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Spermatotoxic effect of diethanolamine: An *in vitro* study

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ABSTRACT

Objective: To determine *in vitro* spermatotoxic effect of diethanolamine on human spermatozoa. **Methods:** For this study, samples were collected from normal healthy donors. After liquefaction, samples were used for preparation of sperm suspension. Sperm suspension was incubated with different concentrations (100–500 μg/mL) of diethanolamine to evaluate sperm parameters such as 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 $P<0.05$. **Results:** The results showed that diethanolamine caused significant decrease in sperm motility and sperm viability which was concentration and time-dependent. Microscopic analysis revealed concentration-dependent increase in various kinds of sperm morphological abnormalities. **Conclusion:** DEA is spermatotoxic which alters structure and function of human spermatozoa and may affect male reproductive health.

1. Introduction

Male fertility has been deteriorated in many countries during last few decades. It is commonly due to poor sperm quality [1,2]. World Health Organization (WHO) estimated that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollutants [3]. General population are unwittingly exposed to numerous harmful chemicals. These harmful chemicals can find their way into the system. Frequent exposure to chemical products put men to higher risks of poor sperm quality.

Diethanolamine is a class of organic compounds that combines the properties of amines and alcohols (alkanolamine) which is a widely used as industrial chemicals [4], agricultural chemicals, metal working fluids and personal care products like cosmetics [5,6], shampoos and hair conditioners. Aqueous DEA solutions are used as solvents for numerous drugs that are administered intravenously [7,8]. It is used in pharmaceutical industries as buffer and stabilizer for certain drugs [9] and also used as raw materials in the production of antihistamines, antimalarials, antibiotics, local anesthetics, antidepressants, and muscle relaxants drugs. DEA is widely used in preparation of diethanolamine salts of long chain fatty acids that are formulated into diethanolamine soaps. General population may be exposed to DEA through cigarette smoking [10]. DEA may be released to the environment in emissions from sites of its manufacture or industrial use and from application of agricultural chemicals.

Humanbeings are exposed to DEA via dermal exposure to consumer products (Soaps, shampoos, and cosmetics) and occupational exposure to DEA may occur by inhalation of vapors and aerosols and by skin contact during the use of DEA in many industries [11,12]. The National Institute for Occupational Safety and Health estimates that the number of workers potentially exposed to DEA is approximately 800 000 per year [13].

DEA is readily absorbed through skin. According to National Toxicology Program DEA was eliminated very slowly in the urine and feces of rats and mice after single intravenous, oral or dermal adminisration[14]. DEA is

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incorporated into membrane phospholipids and interacts with lipid metabolism by inhibiting incorporation of ethanolamine and choline into phospholipid [15]. It has been previously reported that DEA exposure caused alterations in rodent testis [16,17].

DEA was selected for evaluation because of its large scale production and its pattern of use indicate the potential for widespread human exposure. The aim of this study was to evaluate the effect of DEA on sperm parameters such as sperm motility, sperm viability and sperm morphology in *in vitro* condition.

2. Materials and methods

Analytical grade diethanolamine was purchased from Merck specialities Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Semen samples were collected in vials from 10 normal healthy adult donors (age 23-25 years) after two days of abstinence and brought to the laboratory in cold condition. For this *in vitro* study, semen samples with sperm counts above 50 millions/mL with normal morphology, rapid, linear, progressive motility and viability above 50% were considered. After liquefaction semen samples were used for sperm suspension preparation. Sperm suspension was prepared in normal saline (0.9% NaCl) [18]. Also various concentration of DEA was prepared in normal saline (0.9% NaCl). In this experiment control tubes contained 0.5 mL sperm suspension and treated tubes contained 0.5 mL sperm suspension with different concentrations of DEA (100-500 μg/mL). In each tube final volume was made up to 1 mL with addition of normal saline and incubated at 37 ℃ for 60 min.

2.1. Sperm motility

Sperm motility was determined by counting both motile and non motile spermatozoa in at least 10 separate and randomly selected fields. Motility was measured at different time interval (0, 15, 30, 45, 60 min). Percent motility was calculated by following formula [19].

$$\% \text{ Motility} = \frac{\text{Number of motile spermatozoa}}{\text{Total number of spermatozoa}} \times 100$$

2.2. Sperm viability

Sperm viability (using trypan blue) was determined by counting live and dead spermatozoa in at least 10 separate and randomly selected fields. Viability was measured at different time interval (0, 15, 30, 45, 60 min). Percent viability was calculated by following formula [19].

$$\% \text{ Viability} = \frac{\text{Number of live spermatozoa}}{\text{Total number of spermatozoa}} \times 100$$

2.3. Sperm morphology

After completion of sperm motility and sperm viability experiments sperm morphological assessments were carried out by using Gimesa stain [20]. Examination of the stained slides were carried out under light microscope. Total 150 spermatozoa per slide were scored. Percent sperm morphological abnormalities were calculated by following formula.

$$\% \text{ Abnormal sperm morphology} = \frac{\text{Number of abnormal spermatozoa}}{\text{Total number of spermatozoa}} \times 100$$

2.4. Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) followed by Tukey's test using GraphPad prism software. Data is expressed as the means ± 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.

3. Results

Figure 1 shows untreated control sperms with normal head, mid piece and normal tail length. Figure 2-6 show effect of different concentrations of DEA on human spermatozoa. Maximum sperm morphological abnormalities were observed with 500 μg/mL of DEA at 60 min.

Addition of DEA (0-500 μg/mL) to sperm suspension caused significant (P<0.05) decrease in sperm motility *in vitro* as compared with control. This decrease was concentration-dependent (r=0.9621,-0.8829,-0.7388.-0.6857, respectively) as well as time-dependent (r=-0.9994, -0.9603,-0.9639,-0.9156,-0.8597,-0.8427, respectively) (Table 1). Similarly, addition of DEA to sperm suspension caused significant decrease in sperm viability as compared with control which was concentration dependent (r= -0.9561, -0.8825,-0.7799,-0.7060, respectively) as well as time-dependent (r=-0.9955,-0.9792,00.9450,-0.9276, -0.8788, -0.8447, respectively) (Table 2). Addition of DEA to sperm suspension for 60 min also caused significant increase in sperm morphological abnormalities as compared with control. This increase was concentration-dependent (r=0.9974) (Table 3). Table 3 also shows that different kinds of sperm morphological abnormalities (swollen head, round head, bent neck, swollen mid piece, decapitation, coiled tail, tail deformities, head-head agglutination, tail-tail agglutination and head-tail agglutination) increase with increasing concentration of DEA.

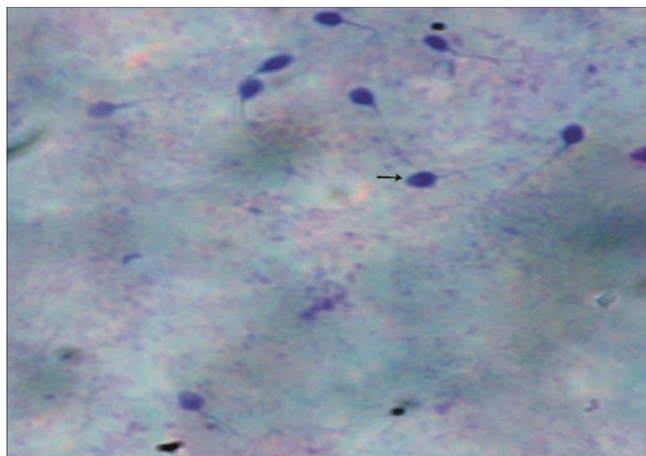

Figure 1. Untreated control sperms showing normal head, mid piece and normal tail length and morphological structure.

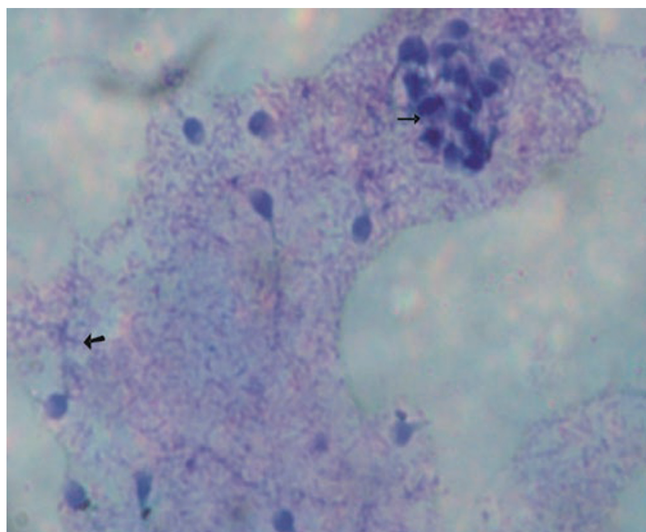

Figure 2. 100 μg/mL DEA treated sperms showing head-head agglutination and tail deformities.

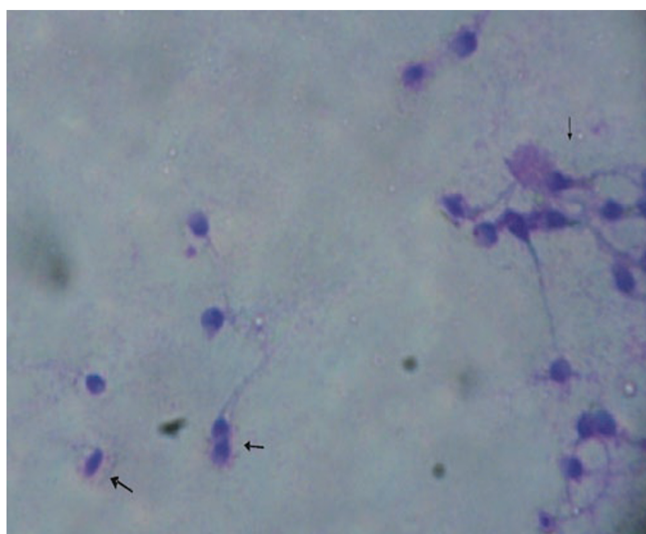

Figure 3. 200 μg/mL DEA treated sperm shows head-head agglutination, head-tail agglutination and bent neck.

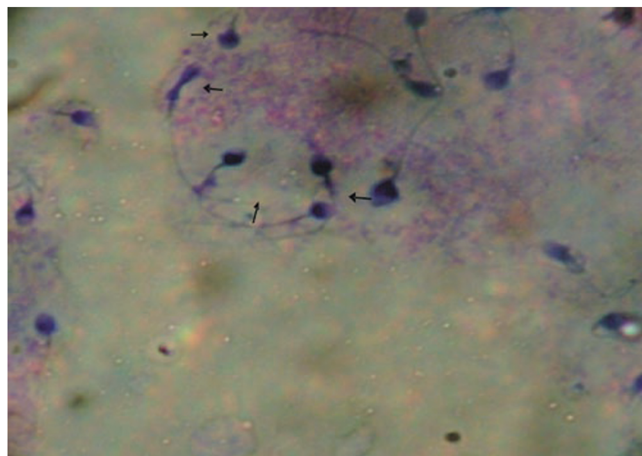

Figure 4. 300 μg/mL DEA treated sperms showing swollen head, swollen mid piece, round head and tail-tail agglutination.

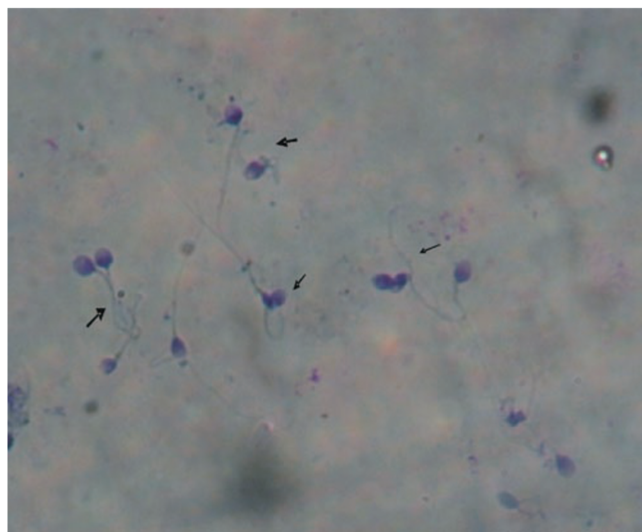

Figure 5. 400 μg/mL DEA treated sperms showing increased head-head agglutination, tail-tail agglutination, coiled tail and other tail deformities.

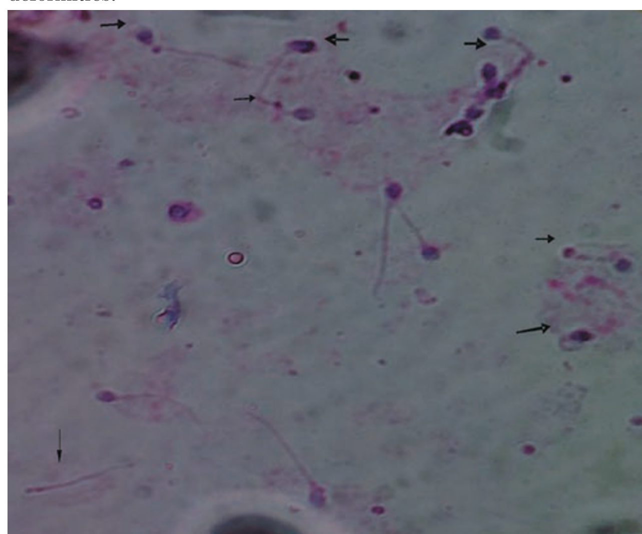

Figure 6. 500 μg/mL DEA treated showing highly increased decapitation, round head, swollen head, bent neck, coiled tail, tail-tail agglutination and other tail deformities.

Table 1
Effect of DEA on percent motility of human spermatozoa *in vitro*.

DEAconcentration (μg/mL)	Duration of treatment (min) motility at 0 min–82.44%				*r* value as per duration
	15	30	45	60	
Control	77.79±0.98	74.08±1.62	70.41±1.99	67.20±2.23	–0.9994
100	61.70±2.24*	30.38±4.21*	9.73±2.13*	3.66±1.44*	–0.9603
200	37.51±2.95*	18.25±3.26*	4.85±0.71*	0.78±0.78*	–0.9639
300	33.31±2.36*	10.65±2.45*	1.36±0.86*	0*	–0.9156
400	23.46±2.78*	4.13±2.27*	0.42±0.42*	0*	–0.8597
500	18.62±4.46*	2.56±1.66*	0.36±0.36*	0*	–0.8427
r value as per concentration	–0.9621	–0.8829	–0.7388	–0.6857	

Values are mean±S.E.M. , n=10. *P<0.05, as compared with control, *r* value shows pearson correlation. (Horizontal is concentration dependent and verical is time dependent).

Table 2
Effect of DEA on percent viability of human spermatozoa *in vitro*

DEA Concentration (μg/mL)	Duration of treatment (min) viability at 0 min–86.32%				*r* value as per duration
	15	30	45	60	
Control	84±0.90	79.89±1.54	77.47±0.74	73.97±1.27	–0.9955
100	63.78±2.08*	35.37±0.97*	17.58±1.88*	6.63±0.38*	–0.9792
200	45.72±1.16*	18.41±2.18*	7.19±0.84*	1.6±0.81*	–0.9450
300	34.67±2.65*	11.99±0.38*	3.65±0.87*	0.51±0.51*	–0.9276
400	31.40±0.73*	6.74±0.59*	1.66±0.21*	0*	–0.8788
500	25.53±1.01*	3.56±0.34*	1.01±0.52*	0*	–0.8447
r value as per concentraton	–0.9561	–0.8825	–0.7799	–0.7060	

Values are mean±S.E.M. , n=10. *P<0.05 as compared with control, *r* value shows pearson correlation. (Horizontal is concentration dependent and verical is time dependent).

Table 3
Effect of DEA on percent various kinds of morphological abnormalities of human spermatozoa *in vitro* at 60 min.

DEA Concentra –tion (μg/mL)	Total abnormality	Normal sperm	% Various kinds of sperm morphological abnoramlities									
			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
Control	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
100	16.44±0.58*	78.00±1.38*	2.66±0.66	2.66±0.38*	1.55±0.22	2.22±0.22	2.66±0.38	2.00±0.38	2.22±0.22	2.44±0.22	2.00±0.38	1.55±0.22
200	29.78±1.73*	63.33±2.52*	5.77±0.58*	4.44±0.22*	2.66±0.38*	3.11±0.80*	3.77±0.44*	2.88±0.22*	3.77±0.44*	4.22±0.22*	2.88±0.22*	3.11±0.58*
300	46.67±2.69*	46.89±2.22*	8.00±0.76*	6.88±0.58*	3.55±0.22*	6.00±0.38*	5.33±0.38*	3.77±0.22*	5.11±0.44*	5.77±0.22*	4.22±0.22*	4.44±0.58*
400	64.22±0.96*	26.88±2.47*	9.77±0.58*	8.00±0.38*	5.33±0.38*	8.00±0.38*	7.55±0.44*	5.11±0.44*	7.77±0.80*	9.33±0.76*	6.66±0.38*	5.55±0.22*
500	79.78±1.17*	13.11±1.55*	12.44±0.58*	10.00±0.38*	6.44±0.44*	8.66±0.38*	10.22±0.58*	5.55±0.44*	9.11±0.44*	10.22±0.58*	7.77±0.22*	6.44±0.58*

Values are mean±S.E.M. , n=10. *P<0.05 as compared with control, *r* value for total abnormality = 0.9974, *r* value shows pearson correlation.

4. Discussion

The present study clearly indicates that addition of DEA to sperm suspension caused concentration and time-dependent reduction in sperm motility in vitro. DEA disturbs the phospholipids metabolism, structure and function [17,21]. Phospholipids are essential for normal membrane structure and function. Phospholipids are the most representative lipid fraction of the sperm cell membrane and phosphatidylcholine and phosphatidylethanolamine are the major components of them[22,23]. Any alterations in phospholipids metabolism cause functional change in mitichondria [24-26] Therefor, alteration in phospholipids composition and mitochondrial function can decrease spermmotility.

Diethanolamine is known to create choline deficiency. It has been previously reported that DEA competitively inhibits the cellular uptake of choline in vitro [27,28]. Choline deficiency include increased generation of free radicals and increased susceptibility to oxidative damage which may

induce DNA damage and alter gene expression [29]. Another possible cause of decrease in sperm motiliy might be due to oxidative damage leading to ultimate death of the cell [30].

Diethanolamine caused concentration and time-dependent reduction in sperm viability. Phospholipids play important role in maintaining membrane integrity and its semi permiability. When spermatozoa stained with trypan blue showed large number of dead spermatozoa due to loss of membrane integrity mainley due to alterations in phospholipids as mention above. Loss of membrane permeability might be another major factor in the loss of sperm motility [31].

DEA also caused various kinds of sperm morphological abnormalites. These morphological abnormalities were depending on concentration of DEA. Alterations in the membrane composition and membrane integrity may alter the sperm morphological changes. These abnormalities affect the sperm's ability to swim and fertilitze the egg. Male fertility depends on normal sperm function and srtucture. The low sperm motility and sperm morphological abnormalities caused male infertility.

It can be concluded from this study that addition of DEA to sperm suspension caused adverse effect on sperm parameters including sperm motility, sperm viability and sperm morphology which may affect male infertiltiy.

Conflict of interest statement

We declare that we have no conflict of interest.

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