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Efficacy of a novel water soluble curcumin derivative versus Tadalafile in mediating erectile function

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Abstract

The present study was conducted to assess the efficacy of a novel curcumin derivative (NCD) versus Tadalafile in erectile signaling. The study was conducted on 15 control male rats and 75 diabetic male rats divided into the following groups:, diabetic, curcumin, NCD, Tadalafile and NCD combined with Tadalafile . Cavernous tissue gene expression levels of heme oxygenase-1 (HO-1), Nrf2, NFK-β, and p38, enzyme activities of heme oxygenase (HO) and nitric oxide synthase (NOS), cGMP and intracavernosal pressure (ICP) were assessed. Results showed that 12 weeks after induction of diabetes, erectile dysfunction (ED) was confirmed by the significant decrease in ICP, a significant decrease in cGMP, NOS, HO enzyme activities, a significant decrease in HO-1 gene and a significant elevation of NFK-β, p38 genes. Administration of all therapeutic interventions led to a significant elevation in ICP, cGMP levels, a significant increase in HO-1 and NOS enzymes, a significant increase in HO-1, and Nrf2 gene expression, and a significant decrease in NFK-β, p38 gene expression. NCD or its combination with Tadalafile showed significant superiority and more prolonged duration of action. In conclusion, NCD could enhance erectile function with more efficacy and more prolonged duration of action.

Keywords: Erectile dysfunction, cGMP, HO-1, Nrf2, p38, NFK-β, NOS, Rats.

1 Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism (Lindberg et al., 2012). Diabetes is the most common endocrine disorder and it was reported that by the year 2010 more than 200 million people worldwide will have DM and 300 million will subsequently have the disease by 2025 (King et al.,1998). Most cases of diabetes mellitus fall into the three broad categories of type 1, type 2 and gestational diabetes. A few other types are described.

In the Indian subcontinent and Southeast Asia, turmeric has traditionally been used as a treatment for inflammation, skin wounds, and tumors. Clinical activity of curcumin has yet to be confirmed; however, in preclinical animal models, curcumin has shown cancer chemopreventive, antineoplastic, and anti-inflammatory properties (Hatcher et al ., 2008). Turmeric extract (curcumin) has been shown to improve blood flow as well as strengthen blood vessels and ability to neutralize free radicals, chemicals that could damage cells. Turmeric extract (curcumin) can ward off cancer and tumorous growths (Aggarawl et al ., 2003).

Stimulation of insulin release and lowering blood glucose level in type II diabetes (Seo et al ., 2008). Erectile response depends on nitric oxide (NO) generated by NO synthase (NOS) enzyme of the nerves and vascular endothelium in the cavernous tissue. A role similar to that of NOS/NO signaling was proved for carbon monoxide (CO) produced by heme oxygenase (HO) enzyme (Abdel Aziz et al.,2009). Interestingly, hydrogen sulfide (H2S) a known vasodilator and smooth muscle relaxant was proved to be an inducer for HO-1 gene expression (Hua et al.,2013, D'Araio . et al., 2013). Moreover, (Abdel aziz et al., 2008). reported that the effect of sildenafil, verdenafil and tadalafil are partially mediated via upregulation of HO enzyme activity and cGMP and their effect is partially inhibited by HO inhibitor. The present study was conducted to compare the molecular and physiological effects of a novel water-soluble curcumin protein conjugates (NCD)

versus natural curcumin and Tadalafile in experimental diabetic model of erectile dysfunction (ED).

2 **Materials and Methods**

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(Jin-Jia Hu et al., 2008). A total number of 90 adult male white albino rats weight (180–200 g) were used in the current study after the approval of the Institutional Animal Care and Use Committee (IACUC).

Experimental diabetes was induced by a single intra peritoneal injection of 65 mg/kg body weight of streptozotocin (STZ) (Paget et al., 1964). The treatment phase of the study lasted twelve weeks according to the findings reported by (Li et al., 2011) who stated that erectile dysfunction is established 12 weeks after diabetic induction in rats. Curcumin dose was chosen according to our previous studies (Abdel Aziz et al.,2007, Zhang et al., 2013). Erectile dysfunction was proved by assessment of intracavernosal pressure (ICP) / mean arterial pressure (MAP) as a physiological index of erectile function. All drug interventions were initiated after 12 weeks of diabetes induction. Animals involve 15 albino male rats served as control group and 75 STZ-induced diabetic rats that were equally divided into the following groups: untreated Diabetic control group, Curcumin group which received 10 mg/kg body weight (B.Wt.) oral dose of natural curcumin, NCD group which received 2 mg/kg B.Wt. single oral dose of NCD, Tadalafile group which received Tadalafile citrate dissolved in distilled water (4 mg/kg; equivalent to 50-mg dose in 70-kg adult man according to Paget's table of experimental studies), rat group that received NCD combined with Tadalafile (NCD+ Tadalafile group) (Paget et al., 1964). Animals were euthanized after 1 hr, 24 hr and 5 days following drug intervention regimens. cavernous tissue cut into small pieces and homogenate in lyses buffer, samples were kept on crushed ice all times during the preparation then kept frozen at -70˚c till analysis. The following parameters were assessed in the cavernous tissue: HO enzyme activity (Abdel Aziz et al., 2008)' NOS activity (Moshag et al., 1995)., cGMP, intracavernosal pressure (ICP) as physiologic assessment of erectile function, and gene expression of HO-1, Nrf2, NFK-B, and p38 gene by quantitative real time PCR (Paget et al.,1964, Abdel Aziz et al., 2012).

3 Results

a. Change in HO-1 gene

Results showed that administration of either natural curcumin, NCD, Tadalafile, or NCD& Tadalafile combination led to a significant elevation in HO-1 gene expression after 24 hr persisting up to 5 days (with natural curcumin, NCD, NCD combination with Tadalafile) with more significant elevation with NCD administration in comparison to natural curcumin and Tadalafile rat groups. Combined NCD with Tadalafile showed significant enhancing effect when compared to Tadalafile alone. (Tables 1&2).

Table (1): HO-1 Gene in rats treated with curcumin, tadalafil after injection with streptozotocinat different periods:

Groups / Period	Mean ±SD	Control	DM	DM+ p.Curc.	DM+ Curc.D.	DM+ Znpp+ Curc.D	DM+ Tada.	DM+ Tada.+ Curc.D
HO-1 gene (1.5 H)	Mean ±SD	0.154a ±0.05	0.14a ±0.02	0.16b ± 0.05	0.19d 0.03	0.14a ±0.04	0.17c ±0.03	0.17c ±0.01
HO-1 gene (24 H)	Mean ±SD	0.150a ±0.05	0.13a ±0.02	0.58b ±0.28	0.89c ±0.13	0.55b ±0.16	0.53b ±0.3	0.98c ±0.01
HO-1 (1WK)	Mean ±SD	0.146a ±0.05	0.12a ±0.02	0.53b ±0.14	0.86c ±0.17	0.53b ±0.17	0.41b ±0.12	0.98c ±0.01

Table (2): Statistical ANOVA one way of HO-1 gene:

HO-1 gene / **Depend. Variable**	**Sum of Squares**	**Mean Square**	**F**	**P value**
Group	12.346	1.543	120.502	0.001
Period	9.285	4.643	362.511	0.001
Group#Period	5.582	0.349	27.241	0.001

b. Change in HO-1 enzyme activity

Results showed that there was a marked decrease in HO enzyme activity in diabetic rat group. Administration of either natural curcumin, NCD, sildenafil, or sildenafil combined with NCD led to a significant elevation in the enzyme activity in comparison to diabetic group that persisted up to 24 hr with all of the above mentioned treatment protocols and up to 5 days with NCD and combined NCD + Tadalafile Combined NCD with Tadalafile showed significant enhancing when compared to sildenafil alone (Tables 3&4).

Table (3): HO-1 enzyme activity in rats treated with curcumin, tadalafil after injection with streptozotocinat different periods :

Groups / Period	Mean ±SD	Control	DM	DM+ p.Curc.	DM+ Curc.D.	DM+ Tada.	DM+ Tada.+ Curc.D
HO-1 (1.5 H)	Mean ±SD	15.00cd ±1.41	7.00a ±1.94	12.00b ±1.05	17.50e ±1.18	19.00c ±1.25	19.00d ±2.91
HO-1 (24 H)	Mean ±SD	14.70c ±1.40	7.00a ±1.94	10.00b ±1.15	15.00c ±1.15	14.90c ±2.47	17.00c ±2.31
HO-1 (1WK)	Mean ±SD	15.10d ±1.42	7.00a ±1.94	7.40b ±1.65	9.50b ±1.08	7.40b ±1.78	12.00c ±2.01

Table (4): Statistical ANOVA one way of HO-1 enzyme:

HO-1 enzyme / Depend. variable	Sum of Squares	Mean Square	F	P value
Group	3224.80	403.10	102.83	0.001
Period	1270.42	635.21	162.05	0.001
Group#Period	988.77	61.79	15.76	0.001

c. Change in cGMP level

Results showed that there was a significant decrease in cGMP levels in the diabetic group. Administration of either curcumin, NCD or Tadalafile or combinations of NCD with Tadalafile led to a significant elevation of cGMP levels that persisted up to one week in comparison to the diabetic group. Administration of Tadalafile alone led to a significant elevation of cGMP levels in comparison to the diabetic group that persisted for 1 hr (Tables 5&6).

Table (5): Means of Tissue cGMP enzyme activity in rats treated with curcumin, tadalafil after injection with streptozotocinat different period s :

Groups / Period	Mean ± SD	Control	DM	DM+ p.Curc.	DM+ Curc.D.	DM+ Tada.	DM+ Tada.+ Curc.D
cGMP (1.5 H)	Mean ± SD	3.53a ± 0.85	0.50b ± 0.13	4.00bc ± 0.82	4.90bcd ± 1.1	4.00d ± 0.67	5.20d ± 1.72
cGMP (24 H)	Mean ± SD	3.58a ± 0.88	0.50b ± 0.13	4.00b ± 0.82	4.90bc ± 1.1	5.00bc ± 0.94	5.20c ± 1.72
cGMP (1WK)	Mean ± SD	3.55a ± 0.86	0.50b ± 0.13	4.10ca ± 0.99	5.20d ± 0.79	0.52cb ± 0.13	5.00d ± 2.58

Table (6): Statistical a nova one way of cGMP enzyme:

cGMP enzyme / Depend. variable	Sum of Squares	Mean Square	F	P value
Group	242.09	0.98	20.57	<0.001
Period	19.50	0.72	.001	1.00
Group#Period	390.6	24.41	1.113	.343

d. change in NOS enzyme activity

Results showed that there was a significant decrease in NOS enzyme activity levels in the diabetic group. There was a significant elevation in NOS activity levels in all treated rat groups that persisted up to 5 days in comparison to the untreated diabetic group (with curcumin, NCD, Tadalafile, NCD combined with Tadalafile). Administration of Tadalafile or its combinations with NCD led to a significant elevation in NOS levels after 24 hr in comparison to NCD rat group (Tables7&8).

Table (7): NOS enzyme activity in rats treated with curcumin, tadalafil after injection with streptozotocinat different periods :

Groups / Period	Mean ± SD	Control	DM	DM+ p.Curc.	DM+ Curc.D.	DM+ Tada.	DM+ Tada.+ Curc.D
NOS (1.5 H)	Mean ± SD	892.0b ± 60.05	726.0a ± 17.03	1191.0c ± 91.55	1218.0cd ± 66.51	1160.0c ± 74.58	1381.0e ± 151.1
NOS (24 H)	Mean ± SD	894.0ab ± 61.05	726.0a ± 17.03	1078.0bc ± 45	1300.0cd ± 80.73	1413.2de ± 486.93	1790.0f ± 322.33
NOS (1WK)	Mean ± SD	812.0a ± 47.56	726.0 ± 39.73	1096.30b ± 52.04	1181.0b ± 75.02	1180.0b ± 74.21	1280.0b ± 290.52

Table (8): Statistical a nova one way of NOS enzyme:

NOS enzyme / Depend. variable	Sum of Squares	Mean Square	F	P value
Group	3224.80	403.10	102.83	0.001
Period	1270.42	635.21	162.05	0.001
Group#Period	988.77	61.79	15.76	0.001

e. Change in ICP

Results showed that there was a significant decrease in ICP/MAP in the diabetic rat group. There was a significant progressive elevation in ICP/MAP that rises with increasing the frequency of electric current stimulation in all treated diabetic rat groups with all treatment regimens in comparison with untreated diabetic rat group. NCD showed superior effects in comparison to curcumin at 0.3 and 0.5 Hz (Tables 9&10).

f. Change in NFK-β

Results showed that there was a significant elevation in NFK-β in the diabetic group. There was a significant decrease in NFK -β in all treated diabetic rat groups with all treatment regimens that persisted up to 5 days in comparison to untreated diabetic group except with Tadalafile group where the effect persisted for 1 hr (Table 11&12).

g. Change in P38

Results showed that there was a significant increase in p38 gene expression in the diabetic group. There was a significant decrease in p38 gene that persisted up to 24 hr in the diabetic rat groups treated with curcumin, NCD, NCD combinations with Tadalafile in comparison to the untreated diabetic group (Table 13&14).

Table (9): ICP in rats treated with curcumin, tadalafil after injection with streptozotocinat different periods :

Groups / Period	Mean ± SD	Control	DM	DM+ p.Curc.	DM+ Curc.D.	DM+ Tada.	DM+ Tada.+ Curc.D
ICP (0.3 HZ)	Mean ± SD	1.00ab ± 0.41	.50a ± 0.23	1.50bc ± 0.35	2.20cd ± 0.73	2.00cd ± 0.76	2.20cd ± 0.69
ICP (0.5 HZ)	Mean ± SD	2.20ab ± 0.73	1.20a ± 0.47	2.30ab ± 0.66	3.50c ± 0.91	3.20bc ± 1.1	3.70c ± 0.97
ICP (0.8 HZ)	Mean ± SD	4.50bc ± 1.63	2.38a ± 0.62	4.20abc ± 1.22	5.10c ± 0.91	5.00c ± 2.05	5.50c ± 0.47
ICP (1 HZ)	Mean ± SD	5.00abc ± 2.05	2.80a ± 0.93	5.50bc ± 2.17	6.00bc ± 2.05	6.50c ± 1.84	6.60c ± 2.27

Table (10): Statistical a nova one way of ICP :

ICP / Depend. variable	**Sum of Squares**	**Mean Square**	**F**	**P value**
ICP (0.3 HZ)	75.74	0.40	13.30	<0.001
ICP (0.5 HZ)	131.80	0.70	13.20	<0.001
ICP (0.8 HZ)	247.86	1.77	7.31	<0.001
ICP (1 HZ)	452.03	3.49	6.03	<0.001

Table (11): NFK -β Gene in rats treated with curcumin, tadalafil after injection with streptozotocinat different periods :

Groups / Period	Mean ± SD	Control	DM	DM+ p.Curc	DM+ Curc.D.	DM+ Znpp+ Curc.D	DM+ Sildenf	DM+ Sildenfil + Curc.D	DM+ Tada.	DM+ Tada.+ Curc.D
NFK-β (1.5 H)	Mean ± SD	0.25a ± 0.02	0.39b ± 0.14	0.29ab ± 0.07	0.24a ± 0.05	0.24a ± 0.1	0.29ab ± 0.1	0.23a ± 0.03	0.26a ± 0.07	0.23a ± 0.02
NFK-β (24 H)	Mean ± SD	0.24a ± 0.02	0.36b ± 0.11	0.29ab ± 0.07	0.24a ± 0.05	0.24a ± 0.06	0.23a ± 0.03	0.23a ± 0.02	0.26a ± 0.07	0.23a ± 0.03
NFK-β (1 WK)	Mean ± SD	0.26a ± 0.03	0.38b ± 0.12	0.29a ± 0.07	0.24a ± 0.05	0.24a ± 0.06	0.23a ± 0.03	0.23a ± 0.02	0.26a ± 0.07	0. 23a ± 0.03

Table (12): Statistical ANOVA one way of NFK-β gene :

NFK-β / Depend. variable	Type III Sum of Squares	Mean Square	F	P value
Group	0.52	0.06	16.19	0.001
Period	0.01	0.01	0.27	**0.**76
Group#Period	0.01	0.01	0.27	0.76

Table (13): p38 Gene in rats treated with curcumin ,, tadalafil after injection with streptozotocinat different periods :

Groups / Period	Mean ±SD	Control	DM	DM+ p.Curc.	DM+ Curc.D.	DM+ Tada.	DM+ Tada.+ Curc.D
P38 (1.5 H)	Mean ±SD	0.13a ±0.06	0.29b ±0.07	0.18a ±0.08	0.15a ±0.03	0.28b ±0.06	0.16a ±0.03
P38 (24 H)	Mean ±SD	0.12a ±0.06	0.29b ±0.07	0.20a ±0.05	0.16a ±0.03	0.30b ±0.08	0.17a ±0.07
P38 (1WK)	Mean ±SD	0.14a ±0.06	0.29b ±0.07	0.26b ±0.05	0.24b ±0.05	0.29b ±0.07	0.26b ±0.05

Table (14): Statistical ANOVA one way of 38 gene:

P38 gene / Dependent Variable	Sum of Squares	Mean Square	F	P value
Group	0.78	0.09	32.96	0.001
Period	0.14	0.07	23.72	**0.**001
Group#Period	0.11	0.01	2.37	0.003

h. Change in Nrf2

Results showed that there was a significant increase in Nrf2 gene expression in diabetic rat groups treated with curcumin, NCD, combinations of NCD with Tadalafile in comparison to the untreated diabetic rat group and the control group (Table 15&16).

Table (15): Nrf2 Gene in rats treated with curcumin , tadalafil after injection with streptozotocinat different periods :

Groups / Period	Mean ±SD	Control	DM	DM+ p.Curc.	DM+ Curc.D.	DM+ Tada.	DM+ Tada.+ Curc.D
Nrf2 (1.5 H)	Mean ±SD	0.251a ±0.03	0.25a ±0.04	0.35ab ±0.11	0.42b ±0.06	0.26a ±0.05	0.42b ±0.07
Nrf2 (24 H)	Mean ±SD	0.254a ±0.03	0.25a ±0.04	0.37b ±0.09	0.45b ±0.06	0.27a ±0.05	0.45b ±0.06
Nrf2 (1WK)	Mean ±SD	0.252a ±0.03	0.25a ±0.04	0.36bc ±0.11	0.43c ±0.07	0.26ab ±0.05	0.43c ±0.06

Table (16): Statistical ANOVA one way of Nrf2 gene:

Nrf2 gene / Dependent Variable	Sum of Squares	Mean Square	F	P value
Group	1.66	0.20	43.81	0.001
Period	0.02	0.01	3.07	**0.**04
Group#Period	0.17	0.01	2.29	0.04

4 Discussion

Erectile response depends on nitric oxide (NO) generated by NO synthase (NOS) enzyme of the nerves and vascular endothelium in the cavernous tissue. NO activates soluble guanylate cyclase (sGC), leading to the production of cyclic guanosine monophosphate (cGMP) which activates cGMP-dependent protein kinase that activates Ca^{2+}/ATPase pump with subsequent activation of Ca^{2+}/K efflux pump extruding Ca^{2+} across the plasma membrane resulting in smooth muscle cell relaxation. A role similar to that of NOS/NO signaling has been proved for carbon monoxide (CO) produced in mammals from heme catabolism by heme oxygenase (HO) enzyme **(**Abdel Aziz et al., 2009). The concept that HO-derived CO could play a role in mediating erectile function acting in synergism with, or as a potentiator for, NOS/NO signaling pathway is gaining momentum. CO/HO signaling pathway has been shown to partially mediate the actions of oral phosphodiesterase-5 inhibitors (PDE5 Is). (Abdel aziz et al. 2007, 2008) reported that the effect of the three available PDE5 Is; sildenafil, verdenafil and tadalafil are partially mediated via upregulation of HO enzyme activity and their effect is partially inhibited following administration of HO inhibitor. Results showed that administration of all drug regimen interventions led to a significant increase in ICP/MAP as compared to the diabetic group. The present result is in agreement with other reported, showed the Endothelial dysfunction is the main a etiologic factor of vasculogenic erectile dysfunction (ED) (Strong TD et al., 2008). In diabetes hyperglycemia affects endothelial functions by synthesis of growth factors and vasoactive agents (Yang et al., 2010). Hyperglycemia activate protein kinase-C (PKC) that induces phosphorylation of p115RhoGEF, a guanine nucleotide exchange factor for Rho GTPase (Xu et al., 2009, Chen et al., 2005). Active RhoA is implicated in arginase induction leading to decrease in nitric oxide (NO) bioavailability (Romero et al., 2008, Abdel Aziz et al., 2009). Furthermore, activated PKC leads to sustained increases in the production of superoxide anion (O2•-) that activates NFK-β and affecting the expression of endothelial NOS (Enos) (Zhang et al., 2008). Biochemical Results by Elisa method showed revealed that there were significant decreases in cavernous tissue cGMP, NOS and hemeoxygenase enzymatic activities in diabetic rats as compared to control rats. These results is agreement with others who found (Wang et al.,

2003, Abdel Aziz et al., 2009) who reported that administration of either curcumin, NCD, Tadalafile or Tadalafile combined with NCD led to significant elevations in cGMP levels, NOS and HO-1 cavernous tissue enzyme levels with significantly higher sustained effect in favor of NCD. The molecular study by Real time RT PCR.the gene expression profile of NFK-β and p38 were significantly increased in STZ-induced diabetic rat. Administration of either natural curcumin or NCD led to a significant lowering effect on their gene expression with more significant superior effects with NCD. Nrf2 gene expression was unchanged in STZ-induced diabetic rats, whereas its levels were significantly elevated with curcumin and NCD. In concordant with these results (Aggarwal et al.,2009) reported stated that curcumin suppresses NFK-β and activates Nrf2 cell-signaling pathways. Reported that HO protects NO through scavenging of reactive oxygen species (ROS), preventing the formation of peroxynitrite and subsequent degradation of NO. (Ahmad et al.,2009) stated that over expression of HO-1 may mediate an increase in eNOS and a decrease in iNOS, with restoration of vascular responses in diabetic rats. Curcumin as an inducer of HO-1 could indirectly potentiate eNOS effects on vascular endothelium. (Williams et al.,2005) stated that CO like NO, acts as a vasorelaxant. HO-1-derived CO has a positive effect on both sGC and cGMP levels in vascular endothelial cells (Aggarwal et al., 2009).

In conclusion, NCD could enhance erectile function in a diabetic model via up-regulation of cavernous tissue levels of HO-1 gene and cGMP. NCD is superior to curcumin with more prolonged duration of action.

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