

# Curcumin ameliorates diethanolamine-induced enzymatic changes in testis of mice

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**Abstract:** The present investigation was an attempt to evaluate the ameliorative effect of curcumin on diethanolamine-induced enzymatic changes in testis of mice. Adult Swiss strain male albino mice were orally administered with diethanolamine (110, 165 and 330 mg/kg body weight) for 45 days. Treatment caused, as compare to control, significant ( $p < 0.05$ ) dose-dependent increase in the activities of acid phosphatase and alkaline phosphatase in testis. On the other hand, activities of adenosine triphosphatase and succinate dehydrogenase significant ( $p < 0.05$ ) dose-dependent decrease in testis as compare to control. Co-treatment of curcumin (10, 25 and 50 mg/kg body weight) along with diethanolamine (330 mg/kg body weight) significantly ameliorates diethanolamine-induced changes in activities of enzymes in testis as compared to diethanolamine alone treated mice. It is concluded from the present study that curcumin exerts protective effect against diethanolamine-induced enzymatic changes in testis of mice.

**Key Words:** Diethanolamine, Curcumin, Acid phosphatase, Alkaline phosphatase, Adenosine triphosphatase, Succinate dehydrogenase, Testis.

## 1. INTRODUCTION:

Diethanolamine (DEA) is an alkanolamine which is reactive and bifunctional, combining both the properties of alcohols and amines. DEA is widely used as industrial chemicals (Wagner, 2006), in agricultural chemicals, metal working fluids and personal care products like cosmetics, shampoos and hair conditioners (CIR, 1983, 1986). It is used in pharmaceutical industries as buffer and stabilizer for certain drugs (Soreat, 1973) and aqueous DEA solutions are also used as solvents for numerous drugs that are administered intravenously (Cavender, 2001). Wide use of DEA in industrial and consumer products may results in its release to the environment (Knaak *et al.*, 1997). General populations may be exposed to DEA through cigarette smoking (Hoffman *et al.*, 1982), consumer products such as soaps, shampoos and cosmetics via dermal exposure 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 (Knaak *et al.*, 1997).

Diethanolamine is metabolized by biosynthetic routes common to endogenous alkanolamines (ethanolamine and choline) and incorporated into phospholipids in liver, kidney, spleen and brain of mice and rats (Mathews *et al.*, 1995). Gamer *et al.* (2008) reported DEA-induced systemic toxicity in liver and kidney by repeated exposure of DEA. Dermal exposure of DEA caused adverse effect on testis and sperms in rats (EI-Mehallavi *et al.*, 2007). DEA also altered choline homeostasis and caused choline deficiency (Leung *et al.*, 2005). It was found that DEA treatment caused biochemical changes consistent with choline deficiency in mice (Lehman-McKeeman *et al.*, 2002). Other effects associated with choline deficiency include increased generation of free radicals and increased susceptibility to oxidative damage (Rushmore *et al.*, 1984).

Curcumin is a major chemical component isolated as yellow pigment of turmeric powder produced from the rhizome of the plant *Curcuma longa* which has been used in India for thousands of years and is a major part of Siddha medicine. The most important feature of curcumin is that it has no side effect and therapeutic agent with multiple beneficial functions (Kamboj, 2000). Curcumin is considered to be an effective antioxidant which acts as a scavenger of oxygen free radicals (Ruby *et al.*, 1995). It also lowers the production of reactive oxygen species (ROS) *in vivo*. (Joe and Lokesh, 1994). Curcumin has also been reported to ameliorate aflatoxin-induced toxicity in mice spermatozoa (Mathuria and Verma, 2007).

The male reproductive system is vulnerable to the effects of occupational chemical agents. DEA is one of these occupational chemical agents. Hence the aim of this study was to evaluate the toxic effect of DEA on testis of mice and its possible amelioration by curcumin.

## 2. MATERIALS AND METHOD:

### Chemicals

Diethanolamine was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and was of analytical grade. Curcumin was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

### Experimental animals

Healthy young inbred Swiss strain male albino mice (*Mus musculus*), weighing approximately 30-35 gm were obtained from Cadila Pharmaceuticals, Ahmedabad. Animals were provided with certified pelleted rodent feed and water *ad libitum* and maintained under laboratory conditions. All animal studies were approved by Institutional Animal Ethics Committee of Gujarat University, Ahmedabad and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg- 167/1999/CPCSEA; 1st December, 1999), New Delhi, India.

### Experimental design

Ninety animals were randomly divided in nine groups and caged separately. Group 1 (untreated control) animals were maintained without any treatment. Animals of group 2 (vehicle control) received olive oil (0.2 mL/animal/day) for 45 days as olive oil was used as vehicle to dissolve curcumin. Animals of group 3 (antidote control) received 50 mg/kg bw/day of curcumin. Animals of group 4, 5 and 6 were orally administered with low dose (110 mg/kg bw/day), mid dose (165 mg/kg bw/day) and high dose (330 mg/kg bw/day) of DEA respectively. Animals of group 7, 8 and 9 were orally administered with 10, 25 and 50 mg/kg bw/day of curcumin respectively along with high dose of DEA.

### Biochemical analysis

After completion of treatment animals were humanly sacrificed by cervical dislocation. Then the testes were quickly dissected out, blotted free of blood and used for determination of enzymatic assays. Alkaline phosphatase (ALP) activity was assayed by the method of Bessey *et al.* (1946) and acid phosphatase (ACP) activity was assayed by the method as described in Sigma Technical Bulletin. The activity of adenosine triphosphatase (ATPase) was assayed by the method of Quinn and White (1968) and succinic dehydrogenase (SDH) activity was assayed by the method of Beatty *et al.* (1966).

### Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) followed by Tukey's test using GraphPad Instant software version 5.03. Data are expressed as the means  $\pm$  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.

### Organoprotective index

The organ protecting activity of curcumin was expressed as organoprotective percentage (O) (Chandan *et al.*, 2007) which was calculated using the formula as mentioned below:

$$O = 1 - \left( \frac{T - V}{C - V} \right) \times 100$$

Where T is the mean value of curcumin along with the DEA, C is the mean value of DEA alone, and V is the mean value of vehicle control animals.

## 3. RESULTS AND DISCUSSION:

**Table 1: Ameliorative effect of curcumin on diethanolamine-induced enzymatic changes in testis of mice**

Experimental groups	ACP	ALP	ATPase	SDH
<b>(I) Control</b>				
1. Untreated	1.07 $\pm$ 0.06	0.45 $\pm$ 0.02	1.89 $\pm$ 0.03	20.15 $\pm$ 0.35
2. Vehicle	0.93 $\pm$ 0.05	0.41 $\pm$ 0.02	1.80 $\pm$ 0.04	20.16 $\pm$ 0.68
3. Antidote	1.02 $\pm$ 0.06	0.42 $\pm$ 0.02	1.85 $\pm$ 0.04	20.95 $\pm$ 0.56

<b>(II) Diethanolamine (DEA)-treated</b>				
4. DEA-LD	1.50±0.03 <sup>a</sup> (40.18)	0.67±0.02 <sup>a</sup> (48.88)	1.69±0.12 (10.58)	17.64±0.42 <sup>a</sup> (12.45)
5. DEA-MD	2.12±0.08 <sup>a</sup> (98.13)	1.05±0.04 <sup>a</sup> (133.33)	1.54±0.04 <sup>a</sup> (18.51)	14.23±0.45 <sup>a</sup> (29.37)
6. DEA-HD	2.87±0.11 <sup>a</sup> (168.22)	1.55±0.04 <sup>a</sup> (244.44)	1.32±0.04 <sup>a</sup> (30.15)	11.44±0.41 <sup>a</sup> (43.22)
<b>(III) Diethanolamine (DEA-HD) + curcumin (C)-Treated</b>				
7. DEA-HD+C10	2.40±0.04 <sup>b</sup> <b>(24.22)</b>	1.24±0.04 <sup>b</sup> <b>(27.19)</b>	1.43±0.03 <b>(22.91)</b>	12.94±0.43 <b>(17.20)</b>
8. DEA-HD+C25	1.94±0.07 <sup>b</sup> <b>(47.93)</b>	0.92±0.03 <sup>b</sup> <b>(55.26)</b>	1.58±0.05 <sup>b</sup> <b>(45.83)</b>	15.19±0.56 <sup>b</sup> <b>(43.00)</b>
9. DEA-HD+C50	1.28±0.03 <sup>b</sup> <b>(81.95)</b>	0.58±0.02 <sup>b</sup> <b>(85.08)</b>	1.76±0.03 <sup>b</sup> <b>(91.66)</b>	18.27±0.36 <sup>b</sup> <b>(78.32)</b>

Values are mean±SEM; n=10.

Values shown in parenthesis indicate:

Italics- Percent change in DEA-treated from untreated control

Bold- Organoprotective index from DEA-HD

Level of significance <sup>a</sup>p<0.05, as compared to untreated control

<sup>b</sup>p<0.05, as compared to DEA-HD-treated

No significant difference was noted between different control groups (Groups 1-3).

**Units:** ACP- μmoles p-nitrophenol released/mg protein/30 min; ALP- μmoles p-nitrophenol released/mg protein/30 min; ATPase- μmoles inorganic phosphate released/mg protein/30 min; SDH- μg formazon formed/mg protein/15 min

Table 1 shows the effect of DEA on activities of enzymes in testis of mice and its possible amelioration by co-treatment of curcumin along with DEA-HD. No significant difference was observed in activities of enzymes in testis of different control groups of animals (Groups 1-3). Oral administration of DEA (Groups 4-6) for 45 days caused significant (p<0.05) increase in ACP (DEA-LD: 40.18%, DEA-MD: 98.13%, DEA-HD: 168.22%) and ALP (DEA-LD: 48.88%, DEA-MD: 133.33%, DEA-HD: 244.44%) as compared to untreated control (Group 1) in testis of mice which was dose-dependent (r=0.9929, 0.9857, respectively). Acid phosphatase is a marker enzyme for the lysosomal integrity (Collins and Lewis, 1971) and important for the tissue reorganization and tissue repair. Increased ACP activity might be due to increased release of lysosomal enzyme by damaging lysosomal integrity causing lysis of cell. Alkaline phosphatase is a marker enzyme for plasma and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan *et al.*, 2004). DEA treatment also causes alteration in endoplasmic reticulum (Blum *et al.*, 1972). This could be the reason for increasing ALP activity. Annau and Manginelli (1950) reported increased ALP activity in liver by DEA treatment.

Diethanolamine treatment also caused significant (p<0.05) dose-dependent decrease in ATPase (r=-0.9976) and SDH (r=-0.9984) activities as compared to untreated control. Maximum alteration observed was up to 30.15% and 43.22% for ATPase and SDH respectively in high dose DEA-treated animals (Group 6). SDH is a key enzyme of mitochondrial krebs cycle and it is mainly concerned with aerobic oxidation of acetyl coA and generation of ATP. ATPase is required for enzymatic hydrolysis of ATP which is important for intracellular transfer of energy. DEA creates choline deficiency which has been discussed earlier. Choline deficiency induces mitochondrial dysfunction by over generation of ROS (Zeisel, 2012). Barbee and Hartung (1979) also reported that DEA-induced alterations in mitochondrial structure and function. Thus, reduction in ATPase and SDH activity could be due to alteration in mitochondria.

Oral administration of curcumin along with DEA-HD (Groups 7-9) caused significant (p<0.05) decrease in ACP and ALP activities as compared to DEA-HD (Group 6) which was dose-dependent (r=-0.9959, -0.9997, respectively). Similarly, co-treatment of curcumin along with DEA-HD caused significant (p<0.05), dose-dependent increase ATPase (r=0.9943) and SDH (r=0.9881) activities. In all these enzyme activities, maximum amelioration was observed in 50 mg/kg bw/day dose of curcumin along with DEA-HD (ACP:81.95%, ALP:85.08%, ATPase:91.66%, SDH:78.32%) as per calculated by organoprotective index (Table 1). Curcumin plus DEA treatment significantly decreased ACP and ALP activities by preventing damage to the tissue. Karmakar and his colleagues (2011) have also reported that curcumin

decreased ACP and ALP activities in arsenic-induced biochemical perturbation in Swiss albino mice. Co-administration of curcumin along with DEA significantly increased ATPase and SDH activities in tissues might be preventing mitochondrial dysfunction from ROS due to its antioxidative property. It has been previously reported that curcumin potentially reduce aluminum-induced oxidative stress and mitochondrial dysfunction in rat brain (Sood *et al.*, 2011).

#### 4. CONCLUSION:

It can be concluded from this study that oral administration of DEA caused enzymatic changes in testis of mice which is significantly ameliorated by curcumin.

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