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Neuroprotective Role of Curcumin from *Curcuma Longa* on Ethanol-induced Brain Damage

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In the present study, curcumin from *Curcuma longa* was screened for neuroprotective activity using ethanol as a model of brain injury. Oral administration of curcumin to rats caused a significant reversal in lipid peroxidation, brain lipids and produced enhancement of glutathione, a non-enzymic antioxidant in ethanol intoxicated rats, revealing that the antioxidative and hypolipidaemic action of curcumin is responsible for its protective role against ethanol induced brain injury. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords: curcumin; neuroprotective; hypolipidaemic; antioxidative.

INTRODUCTION

Spices form an important class of food adjuncts and are used to enhance the sensory quality of food (Gusataffson *et al.*, 1978). Among them *Curcuma longa* along with its primary constituent curcumin is known for its antioxidant and cytoprotective role. Recent studies show that some of the biochemical effects of spices are due to their active principles.

Turmeric (*Curcuma longa* Linn, family: *Zingiberaceae*) has been used as a colouring agent and food additive in Indian culinary preparations from time immemorial (Huang *et al.*, 1994). The active antioxidant principle in *Curcuma longa* has been identified as curcumin (Choiu *et al.*, 1983). The beneficial effects of turmeric which is credited with therapeutic properties have been postulated to be due to curcumin, which has many pharmacological activities such as antiinflammatory (Srimal and Dhawan, 1973), anticancer (Huang *et al.*, 1994), antioxidant properties (Soudamini *et al.*, 1992) and inhibition of lipid peroxidation.

The central nervous system is one of the primary target organs which is most impaired by alcohol. Cognitive impairment and behavioural disturbances are also very prevalent, and for the most part are due to the disruption of central nervous system integrity (Tarter and Alterman, 1984). Ethanol is a powerful neurotoxin and several neurological syndromes have been documented in alcoholics (Freund, 1985). Acetaldehyde produced by the oxidation of ethanol may play a critical role in mediating its pharmacological actions on the central nervous system by penetrating the ventricular walls and stimulating the release of catecholamines in the lateral hypothalamus (Spivak and Amit, 1987).

In the present communication, we have assessed the neuroprotective activity of curcumin against alcohol toxicity by monitoring the lipid peroxidation and brain lipid levels. We have also assessed the level of glutathione in the brain of alcoholic rats fed curcumin.

MATERIALS AND METHODS

Experimental animals. Male albino rats of the Wistar strain (body weight 150–170 g) bred in Central Animal House, Rajah Muthiah Medical College, were used in this study. The animals were fed on pellet diet (Hindustan Lever Limited, Mumbai) and water *ad libitum*.

Chemicals. Curcumin was purchased from CDH(P) Limited, Mumbai. Ethanol was purchased from E. Merck, Darmstadt, F.R. Germany. All other reagents used were of analytical grade.

Experimental procedure. The rats were divided into two groups.

Group I: Control rats (rats given laboratory diet + glucose solution). Equicaloric to ethanol.

Group II: Rats given ethanol 25% (absolute ethanol diluted to 25%) 5 mL each i.e. 9.678 g ethanol/kg body weight using intragastric tube. After a period of 1 month. group II was redivided into two groups.

Group IIa: Rats given 25% ethanol at the dosage mentioned above.

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

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Table 1. Histopathological changes in brain of control, alcohol and alcohol + curcumin treated rats

Microscopic observations	Control	Alcohol	Alcohol + curcumin
Vacuole in neurofil	Absent	Present	Reduced
Spongi form like changes	Absent	Present	Reduced

Histopathological assessment. Animals were perfused with 10% formalin, by means of cardiac puncture and then the brain was removed and stored in formalin. The stored brains were sectioned using a microtome, dehydrated in graded alcohol, embedded in paraffin, sectioned and stained with haematoxylin and eosin.

Biochemical screening. After overnight fast animals were killed by decapitation. The brain was collected in ice cold containers for various estimations. The content-of thiobarbituric acid reactive substances in the brain was estimated by the method of Nichans and Samuelsson (1968) and reduced glutathione in tissue was determined by the method of Beutler and Kelley (1963). Lipid extraction from the brain tissue was carried out according to the procedure of Folch *et al.* (1951). The total cholesterol was estimated by the method of Zak *et al.* (1953), phospholipids by the method of Zilversmit and Davis (1950) and free fatty acids by the method of Folholt and Lund (1973).

Statistical analysis. Statistical analysis was done by Student's *t*-test and values were expressed as mean ± SD (Bennett and Franklin, 1954).

RESULTS

Table 1 shows the histopathological changes in the brain after alcohol treatment. It shows vacuoles in the neurofil and some spongi form like changes (status spongiosis) in the brain. There was also microdysplasia. In the case of curcumin treatment, the changes were much reduced.

The levels of TBARS, cholesterol, phospholipids and free fatty acids were increased significantly in the brain after alcohol treatment. The levels of these parameters were near normal after curcumin treatment (Table 2). The level of reduced glutathione was decreased significantly in the brain of rats fed alcohol (Table 2). The administration of curcumin restored the levels of glutathione to near normal.

DISCUSSION

In our previous study we have observed that curcumin offers protection against alcohol induced changes in the levels of serum lipid and marker enzymes (Rajakrishnan *et al.*, 1998), the indicies of hepatic dysfunction.

Acute alcoholic intoxication leads to cerebral congestion, oedema, petechial haemorrhages (Butterworth *et al.*, 1993) and when cardiovascular degeneration is present, in particular cerebral arteriosclerosis with hypertension, a toxic dose of alcohol may lead to massive haemorrhage or infarction (Allsop and Turner, 1996).

In our present study we have also observed changes in the brain of alcohol treated animals such as vacuoles in the neurofil and spongi form like changes (Fig. 2) as reported earlier by Pieffer *et al.* (1979). Curcumin administration resulted in a reduction in the abnormalities (Fig. 3). Ethanol is a substantial source of energy that exceeds the energy content of carbohydrates or proteins and accounts for half an alcoholic's caloric intake, causing malnutrition, including deficiencies of folate, thiamine and other vitamins (Lieber, 1995) which may cause lesions in the brain leading to memory impairment (Butterworth *et al.*, 1993).

The pathological changes we observed may be due to the above mentioned factors. Administration of curcumin decreases the pathological changes caused by alcohol thereby protecting the brain from the adverse effects of alcohol.

The brain, due to its high rate of oxygen consumption, high phospholipid content with polyunsaturated fatty acids, low levels of vitamin E and selenium dependent glutathione peroxidase activity is more susceptible to peroxidative changes (Suresh Kumar and Menon, 1993).

The increased levels of thiobarbituric acid reactive substances in the brain of rats fed alcohol may be due to the oxidation product of ethanol, acetaldehyde, which may stimulate the release of catecholamines, which in turn produce superoxide radicals (Halliwell and Gutter-

Table 2. Levels of cholesterol, phospholipid, free fatty acids, TBARS and reduced glutathione in brain of control, alcohol and alcohol + curcumin treated rats

Parameter	Control(Group I)	Alcohol(Group IIa)	Alcohol + curcumin(Group IIb)
Cholesterol (mg/100 g tissue)	1531.93 ± 41.22	2031.08 ± 64^{a}	1654.18 ± 41.70^{aA}
Phospholipid (mg/100 g tissue)	1845.46 ± 102.46	2795.08 ± 146.26^{a}	2011.49 ± 111.26^{cA}
Free fatty acids (mg/100 g tissue)	26.69 ± 3.25	53.08 ± 6.95^{a}	39.865 ± 7.28^{bB}
TBARS (mM/100 g tissue)	1.293 ± 0.123	2.983 ± 0.180^{a}	2.408 ± 0.209^{aA}
Reduced glutathione (mg/100 g tissue)	96.00 ± 12.42	42.39 ± 3.82^{a}	65.09 ± 5.89^{aA}

Values are mean ± SD from 6 rats in each group.
Groups IIa and IIb compared with Group I
a$p < 0.001$.
b$p < 0.01$.
c$p < 0.05$.
Group IIa compared with Group IIb
A$p < 0.001$.
B$p < 0.01$.

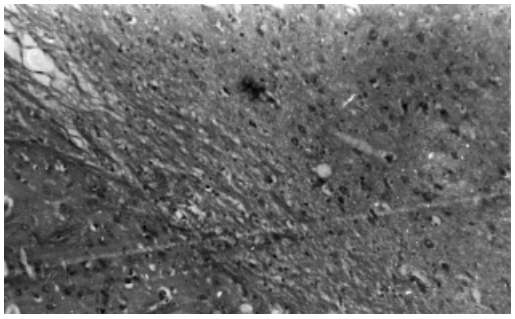

Figure 1. Control rat brain H and E × 200.

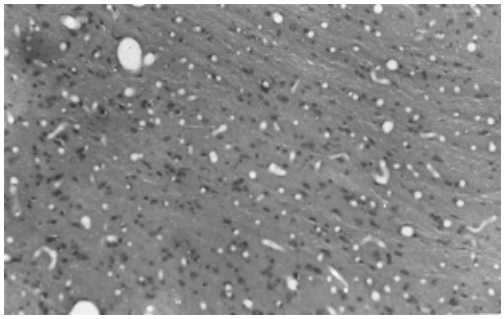

Figure 2. Alcohol treated rat brain H and E × 200.

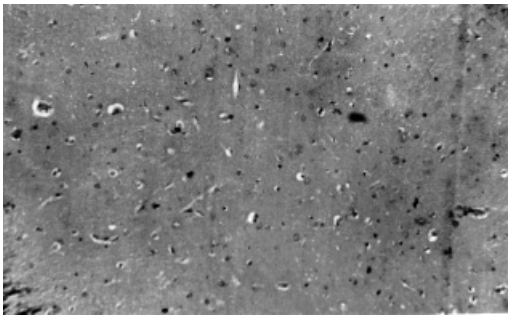

Figure 3. Alcohol + curcumin treated rat brain H and E × 200.

idge, 1985). In this context Thomas *et al.* (1985) have demonstrated that superoxide can promote the release of iron from ferritin which is rich in the brain (Halliwell and Gutteridge, 1985). This release of iron may cause lipid peroxidation. Lipid peroxidation results in membrane disorganization by peroxidizing mainly PUFA, with a decrease in membrane fluidity (Yamada *et al.*, 1985). The uncontrolled peroxidation of biomembranes can thus lead to a profound effect on the membrane structure and may also be responsible for the defective neurotransmission.

On curcumin administration the levels of TBARS decreased significantly in the brain of rats fed alcohol. This may be due to the antioxidant property of curcumin. The antioxidant property of curcumin (diferuloyl methane) may be due its phenolic character.

Glutathione, (γ-glutamyl cysteinyl glycine) a tripeptide, conjugates potentially toxic electrophilic xenobiotics and is therefore an important defence mechanism against certain toxic compounds. If the levels of glutathione in a tissue are lowered, then the tissue can be shown to be more susceptible to injury by various chemicals that would normally be conjugated to GSH. GSH, being the most important biomolecule against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates (Nordmann, 1994).

In our present study the levels of glutathione in the brain decreased in alcohol treated rats. The decreased glutathione content may be due to increased utilization for scavenging the toxic intermediates formed from ethanol. Curcumin treatment caused an enhancement in the GSH content of the brain.

Studies have shown that cytochrome P4502E1 is present in specific areas of the brain (Sohda *et al.*, 1993) and this system metabolizes ethanol and produces acetaldehyde, which has striking effects on the central nervous system. In this context Oetari *et al.*, (1996) indicate that curcumin possesses an inhibitory property towards cyt P-450 in addition to its well-known antioxidant activity which may explain the enhanced level of GSH in brain.

Ethanol is a powerful inducer of hyperlipidaemia and causes changes in the metabolism of serum and tissue lipids (Rajakrishnan *et al.*, 1997). Our present study shows that there is an increase in the levels of cholesterol, phospholipid and free fatty acids.

The increase in the levels of cholesterol may be due to an increase in the activity of hydroxymethyl coenzyme A (HMG-CoA) reductase activity, the rate limiting enzyme in the cholesterol synthesis.

Alcohol permeates the membranes and fluidizes them (Lieber, 1995). The increase in phospholipid content in the brain of rats fed alcohol may be due to an increase in the fluidity of the membrane due to an increase in peroxidation. In this context, Taraschi and Rubin (1985) have demonstrated that there is an alteration in the membrane phospholipid in the brain.

The levels of free fatty acids (FFA) increased in the brain of alcohol treated rats. The higher level of free fatty acids in the brain may be due to extraction of FFA from the plasma. In this context, an increased concentration of FFAs in the plasma has been observed (Kaffarink, 1978). Alterations in membrane saturated and unsaturated fatty acids in brain membrane have also been observed (Sun and Sun, 1985). The increased FFA content in the brain may also be due to increased peroxidation of brain tissue.

Curcumin administration to rats fed alcohol showed a reduction in the level of cholesterol in the brain, the mechanism explaining this may be either due to a decrease in absorption or due to the mobilizing capacity of curcumin from extrahepatic tissue to liver where it is catabolized (Soudamini *et al.*, 1992). Curcumin administration also decreased the levels of phospholipid and free fatty acids. This may be due to the protective effect of curcumin against alcohol induced membrane damage thereby preserving the membrane integrity of the brain from adverse effects of ethanol.

Thus curcumin plays a major role in preventing ethanol induced changes, in the brain.

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