**Effect of *Mucuna pruriens* (Linn.) on Oxidative Stress-Induced Structural Alteration of Corpus Cavernosum in Streptozotocin-Induced Diabetic Rat**

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**ABSTRACT**

**Introduction.** Erectile dysfunction is one of the major secondary complications of diabetes. *Mucuna pruriens* (*M. pruriens*), a leguminous plant identified for its antidiabetic, aphrodisiac, and fertility enhancing properties, has been the choice of Indian traditional medicine.

**Aim.** The objective of the present study was to analyze the efficacy of *M. pruriens* on free radicals-mediated penile tissue alterations in hyperglycemic male rats.

**Methods.** Male albino rats were divided as group I (sham) control, group II (STZ) diabetes-induced (streptozotocin 60 mg/kg of body weight [bw] in 0.1 M citrate buffer), group III (STZ + MP) diabetic rats administered with 200 mg/kg bw of Ethanolic extract of *M. pruriens* seed, group IV (STZ + SIL) diabetic rats administered with 5 mg/kg bw of sildenafil citrate, group V (sham + MP) administered with 200 mg/kg bw of extract alone, and group VI (sham + SIL) administered with 5 mg/kg bw of sildenafil citrate. The *M. pruriens* and sildenafil citrate were given (gavage) once daily for a period of 60 days. At the end of 60 days, the animals were sacrificed and subjected to analysis of reactive oxygen species levels, enzymic and nonenzymic antioxidant levels, levels of NOx, histological, and histomorphometrical study of penile tissue.

**Main Outcome Measures.** Remedial use of *M. pruriens* seed extract on diabetes-induced erectile tissue damage.

**Results.** Significantly high levels of oxidative stress and low levels of antioxidants in the penile tissue seem to contribute to the increased collagen deposition and fibrosis of erectile tissue in STZ rats. Relatively, there was increased damage in STZ + SIL group. Supplementation of *M. pruriens* in STZ + MP group has revealed the potency to overcome oxidative stress, and good preservation of penile histioarchitecture.

**Conclusion.** The Ethanolic extract of *M. pruriens* seed significantly recovered or protected erectile tissue from the oxidative stress-induced degeneration by its antioxidant potentials. These findings propound to serve mankind by the treatment of diabetes-induced erectile dysfunction. **Suresh S and Prakash S. Effect of *Mucuna pruriens* (Linn.) on oxidative stress-induced structural alteration of corpus cavernosum in streptozotocin-induced diabetic rat. J Sex Med.**

**Key Words.** *Mucuna pruriens*; Traditional Medicine; Oxidative Stress; Corpus Cavernosum; Erectile Dysfunction; Sexual Medicine; Diabetes

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**Introduction**

Erectile dysfunction (ED) has been recognized as one of the major secondary complications in diabetic men than age-matched nondiabetic men [1]. A variety of mechanisms have been suggested for the erectile disorders associated with diabetes. Impaired nitric oxide (NO) activity and endothelial dysfunction have been described in diabetic animal models as well as in human volunteers [1,2] such as reduced number of nitrous oxide synthase (NOS)-containing neurons, impairment of NOS activity, and both neurogenic- and endothelium-mediated smooth muscle relaxation [3], increased NO scavenging by reactive oxygen species (ROS) or oxidized low-density lipoprotein [4]. Further, oxidative stress (OS) plays a major role in ED induced by diabetes. Increased ROS
can inactivate the NO by superoxide, resulting in impaired penile NO transmission and smooth muscle relaxation through NO-cyclic guanosine monophosphate (cGMP) pathway [5,6].

*Mucuna pruriens* (*M. pruriens*), a leguminous plant, is identified as herbal medicine for improving fertility in the Indian traditional system of medicine. The plant is rich in alkaloids [7]. Triterpenes and sterols (b-sitosterol, ursolic acid, etc.) are discovered in its root and seeds. The seeds also contain L-3,4-dihydroxyphenylalanine (L-DOPA) [8,9], methionine, tyrosine, lysine, glycine, aspartic acid, glutamic acid, leucine, and serine along with globulins and albumins [10], and compounds such as oleic acid, linoleic acid, and palmitic acid [11–13]. Our previous studies proved that the ethanolic seed extract of *M. pruriens* is a good sexual enhancer and increases the sperm count and motility in normal rat [8] and also in diabetic rat [14]. It also improves viability and reduces structural and functional abnormalities in aged rat sperm from OS [9] and also shown to have hypoglycemic effects [15]. The bioavailability and natural source of essential bioactive compounds in the seed of *M. pruriens* has been the motivating force for the design of this study. In the present work, the therapeutic efficacy of ethanolic seed extract on diabetes-induced OS and ensuing erectile tissue damage was analyzed.

**Materials and Methods**

**Animals**

Albino rats of Wistar strain were used for this study. Twelve-week-old male rats around 225–250 g bw were selected. The study was approved by Institutional Ethical Committee (IAEC no. 01/044/07). Details of the animal maintenance are given elsewhere [14]. The quarantine procedures and the animal maintenance were according to the recommendations of Canadian Council Guide to the Care and Use of Experimental Animals [16] and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (India) Guidelines for laboratory animal facility [17]. Animals were randomly divided into six groups (N = 12) and were subdivided randomly into two set of rats, i.e., histology and histomorphometry (N = 6) and cryosection and biochemical analysis (N = 6). The group I (sham) represented control animals (received citrate buffer), group II (STZ) received single dose of 60 mg/kg bw of streptozotocin (STZ) in 0.1 M citrate buffer, group III (STZ + MP; diabetes induced and treated with ethanolic seed extract of *M. pruriens* 200 mg/kg bw), group IV (STZ + SIL; diabetes induced and treated with sildenafil citrate 5 mg/kg bw), group V (MP; received ethanolic citrate seed extract of *M. pruriens* 200 mg/kg bw), and group VI (SIL) rats were administered with sildenafil citrate (5 mg/kg bw), reference drug that served as positive control. The extract and sildenafil citrate were administrated once daily for 60 days by gavage; the dosage used in the present study was based on our previous studies [8,9].

**Drug Preparations and Phytochemical Screening**

The ethanolic extract of *M. pruriens* seed was used in the present study. Collection of plants and extract preparation, percentage of yield, and phytochemical analysis were described elsewhere [8,9].

**Reactive Oxygen Species (ROS) and Antioxidants**

**Estimation of OH• and H2O2 Generation**

The ROS generation in the penis was assayed using 2′,7′ dichlorofluorescein-diacetate (DCFH-DA; Sigma-Aldrich, St. Louis, MO, USA) according to the methods of Driver et al. [18] The DCFH-DA is a nonpolar compound that after conversion to a polar derivative by intracellular esterases, can rapidly react with ROS to form the highly fluorescent compound dichlorofluorescein (DCF).Briefly, the homogenate was diluted (1:20 ratio) with ice cold Locke’s buffer to obtain a concentration of 5 mg tissue/mL. The reaction mixture (1 mL) containing Locke’s buffer (pH 7.4; 154 mM NaCl, 5.6 mM KCl, 3.6 mM NaHCO3), 2.3 mM CaCl2, 5.6 mM D-glucose, 50 μL of homogenate and 10 μL DCFH-DA (5 μM) was incubated for 15 minutes at room temperature to allow the DCFH-DA to be incorporated into any membrane-bound vesicles and the diacetate group cleaved by esterases. After 30 minutes of further incubation, the conversion of DCFH-DA to the fluorescent product DCF was measured using a spectrofluorimeter with excitation at 484 nm and emission of 530 nm. ROS formation was quantified from a DCF standard curve and data were expressed as pmol DCF formed/minute/mg protein.

**Estimation of Superoxide Anion (O2•−) Generation**

Superoxide generation in the penis was assayed using dihydroethidium (Sigma-Aldrich), methods of Driver et al. [18], with some modifications.
Briefly, rats' penis were homogenized in a buffer containing 50 mM Tris–HCl buffer (pH 7.4) and the homogenate was diluted 1:20 ratio with the same buffer (ice cold) to obtain a concentration of 5 mg tissue/mL. A 50 μL of homogenate and a 10 μL dihydroethidium (5 μM) were incubated for 15 minutes at room temperature to allow the dihydroethidium to be freely permeable to cells and in the presence of O$_2^-•$, is oxidized to red fluorescent ethidium bromide (EtBr), where it is trapped by intercalation with DNA. After 30 minutes of further incubation, the conversion of dihydroethidium to the fluorescent product EtBr was measured using a spectrofluorimeter with 488–610 nm as excitation. Superoxide formation was quantified from EtBr standard curve and data were expressed as pmol EtBr formed/minute/mg protein.

**Estimation of LPO**

The protein content was determined as per the method described by Lowry et al. [19] Lipid peroxidation was measured by the method of Ohkawa et al. [20] The thiobarbituric acid-reactive substance thus measured was expressed as nmol of malondialdehyde (MDA) formed/minute/mg protein in tissue samples.

**Estimation of Nonenzymic Antioxidants**

The level of vitamin E was estimated by the method of Desai [21]. The level of vitamin E was expressed as μg/mg protein. The level of ascorbic acid was estimated by the method of Omaye et al. [22] The level of ascorbic acid was expressed as μg/mg protein. The level of reduced glutathione was measured by the method of Moron et al. [23] The level of glutathione was expressed as μg/mg protein.

**Estimation of Enzymic Antioxidant**

The activity of superoxide dismutase (EC 1.15.1.1, SOD) was assayed according to the method of Marklund and Marklund [24]. The enzyme activity was expressed as unit/mg protein. The activity of catalase (EC 1.11.1.6, CAT) was assayed by the method of Sinha [25]. The activity of CAT was expressed as units/mg protein (one unit is the amount of enzyme that utilizes 1 mole of hydrogen peroxide/minute). The activity of glutathione reductase (EC 1.6.4.2, GR) was determined by the method of Staal and Vegel [27]. Activity of GR was expressed as micromoles of nicotinamide adenine dinucleotide phosphate (NADPH) oxidized/minute/mg protein. This glutathione-S-transferase (EC 2.5.1.1.8, GST) enzyme was assayed by the method of Habig et al. [28] Activity of GST was expressed as unit/mg protein (one unit is the amount of enzyme that conjugate 1 nm of 1-chloro-2,4-dinitrobenzene with GSH/min).

**In-Situ Detection of Reactive Oxygen Species (ROS) Level**

The generation of ROS was determined by using DCFH-DA as a probe (Sigma-Aldrich), by the method of Liu et al. [29] with minor modifications. Unfixed penile tissues were used for cryosectioning (Leica Microsystems, Wetzlar, Germany). Sections were taken at 20 μm thickness and 50 μL DCFH-DA (5 μM in DMSO) was applied to each tissue section and coverslipped. Slides were incubated in a light-protected humidified chamber at 37°C for 30 minutes. After incubation, the conversion of DCFH-DA to DCF by ROS was observed under an epifluorescent microscope (Nikon Corporation, Tokyo, Japan) with 488 and 525 nm as excitation and emission wavelengths, respectively.

**In-Situ Detection of Superoxide Anion**

Intracellular generation of O$_2^-•$ was assessed using dihydroethidium (Sigma-Aldrich) by the method of Bivalacqua et al. [30], dihydroethidium (DHE) is freely permeable to cells and in the presence of O$_2^-•$, DHE is oxidized to red fluorescent EtBr, where it is trapped by intercalation with DNA. The hydroethidine (0.2 μM in phosphate buffer saline [PBS]) was applied to cryosection and coverslipped. Slides were incubated in a light-protected humidified chamber at 37°C for 30 minutes. After incubation, the slides were observed and images were obtained using epifluorescent microscope (Nikon Corporation). Fluorescence was detected at 510–595 nm as excitation and emission.

**Estimation of Nitrite and Nitrate (NOx)**

The modified Griess’s method was used for the determination of nitrite and nitrate levels as indicators of NO in the tissue, and total NO (NOx) levels were determined by the methods described by Miranda et al. (2001) [31]. The NOx levels were obtained using an enzyme-linked immun-
osorbent assay (ELISA) reader by vanadium chloride (VCl₃)/Griess assay. Prior to NOx determination, penile tissues were homogenized in five volumes of phosphate-buffered saline (pH 7.5) and centrifuged at 2,000 x g for 5 minutes. Then, 0.25 mL of 0.3 M NaOH was added to 0.5 mL supernatant. After incubation for 5 minutes at room temperature, 0.25 mL of 5% (w/v) ZnSO₄ was added for deproteinization. This mixture was then centrifuged at 3,000 x g for 20 minutes and supernatants were used for the assays. Experiments were performed at room temperature. Nitrate standard solution was serially diluted. After loading the plate with samples (100 µL), addition of vanadium III chloride (VCl₃; 100 µL) to each well was rapidly followed by addition of Griess reagents, sulphanilamide (50 µL) and N-(1-naphthyl) ethylenediamine dihydrochloride (50 µL). After incubation (usually for 30–45 minutes), samples were measured at 540 nm using ELISA reader. The amount of NOx was expressed as nmol/g tissue.

Morphological and Histopathological Study
Adult animals were sacrificed by an overdose of anesthesia (pentathol sodium 60 mg/kg bw). Immediately after the respiration ceased, the animals were fixed by transcardial perfusion with 4% paraformaldehyde in 0.1M phosphate buffer after flushing the blood with normal saline. The penile tissues were dissected out and the length, breadth, circumference, weight, and volume were estimated. Paraffin sections (Leica Microsystems) were taken at 5 µm thickness and stained with Harris hematoxylin and eosin and trichrome stain (Bancroft and Gamble) [32] using Celestin blue hematoxylin (Central Drug House, Mumbai, India) and methyl blue stains (Central Drug House). The sections were observed under a bright-field microscope (Nikon Corporation, Tokyo, Japan).

Histomorphometry and Stereological Analyses
The conventional stereological principles and accepted morphometric procedures as outlined by Elias and Hyde [33], were used to obtain quantitative information; details of our procedure have been described previously [34]. Stereological analysis was performed by the point count method, superimposing lattice of 121 intersections. Volume density of smooth muscle, sinusoids, endothelium, and numerical density of sinusoids per unit area (723 µm²; area in the section observed with the 10x objective) were quantified and the values were given in relative terms. The thickness of the endothelium and diameter of the sinusoids were estimated using ocular micrometer scale. Mori and Christensen [35] protocol was followed to combat shrinkage during fixation and processing.

Polarizing Microscopic Study of Collagen Deposition
Analysis of the collagen deposition in the penis was done according to the methods of Junqueira et al. [36] Briefly, the paraformaldehyde-fixed paraffin sections were stained using 0.5 g of picrosirius red (Sirius red F3B C.I. 35782; Sigma-Aldrich) in 500 mL of saturated aqueous solution of picric acid. The sections were then washed twice in acidified water (5 mL glacial acetic acid to 1 L of distilled water). The sections were observed for collagen I (red-orange) and collagen III (green) under Olympus polarizing microscope (Tokyo, Japan).

NADPH-Diaphorase Histochemistry
The NADPH diaphorase activity was determined by the methods of Dorfman et al. [37] The cryosections were incubated in a solution containing 0.1% n-NADPH and 0.02% nitroblue tetrazolium diluted in 0.1 M phosphate buffer, pH 7.4, with 0.3% Triton X-100 (HiMedia Laboratories, Mumbai, India), for 60 minutes at 37°C. Negative control sections were performed omitting NADPH in the incubation mixture. The slides were then coverslipped with PBS–glycerol mixture (1:3) and observed under the bright-field microscope (Nikon Corporation, Tokyo, Japan).

Statistical Analysis
The data were subjected to the “one-way ANOVA” by the method of Zar [38]. The significance was determined using “Tukey’s post hoc test” with P < 0.05 considered statistically significant (Microsoft Excel 2003 and SPSS statistical package version 7, SPSS Inc., Chicago, IL, USA).

Results
Estimation of ROS Generation
The conversion of the DCFH-DA into DCF and DHE into EtBr in the presence of ROS is significantly increased in STZ and STZ + SIL rats when compared to the sham. The STZ + MP group showed significantly decreased ROS production when compared to the STZ rats. The STZ + SIL rats did not show recovery from ROS production when compared to STZ rats. No significant changes in ROS production in sham + MP and sham + SIL groups when compared to sham rats (Figure 1A).
Estimation of Lipid Peroxidation (LPO)

The LPO of penile tissue was significantly increased in STZ rats when compared to group I. Supplementation of *M. pruriens* (STZ + MP) significantly reduced LPO levels when compared to STZ group. But in STZ + SIL group, LPO level was not reversed when compared to STZ group. Administration of *M. pruriens* (sham + MP) did not show any significant changes when compared to sham group. However, the sham + SIL rats showed significant increase in LPO when compared to sham group (Figure 1B).

Estimation of Enzymic Antioxidants

The activities of enzymic antioxidants (SOD, CAT, GPx, GST, and GR) were significantly reduced in STZ and STZ + SIL groups when compared to sham. The STZ + MP group showed significant recovery (similar to sham) in enzymic antioxidants levels when compared to STZ rats. The STZ + SIL group did not show any significant recovery when compared to STZ group. The enzymic antioxidants in sham + MP group were significantly increased in GPx but not in the others, whereas sham + SIL group showed significant decrease when compared to sham animals (Figure 2).

Estimation of Nonenzymic Antioxidants

The activity of nonenzymic antioxidants in STZ group had significantly reduced activity when compared to sham group. The STZ + MP showed significant reversal of nonenzymic antioxidants levels when compared to STZ rats. In STZ + SIL group, there was no significant alteration in nonenzymic antioxidants levels when compared to STZ rats. In sham + MP group, there was significantly increase and sham + SIL group showed sig-
significant reduction in nonenzymic antioxidants when compared to sham group (Figure 3).

**Estimation of Nitrite and Nitrate (NOx)**

The NOx levels were found to be significantly decreased in STZ when compared to sham. Supplementation of *M. pruriens* in STZ + MP significantly increased the NOx level (similar to sham) when compared to STZ. The STZ + SIL rats showed increased NOx levels in penis when compared to STZ rats but less than that to the sham group. The sham + MP and sham + SIL groups did not show significant alterations when compared to sham group (Figure 4).

**In-Situ Detection of Reactive Oxygen Species (ROS) Level**

The in-situ localization of free radical production in the penile tissue of STZ and STZ + SIL showed marked increase in the conversion of DCFH-DA into DCF and DHE into EtBr when compared to sham. The STZ + MP group showed marked reduction in these conversions, when compared to STZ. No obvious change in sham + MP and sham + SIL groups (Plate 1).

**Morphological and Histopathological Study**

The morphological measurements of penis showed significant reduction and thinning out of glans penis and the shaft of penis in STZ and STZ + SIL groups when compared to sham group. The STZ + MP group showed marked morphological recovery in both the glans penis and shaft of the penis when compared to STZ. The sham + SIL groups showed no significant difference in morphological parameters. However, there was increase in the length and volume in sham + MP rat’s penis (Plate 2 and Table 1).

The histopathological observations showed alteration in cavernosal architecture such as thickening of the sinusoidal endothelium, aggregation of the blood cells and macrophage in the sinusoidal endothelium, and smooth muscle degeneration in the STZ rats when compared to sham rats. In STZ + MP group, marked improvement was seen with regard to these histopatho-

![Figure 3](image.png)

Figure 3 Shows the activity of nonenzymic antioxidants (Vitamin E, C, and GSH) in penile tissue of control and various experimental groups. Each bar indicate the mean ± SEM of n = 6 of each group. a—sham; b—STZ; c—STZ + MP; $—P < 0.001; #—P < 0.05; NS—not significant.

![Figure 4](image.png)

Figure 4 Shows the nitrate and nitrite levels in penile tissue of control and various experimental groups. Each bar indicate the mean ± SEM of n = 6 of each group. a—sham; b—STZ; c—STZ + MP; $—P < 0.001; #—P < 0.05; NS—not significant.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Morphology of the penis in various experimental groups</th>
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<tr>
<td>Parameters</td>
<td>Sham</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>3.18 ± 0.08</td>
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<tr>
<td>Breadth (cm)</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>0.35 ± 0.05</td>
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<tr>
<td>Weight (mg)</td>
<td>0.37 ± 0.02</td>
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<tr>
<td>Volume (mL)</td>
<td>0.49 ± 0.07</td>
</tr>
<tr>
<td>Circumference (cm)</td>
<td>1.67 ± 0.10</td>
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</tbody>
</table>

^aSham
^bSTZ
^cSTZ + MP
^IP < 0.001
^NS = not significant

J Sex Med **;**:*;**—**
logical changes when compared to STZ group. However, STZ + SIL group showed more degenerative changes when compared to STZ group. The sham + MP rats did not show any changes in penile architecture; however, sham + SIL group showed sinusoidal endothelium thickening and smooth muscle degeneration when compared to sham group (Plate 2).

**Histomorphometry and Stereology**

The morphometrical analyses showed significant increase in diameter, volume of the sinusoids and its lining endothelium and endothelial thickening, and significant reduction in volume of the smooth muscles in STZ when compared to sham group. In STZ + MP group, there was significant recovery in these parameters and these changes were not observed in STZ + SIL rats. The sham + MP group rats showed no significant changes, but sham + SIL group rats showed significant increase in sinusoidal diameter, endothelial thickness but not in the endothelial volume, and smooth muscle component when compared to sham group (Figures 5 and 6).

**Collagen Analysis Masson Trichrome Staining and Picrosirius Red Staining**

The STZ and STZ + SIL groups showed substantial increase in the collagen deposition (blue in trichrome) and collagen I (yellow) and collagen III (green) and with decrease in the collagen IV and smooth muscle proportion (red) in picrosirius red staining when compared to sham group. The STZ + MP group showed decreased collagen (I and III) deposition when compared to the STZ group. The sham + MP rats showed decreased collagen deposition with reduced smooth muscle fiber content when compared to sham group (Plate 3—column A and B).

**NADPH-Diaphorase Histochemistry**

The activity of NADPH—diaphorase was found to be substantially reduced in STZ and STZ + SIL groups when compared to sham. The STZ + MP group showed marked reversal or prevention of NADPH—Diaphorase activity when compared to Sham + MP group rats (Plate 4—column A and B).
STZ rats. In sham + MP group, the NADPH—Diaphorase positive areas were more when compared to sham rats. No obvious changes were seen in sham + SIL group (Plate 3—column C).

Discussion

Erectile dysfunction induced by diabetes is one of the most common secondary complications in diabetic men. Plant-derived drugs are playing an important role in the field of drug discovery. The present study demonstrated the high levels of ROS and MDA in the corpora cavernosa of STZ rats that indicates an imbalance between the oxidants and antioxidants. The high concentration of glucose may cause increased intracellular diacylglycerol levels, which leads to protein kinase C (PKC) activation and production of PKC-mediated phosphorylation of NOS III protein that

Plate 2 (A) Gross anatomy of rat penis in various groups treated with M. pruriens (MP). The STZ rat showed thinning of the glans penis and the shaft (arrow head) of the penis. Supplementation of M. pruriens (STZ + MP) exhibited recovery of both the penile shaft and glans penis (arrow head) compared with STZ rats. In STZ + SIL group supplementation with sildenafil citrate did show severe diabetes-induced thinning of glans and shaft bluing (arrow head) of the penis when compared to STZ rat penis. No such changes seen in M. pruriens alone treated rat penis (sham + MP). (B) Microscopic image of rat penis treated with for M. pruriens, stained with hematoxylin and eosin staining and imaged through light microscopy. The squares inside the micrographs are the regions of magnified images presented in column C. (C) In the STZ rat shows (arrows) thickening of the sinusoidal endothelium with aggregation of the blood cells. In the STZ + MP group, the supplementation of M. pruriens exhibited reduction in terms of both thickening and blood cell aggregation in sinusoidal endothelium (arrow heads) when compared with SIL supplementation (STZ + SIL) in diabetic rat penis (arrows). Continuous exposure of SIL (sham + SIL) also shows thickening of sinusoidal endothelium (arrow) and smooth muscle degeneration. M. pruriens alone treated rats (sham + MP) showing normal histological picture.

Figure 5 Shows the histomorphometrical values of the sinusoidal diameter and the number of the sinusoids in penile tissue of control and various experimental groups. Each bar indicates the mean ± SEM of n = 6 of each group. a—sham; b—STZ; c—STZ + MP; $—P < 0.001; NS—not significant.

Figure 6 Shows the histomorphometrical values of the sinusoidal epithelial thickness and the volume of sinusoids, endothelium, and smooth muscle in penile tissue of control and various experimental groups. Each bar indicates the mean ± SEM of n = 6 of each group. a—sham; b—STZ; c—STZ + MP; $—P < 0.001; NS—not significant.
in turn can reduce the activity of the enzyme [39]. The increased endothelial superoxide production influenced by hyperglycemia can greatly enhance the formation of peroxynitrite in vascular structures causing tetrahydrobiopterin (BH4) oxidation (a NOS III cofactor to dihydrobiopterin) [40,41]. With BH4 deficiency, the NOS III is in an uncoupled state, which means that the electrons flowing from NO III reductase domain to the oxygenase domain are diverted to molecular oxygen rather than to L-arginine [42,43] resulting in production of superoxide rather than NO.

The ROS leads to changes in the cell permeability and loss of membrane integrity [44], which can lead to tissue degeneration, cell death, and irreversible endothelial damage also diminishes NO production [45] and known to increase lipid peroxidation. Thus, the high levels of ROS and MDA creates a constant stressful environment thereby it intensifies the endothelial damage [46,47]. Thus, the supraphysiological levels of circulating glucose induce high OS and their secondary cascades in multifaceted mechanism induce smooth muscle and endothelial degeneration and endothelial thickening in STZ rats. The supplementation of M. pruriens significantly reduces the ROS production and MDA formation in STZ + MP rats by suppressing the ROS production or increase the free radicals scavenging and thus may prevent the self-propagating LPO reaction and the endothelial and smooth muscle damage. However, STZ + SIL rat’s shows increased ROS levels and MDA formation and yield to diabetes-induced OS.

The increased NADH or NADPH oxidization or impaired defense system may have elevated ROS in STZ rat penis. The increased O$_2^•−$ interact with NO to form peroxynitrite, which reacts with tyrosine residues in proteins to form nitroyrside- nilated derivatives [48] that leads to smooth muscle and nerve tissue damage [49]. The activity of SOD is significantly reduced in STZ rat penis. This may be due to the concomitant increase of O$_2^•−$ anion level causing increased peroxynitrite (ONOO⁻) that react with the tyrosyl residue of proteins, which in turn can inactivate the superoxide dismutase and lead to decreased removal of superoxide [50]. Also, elevated glucose increases the degradation or glycosylate the native SOD [51] causing ROS-mediated endothelial damage and quenching of NO and in turn reduction of cGMP. This may lead to peroxide-induced tissue degeneration and cell death [52]. The STZ + MP rats shows significant increase in SOD and catalase synthesis, thereby scavenging the excess radicals before they interact with the NO and tyrosine residues of the proteins and thus M. pruriens protects the normal function of the enzyme in penis, supported by its hypoglycemic effects.

The GPx localized to the cytoplasm is known to be another H$_2$O$_2$ scavenger [53]. Level of GPx was significantly decreased in corpus cavernosum in STZ rat and concomitant increased H$_2$O$_2$ production signifies the defective antioxidant system via reduced biosynthesis or increase in the degradation of GPx in STZ rat penis. The STZ + MP rat showed significantly increased GPx levels and decreased H$_2$O$_2$ in penile tissue, indicating the ability of the extract to enhance the synthesis or prevent the degradation of GPx. Further, it has been suggested that the hydrogen-sharing ability of the extract may convert H$_2$O$_2$ into water molecule [54]. The STZ + SIL rat penis did not show any changes in both GPx and H$_2$O$_2$ levels when compared to STZ rat.

Alpha tocopherol not just acts as an antioxidant and oxygen-free radical scavenger, but also has an independent inhibitory action on PKC in vascular cells [55]. The levels of α-tocopherol are found to be significantly reduced in STZ rat penis, which may be due to the increased ROS-mediated degradation or suppression of synthesis. This may
have enhanced the PKC activity causing cavernosal damage in STZ rats. The supplementation of the *M. pruriens* in STZ + MP group has significantly increased the levels of vitamin E, indicating the potency of the extract to restore the antioxidant defense system and protecting the endothelial and smooth muscles in corpus cavernosum. But STZ + SIL rat penis did not show this protection.

Ascorbic acid (AA) is a naturally occurring major antioxidant, essential for the scavenging of free radicals. The activity of AA has been significantly reduced in the STZ rat that can be due to increased degradation/decreased biosynthesis via diminished activity of L-gulono-gamma-lactone oxidase, a terminal enzyme of AA biosynthesis [56,57] or via increased urine excretion and oxidative loss [58]. Apart from this, the reduced levels of GSH observed in STZ rat can have declined the AA regeneration (GSH-NADPH oxidase-dependent) [58]. The STZ + MP rat shows significantly increased levels of AA and thus improving the antioxidant mechanism. But such protection is not seen in STZ + SIL rat penis.

The NADPH diaphorase staining shows high negativity in STZ rat when compared to control rat. The staining indicates the selective nitrergic nerve degeneration in diabetic erectile tissue as a result of decreased neuronal NOS activity and diminished NO production. This can cause impairment of nitrergic relaxations [2,59,60]. Literature indicates that androgen insufficiency can induce ultrastructural alteration in dorsal nerve or can induce degeneration of cavernous nerves [61,62]. Supplementation of *M. pruriens* in STZ + MP rats has demonstrated more positive staining of NADPH diaphorase, indicating neuronal integrity in the penile tissue. Thus, the dorsal nerve is protected by the influence exhibited by the extract in the form of antioxidant and supported by our earlier observation on antidiabetic property and androgenic effect (by restoration of the pituitary testicular axis) [8,14]. The NADPH diaphorase staining is negative in STZ + SIL rats indicating no signs of nerve recovery and concomitant fibrotic changes. Thus, the increased fibers in the penile tissue can be induced by the loss of corporal smooth muscle cells by the neuropathia-induced apoptosis, a response that can lead to fibrosis of penile corpora cavernosa [63]. The histomorphometrical observation reveals a significant reduction in trabecular smooth muscle content and marked increase in connective tissue deposition in STZ rat penis. This is significantly reduced in STZ + MP groups, thus the present study clearly demonstrates the potential of *M. pruriens* to recover the penile tissue from diabetic-induced fibrotic changes. The STZ + SIL rat penis shows structural alteration with increased collagen depositions.

From the present data, we conclude that the STZ + SIL rats did not overcome the OS-induced structural deformities. This may be a reason why only 50% of the patients are successful with available oral therapy. However, the administration of ethanolic extract of *M. pruriens* seed to STZ rats significantly protected the erectile tissue and also improved penile reflex. These observations serve as a motivating factor for its implication to treat diabetes-induced ED in mankind.

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References
1 Escrig A, Marin R, Abreu P, Gonzalez-Mora JL, Mas M. Changes in mating behavior, erectile function and nitric oxide...


44 Vane JR, Botting RM. The role of chemical mediators released by the endothelium in the control of the cardiovascular system. Int J Tissue React 1992;14:53–64.


