Impact of Essentiale L on ethanol-induced changes in rat brain and erythrocytes

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ABSTRACT

Introduction: The present study was designed to investigate the effect of Essentiale L, a mixture of polyenylphospholipids from soybeans, on oxidative stress in various brain regions, on erythrocytes (RBC) and on RBC membrane composition in ethanol-administered rats.

Methods: Adult male albino rats of body weight 150-170 g were divided into four groups and administered either isocaloric glucose (5 g/kg body weight/day) or ethanol (6 g/kg body weight/day) through oral gavage. Essentiale L was administered to a set of ethanol-fed rats and the control rats at a dosage of 300 mg/kg body weight/day through oral gavage. The treatment protocol was carried out for 45 days. At the end of the experimental period, the animals were sacrificed, and the biochemical parameters related to the lipid profile, oxidative stress and thiol status were assayed in the brain regions, RBC and RBC membrane.

Results: Ethanol administration resulted in increased levels of lipid peroxidation products in RBC and different brain regions, such as the cortex, cerebellum, striatum, hippocampus and hypothalamus, and depletion of enzymatic and non-enzymatic antioxidants and alterations in oxidised glutathione/glutathione(GSSG/GSH) ratio and thiol groups (protein-bound and total), signifying oxidative stress. Ethanol-treated rats also showed significant alterations in protein content and lipid composition in RBC membranes. Significant differences in the relative proportions of hexose, hexosamine and sialic acid of the membranes were observed. Administration of Essentiale L prevented all the alterations induced by ethanol and returned their levels to near-normal.

Conclusion: These findings suggest that Essentiale L, a therapeutic adjunct for liver diseases, also has bioprotective effects on nonhepatic tissues and cells.

Keywords: antioxidants, brain, erythrocytes, erythrocyte membrane, essentiale L, lipid peroxidation products, oxidative stress

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INTRODUCTION

Ethanol, consumed in the form of alcohol, is one of the most abused drugs worldwide. Although it is primarily metabolised in the liver, its detrimental effects on other organ systems, such as the central nervous system, are well documented. Chronic alcohol abuse has been shown to cause degenerative changes in the brain.[1,2] The mechanism, by which ethanol induces alterations in the brain, may be directly due to ethanol or its oxidation products. Ethanol-related neuronal loss has been evidenced in specific regions of the cerebral cortex (superior and frontal association cortex), hypothalamus and cerebellum.[3] Ethanol also interacts with cell membrane constituents, such as proteins and lipids, and compromises the membrane structural integrity and function. Phospholipids (PL) are vital components and are essential for membrane fluidity and transport function. Chronic ethanol consumption depletes PL, especially phosphatidylcholine, in biomembranes.[4]

Essentiale L (Nicholas Piramal India Ltd, Mumbai, India) contains essential PL extracted from soybeans. It is prescribed in India and other countries for the treatment of viral hepatitis and other hepatocellular diseases.[5] The product is a phosphatidylcholine particularly rich in polyunsaturated fatty acids (PUFA) and is therefore termed polyene phosphatidylcholine (PPC). PPC is a mixture of 94%–96% of polyunsaturated phosphatidylcholine, about half of which is dilinoleoylphosphatidylcholine (DLPC), the active species. Differing from mammalian PL, this plant PL contains two unsaturated fatty acids in both 1 and 2 positions of the glycerol backbone. The reacylation of the unsaturated 1-acyl-lysophosphatidylcholine with additional unsaturated fatty acids during intestinal absorption confers high bioavailability.[6]

The hepatoprotective effects of PPC are studied with regard to the antilipid peroxidative and antifibrotic effects in mammalian livers.[7,8] However, its effect on nonhepatic tissues is unknown. The present work reports the effect of

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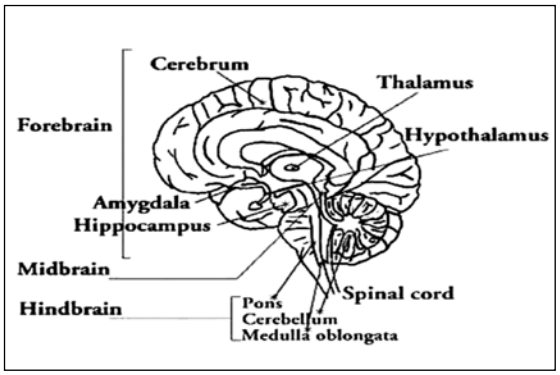

Fig. 1a Diagram shows the median sagittal section of the brain.

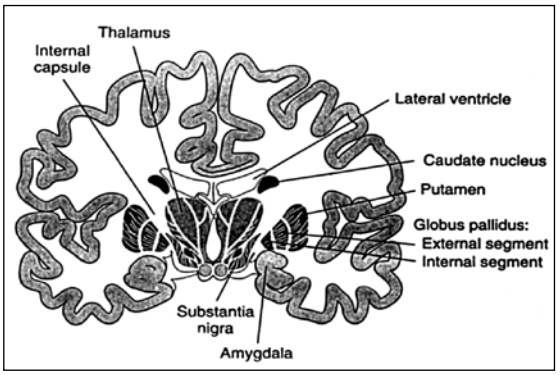

Fig. 1b Diagram of the frontal section of the cerebrum.

Essentiale L on the oxidative stress parameters in red blood cells (RBC) and various brain regions (cortex, cerebellum, striatum, hippocampus and hypothalamus. Figs. 1a & b), and RBC membrane composition. The RBC membrane was chosen for the study owing to its ease of preparation and its susceptibility to ethanol toxicity.

METHODS

Glutathione reductase and 1, 1', 3, 3'-tetraethoxypropane were purchased from Sigma Chemical Company, MO, USA. Essentiale L (Nicholas Piramal India Ltd, Mumbai, India), was purchased from the local pharmacy in Chidambaram, Tamil Nadu, India. All the other chemicals and solvents used in the study were of analytical grade and were purchased from Sisco Research Laboratories (P) Ltd, Mumbai, India. Adult male albino rats of Wistar strain (160–170 g) were purchased and maintained in the Central Animal House, Rajah Muthiah Medical College. They were fed a pellet diet (Kamadhenu Agencies, Bangalore, India) and water *ad libitum*. The animals were housed in polypropylene cages under controlled conditions of 12 h light and dark cycle, 50% humidity and 22°C–25°C. The Institutional Animal Ethical Committee, Rajah Muthiah Medical College, Annamalai Nagar, approved all experimental procedures.

The animals were divided into four groups consisting of six rats each. Group 1 received isocaloric glucose from a 40% glucose solution every day. Group 2 received ethanol (6 g/kg body weight) from a 30% stock solution in two divided doses. Group 3 received ethanol, as in group 2, along with Essentiale L (300 mg/kg body weight). Group 4 received isocaloric glucose, as in group 1, and Essentiale-L, as in group 2. Food and water were provided *ad libitum*. The dosage of Essentiale L was adopted from Dargel et al.[9] Essentiale L and alcohol were administered by oral gavage. At the end of 45 days, the animals were sacrificed by cervical decapitation. Blood was collected from the jugular vein. The body was cut opened and the brain was removed and washed in ice-cold saline. The brain tissue was dissected and the regions, such as the cortex, cerebellum, striatum, hippocampus and hypothalamus, were separated according to the method used by Glowinski and Iverson.[10] The tissue homogenates were prepared in ice-cold Tris-EDTA buffer, pH 7.4, using a mechanically driven Teflon-fitted Potter Elvejhem homogeniser for the total disruption of cells. The homogenates were used for analyses.

The blood samples were centrifuged at 1,000 g-force for 15 minutes. The cells were washed thrice with ice-cold saline. RBC membrane was prepared according to the procedure used by Dodge et al[11] with a modification in the buffer preparation according to Quist.[12] The markers of lipid peroxidation, thiobarbituric acid reactive substances (TBARS)[13] and lipid hydroperoxides (LHP),[14] were quantified in the brain and RBC membrane. The levels of α-tocopherol and ascorbic acid and the activities of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), were assayed in the various brain regions and RBC by standard methods outlined elsewhere.[15] Total thiols, protein-bound thiols and non-protein thiols were measured in various brain regions by the method used by Sedlak and Lindsay.[16] Glutathione (GSH) and oxidised glutathione (GSSG) were measured by the enzymatic method employed by Teitz.[17] The levels of protein-bound sugars, such as hexose,[18] hexosamine[19] and sialic acid,[20] were determined in the RBC membrane. Membrane protein was determined by the method used by Lowry et al,[21] and lipids were extracted using the method employed by Folch et al.[22] Cholesterol[23] and PL[24] were determined in the lipid extracts obtained from the RBC membrane. Values are given as mean ± SD. The differences between the four groups were analysed using ANOVA, followed by Duncan's Multiple Range Test (DMRT). The level of statistical significance was set at $p < 0.05$.

RESULTS

The weight of the various brain regions of the control and experimental rats are shown in Table I. Significant reduction in the weight of various brain regions of the

Table I. The weight (mg) of brain regions of control and experimental animals.

Brain regions	CON	EtOH	EtOH + ESS-L	CON + ESS-L
Cortex	129 ± 12.8	97.5 ± 9.35*	120 ± 11.8**	132 ± 8.21
Cerebellum	102 ± 8.21	86.6 ± 6.83*	98.3 ± 7.17**	107 ± 9.35
Striatum	91.6 ± 4.22	80.3 ± 5.00*	88.5 ± 5.61**	94.8 ± 8.23
Hippocampus	96.1 ± 8.56	80.3 ± 6.86*	90.8 ± 4.95**	99.8 ± 8.23
Hypothalamus	59.1 ± 3.31	48.0 ± 4.14*	56.1 ± 4.66**	61.6 ± 5.39

Data is expressed as mean ± SD of six rats from each group.
CON: control rats (group 1); EtOH: ethanol-fed rats (group 2); EtOH + ESS-L: ethanol-fed rats treated with Essentiale L (group 3); CON + ESS-L: control rats treated with Essentiale L (group 4).
* Significant as compared to CON ($p < 0.05$; ANOVA followed by DMRT)
** Significant as compared to EtOH ($p < 0.05$; ANOVA followed by DMRT)

Table II. Lipid peroxidation indices (mmol/mgHb) and enzymatic antioxidant activities in RBC of control and experimental animals.

Parameters	CON	EtOH	EtOH + ESS-L	CON + ESS-L
TBARS	2.04 ± 0.19	2.99 ± 0.27*	2.22 ± 0.20**	1.96 ± 0.18
LHP	8.85 ± 0.55	13.8 ± 1.21*	9.36 ± 0.84**	8.24 ± 0.71
SOD [a]	2.63 ± 0.15	1.35 ± 0.12*	2.46 ± 0.19**	2.70 ± 0.24
CAT [b]	47.6 ± 3.35	26.6 ± 2.48*	44.5 ± 3.05**	48.8 ± 4.37
GPx [b]	12.9 ± 1.13	8.53 ± 0.64*	11.9 ± 0.94**	13.1 ± 1.11

Data is expressed as mean ± SD of six rats from each group.
CON: control rats (group 1); EtOH: ethanol-fed rats (group 2); EtOH + ESS-L: ethanol-fed rats treated with Essentiale L (group 3); CON + ESS-L: control rats treated with Essentiale L (group 4).
* Significant as compared to CON ($p < 0.05$; ANOVA followed by DMRT)
** Significant as compared to EtOH ($p < 0.05$; ANOVA followed by DMRT)
[a] Unit/mgHb; [b] µmol substrate/min/mgHb

Table III. Levels of lipids (µg/mg protein) in RBC membrane of control and experimental animals.

Parameters	CON	EtOH	EtOH + ESS-L	CON + ESS-L
Cholesterol	145 ± 13.3	171±15.94*	152 ± 15.9**	139 ± 11.8
Phospholipids	257 ± 23.9	198 ± 16.2*	277 ± 26.7**	262 ± 23.2
C/P ratio	0.56 ± 0.02	0.86 ± 0.05*	0.55 ± 0.03**	0.53 ± 0.02

Data is expressed as mean ± SD of six rats from each group.
CON: control rats (group 1); EtOH: ethanol-fed rats (group 2); EtOH + ESS-L: ethanol-fed rats treated with Essentiale L (group 3); CON + ESS-L: control rats treated with Essentiale L (group 4).
C/P: cholesterol/phospholipid
* Significant as compared to CON ($p < 0.05$; ANOVA followed by DMRT)
** Significant as compared to EtOH ($p < 0.05$; ANOVA followed by DMRT)

rats intoxicated from chronic alcohol consumption was observed. Administration of Essentiale L along with alcohol ameliorated the negative effects of ethanol and the values returned to near-normal. The levels of TBARS and LHP in the RBC membrane of control and experimental rats are presented in the Table II. Ethanol-treated rats showed a significant increase in lipid peroxidation products as compared to control rats. Rats administered with Essentiale L and alcohol showed no significant changes in the levels of TBARS and LHP.

Table II also gives the activities of enzymatic antioxidants such as SOD, CAT and GPx in the haemolysate of control and experimental animals. Significant decreases in the activities of these antioxidants were noted, consistent with the observed increase in lipid peroxidation products. Rats co-administered Essentiale L with ethanol showed near-normal levels of these enzymes. Table III represents the levels of lipids in the RBC membrane of control and experimental animals. The levels of cholesterol and PL were respectively higher and lower in the animals intoxicated with ethanol, as compared to untreated normal animals, resulting in a higher cholesterol/PL (C/P) ratio. Administration of Essentiale L to the animals treated with ethanol significantly normalised the levels.

Table IV shows the levels of protein, total sugar and protein-bound sugars in control and experimental rats. There was a significant rise in the levels of total sugar and hexose, and depletion of hexosamine and sialic acid,

Table IV. Levels of protein (% w/w dry weight) and protein bound sugars (µg/mg protein) in RBC membrane of control and experimental animals.

Parameters	CON	EtOH	EtOH+ ESS-L	CON+ ESS-L
Protein	48.24 ± 2.16	37.51 ± 2.53*	46.03 ± 3.04**	48.32 ± 2.17
Hexose	26.0 ± 2.51	35.6 ± 2.93*	27.1 ± 2.37**	24.14 ± 2.05
Hexosamine	35.5 ± 3.04	24.8 ± 2.20*	36.8 ± 3.54**	32.5 ± 3.20
Sialic acid	10.7 ± 0.91	5.2 ± 0.49*	11.1 ± 0.95**	9.72 ± 0.77

Data is expressed as mean ± SD of six rats from each group.
CON: control rats (group 1); EtOH: ethanol-fed rats (group 2); EtOH + ESS-L: ethanol-fed rats treated with Essentiale L (group 3); CON + ESS-L: control rats treated with Essentiale L (group 4).
* Significant as compared to CON ($p < 0.05$; ANOVA followed by DMRT)
** Significant as compared to EtOH ($p < 0.05$; ANOVA followed by DMRT)

Table V. Levels of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LHP) in various brain regions of control and experimental animals.

Parameters	CON	EtOH	EtOH + ESS-L	CON + ESS-L
TBARS (µmol/mg protein)				
Cortex	3.81 ± 0.21	7.70 ± 0.64*	4.06 ± 0.34**	3.47 ± 0.27
Cerebellum	2.50 ± 0.14	3.85 ± 0.27*	2.88 ± 0.17**	2.37 ± 0.12
Striatum	2.90 ± 0.19	5.87 ± 0.45*	3.09 ± 0.26**	2.65 ± 0.21
Hippocampus	2.51 ± 0.21	4.83 ± 0.31*	2.79 ± 0.23**	2.44 ± 0.18
Hypothalamus	3.20 ± 0.24	4.11 ± 0.35*	3.45 ± 0.29**	3.08 ± 0.26
LHP (µmol/mg protein)				
Cortex	2.13 ± 0.14	5..55 ± 0.42*	2.25 ± 0.17**	2.01 ± 0.12
Cerebellum	2.65 ± 0.21	6.52 ± 0.51*	2.83 ± 0.23**	2.49 ± 0.23
Striatum	3.28 ± 0.25	4.75 ± 0.34*	3.33 ± 0.29**	3.20 ± 0.26
Hippocampus	2.67 ± 0.17	6.50 ± 0.52*	2.93 ± 0.21**	2.56 ± 0.19
Hypothalamus	3.08 ± 0.22	6.93 ± 0.54*	3.13 ± 0.27**	2.95 ± 0.21

Data is expressed as mean ± SD of six rats from each group.
CON: control rats (group 1); EtOH: ethanol-fed rats (group 2); EtOH + ESS-L: ethanol-fed rats treated with Essentiale L (group 3); CON + ESS-L: control rats treated with Essentiale L (group 4).
* Significant as compared to CON ($p < 0.05$; ANOVA followed by DMRT)
** Significant as compared to EtOH ($p < 0.05$; ANOVA followed by DMRT)

in animals treated with ethanol. Essentiale L-treated alcoholic rats did not show such alterations. Levels of TBARS and LHP in various brain regions of control and experimental are presented in the Table V. Animals treated with ethanol showed an increase in the lipid peroxidation products possibly by increased free radical production. Administration of Essentiale L along with ethanol reduced the formation of lipid peroxidation products, suggesting a reduction in oxidative stress.

The activities of SOD, CAT and GPx in various brain regions of control and experimental animals are shown in Figs. 2, 3 and 4. Activities of these antioxidant enzymes were lower in the animals treated with chronic ethanol as compared to control animals. Essentiale L, which has potential antioxidant activity, enhanced the antioxidant status of the animals fed with ethanol. The levels of vitamins C and E and GSSG/GSH ratio in various brain regions are given in the Table VI. The GSSG/GSH ratio was significantly increased, while vitamin C and E levels were reduced in the animals administered ethanol, as compared to the control animals (Table VI). Administration of Essentiale L restored the levels to near-normal. The content of total, protein-bound and non-protein-bound thiols in various brain regions of the animals are shown in Table VII. Animals given Essentiale L and ethanol showed an increase in the content of total, protein-bound and non-protein-bound thiols, as compared to those animals given ethanol only. Normal animals treated with Essentiale L alone did not show any significant changes in the parameters measured.

DISCUSSION

Reduction in brain mass is suggestive of organ damage due to ethanol toxicity. A lack of nutrient supply due to malabsorption, which is characteristic of ethanol feeding, could also be a reason for this reduction. The drug, Essentiale L, restored the organ weight to near-normal levels, indicating a bioprotective effect. Oxidative stress is the mainstay of alcohol toxicity. The brain is thought to be susceptible to lipid peroxidation and oxidative injury, since it contains membranes rich in polyunsaturated fatty acids. It is antioxidant-poor compared to other organs

and has a high content of iron. Increased ethanol intake results in oxidative stress in the brain[25] and produces necrotic degeneration of the neurons in the areas of brain most closely associated with the hippocampus.[26] Mandavilli and Rao have reported that regions like the cortex, hypothalamus, hippocampus and striatum, are more susceptible to oxidative stress when compared to the cerebellum.[27] The hippocampus contains large amounts of non-haem iron, which makes this area of the brain particularly susceptible to free-radical induced lipid peroxidation. The formation of fatty acid, ethyl esters, in the brain from ethanol could be damaging to the hippocampus. In the present study, the cortex and hypothalamus were more prone to oxidative stress compared to the other regions, as indicated by increased levels of TBARS and LHP. The cortex region also showed a greater decline in vitamin C levels than other brain regions. The decrease in vitamin E levels was greater in the hippocampus as compared to other regions.

Acetaldehyde, the product of ethanol metabolism and other aldehydes formed during peroxidation of lipids, can bind to sulfhydryl groups of proteins to form stable adducts in tissues. Formation of such adducts is associated with loss of protein function and severity of alcoholic liver damage through humoral and cell-mediated immunological responses. Acetaldehyde-protein adduct formation has been demonstrated in white matter and large neurons of the frontal cortex in ethanol-treated animals.[28] A decrease in the enzymatic antioxidant activities during ethanol intoxication observed in the RBC and various brain regions could be attributed to either inhibition of enzyme synthesis or damage to the enzyme protein.[29] Free radicals can readily react with the amino and sulfhydryl groups of these enzyme proteins. Reports suggest that SOD and CAT are prone to oxidative damage, which will lead to their inactivation.[30] Acetaldehyde, the toxic metabolite, reacts with the selenocysteine residue of GPx, resulting in its deactivation.[31] Protein damage is evident from the reduction in total and protein-bound thiols, and a loss in intracellular thiol homeostasis (this causes an imbalance in the GSSG/GSH ratio). The redox imbalance created by ethanol intoxication, and increased utilisation of antioxidants towards reaction with the free radicals, is responsible for protein damage. Depletion of antioxidant status by ethanol is well documented in the literature and has been reported in brain tissue.[32] The cortex and hippocampus regions showed more pronounced depletion of antioxidants vitamin E and C respectively in ethanol-treated rats as compared to the controls.

Therapeutic application of Essentiale L has protective, curative and regenerative effects on the hepatocytes. It has been suggested that a component of this PL mixture may act as an antioxidant. For instance, PPC normalises

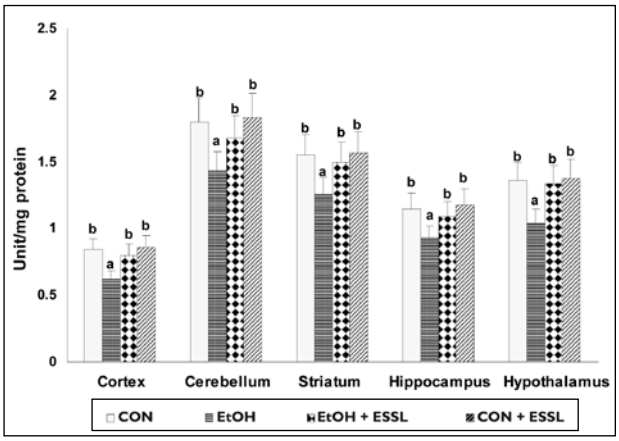

Fig 2. Bar chart shows superoxide dismutase (SOD) activity in various brain regions.
Values are mean ± SD for six animals in each group.
[a] Significant as compared to CON ($p < 0.05$); [b] Significant as compared to EtOH ($p < 0.05$) (DMRT).

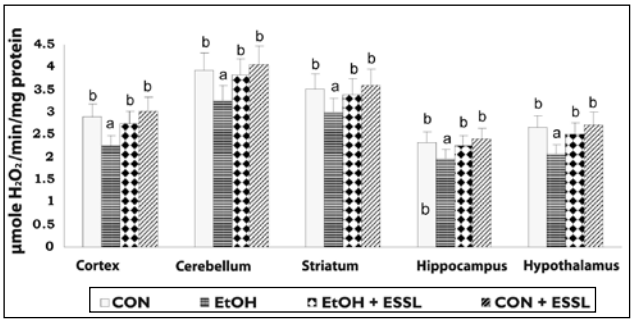

Fig 3. Bar chart shows catalase (CAT) activity in various brain regions.
Values are mean ± SD for six animals in each group.
[a] Significant as compared to CON ($p < 0.05$); [b] Significant as compared to EtOH ($p < 0.05$) (DMRT).

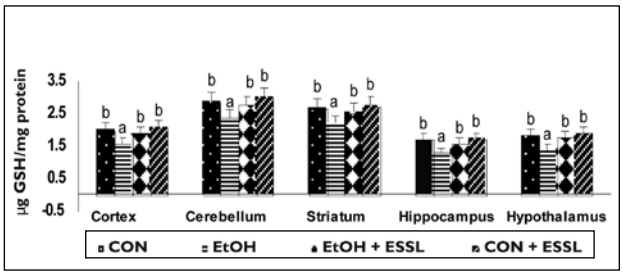

Fig 4. Bar chart shows glutathione peroxidase (GPx) activity in various brain regions.
Values are mean ± SD for six animals in each group.
[a] Significant as compared to CON ($p < 0.05$); [b] Significant as compared to EtOH ($p < 0.05$) (DMRT).

the cellular glutathione and reduces the alcohol-induced oxidative stress in animals.[33] Aleynik et al reported that essential PL, particularly the PPC mixture, decreased lipid peroxidation via the down regulation of CYP2E1.[34] The effect was associated with a reduction of peroxidation products, such as F2-isoprostane and 4-hydroxy nonenal, and a rise in GSH. PPCs maintain and promote the activity of several membrane-bound proteins and enzymes, including Na^+-K^+-ATPase and adenylate cyclase. They

Table VI. Levels of vitamin C, vitamin E and GSSG/GSH ratio in various brain regions of control and experimental animals.

Parameters	CON	EtOH	EtOH + ESS-L	CON + ESS-L
Vitamin-C (μg/g tissue)				
Cortex	464 ± 32.1	347 ± 29.6*	447 ± 34.6**	471 ± 37.8
Cerebellum	344 ± 26.3	289 ± 21.8*	337 ± 31.4**	350 ± 27.6
Striatum	372 ± 29.4	282 ± 19.6*	359 ± 26.3**	382 ± 30.1
Hippocampus	388 ± 27.8	309 ± 21.6*	375 ± 29.3**	395 ± 32.8
Hypothalamus	336 ± 25.4	288 ± 18.4*	324 ± 23.2**	342 ± 21.7
Vitamin-E (μg/g tissue)				
Cortex	19.5 ± 0.98	16.2 ± 1.21*	18.3 ± 1.42**	20.4 ± 1.28
Cerebellum	13.3 ± 0.85	10.1 ± 0.76*	12.8 ± 0.93**	14.2 ± 1.01
Striatum	14.3 ± 1.21	11.0 ± 0.87*	13.7 ± 1.03**	15.2 ± 1.34
Hippocampus	16.2 ± 1.40	11.2 ± 0.92*	15.3 ± 1.16**	17.0 ± 1.52
Hypothalamus	11.7 ± 0.78	9.8 ± 0.64*	11.0 ± 0.85**	12.4 ± 0.97
GSSG/GSH (μg/mg tissue)				
Cortex	0.015 ± 0.01	0.026 ± 0.02*	0.016 ± 0.01**	0.014 ± 0.01
Cerebellum	0.014 ± 0.01	0.031 ± 0.03*	0.015 ± 0.01**	0.013 ± 0.01
Striatum	0.016 ±0.01	0.034 ± 0.02*	0.019 ± 0.01**	0.017 ±0.01
Hippocampus	0.016 ± 0.01	0.033 ± 0.03*	0.018 ± 0.01**	0.015 ± 0.01
Hypothalamus	0.013 ± 0.01	0.025 ± 0.01*	0.015 ± 0.01**	0.014 ± 0.01

Data is expressed as mean ± SD of six rats from each group.
CON: control rats (group 1); EtOH: ethanol-fed rats (group 2); EtOH + ESS-L: ethanol-fed rats treated with Essentiale L (group 3); CON + ESS-L: control rats treated with Essentiale L (group 4).
* Significant as compared to CON ($p < 0.05$; ANOVA followed by DMRT)
** Significant as compared to EtOH ($p < 0.05$; ANOVA followed by DMRT)

are also known to be precursors of cytoprotective agents such as eicosanoids, prostaglandins and antioxidants.[35] Essentiale L restored GSH content and decreased the GSSG/GSH ratio. This is a reflection of increased synthesis of GSH in liver. Synthesis of GSH in the liver requires cysteine, which is obtained from S-adenosyl methionine (SAM) by transulfuration. The formation of PPC requires SAM for methylation. Exogenous PPC could spare SAM for methylation; in other words, administration of PPC decreases SAM utilisation, restores the SAM concentration and leads to the replenishment of GSH.[36]

Elevated levels of cholesterol in plasma and RBC membranes in ethanol-fed rats have been observed by others.[37] The RBC membrane is capable of exchanging its cholesterol with that in plasma. Hence, an increase in cholesterol in RBC membrane reflects a proportionate increase in plasma. PUFA are highly susceptible to lipid peroxidation and this can cause a reduction in PL. PL, the backbone of the cellular membrane, can be strikingly altered by ethanol due to its membrane solubilising effect. In chronic alcohol feeding, decreased levels of RBC membrane-bound PL were reported.[38] In order to maintain a constant C/P ratio, more PL from the plasma lipid pool must be acquired. In spite of this, the RBC is unable to restore the C/P ratio due to increased degradation of PL that contain the PUFA, which are the targets of lipid peroxidation. An increased C/P ratio in the RBC membrane leads to increased rigidity, microviscosity and packing density of the RBC membrane; this accounts for the decreased cell deformability and cell fluidity in alcohol-intoxicated rats. Essentiale L has been shown to attenuate the ethanol-induced alterations in liver lipids and lipid metabolism.[39,40] Dietary PPC and DLPC had hypolipidaemic effects in alloxan-diabetic rats and in cholesterol-fed rabbits.[41,42] A likely mechanism for the effect of PPC on lipid levels could be the correction of the ethanol-induced impairment in FFA oxidation and biosynthesis.

The cell surface contains complex carbohydrates that play a crucial role in many important biological functions. Protein-bound hexose in the cell membrane provides hydrophobic areas, whereas the protein-bound hexosamine provides the cationic charges on the cell membrane cell surface and makes the membrane more polar. Sialic acid, which accounts for 95% of the negative charges on the membrane surface of the RBC, is crucial for the survival of mammalian RBC in circulation. Chronic ethanol administration affects the synthesis, intracellular transport, subcellular distribution and secretion of the glycoproteins. Ethanol alters the carbohydrate moiety of the proteins and increases cell surface glycoproteins containing terminal non-reduced mannose in cultured astrocytes.[43] The observed decrease in protein-bound hexosamine and sialic acid may be due to defective enzymatic glycation and/or enhanced removal and shedding of carbohydrate moieties from the proteins under alcoholic conditions as compared

Table VII. Levels of total (T-SH), protein-bound (P-SH) and non-protein bound thiol (NP- SH) in various brain regions of control and experimental animals.

Parameters	CON	EtOH	EtOH + ESS-L	CON + ESS-L
T-SH (μg/mg tissue)				
Cortex	483 ± 32.1	341 ± 29.6*	465 ± 34.6**	492 ± 37.8
Cerebellum	369 ± 26.3	297 ± 21.8*	353 ± 31.4**	378 ± 27.6
Striatum	397 ± 29.4	299 ± 19.6*	380 ± 26.3**	402 ± 30.1
Hippocampus	360 ± 27.8	289 ± 21.6*	342 ± 29.3**	372 ± 32.8
Hypothalamus	320 ± 25.4	253 ± 18.4*	305 ± 23.2**	329 ± 21.7
P-SH (μg/mg tissue)				
Cortex	298 ± 19.4	209 ± 16.7*	291 ± 21.4**	301 ± 25.6
Cerebellum	239 ± 21.7	186 ± 19.7*	228 ± 22.4**	242 ± 23.6
Striatum	284 ± 24.4	215 ± 17.8*	272 ± 21.7**	286 ± 26.3
Hippocampus	216 ± 15.2	186 ± 12.7*	207 ± 14.1**	222 ± 16.2
Hypothalamus	191 ± 9.6	144 ± 7.8*	183 ± 9.2**	195 ± 11.3
NP-SH (μg/mg tissue)				
Cortex	185 ± 12.7	132 ± 8.4*	174 ± 10.5**	191 ± 13.7
Cerebellum	130 ± 9.6	111 ± 6.9*	125 ± 7.2**	136 ± 8.4
Striatum	113 ± 8.3	84 ± 5.6*	108 ± 5.8**	116 ± 8.6
Hippocampus	144 ± 10.2	103 ± 4.3*	135 ± 7.8**	150 ± 9.8
Hypothalamus	129 ± 7.1	109 ± 5.4*	122 ± 6.3**	134 ± 4.8

Data is expressed as mean ± SD of six rats from each group.
CON: control rats (group 1); EtOH: ethanol-fed rats (group 2); EtOH + ESS-L: ethanol-fed rats treated with Essentiale L (group 3); CON + ESS-L: control rats treated with Essentiale L (group 4).
* Significant as compared to CON ($p < 0.05$; ANOVA followed by DMRT)
** Significant as compared to EtOH ($p < 0.05$; ANOVA followed by DMRT)

to controls.

The findings of the study show that administration of Essentiale L, which has been used favourably in acute and chronic hepatitis, fatty liver and cirrhosis, alleviates oxidative stress in the rat brain and RBC membrane, and also preserves the integrity of the RBC membrane in ethanol-treated rats. These effects could be associated with the membrane stabilising effects and repletion of antioxidant defense system by Essentiale L. The consumption of Essentiale L has health benefits, and the therapeutic efficacy of the drug is not restricted to the liver. Confirmatory studies on histological localisation of oxidative stress markers, like lipofuscin and 4-hydroxynonenal, and their effects on macromolecular targets, including DNA, are needed.

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