Diethanolamine-Induced Hepatic Injury and Its Amelioration by Curcumin

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ABSTRACT

Aim: Present investigation was an attempt to evaluate diethanolamine (DEA) induced-hepatic injury and its amelioration by curcumin.

Background: Diethanolamine is an organic compound which is not reported to occur naturally. It is used in ample number of cosmetic as well a personal care products such as shampoos, hand washes and conditioners. It is also used in pharmaceutical industries as outer covering of capsulated drugs and as pH stabilizer and also in buffer solutions. It produces cumulative toxicity.

Materials and Methods: Swiss strain male albino mice were orally administered with three different doses of DEA (110, 165, 330 mg/kg body weight) for 30 days. Curcumin was orally administered in three doses (10, 20, 30 mg/kg body weight) along with the high dose of DEA for 30 days on completion of treatment, animals were sacrificed and blood was collected by cardiac puncture, serum was separated to assess liver marker enzymes such as AST, ALT, ACP and ALP.

Results: Results revealed that DEA causes significant ($P<0.05$) alterations in liver marker enzymes in serum as compared to untreated control groups indicating leaking of enzymes into serum due to hepatic injury. Curcumin-treated groups caused significant amelioration as compared to DEA-treated groups. Curcumin-treated groups showed significant ($p<0.05$) decrease in serum AST, ALT and ACP, ALP levels. Results also revealed that curcumin protects from liver injury by inhibiting cellular damage and leaking of enzymes in serum.

Conclusion: Diethanolamine causes cell damage and injury to liver tissues and the toxicity generated by diethanolamine can be ameliorated by curcumin.

Keywords: Diethanolamine; Hepatic Injury; Liver Marker Enzymes; Curcumin

Abbreviation: DEA-diethanolamine ACP- acid phosphatase; ALP- alkaline phosphatase; AST- aspartate aminotransferase; ALT- alanine aminotransferase; MEA- monoethanolamine

INTRODUCTION

Diethanolamine is an organic compound with slight ammonia like odour. It is colourless, solid or liquid, water soluble and viscous fluid. It contains two functional groups that are alcohol and amine and so is highly reactive [1]. Diethanolamine is used in ample number of cosmetics and personal care products as an emulsifier to provide lathery smooth texture to products like shampoos, lotions, hand washes, and conditioners [2,3]. It is also used in pharmaceutical industries as drug stabilizer, pH adjuster and as a plasticizer in outer covering of capsulated drugs [4,5]. Diethanolamine has cumulative toxicity, upon repeated exposure it gets accumulated into liver and kidney [6].

Curcumin is a marvel molecule that is widely used to treat many diseased conditions as well as to prevent some adverse diseased conditions. It is a natural food colouring agent. It is yellow coloured active component of curcuma longa plant root. It has anti-inflammatory [7], antiseptic, anti-carcinogenic [8,9], anti-fungal, anti-bacterial and many more medicinal values and antioxidant properties [10].

Present study was an attempt to evaluate diethanolamine-induced hepatic injury and its amelioration by curcumin

MATERIALS AND METHODS

Experimental animals

In this study, inbred healthy adult Swiss strain male albino mice weighing 30-35 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

Analytical grade diethanolamine and curcumin were procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Hi Media Research Laboratories Pvt. Ltd., Mumbai, India respectively. Olive oil was obtained from Figaro, Madrid, Spain. All the other chemicals used were of analytical grade

Study Design

Ninety animals were randomly divided into nine groups and caged separately. Animals of group I were maintained without any treatment (Untreated control). Group II (vehicle control) animals received olive oil (0.2 ml/animal/day) for 30 days. Curcumin was dissolved in olive oil hence it was used in vehicle control. Group III (antidote control) animals received 30 mg/kg body weight/ day curcumin orally for 30 days. Group IV, V and VI animals received 110, 165 and 330 mg/kg body weight/day of DEA respectively. In addition to high dose of DEA (330 mg/kg bw/animal/day), animals of group VII, VIII and IX received 10, 20 and 30 mg/kg body weight/day curcumin. Doses were orally administered by gavage using stomach tube. Animals were sacrificed on 31st day to assess different biochemical changes.

BIOCHEMICAL ANALYSIS

Alanine transaminase (E.C.2.6.1.2) activity

The alanine transaminase (ALT) activity in serum was assayed by the method of Reitman and Frankel (1957) [11]. A buffered solution of α – ketoglutarate and L- alanine was made to react with the liver homogenate for 30 min. The pyruvate formed from L- alanine by the enzymatic reaction reacts with 2, 4-dinitrophenyl hydrazine (DNPH) in alkaline medium was measured at 540 nm. The enzymatic activity was expressed as mU/mg protein/30 min in case of liver and mU/mL in case of serum.

Aspartate transaminase (E.C.2.6.1.1) activity

The aspartate transaminase (AST) activity in serum was assayed by the method of Reitman and Frankel (1957) [11]. Assay method is similar as described in ALT activity assay, except buffered solution contained L-aspartate instead of L-alanine and allowed to react for 1 h. The enzymatic activity was expressed as mU/mg protein/60 min in case of liver and mU/mL in case of serum.

Aspartate transaminase (E.C.2.6.1.1) activity

The alkaline phosphatase (ALP) activity in serum was determined by the method of Bessey et al. (1946) [12]. Alkaline phosphatase at optimum pH 10.5 catalyzes the hydrolysis of p-nitrophenyl phosphate (disodium salt) to p-nitrophenol and inorganic phosphate. The liberated p-nitrophenol reacts with sodium hydroxide to form yellow colored complex which was measured at 410 nm. The ALP activity was expressed as µmoles p-nitrophenol released/mg protein/30 min in liver and IU/mL in serum.

Acid phosphatase (E.C.3.1.3.2) activity

The acid phosphatase (ACP) activity was assayed in serum by the method as described in Sigma Technical Bulletin (Sigma Technical Bulletin, MO, USA). Acid phosphatase at optimum pH 4.8 catalyzes the hydrolysis of p-nitrophenyl phosphate (disodium salt) to p-nitrophenol and inorganic phosphate. The liberated p-nitrophenol reacts with sodium hydroxide to form a yellow colored complex which was measured at 420 nm. The enzyme activity was expressed as µmoles p-nitrophenol released/mg protein/30 min in liver and IU/mL in serum.

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 and toxin with antidote-treated samples.

Results

Table 1: Experimental protocol

Sr. No.	Experimental groups	No. of animals Treated	Duration of treatment (days)	Necropsy
Control groups				
I	Untreated control	10	30	31st
II	Vehicle control (0.2 ml olive oil/animal/day)	10	30	31st
III	Antidote control (30 mg curcumin /kg bw/day)	10	30	31st
Diethanolamine (DEA)-treated groups				
IV	DEA-LD (110 mg/kg body weight/day)	10	30	31st
V	DEA-MD (165 mg/kg body weight/day)	10	30	31st
VII	DEA-HD (330 mg/kg body weight/day)	10	30	31st
Diethanolamine (DEA) (HD)+curcumin-treated groups				
VII	DEA-HD + curcumin (10 mg/kg body weight/day)	10	30	31st
VIII	DEA-HD + curcumin (20 mg/kg body weight/day)	10	30	31st
IX	DEA-HD + curcumin (30 mg/kg body weight/day)	10	30	31st

60.636±0.242b

Sr. No.	Experimental groups	AST	ALT	ACP	ALP
Control groups					
I	Untreated control	32.184 ±0.232	11.622 ±0.204	0.199 ±0.010	0.308 ±0.011
II	Vehicle control (0.2 ml olive oil/animal/day)	31.853 ±0.332	11.447 ±0.161	0.196 ±0.010	0.297 ±0.013
III	Antidote control (30 mg curcumin /kg bw/day)	31.694 ±0.232	11.599 ±0.185	0.201 ±0.012	0.303 ±0.015
Diethanolamine (DEA)-treated groups					
IV	DEA-LD (110 mg/kg body weight/day)	48.372 ±0.425[a]	20.508 ±0.136a	0.279 ±0.011[a]	0.473 ±0.013a
V	DEA-MD (165 mg/kg body weight/day)	62.761 ±0.240[a]	28.293 ±0.125[a]	0.381 ±0.010[a]	0.593 ±0.013[a]
VII	DEA-HD (330 mg/kg body weight/day)	74.897 ±0.209[a]	39.532 ±0.146[a]	0.497 ±0.009[a]	0.737 ±0.014[a]
Diethanolamine (DEA) (HD)+curcumin-treated groups					
VII	DEA-HD + curcumin (10 mg/kg body weight/day)	60.636 ±0.242[b]	30.945 ±0.251[b]	0.393 ±0.009[b]	0.600 ±0.011[b]
VIII	DEA-HD + curcumin (20 mg/kg body weight/day)	46.571 ±0.283[b]	21.487 ±0.307[b]	0.316 ±0.009[b]	0.493 ±0.009[b]
IX	DEA-HD + curcumin (30 mg/kg body weight/day)	33.657 ±0.164[b]	12.459 ±0.281[b]	0.269 ±0.010[b]	0.309 ±0.010[b]

Values are expressed as means ± S.E.M.; n=10,

Level of significance: ap<0.05, as compared to untreated control (Group I)

bp<0.05, as compared to toxin-treated groups (Group I, II and III)

No significant difference was noted between untreated and vehicle control groups (Group I, II and III)

Units: Alanine transaminase activity (ALT): mU/mg protein/30 min; Aspartate transaminase activity (AST): mU/mg protein/30 min; Acid phosphatase (ACP): µmoles p- nitrophenol released/mg protein/30 min; Alkaline phosphatase (ALP): µmoles p- nitrophenol released/mg protein/30 min: Protein: gm/dl

Discussion

Treatment of diethanolamine for 30 days caused elevated levels of liver marker enzymes that are mainly AST, ALT, ACP and ALP in dose dependent manner (table-1). It also reduces protein content [13] Shaarawy et al., (2009) also found N-Nitrosodiethylamine (NDEA)-induced increase AST, ALT and ALP levels in serum. ALT is more specific for liver then AST both these enzymes are present mainly in liver. The release of liver specific enzymes is due to liver injury which is the result of increased free radical generation and disturbed antioxidant system which includes enzymatic (SOD, CAT, GPx) and non-enzymatic (GSH and TAA) antioxidant. Prior study on liver supports current findings which states DEA treatment for 30 days caused oxidative stress and reduced enzymatic and non-enzymatic antioxidants in mice liver.

In addition to that DEA also increases lipid peroxidation [14]. High dose of DEA caused hepatic steatosis this might be due to the presence of alcohol group in the form of two ethanol groups present in DEA [15] De Level and colleagues also observed ethanol induced fatty liver in mice model as well as elevated levels of AST, ALT and deposition of lipid in liver which supports present results. Oxidative stress often causes cell damage affecting structural and functional integrity of cells which ultimately leads to release of enzymes from cytoplasm to blood [16]. This observation is supported by the report of Vermaulen et al. (1992) [17] which stated that ALT, AST, GGT and ALP are normally located in the cytoplasm and released into circulation after cellular damage. (Diethanolamine contains amine and alcohol groups. ALT and AST are aminotransferases which transfers amino group from one to another group present investigation assumes that AST and ALT may transfer amine group from diethanolamine and converts DEA into 2 ethanol molecules which may produce effects same as ethanol or alcohol. Another study has reported that DEA is substituted into monoethanolamine (MEA) [18] .Co-administration of curcumin (10, 20 and 30 mg /bd /day) for 30 days significantly ($p<0.05$) reduce the activity of AST, ALT, ALP and ACP in serum (Table-2). Administration of curcumin also resulted into increased protein levels. Curcumin restores structural and functional integrity of cells (Figure-3) and prevents leaking of enzymes which suggest that curcumin significantly inhibits hepatic injury induced by DEA. Abarikwu et al., 2016 [19] supports these findings suggesting that curcumin protects from oxidative stress, suppression of glutathione antioxidant defences, hepatic and renal damage in rats. Xiong et al., 2015 [20] also reported that Curcumin attenuates ethanol-induced liver injury by inhibiting oxidative stress via mitogen-activated protein kinase/nuclear factor E-2related factor 2 pathway in mice.

Conclusion

On the basis of the results obtained in the present study, conclusions can be made that diethanolamine causes hepatic injury and use of active component curcumin exhibited significant ameliorative potency against diethanolamine-induced hepatic injury. The ameliorative activity may be related to the presence of phenols and flavonoids in the curcumin.

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REFRENCES

1. Environment protection Agency (EPA). Diethanolamine, Hazard Summary-Created in April 1992; Revised in January-2000. https://goo.gl/i9KRyx
2. CIR Cosmetic Ingredients Review. 8 Final Report on the Safety Assessment of Triethanolamine, Diethanolamine, and Monoethanolamine. J Am Coll Toxicol. 1983; 2: 183–235. https://goo.gl/P8sqKe
3. CIR Cosmetic Ingredients Review. Final report on the safety assessment of cocamide DEA, lauramide DEA, linoleamiede DEA, and oleamide DEA. J Am Coll Toxicol. 1986; 5: 415-454. https://goo.gl/kUtUu5
4. Wagner P. 2006. Reassessment of Diethanolamine. United States Environmental Protection Agency. Washington DC. 111: 42-2.
5. DOW Chemical Company Diethanolamine Technical Data Sheet, the Dow Chemical Company. 2014; 111: 01411-1204.
6. Mathews JM, Garner CE, Black SL, Matthews HB. Diethanolamine absorption, metabolism and disposition in rat and mouse following oral, intravenous and dermal administration. Xenobiotica. 1997; 27: 733-746. https://goo.gl/3UFDSA
7. Chainani Wu N. Safety and anti-inflammatory activity of curcumin: a component of turmeric (Curcuma longa). J Altern Complement Med. 2003; 9: 161-168. https://goo.gl/ar3MFo
8. Chan W, Wu H. Protective effects of curcumin on methylglyoxal-induced oxidative DNA damage and cell injury in human mononuclear cells. Acta Pharmacologica Sinica. 2006; 27: 1192-1198. https://goo.gl/pK1gqC
9. Choi H, Chun Y, Kim S, Kim M, Park J. Curcumin inhibits hypoxia inducible factor-1 by degrading aryl hydrocarbon receptor nuclear translocator: a mechanismof tumor growth inhibition. Mol. Pharmacol. 2006; 70: 1664-1671. https://goo.gl/DY7qJC
10. Kalpana C, Menon VP. Curcumin ameliorates oxidative stress duringnicotine-induced lung toxicity in Wistar rats. Ital J Biochem. 2004; 53: 82-86. https://goo.gl/hs9UGM
11. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamaic oxaloacetic acid and glutamic pyruvate transaminase. Am J clin Pathol. 1957; 28: 56-63. https://goo.gl/a4QJhw
12. Bessy O A, Lowry O H, Bririck N J. A method for the rapid determination of alkaline phosphatase in 5 cu mm serum. J Biol Chem. 1946; 164: 321-329. https://goo.gl/cbD6mG
13. Shaarawy SM, Tohamy AA, Elgendy SM, Elmageed ZYA, Bahnasy A, Mohamed MS, Kandil E, Matrougui K. Protective Effects of Garlic and Silymarin on NDEA-Induced Rats Hepatotoxicity. Int J Biol Sci. 2009; 5: 549-557. https://goo.gl/qP6tiL
14. Doctor H, Samova s, Verma R.J. Oxidative stress caused by diethanolamine in liver of mice and its amelioration by curcumin. WJPPS. 2016; 5: 5797-805.
15. Jin Gu, Yongxian Zhang, Daqian Xu, Zilong Zhao, Yuxue Zhang, Yi Pan, Peijuan Cao, et al. Ethanol-induced hepatic steatosis is modulated by glycogen level in the liver. J Lipid Res. 2015; 56: 1329-1339. https://goo.gl/Kect8s
16. De Level LD, Wang X, Kuhlenkamp JF, Kaplowitz N. Toxicity of azathioprine and monocrotaline in murrine sinusoidal endothelial cells and hepathocites: the role of glutathione and relevance to hepatic veno-occlusive disease: Hepatology. 1996; 23: 589-599. https://goo.gl/wpytN9
17. Vermuelen NPE, Bessems JGM, Van de straat R. Molecular aspects of paraceutamol–induced hepatotoxity and its mechanism–based prevention. Drug Metab Rev. 1992; 24: 367-407. https://goo.gl/sVR6sb
18. Knaak JB, Leung HW, Stott WT, Busch J, Bilsky J. Toxicology of mono-, di-, and triethanolamine. Rev Environ Contam Toxicol. 1997; 149: 1-86. https://goo.gl/rSL3VG
19. Abarikwu SO, Durojaiye M, Alabi A, Asonye B, Akiri O. Curcumin protects against gallic acid-induced oxidative stress, suppression of glutathione antioxidant defences, hepatic and renal damage in rats. Ren Fail. 2016; 38: 321-329. https://goo.gl/Ayaosx
20. Xiong ZE, Dong Wg, Wang BY, Tong, QY, Li ZY. Curcumin attenuates chronic ethanol-induced liver injury by inhibition of oxidative stress via mitogen-activated protein kinase/nuclear factor E2-related factor 2 pathway in mice. Pharmacogn Mag. 2015; 44: 705-715. https://goo.gl/Xm4Rg4