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Changes in the prostaglandin levels in alcohol toxicity: Effect of curcumin and N-acetylcysteine

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The present study was undertaken to evaluate the potential role of curcumin, the antioxidant principal from Curcuma longa *Linn., and the sulphur-containing amino acid N-acetylcysteine against ethanol-induced changes in the levels of prostanoids. Biochemical assessment of liver damage was done by measuring the activities of serum enzymes (i.e., aspartate transaminase and alkaline phosphatase), which were significantly increased in rats fed ethanol, whereas the elevated levels of these enzymes were decreased after curcumin and N-acetylcysteine treatment to rats fed ethanol. We observed a significant increase in the levels of prostaglandins E_1, E_2, $F_{2\alpha}$, and D_2 in liver, kidney, and brain. Administration of curcumin and N-acetylcysteine was shown to decrease the level of these prostanoids in the tissue studied.* (J. Nutr. Biochem. 11:509–514, 2000) *© Elsevier Science Inc. 2000. All rights reserved.*

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Introduction

Eicosanoids are the oxygenated derivatives of arachidonic acid (20:4) or similar polyunsaturated fatty acids (PUFA) precursors. They are derived from PUFA by the cycloxygenase or lipoxygenase pathways.[1] Major eicosanoids are prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), lipoxins (LXs), and hydroperoxy- and hydroxy-eicosatetraenoic acid (HPETEs and HETEs). These are not stored in the body, but are synthesized when required and have diverse and potent biological activities.

Eicosanoids play an important role in liver function and liver pathophysiology.[2–4] The liver is a source of eicosanoid production,[2] a target organ for their actions,[5] and a major site for their metabolic inactivation.[6] PGs, TXs, and LTs may also originate from extrahepatic cells[7] and non-paranchymal cells within the liver.[8]

The intracellular concentration of free arachidonate is low because most of this PUFA is esterified in the carbon-2 position of the glycerol moiety of phospholipids in cell membranes.[9]

Almost all cells of the body are able to produce prostanoids. PGD_2, PGE_2, $PGF_{2\alpha}$, and TXA_2 are mainly formed in liver by nonparenchymal cells such as kupffer cells and sinusoidal endothelial cells.[10] Prostaglandin endoperoxide synthase catalyzes the initial reaction in prostanoid biosynthesis. This microsomal enzyme contains cycloxygenase and peroxidase activity and requires for its enzymatic activity molecular oxygen and heme.[11]

Previous study has shown that cycloxygenase products of arachidonic acid are present in much higher concentrations in inflamed tissue than in healthy tissue.[12] Elmer and George[13] reported that the pharmacological effects of ethanol may be, in part, due to increased membrane fluidity, resulting in increased phospholipase A_2 (PLA_2) activity and the subsequent conversion of released arachidonic acid into pharmacologically relevant eicosanoids. Chronic ethanol ingestion shows a marked decrease in erythrocytes and platelets, docasatetraenoic acid, and arachidonic acid.[1]

Curcumin and N-acetylcysteine have many biological properties including antioxidant, anti-inflammatory, and hypolipidemic properties.[14–16] The objective of the present

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Table 1 Experimental design

Group	No. of rats	Treatment and duration		Dose administered
		1–30 days	31–60 days	
1. Control	10	Glucose	Glucose	19.356 g kg^{-1} body weight
2. Curcumin	10	Curcumin	Curcumin	80 mg kg^{-1} body weight
3. NAC	10	NAC	NAC	150 mg kg^{-1} body weight
4a. Alcohol	10	25% ethanol	25% ethanol	9.678 g kg^{-1} body weight
4b. Alcohol+ curcumin	10	25% ethanol	25% ethanol + curcumin	9.678 g kg^{-1} body weight ethanol and 80 mg kg^{-1} body weight curcumin
4c. Alcohol + NAC	10	25% ethanol	25% ethanol + NAC	9.678 g kg^{-1} body weight ethanol and 150 mg kg^{-1} body weight NAC

NAC–N-acetylcysteine.

study was to know the role, if any, played by curcumin and N-acetylcysteine on the PG composition in various tissues in alcohol-fed rats.

Methods

Experimental animals

Adult male Wistar rats (body weights between 150–170 g) obtained from Central Animal House (Faculty of Medicine, Annamalai University, Tamil Nadu, India) were kept at room temperature (30 ± 2°C) under seminatural light/dark conditions. All the rats were fed commercial diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

Materials

Alcohol was purchased from E. Merck (Daramstadt, Germany) and N-acetylcysteine and PG standards (PGE_1, PGE_2, $PGF_{2\alpha}$, and PGD_2) were purchased from Sigma Chemical Company (St. Louis, MO USA). All other reagents used were of high performance liquid chromatography (HPLC) grade.

Animals and treatment

The rats were divided into the following groups (see *Table 1*).

Group 1: Control rats (Rats given a laboratory diet and glucose solution equivalent to calorific value of ethanol (19.356 g of glucose/kg body weight).

Group 2: Rats given curcumin alone (80 mg/kg body weight) as an aqueous solution using an intragastric tube.[17]

Group 3: Rats given N-acetylcysteine (150 mg/kg body weight) as an aqueous solution using an intragastric tube.[18]

Group 4: Rats given 25% ethanol (absolute ethanol diluted to 25%), 5 ml each, (i.e., 9.678 g ethanol/kg body weight) using an intragastric tube. After a period of 1 month, Group 4 was subdivided into three groups.

Group 4a: Rats given 25% ethanol as dosage mentioned above.

Group 4b: Rats given 25% ethanol and curcumin (80 mg/kg body weight) mixed along with ethanol.

Group 4c: Rats given 25% ethanol and N-acetylcysteine (150 mg/kg body weight) as an aqueous solution using an intragastric tube.

After the experimental period (60 days), rats were stunned by a blow at the back of the neck and killed by decapitation.

Sample preparation

Blood for enzyme analysis was collected by sinocular puncture in ependorf tubes. After centrifugation at 3,000 × *g* for 15 min, serum was removed for analysis. Aspartate transaminase (AST) and alkaline phosphatase (ALP) in serum was determined by the methods of Reitman and Frankel[19] and King and Armstrong,[20] respectively, using a diagnostic kit.

Phospholipid and fatty acid analysis

Liver, kidney, and brain for phospholipid and fatty acid analysis were dried between the folds of filter paper, weighed, and transferred to 2:1 choloroform/methanol (vol/vol) for extraction of lipids according to the procedure of Folch et al.[21] Total phospholipids were quantified by phosphorous assay[22] and fatty acid composition was performed according to the method of Morrison and Smith.[23]

Fatty acid analysis was performed by using a Tracer 540 gas chromatograph (2 m long × 2 mm i.d.) packed with 10% cilar on chromosorb W, 80/100 mesh. Separated fatty acids were identified by the comparison of retention times with those obtained by the separation of a mixture of standard fatty acids. Measurement of peak areas and data processing were carried out by an electronic integrator. Individual fatty acids were expressed as a percentage of total fatty acids per 100 mg tissue.

Separation of PGs

PGs from tissues were extracted by the method of Powell.[24] To the tissue samples, a known concentration of the standards was added and processed along with the test samples, and the recovery was measured and found to be more than 95%. The minimum detection was found to be at nanogram level. Tissues transferred to ice-cold chloroform/methanol (1:1) were homogenized and centrifuged, and the supernatant was evaporated using nitrogen gas. Ethanol (1.5 ml) was added and the mixture was allowed to stand for 5 min. Water (10 ml) was then added and acidified to pH 3.0 with 1N HCl, and passed through sep-pak containing ODS

Table 2 (Initial weight, final weight, and gain in weight of animals in different experimental groups

Groups	Initial weight	Final weight	Weight gain
	(g)		
1. Control	163.7 ± 8.36*	291.7 ± 11.86	127.1 ± 12.36^{abh,†}
2. Curcumin	160.2 ± 7.38	278.31 ± 10.11	118.6 ± 11.86^{cfh}
3. NAC	158.9 ± 8.56	285.4 ± 11.03	126.4 ± 8.36^{aefg}
4a. Alcohol	168.7 ± 10.11	251.80 ± 8.86	83.02 ± 11.10
4b. Alcohol + curcumin	155.9 ± 9.11	265.8 ± 7.89	108.9 ± 7.09^{dg}
4c. Alcohol + NAC	160.7 ± 8.89	281.3 ± 8.86	120.63 ± 9.91^{bcde}

*Values are mean ± SD from 6 rats in each group.
†Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's Multiple Range Test).
NAC–N-acetylcysteine.

Silica (Waters C_{18} column 10 × 10mm) using a 20-ml syringe. C_{18} column was pretreated with 20 ml ethanol followed by 20 ml water before use. The column was eluted successively with 20 ml ethanol/water (15:85), 20 ml water, 20 ml petroleum ether, and 10 ml ethyl acetate. The ethyl acetate fraction was collected and evaporated under nitrogen.

Separation and detection of PGs by HPLC

Different types of PGs were separated in HPLC by a modified method of Rolling et al.[25] The solvent used was acetonitrile: 0.0174 M orthophosphoric acid (40:60), pH 2.5. Flow rate was adjusted to 1 ml/min. The HPLC system consisted of SPD-6AV UV-VIS spectrophotometric detector (LC-6AD liquid chromatopac); the column was SP-HPLC, C18-column (250 × 4.6 mm, Nucleosil-0.5 μm, Shimadzu, Japan). The wavelength of the detector was set at 196 nm. Retention time of peaks was compared with those of the standards.

Estimation of total protein in the dry tissue

Total protein in the dry defatted tissue was estimated by microkjeldahl digestion followed by Nesslerisation.[26]

Statistical analysis

Statistical analysis was done by analysis of variance followed by Duncan's Multiple Range Test.[27]

Results

Changes in body weight

Ethanol administration produced less weight gain (*Table 2*) as indicated by emaciation, even though the eating habits of animals in various groups were similar.

Changes in the activities of serum AST and ALP

The activities of serum AST and ALP are given in *Table 3*. The activities of the serum marker enzymes were increased significantly in rats fed alcohol, whereas the activities decreased significantly in rats fed curcumin and N-acetylcysteine along with ethanol.

Table 3 Changes in the activities of serum AST and ALP in different experimental groups

Groups	AST (U/L)	ALP (U/L)
Control	7.9 ± 1.8^{ab,*,†}	126.8 ± 6.02^{ab}
Alcohol	59.7 ± 3.9	193.6 ± 6.11
A + cur	36.8 ± 1.44^{c}	140.6 ± 3.1^{d}
Cur	9.5 ± 1.77^{ab}	128.7 ± 5.9^{ac}
A + NAC	33.7 ± 2.11^{c}	138.6 ± 3.02^{d}
NAC	8.9 ± 1.4^{bd}	127.1 ± 3.89^{bc}

*Values are mean ± SD from 6 rats in each group.
†Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's Multiple Range Test).
AST–aspartatetransaminase. ALP–alkaline phosphatase. A + cur–alcohol + curcumin. Cur–curcumin. A + NAC–alcohol + N-acetylcysteine. NAC–N-acetylcysteine.

Changes in the concentration of phospholipid and arachidonic acid in liver, kidney, and brain

The levels of phospholipids and arachidonic acid were decreased significantly in the tissues of rats fed ethanol when compared with that of control rats, whereas the levels were increased when curcumin and NAC were administered to rats fed ethanol when compared to the group being given ethanol only (*Tables 4, 5, and 6).*

Changes in the concentration of PGs in liver, kidney, and brain

Concentration of PGs (i.e., PGE_1, PGE_2, $PGF_{2\alpha}$, and PGD_2) was significantly increased in liver (*Table 4*), kidney (*Table 5*), and brain (*Table 6*) of rats fed alcohol, whereas the levels were decreased significantly in rats fed alcohol and curcumin, and alcohol and N-acetylcysteine. The administration of curcumin alone was shown to increase the level of PGE_2, PGE_1, and PED_2 in liver, and $PGF_{2\alpha}$, PGE_1, and PGD_2 in kidney. In brain, the levels of PGE_1 and PGE_2 were increased. The administration of N-acetylcysteine alone had no effect on the levels of PGs in the liver, kidney, and brain.

Discussion

Damage to the liver after ethanol ingestion is a well-known phenomenon, and the obvious sign of hepatic injury is the leakage of cellular enzymes into plasma.[28] Raised serum enzyme activities in alcohol-induced toxicity can be attributed to enhanced hepatic generation of oxygen-derived species at various subcellular sites due to the oxidation of ethanol,[29] and oxygen-derived species mediate the cell membrane damage, forming lipid hydroperoxides[30] and ultimately leading to the loss of functional integrity of the membrane and cell death. Previous reports showed that when hepatocytes were exposed to ethanol, the cellular function was perturbed and leakage of AST and lactate dehydrogenase was increased; ethanol also affects structural and functional changes in the mitochondria and increases the membrane permeability,[31] thus leading to the leakage of

Table 4 Changes in the concentrations of phospholipid, arachidonic acid, and prostaglandin in liver in different experimental groups

Group	Phospholipid (mg/100 g tissue)	Arachidonic acid (percentage of AA/100 mg tissue)	$PGF_{2\alpha}$	PGE_2	PGE_1	PGD_2
			(μg/g protein			
Control	2,155.09 ± 107.33[af,*,†]	23.73 ± 0.746[ad]	0.303 ± 0.07[abd]	0.112 ± 0.015	0.034 ± 0.010	0.508 ± 0.050[b]
Alcohol	1,358.47 ± 63.10	16.36 ± 0.575	1.074 ± 0.128	1.096 ± 0.113	0.404 ± 0.047	3.011 ± 0.204
A + cur	1,802.11 ± 101.11[bc]	19.15 ± 0.866[c]	0.826 ± 0.075	0.877 ± 0.080	0.174 ± 0.013[a]	1.074 ± 0.113[a]
Cur	1,787.11 ± 90.63[bd]	21.28 ± 0.956[c]	0.383 ± 0.054[ae]	0.308 ± 0.057[a]	0.234 ± 0.044	0.979 ± 0.083[a]
A + NAC	1,998.13 ± 110.66[edcf]	22.85 ± 0.651[ab]	0.296 ± 0.068[bce]	0.483 ± 0.051	0.135 ± 0.025[a]	0.676 ± 0.053
NAC	2,063.89 ± 109.87[ae]	24.87 ± 0.966[bd]	0.221 ± 0.056[cd]	0.228 ± 0.042[a]	0.092 ± 0.012	0.385 ± 0.039[b]

*Values are mean ± SD from 6 rats in each group.
†Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's Multiple Range Test).
AA–arachidonic acid. PG–prostaglandin. A + cur–Alcohol + curcumin. Cur–curcumin. A + NAC–alcohol + N-acetylcysteine. NAC–N-acetylcysteine.

enzymes into circulation. The observed decrease in serum AST and ALP activities after curcumin and N-acetylcysteine administration shows that curcumin and N-acetylcysteine preserve, to some extent, the structural integrity of the liver from the toxic effects of alcohol.

We reported previously that ethanol administration resulted in both biochemical and histopathological changes in liver, kidney, and brain, which were reverted back when curcumin (80 mg/kg body weight) and N-acetylcysteine (150 mg/kg body weight) were administered to rats fed ethanol.[32]

Free radical mechanisms are involved in ethanol-induced damage in various extra hepatic tissues, especially kidney, heart, brain, testes, and so forth,[33] and we also observed previously that the level of lipid peroxidation product thiobarbituric acid-reactive substances increased significantly in the kidney and brain.[18,32] There are also reports that showed that ethanol increased eicosanoids formation in brain[34] and, hence, we studied the levels of PGs and arachidonic acid in kidney and brain in addition to liver.

Researchers have shown that alcohol-induced changes can cause a significant decrease in the level of arachidonic acid in human plasma, platelets, erythrocytes, and liver tissues.[35–38] Arachidonic acid (20:4) is released from cell membrane phospholipids by the action of calcium-dependent PLA_2.[39] There are basically two pathways for arachidonic acid metabolism. One is the cycloxygenase pathway, which produces PGs, TXs, and prostacyclins.[40] The other pathway results in the production of a variety of biologically active HETE acids and LTs, which are known mediators of the acute vascular changes that accompany the process of inflammation.[41,42] Previous reports have shown that cycloxygenase products of arachidonic acid are present in much higher concentrations in inflamed tissues than in healthy tissues.[12]

Previous studies[43,44] have also demonstrated that chronic exposure to ethanol leads to a progressive increase in membrane PLA_2 activity. This increase in PLA_2 activity increases the breakdown of phospholipids to arachidonic acid, which is subsequently converted to pharmacologically relevant eicosanoids. We also observed decreased levels of phospholipids and arachidonic acid in ethanol-treated rats. Further, this decrease may be due to channeling of arachidonic acid for the production of PGs. Studies have shown that arachidonic acid hydrolyzed from phospholipids is utilized for the production of eicosanoids.[45] Curcumin and N-acetylcysteine have shown to inhibit the production of arachidonic acid in the liver, kidney, and brain. Curcumin also has an inhibitory effect on PLA_2 activity.[46] This can result in decreased hydrolysis of phospholipids. We observed that after curcumin administration, the level of phospholipids remained near normal.

Alcohol administration was shown to increase the levels of messenger RNA for cycloxygenase.[47] This enhanced the production of PGs. In our study, we observed a significant increase in the level of PGE_1, PGE_2, $PGF_{2\alpha}$, and PGD_2 in liver, kidney, and brain in alcohol-fed rats. Maternal infusion of ethanol causes an increase in PGE concentrations of fetal cerebral cortex.[48] Randall et al.[49] reported that in-

Table 5 Changes in the concentrations of phospholipid, arachidonic acid, and prostaglandin in kidney in different experimental groups

Group	Phospholipid (mg/100 g tissue)	Arachidonic acid (percentage of AA/100 mg tissue)	$PGF_{2\alpha}$	PGE_2	PGE_1	PGD_2
			(μg/g protein			
Control	2,014.62 ± 111.12[ab,*,†]	13.04 ± 0.606[a]	0.169 ± 0.044[ac]	0.106 ± 0.012[bcd]	0.115 ± 0.014	0.425 ± 0.045[a]
Alcohol	1,567.26 ± 94.31	6.34 ± 0.621	0.925 ± 0.065	0.580 ± 0.077	0.315 ± 0.094	1.443 ± 0.881
A + cur	1,802.11 ± 101.11[c]	8.21 ± 0.875	0.528 ± 0.076	0.324 ± 0.069	0.100 ± 0.011	0.893 ± 0.071
Cur	1,813.26 ± 87.63[be]	10.42 ± 0.912[b]	0.329 ± 0.10[a]	0.128 ± 0.032[abe]	0.118 ± 0.035	0.541 ± 0.067
A + NAC	1,989.34 ± 97.36[bcd]	10.69 ± 0.635[b]	0.244 ± 0.066[ab]	0.173 ± 0.058[ad]	0.074 ± 0.012	0.361 ± 0.063[a]
NAC	2,063.71 ± 101.26[ac]	12.77 ± 0.585[a]	0.889 ± 0.023[c]	0.097 ± 0.020[ce]	0.073 ± 0.014	0.254 ± 0.077

*Values are mean ± SD from 6 rats in each group.
†Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's Multiple Range Test).
AA–arachidonic acid. PG–prostaglandin. A + cur–Alcohol + curcumin. Cur–curcumin. A + NAC–alcohol + N-acetylcysteine. NAC–N-acetylcysteine.

Table 6 Changes in the concentrations of phospholipid, arachidonic acid, and prostaglandin in brain in different experimental groups

Group	Phospholipid (mg/100 g tissue)	Arachidonic acid (percentage of AA/100 mg tissue)	$PGF_{2\alpha}$	PGE_2	PGE_1	PGD_2
			(μg/g protein			
Control	1845.46 ± 102.46[d,*,†]	15.34 ± 0.619[a]	0.421 ± 0.044[bcd]	0.460 ± 0.054	1.731 ± 0.099[a]	0.087 ± 0.012[bcd]
Alcohol	2795.08 ± 146.26	9.926 ± 0.857	3.322 ± 0.448	1.068 ± 0.097	7.05 ± 0.790	0.309 ± 0.057
A + cur	2011.49 ± 111.26[ac]	14.09 ± 0.673[b]	1.439 ± 0.118	0.663 ± 0.063	5.509 ± 0.450	0.088 ± 0.010[abe]
Cur	2166.73 ± 109.76[ab]	13.64 ± 0.732[b]	0.585 ± 0.057[ad]	0.212 ± 0.075[a]	3.176 ± 0.530	0.119 ± 0.014[ad]
A + NAC	2111.77 ± 100.1[bc]	15.68 ± 0.621[ac]	0.526 ± 0.074[ab]	0.356 ± 0.044	1.767 ± 0.381[a]	0.024 ± 0.009
NAC	1802.11 ± 93.77[d]	16.43 ± 0.520[c]	0.196 ± 0.061[c]	0.197 ± 0.050[a]	1.097 ± 0.144	0.082 ± 0.011[ce]

*Values are mean ± SD from 6 rats in each group.
†Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's Multiple Range Test).
AA–arachidonic acid. PG–prostaglandin. A + cur–Alcohol + curcumin. Cur–curcumin. A + NAC–alcohol + N-acetylcysteine. NAC–N-acetylcysteine.

creased production of PGs occurs when isolated placental cotyledons were perfused with ethanol.

Tumor necrosis factor α (TNF-α) is a central mediator of inflammatory response involved in the pathogenesis of acute and chronic alcoholic liver disease.[50] Reports have shown that PGE_2 and its derivatives have hepatoprotective effects from their ability to suppress TNF-α production in macrophages and kupffers cells accumulated at the site of inflammation.[51,52] Thus, the increase of PGE_2 production may be a mechanism to prevent the toxic effect of ethanol.

The administration of curcumin alone to rats increases PGE_2, PGE_1, and PGD_2 in liver, $PGF_2\alpha$ in kidney, and PGE_1 and PGD_2 in brain; this may be due to long-term administration of curcumin. Previous reports showed that curcumin at higher concentrations depleted cellular glutathione levels and enhanced leakage of lactate dehydrogenase when hepatocytes were treated with curcumin.[53] Thus, curcumin at the dosage of 80 mg/kg body weight prevents ethanol-induced damages but can become cytoxic itself at this dosage when administered alone for a long period.

Curcumin and N-acetylcysteine were shown to decrease the level of PGs in all the tissues studied in alcohol-fed rats.

Curcumin was shown to decrease the PG formation (PGE_2, $PGF_{2\alpha}$, PGD_2, 6 keto-PGF_1 and TXB_2) in azoxymethane-induced colon carcinogenesis.[54] The mechanism by which curcumin decreases the production of PGs is directly due to inhibiting the activities of PLA_2, cycloxygenase, and lipoxygenase.[55] Further, in curcumin-fed animals, the levels of PGs are lower than in alcohol-fed animals. This decrease in PGs suggests that increased arachidonic acid may be utilized for the synthesis of phospholipids, which can be utilized for plasma membrane synthesis. In the present study, we administered curcumin after 1 month of ethanol administration. Because ethanol may have resulted in membrane hydrolysis and dysfunction, curcumin administration results in the resynthesis of plasma membrane, as evident from decreased PG synthesis, increased arachidonic acid level, and increased phospholipid concentration. Thus, curcumin helps in maintaining the membrane structure, integrity, and function. N-acetylcysteine was also shown to decrease the level of PGs in the liver, kidney, and brain. Previous studies have shown that N-acetylcysteine, an amino acid derivative, helps to restore the levels of phospholipids to near normal during ethanol-induced toxicity,[56] thus preventing the breakdown of phospholipids to arachidonic acid and to the pharmacologically relevant eicosanoids.

Thus, the present study shows that both curcumin and N-acetylcysteine offer protection to the liver, kidney, and brain by decreasing PG synthesis, decreasing phospholipid hydrolysis, and increasing phospholipid synthesis. This helps in maintaining the plasma membrane structure, integrity, and function.

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