Efficacy of ellagic acid and sildenafil in diabetes-induced sexual dysfunction

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ABSTRACT

Background: Diabetes induced sexual dysfunction is a leading cause of male sexual disorder and an early indicator of cardiovascular complication. Reactive oxygen species generated in body during diabetes is a main causative factor for erectile dysfunction, a sexual dysfunction. Adjuvant antioxidant therapy along with phosphodiesterases type 5 enzyme inhibitor (PDE5i) is more effective than PDE5i alone. **Objective:** The aim of the study was to investigate efficacy of ellagic acid a known antioxidant and sildenafil in diabetes induced erectile dysfunction. **Materials and Methods:** Type 1 diabetes was induced in male rats and rats were treated with ellagic acid (50 mg/kg, p.o.) and a combination of ellagic acid (50 mg/kg, p.o.) and sildenafil (5 mg/kg, p.o.), a PDE5i for 28 days. Sexual function was observed in diabetic rat and compared with those of treatment group and normal rats. Effect of ellagic acid was studied on *advanced glycation end products* (AGE) and isolated rat corpus cavernosum in vitro. **Results:** Sexual function of diabetic rats was found to be reduced and ellegic acid treatment could preserve sexual function of diabetic rats to some extent. Ellagic acid + sildenafil treatment was more efficient in management of diabetes induced sexual dysfunction. Ellagic acid inhibited (AGE) *in vitro* implying its role in reducing oxidative stress in diabetes. The polyphenol could not increase sexual function in normal rats and relax isolated rat corpus cavernosum smooth muscle significantly. **Conclusion:** The study proves usefulness of adjuvant antioxidant therapy in the management of erectile dysfunction in diabetes.

Key words: Diabetes, ellagic acid, sexual dysfunction, sildenafil, AGE, corpus cavernosum smooth muscle

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INTRODUCTION

Diabetes is a debilitating metabolic disorder that affects more than 100 million people worldwide. The disease affects many physiological systems, decreases quality of life and is responsible for development of cardiovascular complications in patients.[1]

Diabetic men are three times more susceptible to suffer from erectile dysfunction (ED) than non-diabetic men and 50-75% of such diabetic men reports some degree of ED. Severity of ED in diabetic men increases with the progression of diabetes. The main reason for development of sexual dysfunction is co-related to endothelial dysfunction that arises due to excessive production of reactive oxygen species (ROS).[2,3]

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ED, a type of male sexual disorder, is primarily treated by Phosphodiesterase 5 inhibitors (PDE5is) but the medicines are not 100% effective and failure of PDE5i (s) has been reported for diabetes-induced sexual dysfunction. Adjuvant therapy of an antioxidant with a PDE5i is reported to increase efficacy of PDE5i.[4]

Ellagic acid (EA), a dimeric derivative of gallic acid is found in numerous fruits and vegetables including different kind of berries, walnuts, peanuts, pomegranates, wolfberry and other plant foods in either its free form, as EA-glycosides, or bound as ellagitannins.[5] Ellagic acid was also reported to be present in extract of *Terminalia chebula* fruit.[6] It is believed to have antioxidant, antidiabetic, anti-inflammatory, anti-carcinogen and antimutagenic properties.[7,8] The objective of this experiment was to study the efficacy of ellagic acid and sildenafil in diabetes-induced sexual dysfunction. In addition, the effect of ellagic acid was studied on advanced glycation end-product (AGE) and isolated rat corpus cavernosum smooth muscles (CCSMs) from rat penile tissue *in vitro*.

MATERIALS AND METHODS

Chemicals

Streptozotocin/STZ (Sigma-Aldrich, Co., USA), ketamine hydrochloride (Neon laboratories Laboratories Limited, India), xylazine (Indian Immunologicals Limited, India), povidone-iodine solution (Cipla, India), catgut (absorbable surgical suture) suture of size 3-0 size with curved needle (Johnson and Johnson Limited, India), mersilk (non-absorbable surgical suture) suture of size 3-0 with curved needle (Johnson and Johnson Limited, India), diethyl stilbestrol (Penta Pharmaceuticals, India), progesterone (Sun Pharmaceutical Ind. Ltd, India), NaCl (Himedia, India), KCl (Rankem, India), KH_2PO_4 (Qualigen Fine Chemicals, India), $MgSO_4$ (Reachem Chemicals Private Limited, India), $CaCl_2$ (Qualigen Fine Chemicals, India), $NaHCO_3$ (SD Fine Chemicals Limited, India), Glucose (Himedia, India), phenylephrine (Sigma-Aldrich, Co., USA) and glucose estimation kit (Autospan, India) were procured. Ellagic acid and sildenafil were gift samples from Sami Labs and Watson Pharma. Pvt. Ltd, India respectively. All other chemicals and reagents used were of analytical grade.

Animals

Healthy male Wistar rats weighing about 175-225 g were used in the study. The use of animals in these experiments was authorized by institutional animal ethics committee (IAEC, reference number: AACP/P-49). All the animals were housed in an air-conditioned room at 24 ± 1°C with a 12 h light/dark cycle and allowed *ad libitum* access to water and standard pelleted diet.

Induction of type-1 diabetes

Streptozotocin (STZ) was dissolved in ice cold 0.1 M sodium citrate buffer just prior to use and injected at a dose of 55 mg/kg, i.p. to male rats.[9] 0.1 M sodium citrate buffer without STZ was used for control animals.

After 48 h of STZ administration, rats with fasting serum glucose more than 250 mg/dL were selected. Rats were divided into five groups as follows:

- Group I - normal control treated with vehicle (0.2% w/v dimethyl sulfoxide in water)
- Group II - diabetic control treated with vehicle
- Group III - diabetic rats treated with ellagic acid 50 mg/kg, p.o
- Group IV - diabetic rat treated with sildenafil 5 mg/kg, p.o
- Group V - diabetic rats dosed with ellagic acid 50 mg/kg + sildenafil 5 mg/kg, p.o.

Ellagic acid and sildenafil were dissolved in 0.2% w/v dimethyl sulfoxide and administered to diabetic rats orally using per oral tube daily for 30 days after confirmation of diabetes. The solutions were freshly prepared every day before dosing the animals.

Blood glucose level of rats

Blood glucose was monitored to estimate the efficacy of the treatments on the rats using an *in vitro* assay. The assay was based on the principle as described below.

Glucose oxidase (GOD) oxidises glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, hydrogen peroxide is coupled with phenol and 4-aminoantipyrine (4-AAP) to form a colored dye, quinoneimine. Absorbance of colored dye is measured at 505 nm and is directly proportional to glucose concentration of the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

Blood samples (100 μL) were collected in micro-centrifuge tube from retro orbital plexus of rats after 12 h of fasting. The samples were allowed to stand for 15 min and then centrifuged (10000 rpm for 10 min at 37°C) to separate serum from other blood components. The serum samples (10 μL) containing unknown quantity of glucose were collected in other micro-centrifuge tubes and 1000 μL of reagent 1 consisting up phosphate buffer, glucose oxidase, 4-AAP, phenol and peroxidase was added to each micro-centrifuge tubes. Similarly 10 μL of standard glucose solution (100 mg/dL, reagent 2) was added to 1000 μL of reagent 1 in a separate micro-centrifuge tube. The contents of the reaction were then incubated for 10 min at 37°C and absorbance was read at 505 nm using Artos Semi Auto Analyzer. Absorbance of a blank containing only reagent 1 was also studied.[10,11] The procedures were performed in triplicate.

Sexual function of diabetic rat

Ovariectomy of female rat

Ovariectomised female rats were used to reduce the usage of female rats in the study. Ovariectomy was performed as described by Khajuria *et al.*[12] Briefly, female rats were anesthetized by ketamine (75 mg/kg body weight) and xylazine (10 mg/kg body weight). The area below the second right breast (near to tail) was chosen for operation and hair was removed using a surgical scissor. Area to be operated was washed with chlorhexidine scrub and ethanol 70%, respectively. Povidone-iodine solution was then applied to the area to disinfect the skin. A 1.5 cm incision was performed on the skin transversally by a scalpel blade (number 11). Peritoneal and abdominal muscles were then accessed to make a 1 cm incision

using an iris scissor. Subsequently, right ovary and the associated fat was located and exteriorized by gentle pulling. Left ovary was also exteriorized through the same incision. The connection between the fallopian tube and the uterine horn was tied (by non absorbable suture), and cut to remove the ovary. Peritoneal cavity was stitched with absorbable suture and the skin was stitched using non-absorbable suture (size 3-0). Aseptic conditions were maintained throughout the operation and after the operation the animal were covered with paper in order to avoid hypothermia. The animals were kept individually in separate cages and the wounds were applied with Povidone-iodine solution twice a day. Tramadol at a dose 30 mg/kg was used orally as analgesic. The animals were placed on the cages containing filter paper as bedding which was changed every day and cages were cleaned with Dettol® (disinfectant) water every day for 10 days. After 10 days wounds were found to be healed, therefore, animals were again regrouped suitably.

Sexual behavior study

Sexual behavior study was conducted as per published literature with slight modification.[13,14] Briefly, the male rats were placed in a wooden cage (45 × 50 × 35 cm) having glass covering and illuminated by red light. The training was provided after 5 pm every day for 30 min. The rats were allowed to acclimatize to the environment for 10 min before a female rat was placed in the cage, following which sexual behavior was noted.

Mount Latency (ML) : Time from the introduction of female into the cage of the male up to the first mount.
Intromission Latency (IL): Time from the introduction of the female up to the first intromission by the male.
Ejaculatory Latency (EL): Time from the first intromission to the ejaculation.
Mount Frequency (MF) : Number of mounts per given period of time
Intromission Frequency (IF) : Number of intromission per given period of time
Post-ejaculatory Interval (PEI) : Time from end of first ejaculation to start of next intromission.

After training the male rats three times on different days, the male rats those failed to perform intromission within 5 min were considered as sexually inactive and were excluded from the study. Sexual activity of a male rat was observed in presence of a female rat in estrous phase. Estrous phase was induced in ovariectomised female rat by administering diethylstilbestrol (1 mg/kg, p.o.) 48 h prior, and progesterone (5 mg/kg, s.c.) 4 hour prior to the study. All male rats (group I: Normal rat treated with vehicle, group group II: Diabetic rats treated with vehicle, group III: Diabetic rats treated ellagic acid, group IV: Diabetic rats treated with sildenafil, group V: Diabetic rats treated with both ellagic acid and sildenafil) were treated once daily, p.o., for 30 days and change in sexual behavior was observed in

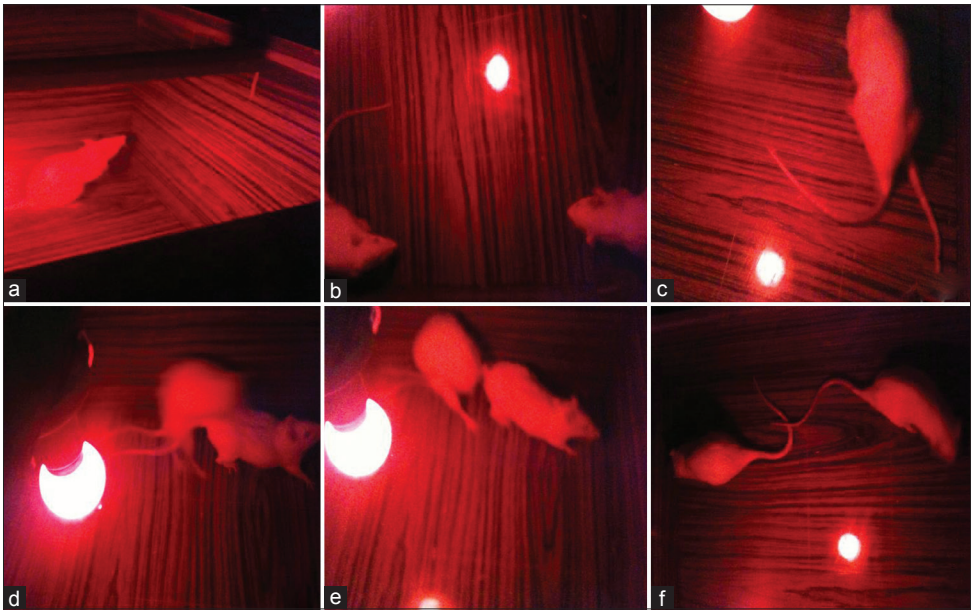

Figure 1: Different stages of male rat sexual behavior study. The study was performed in a wooden box with glass cover and illuminated with red light during evening hours. (a) Male rat accustoms to the new environment for 10 min. (b) After introduction of female rat, male rat approaches female rat. (c) Male rat mounts on female rat. (d) Female rat takes lordosis posture and male rat starts intromission. (e) Male rat autogrooms after intromission and performs intromission again. (f) After ejaculation, male rat takes rest before another sexual cycle (post-ejaculatory period)

Figure 2: Vaginal plug can be found in the vagina of female rat or on the floor of sexual behavior study chamber. It confirms ejaculation by male rats

normal as well as diabetic male rats on 0 (before dosing), 15th and 30th day. After observing the activity of a pair of animals for approximately 20 min, they were gently removed from the chamber. Urine, vaginal plug (coagulated sperm in vaginal) and feces were cleaned, if any. The chamber was then cleaned with methanol-soaked cotton to remove traces of urine smell as it might decrease sexual activity of next pair of rats [Figures 1 and 2].

Effect of ellagic acid on AGE

Assay was carried out as per the available literature.[15] Briefly, bovine serum albumin (l0 mg/mL) was incubated with glucose (500 mM) in phosphate buffered-saline (PBS) (5 mL, pH 7.4) and extract containing 0.02% sodium azide at 37°C. All the reagent and samples were sterilized by filtration through 0.2 μm membrane filters. The protein, the sugar and the prospective inhibitor were included in the mixture simultaneously. Aminoguanidine was used as an inhibitor (positive control). Reactions without any inhibitor were also set up for baseline values. Each solution was kept in the dark in a capped tube. After 7 days of incubation, fluorescence intensities (excitation wavelength of 370 nm and emission wave-length of 440 nm) were measured for different solutions. Percent inhibition was calculated as follows

$$\text{Inhibition } \% = \left[1 - \frac{(\text{As} - \text{Ab})}{(\text{Ac} - \text{Ab})}\right] \times 100$$

where, As = fluorescence of the incubated mixture with drug; Ac, Ab = the fluorescence of the incubated mixture with aminoguanidine as a positive control and the fluorescence of incubated mixture without sample as a blank control, respectively. The assay was performed in triplicate.

Effect of ellagic acid on isolated rat penile tissue

Corpus cavernosum smooth muscles/CCSMs (3 × 3 × 15 mm) were isolated from shaft of rat penile tissues under anesthesia (ketamine 60 mg/kg, xylazine 8 mg/kg; i.p.) and then rats were sacrificed by cervical dislocation. The CCSMs were washed in warm modified Krebs-Henseleit/KH solution (composition in mM: 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.5 $CaCl_2$, 25 $NaHCO_3$, 11 glucose) and tunica albuginea covering each CCSM was partially removed microsurgically. Each CCSM was mounted in a single channel of 4 channel organ bath (Panlab, Spain) between a steel hook at the bottom and force transducer (Model no: MLT0201; ADInstruments, Australia) at the top using two cotton threads. The tissues were maintained in KH solution at 37°C, aerated with carbogen gas (95% O_2 + 5% CO_2) and subjected to 500 mg of tension throughout the experiment. The tissues were washed every 15 min for 1 hour and contracted with 3 μM phenylephrine (a α1 adrenergic receptor agonist). Effects of different concentrations of ellagic acid (0.1-100 μg/mL) and sildenafil ((0.1-100 μg/mL) were observed in pre-contracted CCSMs in the interval of 6 minutes. The effects were monitored with PowerLab/8SP data acquisition system (Chart software, version 7.0; ADInstruments, Australia).[6,16]

Statistical significance

Statistical significance for sexual behavior study was evaluated using one-way ANOVA followed by Tukey's multiple comparison test of pairs. Effects of ellagic acid and sildenafil on isolated rat CCSMs were calculated using one-way ANOVA followed by Dunnett's test using Graphpad Prism (version 6). Effect of ellagic acid and aminoguanidine on AGE was expressed as mean ± standard deviation (MSD) of three readings.

RESULTS

Ellagic acid at 50 mg/kg was found to have anti-diabetic effect and increased sexual function of diabetic rats that had decreased after STZ exposure. Sildenafil at 5 mg/kg also increased sexual function of diabetic rats and as expected was better than ellagic acid. Combination of sildenafil (5 mg/kg) and ellagic acid (50 mg/kg) showed a better effect than either of them suggesting possible additive effect of the two compounds in our study.

Observing effect of treatment on blood glucose level of rats

Serum glucose levels in STZ-induced diabetic rats after 30 days of induction of diabetes was 349.79 ± 4.24 mg/dL which was significantly higher than the serum glucose levels of 111.05 ± 2.80 mg/dL in normal rats.

Serum glucose levels in STZ diabetic rats administered with ellagic acid 50 mg/kg p.o. was 248.25 ± 15.65 mg/dL which was significantly lower than that of diabetic control (349.79 ± 4.24 mg/dL). There was no observable effect of sildenafil on blood glucose level.

Sexual function of diabetic rat

Prior to dosing, a decreased sexual motivation of diabetic control rat was observed as was evident by increase in mount latency. Similarly, sexual function of diabetic rat had decreased as there was decrease in intromission frequency and ejaculation latency, and increase in post-ejaculatory interval. Dosing with ellagic acid, sildenafil and combination of both ellagic acid and sildenafil increased sexual function of diabetic rats. Sildenafil was more efficacious than ellagic acid while a combination of both ellagic acid and sildenafil was most effective in the management of diabetes induced sexual dysfunction [Table 1]. Ellagic acid did not increase sexual behavior of normal male rats (data not given).

Effect of ellagic acid on AGE

Ellagic acid (10-100 μg/mL) inhibited the glycation of bovine serum albumin and subsequent formation of fluorescent glycated products in a concentration-dependent manner. IC_{50} values of ellagic acid and aminoguanidine (standard inhibitor) for inhibition of AGE were found to be 55 and 50 μg/mL, respectively [Figure 3].

Effect of ellagic acid on isolated rat penile tissue

Ellagic acid was able to relax isolated rat CCSM but the relaxation was not statistically significant ($P > 0.05$) whereas sildenafil relaxed CCSMs significantly ($P < 0.05$). Data is presented in Figure 4.

DISCUSSION

Ellagic acid was found to have anti-diabetic effect as rats treated with ellagic acid had reduced blood sugar level and it also inhibited formation of AGE. Ellagic acid was also effective in increasing sexual function in diabetic rat. more than 50% of diabetics are likely to suffer from sexual dysfunction.[2]

Around 80% of ED cases have organic causes where as remaining cases are due to psychogenic causes. Organic causes of ED may include hormonal (testosterone) changes, damage to penile nerve supply (accident and/operation in pelvic region), cigarettes smoking, diseases (diabetes and blood pressure) etc., The pathology for development of ED is impaired penile arterial inflow, corporal veno-occlusive dysfunction, or corporal tone derangement. The ultimate reason for ED is due to impaired endothelial and smooth muscle relaxation. ED is primarily a vascular disease and considered as an early manifestation of endothelial dysfunction.[3]

Relaxation of penile smooth muscle and arteries is primarily mediated by nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway: Nitric oxide synthase (NOS) converts L-arginine to NO that diffuses into penile smooth muscle to stimulate soluble guanylyl cyclase (sGC) to produce cGMP that relaxes the smooth muscle and it is manifested as penile erection. RhoA/Rho-kinase pathway is one of the major pathways those control contraction of CCSMs (penile smooth muscles). Alteration in NO/cGMP and RhoA/Rho-kinase pathway in diabetes leads to development of ED [Figure 5].[3]

Endothelial nitric oxide synthase (eNOS) expression and

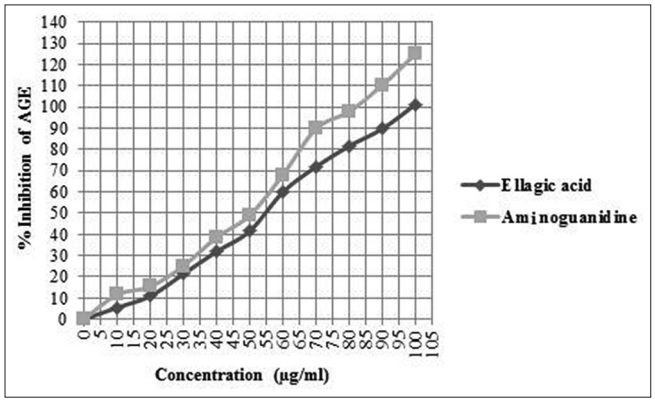

Figure 3: *In vitro* estimation of inhibition of advanced glycated end products. The data shows mean ± standard deviation of three readings

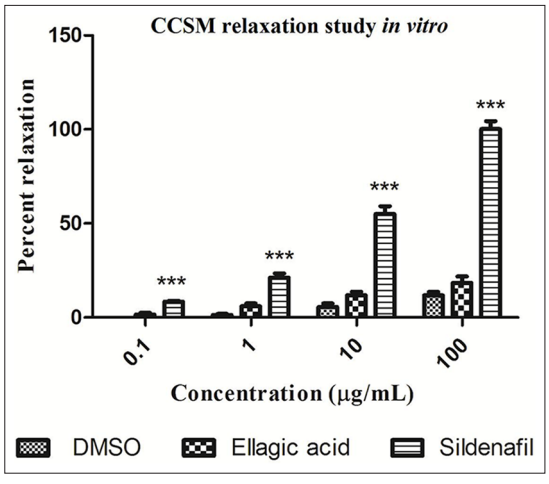

Figure 4: Effect of vehicle, ellagic acid and sildenafil on the isolated corpus cavernosum smooth muscle (CCSM) of rat penile tissue. Relaxant effect of ellagic acid on phenylephrine precontracted CCSM was not significant statistically where as sildenafil could relax CCSM upto 100% (***$P < 0.001$)

activity decreases in diabetic penis which may be due to decrease in eNOS substrate (L-arginine) and inactivation of eNOS cofactor tetrahydrobiopterin (BH_4). Hyperglycemia resulting from diabetes impairs eNOS phosphorylation that increases enzyme's catalytic activity thus increasing NO production (and subsequently ROS). Heat shock protein (Hsp) 90 acts as a major protein activator of eNOS and mechanism of this activation involves calmodulin-dependent release of eNOS from caveolin-1, recruitment of eNOS and Akt to adjacent regions on Hsp etc., Expression of caveolin-1 decreases in the penis of diabetic rat in comparison to that of non-diabetic rat. eNOS (as well as neuronal NOS and inducible NOS) can switch from a NO-producing enzyme to a superoxide-producing enzyme (monomerization), known as eNOS uncoupling. This might be due to oxidation of zinc thiolate cluster of eNOS and cofactor BH_4 and decreased availability of L-arginine. Oxidative stress, an imbalance between production (by NADPH oxidase, eNOS uncoupling and mitochondrial electron-transport chain) and elimination of ROS, is a major limiting factor in diabetes. Superoxide forms peroxynitrite after reacting with NO, this decreases NO availability resulting in ED, a male sexual dysfunction [Figure 5].[1,3]

One of the reasons for development of ED could be due to increase in Rho-kinase expression and activity in rat CCSMs. Rho-kinase activity was found to be increased in diabetic rat penis and the enzyme suppressed eNOS activity.[17] ROS were reported to activate Rho-kinase[18-21] and antioxidant potential of ellagic acid might be responsible for the increase in sexual function of diabetic rats by decreasing deleterious effect of ROS [Figure 5]. However, ellagic acid could not increase sexual function of normal rats and did not relax

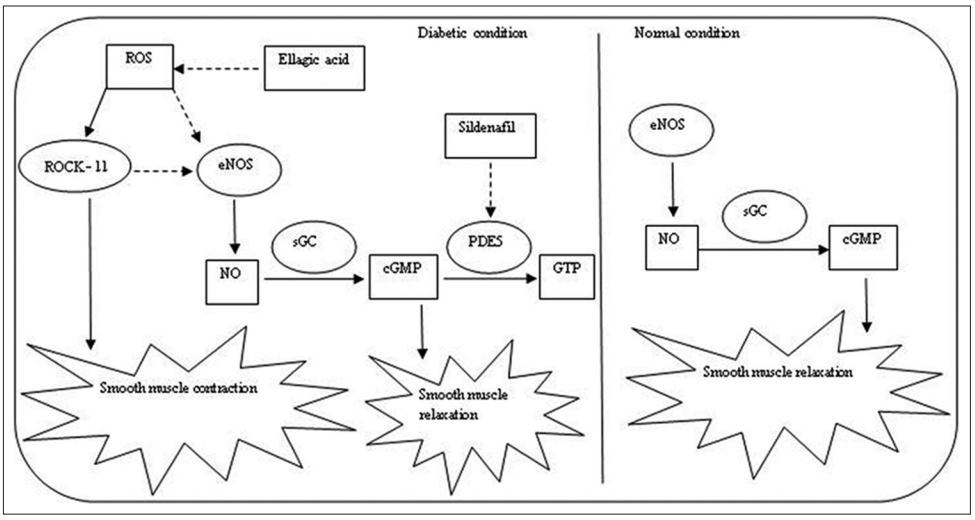

Figure 5: In normal condition, nitric oxide (NO) generated by endothelial nitric oxide synthase (eNOS) stimulates soluble guanyl cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP) that relaxes CCSM. But, in diabetic condition Rho-kinase 2 (ROCK-II) is activated by increased reactive oxygen species and the enzyme increases the contractility of CCSM. In addition, ROS inhibits eNOS ultimately decreasing CCSM relaxation. Ellagic acid increases sexual function in diabetic rat probably by inhibiting ROS and sildenafil increases sexual function by inhibiting PDE5 that metabolizes cGMP

Table 1: Effect of treatment on sexual behavior of diabetic rats

Sexual activities (units)	Normal rat (*n*=6)		Diabetic rat (*n*=6)							
	Vehicle		Control rat (vehicle)		Ellagic acid 50 mg/kg		Sildenafil 5 mg/kg		Ellagic acid 50 mg/kg+Sildenafil 5 mg/kg	
	0 day	30th day	0 day	30th day	0 day	30th day	0 day	30th day	0 day	30th day
ML (seconds)	42.7±3.8	45.3±3.5	46.8±4.8	141.2±6.1$	42.0±4.6	78.3±2.7[b]	39.2±4.2	44.5±3.1[c,d]	39.5±4.5	37.3±2.1[f,g]
IL (seconds)	47.3±3.5	50.7±2.0	53.2±3.1	168.2±7.1$	48.3±3.1	120.7±4.2[b]	47.3±4.5	71.3±4.6[c,d]	54.8±4.0	50.5±2.9[f,g,h]
EL (seconds)	418.7±10.7	415.3±14.3	431.8±10.8	300.7±10.6$	433.2±17.8	368.8±12.8[a]	421.3±15.3	407.7±17.7[c]	415.7±18.7	422.7±17.4[f]
MF (number)	9.3±1.3	8.5±0.6	10.2±1.1	4.5±0.7$	10.0±1.2	7.2±0.3[a]	10.8±1.4	9.3±0.6[c]	10.5±1.1	12.2±0.5[f,g,h]
IF (number)	11.0±1.1	10.5±0.8	10.7±0.9	3.7±0.5$	10.5±0.8	6.5±0.6[a]	11.0±0.6	9.0±0.7[c]	9.8±0.8	10.8±0.7[e,g]
PEI (seconds)	371.3±9.3	359.2±15.1	372.7±10.9	891.5±17.6$	379.0±12.3	586.2±14.3[b]	363.8±11.6	448.3±4.8[c,d]	369.3±13.0	371.5±10.8[f,g,i]

ML: Mount latency; IL: Intromission latency; EL: Ejaculation latency; MF: Mount frequency; IF: Intromission frequency, and PEI: Post-ejaculatory interval. *n*=Number of rats. Sexual function of diabetic control rats was reduced significantly ($P<0.001) in comparison to normal rat on 30th day after induction of diabetes. Treatment of ellagic acid increased sexual function of diabetic rats significantly ([a]$P<0.05, [b]$P<0.001) with respect to diabetic control rat. Sildenafil administration also increased sexual function of diabetic rat significantly ([c]$P<0.001) in comparison to diabetic control rat. Effect of sildenafil in maintaining sexual function was more significant than ellagic acid (d $P<0.001). Combination of ellagic acid and sildenafil was most efficient in increasing the sexual function in diabetic rats when compared with diabetic control rat ([e]$P<0.05, [f]$P<0.001), diabetic rat treated with ellagic acid ([g]$P<0.001) and sildenafil ([h]$P<0.05, [i]$P<0.01)

isolated corpus cavernosum smooth muscle significantly up to a dose of 100 μg/mL. Therefore, ellagic acid may not increase sexual function in healthy men. However, it could be useful in the management of diabetes-induced ED.

Sildenafil was reported to increase the level of cGMP in CCSM by inhibiting metabolism of cGMP by Phosphodiesterases 5 (PDE5). The PDE5 inhibitor was also reported to increase sexual function in diabetics.[22] In our experiment, we also observed that sildenafil increases sexual function of diabetic rats. Sildenafil's efficacy, at least in part, could be due to its antioxidant potential also.[23] A combination of ellagic acid (50 mg/kg) and sildenafil (5 mg/kg) was most efficient in increasing sexual function in diabetic rats [Table 1].

CONCLUSION

Combination of PDE5 inhibitor and ellagic acid (antioxidant) was more efficient in reducing diabetes-induced erectile dysfunction (ED) than individual treatments. The combination may be useful in the management of diabetes-induced ED and could be considered for evaluation at clinical levels in future.

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