Original Article

Effect of *Cassia auriculata* leaf extract on lipids in rats with alcoholic liver injury

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We studied the effect of administering *Cassia auriculata* leaf extract to rats with experimentally induced liver damage. Hepatotoxicity was induced by administering 9.875 g/kg bodyweight ethanol for 30 days by intragastric intubation. *C. auriculata* leaf extract was administered at a dose of 250 mg/kg bodyweight daily in one group and 500 mg/kg bodyweight daily in another group of alcohol-treated rats. All rats were fed with standard pellets. The control rats were also given isocaloric glucose solution. The average bodyweight gain was significantly lower in alcohol-treated rats, but improved on supplementation with *C. auriculata* leaf extract. Alcohol supplementation significantly elevated the cholesterol, phospholipid and triglyceride concentration in the liver, brain, kidney and intestine, as compared with those of the normal control rats. Treatment with *C. auriculata* leaf extract and alcohol significantly lowered the tissue lipid levels to almost normal levels. Microscopic examination of alcohol-treated rat liver showed inflammatory cell infiltrates and fatty changes, which were reversed on treatment with *C. auriculata* leaf extract. Similarly, alcohol-treated rat brain demonstrated spongiosis, which was markedly reduced on treatment with *C. auriculata*. In conclusion, this study shows that treatment with *C. auriculata* leaf extract has a lipid-lowering effect in rats with experimentally induced, alcohol-related liver damage. This is associated with a reversal of steatosis in the liver and of spongiosis in the brain. The mechanism of *C. auriculata* leaf extract lipid-lowering potential is unclear.

Key words: alcohol, *Cassia auriculata*, hepatotoxicity, hypolipidemia, India.

Introduction

Ethanol is a powerful inducer of hyperlipidemia in both animals and humans.[1] It also causes a change in the metabolism of lipoproteins.[2] Marked alterations in lipid metabolism have been reported in chronic alcohol feeding.[3] In addition, Remla *et al.*[4] have reported that administration of ethanol to rats causes changes in the metabolism of serum and tissue lipids. The lipid abnormalities seen after alcohol consumption include alterations in the level of cholesterol, fatty acid esters, cholesterol esters and, particularly, the fatty acyl composition of membrane phospholipids.[5]

There is a resurgence of interest in herbal medicine for the treatment of various ailments, chiefly because of the prohibitive cost of allopathic drugs, their unavailability in remote areas and the popular belief that naturally occurring products are without any adverse side-effects. Various spices and herbs used in the traditional Indian system of medicine, 'Ayurvedha', have anti-thrombotic, anti-atherosclerotic, hypoglycaemic, hypolipidemic, anti-inflammatory and anti-arthritic properties.[6]

Cassia auriculata Linn., known locally as 'avaram' and belonging to the family Caesalpiniaceae, has been used for the treatment of ulcers, leprosy, skin and liver diseases. A literature survey using Medline failed to identify a definite mechanism of action for its anecdotal benefits. The present study was undertaken to evaluate the effect of *C. auriculata* leaf extract on lipid metabolism in alcohol-induced hepatic damage, as well as on histomorphological changes in the liver and brain in the animal model.

Materials and methods

Ammonium molybdate, sodium metaperiodate, acetyl acetone and 1-amino, 2-naphthol, 4-sulphonic acid (ANSA) were obtained from the Sigma Chemical Company (St Louis, MO, USA). Ethanol was obtained from Nellikuppam (Cuddalore District, South India). All other chemicals and solvents were of analytical grade and purchased from Central Drug House (Mumbai, India).

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Plant material

Fresh *C. auriculata* leaves was collected from Kavarapattu, Chidambaram Taluk, South India. The leaves were thoroughly dried under shade and powdered. A suspension of 100 g in 200 mL distilled water was stirred magnetically overnight at room temperature (approximately 27°C). This was repeated three times. The extract was evaporated to dryness under reduced pressure in a rotary evaporator. The yield was 26.9% (w/w) in terms of dry starting material.

Thirty healthy, male, adult Wistar rats (150–170 g) were procured from the Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. They were housed in plastic cages with filter tops under controlled conditions of a 12 h light/12 h dark cycle, 50% humidity and 28°C. They all received a standard pellet diet (Lipton Lever, Mumbai, India) and water ad libitum.

The animals were divided into five groups of six rats each. Groups 1 and 2 received a normal diet of standard pellets and isocaloric glucose from a 40% glucose solution. Liver cell damage was induced in rats of Groups 3, 4 and 5 by administering 5 mL of 25% ethanol (2.5 mL in the morning and 2.5 mL in the afternoon), equivalent to 9.875 g/kg bodyweight as an aqueous solution, using an intragastric tube for 30 days.[7,8] At the end of this period the animals were treated as follows for the next 30 days.

Group 1. Experimental control rats continued to receive a standard pellet diet (15 g/150 g bodyweight/day) and isocaloric glucose from a 40% glucose solution daily by intragastric intubation.

Group 2. Control rats continued to receive a standard pellet diet (15 g/150 g bodyweight/day) and isocaloric glucose from a 40% glucose solution daily by intragastric intubation. In addition, they received *C. auriculata* leaf extract, at a dose of 250 mg/kg bodyweight, in distilled water.

Group 3. Rats received a standard pellet diet (15 g/150 g bodyweight/day), with 25% ethanol.

Group 4. Rats received a standard pellet diet (15 g/150 g bodyweight/day), with 25% ethanol and also with 250 mg/kg bodyweight of *C. auriculata* leaf extract in distilled water by intragastric intubation.

Group 5. Rats received a standard pellet diet (15 g/150 g bodyweight/day), with 25% ethanol and also with 500 mg/kg bodyweight of *C. auriculata* leaf extract in distilled water by intragastric intubation.

The total duration of the experiment was 60 days, at the end of which, some of the animals were anaesthetised using light ether and killed by cervical decapitation. Blood was collected and processed for the assays of aspartate transaminase (AST; EC 2.6.1.1, Sigma)[9] alanine transaminase (ALT; EC 2.6.1.2, Sigma)[9] and alkaline phosphatase (ALP; 3.1.3.1, Sigma).[10] Liver, brain, kidney and intestine were cleared of adhering fat, weighed accurately and used for lipid extraction. Lipids were extracted from tissue as described previously by Folch *et al.*,[11] and the tissue concentrations of total cholesterol,[12] phospholipids[13] and triglycerides[14] were measured. The rest of the animals were subjected to whole-body perfusion using normal saline and 10% formalin under light ether anaesthesia. The brain and liver were removed and stored immediately in 10% formalin for histopathological examination. The tissues were then embedded in paraffin, thinly sectioned using a microtome, stained with haematoxylin and eosin (H&E) and mounted in neutral disterene dibutyl phthalate xylene (DPX) medium and examined by light microscopy.

All of the grouped data were evaluated statistically and the significance of changes caused by the various treatments was determined using the Student's *t*-test.[15] The results are expressed as Mean ± SD of six rats from each group. A one-way ANOVA was carried out wherever appropriate. The level of statistical significance was set at $P < 0.01$.

Results

Table 1 shows the average weight gained by the rats during the experimental period of 60 days. The final bodyweights of the alcohol-treated rats (Group 3) were significantly lower than those of the control group (Group 1). Treatment with *C. auriculata* leaf extract along with alcohol (Groups 4 and 5) improved the gain in bodyweight significantly. Supplementation with *C. auriculata* leaf extract to control rats (Group 2) did not show any significant change in the amount of weight gained.

The concentrations of AST, ALT and ALP are shown in Table 2. The levels of AST, ALT and ALP in the serum were significantly higher in rats receiving alcohol (Group 3) than in the normal control rats (Group 1; $P < 0.01$). Control rats supplemented with *C. auriculata* leaf extract (Group 2) did not show any significant change in the activities of these enzymes. Treatment with *C. auriculata* at a dose of 250 mg or 500 mg/kg bodyweight, along with alcohol (Groups 4 and 5), showed significantly reduced levels of AST, ALT and ALP as compared with those of the unsupplemented alcohol-treated rats (Group 3). Table 3 highlights the concentrations of cholesterol and phospholipids in liver, brain, kidney and intestine. Cholesterol and phospholipid levels were significantly higher in the alcohol-treated rats (Group 3) compared with

Table 1. Average weight gain by the rats during the experimental period of 8 weeks

Group	Initial weight (g)	Final weight (g)
1	135.00 ± 10.80	222.00 ± 8.53
2	132.00 ± 83.00	205.00 ± 10.80†*
3	145.00 ± 10.60	162.00 ± 8.53†**
4	144.00 ± 10.57	190.00 ± 10.82‡*
5	145.00 ± 10.80	202.00 ± 8.5‡**
F-ratio	1.14	27.40***

Values are the Mean ± SD of six rats from each group. †Compared with Group 1; ‡Compared with Group 3. Statistical significance (Student's *t*-test): *$P < 0.05$; **$P < 0.001$. Statistical significance (ANOVA): ***$P < 0.01$.

those of the control rats (Group 1; $P < 0.01$). Treatment with *C. auriculata* leaf extract at 250 or 500 mg/kg bodyweight in the alcohol-supplemented rats (Groups 4 and 5) resulted in significantly lower levels of cholesterol and phospholipids, compared with the alcohol-treated rats who did not receive the *C. auriculata* leaf extract (Group 3; $P < 0.01$). Supplementation with *C. auriculata* leaf extract to the control rats (Group 2) did not produce any significant change in the concentration of phospholipids or cholesterol.

Table 4 show the concentrations of triglycerides in liver, brain, kidney and intestine. The concentrations of the triglycerides were significantly higher in alcohol-treated rats (Group 3) as compared with those of control rats (Group 1; $P < 0.01$). Treatment with *C. auriculata* leaf extract at 250 or 500 mg/kg bodyweight, along with alcohol (Groups 4 and 5), significantly lowered the levels of triglycerides, compared with the alcohol-treated rats who did not receive the *C. auriculata* leaf extract (Group 3; $P < 0.01$). Supplementation with *C. auriculata* leaf extract to the control rats (Group 2) did not produce any significant change in the concentration of triglycerides.

Microscopic findings

In the alcohol-treated rat liver, the involvement of the liver was uniform. Fatty changes of both macro- and microvesicular type, and mononuclear cell infiltrates were observed in all fields (Fig 1).

The liver of the alcohol-treated rats who received 250 mg/kg bodyweight of *C. auriculata* leaf extract showed only focal areas of fatty changes. Moreover, the fatty changes appeared to be greatly reduced. Mononuclear inflammatory cell infiltrates were present, but not to the extent seen in the liver of those rats treated with alcohol only (Fig. 2). The liver of alcohol-treated rats who received 500 mg/kg bodyweight of *C. auriculata* leaf extract showed loss of individual hepatocytes by degeneration and the space were the cell had originally been appeared empty, but there was no evidence of fatty change (Fig. 3). Control rats treated with *C. auriculata* demonstrated normal liver morphology (Figs 4,5).

The brain tissue in alcohol-treated rats showed spongiosis, which was not evident in rats treated with *C. auriculata* leaf extract (Figs 6–8). Brain tissue in control rats treated with *C. auriculata* revealed normal histology (Figs 9,10).

Discussion

The liver plays a central role in coordinating various metabolic functions of the body. Alcohol is an important cause of various liver diseases. Hepatocyte injury reduces the capacity of this major storage site and causes release of vitamins (coenzymes) into the circulation in the form of holoenzymes.

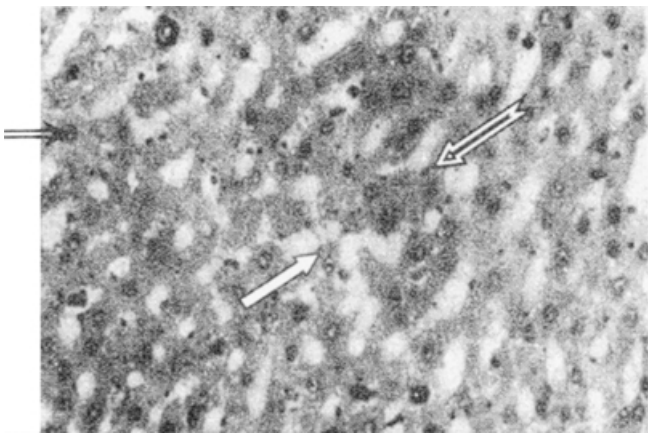

Figure 1. Liver of alcoholic rat. Fatty changes of macrovesicular type (white arrow with black line), microvesicular type (white arrow) and mononuclear cell infiltrates (black-headed arrow) were observed (H&E).

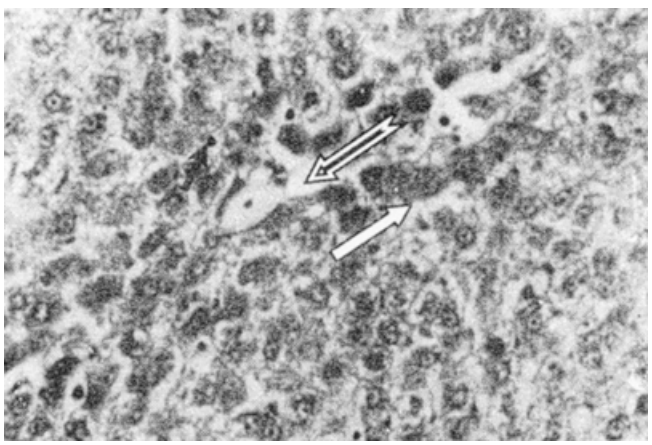

Figure 2. Liver of alcoholic rat treated with 250 mg/kg bodyweight *Cassia auriculata* leaf extract. Arrows indicate focal areas of fatty changes and significantly reduced amounts of mononuclear cell infiltrates (H&E).

Table 2. Effect of *Cassia auriculata* on serum AST, ALT and ALP of the control and experimental rats

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
1	73.70 ± 7.16	22.16 ± 1.49	75.29 ± 7.02
2	72.29 ± 7.42	25.59 ± 2.73	75.16 ± 7.65
3	84.01 ± 8.56†**	55.43 ± 5.60†**	97.69 ± 9.49†**
4	74.25 ± 7.88‡**	35.89 ± 3.49‡**	85.14 ± 8.98‡***
5	73.27 ± 7.11‡**	25.30 ± 1.56‡***	76.81 ± 7.12‡***
	1.97	54.26****	10.75****

Values are the Mean ± SD of six rats from each group. ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase. †Compared with Group 1; ‡Compared with Group 3. Statistical significance (Student's *t*-test): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Statistical significance (ANOVA): ****$P < 0.01$.

Table 3. Effect of *Cassia auriculata* on phospholipids and cholesterol in tissues of the control and experimental rats

Group	Phospholipids (µg/mg tissue)				Cholesterol (µg/mg tissue)			
	Liver	Brain	Kidney	Intestine	Liver	Brain	Kidney	Intestine
1	21.98 ± 1.64	23.38 ± 2.11	15.16 ± 1.76	6.70 ± 1.26	2.84 ± 0.74	6.69 ± 0.51	5.11 ± 0.71	3.31 ± 0.63
2	19.74 ± 1.92	23.45 ± 2.18	14.84 ± 1.62	7.46 ± 1.26	2.53 ± 0.86	6.70 ± 0.74	5.71 ± 0.67	3.16 ± 0.86
3	34.84 ± 2.62†***	38.28 ± 2.16†***	20.61 ± 2.20†**	9.77 ± 1.45†**	5.06 ± 0.87†**	9.53 ± 0.82†**	8.03 ± 0.54†***	6.34 ± 0.54†***
4	27.01 ± 1.72‡**	33.46 ± 2.18‡*	16.41 ± 1.98‡*	8.66 ± 1.21‡*	3.58 ± 0.54‡*	8.00 ± 0.94‡***	6.20 ± 1.12‡*	5.62 ± 0.90‡*
5	24.88 ± 2.58‡*	26.95 ± 1.75‡***	16.56 ± 2.06‡*	8.33 ± 1.09‡*	3.10 ± 0.57‡**	7.61 ± 0.74‡***	6.46 ± 0.65‡**	4.25 ± 0.90‡**
F-ratio****	39.00	49.67	7.00	3.82	5.59	4.96	4.98	14.45

Values are the Mean ± SD of six rats from each group. †Compared with Group 1; ‡Compared with Group 3. Statistical significance (Student's *t*-test): $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Statistical significance (ANOVA): $****P < 0.01$.

Figure 3. Liver of alcoholic rat treated with 500 mg/kg bodyweight *Cassia auriculata* leaf extract. Arrow indicates hepatocyte dropout. Fatty changes were markedly reduced (H&E).

Figure 4. Liver of control rats (H&E).

Figure 5. Liver of control rat treated with *Cassia auriculata* leaf extract (H&E).

Table 4. Effect of *Cassia auriculata* on triglycerides in tissues of the control and experimental rats

Group	Triglycerides (mg/g tissue)			
	Liver	Brain	Kidney	Intestine
1	3.64 ± 1.26	3.72 ± 1.08	4.41 ± 1.17	3.78 ± 1.05
2	3.47 ± 0.40	3.67 ± 1.02	4.43 ± 1.00	3.60 ± 1.00
3	7.58 ± 1.22†***	8.26 ± 1.24†***	6.53 ± 1.24†***	6.40 ± 0.994†***
4	5.70 ± 0.87‡*	5.85 ± 1.41‡**	4.55 ± 1.23‡*	4.28 ± 1.05‡**
5	4.62 ± 1.12*	4.41 ± 1.21‡***	4.38 ± 1.10‡*	4.51 ± 1.23‡**
F-ratio****	11.48	11.20	3.25	4.15

Values are the Mean ± SD of six rats from each group. †Compared with Group 1; ‡Compared with Group 3. Statistical significance (Student's *t*-test): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Statistical significance (ANOVA): ****$P < 0.01$.

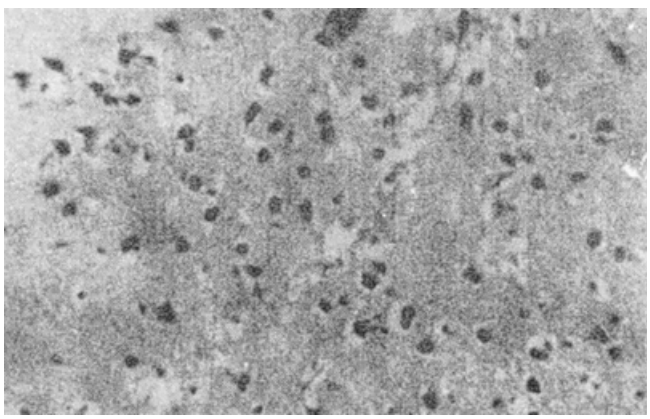

Figure 6. Brain of alcoholic rat showing spongiosis (H&E).

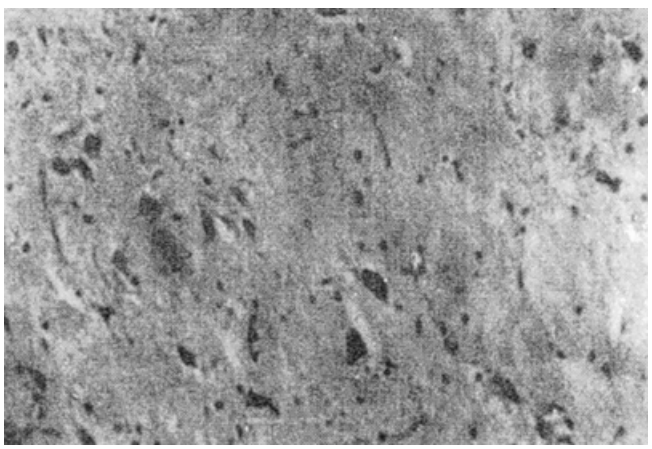

Figure 8. Brain of alcoholic rat treated with 500 mg/kg bodyweight *Cassia auriculata* leaf extract. Spongiosis is markedly reduced (H&E).

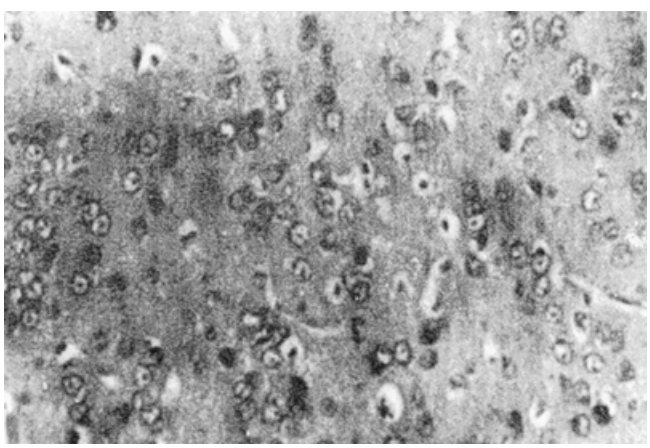

Figure 7. Brain of alcoholic rat treated with 250 mg/kg bodyweight *Cassia auriculata* leaf extract showing spongiosis.

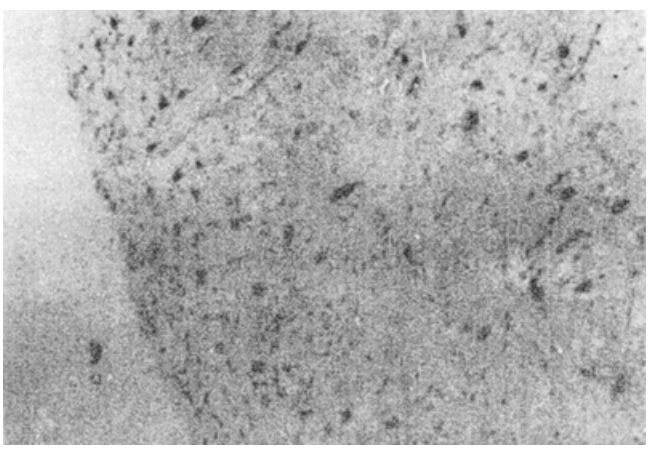

Figure 9. Brain of control rat (H&E).

On *C. auriculata* leaf extract administration to alcoholic rats, we observed decreased activities of serum AST, ALT and ALP. This shows that *C. auriculata* leaf extract does, to an extent, preserve the structural integrity of hepatocytes, thereby protecting the liver from the adverse effects of ethanol.[16]

The average weight gain of the rats during the experimental period was significantly reduced in alcohol-treated rats, compared with the control rats. Rajakrishnan *et al.*[17] also observed a decrease in weight gain on alcohol treatment. But supplementation with *C. auriculata* leaf extract along with alcohol showed a significant improvement in the amount of weight gained, emphasising the beneficial effect of *C. auriculata* leaf extract.

The interaction of ethanol with lipid metabolism is complex. When ethanol is present, it becomes the preferred fuel for the liver and displaces fat as a source of energy. This blocks fat oxidation and favours fat accumulation.[18] The accumulation of fat in the liver acts as a stimulus for the secretion of lipoproteins into the bloodstream and the development of

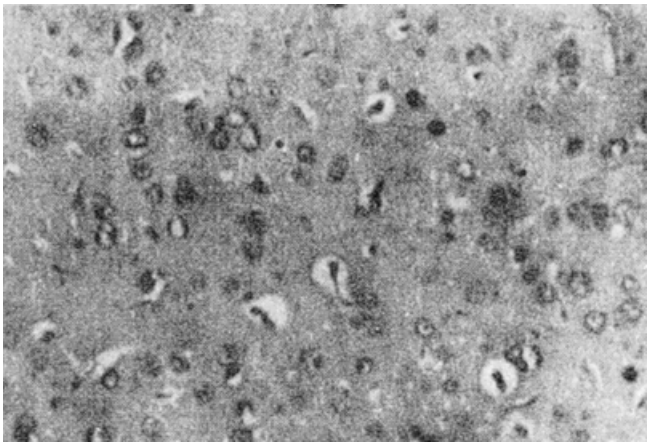

Figure 10. Brain of control rat treated with *Cassia auriculata* leaf extract (H&E).

hyperlipidemia. Hyperlipidemia may also be caused by proliferation of the endoplasmic reticulum after chronic ethanol consumption and the associated increase in the activities of enzymes involved in the assembly of triglycerides and lipoproteins.[19] A number of studies have shown that the plasma and tissue cholesterol level increases with alcohol consumption.[20] Our observations also correlate with the previous researchers in that there was a significant increase with the cholesterol levels of the liver, brain, kidney and intestine of alcohol-treated rats. Moreover, this study demonstrates lower hepatic cholesterol levels in *C. auriculata* leaf extract-treated alcoholic rats. Many plant products are known to elevate the activity of cholesterol 7-α hydroxylase,[21] thereby enhancing cholesterol degradation. *C. auriculata* leaf extract may also play a significant role in elevating the activity of cholesterol 7-α hydroxylase. Thus, the hypocholesterolemic effect of *C. auriculata* leaf extract may be due to an enhanced degradation of cholesterol.

Various studies have shown that ethanol exposure induces changes in various lipid constituents of the brain and erythrocyte membranes, such as cholesterol,[22] cholesterol esters,[23] fatty acid acyl esters[24] and, particularly, the fatty acyl composition of membrane phospholipids.[25] It is conceivable that many of the actions of ethanol lead to the disordering of phospholipid acyl chains in the membrane and the subsequent development of resistance to ethanol, which is in turn associated with the changes in enzymes that govern phospholipid metabolism, such as phospholipase A2 and acyl transferases. These enzymes may participate in several key events that determine the turnover of phospholipids in cell membranes, specifically the deacylation and reacylation cycle,[26] the biosynthesis of eicosanoids and signal transduction across membranes.[27] We observed a significantly reduced level of phospholipids in rats receiving alcohol plus *C. auriculata* leaf extract. This may be due to increased degradation of phospholipids in the presence of *C. auriculata* leaf extract.

Fatty acids from different sources can accumulate as triglycerides in the liver as a consequence of a variety of metabolic disturbances. These include enhanced hepatic lipogenesis, decreased hepatic release of lipoproteins, increased mobilisation of peripheral fat, enhanced hepatic uptake of circulating lipids and, most importantly, decreased fatty acid oxidation, whether as a function of reduced citric acid cycle activity secondary to the altered redox potential, or as a result of permanent changes in mitochondrial structure and function.[28] In cultured hepatocytes, the increased intracellular accumulation of triacyl glycerol in the presence of ethanol was accounted for quantitatively by increased fatty acid uptake, decreased fatty acid oxidation in the tricarboxylic acid cycle and decreased lipoprotein secretion.[29] Both acute and chronic ethanol consumption can induce hypertriglyceridemia.[30] Brodie *et al.*[31] showed that a single oral dose of ethanol to rats induced accumulation of liver triglycerides. Antonenkov *et al.*[32] also reported that chronic ingestion of ethanol results in moderate hypercholesterolemia, hypertriglyceridemia and increased concentration of lipids in the liver. In the present study, we observed elevated triglyceride levels in alcohol-treated rats. Moreover, treatment with *C. auriculata* leaf extract to alcohol-treated rats decreased the tissue triglyceride levels significantly, possibly because *C. auriculata* leaf extract inhibits enzymes of fatty acid oxidation and enhances the activity of lipogenic enzymes. In this context, spices and other medicinal plants are also known to have similar hypolipidemic effects.[33–35]

Significant pathomorphological alterations in the liver and brain were observed in alcohol-treated rats. Alcohol supplementation is known to damage the liver and brain. These changes can alter the properties of the cell. The microscopic changes observed in the liver of alcohol-treated rats were predominant in the centrilobular region. Hepatic damage may be partially attributed to cytochrome-P_{450}-generated metabolic cytochrome-P_{450}-dependent enzyme activities in liver that tend to be present at their greatest concentration near the central vein, and lowest near the peripheral sites.[36] Treating alcohol-treated rats with *C. auriculata* leaf extract reduced the level of fatty changes and improved the histomorphology of the liver.

Microdysplasia and spongioform changes have been demonstrated in the hypothalamic and thalamic regions of the brains of alcohol-treated rats.[37] These are indicative of local brain development disorders. In this study, we observed spongiosis in the brain of the alcohol-treated rats, which was reversed on treatment with *C. auriculata* leaf extract.

Control rats were also treated with *C. auriculata* leaf extract to examine the role of *C. auriculata* leaf extract per se under controlled conditions, and to evaluate statistically the extent of benefit it offers in alcohol-induced hyperlipidemia. Data observed in the present study did not show any significant effect on tissue lipids when *C. auriculata* leaf extract was administered to control rats.

Thus, our results demonstrate that *C. auriculata* leaf extract has a hypolipidemic effect in an animal model of alcohol-induced hyperlipidemia. *C. auriculata* leaf extract treatment showed significant improvement in the histopathological changes observed in the liver and brain of alcohol

supplemented rats. Further studies in our laboratory are being done to unravel the exact mechanism by which *C. auriculata* leaf extract treatment lowers the levels of tissue lipids.

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