Effects of a Water-Soluble Curcumin Protein Conjugate vs. Pure Curcumin in a Diabetic Model of Erectile Dysfunction

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ABSTRACT

Introduction. Curcumin is involved in erectile signaling via elevation of cyclic guanosine monophosphate (cGMP).

Aim. Assessment of the effects of water-soluble curcumin in erectile dysfunction (ED).

Methods. One hundred twenty male white albino rats were divided into: 1st and 2nd control groups with or without administration of Zinc protoporphyrin (ZnPP), 3rd and 4th diabetic groups with or without ZnPP, 5th diabetic group on single oral dose of pure curcumin, 6th diabetic group on pure curcumin administered daily for 12 weeks, 7th and 8th diabetic groups on single dose of water-soluble curcumin administered with or without ZnPP, 9th and 10th diabetic groups on water-soluble curcumin administered daily for 12 weeks with or without ZnPP. All curcumin dosage schedules were administered after induction of diabetes.

Main Outcome Measures. Quantitative gene expression of endothelial nitric oxide synthase (eNOS), neuronal NOS (nNOS), inducible NOS (iNOS), heme oxygenase-1 (HO-1), nuclear transcription factor-erythroid2 (Nrf2), NF-KB, and p38. Cavernous tissue levels of HO and NOS enzyme activities, cGMP and intracavernosal pressure (ICP).

Results. Twelve weeks after induction of diabetes, ED was confirmed by the significant decrease in ICP. There was a significant decrease in cGMP, NOS, HO enzymes, a significant decrease in eNOS, nNOS, HO-1 genes and a significant elevation of NF-KB, p38, iNOS genes. Administration of pure curcumin or its water-soluble conjugate led to a significant elevation in ICP, cGMP levels, a significant increase in HO-1 and NOS enzymes, a significant increase in eNOS, nNOS, HO-1, and Nrf2 genes, and a significant decrease in NF-KB, p38, and iNOS genes. Water-soluble curcumin showed significant superiority and more prolonged duration of action. Repeated doses regimens were superior to single dose regimen. Administration of ZnPP significantly reduced HO enzyme, cGMP, ICP/ mean arterial pressure (MAP), HO-1 genes in diabetic groups.

Conclusion. Water-soluble curcumin could enhance erectile function with more effectiveness and with more prolonged duration of action. 

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Key Words. Diferuloylmethane; cGMP; Heme Oxygenase; Erectile Dysfunction; Diabetes Mellitus; Intracavernosal Pressure
Introduction

Endothelial dysfunction plays a preponderant role in a considerable number of vasculogenic erectile dysfunction (ED) cases. The endothelium is considered a primary mediator of vascular blood flow and muscle tone [1–4]. In diabetes, endothelial dysfunction is regarded as important factor in the pathogenesis of diabetic micro- and macroangiopathy. There are three main sources contributing to endothelial dysfunction in diabetes: hyperglycemia which influences endothelial cell functions indirectly by the synthesis of growth factors, vasoactive agents, and the components of the metabolic syndrome [5].

Numerous studies showed that high glucose levels in diabetes activate protein kinase-C (PKC) in vascular endothelial cells [6]. Hyperglycemia causes de novo synthesis of diacylglycerol, leading to the activation of PKC, a pathway now demonstrated in all vascular tissues involved in diabetic complications [7]. The reported deleterious effect of PKC on endothelial function is partially mediated by inducing phosphorylation of p115RhoGEF [8], a guanine nucleotide exchange factor for Rho GTPase [9]. Because active RhoA is implicated in arginase induction [10], it was suggested that PKC might also be involved in regulation of arginase activity leading to decrease in nitric oxide (NO) bioavailability because arginase compete with NO synthase (NOS) for a common substrate: arginine.

In addition, activated PKC results in sustained increases in the production of superoxide anion \( (\text{O}_2^-) \) and induces oxidative damage to diabetic blood vessels. It also induces a number of pathogenic consequences by activating NF-\( \kappa \)B and affecting the expression of endothelial NOS (eNOS), endothelin-1 (ET-1), vascular endothelial growth factor, transforming growth factor-\( \beta \) and plasminogen activator inhibitor-1 [11]. Rungsseesantivanon et al. [12] stated that curcumin supplementation could improve diabetes-induced endothelial dysfunction significantly in relation to its potential to decrease superoxide production and PKC inhibition. This finding was also reported by Balasubramanyam et al. [13], who confirmed that the dose-dependent ROS inhibitory effect of curcumin interfered mechanistically with PKC activity. Okamoto et al. [14] stated that curcumin completely prevents the advanced glycation end products (AGE) and AGE-receptors-induced increase in NF-\( \kappa \)B and activator protein 1 (AP-1) activity and blocks microvascular complications in diabetes.

Different mechanisms of endothelial dysfunction in diabetes and effects of curcumin on different signaling molecules affecting endothelial functions are illustrated in Figures 1 and 2.

Abdel Aziz et al. [2] reported that a novel water-soluble derivative of curcumin could mediate erectile function via induction of heme oxygenase-1 (HO-1) enzyme with subsequent upregulation of cyclic guanosine monophosphate (cGMP) levels in cavernous tissue. The inducible enzyme HO-1 metabolizes heme to biliverdin and carbon monoxide (CO). Several studies proved that CO exhibits physiological properties similar to NO mediated in part by the ability of CO to act as an activator of soluble guanylate cyclase (sGC) [10]. Abdel Aziz et al. [15–20] proved that HO and its product CO dominates NO as a signaling molecule in erectile function and could partially mediate the actions of phosphodiesterase inhibitors.

Curcumin is one of the nuclear transcription factor-erythroid2-related factor 2-Kelch-like ECH-associated Protein1 (Keap1)-antioxidant-response element (Nrf2-Keap1-ARE) signaling pathway activators inducing de novo synthesis of phase II detoxifying genes including HO-1 gene [21–24]. Curcumin effect on erectile function could be mediated via upregulation of HO-1. Andreadi et al. [22] showed the involvement of Nrf2, AP-1 (via extracellular signal-regulated kinases; ERK), protein 38 mitogen-activated protein kinases; p38, and NF-\( \kappa \)B in induction of HO-1 by dietary polyphenols including curcumin [25–27]. Xu et al. [28], stated that curcumin induces relaxation of isolated precontracted porcine coronary arteries and this effect is partially dependent on the action of NO, cGMP, and adrenergic \( \beta \)-receptor. Hence, it is reasonable to assume that the relaxing effect of curcumin on porcine coronary arteries might be related to its antioxidant effect which can protect the coronary arteries from oxidative stress by blocking the production of superoxide anion and promote NO release. Moreover, Schini-Kerth et al. [29], stated that several polyphenols as curcumin are able to induce NO-mediated endothelium-dependent relaxations in a large number of arteries including the coronary artery; they can also induce endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxations in some of these arteries.

Clinical trials in humans indicated that the systemic bioavailability of orally administered curcumin is relatively low [30]. Curcumin and its metabolites could not be detected in plasma at doses lower than 3.6 g/day. Several water-soluble
Curcumin derivatives were prepared to achieve clinically efficient systemic bioavailability.

The present study was conducted to compare the molecular and physiological effects of a novel member of a series of water-soluble curcumin protein conjugates vs. pure curcumin in an experimental diabetic model of ED, with assessment of the molecular signaling targets of pure curcumin and its water-soluble protein conjugate was also conducted.

**Methods**

The study was conducted in the Unit of Biochemistry and Molecular Biology (UBMB), Faculty of Medicine, Cairo University. Curcumin protein conjugate was presented free of charge to the participating researchers as a personal nonprofit scientific donation to help advancement of cooperation in national medical research. The novel curcumin conjugate is registered as international patent protected by the rights of “The Patent Cooperation Treaty” under: (PCT/EG2010/000008, Published Patent Pending, WO 2011/100984) and is the personal property of its inventors.

One hundred and twenty adult male white albino rats (Cux1: HEL1) of matched age and weight (180–200 g) were included in the study after the approval of the Institutional Animal Care and Use Committee.

Experimental rat groups were preferably classified into: single and repeated doses regimen groups and subgroups.
Animals were equally divided into 10 groups: 1st normal control group, 2nd streptozotocin (STZ)-induced diabetic group, 3rd STZ-induced diabetic rat group on 10 mg/kg body weight (BW) single oral dose of pure curcumin administered 12 weeks after induction of diabetes, 4th STZ-induced diabetic rat group on the same oral dose of pure curcumin administered daily for 12 weeks following induction of diabetes, 5th and 6th STZ-induced diabetic rat groups on 2 mg/kg BW single oral dose of curcumin protein conjugate administered 12 weeks after induction of diabetes with or without administration of 50 mg/kg BW intraperitoneal (IP) Zinc protoporphyrin (ZnPP) as HO inhibitor, 7th and 8th STZ-induced diabetic rat groups on the same dose of curcumin protein conjugate (water-soluble curcumin) administered daily for 12 weeks following induction of diabetes with or without administration of ZnPP, 9th group involved control rats that received ZnPP, 10th group involved STZ-induced diabetic rats that received ZnPP. Animals were euthanized 24 hours, 48 hours, and 1 week after the last administered dose of pure curcumin or its conjugate. Diabetes was induced by a single IP injection of 65 mg/kg BW STZ dissolved in 0.1 mM sodium citrate buffer, pH 4.8 and left untreated [31]. Diabetes induction was confirmed by assessment of blood glucose after 1 week and followed by assessment of glycated hemoglobin 12 weeks after induction of diabetes. The treatment phase of the study lasted 12 weeks according to the findings reported by Li et al. [32], who stated that ED is established 12 weeks after diabetic induction in rats. Curcumin and ZnPP doses were chosen according to previous studies [1,2]. Wash-off period of curcumin was 1 week as assessed in a previous study conducted by Abdel Aziz et al. [2]. ZnPP was administered as a single dose for the specified rat groups.

Animals were euthanized after 24 hours, 48 hours, and 1 week of the starting treatment. The following parameters were assessed: intracavernosal HO-1 gene expression [20], HO-1 enzyme activity [20], NOS activity [33], cGMP [2], intracavernosal pressure (ICP) [3] as physiologic assessment of erectile function, and gene expression of inducible NOS (iNOS), eNOS, neuronal NOS (nNOS), Nrf2, NF-κB, and p38 gene by quantitative real-time PCR.
Real-Time Quantitative Assessment of HO-1, iNOS, eNOS, nNOS, Nrf2, NF-kB, and p38 Genes Expression

Total RNA was extracted from cavernous tissue homogenate using RNeasy purification reagent (Qiagen, Valencia, CA, USA). cDNA was generated from 5 μg of total RNA extracted with 1 μL (20 pmol) antisense primer and 0.8 μL superscript avian myeloblastosis virus (AMV) reverse transcriptase for 60 minutes at 37°C. Quantification of gene expression was conducted using universal probe library sets based real-time polymerase chain reaction (PCR) (Roche Diagnostics, Indianapolis, IN, USA). Selection of genes-specific probes and primers was done using the online ProbeFinder software (Roche Diagnostics) and the real-time PCR design assay of Roche Diagnostics found in their website: http://www.universalprobelibrary.com. Primers used for quantitative real time PCR are shown in Table 1. Hypoxanthine phosphoribosyltransferase 1 was used as a positive control housekeeping gene. FastStart Universal Probe Master mix was used in LightCycler® 480 Instrument (Roche Applied Science, Indianapolis, IN, USA). Briefly, in the LightCycler® 480, a total reaction volume of 20 μL was prepared, of which 2 μL (equivalent to 5 μg RNA) of starting RNA material was included for RT-PCR, a final concentration of 0.5 μM of each forward and reverse primer and 0.2 μM the TaqMan probe was used. Cycling conditions involve reverse transcription at 50°C for 30 minutes; enzyme activation at 95°C for 15 minutes, followed by 50 cycles of 95°C for 10 seconds and 60°C for 60 seconds. LightCycler® 480 RT-PCR data were analyzed using LightCycler1.2 version 3.5 software (Roche Diagnostics) using the second derivative maximum method. Successfully amplified targets are expressed in Ct values, or the cycle at which the target amplicon is initially detected above background fluorescence levels as determined by the instrument software. Each sample RT-PCR was performed minimally in duplicate, and the mean Ct value with standard deviation was reported.

NOS Enzyme Activity Assay

NOS enzyme activity was assayed in cavernous tissue by kit supplied by OXIS, International, Inc. (Portland, OR, USA) according to manufacturer’s recommendations. In brief, nitrites and nitrates were used as markers for assay of NOS enzyme activity. Nitrate reductase is used for enzymatic reduction of nitrate to nitrite. Spectrophotometric quantitation at 540 nm of nitrite using the Griess Reagents was conducted as described by Moshag et al. [33].

cGMP Assays

Frozen cavernous tissue (CC) samples (50 mg) stored in a specific lysis buffer were grounded with a stainless steel mortar then homogenized and centrifuged at 600 g at 4°C for 10 minutes. The supernatant was used for cGMP assay using the ELISA kit supplied by R&D Systems, Inc. (Minneapolis, MN, USA).

HO Enzyme Activity Assay

CC (50 mg) homogenized samples were incubated with heme (50 mmol/L), rat liver cytosol (5 mg/mL), MgCl2 (2 mM/L), glucose-6-phosphate dehydrogenase (1U), glucose-6-phosphate (2 mM/L), and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0.8 mM/L) in 0.5 mL of 0.1 M/L phosphate buffer saline (pH 7.4) for 60 minutes at 37°C. The reaction was stopped by putting the tubes on ice, and the reaction solution was extracted with chloroform. The rate of bilirubin formation was monitored at 464 nm and 520 nm by a spectrophotometer [34].

ICP Assessment

Measuring ICP changes elicited by electrical stimulation of the cavernous nerve (CN) was performed in rats according to the method previously described [35,36]. Briefly, rats were anesthetized by IP injection of thiopental (50 mg/kg). The skin

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primer sequences used for quantitative RT-PCR</th>
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<tr>
<td>Beta actin</td>
<td>Forward primer ACCAGTTGCCATGATGAGGACTGGG</td>
</tr>
<tr>
<td>HO-1</td>
<td>GenBank Accession Number: NM_031144.2</td>
</tr>
<tr>
<td>Nrf2</td>
<td>Forward primer TGGACAGCCGGGGCCGCTGAGG</td>
</tr>
<tr>
<td>P38</td>
<td>Reverse primer TGCTTGGGAGTGCTGGCT</td>
</tr>
<tr>
<td>NF-kB</td>
<td>GenBank Accession Number: NM_031789.1</td>
</tr>
<tr>
<td>eNOS</td>
<td>Forward primer GACCCCGGCTGAGGGACGAG</td>
</tr>
<tr>
<td>nNOS</td>
<td>Reverse primer CACCCCGGCTGAGGGACGAG</td>
</tr>
<tr>
<td>iNOS</td>
<td>GenBank Accession Number: NM_021832.2</td>
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overlying the penis was incised and the prepuce was degloved to expose the corpora cavernosa. A 26-gauge needle filled with heparinized saline was carefully inserted into the corpus cavernosum on one side to measure the ICP. The needle was inserted at mid-length of the penile shaft pointing toward its base. The needle was sealed to polyethylene 50 tubing and connected to Gould-Statham pressure transducer (Oxnard, CA, USA) and ICP was displayed on a Grass polygraph (Model 7D, Grass Inst. Co., MA, USA). Through a lower suprapubic midline incision, the lateral prostate was dissected and the major pelvic ganglion identified [37,38]. The CN was unilaterally freed from its facial attachments and stimulated electrically using a bipolar platinum electrode placed 3 to 4 mm distal to the major pelvic ganglion [39]. The two poles of the electrode were separated by 2 mm and the electrode was connected to an electronic stimulator (Letica, Panlab, Model 12106/150, Barcelona, Spain). The CN was stimulated for 1 minute; the pulse parameters were at 5 V at 1 millisecond duration, and 0.3–5 Hz frequency. Each period of stimulation was separated by a resting period of 5 minutes. A frequency response curve was obtained where the maximum rise in ICP during nerve

Figure 3 Heme oxygenase (HO) enzyme activity (pmol bilirubin/mg protein) in rat cavernous tissue. *Significant difference in comparison with control rats, #significant difference in comparison with diabetic rats.
stimulation was measured and compared statistically with healthy control at each frequency.

**Statistical Analysis**
Statistical Package for Social Studies (SPSS) program version 16.0.1 (SPSS Inc., Chicago, IL, USA) was used. Numerical data were expressed as mean ± standard deviation. For comparisons between treatment groups, the null hypothesis was tested by a single-factor ANOVA for multiple groups or unpaired t-test for two groups. Comparisons were considered statistically significant if \( P < 0.05 \).

**Results**
Twelve weeks after experimentally induced diabetes in rat, ED was confirmed by assessment of ICP which was found to be significantly decreased in comparison with control rats. This was accompanied with a significant decrease in cavernous tissue-cGMP levels, NOS, HO enzyme activities (Figures 3–5), a significant decrease in gene expression of eNOS, nNOS, and HO-1 and a significant increase in gene expression of NF-κB, p38, and iNOS in STZ-induced diabetic rats in comparison with the control group (Figures 6–14).
Administration of either pure curcumin or its water-soluble protein conjugate led to a significant elevation in ICP, cGMP levels at all time intervals, a significant increase in enzymatic activities of HO-1 (up to 48 hours with pure curcumin and up to 1 week with curcumin conjugate in the single dose regimen and up to 1 week with both curcumin formulae in the repeated dose regimen) and NOS (up to 24 hours with pure curcumin and up to 48 hours with curcumin conjugate in the single dose regimen, up to 48 hours with pure curcumin and up to 1 week with curcumin conjugate in the repeated dose regimen) (Figures 3–5, 10, 11), a significant increase in genes expression of eNOS, nNOS, HO-1, and Nrf2 (Figures 6–8, 13) and a significant decrease in iNOS, NF-κB, and p38 genes expression (Figures 9, 12, 14) in comparison with untreated induced diabetic rat group but not at all time intervals, for example; p38 gene expression was significantly repressed up to 48 hours in the single dose regimen and for up to 1 week in the repeated dose regimen with both curcumin formulae.
Water-soluble protein conjugate showed significant superiority to the pure curcumin regarding most of the studied parameters with more prolonged duration of action up to 1 week after curcumin conjugate administration. Repeated doses regimens were superior to single dose regimen regarding most of the studied parameters. For example, the effect on iNOS and nNOS genes expression is prominent up to 48 hours in the single dose regimen and up to 1 week in the repeated dose regimen.

Administration of ZnPP to control rats significantly reduced the HO-1 gene expression and HO enzyme activity in comparison with untreated control rats. Moreover, ZnPP significantly reduced HO enzyme activity, cGMP, ICP/MAP, HO-1 relative gene expression in STZ-induced diabetic rat groups that received curcumin conjugates either single or repeated doses in comparison with their corresponding STZ-induced diabetic rat groups that did not receive ZnPP. ZnPP has no effect on the other parameters.

**Discussion**
In the present study, comparative assessment of the molecular and physiological effects of a water-soluble curcumin protein conjugate vs. pure curcumin...
cumin in an experimental diabetic model of ED was conducted. Results revealed that in diabetic rats, there were significant decreases in cavernous tissue cGMP, NOS, and HO enzymatic activities. There was a significant decrease in intracavernous pressure as an index of erectile function in STZ-induced diabetic rats as compared with control rats. These results coincided with previous findings reported by Abdel Aziz et al. [40] who reported that the decline in erectile function in diabetes could be attributed to the decrease in HO-1 gene expression, HO enzyme activity with subsequent decline in cGMP cavernous tissue levels and decline in ICP. Diabetes-associated decline in erectile function is attributed mainly to endothelial dysfunction. Recent studies on conditions associated with endothelial dysfunction had demonstrated that endothelial dysfunction is a major factor contributing to vasculogenic ED cases [41,42].

Factors contributing to diabetic endothelial dysfunction include the effects of oxidative stress and decreased NO bioavailability. Yang et al. [5] stated that vascular arginase activity is increased in diabetic rats and that both arginase and eNOS

![Real-time PCR of endothelial nitric oxide synthase (eNOS) gene expressed in Ct values relative to housekeeping gene. *Significant difference in comparison with control rats, #significant difference in comparison with diabetic rats.](image-url)
compete for their common substrate, L-arginine, with subsequent decrease in NO bioavailability in the cavernous tissue [10,43]. Moreover, glucose-induced alterations in cellular metabolism that may account for endothelial dysfunction involve activation of PKC which may also be involved in regulation of arginase activity. Furthermore, the generation of tumor necrosis factor-α (TNF-α) is increased during diabetes and this cytokine has been shown to upregulate the expression of arginase in endothelial cells [44]. Moreover, TNF can affect NO production by decreasing eNOS expression [45] and increase the production of ROS in neutrophils and endothelial cells through NADPH oxidase [46], xanthine oxidase, and uncoupled NOS [5]. These findings coincide with our results that demonstrated decreased NOS activity and decreased gene expression of eNOS and nNOS in the cavernous tissue of diabetic rats as compared with the control group. On the contrary, iNOS was significantly increased. Wan et al. [47], reported similar findings.

Musicki et al. [48] stated that diabetes-associated ED has been attributed to a reduction in
the number of NOS-containing nerves, the impairment of NOS activity, and both neurogenic- and endothelium-mediated smooth muscle relaxation [49–51], and also to downregulation of the mediators downstream from NO, such as cGMP [52] and cGMP-dependent protein kinase-1 [53], in the corpus cavernosum. Several mechanisms of impaired endothelial function in the diabetic penis have been described that contribute to vasculogenic ED. They include reduced eNOS expression, decreased eNOS activity and impaired eNOS phosphorylation, increased oxidative stress, and increased activity of the RhoA/Rho-kinase signaling pathway.

Furthermore, Hurdag et al. [54] stated that in erectile function, eNOS and nNOS may have a significant role whereas an increase in iNOS may have a deleterious effect via increase in peroxynitrite formation with decrease in nitrate bioavailability. Reactive oxygen species (ROS) play an important role in vascular dysfunction in diabetes. Overproduction of superoxide can lead to scaveng-
ing of NO and to its reduced bioavailability [55]. ROS have been implicated in increased arginase activity and gene expression. Arginase activation can cause uncoupling of eNOS by reducing the supply of L-arginine. The uncoupled eNOS uses molecular oxygen to produce superoxide, thereby further reducing NO and increasing ROS formation. These facts explain our results that demonstrated decreased erectile function as indicated by decreased NOS enzyme activity, eNOS and nNOS gene expression as well as decreased intracavernous pressure in STZ-induced diabetic rat group.

Administration of either pure curcumin or its water-soluble protein conjugate led to significant elevations in cGMP levels, NOS and HO-1 cavernous tissue enzyme levels with significantly higher efficacy in favor of the water-soluble curcumin protein conjugate.

These findings could be explained by improvement in diabetes-induced endothelial dysfunction. Rungseesantivanon et al. [12] stated that curcumin supplementation could improve diabetes-induced endothelial dysfunction via decrease in vascular superoxide production and PKC inhibition. Moreover, a clinical study by Usharani et al. [56] showed that curcumin administration significantly reduced the levels of malondialdehyde, ET-1, interleukin-6, and TNF-α in type 2 diabetes patients. Therefore, the molecular mechanisms of curcumin-mediated increases in vascular NO bioavailability might be enhanced by its antioxidant properties and by its anti-inflammatory effects. Moreover, Ahmad et al. [57] and Ryter et al. [58] reported that HO protects NO through scavenging of ROS, preventing the formation of peroxynitrite and its subsequent degradation and confirming that CO tissue levels parallel NO levels. Ahmad et al. [57] stated that overexpression of HO-1 may mediate an increase in eNOS and a decrease in iNOS, potentially contributing to restoration of vascular responses in diabetic rats. Curcumin as an inducer of HO-1 could indirectly potentiate eNOS effects on vascular endothelium. More recently, Abdel Aziz et al. [16] stated that CO-derived HO-1 could mediate erectile signaling via upregulation of cGMP, the signaling molecule of erectile function. CO relaxes the vascular smooth muscles and promotes

![Figure 10 Intracavernosal pressure/mean arterial pressure in response to 0.3, 0.5, 0.8, 1.0 Hertz. *Significant difference in comparison with control rats, #significant difference in comparison with diabetic rats.](image-url)
relaxation linked to activation of sGC [59,60], stimulation of calcium-activated potassium channels that is involved in the regulation of membrane potential and tone in small myogenically active arterioles [61], or inhibition of endothelin release from endothelial cells [62]. CO of vascular origin is thought to serve a vasodilatory function based on reports that metalloporphyrins that inhibit HO bring about vasoconstriction or increased vascular reactivity to constrictor agonists [63,64]. Ryter et al. [58] stated that CO like NO acts as a vasorelaxant and may regulate other vascular functions such as platelet aggregation and smooth muscle proliferation. The inhibition of NOS by L-Nitro-arginine methyl ester (L-NAME) had no effect on HO enzyme activity and cGMP. This finding indicates that the HO/CO system might supervene, complement, or substitute the NOS/NO system in cavernous tissue. The role of CO as a NO-like signaling molecule has been supported from studies on HO and NOS knockouts. HO-1-derived CO has a positive effect on both sGC and cGMP levels in vascular endothelial cells [1,63,65].

Administration of ZnPP as HO inhibitor to control rats significantly reduced the HO-1 gene expression and HO enzyme activity in comparison with untreated control rats. Moreover, ZnPP significantly reduced HO enzyme activity, cGMP, ICP/MAP, HO-1 relative gene expression in diabetic rat groups that received curcumin conjugates either single or repeated doses in comparison with their corresponding diabetic rat groups that did not receive ZnPP. These findings support our hypothesis that HO/CO system might supervene, complement, or substitute the NOS/NO system.

In the present study, gene expression profile of NF-κB and p38 were significantly increased in STZ-induced diabetic rat. Administration of either pure curcumin or water-soluble protein curcumin conjugate led to a significant lowering effect on their gene expression with more significant effects with water-soluble curcumin protein conjugate than with pure curcumin treated animals and more significant effect with repeated doses than single dose regimens. Moreover, Nrf2 gene expression was unchanged in STZ-induced dia-

Figure 11  Intracavernosal pressure/mean arterial pressure in response to 2, 3, 4, 5 Hertz. *Significant difference in comparison with control rats, #significant difference in comparison with diabetic rats.
betic rats, whereas its levels were significantly elevated with pure curcumin and its water-soluble conjugate. Aggarwal and Sung [66] stated that curcumin suppresses the proinflammatory transcription factors nuclear factor-kappa B and signal transducer and activators of transcription-3, and it activates peroxisome proliferator-activated receptor-gamma and Nrf2 cell-signaling pathways, thus leading to downregulation of adipokines in several tissues and upregulation of adiponectin and other gene products.

Several studies showed the upstream signaling networks involved in HO-1 induction by curcumin involves Nrf2, AP-1, p38, and NF-KB [22–26]. Moreover, the pharmacokinetics of curcumin have recently been found to be mediated via downstream signaling networks such as the NF-KB and MAPK/ERK pathways [39].

In conclusion, the water-soluble curcumin protein conjugate could enhance erectile function in a diabetic model via upregulation of cavernous tissue levels of HO-1 gene and cGMP. Curcumin protein conjugate is superior to pure curcumin with more prolonged duration of action. Molecular signaling of curcumin protein conjugate involve upregulation of gene expression of eNOS, nNOS,
HO-1, and Nrf2 genes and downregulation of gene expression of NF-κB, p38, and iNOS genes.

Acknowledgment

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Conflict of Interest: None.

Figure 14 Real-time PCR of p38 gene expressed in Ct values relative to housekeeping gene. *Significant difference in comparison with control rats, #significant difference in comparison with diabetic rats. 

Statement of Authorship

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(b) Acquisition of Data
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