BASIC SCIENCE

Antioxidant Potential and Ability of Phloroglucinol to Decrease Formation of Advanced Glycation End Products Increase Efficacy of Sildenafil in Diabetes-Induced Sexual Dysfunction of Rats

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ABSTRACT

Introduction: Diabetes-induced sexual dysfunction is associated with an increase in oxidative stress. Scavengers of reactive oxygen species (ROS) have been shown to reduce oxidative stress and aid in the management of sexual dysfunction in diabetes.

Aim: The aim of the study was to test the hypothesis that antioxidant, which scavenge ROS and reduce formation of advanced glycation end products (AGEs), can potentiate efficacy of phosphodiesterase type 5 inhibitors in diabetes-induced sexual dysfunction that is associated with oxidative stress.

Materials and Methods: Effect of phloroglucinol and sildenafil on serum glucose level, sexual function, penile smooth muscle : collagen ratio, and phenylephrine precontracted corpus cavernosum smooth muscle (CCSM) was studied. The ability of phloroglucinol to reduce the formation of AGEs and its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) was also evaluated.

Main Outcome Measures: Antioxidant potential of phloroglucinol was studied in addition to its effect on diabetes-induced sexual dysfunction in presence and absence of sildenafil.

Results: Phloroglucinol (50 mg/kg, p.o.) significantly decreased serum glucose level and increased sexual function in streptozotocin-induced diabetic rats when compared with diabetic control rats. Sildenafil (5 mg/kg, p.o.) had no effect on glycemia but significantly increased sexual function of diabetic rats. Coadministration of phloroglucinol increased the efficacy of sildenafil by improving sexual function. Treatment of diabetic rats with phloroglucinol + sildenafil maintained smooth muscle : collagen levels similar to that of normal rat penile tissue. Phloroglucinol decreased formation of AGEs and significantly scavenged DPPH radical activity in vitro. Sildenafil relaxed isolated CCSM of normal rat and diabetic rat significantly, but phloroglucinol did not show any significant effect. Phloroglucinol also inhibited human CYP3A4 enzyme activity in vitro.

Conclusion: Phloroglucinol coadministration increases efficacy of sildenafil in diabetes-induced sexual dysfunction. However, further studies are required to ascertain the benefits of phloroglucinol owing to its undesirable CYP3A4 inhibition activity.

Key Words: Phloroglucinol; Diabetes-Induced Erectile Dysfunction; Sildenafil; CYP3A4; AGEs; Antioxidant

Received June 3, 2015. Accepted September 15, 2015.

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http://dx.doi.org/10.1016/j.esxm.2015.12.002

INTRODUCTION

Diabetes mellitus is a metabolic disorder that is characterized by increased blood sugar levels (hyperglycemia). India is home to largest number of patients with diabetes in the world, and the number is expected to reach 87 million by 2030.[1] Type 1 diabetes is characterized by lack of insulin secretion from pancreas.[2] Diabetic complications include nephropathy, neuropathy, retinopathy, cardiovascular disease, and sexual dysfunction.[3,4] Sexual

dysfunction is an indirect indicator of cardiovascular disorder[5] and can lead to loss of self-esteem.[6]

Oxidative stress associated with hyperglycemia damages the penile smooth muscle and nerves that leads to sexual dysfunction.[7,8] Though, phosphodiesterase inhibitors such as sildenafil is preferred medicine for managing erectile dysfunction (ED), a male sexual dysfunction in diabetics, but it is reported not to be 100% efficacious.[9,10] Co-treatment of an antioxidant and sildenafil has been reported to increase efficacy of sildenafil.[7,11]

We evaluated the effect of co-treatment of phloroglucinol, an antioxidant and sildenafil on sexual function of streptozotocin-induced type 1 diabetic rats and compared it with the effect of phloroglucinol and sildenafil treatment under same physiological condition.[7,11,12] Effect of sildenafil and phloroglucinol on phenylephrine precontracted isolated rat corpus cavernosum smooth muscles (CCSMs) was studied to evaluate their erectogenic potential in normal rats.[11] Phloroglucinol (1,3,5-trihydroxybenzene), a polyphenol, is the monomeric building unit of phlorotannins, phenolic compounds known only from brown algae (*Phaeophyceae*).[13] Polyphenols are known to reduce oxidative stress by scavenging reactive oxygen species, chelating iron, and modulating various enzymes.[14] Increase in formation and accumulation of AGEs in corpus cavernosum in diabetes was linked to ED.[15] Therefore, we studied the effect of phloroglucinol on formation of AGEs in vitro.[16] Antioxidant potential of phloroglucinol was evaluated by studying its effect on scavenging of DPPH and nitric oxide (NO).[17] Effect of phloroglucinol was studied on activity of cytochrome P450 3A4 (CYP3A4), which metabolizes sildenafil to find out possible pholoroglucinol−sildenafil interaction.[18]

MATERIALS AND METHODS

Materials

Phloroglucinol and streptozotocin (STZ) were purchased from Sigma-Aldrich (St Louis, MO, USA), whereas glucose estimation kit was procured from Autospan (Surat, India). Human CYP3A4 substrate Benzyloxy-4-(trifluoromethyl) coumarin (BFC) was collected from ChemBridge Corporation (San Diego, CA, USA), whereas enzyme and BFC metabolite: 7-Hydroxy-4-(trifluoromethyl) coumarin (HFC) was from Corning Inc (Fremont, CA, USA). Other reagent and chemicals procured were of analytical grade.

Animals

Three-month-old adult male and female Wistar rats weighing about 175−225 g were used in the study. Thirty male rats and equal number of female rats were used for the study. The use of animals in these experiments was approved by Institutional Animal Ethical Committee of Al-Ameen College of Pharmacy. Experiments on rats were conducted in accordance with the *Committee for the Purpose of Control and Supervision of Experiments on Animals* guidelines. Rats were maintained under controlled temperature at 25 ± 2°C and relative humidity of 45−55% with an alternating 12 h light/dark cycle (light ON 6:00−18:00 h). The animals were reared and maintained with free access to food and water throughout the day.

Induction of Type 1 Diabetes and Drug Treatment

Streptozotocin was dissolved in ice-cold 0.1 M sodium citrate buffer just prior to use and injected intraperitoneally to male rats at a dose of 55 mg/kg.[7,11] Three days after administration of STZ, fasting serum glucose level of overnight-fasted rats was analyzed as per procedure mentioned in glucose estimation kit (Autospan, India) to confirm induction of diabetes.[11] This day was considered as day 0, following which drug treatment was started.

Phloroglucinol was dissolved in distilled water and administered daily to rats orally using per oral tube. The test solutions were freshly prepared every day before dosing the animals. Diabetic rats were administered with phloroglucinol for 4 weeks post-induction of diabetes. Male rats were divided into five groups as follows, each group containing six animals.

Group 1: Negative control, normal rats (water 4 mL/kg, p.o.)

Group 2: Positive control, diabetic rats (vehicle 4 mL/kg, p.o. for 4 weeks)

Group 3: Diabetic rats administered with phloroglucinol (50 mg/kg, p.o. for 4 weeks)

Group 4: Diabetic rats administered with sildenafil (5 mg/kg, p.o. for 4 weeks)

Group 5: Diabetic rats administered with phloroglucinol (50 mg/kg, p.o.) + sildenafil (5 mg/kg, p.o.) for 4 weeks

Though study started with six animals in each group, few animals were excluded from statistical calculation due to development of sexual dysfunction and inability of individual treatment to elevate sexual function in all animals.

Sexual Behavior Study of Rats

Before induction of diabetes, all the male rats were trained thrice for sexual activity in presence of ovariectomized estrous female rats in an observation chamber on different days. The male rats that did not perform intromission (vaginal penetration) within 10 minutes of introduction of female rats were removed from the study and those rats were replaced by sexually active male rats. Female rats were treated with diethylstilbestrol (1 mg/kg, p.o., administered 48 hours prior to sexual behavior study) and progesterone (5 mg/kg, s.c., administered 4 hours prior to the sexual behavior study) to induce artificial estrous phase. Female rats that did not exhibit lordosis posture were not paired with male rats for sexual behavior study.[19,20]

Sexual behavior study was performed in evening and night on day 0 and 28. The study was performed as per published literature. Briefly, after male rat accustoms to observation chamber (5−10 minutes), a female rat was introduced and following sexual behavior parameters were observed.[19,20]