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DIETHANOLAMINE-INDUCED HEPATIC STEATOSIS IN MICE AND ITS AMELIORATION BY CURCUMIN

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Abstract: Extensive use of chemicals in personal care products has led to many health issues. Diethanolamine is one of such harmful chemicals containing two highly functional groups alcohol and amine that requires toxicological evaluation and its mitigation. Swiss strain albino mice were used and divided into different control and treated groups. Different doses of DEA (110, 165 and 330 mg/kg body weight/day) were orally administered for 30 days. Biochemical and histopathological assessments were performed at the end of the treatment. Results collectively revealed body weight loss as well as significant increase in absolute and relative liver weight in DEA-treated groups. Biochemical analysis revealed that DEA treatment further promotes significant ($P<0.05$), dose-dependent increase in lipid and cholesterol contents and also cause decrease in protein and glycogen content. Histopathological assessment confirms vacuole formation due to accumulation of lipid within the liver tissue. Administration of curcumin (10, 20 and 30 mg/kg body weight/day) along with high dose of DEA (330 mg/kg body weight/day) showed improved values of lipid, cholesterol, protein and glycogen contents. It also helped retaining normal histological structure of liver. Observations in all groups and results indicate DEA-treatment causes hepatic steatosis and treatment of curcumin attenuated effect of DEA that is due to its potential antioxidant properties.

Keywords: diethanolamine; hepatic steatosis; curcumin; histopathology; amelioration

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Introduction

Diethanolamine (DEA) is a colourless liquid or solid organic compound having slight ammonia like odour.[1] It is used as an emulsifier to provide leathery smooth texture to products like shampoos, conditioners, lotions and hand washes.[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 as it gets accumulated into liver and kidney.[6]

Since DEA contains two functional groups hydroxyl and amine, it is a highly reactive compound. After administering DEA into body the question arises that whether the toxicity generated to liver is due to either DEA as a whole compound itself or its functional groups. Ethanol may produce alcoholic diseased conditions as DEA contains two hydroxyl groups in its structure. Moreover, the ethanolamine metabolism pathway suggests that primary step of ethanolamine metabolism pathway is deamination leading to the hypothesis that diethanolamine may produce the effect of ethanol.

Curcumin is a bioactive component of turmeric root. It is distinguishes by number of medicinal properties such as anti-inflammatory, antioxidant,[7] anti-carcinogenic, anti-septic, anti-fungal and anti-bacterial properties.[8,9]

Present study was an attempt to evaluate protective effect of curcumin against diethanolamine-induced hepatic steatosis.

Materials and methods

Experimental animals

In this study, healthy Swiss strain male albino mice weighing 30-35 g were obtained from Cadila Research Centre, Ahmedabad, India. Animals were housed in an air - conditioned room at a temperature of 25±2º C 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. Mice were acclimatized to the laboratory conditions for two weeks prior to the treatments. All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiment on Animals (Reg- 167/GO/ReBi/S/99CPCSEA), 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 experimental groups and caged separately. Each group contained 10 mice. All experimental animals were subjected to overnight fasting prior to the treatment. Animals of group one were maintained without any treatment (Untreated control). Second group (vehicle control) received olive oil (0.2 ml/animal/day). Curcumin was dissolved in olive oil hence it was used as vehicle control. Third group (antidote control) animals received 30 mg/kg body weight/day curcumin. Fourth, fifth and sixth group of 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 seven, eight and nine received 10, 20 and 30 mg/kg body weight/day curcumin

(Table-1). Doses of DEA were based on LD_{50} values (3300). Doses of curcumin used according with our earlier study.[10] All doses were orally administered using stomach tube for the duration of 30 days. On completion of treatment animals were humanely sacrificed, liver was immediately isolated, blotted free of blood and used for histopathological and biochemical studies.

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 body weigh/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 (C 10) (10 mg/kg body weight/day)	10	30	31st
VIII	DEA-HD + curcumin (C 20) (20 mg/kg body weight/day)	10	30	31st
IX	DEA-HD + curcumin (C 30) (30 mg/kg body weight/day)	10	30	31st

Biochemical analysis

The glycogen content in the liver was estimated by the method of Seifter *et al*. (1950).[11] The glycogen present in tissue is converted to glucose, which reacts with anthrone reagent to give a green coloured product. The colour intensity was read at 620 nm which was directly proportional to glycogen content in liver. Total lipid content of the liver was estimated by the method of Fringes *et al*. (1972).[12] When lipid containing solutions are heated with sulphuric acid followed by addition of vanillin and phosphoric acid it produces pink colour whose optical density is measured at 530 nm. The concentration of cholesterol was estimated in the liver by the method of Zlatki *et al*. (1953).[13] Cholesterol forms a coloured complex with ferric chloride ($FeCl_3$) in the presence of concentrated sulphuric acid and glacial acetic acid which can be measured at 540 nm.

Protein content in liver was estimated by the method of Lowry *et al*. (1951)[14] using bovine serum albumin as a standard. Protein reacts with phenol reagent of Folin Ciocalteu, resulting in a deep blue colouration. The colour development is due to two reactions occurring simultaneously i.e., the reaction of alkaline copper sulphate solution with peptide bonds and reaction of phosphomolybdic and phosphotungstic acids by aromatic amino acids present in the protein. The blue colour that develops is quantitatively proportional to the total protein, which was measured at 540 nm.

Histopathological study

For histopathological evaluations, liver tissue was quickly isolated from all experimental animals. Liver tissues were preserved in 10% formaldehyde. Preserved tissues were processed and embedded in paraffin. Sections were mounted on slides for hematoxylin and eosin staining. All

sections were examined using a light microscope and photographed by a camera.

Statistical analysis

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).The results were expressed as the mean ± SEM. Statistical significance was accepted with $p< 0.05$. Correlation coefficient was measured to estimate the strength of linear association between the two variables. Pearson's correlation analysis was used to find the correlation between untreated and DEA-treated groups.

Results

Body weight

No significant changes in body weight were observed between different control groups of animals fed with normal diet (Table-2). Significant ($p<0.05$), decrease in body weight of mice fed with normal diet was observed in DEA-treated animals (Groups IV-VI) as compared to untreated control groups of mice (Group I) fed with normal diet. Significant recovery in body weight gain was observed in curcumin-treated groups (Group VII-IX).

Absolute and relative weight

No significant changes were noted in absolute and relative weight of different control groups of animals (Table- 3) fed with normal diet. Increase in absolute and relative weight of liver was observed in DEA-treated groups as compared to untreated control group of animals fed with normal diet. Recovery in DEA-induced changes in absolute and relative weights of liver

was observed in curcumin supplemented groups along with DEA-HD (Groups VII-IX) caused significant ($p<0.05$) as compared to DEA-HD alone treated groups of mice (Group VI). These effects were dose-dependent. The amelioration was maximum with 30 mg/kg bw/ day of curcumin along with high dose of DEA.

Biochemical analysis

Protein content

No significant reduction in protein content of untreated and vehicle control groups was observed. Protein content in DEA-treated groups of animals were found to reduce significantly ($p<0.05$) as compared to animals of different control groups. Oral administration of DEA for 30 days caused reduction by 22.72% (LD), 41.38% (MD) and 57.10% (HD). The effect was dose-dependent ($r= 0.9312$).

Protein content significantly ($p<0.05$) normalizes in curcumin along with DEA-treated groups. Hepatoprotective index shows increase in protein values up to 27.68% in LD, 50.84% in MD and 92.80% in HD (Table 3.11).

Total lipid content

No significant increase in total lipid content was observed between different control groups of animals. Oral administration of DEA for 30 days caused significant ($p < 0.05$) elevation in hepatic lipid content as compared to control groups (group- I-III). (Table 3)**.** The effect was dose-dependent ($r= 0.9852$) LD (92.09%); MD (170.93 %:) and HD (286.24%)

Administration of curcumin along with high dose of DEA (Group VII-IX) caused significant ($p<0.05$), as well as dose-dependent decrease in lipid ($r= 0.9852$) content as compared to DEA high dose alone treated group (Group VI). The protection induced by curcumin against DEA-induced

decrease in lipid content was (C10: 31.29, C20: 74.78 and C30 96.60). Maximum protection was achieved at the high dose of curcumin (Table 3).

Cholesterol content

Cholesterol content of animals of (Group I-III) showed no significant alterations in cholesterol content. While, oral administration of DEA for 30 days significantly ($p<0.05$) and dose-dependently ($r= 0.9668$) increased hepatic cholesterol content. Percent increase in cholesterol content by LD, MD and HD was 66.13%, 113.95% and (191.99%) respectively.

Administration of curcumin along with high dose of DEA (Groups VII-IX) caused significant ($p<0.05$), decrease in cholesterol content as compared to DEA high dose alone treated group (group 6). Similarly, cholesterol values manages to be normal in LD (31.16%), MD (67.87%) and HD (96.38%). (Table 4).

Glycogen content

Table 4 shows the effect of DEA administration on glycogen content in the liver of mice. Results revealed that oral administration of three different doses of DEA caused significant ($p<0.05$), dose-dependent ($r= 0.9949$) decrease in hepatic glycogen content. DEA reduced the glycogen content by 32.01% (HD), 63.83% (MD) and 97.60% (LD). No significant difference was observed in glycogen content of different control groups of animals.

Glycogen content in the liver of curcumin treated group of mice (group VII-IX) was found to be restored back because of protective effect of curcumin. Hepatoprotective index showed 32.01%, 63.83% and 97.60% protection denoted by C10, C20 and C30 respectively (Table 3).

Table 2. The effect of diethanolamine on body weight (g) of mice and its amelioration by curcumin.

Sr. no.	Experimental groups	Days of treatment	
		0 day	30th day
	Control groups		
I	Untreated control	32.6±0.3	34.4±0.4
II	Vehicle control	31.9±0.4	34.4±0.4
III	Antidote control	32.3±0.4	34.9±0.5
	DEA-treated groups		
IV	DEA (LD)	32±0.5	30.3±0.4 [a]
V	DEA(MD)	32.4±0.5	29.6±0.5[a]
VI	DEA (HD)	32.2±0.4	26.6 ±0.4[a]
	DEA-HD+Curcumin-treated groups		
VII	DEA-HD+C10	32±0.3	28.9±0.4[b]
VIII	DEA-HD+C20	33.3±0.3	32±0.3[b]
IX	DEA-HD+C30	31.4±0.3	33.5±0.3[b]

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

Level of significance: [a]$p<0.05$, as compared to untreated control (Group I)

[b]$p<0.05$, as compared to DEA HD-treated groups (Group IV-VI)

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

Table 3. The effect of diethanolamine on absolute and relative weight of liver of mice and its amelioration by curcumin.

Experimental groups	Absolute weight (gm)	Relative weight (gm /100gm body weight)
	Control	
Untreated control	1.707±0.131	4.639±0.292
Vehicle control	1.939±0.120	5.601±0.542
Antidote control	1.786±0.127	4.829±0.310
	DEA- treated groups	
DEA(LD)	2.220±0.201[a]	6.916±0.383[a]
DEA(MD)	2.732±0.184[a]	8.240±0.514[a]
DEA(HD)	3.844±0.112[a]	9.543±0.481[a]
	DEA-HD + Curcumin	
DEA-HD+C5	2.705±0.340[b]	8.067±0.543[b]
DEA-HD+C10	2.212±0.124[b]	6.230±0.423[b]
DEA-HD+C25	1.951±0.132[b]	5.623±0.348[b]

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

Level of significance: [a]$p<0.05$, as compared to untreated control (Group I)

[b]$p<0.05$, as compared to DEA-treated groups (Group IV-VI).

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

Table 4. The effect of diethanolamine on metabolic profile (in mice liver) and its amelioration by curcumin.

Experimental groups		Lipid	Cholesterol	Glycogen	Protein
Control groups					
I	Untreated control	4.036±0.044	0.437±0.014	1602±8.6	17.825±0.237
II	Vehicle control	4.115±0.043	0.445±0.016	1617±7.5	18.643±0.172
III	Antidote control	4.060±0.192	0.436±0.018	1613±7.7	18.932±0.334
DEA-treated groups					
IV	DEA-LD	7.753±0.158[a] (92.09)	0.726±0.012[a] (66.13)	1320±6.7[a] (17.60)	13.774±.0.334[a] (22.726)
V	DEA-MD	10.935±0.274[a] (170.93)	0.935±0.021[a] (113.95)	919±6.9[a] (42.63)	10.448±0.412[a] (41.38)
VI	DEA-HD	15.589±0.189[a] (286.24)	1.276±0.026[a] (191.99)	533±11.9[a] (66.72)	7.646±0.521[a] (57.10)
DEA-HD +curcumin-treated groups					
VII	DEA-HD+C10	11.998±0.095[b] *(31.29)*	1.017±0.013[b] *(31.16)*	880±10.0[b] *(32.01)*	10.690±0.323[b] *(27.68)*
VIII	DEA-HD+C20	7.008±0.193[b] *(74.78)*	0.712±0.010[b] *(67.87)*	1225±12.2[b] *(63.83)*	13.237±0.423[b] *(50.84)*
IX	DEA-HD+C30	4.505±0.129[b] *(96.60)*	0.475±0.019[b] *(96.38)*	1591±6.5[b] *(97.60)*	17.852±0.316[b] *(92.80)*

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

Level of significance: [a]$p<0.05$, as compared to untreated control (Group I)

[b]$p<0.05$, as compared to DEA-treated groups (Group IV-VI)

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

Units: Protein: mg/100 mg tissue weight**; Glycogen:** µg/100 mg tissue weight**; Total lipid:** mg/100 mg tissue weight**; Cholesterol:** mg/100 mg tissue weight**;**

Histopathology

No histopathological alteration was observed in liver tissue of the control groups. Intact cellular structure and clear hepatocytes were noticed (Figure 1).

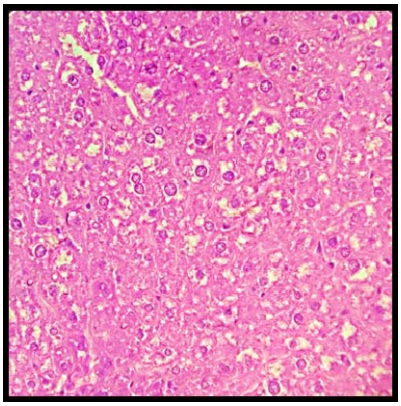

Figure 1. Transverse section (T.S.) of liver of untreated control group showing normal histology of tissue.

Histopathological evaluation showed high dose of DEA (Group-VI) for 30 days caused considerable cellular necrosis, ballooning or large vacuole formation, cellular degeneration and cellular infiltration of the liver. Condition known as hepatic steatosis was observed (Figure 2). Macrovesicular steatatosis (large fat vacuoles) and microvesicular steatosis were observed, amongst this two, macrovesicular steatosis is more common form of fatty degeneration and may be caused by oversupply of lipids. Microvesicular steatosis is characterized by small intracytoplasmic fat vacuoles which accumulate in the cell.

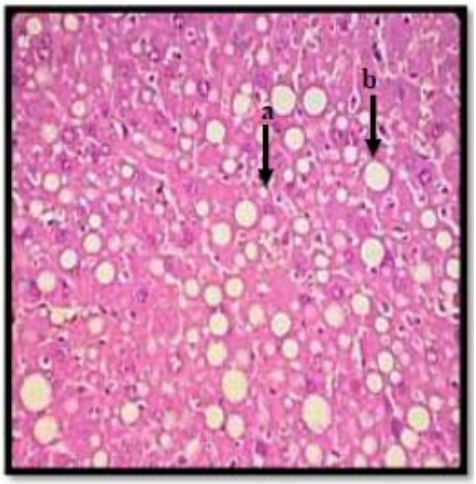

Figure 2. T.S. of liver of mice treated with DEA- high dose showing degeneration and vacuoles: a-microvesicle, b-macrovesicle.

Histopathological evaluation revealed that curcumin -treatment along with high dose of DEA, showed normal histology of liver as compared to DEA-HD treated liver tissue. No cellular necrosis or fatty infiltration was observed (Figure 3).

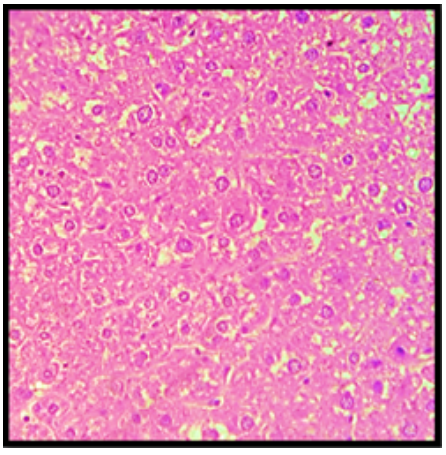

Figure 3. T.S. of liver of curcumin and DEA-HD-treated group showing normal histology.

Discussion

Chemicals that are used in routine can cause toxic effects in biological system and affect homeostasis in various aspects. Liver plays a pivotal role in detoxification and largely exposed to various toxicants that has been exposed to body. If liver is damaged it will be difficult to maintain homeostasis. Moreover, allopathic medicines also tend to damage liver so that substitute to such medicines should be promoted. Herbal medicines that has been used traditionally which have no or least side effects are suitable to treat diseases.

Oral administration of DEA for 30 days triggered significant reduction in body weight of mice (Table 3). Reduction in body weight might be due to reduced feed intake. Comparable results were reported by NTP (1992)[15] and (Melnick *et al*., 1994a)[16] in mice. Though, co-treatment of curcumin along with DEA improved body weight. This protective effect might be due to normalization of food intake and metabolism. Sharma and collogues (2011)[17] also reported protective effect of curcumin on body weight gain on aflatoxin-treated mice.

Oral administration of DEA caused significant increase in absolute and relative liver weight. This could be a consequence of significant increase in both lipid and cholesterol contents (Table 4). Histopathological studies also revealed fatty infiltration in liver of DEA-treated mice (Figure 2). Effect was dose-dependent. DEA-treatment caused significant reduction in protein and glycogen contents in liver of mice. It could be due to effect of DEA on DNA, RNA and protein expression. Earlier studies reported reduction in protein content in liver of DEA-treated mice. Administration of curcumin ameliorated effect of DEA due to antioxidant properties of curcumin. It might be due to reduced use of lipids as fuels and inability to

produce lipoproteins that transport lipids out of the liver. The two alcohol groups present in diethanolamine in the form of ethanol may be responsible for inducing hepatic steatosis.[18]

Histopathological evaluation of curcumin-treatment along with high dose of DEA, indicated that the treatment of curcumin aid into maintaining normal histology of liver (Figure 3). Biochemical studies also revealed significant reduction in lipid and cholesterol contents in liver of DEA-HD along with curcumin-treated mice (Table 4).

Platel and Shrinivasan in 1996, stated that curcumin stimulates digestion of fats and carbohydrates in animal models.[19] The observations of Soni and Kuttan in 1992 supports the present results which suggests that oral dose of turmeric and curcumin extracts alter serum lipids, specially decreasing LDL cholesterol, total cholesterol, and LDL peroxidation.[20] While increasing HDL cholesterol in humans. Curcumin obstructs intestinal cholesterol-uptake, rises the conversion of cholesterol into bile acids through increasing the activity of hepatic cholesterol-7-alpha-hydroxylase which is the rate limiting enzyme in synthesis of bile acids that increases secretion of bile acid.[21] Curcumin possesses hepatoprotective and choleretic properties. Curcumin has been demonstrated *in-vivo* to prevent lipid peroxidation from diverse agents such as carbon tetrachloride, and aflatoxin from aspergillus parasiticus.[20] In animal models, curcumin is a potent choleretic, increasing bile output by almost 100%.[22] Turmeric and curcumin has been historically used as a carminative and digestive. In vivo, curcumin ameliorated HFD-induced body weight gain and fat accumulation in liver or adipose tissues.[23]

Conclusion

The present investigation and its results conclude that orally administered of high dose of diethanolamine contributes to lipid accumulation in liver tissues leading to the condition called hepatic steatosis. Such conditions can be mitigated by the use of potent antioxidant curcumin which reduces the risk of hepatic steatosis in dose-dependent manner.

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