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Journal of Nutritional Biochemistry 14 (2003) 452–458

THE JOURNAL OF Nutritional Biochemistry

Activity of *Cassia auriculata* leaf extract in rats with alcoholic liver injury

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Received 22 January 2002; received in revised form 13 January 2003; accepted 14 April 2003

Abstract

This study was undertaken to investigate the effect of *Cassia auriculata* leaf extract on tissue lipid peroxidation and antioxidant status in experimental hepatotoxicity. Administering ethanol to rats for 60 days resulted in significantly elevated levels of serum total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) as compared with those of the experimental control rats. Significantly elevated levels of tissue thiobarbituric acid reactive substances (TBARS), hydroperoxides and lowered activities of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were also observed on alcohol treatment as compared with those of experimental control rats. Concentration of serum non-enzymic antioxidants such as vitamin E and vitamin C were also significantly lowered on alcohol supplementation. Treatment with *Cassia auriculata* leaf extract at a dose of 250 mg kg^{-1} body weight and 500 mg kg^{-1} body weight to rats administered alcohol, lowered the levels of TBARS and hydroperoxides and elevated the activities of SOD and CAT and the levels of reduced GSH in the liver, brain, kidney and intestine significantly compared to unsupplemented alcohol treated rats. *Cassia auriculata* leaf extract treatment restored the serum vitamin E, and vitamin C levels also to near those of the experimental control animals. Our data indicate that supplementation with *Cassia auriculata* leaf extract can offer protection against free radical mediated oxidative stress in experimental hepatotoxicity. In addition, histopathological studies of the liver and brain confirmed the beneficial role of *Cassia auriculata* leaf extract.

Keywords: Antioxidants; *Cassia auriculata*; Hepatotoxicity; Lipid peroxidation; Oxidative stress

1. Introduction

Ethanol is a fat-soluble non-electrolyte, which is readily absorbed from the gastrointestinal tract, diffuses rapidly into circulation and is distributed uniformly throughout the body [1]. Ethanol is almost exclusively metabolized in the body by enzyme catalyzed oxidative processes. The acetaldehyde formed is further oxidized to acetate, which is then converted to carbon dioxide via the citric acid cycle [2]. Ethanol or its metabolites can also cause auto-oxidation of the hepatic cells either by acting as a pro-oxidant or by reducing the anti-oxidant levels resulting in marked hepatotoxicity [3]. Lipid peroxidation and associated membrane damage is a key feature in alcoholic liver injury [4].

The protective action of antioxidants is usually due to the inhibition of free radical-induced chain reaction and the resultant prevention of peroxidative deterioration of structural lipids in membranous organelles. Circulating antioxidants mainly vitamin C and vitamin E and tissue enzymic and non-enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) play an important role in alleviating tissue damage due to the formation of free radicals [5].

Spices and vegetables possess antioxidant activity and thus in addition to imparting flavor to the food, they possess potential health benefits by inhibiting lipid peroxidation [6]. *Cassia auriculata* Linn., locally known as ‘avaram,’ belonging to the family caesalpiniaceae, grows abundantly and widely all over India. The leaves of this plant have been used in the traditional system of Indian medicine for the treatment of jaundice and liver diseases.

Literature survey shows that no sufficient work has been done to study its mechanism of action. The present study

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0955-2863/03/$ – see front matter
doi:10.1016/S0955-2863(03)00053-6

was planned to evaluate the effect of *Cassia auriculata* leaf extract on liver function and also to unravel its role on tissue lipid peroxidation and on the antioxidant levels in rats administered alcohol. The findings are compared with those of the control and unsupplemented alcohol treated rats.

2. Materials and methods

2.1. Chemicals

1, 1′, 3, 3′ tetra methoxy propane, glutathione, thiobarbituric acid and 5, 5′ dithio - dinitro bisbenzoic acid were obtained from the Sigma Chemical Company, MO, St. Louis, USA. Phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), and reduced nicotinamide adenine dinulceotide (NADH), were purchased from SISCO Research Laboratories (P) Ltd, Mumbai, India. Ethanol was obtained from Nellikuppam, Cuddalore District, South India. All other chemicals and solvents were purchased from Central Drug House (P) Ltd, Mumbai, India.

2.2. Plant material

Fresh *Cassia auriculata* leaves were collected from Kavarapattu, Chidambaram Taluk, South India. The leaves were dried thoroughly under shade and powdered. A suspension of 100 g in 200ml distilled water was stirred magnetically overnight at room temperature. This was repeated three times. The extract was evaporated to dryness under reduced pressure in a rotary evaporator. The residual extract was dissolved in distilled water and used for the study.

2.3. Animals

50 male healthy adult Wistar rats (150–170g, age 90 days) were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. They were housed in plastic cages with filter tops under controlled conditions of a 12h light/12h dark cycle, 50% humidity and 28°C.

2.4. Experimental design

The animals were divided into 5 groups of 10 rats each. All the rats received standard pellet diet. (Lipton Lever Ltd, Mumbai, India) and water *ad libitum.* The standard pellet diet comprised of 20% crude protein, 5% fat, 4% crude fiber, 8% ash, 2% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract. Liver cell damage was induced to rats in groups 3,4 and 5 by giving 20% ethanol, 5ml each (2.5ml in the forenoon and 2.5ml in the afternoon) i.e., 7.9g/kg body weight as an aqueous solution using an intragastric tube daily for 30 days. At the end of this period the animals were treated as follows for the next 30 days.

The animals were monitored closely everyday. The rat food was weighed daily before and after feeding the animals to determine the daily food intake. The animals consumed approximately 15g feed/150g body weight per day. Food consumption did not vary significantly between or within the groups.

The total experimental duration was 60 days, at the end of which, the animals were anesthetized using light ether and sacrificed by cervical decapitation. Blood was collected and processed for the estimation of bilirubin [7], aspartate transaminase (AST, EC.2.6.1.1), alanine transaminase (ALT, EC.2.6.1.2) [8], alkaline phosphatase(ALP, EC.3.1.3.1) [9], vitamin E [10] and vitamin C [11]. Liver, brain, kidney and intestine were cleared of adhering fat weighed accurately and used for the estimation of thiobarbituric acid reactive substances (TBARS) [12], hydroperoxides [13], superoxide dismutase (SOD, EC.1.15.1.1) [14], catalase(CAT, EC.1.11.1.6) [15], and reduced glutathione (GSH) [16]. Protein was estimated in the tissues by the method of Lowry et al. [17].

2.5. Statistical analysis

All the grouped data were statistically evaluated and significance of changes caused by the various treatments was determined using Tukey's test. The results are expressed as mean ± SD of 6 rats from each group. A one way ANOVA was done wherever appropriate [18]. The level of statistical significance was set at $p < 0.01$.

3. Results

The concentration of serum bilirubin and the activities of AST, ALT and ALP of control and experimental animals are given in Table 1. Treatment with *Cassia auriculata* to control rats (Group 2) did not significantly alter the serum bilirubin levels (total and direct) and the activities of AST, ALT and ALP as compared with the experimental control rats, whereas bilirubin, AST, ALT and ALP activities were significantly elevated in the alcohol treated rats (Group 3) as compared with those of the experimental control (Group 1) ($P < 0.01$). Treatment with *Cassia auriculata* along with alcohol (Groups 4 and 5) showed significantly reduced levels of serum bilirubin, AST, ALT and ALP as compared with those of the unsupplemented alcohol treated rats (Group 3).

The concentration of tissue TBARS and hydroperoxides of control and experimental animals are given in Table 2. TBARS and hydroperoxides level in the liver, brain, kidney and intestine of rats on alcohol supplementation (Group 3) was significantly higher as compared with those of the experimental control rats (Group 1) ($p < 0.01$). Treatment with *Cassia auriculata* leaf extract to control rats (Group 2) did not alter the TBARS and hydroperoxides level. Supplementation with *Cassia auriculata* leaf extract to rats on

Table 1
Effect of *Cassia auriculata* on serum bilirubin, AST, ALT and ALP of the control and experimental rats

Groups	AST (I.U/Litre)	ALT (I.U/Litre)	ALP (I.U/Litre)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Group 1	73.7 ± 4.16^{a}	22.16 ± 1.49^{af}	75.29 ± 2.02^{af}	1.57 ± 0.06^{afi}	1.19 ± 0.50^{afh}
Group 2	72.29 ± 3.42^{a}	25.59 ± 2.73^{bf}	75.16 ± 1.65^{bf}	0.91 ± 0.13^{bfg}	0.52 ± 0.13^{bgh}
Group 3	84.01 ± 2.56^{b}	55.43 ± 2.60^{c}	97.69 ± 2.49^{cg}	3.16 ± 0.68^{cg}	1.98 ± 0.23^{c}
Group 4	74.25 ± 1.88^{a}	35.89 ± 2.49^{d}	85.14 ± 1.98^{dg}	2.23 ± 0.39^{dg}	1.13 ± 0.34^{dfg}
Group 5	73.27 ± 2.11^{a}	25.3 ± 1.56^{ef}	76.81 ± 2.12^{ef}	1.80 ± 0.39^{ef}	0.99 ± 0.16^{efgh}
F - ratio	1.97	54.26^{σ}	10.75^{σ}	13.63^{σ}	13.81^{σ}

Values are mean ± SD of six rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Tukey's test)
$^{\sigma}$ $p < 0.01$ (ANOVA)

alcohol treatment (groups 4 and 5) lowered the TBARS and hydroperoxide levels.

The activities of SOD and CAT in the tissues of control and experimental animals are given in Table 3. SOD and CAT activities in the liver, brain, kidney and intestine of rats on alcohol supplementation (Group 3) were significantly lower than the experimental control rats (Group 1) ($p < 0.01$). Treatment with *Cassia auriculata* to control rats (Group 2) did not alter significantly the SOD and CAT activities as compared to experimental control (Group 1), whereas administering *Cassia auriculata* leaf extract to alcohol treated rats (Group 4) significantly elevated their activities as compared with those on alcohol treatment alone.

The concentration of GSH was significantly lower in the liver, brain, kidney and intestine of animals supplemented with alcohol (Group 3) than in experimental control rats (Group 1) ($p < 0.01$) (Table 4). GSH values did not alter significantly on treatment with *Cassia auriculata* leaf extract to control rats (Group 2). Administering *Cassia auriculata* leaf extract at 250 or 500 mg kg^{-1} body weight along with alcohol elevated the concentration of reduced GSH significantly in these tissues as compared with those of the unsupplemented alcohol treated rats (Group 3).

Table 2
Effect of *Cassia auriculata* on TBARS and hydroperoxides in tissues of the control and experimental rats

Groups	TBARS (mM/g tissue)				Hydroperoxides (μM/mg tissue)			
	Liver	Brain	Kidney	Intestine	Liver	Brain	Kidney	Intestine
Group 1	0.59 ± 0.21^{a}	0.54 ± 0.11^{a}	0.56 ± 0.10^{a}	0.57 ± 0.10^{a}	1.01 ± 0.50^{abc}	0.94 ± 0.35^{a}	0.71 ± 0.28^{a}	0.31 ± 0.81^{ac}
Group 2	0.56 ± 0.13^{a}	0.54 ± 0.11^{a}	0.53 ± 0.09^{a}	0.54 ± 0.09^{a}	0.71 ± 0.25^{ab}	0.68 ± 0.25^{a}	0.68 ± 0.24^{a}	0.29 ± 0.16^{ac}
Group 3	2.23 ± 0.42^{b}	1.81 ± 0.16^{b}	1.81 ± 0.15^{b}	2.33 ± 0.43^{b}	3.76 ± 0.75^{c}	4.09 ± 0.83^{b}	3.38 ± 0.24^{b}	3.34 ± 0.47^{b}
Group 4	1.18 ± 0.38^{a}	0.70 ± 0.15^{a}	0.62 ± 0.16^{a}	3.80 ± 0.15^{a}	1.8 ± 0.75^{ac}	1.44 ± 0.32^{a}	3.38 ± 0.32^{a}	0.98 ± 0.38^{c}
Group 5	0.77 ± 0.14^{a}	0.69 ± 0.14^{a}	0.69 ± 0.13^{a}	0.70 ± 0.13^{a}	1.38 ± 0.44^{abc}	1.36 ± 0.48^{a}	1.10 ± 0.28^{a}	0.58 ± 0.15^{ac}
F - ratio	36.96^{σ}	75.15^{σ}	84.17^{σ}	55.80^{σ}	21.53^{σ}	35.40^{σ}	52.91^{σ}	72.124

Values are mean ± SD of six rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Tukey's test)
$^{\sigma}$ $p < 0.01$ (ANOVA)

Table 3
Effect of *Cassia auriculata* on SOD and CAT in tissues of the control and experimental rats

Groups	SOD (U */min/mg protein)				CAT (μM of H_2O_2 decomposed /min/mg protein)			
	Liver	Brain	Kidney	Intestine	Liver	Brain	Kidney	Intestine
Group 1	6.06 ± 0.78^{ab}	8.76 ± 0.77^{ad}	3.00 ± 0.81^{ab}	6.30 ± 0.93^{a}	83.08 ± 0.87^{a}	2.99 ± 0.79^{ad}	44.08 ± 0.81^{a}	76.93 ± 0.50^{ad}
Group 2	5.81 ± 0.74^{abd}	8.39 ± 0.85^{ad}	2.91 ± 0.98^{ab}	4.34 ± 0.91^{b}	83.59 ± 1.19^{a}	2.46 ± 0.83^{acd}	44.56 ± 1.36^{a}	77.62 ± 1.13^{ad}
Group 3	2.93 ± 0.82^{ce}	4.27 ± 0.82^{bc}	1.59 ± 0.04^{bc}	1.84 ± 0.74^{c}	71.76 ± 0.77^{b}	1.19 ± 0.29^{bc}	32.61 ± 0.93^{b}	63.00 ± 0.97^{b}
Group 4	4.52 ± 0.19^{bde}	6.42 ± 0.92^{bcd}	2.26 ± 0.04^{abc}	2.39 ± 0.03^{b}	82.52 ± 0.97^{a}	1.65 ± 0.70^{acd}	42.84 ± 1.08^{a}	74.03 ± 0.85^{cd}
Group 5	4.21 ± 0.84^{cde}	6.46 ± 0.86^{acd}	2.39 ± 0.03^{abc}	3.47 ± 0.41^{b}	83.35 ± 1.02^{a}	2.76 ± 0.86^{ad}	43.59 ± 1.22^{a}	76.44 ± 0.79^{ad}
F - ratio	12.06^{σ}	22.45^{σ}	1.62^{σ}	18.32^{σ}	136.94^{σ}	5.56^{σ}	104.11^{σ}	157.41^{σ}

* Enzyme concentration required to inhibit optical density at 560 nm of chromogen produced by 50% in / min
Values are mean ± SD of six rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Tukey's test)
$^{\sigma}$ $p < 0.01$ (ANOVA)

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

Groups	GSH (mM/g tissue)			
	Liver	Brain	Kidney	Intestine
Group 1	22.5 ± 1.05^{af}	17.2 ± 1.08^{a}	17.47 ± 1.71^{a}	10.00 ± 1.02^{ad}
Group 2	21.3 ± 1.04^{bf}	17.5 ± 1.30^{a}	16.28 ± 1.38^{a}	9.66 ± 0.76^{acd}
Group 3	14.0 ± 0.77^{c}	13.92 ± 1.37^{bc}	14.3 ± 1.08^{b}	7.71 ± 0.65^{bc}
Group 4	17.47 ± 1.08^{db}	15.95 ± 1.01^{ac}	16.65 ± 0.81^{a}	9.07 ± 0.83^{acd}
Group 5	18.55 ± 1.22^{eh}	16.92 ± 1.34^{a}	16.28 ± 1.28^{a}	9.6 ± 0.81^{acd}
F-ratio	51.72^{σ}	7.49^{σ}	7.37^{σ}	3.02^{σ}

Values are mean ± SD of six rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Tukey's test)
$^{\sigma}$ $p < 0.01$ (ANOVA)

Vitamin E and vitamin C levels in the serum were significantly lower in rats on alcohol treatment (Group 3) ($p < 0.01$) (Table 5). There were significant elevation of serum vitamin E and vitamin C levels on treatment with *Cassia auriculata* leaf extract to rats on alcohol supplementation (Groups 4 and 5) as compared with the experimental control and unsupplemented alcohol treated rats.

In the alcohol treated rat liver, the involvement of the liver was uniform (tautology). Fatty changes of both macro and micro vesicular type and mononuclear cell infiltrates were observed in all the fields (Fig. 3).

The liver of the alcohol treated rats who received 250 mg/kg body weight of *Cassia auriculata* leaf extract showed fatty changes that were greatly reduced and confined to only focal areas. Mononuclear inflammatory cell infiltrates were present but not to the extent seen in the liver of those rats treated with only alcohol (Fig. 4).

The liver of alcohol treated rats who received 500 mg/kg body weight of *Cassia auriculata* leaf extract showed loss of individual hepatocytes by degeneration and the space appeared empty, but there was no evidence of fatty change (Fig. 5). Control rats treated with *Cassia auriculata* demonstrated normal liver morphology (Fig. 1 and 2).

The brain tissue in alcohol treated rats showed spongiosis, which was not evident in rats treated with *Cassia auriculata* leaf extract (Figs. 8, 9 and 10). Brain tissue in control rats treated with *Cassia auriculata* revealed normal histology (Figs. 6 and 7).

Table 5
Effect of *Cassia auriculata* on serum vitamin e and vitamin c of the control and experimental rats

Groups	Vitamin E (mg/dl)	Vitamin C (mg/dl)
Group 1	2.20 ± 0.41^{a}	2.35 ± 0.43^{abd}
Group 2	2.27 ± 0.47^{a}	2.88 ± 0.75^{ab}
Group 3	0.81 ± 0.04^{b}	0.94 ± 0.04^{cd}
Group 4	1.46 ± 0.62^{ab}	1.66 ± 0.09^{acd}
Group 5	1.06 ± 0.66^{ab}	1.74 ± 0.06^{acd}
F-ratio	6.32^{σ}	17.59^{σ}

Values are mean ± SD of six rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Tukey's test)
$^{\sigma}$ $p < 0.01$ (ANOVA)

4. Discussion

Chronic consumption of ethanol causes injury to the liver cells. Serum bilirubin and the activities of AST and ALT are the most sensitive tests employed in the diagnosis of hepatic diseases [19]. We have observed increased activities of serum AST, ALT and ALP in alcohol treated rats. This can be attributed to the damaged structural integrity of the

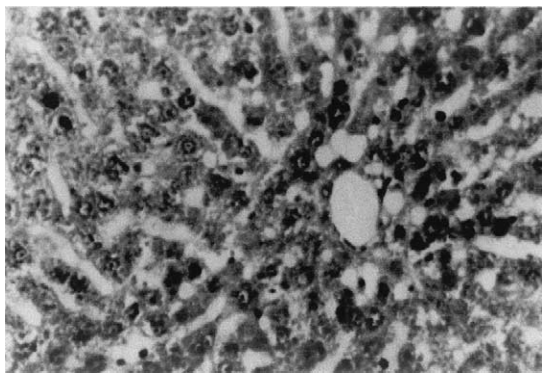

Fig. 1. Liver Control Rats: H & E × 20

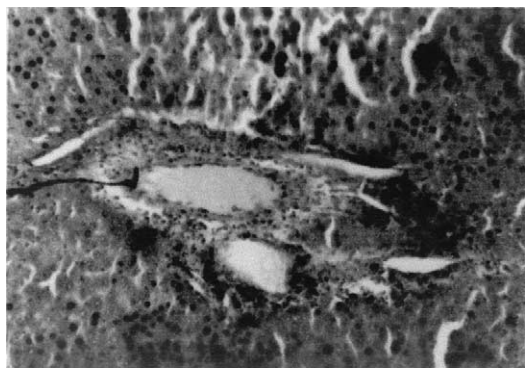

Fig. 2. Liver and control rat treated with *Cassia auriculata* leaf extract: H & E × 20.

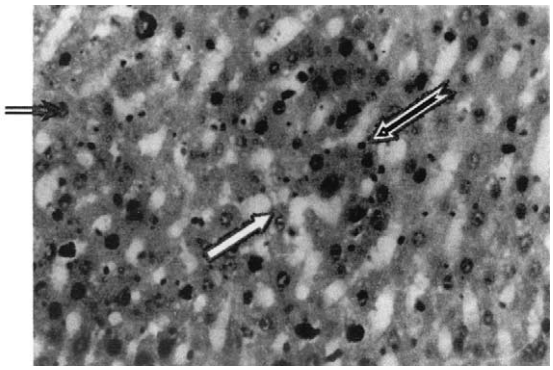

Fig. 3. Liver of alcoholic rat: Arrow indicates fatty changes of macrovesicular type (⟹), microvesicular type (⟹) and mononuclear cell infiltrates (⇨). H & E × 20.

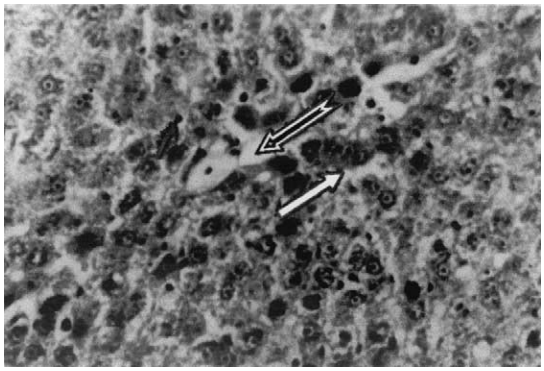

Fig. 4. Liver of alcoholic rat treated with 250 mg/kg body weight *Cassie auriculata* leaf extract: Arrows indicate focal areas of fatty changes (⟸) and significantly reduced amounts of mononuclear cell infiltrates (⟹). H & E × 20.

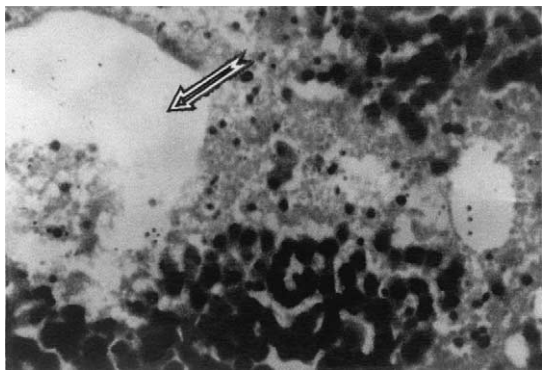

Fig. 5. Liver of alcoholic rat treated with 500 mg/kg body weight *Cassia auriculata* leaf extract: Arrow indicates hepatocyte drop out (⟸). Fatty changes are markedly reduced. H & E × 20.

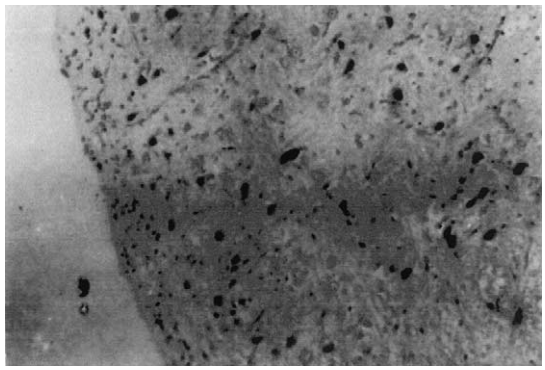

Fig. 6. Brain of control rat: H & E × 20.

hepatic cells, because the enzyme ALP is located in the cytoplasm and is released into circulation after cellular damage [20]. If injury involves other organelles, such as mitochondria then the soluble enzymes such as AST compartmented will also be similarly released indicating that alcohol consumption causes both plasma membrane and organelle membrane damage. On adminstering *Cassia auriculata* leaf extract to alcoholic rats we have observed decreased levels of serum AST, ALT and ALP. This shows that *Cassia auriculata* leaf extract, to an extent preserves the structural integrity of the liver from the adverse effects of ethanol.

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity [5]. In our study, there was significantly elevated levels of TBARS and hydroperoxides in the liver, brain, kidney and intestine of rats on alcohol treatment (Group 3). These results are in agreement with the observations of previous researchers [21].

Superoxide ion (O_2^-) and hydroxyl radical are known to cause marked injuries to the surrounding tissues and organs. Any natural or synthetic compound with antioxidant properties may help to alleviate the liver damage totally or partially. Therefore removing superoxide ion and hydroxyl radical is probably one of the most effective defense mechanisms against a variety of diseases. Activities of SOD and CAT the two enzymes that help to scavenge superoxide ions and hydroxyl ions respectively were significantly lower in alcohol administered rats as compared with those of the experimental rats. Lowered activities of SOD and CAT will result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of cell membrane integrity and membrane function [22,23,24]. The observed restoration of the SOD and CAT activities in our study on treatment with *Cassia auriculata* leaf extract may be due to the direct stimulatory effect of *Cassia auriculata* leaf extract on SOD and CAT. The decrease in the tissue lipid peroxidation on *Cassia auriculata* treatment can also be correlated with the elevated SOD and CAT activities.

Glutathione (GSH) is a major non-protein thiol in living organisms which plays a central role in co-ordinating the antioxidant defense processes in our body. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reactions [25]. Rats on alcohol treatment had reduced levels of tissue GSH as compared to the experimental control. Ethanol administration induces a loss of glutathione from the liver and a decrease in its hepatic content [26]. These lowered levels

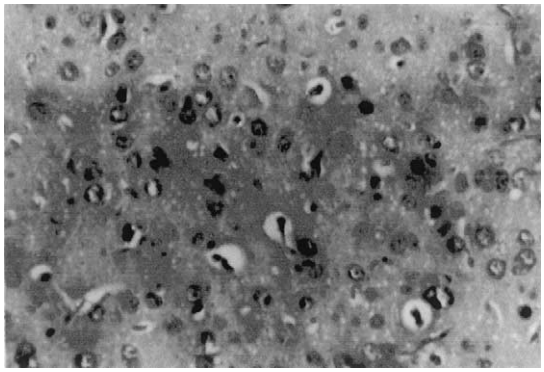

Fig. 7. Brain of control rat treated with *Cassia auriculata* leaf extract: H & E × 20.

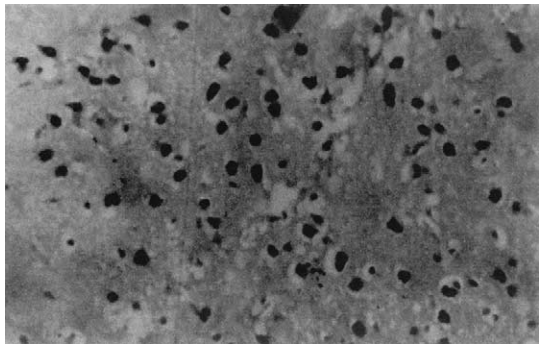

Fig. 8. Brain of alcoholic rat: H & E × 20. Shows spongiosis.

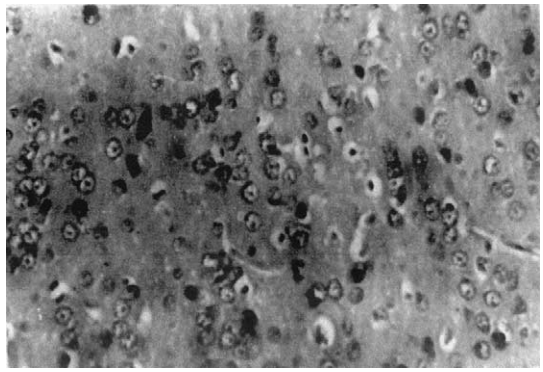

Fig. 9. Brain of alcoholic rat treated with 250 mg/kg body weight *Cassia auriculata* leaf extract: H & E × 20. Shows spongiosis.

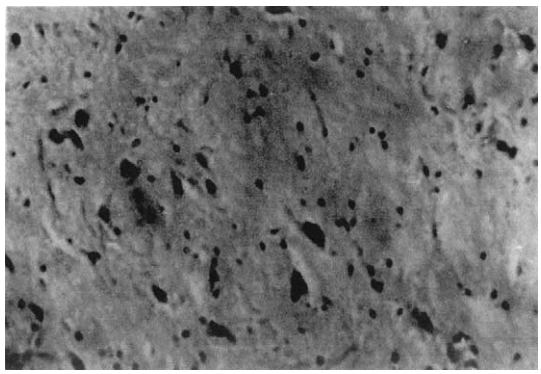

Fig. 10. Brain of alcoholic rat treated with 500 mg/kg body weight *Cassia auriculata* leaf extract: H & E × 20. Spongiosis is markedly reduced.

may be due to the increased utilization of GSH by antioxidant enzymes such as glutathione peroxidase which scavenge H_2O_2 [27]. The glutathione-depleted state, particularly during oxidative stress of hepatocytes causes a rise in the cytosolic Ca^{2+} concentration and in addition leads to protein thiol oxidation. Administering *Cassia auriculata* leaf extract to alcohol treated rats helped to restore the GSH levels to near those of the experimental control rats.

Circulatory antioxidants such as vitamin E and vitamin C are non-enzymic free radical scavengers. Their synergestic action in scavenging oxygen derived free radicals is well documented [28]. Vitamin E reacts with lipid peroxy radicals acting as a chain terminator of lipid peroxidation while vitamin C helps to maintain the level of vitamin E at optimum concentrations. Serum levels of vitamin E and vitamin C in our study were significantly reduced in alcohol treated rats than in the experimental control rats. Supplementation with *Cassia auriculata* leaf extract at a dose of 250 or 500 mg kg^{-1} body weight to alcohol treated rats resulted in near normal serum levels of vitamin E and vitamin C.

Moreover, significant pathomorphological alterations in the liver and brain were observed in alcohol treated rats. (Figs. 3 and 8). 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 having reduced oxygen perfusion. Hepatic damage may be partially attributed to cytochrome-P_{450} generated metabolic cytochrome-P_{450} dependant enzyme activities in liver that tends to be present in greatest concentration near the central vein and lowest near the peripheral sites [29]. Treatment with *Cassia auriculata* leaf extract to alcohol treated rats reduced the fatty changes and improved the histomorphology of the liver.

Spongiosis was demonstrated in the hypothalamic and thalamic regions of the brain of alcohol treated rats (Figure 8) [30]. These are indications of local disorders of brain development. In this study we observed spongiosis in the brain of the alcohol treated rats, which was reversed on *Cassia auriculata* leaf extract treatment.

Multiple mechanisms may interplay in the antioxidant role of *Cassia auriculata* leaf extract. *Cassia auriculata* leaf extract has been reported to have a hypolipidemic effect in alcohol treated rats [31]. Presence of flavonoids in *Cassia auriculata* leaf extract may contribute to its antiperoxidative properties [32].

5. Conclusion

Cassia auriculata leaf extract has a protective action against alcohol induced oxidative stress to the cells as evi-

denced by the lowered tissue lipid peroxidation and elevated levels of the enzymic and non-enzymic antioxidants. This effect was more pronounced when the dose of *Cassia auriculata* leaf extract was 500 mg kg^{-1} body weight. In addition, lowered levels of serum bilirubin, transaminases and ALP demonstrate that *Cassia auriculata* leaf extract prevents parenchymal cell damage.

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