DIETHANOLAMINE-INDUCED CYTOTOXICITY ON ERYTHROCYTES AND ITS AMELIORATION BY CURCUMIN: AN *IN VITRO* ANALYSIS.

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ABSTRACT

Diethanolamine is highly reactive compound with two functional groups alcohol and amine. It is used in pharmaceutical, cosmetic and agricultural industries. It is well known for its cumulative toxicity. Plant based antioxidants are well known since ancient times for its protective effect against toxicity generated by toxicants. Curcumin is a potent antioxidant active compound of turmeric plant root, known for its health benefits. Diethanolamine cytotoxicity was examined in human red blood corpuscles. Intravenous blood samples were collected from healthy volunteers to study hemolysis. RBC suspension was prepared in phosphate buffer saline and was incubated with various doses of DEA (25-1000 *µg/ml*) and varying concentrations of curcumin (25-100 *µg/ml*) at 37 *ºC* for 1 hour. Amount of hemolysis were noted. Statistical analysis was performed using the analysis of variance (ANOVA) followed by Dunnett's test and the level of significance was accepted with *p<0.05. The results revealed that DEA caused concentration-dependent increase in hemolysis. Whereas curcumin treatment significantly reduced the amount of hemolysis induced by DEA. Ameliorative potency of curcumin may be because of its antioxidant properties that aids into human health.

Key words: hemolysis, diethanolamine, amelioration, curcumin, cytotoxicity

1. INTRODUCTION

Diethanolamine is a synthetic compound, often abbreviated as DEA. It is used in industrial gas purification to remove acid gases, as an anticorrosion agent in metalworking fluids and in preparation of agricultural chemicals[1]. It is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, hair conditioners and shampoos[2, 3]. Aqueous DEA solutions are used as solvents for numerous drugs that are administered intravenously. It is highly reactive with two functional groups alcohol and amine[4].

Curcumin is an active component of turmeric. Major Phytoconstituents of turmeric are curcuminoids that generally make up approximately 1–6% of turmeric by dry weight[4].

It has enumerate antioxidant properties and lot of health benefits. It is a natural compound obtained from the herb *Curcuma longa*, exerts various pharmacological properties such as anti-inflammation, antioxidant, anticancer and antimicrobial [5, 6].

The purpose of this study was to evaluate the effect of Diethanolamine (DEA) on erythrocytes and its amelioration by curcumin.

Hypotheses Proposed:

- DEA may not be causing significant, concentration and time-dependent effect on erythrocyte: *in vitro*.
- Curcumin may not be causing significant, concentration- and time-dependent amelioration on DEA induced toxicity on erythrocyte: *in vitro*.

2. MATERIAL AND METHODS

Diethanolamine was purchased from Sigma Research Laboratories, Mumbai, India. Curcumin was purchased from Hi-media Laboratories, Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Preparation of RBC suspension:

Intravenous blood samples were collected from healthy volunteers in EDTA vials. Blood samples were diluted with phosphate buffer saline (PBS) and centrifuged at 1000×g for 10 min. RBC pellets were washed thrice and diluted with PBS to get cell density of $2x10^4$ cells/ml[7, 8]. Doses of DEA were also prepared in PBS

Evaluation of DEA toxicity on red blood corpuscle

To study the effect of DEA on erythrocyte suspension, following sets of tubes were prepared;

(i) Control tubes containing 2 ml of RBC suspension and 2 mL of PBS.

(ii) 100% hemolysis tubes containing 2 mL of RBC suspension and 2 ml of distilled water.

(iii) DEA-treated tubes containing 2 ml of RBC suspension and 100 to 1000 µg/ml DEA.

The total volume of each tube was made to 4 ml with addition of PBS.

Ameliorative effect of curcumin on DEA induced cytotoxicity

To study the ameliorative effect of curcumin on erythrocyte suspension, following sets of tubes were prepared;

(i) Control tubes containing 2 ml of RBC suspension and 2 mL of PBS.

(ii) 100% hemolysis tubes containing 2 mL of RBC suspension and 2 ml of distilled water.

(iii) Antidote control tubes containing 2 ml of RBC suspension and 100 µg/ml of curcumin.

(iv) Curcumin-treated tubes containing 2 ml of RBC suspension along with 25 to 100 µg/ml curcumin + 800 µg/ml DEA.

The total volume of each tube was made to 4 ml with addition of PBS.

The incubation medium containing RBC suspension were mixed gently and incubated at 37ºC for 1 h with intermittent shaking. Thereafter the tubes were centrifuged at 1000×g for 10 min. The color density of supernatant was measured spectrophotometrically at 540 nm.

2.1 Statistical Analysis

Statistical analysis was performed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using Graph Pad prism software. Data is expressed as the means ± S.E.M. Accepted significance level was *$p<0.05$.

3. RESULTS AND DISCUSSION

Table 3.1 Effect of DEA on hemolysis and amelioration by curcumin.

Groups	DEA conc. (µg/ml)	Hemolysis
Control groups		
I	Untreated control	0.144±0.011
II	100% hemolysis	2.881±0.047
III	Antidote control	0.195 ± 0.002
DEA-treatment		
IV	DEA 100	0.356±0.009[a] (59.55)
V	DEA 200	0.568±0.013 [a] (74.64)
VI	DEA 300	1.009±0.023 [a] (85.72)
VII	DEA 400	1.362±0.012 [a] (89.42)
VIII	DEA 500	1.764±0.015 [a] (91.83)
IX	DEA 600	1.935±0.026 [a] (92.55)
X	DEA 700	2.319±0.020 [a] (93.79)
XI	DEA 800	2.789 ± 0.058 [a] (94.83)
DEA-HD+curcumin		
XII	DEA 800 +25 curcumin	2.092 ± 0.022 [b] (24.99)
XIII	DEA 800 +50 curcumin	1.400 ± 0.042[b] (49.80)
XIV	DEA 800 +75 curcumin	0.987 ± 0.027[b] (64.61)
XV	DEA 800 +100 curcumin	0.199 ± 0.006[b] (92.86)

Significant at the level

[a]$p<0.05$ as compared to control

[b]$p<0.05$ as compared to DEA-HD

Diethanolamine cytotoxicity was examined in human red blood corpuscles. In control tubes the supernatant remained clear and RBC settled in the bottom of the tubes were normal. With increasing concentration (100-1000 µg/ml) of DEA, amount of intact RBCs settling down at the bottom of the tube gets reduced drastically resulting in reddish supernatant which indicates hemolysis. Addition of DEA to RBC suspension resulted in significant ($p<0.05$) increase in hemolysis (59.55%, 74.64%, 85.72%, 89.42%, 91.83%, 92.55%, 93.79% and 94.83% respectively). Results revealed that DEA induces hemolysis in a concentration-dependent manner ($r=0.9917$). 800 µg/ml concentration was found to exert maximum effect showing 94.83 % hemolysis as compared to control.

Table 3.1 shows ameliorative effect of curcumin on DEA-induced hemolysis. No significant changes in hemolysis was observed in untreated control as well as in antidote control tubes. However, concurrent addition of curcumin (25-100 µg/ml) into RBC suspension resulted in significant ($p<0.05$) decrease (24.99% 49.80%,

64.61% and 92.86% respectively), from high dose of DEA (800 µg /ml) (94.83%) in hemolysis. The effect was concentration-dependent (r=0.9910). This protective effect was highest at 100 µg/ml concentration of curcumin.

Hemolysis may be because of influx of DEA into the cells triggering alteration in RBC membrane, swelling and eventual cell lysis. However, the exact mechanism of action is not clearly understood. DEA disturbs phospholipids function, structure and metabolism[9]. RBC membrane contains 60% phospholipids of the total lipid components. Lipid composition is important for membrane permeability and fluidity. DEA is known to create choline deficiency. Choline is essential nutrient for proper cell growth and function. It has been previously reported that DEA competitively inhibits the cellular uptake of choline *in vitro* [10, 11]. Choline deficiency include increased generation of free radicals and increased susceptibility to oxidative damage[12]. Thus one of the reasons of hemolysis might be due to oxidative damage and alteration of phospholipid membrane.

Another possible cause of hemolysis may be increased lipid peroxidation that damages cell membrane and leads to oxidative damage. Panchal S and Verma R.J. also found similar results, supporting present findings. Earlier studies on reproductive system also reported that DEA induces lipid peroxidation in *in vivo* and *in vitro* conditions as well [13].

Eastman (1989) reported that DEA reduced hemoglobin and hematocrit with an increased white blood cell count in male albino rats fed with diet containing 0.01, 0.1 and 1% DEA.[14] NTP reported dose-dependent decrease in erythrocyte and reticulocyte counts, mean corpuscular volume (MCV), hemoglobin concentration and hematocrit in their oral and dermal studies (NTP, 1992) [15] which supports the present findings that reveals addition of DEA to RBC suspension causes significant ($p<0.05$) increase in hemolysis. This could be due to hypotonic nature of DEA that might have caused swelling and ultimately bursting of RBCs that result in hemolysis.

Curcumin plays crucial role in preventing oxidative stress by quenching ROS and lowering lipid peroxidation [16]. Treatment of curcumin protected against diethanolamine-induced oxidative stress by increasing enzymatic (GPx, SOD and CAT) and non-enzymatic (glutathione and ascorbic acid) antioxidants that significantly ($P<0.05$) lowers lipid peroxidation and helps evicting ROS. Similar kind of results were reported in liver and kidney induced by gallic acid [16].

4. CONCLUSION

Present *in vitro* study revealed that Exposure of diethanolamine causes hemolysis in erythrocyte. The effect was dose-dependent. Furthermore, curcumin is effective to ameliorate DEA –induced hemolysis.

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