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# Effect of Hydroalcoholic Extract of *Chlorophytum borivilianum* Tubers in Alleviating the Diabetic Impotency in Streptozotocin Induced Male Diabetic Rats

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## ABSTRACT

The prevalence of diabetes mellitus (DM) has continued to grow over the past decade, and it is an important cause of organic impotence. In the present study we investigated the effect of hydroalcoholic extract of tubers of *Chlorophytum borivilianum* (CB) in alleviating the diabetic impotency in rats. Male wistar albino rats were classified into two groups as normal control and diabetic group. Normal control group rats were injected with 0.1 M citrate phosphate buffer (0.1 ml, i.p) while in diabetic group, DM was induced by intraperitoneal injection of streptozotocin (STZ; 60 mg/kg) freshly dissolved in 0.1 M citrate phosphate buffer (pH 4.5). On confirming the diabetic state, the rats in diabetic group were individually tested for sexual potency by pairing them with pro-oestrus female rat. Diabetic rats showed a significant decline in the sexual potency as compared to the normal control groups. Following this, the diabetic rats were divided into four groups and treated orally as: diabetic control group with distilled water 2 ml/day, CB-100 group with CB 100 mg/kg/day, CB-300 group with CB 300 mg/kg/day and standard group with sildenafil citrate 4 mg/kg/day for 14 days. Their sexual behavior was evaluated on 15$^{th}$ day by pairing with a pro-estrous female rat and evaluated for CB, at 100 mg/kg, for sexual vigor and sexual arousal as compared to diabetic control rats. The study revealed dose dependent improvement in all the parameters of sexual behavior were enhanced against diabetic control group, reflecting increased potency.

**Keywords:** *Chlorophytum borivilianum*; diabetes mellitus; Impotency; streptozotocin

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease that has deleterious effects on male reproductive function, possibly through an increase in oxidative stress and reduction of endogenous antioxidants (1, 2). Antioxidants have been shown to reduce the risk of diabetes onset, improve glucose disposal and improve some of the associated complications (2). The prevalence of DM has continued to grow over the past decade, and the disease increases the risk of erectile dysfunction (ED) (3). Patients with DM were 3 times more likely to develop ED than those who did not have DM. The prevalence for ED in these patients was as high as 75% (4). Studies also found that, harms cavernosal innervation and endothelial function, both of which are important for erectile function, along with decrease in nitric oxide production (5).

*Chlorophytum borivilianum* San. F. (Liliaceae) is a traditional perennial herbaceous medicinal plant commonly known as safed musli (6). Tribals in India have used safed musli since ages for enhancing their virility (7). It contains proteins (8-9%), carbohydrates (41%), root fibers (4%),

saponins (2- 17%), minerals and vitamins. Saponin is the chief medicinal compound present in the roots. Moreover Saponins and alkaloids present in the plant are the primary source of its significant medicinal properties (6). It is scientifically documented for its antistress (8), antimicrobial (9), analgesic (10), anti-inflammatory (11) and immunomodulatory activities (12). Also, it has been traditionally acclaimed and advocated for its aphrodisiac activity (13, 14). However, its role in diabetic impotency has not been studied. In light of this, the present investigation was carried out to study the effect of hydroalcoholic extract of *Chlorophytum borivilianum* in alleviating the diabetic impotency in streptozotocin induced male diabetic rats since STZ is known to induce changes in sexual function through its ability to induce DM (15).

## MATERIALS AND METHODS

### Plant material

The hydroalcoholic extract of tubers of *Chlorophytum borivilianum* was received as a gift sample (SME/8006) from Green Chem, Bangalore, India.

### Chemicals and drugs

Sildenafil citrate (Viagra®) tablet, ketamine injection (Hypnoket), Sesame oil and propylene glycol were purchased from the local market. Streptozotocin was purchased from Vijay Chemicals, Pune. Estradiol benzoate and Progesterone AR grade were purchased from the Rajesh Chemicals, Mumbai.

### Animals

Healthy male and female wistar rats (150-200g) and male swiss albino mice (18-22 g) were obtained from Yash Farms, Pune and were housed in CPCSEA approved animal house in groups of six in polypropylene cages. They were maintained at 25 ± 2° C, relative humidity of 45 to 55% and under standard environmental conditions (12 hrs light 12 hrs dark cycle). The animals had free access to food (Chakan Oil Mills, Pune, India) and water *ad libitum*. All the procedures were performed in accordance with the Institutional Ethical Committee constituted as per the directions of the CPCSEA (CPCSEA/IAEC/PC-05/07-2K8). The study was carried out between 9.00 am and 4.00 pm.

### Acute toxicity test

Acute toxicity study was performed in healthy adult male albino mice (18-22 g) as per guidelines (AOT 425) suggested by the Organization for Economical Co-operation and Development. The mice were observed continuously for 2 h for behavioral and autonomic profiles and for any other sign of toxicity or mortality up to a period of seven days.

### Surgery

All female rats were ovariectomised (OVX) 30 days prior to testing using standard aseptic surgical techniques and under deep anesthesia, induced by intraperitonial administration of 100 mg/kg ketamine, in a volume of 0.1ml/kg. All females received at least one week of postoperative care prior to initiation of experiment.

### Induction of behavioral estrus

For induction of behavioral estrus, OVX female rats were subcutaneously (SC) administered with 25 µg estradiol benzoate (EB; in 0.1 ml sesame oil) 48 h prior to behavioral testing and 500 µg of progesterone (P; in 0.1 ml propylene glycol) 5 h before testing (16).

### Selection of male rats for inclusion in the study

To make sexually experienced, male rats were given 4 training sessions (twice a week for 2 weeks) with receptive females for the period of 30 min. Only males displaying at least 2 ejaculations during the 4 training test sessions were included in the study (17).

### Statistical analysis

The results are expressed as mean ± SEM. Comparison between the groups were made by one way analysis of variance (ANOVA) followed by Dunnett's 't' test.

## EXPERIMENTAL PROTOCOL

All male rats were injected with STZ freshly dissolved in 0.1 M citrate phosphate buffer (pH 4.5) except 6 rats of normal control group which received equal volume of 0.1 M citrate phosphate buffer. In rats injected with STZ, the diabetic state was verified by collecting blood samples retro-orbitally 3 days after injection and estimating blood glucose levels. After two weeks, these rats were individually paired with pro-estrus female rats and were observed for sexual behavioral parameters like mount latency (ML), intromission latency (IL), ejaculation latency (EL), mount frequency (MF), intromission frequency (IF), post ejaculatory interval (PEI) and hit rate (HR) for 30 min. After this, the rats in diabetic group were subdivided into four groups (n=6) and treated orally for 14 days as: diabetic control (DM) group received distilled water 2 ml/day; CB-100 group received CB-100 mg/kg/day;

CB-300 group received CB-300 mg/kg/day and standard group received sildenafil citrate 4 mg/kg/day. At the end of respective treatments, the male rats were individually placed in the copulatory arena. They were given 10 min adaptation periods, after which an OVX estrous female rat was placed in the arena and the male rats were observed for aforementioned sexual behavior parameters either until ejaculation or a maximum for 30 min.

## RESULTS

### Acute oral toxicity test

All mice were free of any toxicity as per acceptable range given by the OECD guidelines up to the dose of 2000 mg/kg. From this data and pilot study reports; two different doses 100 and 300 mg/kg were selected for further study.

To confirm the onset of diabetes, blood samples were collected retroorbitally 3 days after induction of DM by STZ. Serum glucose levels were significantly higher in the DM group (220.69±0.4223 mg/dl) as compared with controls (85.56±0.7017 mg/dl).

### Effect of DM on sexual behavior

DM affects adversely and significantly all parameters of sexual behavior (Table 1). Comparing rats of diabetic group with those in control group, it was found that DM reduced the percentage of rats that could achieve mounting (79.16 versus 100%), reduced the average number of mounts (10.86 versus 23 in control), and significantly prolonged the mount latency (3.64 ± 0.098 versus 0.99 ± 0.093 minutes). The percentage of rats that achieved intromission and the average number of intromissions were less in the DM group (62.5% and 7.6 versus 100% and 19.16 in control, respectively). The intromission latency was significantly prolonged by DM (6.5 ± 0.11 versus 2.23 ± 0.21 in control). Similarly, the percentage of rats that achieved ejaculation and their latency values were adversely and significantly affected by DM (Table 1). Accordingly, the hit rate was significantly reduced in the DM group compared with the control group (0.695 ± 0.014 versus 0.820 ± 0.011).

**Table I: Effect of diabetes mellitus on sexual behavior of the adult male rat**

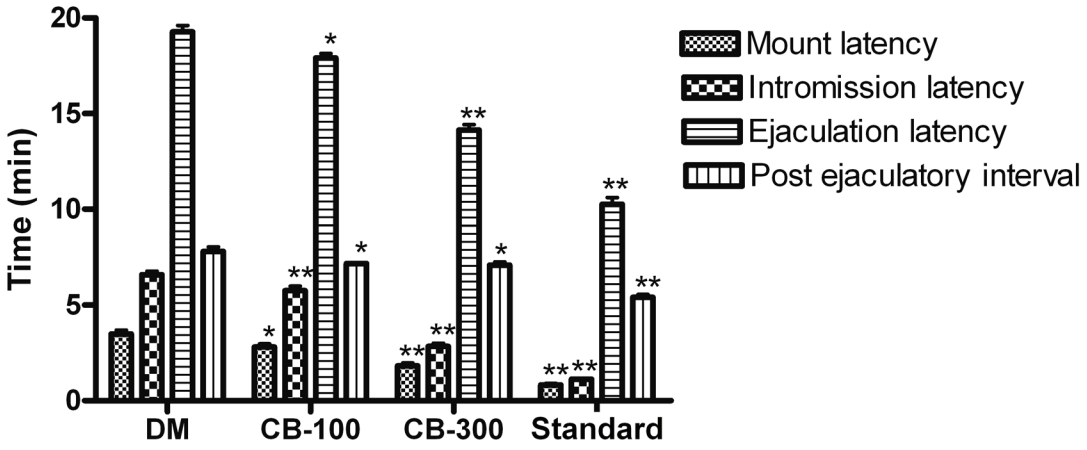
| Parameter | Control (n = 6) | Diabetic (n = 24) |
|---|---|---|
| Mount | | |
| No | 6 (100%) | 19 (79.16%) |
| Latency (min) | 0.99 ± 0.093 | 3.64 ± 0.098** |
| Intromission | | |
| No | 6 (100%) | 15 (62.5%) |
| Latency (min) | 2.23 ± 0.21 | 6.5 ± 0.11** |
| Ejaculation | | |
| No | 6 (100%) | 9 (37.5%) |
| Latency (min) | 4.85 ± 0.45 | 19.07± 0.25** |
| Post ejaculatory interval (min) | 7.24 ± 0.042 | 7.74 ± 0.173* |
| Hit rate | 0.820 ± 0.011 | 0.695 ± 0.014** |

*# The mating test continued for 30 min. Data was analyzed by ANOVA followed by dunnett's 't' test. Values are expressed as mean ± SEM.*
**P<0.05,*
***P<0.01.*

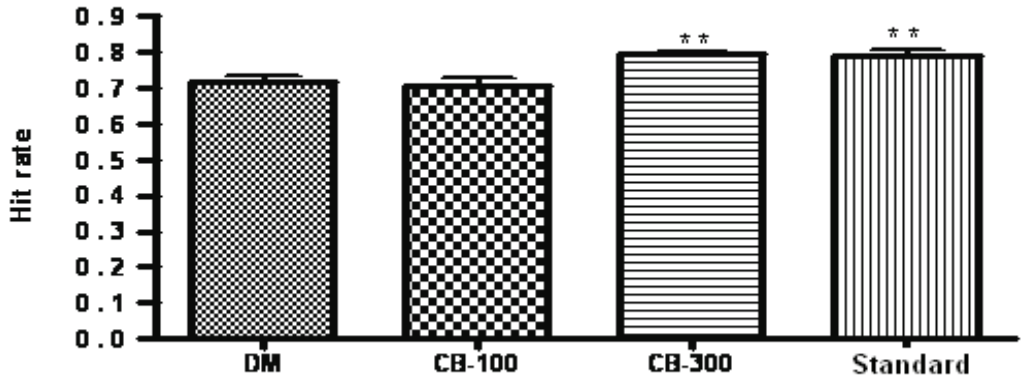
### Effect of drug treatment on diabetic impotency

*Mount latency (ML):*

ML was decreased significantly after 14 days of drug treatment in both the dose groups 100 mg/kg and 300




**"Figure 1:** Effect of CB extract and Sildenafil citrate, a reference standard on ML, IL, EL and PEI". n=6, Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's' test. *P<0.05, **P<0.01 versus DM




**"Figure 2:** Effect of CB extract and sildenafil citrate, a reference standard on hit rate". n=6, Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnetts 't' test. *P<0.05, **P<0.01 versus DM.

mg/kg of CB. Moreover, CB-300 mg/kg was found to be more effective (P<0.01) as compared to CB-100 mg/kg (P<0.05) (Fig. 1).

*Intromission latency (IL):*

The rats treated with CB-100 mg/kg and CB-300 mg/kg showed significant reduction in IL in comparison to standard group, which showed maximum decrease as compared to the diabetic control group. Moreover, CB-100 mg/kg and CB-300 mg/kg were equipotent in alleviating diabetic impotency (Fig. 1).

*Ejaculation latency (EL):*

EL was decreased in both, CB-100 mg/kg (p<0.05) and CB-300 mg/kg (p<0.01) treated rats as compared to the diabetic control rats, whereas standard group showed maximum decrease (Fig. 1).

*Post ejaculatory interval (PEI):*

CB at both the doses significantly (P<0.05) reduced the PEI, while standard group showed maximum reduction (P<0.01) as compared to that of the diabetic control group (Fig. 1).

*Hit rate (HR):*

Hit rate (the number of intromissions divided by the sum of mounts with or without intromissions) was significantly (P<0.01) increased by both CB-300 mg/kg and standard group as compared to diabetic control rats while CB-100 mg/kg was not significant in this regard (Fig. 2).

## DISCUSSION

Streptozotocin is a broad spectrum antibiotic derived from *Streptomyces achromogenes* and was shown to be a potent methylating agent reacting with DNA to form methylated purines. Significant methylation of DNA of β cells of the pancreas causing their destruction is mechanism responsible for the diabetogenic effect of STZ (18). In diabetes, chronic hyperglycemia, directly or indirectly due to associated risk factors, may participate in the development of erectile dysfunction (19). Although the nature of diabetic impotence is multifactorial, several studies have reported the incidence of vascular erectile dysfunction to be as high as 87% (20).

Although erectile dysfunction is a widely investigated phenomenon, no absolute standard of measurement of potency is available in animals or man (21). However, STZ treated rats have been reported to be a relevant model to study the effect of DM on sexual dysfunction (22) and mating tests. Its parameters have been accepted as a standard methodology used to interpret the results too (21).

ML and IL are indices of sexual motivation (libido) and sexual arousal (14). In our study, DM adversely and significantly affected the percentage and the values of ML and IL. Many experimental and clinical studies have revealed that libido was adversely affected in DM (23, 24). Our data showed agreement with these findings.

The ejaculation represents the motor culmination of a genital reflex of which the penile intromission (sensory), are the afferent component. So, EL may be looked on as an index of estimating changes in the threshold of

response of genital reflex (sensory) and of their level of excitability (25). In our study, 37.5% of diabetic animals achieved ejaculation compared with 100% of the control. Moreover, there is a statistically significant prolongation of ejaculatory latency of diabetic rats that did ejaculate when compared with the control value.

The hit rate is the ratio between number of true intromissions divided by the sum of true and false (mount without penile intromission) intromissions. The false intromission could be regarded as badly focused copulatory activities, and it seems to indicate a motor incoordination. Therefore, hit rate is the most direct measure of penile competence and could be used as a parameter to assess the motor pathway of peripheral neurogenic function (15). The hit rate was significantly lowered in diabetic rats compared with control. Therefore both the sensory (ejaculatory latency) and motor (hit rate) indicators of peripheral neurogenic states were adversely and significantly affected by DM, which indicate sensory and motor impairment in these animals.

*Chlolorophytum borivilianum* at both the doses significantly decreased the mount latency in addition with intromission and ejaculation latencies, whereas the sildenafil, reference standard being most effective compared with diabetic control group. PEI is the time period immediately after ejaculation during which sexual activity is minimal or negligible. It is indicative of sexual vigor (14). CB-100 mg/kg and CB-300 mg/kg significantly reduced the PEI and enhanced the sexual vigor. The standard drug was most effective in this regard. Hit rate was significantly increased by CB-300 mg/kg, while CB-100 mg/kg was not effective in this regard as compared to the diabetic control rats. Thus, CB-300 mg/kg effectively improved sensory motor impairments and increased potency in diabetic rats by reducing ejaculation latency and increasing hit rate.

Many researchers reported the deleterious effect of DM on male reproductive function, possibly through an increase in oxidative stress and reduction of endogenous antioxidants (1, 2). Since *Chlorophytum borivilianum* has been reported for its antioxidant activity (26), reduction in oxidative stress associated with free radicals and reactive oxygen species (ROS) could be its probable mechanism in alleviating impotency in diabetic rats. Fructans have been reported for their ability to alleviate diabetes by normalizing the blood glucose level and serve as source of energy. Therefore, the presence of fructans in the herb may have a major role to play in reducing glucose level in diabetic individuals (7). In a recent study, Chakraborthy and Aeri, 2008 reported the antidiabetic and antihyperlipidaemic activity of alcoholic extract of *Chlorophytum borivilianum* roots in alloxan induced diabetic albino rats. The mechanism of this antidiabetic activity was attributed to the potentiation of the insulin effect of plasma by increasing the secretion of insulin from the β- cells of islets of Langerhans (6). The improvement in diabetic state by aforementioned mechanisms could be the probable way by which CB had alleviated diabetic impotency. CB has shown antidiabetic effect towards both alloxan as well as STZ model, which have different mechanisms of induction of DM. This further suggest putative mechanism of action which may be due to more than one phytochemical acting synergistically. However further investigation is required to establish the exact mechanism of protective effect of *Chlorophytum borivilianum* in diabetic impotency.

## CONCLUSION

*Chlorophytum borivilianum* enhances the sexual arousal, vigor and libido in diabetic wistar rats and hence has a very promising future as a potent and a safe herbal supplement as aphrodisiac for diabetic patients.

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## REFERENCES

1. Amaral S., Moreno A.J., Santos M.S., Seic R. and Santos J.R. Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. *Theriogenology*. **66**: 2056–2067 (2006).
2. McCune L.M. and Johns T. Antioxidant activity relates to plant part, life form and growing condition in some diabetes remedies. *J Ethnopharmacol*. **112**: 461–469 (2007).
3. Demir T., Comlekci A., Demir O., Gulcu A., Caliskan S., Argun L., Secil M., Yesil S. and Esen A. A possible new risk factor in diabetic patients with erectile dysfunction: Homocysteinemia. *J Diab Compli*. **22**: 395–399 (2008).
4. Tamler R. Diabetes, Obesity and Erectile Dysfunction. *Gen Med*. **6**: 4–16 (2009).
5. Park K., Ryu K.S., Li W.J., Kim S.W. and Paick J.S. Chronic Treatment with a Type 5 Phosphodiesterase Inhibitor Suppresses Apoptosis of Corporal Smooth Muscle by Potentiating Akt Signalling in a Rat Model of Diabetic Erectile Dysfunction. *European Urol*. **53**: 1282–1289 (2008).
6. Chakraborthy G.S. and Aeri V. Antidiabetic And Antihyperlipidaemic Effect of Alcoholic extract of *Chlorophytum borivilianum* Roots in Alloxan Induced Diabetic Albino Rats. *J Pharm Res*. **1**(1): 29–33 (2008).
7. Thakur M. and Dixit V.K. A Review on Some Important Medicinal Plants of *Chlorophytum spp*. *Pharmacog Rev*. **2**(3): 169–172 (2008).
8. Gopalkrishna B., Akki and Patil S.H. Preliminary phytochemical investigation and in-vitro antistress activity of safed musli – *Chlorophytum borivilianum*. *Indiam Drugs*. **43**(11): 878–80 (2006).
9. Deore S.L. and Khadabadi S.S. In-vitro antimicrobial studies of *Chlorophytum borivilianum* (liliaceae) root extracts. *Asian. J. Microbiol. Biotech. Env. Sc*. **9**(4): 807–09 (2007).
10. Panda S.K., Sai S.C. and Bhatnagar S.P. Studies on hypoglycaemic and analgesic activities of *Chlorophytum borivilianum* Sant & Ferz. *J Natural Rem*. **7** (1): 31–6 (2007).

11. Deore S.L. and Khadabadi S.S. Antiinflammatory and antioxidant activity of *Chlorophytum borivilianum* root extracts. *Asian J Chem*. **20**(2): 983–86 (2008).
12. Thakur M., Bhargava S. and Dixit V.K. Immunomodulatory activity of *Chlorophytum borivilianum* Sant. F. *eCAM*. **4**(4): 419–23 (2007).
13. Thakur M. and Dixit V.K. Effect of *C.borivilianum* on androgenic and sexual behavior of male rats. *Indian Drugs*. **43**: 300–06 (2006).
14. Kenjale R., Shah R. and Sathaye S. Effect of *Chlorophytum borivilianum* on Sexual Behavior and Sperm count in male rats. *Phytother Res*. **22**: 796–801 (2008).
15. Hassan A.A., Hassouna M.M., Taketo T., Gagnon C. and Elhilali M.M. The effects of diabetes on sexual behavior and reproductive tract function in male rats. *J Urol*. **149**: 148–154 (1993).
16. Lopez H.H., Wurzel G. and Ragen B. The effect of acute bupropion on sexual motivation and behavior in the female rat. Pharmacol Biochem Behav. **87**: 368–379 (2007).
17. Rossler A.S., Pfaus J.G., Kia H.K., Bernabe J., Alexandre L. and Giuliano F. The melanocortin agonist, melanotan II, enhances proceptive sexual behaviors in the female rat. *Pharmacol Biochem Behav*. **85**: 514–521 (2006).
18. Bennett R.A. and Pegg A.E. Alkylation of DNA in rat tissues following administration of streptozotocin. *Cancer Res*. **41**: 2786–2790 (1981).
19. Marfella R., Esposito K., Giunta R., Coppola G., De Angelis L. and Farzati B. Circulating adhesion molecules in humans: Role of hyperglycemia and hyperinsulinemia. *Circulation*. **101**: 2247–2251 (2000).
20. Korenman S.G. New insights into erectile dysfunction: A practical approach. *American J Med*. **105**: 135–144 (1998).
21. Heaton J.P.W. and Varrin S.J. The impact of alcohol ingestion on erections in rats as measured by a novel bioassay. *J Urol*. **145**: 192–194 (1991).
22. Crowe R., Lincoln J., Blacklay P.F., Pryor J.P., Lumly J.S. and Burnstock G. Vasoactive intestinal polypeptide-like immunoreactive nerves in diabetic penis. A comparison between streptozotocin-treated rats and man. *Diabetes*. **32**: 1075–1077 (1983).
23. Steger R.W. and Kienasty S.G. Effect of continuous versus delayed insulin replacement on sex behavior and neuroendocrine function in diabetic male rats. *Diabetes*. **39**: 942–948 (1990).
24. Jensen S.B., Hagen C., Froland A., andPedersen P.B. Sexual function and pituitary axis in insulin treated diabetic men. *Acta Med Stand Suppl*. **624**: 65–68 (1979).
25. Elabbady A., Hassouna M. and Elhilai M. Apomorphine versus mating behavior in testing erectile capabilities of diabetic rats. *Urology*. **45**(4): 715–719 (1995).
26. Oudhia P. Problems perceived by Safed Moosli (*Chlorophytum borivilianum*) growers of Chattisgarh (India) region: A study, *Journal of Medicinal and Aromatic. Plants Sciences*22/4A & 23/1A, 396–399. (2001)