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Protective Effect of Curcumin on Diethanolamine-Induced Toxic Effects on Human Spermatozoa: An *in Vitro* Study

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Abstract

Curcumin is a yellow pigment from Curcuma longa which has desirable preventive or putative therapeutic properties. In this study protective effect of curcumin on diethanolamine-induced toxicity on human spermatozoa in in vitro condition was investigated. For this study samples were collected from normal healthy donors. After liquefaction, samples were used for preparation of sperm suspension to evaluate sperm motility, sperm viability and sperm morphology. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's test and the level of significance was accepted with p<0.05. When sperm suspension was treated with diethanolamine (300 μ g/ml) it caused significant decrease in sperm motility and sperm viability as compared to control. Treatment also caused significantly increased different kinds of sperm morphological abnormalities as compared to control. Addition of different concentrations (10-40 μ g/ml) of curcumin to sperm suspension along with diethanolamine caused significant increase in sperm motility and sperm viability as compared to control. Addition different concentrations (10-40 μ g/ml) of curcumin to sperm suspension along with diethanolamine caused significant increase in sperm motility and sperm viability as compared to created which was time-dependent as well as concentration-dependent. As compared to treated, concentration-dependent decrease in various kinds of morphological abnormalities were also observed. This findings clearly indicate that curcumin ameliorates diethanolamine-induced spermatotoxic effect on human spermatozoa.

Keywords: Diethanolamine (DEA), curcumin, spermatozoa, motility, viability, morphology.

Introduction

Diethanolamine (DEA) is an alkanolamine which unites both the properties of amines and alcohol. DEA is widely used as industrial chemicals¹, agricultural chemicals, metal working fluids and personal care products like cosmetics^{2,3}, shampoos and hair conditioners. It is used in pharmaceutical industries as buffer and stabilizer for certain drugs⁴ and also used as raw materials in the production of some drugs. The most common dermal exposure to DEA in humanbeings are from consumer products such as soaps, shampoos, cosmetics, detergents and other surfactants that contain DEA or fatty acid conjugates of DEA⁵. Occupational exposure to DEA occurs by the use of lubricating liquids in industrial processes⁵. Human exposure to DEA is also possible through cigarette smoking⁶.

Approximately 800,000 workers are potentially exposed to DEA per year, estimated by the National Institute for Occupational Safety and Health⁵. DEA is readily absorbed through skin. It can be incorporated into phospholipids and can inhibit synthesis of phospholipid derivatives of choline and ethanolamine⁷. It has been previously reported that DEA alters cell proliferation, choline metabolism and increase rate of apoptosis in *in vitro* condition⁸. DEA also alters DNA methylation in mouse hepatocytes⁹. Oral and dermal exposure to DEA caused alterations in rodent testis¹⁰⁻¹¹.

Herbal medicines have been used from ancient times to cure large number of diseases. About 70-80% of the world

populations, mainly in developing countries use herbal medicine for primary health care¹², because these herbal drugs have no side effect besides being cheap and easily locally available¹³. Curcumin is a polyphenol major chemical component of turmeric power, produced from the rhizome of the plant $longa^{14}$. Curcuma Curcuminposseseswide variety of pharmacological activities such asanti-inflammatory¹⁵, anti-platelet¹⁶, antioxidant¹⁷, cancer chemopreventing¹⁸, anti cancer¹⁹, antimutagenic²⁰ and anti-HIV²¹. The most important feature of curcumin is that it has no side effects and therapeutic agent with multiple beneficial functions²². Protective effect of curcumin on aflatoxin-induced lipid peroxidation in testis of mice and toxicity in mice spermatozoa have also been reported^{23,24}. Deviet al.²⁵ also reported that curcumin shows protective role against chromium-induced genotoxicity in germ cells of male mice.

Infertility is a widespread problem. The semen of the average man today has half the number of sperm with poorer quality, than 50 years ago. Hence, in present investigation, studies were carried out to evaluate the protective effect of curcumin on DEA-induced spermatotoxic effect on human spermatozoa.

Material and Methods

Semen samples were obtained in vials from 10 normal healthy adult volunteers of age 25-30 years after 2 days of sexual abstinence and brought to the laboratory in cold condition for semen analysis. Semen analysis was done after liquefaction. For this study semen samples with sperm counts above 50 million/ml with normal morphology, rapid, linear, progressive motility and viability above 50% were considered. After analysis, semen samples were used for sperm suspension preparation in normal saline (0.9% NaCl). DEA and curcumin were also prepared in normal saline (0.9% Nacl)^{26,27}.

Study Design: For evaluation of toxic effects of DEA on human spermatozoa following sets of tubes were prepared. i. Control tubes containing 0.5 mL sperm suspension, ii. DEA-treated tubes containing 0.5 mL sperm suspension and 300 μ g/mL DEA, iii. Antidote control tubes containing 0.5 mL sperm suspension and 40 μ g/mLcurcumin, iv. DEA and curcumin-treated tubes containing 0.5 mL sperm suspension, 300 μ g/mL DEA and 10-40 μ g/mLcurcumin.

In each tubes final volume was made upto 1 mL with addition of normal saline and incubated at 37°C for 60 min to evaluate sperm motility, sperm viability and sperm morphological abnormalities.

Sperm motility: Sperm motility was measured at different time interval (0, 15, 30, 45, 60) by counting both motile and non-motile spermatozoa in at least 10 separate randomly selected fields. Percent motility was calculated by following formula²⁸.

% Motility = $\underline{\text{Number of motile spermatozoa x 100}}$ Total number of spermatozoa

Sperm viability: Sperm viability at different time interval (0, 15, 30, 45, 60) was measured by counting live and dead spermatozoa after trypan blue staining in at least 10 separate randomly selected fields. Percent viability was calculated by following formula²⁸.

% Viability = <u>Number of live spermatozoa x 100</u> Total number of spermatozoa

Sperm morphology: Sperm morphology was determined by using Giemsa stain²⁹. Total 150 spermatozoa were scored per slide. Percent sperm morphology abnormalities were calculated by following formula.

% Abnormal sperm = <u>Number of abnormal spermatozoa x 100</u> Morphology Total number of spermatozoa

Statistical Analysis: Statistical analysis was done by analysis of variance (ANOVA) followed by Tukey's test using GraphPad prism software. Data are expressed as the mean \pm S.E.M. The level of significance was accepted with * p<0.05. Pearson's correlation analysis was used to determine the correlation between control and treated.

Results and Discussion

Addition of DEA to sperm suspension caused significant (p<0.05) decrease in sperm motility as compared to control in *in*

vitro condition. This effect was time-dependent (r=-0.9157) (table 1). DEA also caused significant, time-dependent (r=-9276) decrease in sperm viability as compared to control (table 2). DEA treated spermatozoa showed different kinds of morphological abnormalities such asswollen head, round head, bent neck, swollen mid piece, decapitation, coiled tail, tail deformities, head-head agglutination, tail-tail agglutination and head-tail agglutination as compared to control at 60 min (table 3). DEA is known to alter phospholipid metabolism, structure and function^{11,30}. Phospholipids are most representative component of sperm cell membrane. It has been reported that DEA caused structural and functional changes in mitochondria by altering phospholipid metabolism³¹. Spermatozoa are rich in mitochondria because they need constant supply of energy for motility. Any alteration in mitochondria caused decrease in sperm motility. It has been previously mention that DEA alter the synthesis of phospholipid derivatives of choline and ethanolamine which are essential for lipid metabolism⁷. DEA competitively inhibits the cellular uptake of choline in invitro condition^{32,33}.

Floyd et al.³⁴ also reported that choline deficiency increased generation of free radicals and also increased susceptibility to oxidative damage which may induce DNA damage and alters gene expression. Oxidative damage leads to ultimate death of the cell and decrease the sperm motility³⁵. So oxidative stress is one major factor that affect sperm motility and affect fertility status³⁶. Alterations in phospholipids affect the membrane integrity and its nature of semi- permeability. When the spermatozoa were stained with trypan blue, it showed large number of dead spermatozoa due to alterations in membrane integrity. Another major factor for loss of sperm motility is loss of membrane permeability. DEA also caused various kinds of sperm morphological abnormalities by causing DNA damage through oxidative stress. DNA and phospholipids are main major components target for free radicals. Alterations in membrane integrity also affect the sperm morphology. Thus DEA affect sperm function and structure and eventuallyfertility status.

Addition of curcumin (10-40 µg/ml) along with DEA significantly ameliorates DEA-induced reduction in sperm motility as compared to treated. This ameliorative effect was dose-dependent (r=0.9082, 8346, 833, 8376) and timedependent (r=-0.9924, -0.9982, -0.9939, -0.9919) (table 1). Similarly, curcumin also ameliorates DEA-induced reduction in sperm viability as compared to treated. This ameliorative effect was dose-dependent (r=0.9179, 0.8328, 0.8191, 0.825) and time-dependent (r=-0.9912, -0.9746, -0.9876 -0.9936) (table 2). Curcumin also significantly decreased sperm morphological abnormalities as compared to treated (r=-0.9979) (table 3). There is no difference between control and antidote control in sperm motility, sperm viability and sperm morphology (Table 1,2,3). Antioxidants are the major defence factors against oxidative stress induced by free radicals³⁷. Curcumin is an effective antioxidant which has unique conjugated structure, includes two methoxylated phenols and enol form of diketone. This unique structure of curcumin shows typical radical trapping ability as a chain-breaking antioxidant³⁸. By this trapping ability curcumin protect spermatozoa from free radicals and increase motility and viability. Chan and Wu³⁹ reported thatcurcumin

showed protective effect on methylglyoxal-induced oxidative DNA damage and cell injury in human mononuclear cells. Curcuminmay also protect spermatozoa from morphological abnormalities by preventing oxidative DNA damage.

Table-1
Ameliorative effect of curcumin on DEA-induced changes in motility of human spermatozoa in vitro

DEA	Curcumin	Duration of treatment (min)						
concentration (µg/ml)	(µg/ml)	15	30	45	60	per duration		
1. Control								
0(control)	0	77.78±0.98	74.08±1.63	70.40±1.99	67.20±2.23	-0.9994		
0(Antidote control)	40	75.96±1.24	73.96±1.32	69.62±1.27	65.31±0.86	-0.988		
2.Diethanolamine-treated								
300	0	33.31 ± 2.36^{a} 10.65 $\pm2.45^{a}$ 1.36 $\pm0.86^{a}$ 0 ^a						
3.Diethanolamine + curcumin-treated								
300	10	56.14 ± 0.82^{b}	54.02 ± 1.19^{b}	50.91 ± 0.55^{b}	47.12 ± 0.53^{b}	-0.9924		
300	20	60.25 ± 0.83^{b}	57.12 ± 0.76^{b}	53.65 ± 0.89^{b}	51.13 ± 0.83^{b}	-0.9982		
300	30	64.12 ± 1.11^{b}	61.88 ± 0.63^{b}	59.38 ± 0.63^{b}	57.98 ± 0.54^{b}	-0.9939		
300	40	69.20 ± 0.92^{b}	65.84 ± 0.73^{b}	64.00 ± 0.85^{b}	60.13 ± 1.44^{b}	-0.9919		
r value as per concentration		0.9082	0.8346	0.833	0.8378			

Sperm motility at 0 min was 82.44%, Values are mean \pm S.E.M., n=10, ^{*a*}p<0.05, as compared to control, ^{*b*}p<0.05, as compared to to toxin-treated, r value shows Pearson correlation. (Horizontal is concentration-dependent and vertical is time- dependent).

Amelio	orative effect of	curcumin on DEA-i	Table-2 induced changes in	viability of human	spermatozoa <i>in vit</i>	ro				
DEA	Curcumin		r value as							
Concentration µg/ml	(µg/ml)	15	30	45	60	per duration				
	-	1	1. Control			1				
0(control)	0	0 84.00±0.90		77.46±0.74	73.97±1.27	-0.9956				
0(Antidote Control)	40	81.65±0.72 78.63±0.69 75.82±1.48 70.52±1.19								
	2. Diethanolamine-treated									
300	300 0 34.67 ± 3.59^{a} 11.98 ± 0.38^{a} 3.65 ± 0.87^{a} 0.51 ± 0.51^{a}									
		3. Diethand	olamine+Curcumin	n-treated						
300	10	59.08 ± 0.79^{b}	57.38±0.61 ^b	54.13±0.45 ^b	50.94 ± 0.55^{b}	-0.9912				
300	20	$61.74 \pm .0.95^{b}$	60.86 ± 0.86^{b}	57.03±1.45 ^b	53.79 ± 0.47^{b}	-0.9746				
300	30	67.23 ± 0.92^{b}	63.43 ± 1.00^{b}	61.93±1.21 ^b	58.93±0.75 ^b	-0.9876				
300	0 40 73.76 ± 0.98^{b} 70.33 ± 0.93^{b} 65.68 ± 0.51^{b} 63.26 ± 0.59^{b}					-0.9936				
r value as per concentraton		0.9179	0.8328	0.8191	0.825					

Sperm viability at 0 min was 86.32%, Values are mean \pm S.E.M., n=10, ^{*a*}p<0.05, as compared to control, ^{*b*}p<0.05, as compared to toxin-treated, r value shows Pearson correlation. (Horizontal is concentration-dependent and vertical is time- dependent)

Ameli	orative	effect of	curcumit	n on DEA	A-induce	ed chang	ges in mo	rpholog	gy of hu	man spe	ermatozo	a <i>in vitro</i> al	t 60 min
			% Various kinds of sperm morphological abnoramalities										
DEA Concentra-tior (µg/ml)	Curcumin (µg/ml)	Total abnormality	Normal sperm	Swollen head	Round head	Bent neck	Swollen Mid piece	Coiled tail	Tail Deformities	Decapit-ation	Head-Head Aggluti nation	Head-Tail Aggluti nation	Tail- Tail Aggluti nation
		-		-	-	1. (Control		-	-			
0 control	0	4.88± 0.58	90.44± 1.45	0.88± 0.58	0.44± 0.22	0.66± 0.38	0.88± 0.22	1.33± 0.38	0.88± 0.58	1.33± 0.38	1.33± 0.01	0.88± 0.44	0.88± 0.22
0 Antidote Control	40	5.77± 0.44	88.22± 1.35	1.11± 0.58	0.66± 0.38	0.88± 0.22	1.11± 0.22	1.33± 0.38	1.11± 0.44	1.55± 0.22	1.55± 0.22	1.11± 0.22	1.11± 0.22
					2. I	Diethano	lamine-	treated					
300	0	45.56± 2.12 ^a	46.89 ± 2.22^{a}	8.00 ± 0.76^{a}	6.88 ± 0.58^{a}	$\begin{array}{c} 3.11 \pm \\ 0.58^a \end{array}$	6.00 ± 0.38^{a}	5.33 ± 0.38^{a}	3.77 ± 0.22^{a}	5.11 ± 0.44^{a}	5.77 ± 0.22^{a}	4.22 ± 0.22^{a}	$\begin{array}{c} 4.44 \pm \\ 0.58^a \end{array}$
	•	r		3	. Dietha	nolamin	e + curc	umin-tro	eated				
300	10	37.33± 1.01 ^b	53.78± 1.17	7.33± 0.76	5.11± 0.22b	3.55± 0.22	5.33± 0.66	4.44± 0.44	2.88± 0.58	4.88± 0.22	4.66± 0.38	3.77± 0.44	4.22± 0.22
300	20	26.22 ± 1.55^{b}	67.33 ± 1.38^{b}	5.77± 0.58	3.66 ± 0.33^{b}	2.44± 0.44	3.11 ± 0.58^{b}	3.11 ± 0.44^{b}	2.00± 0.38	3.33 ± 0.38^{b}	3.33 ± 0.38^{b}	2.88± 0.22	3.11± 0.22
300	30	17.11 ± 0.80^{b}	79.33 ± 0.66^{b}	3.55 ± 0.44^{b}	2.22 ± 0.22^{b}	1.77± 0.44	2.00 ± 0.38^{b}	2.00 ± 0.38^{b}	1.33 ± 0.38^{b}	2.22 ± 0.22^{b}	2.00± 0.38 ^b	1.55± 0.22 ^b	2.00 ± 0.38^{b}
300	40	9.77 ± 0.96^{b}	86.44 ± 1.35^{b}	2.66 ± 0.38^{b}	1.11 ± 0.22^{b}	0.88 ± 0.22^{b}	1.33 ± 0.38^{b}	1.55 ± 0.22^{b}	1.11 ± 0.22^{b}	1.55 ± 0.22^{b}	1.55 ± 0.22^{b}	1.11 ± 0.22^{b}	1.11± 0.22 ^b

	Table-3
1	Ameliorative effect of curcumin on DEA-induced changes in morphology of human spermatozoa in vitro at 60 min

Values are mean \pm S.E.M., n=10, ^{*a*}p<0.05, as compared to control, ^{*b*}p<0.05, as compared to toxin-treated, r value of Total sperm morphological abnormality= -0.9979

Conclusion

It can be concluded from this study that DEA cause significant decrease in sperm motility and sperm viability. DEA also caused significant increase in sperm morphological abnormalities and may cause male fertility. Treatments with curcumin along with DEA improve the sperm characteristics and eventually improve the male fertility status. Our findings suggest that it may be possible to use dietary curcumin for prevention ofmale infertility.

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