm^3$) 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 were subjected to analysis using freeware version of Etholog v 2.2.5© EB Ottoni,[14] (Sao Paulo) run on Windows XP.

Collection, extraction and characterization

Rhizomes of CO were collected in and around the regions of Sagar MP (India), and identified at the Department of Botany Dr H S Gour University, Sagar. A voucher specimen of the same has been deposited (no. MKNS-Pharma-2008). Rhizomes were dried in sunlight and coarsely powdered. Powdered drug passing through 60 μm mesh size and retained on 80 μm mesh size was fed in a Soxhlet extractor (Mahendra Scientific, Kanpur, India) and defatted with petroleum ether 60–80 °C. Defatted drug was further extracted with deionized water and the aqueous extract was subjected to lyophilization. The % yield of aqueous extract was found to be nearly 29% of crude material used for extraction. CO extract was characterized by high-performance thin-layer chromatography and high-performance liquid chromatography with mass detection. High-performance thin-layer chromatography chromatogram against gallic acid as standard was developed.[15] Standardization of the extract was carried out to ensure the composition and uniformity of the extract for future reproducibility of phytochemical parameters. CO extract administered to rats was also found to contain appreciable amounts of phenolic compounds, quercetin, epigallocatechin and a small amount of saponins.

In vivo *studies*

Effect on androgenic activity and sexual behavior.

Two rats from each group were killed on day 0 and

the rest were killed on day 28 of experimentation. The weight of body and the secondary sexual organs (testis, seminal vesicles and epididymis) of all the groups (group I–VI) were determined 28 days after the treatment as mentioned previously.[16]

Measurement of the T level in blood was undertaken using high-performance liquid chromatography (Shimadzu, Kyoto, Japan). Blood samples of the male rats, which were killed on day 28 of treatment, were collected in heparinized vials. The samples were then centrifuged at 3000 *g* for 10 min. Plasma (1 ml) mixed with 100 µl of propyl paraben (3 mg ml^{-1} in methanol) used as an internal standard. Following this, the steroids from plasma were extracted into 5 ml diethyl ether–dichloromethane (60/40 v/v), mixed and immediately centrifuged for 5 min. The organic phase thus obtained was vortex mixed with 1 ml high-performance liquid chromatography-grade water. After centrifugation, the organic phase (3 ml) was evaporated at room temperature under nitrogen. The residue was re-dissolved in 100 µl of methanol–water (80:20 v/v).[16] The guard column and the column were equilibrated using high-performance liquid chromatography-grade methanol–water (80:20 v/v) at a flow rate of 0.4 ml min^{-1}. Separations were made at a temperature of 28 °C, in a SepservC18 column (Berlin, Germany). A Shimadzu- SPD MXA 6 (Shimadzu) system controller was used to flush the mobile phase and the steroids were assessed using the Shimadzu ES detector at a fixed wavelength of 280 nm.[17]

Effect on sexual functioning. 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 *viz.* Mount and intromission latency, which was calculated as the time from the introduction of female to the occurrence of first mount and intromission, respectively.[18] Post ejaculatory interval was calculated as time from ejaculation until next intromission. Other principle parameters determined in this study were mount, intromission and ejaculatory frequencies.[19,20] Determination of attraction toward sexually receptive female was carried out using the methods reported by Ang and Ngai[16] modified by Thakur *et al.*[21] On day 28 of treatment, receptive female rat was placed in a cage that had a wooden barrier of 15 cm separating male and female compartments that could be passed by a motivated male rat. Hesitation time was recorded according to the methodology reported by Thakur *et al.*[5]

Penile erection (PE) was determined on day 28 of the experiment using the method reported by Islam *et al.*[19] The rats of all the groups were given the respective treatment with CO, Sil_5 or $Glib_{600}$ 30 min before experimentation. They were placed in observation cages (six at a time) and continuously observed for a period of 30 min. The PE was recorded when the rats bent down to lick their erect penis. Penile erection index (PI) was determined by multiplying the percentage of rats per treatment group exhibiting at least one episode of PE during 30 min observation period with the mean number of PE.[21]

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

Epididymal sperm count. After the PI determination and cervical decapitation, left and right epididymis of all the rats were taken into 1 ml of 1% sodium citrate solution (pH = 7.2) and squashed thoroughly with the help of needle and forceps until a milky suspension was obtained. The solution was filtered through 80 µm mesh and the filtrate was stained with 1% aqueous eosin Y. The liquid was collected in a leukocyte pipette and subjected to counting of mature sperms as per the standard procedure.[22]

Seminal fructose content. Seminal vesicles of decapitated rats were weighed, minced and homogenized using a tissue homogenizer. The fructose content was measured by a spectrophotometer using resorcinol reagent.[23]

Statistical analysis
Raw data collection and analysis was carried out by a researcher who was blinded to the grouping of the animals. Results are reported as mean ± s.e. The treated groups were compared with control by analysis of variance following Dunnet's test. Significance level was set at $P < 0.05$ and confidence level at 95%. Statistical analysis was carried out using Instat v 2.1 (Graphpad Software Inc., La Jolla, CA, USA). A regression analysis of prostate weight, sperm count and mount, intromission and ejaculation frequencies vs serum T level was performed to determine the dependence between various parameters on T level.

Results

Effect of treatment on b.w. and blood glucose levels
Hyperglycemia was confirmed in rats of all the groups 96 h past STZ injection (glucose content > 250 mg dl^{-1}). On day 28 of experiment a significantly considerable decrease in blood glucose level was observed in CO-treated groups. In CO100-treated group the blood glucose level was 119.12 ± 4.18 mg dl^{-1}, whereas it was 117.63 ± 2.81 mg dl^{-1} in CO200 group. In STZ + Gib600 treated it was found to be 119.61 ± 2.87, whereas in STZ control the

Table 1 Effect of various treatments on body/organ weights and blood glucose levels

Group	*Weight of animal (g)*		*Weight of testes (g)*		*Weight of prostate (mg)*		*Blood glucose level in mg dl^{-1} (96 h after STZ i.p.)*[a]	
	0 Days	*28 Days*	*0 Days*	*28 Days*	*0 Days*	*28 Days*	*0 Day*	*28 Days*
Control	221.5 ± 0.84	224.1 ± 0.84**	0.86 ± 0.01	0.88 ± 0.01*	95.1 ± 1.5	96.6 ± 1.4**	105.0 ± 2.1	106.2 ± 4.3**
STZ_{50}	220.1 ± 0.61	192.6 ± 0.82	0.84 ± 0.1	0.70 ± 0.08	96.5 ± 1.2	78.1 ± 2.1	229.1 ± 1.2	233.15 ± 3.01
STZ_{50} + CO100	223.2 ± 0.8	208.1 ± 1.9**	0.84 ± 0.01	0.77 ± 0.02	95.4 ± 0.6	89.1 ± 1.7**	237.0 ± 1.12	119.12 ± 4.18**
STZ_{50} + CO200	222.1 ± 0.13	213.1 ± 0.2**	0.85 ± 0.02	0.88 ± 0.03*	97.1 ± 1.1	98.1 ± 1.1**	238.0 ± 1.1	117.63 ± 2.81**
STZ_{50} + $Glib_{600}$	221.9 ± 0.78	197.7 ± 1.94*	0.85 ± 0.03	0.79 ± 0.05	97.4 ± 1.6	84.1 ± 0.8*	236.0 ± 1.4	119.61 ± 2.87**
STZ_{50} + Sil_5	212.16 ± 0.67	196.4 ± 0.18*	0.86 ± 0.03	0.78 ± 0.03	96.5 ± 1.1	82.1 ± 1.4	256.0 ± 3.1	229.0 ± 4.5
F value		133.62		2.59		29.85		263.81

Control: no treatment administered vehicle only.
STZ_{50}: 50 mg kg^{-1} b.w. streptozotocin i.p. once.
STZ_{50} + CO100: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 100 mg kg^{-1} b.w. of *Curculigo orchioides* extract orally.
STZ_{50} + CO200: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 200 mg kg^{-1} b.w. of *C. orchioides* extract orally.
STZ_{50} + $Glib_{600}$: 50 mg kg^{-1} b.w. streptozotocin i.p. once and glibenclamide 600 μg kg^{-1} b.w. i.p.
STZ_{50} + Sil_5: 50 mg kg^{-1} b.w. streptozotocin i.p. once and sildenafil citrate 5 mg kg^{-1} b.w. i.p.
*P<0.05, **P<0.01.
*,**refers to significance calculated after one-way analysis of variance followed by Dunnet's test, all the groups in the same column were compared to (hyperglycemic control) STZ_{50} group.

blood glucose content was 233.15 ± 3.01 mg dl^{-1}. Sildenafil treatment had no effect on blood glucose levels (Table 1).

The effects of extract on the body and sexual organ weight have been shown in Table 1. Compared with a loss of nearly 28 g in hyperglycemic control group, there was a significant amelioration of b.w. loss by CO100 (~15 g P<0.05) and CO200 (~9 g P<0.01). In contrast no significant amelioration of b.w. loss was observed in case of glibenclamide and sildenafil treatment.

Diabetes resulted in a reduced serum T levels as evidenced by the loss of testicular weights and subsequently the serum T level in male rats. The data has been presented as Figure 1. In case of STZ group serum T level (ng ml^{-1}) was found to be 5.6 ± 0.6, it was 9.8 ± 0.6 ng ml^{-1} (P<0.05) and 10.7 ± 0.7 (P<0.05) in case of CO100 and CO200, respectively. A nonsignificant improvement in serum T level was observed in glibenclamide and sildenafil-treated groups 6.9 ± 1.2 and 5.9 ± 1.1, respectively. Serum T level was 10.2 ± 1.6 in case of untreated control group animals.

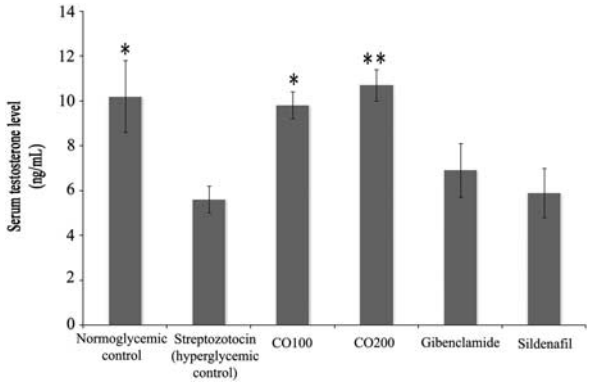

Figure 1 Determination of the serum testosterone levels (ng ml^{-1}) with various treatments. Normoglycemic control: administered vehicle only. Streptozotocin: 50 mg kg^{-1} b.w. streptozotocin i.p. once (hyperglycemic control). CO100: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 100 mg kg^{-1} b.w. of *Curculigo orchioides* extract p.o., daily (CO100). CO200: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 200 mg kg^{-1} b.w. of p.o., daily (CO200). Gibenclamide: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 600 μg kg^{-1} b.w. of Gibenclamide i.p (positive control for hyperglycemia) daily. Sildenafil: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 5 mg kg^{-1} b.w. of sildenafil citrate i.p (positive control) daily. *P<0.05, **P<0.001 compared with hyperglycemic control.

Effect on sexual behavior

Hesitation time was increased to 520 ± 12 s in case of diabetic rats. It was 330 ± 8 s in case of control group. The hesitation time was reduced by ~65% (P<0.05) in case of CO100 and ~74% (P<0.01) in case of CO200-treated group (Table 2). For glibenclamide and sildenafil-treated group the hesitation time was found to be 401 ± 5 and 464 ± 6 s (P>0.05). A comparison of CO200 with sildenafil and glibenclamide-treated group showed a significant reduction in hesitation time of CO200 (P<0.05). CO extract (200 mg kg^{-1}) alone is also effective in lowering the hesitation time in normal rats as well and the data has been reported previously by Thakur *et al*.[5]

The mount, intromission and ejaculatory frequencies were considerably reduced in hyperglycemic rats (STZ50) in comparison with the normoglycemic control group (P<0.01). In diabetic animals treated with CO, a dose-dependent normalization of sexual behavior compared with the STZ50 group was observed. For CO200 group the mount (12.1 ± 2.4),

Table 2 Effect of various treatments on sexual behavior, sperm count and seminal fructose content in male rats after 28 days of treatment

Parameters	*Treated groups*						
	Control	STZ_{50}	$STZ_{50}+CO100$	$STZ_{50}+CO200$	$STZ_{50}+Glib_{600}$	$STZ_{50}+Sil_5$	F *value*
Mount latency (ML) (mean time ± s.e. in s)	169.9 ± 12.6*	271.2 ± 11.2	141.4 ± 3.1**	128.1 ± 3.2**	242.2 ± 8.2	277.1 ± 5.6	66.02
Intromission latency (IL) (mean time ± s.e. in s)	313.2 ± 10.8*	412.6 ± 7.4	281.3 ± 5.9**	262.7 ± 6.4**	404.2 ± 7.1	409.1 ± 10.8	69.51
Post ejaculatory latency (PEL) (mean time ± s.e. in s)	496.7 ± 5.8**	761.0 ± 23	470.9 ± 14.3**	426.6 ± 7.4**	673 ± 21.0*	686 ± 12.0*	80.80
Hesitation time (mean time ± s.e. in s)	330 ± 8**	520 ± 12	181 ± 6**	138 ± 7**	401 ± 5	464 ± 6	382.47
Intromission frequency (IF)	6.2 ± 0.1**	2.1 ± 0.4	6.8 ± 0.8**	7.2 ± 1.0**	3.4 ± 0.3	3.6 ± 1.2	8.03
Ejaculation frequency (EF)	3.1 ± 0.9*	0.2 ± 0.01	3.5 ± 0.8**	3.9 ± 1.1**	2.1 ± 0.02*	2.8 ± 0.01*	3.96
Mount frequency (MF)	15.6 ± 3.9*	4.8 ± 2.9	15.8 ± 0.6**	12.1 ± 2.4*	7.3 ± 1.4	8.0 ± 0.7	3.93
Penile erection index (PI)	21.2 ± 1.1**	6.2 ± 1.6	36.1 ± 2.8**	38.2 ± 1.4**	10.2 ± 1.8	31.9 ± 1.6*	57.34
Epididymal sperm count ($\times 10^6$)	110.1 ± 2.6**	75.2 ± 3.7	112.2 ± 4.5**	113.3 ± 3.4**	84.3 ± 2.2	79.2 ± 5.4	22.43

Control: no treatment administered vehicle only.
STZ_{50}: 50 mg kg^{-1} b.w. streptozotocin i.p. once.
$STZ_{50}+CO100$: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 100 mg kg^{-1} b.w. of *Curculigo orchioides* extract orally.
$STZ_{50}+CO200$: 50 mg kg^{-1} b.w. streptozotocin i.p. once and 200 mg kg^{-1} b.w. of *C. orchioides* extract orally.
$STZ_{50}+Glib_{600}$: 50 mg kg^{-1} b.w. streptozotocin i.p. once and glibenclamide 600 μg kg^{-1} b.w. i.p.
$STZ_{50}+Sil_5$: 50 mg kg^{-1} b.w. streptozotocin i.p. once and sildenafil citrate 5 mg kg^{-1} b.w. i.p.
*$P<0.05$, **$P<0.01$.
*,** refers to significance calculated after one-way analysis of variance followed by Dunnet's test, all the groups in the same row were compared with (hyperglycemic control) STZ_{50} group.

intromission (7.2 ± 1.0) and ejaculatory frequencies (3.9 ± 1.1) were marginally lower or equal to in normo-glycemic control animals. Glibenclamide treatment was the next to follow showing a nonsignificant improvement of intromission frequency (3.4 ± 0.3) and a significant improvement in ejaculation frequency (2.1 ± 0.02) akin to sildenafil treatment, which resulted in an improvement of ejaculation and intromission frequency. For sildenafil-treated group the effects observed in case of mount frequency were nonsignificant (Table 2). A statistical comparison of CO100 and CO200 vs sildenafil-treated group for ejaculation frequency did not show a significant variation ($P>0.05$).

A significant increase in mount, intromission and post-ejaculatory latency was observed in case of STZ50 group compared with normal control group animals. The mount and intromission latency were significantly reduced ($P<0.01$) in CO100 and CO200 group, whereas no significant improvement was observed in case of glibenclamide and sildenafil-treated groups. In case of post-ejaculatory latency, a significant improvement was observed in CO100, CO200 ($P<0.01$) and sildenafil- and glibenclamide-treated groups ($P<0.05$; Table 2).

An evaluation of PI demonstrated that erectile function was markedly reduced in diabetic STZ group (6.2 ± 1.6), which was significantly lower than a normal value of 21.2 ± 1.1 ($P<0.01$ compared with STZ50) in control group animals. Treatment with glibenclamide resulted in a nonsignificant improvement in PI (10.2 ± 1.8). Contrastingly the values for PI were 36.1 ± 2.8 ($P<0.01$) and 38.2 ± 1.4 ($P<0.01$) in case of CO100 and CO200-treated rats. Treatment with sildenafil was also significantly effective, and the PI value was 31.9 ± 1.6 ($P<0.01$; Table 2).

Semen parameters and sperm count

The epididymal sperm count was found to be 110.1 ± 2.6 million ($P<0.05$) in case of normoglycemic control group. Sustained hyperglycemia resulted in a decrease in epididymal sperm count in hyperglycemic control group (STZ) rats (75.2 ± 3.7 million). The values were found to be 112.2 ± 4.5 million ($P<0.05$) in case of CO100 and 113.3 ± 3.4 million ($P<0.01$) in case of CO200-treated animals. Glibenclamide and sildenafil treatment resulted in a sperm count of 84.3 ± 2.2 million and 79.2 ± 5.4 million, respectively (Table 2).

The data for seminal fructose content also followed the same pattern as in case of epididymal sperm count. Normoglycemic rats (control group) had a seminal fructose level of 0.7 ± 0.02 mg g^{-1} weight of seminal vesicles (mg g^{-1}), which was reduced by >50% in case of STZ (0.3 ± 0.04 mg g^{-1}), the value for sildenafil-treated group was similar to STZ and was found to be 0.3 ± 0.02 mg g^{-1}. Contrastingly improved values for seminal fructose content were observed in CO100, CO200 and glibenclamide-treated groups, which showed a value of 0.5 ± 0.02, 0.5 ± 0.01 and 0.6 ± 0.04 mg g^{-1}, respectively. Although the values observed in case of CO100, CO200 and glibenclamide-treated groups were significantly higher than STZ group no significant improvement was observed in sildenafil-treated group.

Furthermore a regression analysis of various parameters (*viz.* prostate weight, sperm count and mount, intromission and ejaculation frequencies) vs serum T level was also carried out. The analysis suggested a strong correlation of T with sperm count and intromission frequency ($r^2 > 0.96$), while a dependence of prostate weight and ejaculation frequencies were also established ($r^2 > 0.88$). Although no dependence between serum T level and seminal fructose content could be established.

Discussion

Sustained hyperglycemia or diabetes has been considered as a major cause of sexual dysfunction in male population globally.[24] Degeneration of testicular functions as well as reduced serum T levels is one of the leading harmful effects of diabetes. One of the mechanisms underlying this is oxidative damage by reactive oxygen species.[25] Although hypoglycemic agents such as glibenclamide, glipizide may assist in better management of diabetic condition and as a consequence ameliorate the sexual dysfunction in part, still the improvement is only marginal. Reports on clinical as well as experimental studies clearly demonstrate that in case of sexual dysfunction associated with diabetic condition the treatment with hypoglycemic agent alone were not successful in improving the sexual performance or libido.[25,26] It has been implicated that hyperglycemia results in nearly 50% reduction of serum T and 30–45% reduction in sperm count in rats.[27–29] This was also confirmed in the presently reported study. The study confirmed that treatment with glibenclamide alone did not have prominent effect on semen parameters and T level. Furthermore, an improved but statistically nonsignificant effect was observed for ejaculation frequency with glibenclamide treatment, thus suggesting that lowering of blood glucose level can only marginally affect sexual performance in diabetic subjects.

In this study it was also demonstrated that the anabolic effect of CO is exhibited in a dose-dependent manner. This can be a contributing factor in amelioration of b.w. loss associated with hyperglycemia.[6] No such effect was observed in case of glibenclamide or sildenafil treatment. The difference in secondary sexual organ weights could mainly be contributed to the gain in b.w., which may be attributed to the presence of anabolic steroids and steroidal saponins present in the CO extract.

Sildenafil treatment was effective in improving PE, ejaculation frequency and reducing the post-ejaculatory latency but no significant effect on anabolic and other behavior parameters was evidenced, thus, implying that phosphodiesterase inhibitor does not have a prominent effect on sexual behavior parameters like mounting or intromission in general. They are mostly effective in improving erectile function.

Diabetic rats had a low ejaculatory/intromission frequency, high mount/post-ejaculatory latency and PI which was in agreement with the earlier reports that diabetes is associated with reduction in male copulatory behavior.[12,18] Although it is reported that STZ treatment reduced plasma T levels in rats, this factor alone does not appear responsible for changes in copulatory behavior as T replacement did not reverse the adverse effects of diabetes on sexual behavior.[26] Although STZ-induced copulatory dysfunction might be due to reduced T responsiveness, copulatory dysfunction in the diabetic state is also a result of direct or indirect action of insulin and/or glucose on the adrenergic complex.[26,29] STZ-treated rats were hesitant and appeared to be disinterested in sexual activity for nearly 8 min as evidenced by their hesitation time. STZ group rats appeared stressed and anxious without any sufficient sexual motivation, and this could be a reason for the low sexual behavior parameters observed in this study. The ability of CO extract to restore the sexual function, therefore, could be hypothesized to the improvement of spermatogenesis via steroid synthesis. It may also be a possibility that administration of CO is effective in ensuring the better availability of hormone to gonads.[6] Previous reports on the ability of CO in reducing glucose levels, along with its anabolic and antioxidant potential[30–32] could provide some insight into the possible mechanism behind the restoration of normal copulatory rate in diabetic animals. In concordance it can be implicated that the multifaceted effect of CO extract acting at different levels of endocrine system accompanied by prevention and amelioration of oxidative damage may be hypothesized as a major contributor to the observed effects. In the light of above mentioned evidences, it can be stated that the plant CO holds promise for effective management of diabetes-induced sexual dysfunction and our study also provides some evidence for the folkloric claim of using CO in diabetes-induced sexual and ED.

It is worth mentioning that there are a few limitations of this study, and therefore needs caution before extrapolation to clinical situation. First, a clinical trial with a larger human population needs to be carried out to validate the clinical potential of CO in sexual dysfunction.[3] Second, it would be fruitful to evaluate the effects of CO on *in vivo* glucose metabolism in detail and its correlation with the steroid biogenesis.[5,6] Further studies on genetically modified rats mimicking human diabetic conditions may be worth a trial to get a better insight into the possible mechanism by which CO ameliorates and improve sexual and reproductive parameters in hyperglycemic conditions. Effects on libido and sexual motivation may also be well determined only if the studies are carried out on a larger scale at clinical level.

Conflict of interest

The authors declare no conflict of interest.

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