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DIETHANOLAMINE-INDUCED OXIDATIVE STRESS AND ITS MITIGATION BY CURCUMIN: AN *IN VITRO* STUDY

[1]Hetal Doctor, [2]Sanman Samova, [3]R.J Verma

Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380 009, India

Abstract: **This study intended to evaluate diethanolamine-induced toxicity and its possible mitigation by potent antioxidant curcumin. Diethanolamine is an organic compound and highly reactive with two functional groups, alcohol and amine. It is used in many personal care products as well as in pharmaceutical industries. Lipid peroxidation was measured by the change in optical density of the prepared solutions. Homogenates prepared from liver of healthy mice were treated with varying concentrations of DEA (25 to 200 µg/ml) with and without curcumin (0.5 to 2.5 µg/ml). Significant ($p<0.05$) increase in lipid peroxidation and remarkable decrease in protein content was noted in DEA-treated homogenates as compared to control. However, addition of diethanolamine (200 µg/ml) along with curcumin (0.5 to 2.5 µg/ml) caused significant ($p<0.05$) decrease in lipid peroxidation as well as increase in protein content. These findings suggest that curcumin significantly ameliorate diethanolamine-induced lipid peroxidation and protein content which is due to its antioxidant property.**

Keywords: **diethanolamine, oxidative stress, mitigation, curcumin.**

1. INTRODUCTION

Diethanolamine often abbreviated as (DEA) is an organic compound synthesized from a reaction of ethylene oxide and ammonia. Diethanolamine is not known to occur naturally in contrast to naturally-occurring ethanolamine (monoethanolamine), a common head group found in cell membrane phospholipids that is synthesized endogenously. Diethanolamine contains two functional groups (alcohol and amine) and is highly reactive. It is colourless, water soluble, viscous liquid above its melting point of 28^{o}C or white solid below its melting point. It is an alkanolamine with slight ammonia like odour [1].

Diethanolamine is used in ample number of personal care products such as shampoos, soaps, lotions, conditioners, hand washes, etc [2,3]. It is used as an emulsifier and foaming agent to give consumer product its leathery smooth and thick texture. It is also used in pharmaceutical industries as buffer and stabilizer in drugs and in an outer coating of drugs [4]. Aqueous DEA solutions are used as solvents for various drugs that are administered intravenously [5]. It is also used as chemical intermediates for herbicide, molluscide, fungicide, and algaecide products [6]. On repeated exposure, DEA shows cumulative toxicity. Liver and kidney are the vital organs where DEA gets accumulated in higher concentration.

Curcuma longa is a rhizomatous herbaceous perennial plant [7]. Curcumin, also known as diferuloylmethane, is the active component found in the rhizome of *Curcuma longa* locally known as turmeric plant [8]. Curcumin is insoluble in water and is an orange yellow coloured compound. **Turmeric has been traditionally used in Asian countries as a medical herb due to its [9],** wide variety of action which includes antioxidant, anti-inflammatory[10] **(Chanini, 2003) antimutagenic, antimicrobial, anticancer[11],[12]** anticoagulant, **pain [13], and to help in the management of inflammatory and**

degenerative eye conditions [14, 15]. In addition, it has been shown to benefit the kidneys [16]. Hypotensive and hypocholesteremic activities. Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects [17].

While there appear to be countless therapeutic benefits to curcumin supplementation, most of these benefits are due to its antioxidant property [18,19,20].

The present investigation was an attempt to evaluate effects of DEA on liver and its possible amelioration by antioxidant curcumin *in vitro*.

2. MATERIALS AND METHODS

Experimental animals: In this study, inbred healthy adult Swiss strain male albino mice weighing 30-40 gm were obtained from Cadila Research Center, Ahmedabad, India. Animals were kept in the Animal House of Zoology Department of Gujarat University, Ahmedabad, India. They were housed in an air-conditioned room at a temperature of 25±2ºC and 50-55% relative humidity with a 12 h light/dark cycle throughout the experiment. Animals were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and potable water *ad libitum*. All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiment on Animals (Reg. – 167/1999/CPCSEA), New Delhi, India. Animals were handled According to the guidelines published by Indian National Science Academy, New Delhi, India (1991).

Chemicals: Diethanolamine was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India while curcumin was obtained from Hi-Media Research Laboratories Pvt. Ltd., Mumbai, India. All the other chemicals used were of analytical grade.

Lipid peroxidation assay:

Liver was isolated from mice; the liver lobes were washed with normal saline and blotted free from blood with the help of blotting paper. Liver lobes were cut into small pieces. Certain amount of tissue was homogenized with cold phosphate buffered saline (pH 7.4) in glass-Teflon homogenizing tubes to evaluate lipid peroxidation. Reaction mixture was prepared by adding 0.2ml homogenate, 0.1ml H_2O_2 and varying concentrations of DEA (25-200 µg/ml) in each tube. Reaction was initiated by addition of H_2O_2. The reaction mixture was incubated for some time at 37^o C. This reaction mixture was **Lipid peroxidation in mice liver homogenate was measured *in vitro* by measuring the formation of thiobarbituric acid reactive substances (TBARS) by using standard method of Ohkawa *et al*., 1979 [21] with minor modifications with the help of spectrophotometer** using 8.1% sodium dodecyl sulphate, 20% acetic acid and 1% thiobarbituric acid (TBA) solution. The final volume was balanced with 0.1 M phosphate buffered saline. The solution was mixed properly and heated in a water bath at 95±2ºC for 60 min. The tubes were cooled and solutions were centrifuged at 1000 g for 15min. The solution turned into pinkish colour gradually which was measured spectrophotometrically at 532 nm. A high dose of 200 µg/ml was selected as desired dose as it produced maximum lipid peroxidation. Protein content was measured by the method of **Lowry *et al* (1951) [22]** with slight modifications and optical density was recorded at 540nm.

Analysis of concentration-dependent ameliorative potency of curcumin:

Different concentrations of curcumin (0.5 to 2.5 mg/ml) were added to homogenate preparations along with the high dose of DEA (200 µg/ml) and formation of MDA/ lipid peroxidation and protein content was measured spectrophotometrically at 532 and 540nm respectively.

Statistical analysis:

The results were expressed as mean ± SEM. The data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey's *post hoc* test in Graph pad prism 5 (graph pad, software, USA). Statistically significance was accepted with $p< 0.05$. Correlation Coefficient was measured to estimate the strength of linear association between two Variables. Pearson's correlation analysis was used to find the correlation between untreated and toxin treated-samples.

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3. RESULTS AND DISCUSSION

Table 1: Effect of diethanolamine on lipid peroxidation

Sr.no.	Concentrations µg/ml	Lipid peroxidation
Control groups		
I	**Control**	**1.268 ± 0.063**
II	**H_2O_2 control**	**1.513 ± 0.002**
Treated groups		
III	**25**	**2.293 ± 0.063** ^{*a}
IV	**50**	**3.195 ± 0.043** ^{*a}
V	**100**	**4.111 ± 0.037** ^{*a}
VI	**150**	**5.303 ± 0.087** ^{*a}
VII	**200**	**7.256 ± 0.145** ^{*a}

Values are mean ± S.E.M., n=10

Significant at the level:

As compared with untreated control^{a}*p<0.05,

No significant difference was noted between untreated control and H_2O_2 control

Units: LPO- nmoles MDA formed/mg protein/60 min.

Table 2: Effect of diethanolamine on protein content

Sr.no.	Concentrations (µg/ml)	Protein
Control		
I	**Control**	**20.580 ± 0.951**
DEA-treated		
II	**25**	**17.222 ± 0.172** ^{*a}
III	**50**	**14.778 ± 0.105** ^{*a}
IV	**100**	**10.705 ± 0.120** ^{*a}
V	**150**	**7.332 ± 0.038**
VI	**200**	**4.889 ± 0.139** ^{*a}

Values are mean ± S.E.M., n=10

Significant at the level:

As compared with untreated control^{a}*p<0.05,

No significant difference was noted between untreated control and DEA-treated groups

Units: Protein-mg/100mg tissue weight.

Table 3: Curcumin ameliorates diethanolamine-induced lipid peroxidation and protein content

Sr. No.	Experimental groups	Lipid peroxidation	Protein
Control groups			
I	**Control**	**1.586 ± 0.042**	**20.58 ± 0.951**
II	**H_2o_2 control (0.1ml)**	**1.781 ± 0.092**	**-**
III	**Antidote control (0.2µg/ml curcumin)**	**1.594 ± 0.034**	**20.074 ± 0.036**
DEA-HD (200 µg/ml)			
IV	**DEA-HD(200 µg)**	**7.256 ± 0.145** ^{*a}	**1.256 ± 0.143**
DEA-HD (200µg/ml) + curcumin (C)			
V	**DEA + C 0.5 µg/ml**	**5.148 ± 0.044** ^{*b}	**6.296 ± 0.105** ^{*b}
VI	**DEA + C 1 µg/ml**	**3.703 ± 0.167** ^{*b}	**9.382 ± 0.102** ^{*b}

VII	**DEA + C 1.5 µg/ml**	**2.736 ± 0.015^{*b}**	**12.173 ± 0.052^{*b}**
VIII	**DEA + C 2.0 µg/ml**	**1.087 ± 0.084^{*b}**	**15.030 ± 0.016^{*b}**
IX	**DEA + C 2.5 µg/ml**	**0.215 ± 0.011^{*b}**	**19.963 ± 0.022^{*b}**

Values are mean ± S.E.M., n=10

Significant at the level:

As compared with untreated control[a]*p<0.05,

As compared with toxin [b]*p<0.05.

No significant difference was noted between untreated control, H_2O_2 control and antidote control

Units: LPO- nmoles MDA formed/mg protein/60 min. protein: mg/100mg tissue weight.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular disease, inflammatory conditions, cancer and ageing[13]. Antioxidants offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms, antioxidants thus prevents diseases. The peroxidation of membrane lipids inhibited by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl perferryl complex (or) through OH radicals by Fenton reaction there by initiating a cascade of oxidative reactions.

Unsaturated lipids in liver tissue are very susceptible to peroxidation when they are exposed to reactive oxygen species (ROS). In the present investigation the liver tissue is incubated in presence of ROS generating system H_2O_2 and examined the effect on the tissue homogenates by measuring the optical density (OD) at 532nm. The results of the investigations revealed that addition of diethanolamine to homogenates caused concentration-dependent increase in H_2O_2 induced lipid peroxidation (table-1). It might be due to action of DEA on plasma membrane resulting into lipid peroxidation by generating free radicals. *In vivo* studies have also reported DEA-induced lipid peroxidation in liver of mice in a dose-dependent manner [23] **(Doctor *et al.*,2016)**. Diethanolamine also reduces protein content in *in vitro* conditions in concentration-dependent manner (table-2).

When these homogenate preparations were treated with curcumin, it showed dose dependent ameliorative response. Curcumin along with the high dose (200 µg) of DEA showed concentration dependent reduction in lipid peroxidation (table -3). Former studies have reported that curcumin inhibits 97.3% lipid peroxidation at 15µg/ml concentration, in free radical scavenging activity assay [24] **(Ak, and Gulcin, 2008)**. It is due to high efficiency of curcumin as an antioxidant that has tremendous radical scavenging activity and many other antioxidant properties[25] **(Choi et al., 2006)**. The previous *in vitro* study carried out by Palani samy hari prasad and N.Ramakrishnan (2012) [26] on lipid peroxidation assay using chicken homogenate supports the present findings.

Curcumin also elevates protein content by restoring normal enzymatic and non-enzymatic antioxidant activities that helps reducing oxidative stress and also helps converting harmful toxicants into non-toxic forms that can be easily eliminated from body.[27] **Mathuria and Verma (2007)** carried out similar study on aflatoxin induced lipid peroxidation in liver, kidney and testis and its amelioration using curcumin. Curcumin that is an active component of *curcuma longa* plant showed significant ameliorative potential proving that daily use of curcumin in diet can reduce risk of oxidative stress generated by xenobiotics compounds. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases.

4. CONCLUSION

Curcumim had potent lipid peroxidation inhibition activity. On the basis of the results obtained in the present study, we conclude that the active component curcumin exhibited significant *in vitro* lipid peroxide inhibition activity against diethanolamine-induced oxidative stress. The activity may be related to the presence of phenols and flavonoids in the curcumin.

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REFERENCES

[1] Environment protection Agency. (2000). Diethanolamine, Hazard Summary-Created in April 1992; Revised in January 2000

[2] CIR Cosmetic Ingredients Review. (1983). Final report on the safety assessment of triethanolamine, diethanolamine, and monoethanolamine. J. Am. Coll. Toxicol., 2: 183–235.

[3] CIR Cosmetic Ingredients Review. (1986). Final report on the safety assessment of cocamide DEA, lauramide DEA, linoleamiede DEA, and oleamide DEA. J. Am. Coll. Toxicol., 5: 415–454.

[4] Wagner, P. (2006). Reassessment of Diethanolamine. (CAS Reg. No.111-42-2) United States Environmental Protection Agency. Washington, D.C. 20460.

[5] Cavender, F.L. (2001) Aliphatic and Acyclic Amines, in Patty's Toxicology (volume 6), 5'h edition. John Wiley & Sons, Inc. New York.

[6] DOW Chemical Company (2014) Diethanolamine Technical Data Sheet, The Dow Chemical Company, Form No. 111-01411-1204 AMS

[7] Priyadarsini, K.I. (**2014).** The chemistry of curcumin: From extraction to therapeutic agent. Molecules, 19, 20091–20112.

[8] Aggarwal, B.B.; Kumar, A.; Bharti, A.C. (**2003).** Anticancer potential of curcumin: Preclinical and clinical studies. Anticancer Res., 23, 363–398.

[9] Lestari, M.L.; Indrayanto, G. Curcumin. Profiles Drug Subst. Excip. Relat. Methodol. **2014**, 39, 113–204.

[10] Chainani-Wu, N. (2003). Safety and anti-inflammatory activity of curcumin: a component of turmeric (*Curcuma longa*). *J. Altern. Complement. Med.*, **9**: 161-168.

[11] Mahady, G.B.; Pendland, S.L.; Yun, G.; Lu, Z.Z. **(2002).** Turmeric (Curcuma longa) and curcumin inhibit the growth of Helicobacter pylori, a group 1 carcinogen. Anticancer Res., 22, 4179–4181.

[12] Reddy, R.C.; Vatsala, P.G.; Keshamouni, V.G.; Padmanaban, G.; Rangarajan, P.N. (**2005).** Curcumin for malaria therapy. Biochem. Biophys. Res. Commun., 326, 472–474.

[13] Vera-Ramirez, L.; Perez-Lopez, P.; Varela-Lopez, A.; Ramirez-Tortosa, M.; Battino, M.; Quiles, J.L. **(2013).** Curcumin and liver disease. Biofactors, 39, 88–100.

[14] Wright, L.E.; Frye, J.B.; Gorti, B.; Timmermann, B.N.; Funk, J.L. **(2013).** Bioactivity of turmeric-derived curcuminoids and related metabolites in breast cancer. Curr. Pharm. Des., 19, 6218–6225.

[15] Kuptniratsaikul, V.; Dajpratham, P.; Taechaarpornkul, W.; Buntragulpoontawee, M.; Lukkanapichonchut, P.; Chootip, C.; Saengsuwan, J.; Tantayakom, K.; Laongpech, S. (**2014).** Efficacy and safety of Curcuma domestica extracts compared with ibuprofen in patients with knee osteoarthritis: A multicenter study. Clin. Interv. Aging, 9, 451–458. [CrossRef] [PubMed]

[16] Mazzolani, F.; Togni, S. **(2013).** Oral administration of a curcumin-phospholipid delivery system for the treatment of central serous chorioretinopathy: A 12-month follow-up study. Clin. Ophthalmol., 7, 939–945. [CrossRef]

[17] Allegri, P.; Mastromarino, A.; Neri, P. (**2010).** Management of chronic anterior uveitis relapses: Efficacy of oral phospholipidic curcumin treatment. Long-term follow-up. Clin. Ophthalmol., 4, 1201–1206. [CrossRef]

[18] Trujillo, J.; Chirino, Y.I.; Molina-Jijón, E.; Andérica-Romero, A.C.; Tapia, E.; Pedraza-Chaverrí, J. **(2013).** Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol., 1, 448–456. [CrossRef]

[19] Gupta, S.C.; Patchva, S.; Aggarwal, B.B. (2013). Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials. AAPS J., 15, 195–218.

[20] Aggarwal, B.B.; Harikumar, K.B. (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int. J. Biochem. Cell Biol, 41, 40–59.

[21] Ohkawa H., Ohishi N. and Yagi K. (1979) Anal Biochem. 95, 351.

[22] Lowry, O.H., Rose-Brough, N.J., Farr, A.L., and Randell, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 4: 492-501.

[23] Doctor H., Samova, S. and Verma R.J. (2016). Oxidative stress caused by diethanolamine in liver of mice and its amelioration by curcumin. *WJPPS* 5(5):797-805.

[24] Palani samy hariprasad and N. Ramakrishnan (2012). In vitro lipid peroxidation assay of Rumex vesicarius L. *IJPPS* Vol.4, supp1.

[25] Ak and Gulcin. (2008). Antioxidant and free radical scavenging properties of curcumin. *Chemio-biological interactions.*

[26] Choi, H., Chun, Y., Kim, S., Kim, M. and Park, J. (2006). Curcumin inhibits hypoxia inducible factor-1 by degrading aryl hydrocarbon receptor nuclear translocator: a mechanismof tumor growth inhibition. *Mol. Pharmacol*., **70(5)**: 1664–1671.

[27] Mathuria and verma (2007). Curcumin ameliorates afflatoxin-induced lipid peroxidation in liver, kidney and testis of mice an in vitro study. *Actapoloniae pharmaceutica*, 64(5):413-6.

[28] Panahi, Y.; Hosseini, M.S.; Khalili, N.; Naimi, E.; Simental-Mendia, L.E.; Majeed, M.; Sahebkar, A. Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial. Biomed. Pharmacother. **2016**, 82, 578–582. [CrossRef] [PubMed]

[29] Chan, W. and Wu, H. (2006). Protective effects of curcumin on methylglyoxal-induced oxidative DNA damage and cell injury in human mononuclear cells, *Acta Pharmacologica Sinica.*, **27 (9)**: 1192-1198.

[30] Dow Chemical Company (2010). Sales Specification Diethanolamine, Submitted by the council on Mar 3, 2011 (1 P).

[31] Dow Chemical Company (1998). The Specifier's Guide to Buying and Applying Ethanolamines, Midland, MI Food and Drug Administration (FDA).Frequency of use of cosmetic ingredients.FDA Database. 2011. Washington, DC:FDA.Updated Feb 25.

[32] Ethanolamines Overview, The Dow Chemical Company, Form No. 111-01375-0103 AMS

[33] Chattopadhyay I., Biswas K., Bandyopadhyay U., and Banerjee R. K., Turmeric and curcumin: Biological actions and medicinal applications. *Current Science*, Vol. 87, No. 1, 2004.

[34] Lerche-Langrand C, Toutain HJ. Precision-cut liver slices: characteristics and use for in vitro pharmaco-toxicology. *Toxicology*. 2000;153:221–253