Pharmacognosy Research Vol 1, Issue 1, Jan-Feb, 2009 Page 25-34
A Supplement to Pharmacognosy Magazine – [Phcog Mag.]
Received: 15th February, 2008
Modified: 23rd June, 2008
Accepted: 23rd June, 2008

PHCOG RES.: Research Article

Effect of the Fractions of *Coccinia grandis* on Ethanol-Induced Cerebral Oxidative Stress in Rats

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ABSTRACT

The present study was aimed at investigating the effect of the various fractions of hydromethanol extract of the leaves of *Coccinia grandis* (Cucurbitaceae) against ethanol-induced cerebral oxidative stress in rats. The leaves of this species is used in traditional medicine for treating jaundice, bronchitis, skin eruptions, burns, rheumatism, syphilis, gonorrhoea, etc. Cerebral oxidative stress was induced in rats by the administration of 20% ethanol (5 ml/100 g b.w.) for 28 days. The pet-ether (PEF), chloroform (CF), ethylacetate (EAF) and residual (RF) fractions at a dose of 200 mg/kg b.w. were simultaneously administered with ethanol orally for 28 days. Vitamin E at a dose of 100 mg/kg orally was used as the standard. Administration of ethanol resulted in a significant increase in the activities of serum transaminases, alkaline phosphatase, uric acid and lipid levels. In addition, there was a significant elevation in the levels of malondialdehyde and lipid hydroperoxides and a reduction in the activities of enzymatic and non-enzymatic antioxidants in the brain. Simultaneous administration of the fractions prevented the enzymatic leakage and the rise in uric acid and lipid levels. All the fractions (except the residual fraction) prevented the peroxidative damage caused by ethanol, which is evidenced from the improved antioxidant potential. Further, histopathological examination of the brain tissue revealed that the fractions offered significant protection against ethanol toxicity. Among the fractions tested, the chloroform fraction exhibited appreciable antioxidant property, which was almost comparable with the standard Vitamin E. These results suggest that the leaves of *Coccinia grandis* exhibit significant antioxidant activity in ethanol-treated rats.

KEYWORDS antioxidant enzymes, *Coccinia grandis*, ethanol, hyperlipidemia, lipid peroxidation

INTRODUCTION

Alcohol is the most frequently abused drug throughout the world. It is a powerful neurotoxin associated with significant morbidity and mortality (1). Chronic alcoholism leads to an alteration in the brain structure and function and severe neurodegeneration mediated by reactive oxygen species (2). Alcohol-related oxidative stress is linked to the metabolism of ethanol by microsomal ethanol oxidizing systems (MEOS), alcohol dehydrogenase and NADPH oxidase reaction and also due to the toxic effects of acetaldehyde, the product of ethanol oxidation. Furthermore, ethanol itself is converted to ethyl and 1-hydroxyethyl radicals (3, 4). Metabolism of ethanol leads to oxidative stress through the generation of reactive oxygen species such as superoxide anion radical, hydroxyl radical and singlet oxygen which adversely alter the polyunsaturated fatty acids of the cell membrane leading to lipid peroxidation and a depletion in protective antioxidant potential, thereby resulting in membrane damage (5). In addition, ethanol increases triglyceride and cholesterol levels, thus inducing an imbalance in lipid metabolism in liver, brain, heart, kidney and other organs leading to lipid peroxidation (6). Thus, ethanol modifies the physiological balance between antioxidant and pro-oxidant mechanisms

contributing to oxidative stress. Recent research has focused on the development of antioxidant drugs that may delay or minimize neurodegeneration (7).

Many herbs and medicinal plants are rich natural sources of antioxidants. *Coccinia grandis* L. Voigt. commonly known as "Ivy gourd" in English is a tropical plant belonging to the family Cucurbitaceae. It has been found in many countries in Asia and Africa. The roots, stems, leaves and whole plant of *C. grandis* are used in the treatment of jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, eye infections, allergy, syphilis, gonorrhoea, etc. (8, 9). The leaves of this species are widely used in Indian folk medicine for reducing the amount of sugar in urine of patients suffering from diabetes mellitus. Literature suggests the use of this plant in the treatment of diabetes (10). The crude hydromethanol extract of the leaves of *C. grandis* has been reported for its xanthine oxidase inhibitory and hypouricaemic activities (11) and the various fractions of the hydromethanol extract of the leaves of *C. grandis* possessed significant *in vitro* antioxidant activity (12). To our knowledge, there are no available reports on the effect of leaves of *C. grandis* against ethanol-induced cerebral oxidative stress. Hence, the objective of the present work is to study the protective effect of the various fractions of *C. grandis* hydromethanol leaf extract against ethanol-induced cerebral oxidative stress in rats.

MATERIALS AND METHODS

Plant material

Leaves of *Coccinia grandis* were collected from Coimbatore district, Tamil Nadu, during the month of May 2006. The plant was identified and authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore, India, where a voucher specimen (No. BSI/SC/5/23/06-07/Tech.1951) of the plant has been kept in the herbarium.

Preparation of extract and fractionation

Fresh leaves of the plant were dried in shade under room temperature and powdered mechanically and sieved through No. 20 mesh sieve. About 500 g of the leaf powder was extracted with 2.5 l of methanol: water (7:3) by maceration at room temperature for 4 h using a mechanical shaker. The hydromethanol extract (27%) was partitioned separately against petroleum ether, chloroform and ethyl acetate separately in the order of increasing polarity. The fractions were dried at 40°C under vacuum and the percentage yield of the fractions was petroleum ether (2%), chloroform (1.2%), ethyl acetate (1.4%) and residual fractions (18%).

Animals

Albino rats of *Wistar* strain of either sex weighing between 150-200 g were used for the study. They were housed in standard cages at room temperature (23 ± 2°C) and provided with food and drinking water *ad libitum*. All animal procedures described were reviewed and approved by the University Animal Ethical Committee.

Drugs and chemicals

Absolute ethanol, 2–2' dipyridyl, *O*-dianisidine and Vitamin E were obtained from Himedia Laboratories Ltd., Mumbai, India. Butylated hydroxytoluene, oxidized glutathione, epinephrine and 5, 5'-Dithiobis-(2-nitrobenzoic acid) were obtained from Sisco Research Laboratories Pvt., Ltd., Mumbai, India. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Acute toxicity studies

Swiss albino mice (20-30 g) maintained under standard laboratory conditions were used. A total of five animals were used which received a single oral dose (2000 mg/kg, b.w.) of the fraction. Animals were kept overnight fasting prior to drug administration. After the administration of the fraction, food was withheld for further 3-4 h. Animals were observed individually atleast once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and CNS (drowsiness, gait, tremors and convulsions) changes (13).

Selection of dose of the fraction

LD_{50} was determined as per OECD guidelines for fixing the dose for biological evaluation. The LD_{50} of the fractions as per OECD guidelines falls under category 4 values with no signs of acute toxicity at doses of 2000 mg/kg. The biological evaluation of the fractions was carried out at a dose of 200 mg/kg body weight.

Experimental design

Cerebral oxidative stress was induced in rats by feeding 20% ethanol (5 ml/100 g b.w.) for a period of 28 days (14, 15). Animals were divided into 6 groups

consisting of six animals each and were kept overnight fasting prior to drug administration. Group I received normal saline (10 ml/kg b.w., orally) and served as solvent control. Group II received 20% ethanol (5 ml/100 g, b.w) orally and served as alcohol control. Groups III-VI received petroleum ether (PEF), chloroform (CF), ethyl acetate (EAF) and residual fractions (RF) of *C. grandis* respectively at a dose of 200 mg/kg b.w., orally. Group VII received Vitamin E at a dose of 100 mg/kg b.w. orally and served as the standard (16). The fractions and the reference drug were suspended in 20% ethanol and administered for a period of 28 days.

On the 29th day, blood was collected by retro-orbital puncture under mild ether anesthesia. The levels of serum triglycerides, cholesterol, lipoproteins, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and uric acid were determined with standard kits using a semi-autoanalyser. Immediately after blood collection, the animals were sacrificed by cervical dislocation and brain was removed, homogenized with 0.1M ice-cold phosphate buffer (pH 7.4) and centrifuged at 5000 g for 10 min and the resulting supernatant was used for the estimation of lipid peroxidation (LPO) and enzymatic and non-enzymatic antioxidants.

The amount of total protein present in the tissue was estimated by the method of Lowry et al. (17). Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LH) were measured by the method of Nichans and Samuelson (18). Superoxide dismutase (SOD) activity was determined by the inhibition of autocatalyzed adrenochrome formation in the presence of the homogenate at 480 nm (19). Catalase (CAT) activity was estimated by the catalysis of hydrogen peroxide (H_2O_2) to H_2O in an incubation mixture adjusted to pH 7.0 and recorded at 254 nm (20). Gutathione reductase (GSSH) activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm (21). Glutathione peroxidase (GPx) activity was measured by the procedure of Paglia and Valentine (22). Peroxidase (Px) activity was measured spectophotometrically by following the change in absorbance at 460 nm due to *O*-dianisidine oxidation in the presence of H_2O_2 and enzyme (23). The estimation of reduced glutathione (GSH) was based on the reaction of GSH with dithionitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm (24). The estimation of α-Tocopherol was based on the Emmerie Engel reaction which in turn was based on the reduction of ferric to ferrous ion by α-Tocopherol which forms a red coloured complex with 2, 2'-dipyridyl (25).

Histopathological studies

A portion of brain tissue in each group was fixed with 10% formaldehyde solution, dehydrated in gradually increasing concentrations of ethanol (50-100%), cleared in xylene and embedded in paraffin for histopathological studies. Brain sections of 5 µm thickness were prepared. Haemotoxylin and eosin were used for staining and later the microscopic slides of brain tissue were photographed under 100X magnification.

Statistical analysis

Results are expressed as mean ± S.E.M. for 6 rats in each group. Statistical analysis of the results was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance set at $P<0.05$.

RESULTS

In acute toxicity studies, it was found that the animals were safe upto a maximum dose 2000 mg/kg of body weight. There were no changes in normal behavioural pattern and no signs and symptoms of toxicity and mortality were observed.

The effect of ethanol and the various fractions on body weight is summarized in Table 1. Basal values in control and experimental animals were not significantly different. The body weight of normal controls significantly increased by approximately 14.9% over their basal values after 28 days of treatment, whereas there was a significant ($P<0.01$) decrease in the body weight of ethanol-treated rats (7.4%). Administration of the PEF, CF, EAF and RF of *C. grandis* caused a significant weight gain of 7.1, 10.9, 6.6 and 5.2% respectively, on 28th day. Treatment with Vitamin E produced 11.1% increase in weight gain; however, the weight gain of all treated groups remained lower than the normal control.

The concentration of uric acid and the activities of serum marker enzymes like AST, ALT and ALP were significantly ($P<0.01$) increased in ethanol-fed rats when compared to control rats. Rats fed simultaneously with ethanol and the pet-ether, chloroform and ethyl acetate fractions showed a marked reduction in uric acid level and the activities of AST, ALT and ALP as compared with rats fed only with ethanol. The effect produced by the residual fraction was insignificant ($P>0.05$) compared to

ethanol fed rats. Simultaneous treatment with Vitamin E prevented the increase in uric acid and enzyme activities (Table 2).

Animals fed with ethanol showed a significant (P<0.01) increase in the levels of triglycerides, cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and a decrease in high-density lipoprotein (HDL). Administration of the various fractions of *C. grandis* (except the residual fraction) showed a marked reduction in serum lipid parameters as compared to ethanol treated rats. The lipid levels of the animals treated with Vitamin E was almost similar to control rats (Table 3).

There was a significant (P<0.01) increase in the concentrations of MDA and LH and a decrease in protein content in the brain tissues of rats fed with ethanol when compared with solvent control. Rats fed ethanol simultaneously with the pet-ether, chloroform and ethyl acetate fractions showed near-normal levels of tissue MDA, LH and protein content which is almost similar to the Vitamin E treated group. Treatment with the residual fraction did not show any significant reduction in the end products of lipid peroxidation when compared to the ethanol control (Table 4).

The activities of enzymatic antioxidants in brain tissue of rats fed chronically with ethanol were significantly (P<0.01) lower when compared with control. Rats fed with the pet-ether, chloroform and ethyl acetate fractions of *C. grandis* and Vitamin E displayed increased activities as compared to ethanol-fed rats. Treatment with the residual fraction did not show any significant increase in the activities of the above enzymes (Table 5).

The levels of non-enzymatic antioxidants like GSH and α-Tocopherol in the brain of control and experimental animals are presented in table 6. Animals treated with ethanol showed a significant (P<0.01) reduction in the concentrations of GSH and α-Tocopherol when compared to normal rats. Rats fed simultaneously with ethanol and the pet-ether, chloroform and ethyl acetate fractions of *C. grandis* (except the residual fraction) showed a significant (P<0.01) elevation in GSH and α-Tocopherol. This effect was almost similar to the Vitamin E treated rats (Table 6).

The results of the histopathological studies are presented in Figures 1-7. Figure 1 shows the brain section of control rats showing normal glial tissue and brain architecture. The brain of ethanol-treated rats showed marked gliosis and there is no evidence of necrosis (Figure 2). On the other hand, rats treated with the pet-ether, choloform and ethyl acetate fractions of *C. grandis* showed normal glial tissue with limited gliosis (Figures 3-5). Rats treated with the residual fraction did not reverse the gliosis produced by the administration of ethanol (Figure 6). Rats treated with vitamin E showed almost normal architecture of brain with normal glial tissue and no gliosis, which is similar to the control rats (Figure 7).

DISCUSSION

There was a reduction in body weight in rats fed with ethanol, which may be due to the reduced body fat mass and reduction of adipose tissue (26). Rats treated with the fractions of *C. grandis* showed significant gain in body weight similar to the control rats. This may be due to the attenuation of malnutrition caused by ethanol. The elevated activities of serum marker enzymes in ethanol-treated rats indicate mitochondrial injury (27). Metabolism of ethanol to acetaldehyde leads to the conversion of a portion of tissue xanthine dehydrogenase (XDH) to xanthine oxidase (XO), causing an elevation in serum uric acid level. Likewise conversion might occur due to an increase in cellular NADH as a result of ethanol oxidation with subsequent production of superoxide anion radicals, which could contribute to the enhancement of uric acid (28). Rats fed with ethanol showed hypertriglyceridemia, which is due to the reduced activity of the enzyme lipoprotein lipase and triglyceride lipase, thus resulting in the decreased uptake of triglycerides from serum and causing it's accumulation in the tissues (29). Also, ethanol administration showed an elevation in cholesterol level due to the increased activity of the enzyme ß-hydroxymethylglutaryl CoA (HMG CoA) which catalyses the rate limiting step in cholesterol biosynthesis leading to increased cholesterol synthesis in tissue and excess leaking out of cholesterol into the blood (30). We observed a significant reduction in the levels of serum marker enzymes, uric acid and lipids in rats receiving ethanol and the fractions simultaneously, thus showing the beneficial effect of the leaves against ethanol toxicity.

Chronic ethanol ingestion is known to promote oxidative stress mediated through LPO by the generation of free radicals (31). In addition, iron, which is present in certain regions of brain catalyses the generation of oxygen derived free radicals (32). LPO is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane lipids. The high vulnerability of the brain to oxidative damage is due to its high lipid

Table 1: Effect of the fractions of Coccinia grandis on body weight in control and experimental animals

Group	Dose (*p.o.*)	Body weight (g)	
		Initial	Final
Control (Normal saline)	10 ml/kg	141.6 ± 4.7	162.2 ± 4.2 [a] (↑14.9)
Alcohol control (20% ethanol)	5 ml/100g	140 ± 4.4	129.6 ± 3.8 [a] (↓7.4)
PEF	200 mg/kg	141 ± 5.7	151.1 ± 4.9 [b] (↑7.1)
CF	200 mg/kg	142.6 ± 8.3	157.6 ± 7.6 [a] (↑10.9)
EAF	200 mg/kg	143.6± 2.4	153.6 ± 2.1 [a] (↑6.6)
RF	200 mg/kg	142 ± 6.3	149.5 ± 4.6 [b] (↑5.2)
Vitamin E	100 mg/kg	143 ± 4.2	159 ± 3.2 [a] (↑11.1)

Values are mean ± SEM; n = 6 in each group. Ethanol control was compared with normal control and treated group was compared with ethanol control on 28th day. Values in parentheses are the percent increase (↑) or decrease (↓) from their corresponding basal value. Values are statistically significant at [a]P<0.01 and [b]P<0.05 (one way ANOVA followed by Dunnett's test).

Table 2 : Effect of the fractions of Coccinia grandis on serum marker enzymes and uric acid in control and experimental animals

Group	Dose (*p.o.*)	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)	Uric acid (mg/dl)
Control (Normal saline)	10 ml/kg	899.2 ± 61.9	972.6 ± 43.5	307 ± 56.9	0.19 ± 0.05
Alcohol control (20% ethanol)	5 ml/100g	5519.6 ±95.6[a]	1809.1 ± 23.6[a]	1689 ± 31.5 [a]	7.14 ± 0.32 [a]
PEF	200 mg/kg	1262.2 ±56.2[b]	1056.9 ±20.2[b]	1182.4±23.9[b]	3.0 ± 0.47[b]
CF	200 mg/kg	1162.2 ± 136[b]	1293.2 ±49.4[b]	790.3 ± 116[b]	4.98 ± 0.65[b]
EAF	200 mg/kg	1723.6 ± 215[b]	1330.6±12.56[b]	1217.8±15.06[b]	2.09 ± 0.08[b]
RF	200 mg/kg	4052.6 ±65.8[c]	1624.2 ± 27.7[c]	1440.6 ± 9.8[c]	5.64 ± 0.5[c]
Vitamin E	100 mg/kg	1139.2 ±55.7[b]	1037.8 ± 50.0[b]	348.3 ± 25.8[b]	1.0 ± 0.01[b]

Values are expressed as mean ± SEM; n = 6 in each group. [a]P<0.01 when compared to control; [b]P<0.01 and [c]P>0.05 when compared to ethanol control (one way ANOVA followed by Dunnett's test).

Table 3 : Effect of the fractions of Coccinia grandis on lipid profile in control and experimental animals

Group	Dose (*p.o.*)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (Normal saline)	10 ml/kg	20.87 ± 0.57	29.7 ± 1.57	199.6 ± 16.6	174.05 ± 15.8	4.17 ± 0.11
Alcohol control (20% ethanol)	5 ml/100g	90.21 ± 1.06[a]	330 ± 35.3[a]	32.48 ± 4.22[a]	238.2 ± 37.5[a]	18.04 ± 0.21[a]
PEF	200 mg/kg	21.51 ± 3.33[b]	32.43 ± 5.2[b]	58.28 ± 16.72[b]	9.48 ± 4.2[b]	4.3 ± 0.6[b]
CF	200 mg/kg	28.72 ± 3.78[b]	37.83 ± 3.63[b]	174.08 ± 24.4[b]	34.4 ± 13.33[b]	5.7 ± 0.8[b]
EAF	200 mg/kg	21.45 ± 1.0[b]	61.04 ± 3.52[b]	68.94 ± 1.37[b]	31.22 ± 9.4[b]	4.29 ± 0.2[b]
RF	200 mg/kg	74.05 ± 2.0[c]	170.6 ± 6.34[b]	36.68 ± 0.89[c]	119.1 ± 1.2[c]	14.8 ± 0.9[c]
Vitamin E	100 mg/kg	21.08 ± 0.6[b]	29.9 ± 1.71[b]	186.8 ± 15.5[b]	161.12 ± 14.9[b]	4.21 ± 0.13[b]

Values are expressed as mean ± SEM; n = 6 in each group. [a]P<0.01 when compared to control; [b]P<0.01 and [c]P>0.05 when compared to alcohol control (one way ANOVA followed by Dunnett's test).

Table 4 : Effect of fractions of Coccinia grandis on tissue protein, MDA and LH in control and experimental animals

Group	**Dose (*p.o.*)**	**Protein** (mmoles/min/ mg wet tissue)	**MDA** (nmoles/min/ mg protein)	**LH** (nmoles/min/ mg protein)
Control (Normal saline)	10 ml/kg	3666.6 ± 149	0.175 ± 0.01	0.55 ± 0.06
Alcohol control (20% ethanol)	5 ml/100g	1750.8 ± 668[a]	2.035 ± 0.35[a]	5.24 ± 0.90[a]
PEF	200 mg/kg	3055.5 ± 633.4[b]	0.93 ± 0.2[b]	1.11 ± 0.02[b]
CF	200 mg/kg	3611.1 ± 633.4[b]	0.41 ± 0.09[b]	0.88 ± 0.07[b]
EAF	200 mg/kg	2916.6 ± 186.3[b]	1.2 ± 0.03[c]	1.52 ± 0.06[b]
RF	200 mg/kg	2117.5 ± 181.7[c]	1.2 ± 0.06[c]	2.86 ± 0.15[c]
Vitamin E	100 mg/kg	3638.8 ± 25[b]	0.25 ± 0.018[b]	0.7 ± 0.03[b]

Values are expressed as mean ± SEM; n = 6 in each group [a]P<0.01 when compared to control; [b]P<0.01 and [c]P>0.05 when compared to ethanol control (one way ANOVA followed by Dunnett's test)

Table 5 : Effect of fractions of Coccinia grandis on tissue enzymatic antioxidants in experimental animals

Group	**Dose (*p.o.*)**	**Catalase** (µmoles/min/ mg protein)	**GPx** (nmoles/min/ mg protein)	**SOD** (nmoles/min/ mg protein)	**GSSH** (nmoles/min/ mg protein)	**Peroxidase** (nmoles/min/ mg protein)
Control (Normal saline)	10 ml/kg	29.4 ± 1.29	43.4 ± 1.23	27.1 ± 1.45	19.6 ± 1.45	33.5 ± 1.1
Alcohol control (20% ethanol)	5 ml/100g	13.51 ± 2.7[a]	32.7 ± 4.4[a]	10.88 ± 1.5[a]	9.14 ± 1.88[a]	9.9 ± 1.5[a]
PEF	200 mg/kg	17.21 ± 3.29	34.77 ± 5.0[b]	17.22 ± 3.29[c]	18.3 ± 1.2[b]	13.85 ± 2.9[c]
CF	200 mg/kg	28.86 ± 5.13[c]	36.15 ± 3.21[b]	24.02 ± 1.14[b]	18.38 ± 0.98[b]	31.7 ± 2.0[b]
EAF	200 mg/kg	16.09 ± 0.63	34.25 ± 0.33[b]	15.12 ± 1.46	17.32 ± 0.87[b]	12.9 ± 0.9

RF	200 mg/kg	13.13 ± 0.53	31.0 ± 1.94	11.4 ± 1.06	11.85 ± 0.7	10.9 ± 0.9
Vitamin E	100 mg/kg	29.01 ± 0.7[b]	41.9 ± 0.57[b]	26.3 ± 0.56[b]	18.6 ± 0.3[b]	31.9 ± 0.6[b]

Values are expressed as mean ± SEM; n = 6 in each group. [a]P<0.01 when compared to control; [b]P<0.01 and [c]P>0.05 when compared to ethanol control (one way ANOVA followed by Dunnett's test).

Table 6 : Effect of fractions of Coccinia grandis on tissue non-enzymatic antioxidants in control and experimental animals

Group	**Dose (*p.o.*)**	**GSH** (nmoles/min/mg protein)	**α-Tocopherol** (mg/mg protein)
Control (Normal saline)	10 ml/kg	35.3 ± 0.94	5.3 ± 0.1
Alcohol control (20% ethanol)	5 ml/100g	17.61 ± 3.6 [a]	3.12 ± 0.11 [a]
PEF	200 mg/kg	28.92 ± 3.67[b]	4.17 ± 0.07[b]
CF	200 mg/kg	30.11 ± 1.8[b]	5.16 ± 0.11[b]
EAF	200 mg/kg	26.7 ± 1.1[c]	3.45 ± 0.106 [c]
RF	200 mg/kg	18.9 ± 0.9 [c]	3.14 ± 0.1 [c]
Vitamin E	100 mg/kg	34.6 ± 0.31[b]	5.36 ± 0.09[b]

Values are expressed as mean±SEM; n = 6 in each group. [a]P<0.01 when compared to control; [b]P<0.01 and [c]P<0.05 when compared to ethanol control (one way ANOVA followed by Dunnett's test).

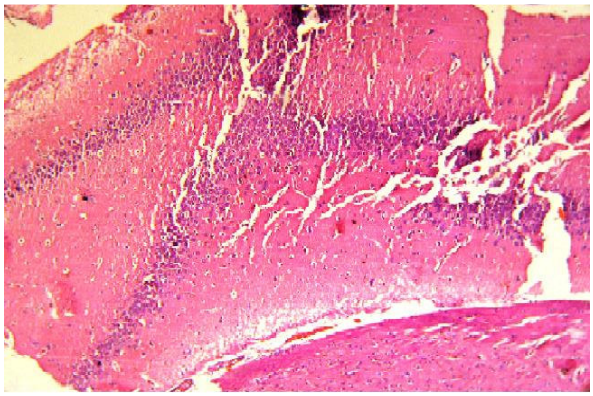

Figure 1 : Section of brain obtained from control rats showing normal glial tissue (Haematoxylin and eosin, 100x)

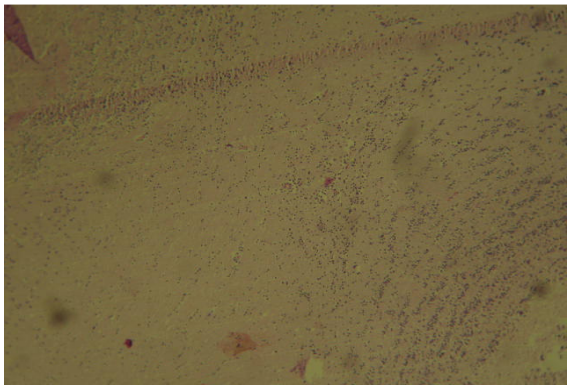

Figure 2 : Section of brain obtained from rats treated with ethanol showing gliosis (Haematoxylin and eosin, 100x)

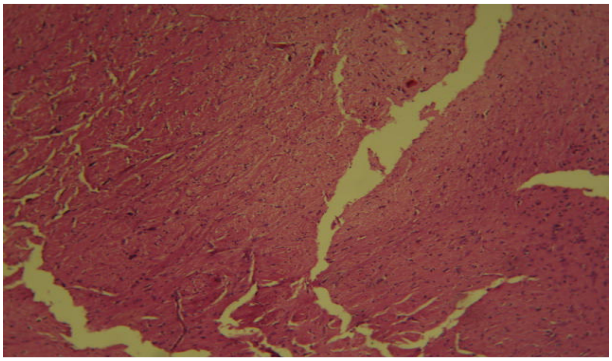

Figure 3 : Section of brain obtained from rats treated with ethanol + PEF showing normal glial tissue with one focus of gliosis (Haematoxylin and eosin, 100x)

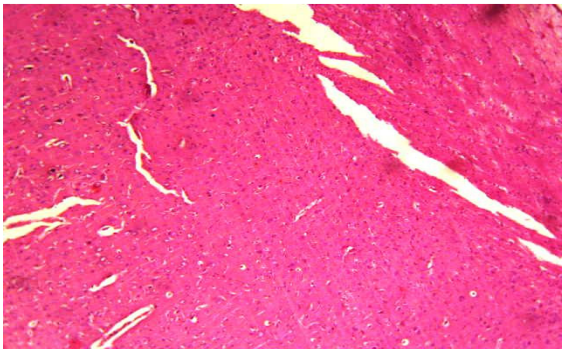

Figure 4 : Section of brain obtained from rats treated with ethanol + CF showing normal glial tissue and no gliosis (Haematoxylin and eosin, 100x)

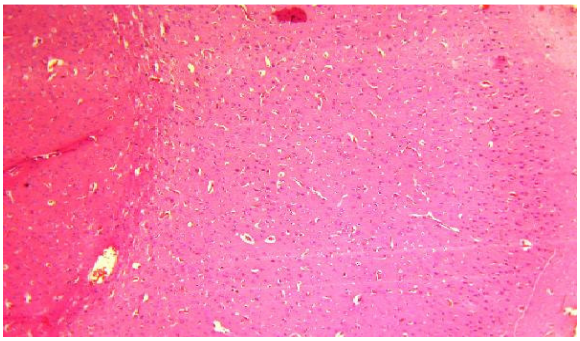

Figure 5 : Section of brain obtained from rats treated with ethanol + EAF showing normal glial tissue with one focus of gliosis and choroid epithelium (Haematoxylin and eosin, 100x)

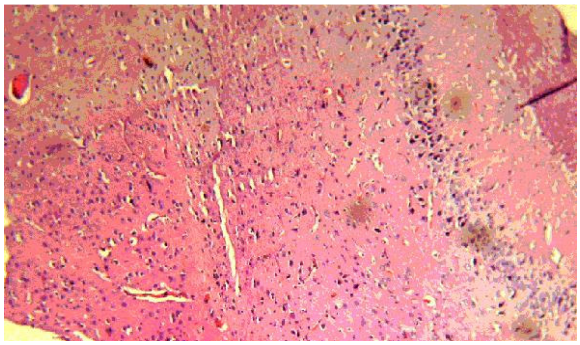

Figure 6 : Section of brain obtained from rats treated with ethanol + RF showing gliosis and rosenthal fibres (Haematoxylin and eosin, 100x)

content. The results of the present study show that chronic ethanol treatment elicits an increase in lipid peroxidation as evidenced by an elevation in the levels of MDA and LH in rat brain. Simultaneous treatment of rats with the fractions inhibited the deleterious process of lipid peroxidation by reducing the end products of LPO, demonstrating the antiperoxidative effect of the leaves of *C. grandis* on ethanol toxicity. Vitamin E as a lipid soluble, chain breaking antioxidant prevented the production of MDA and LH by scavenging the free radicals in biological membranes.

Increased LPO in ethanol treated rats result in a depletion of the cellular antioxidant defense system. SOD protects the membranes and biological structures against the damage caused by superoxide anion radical ($O_2^{\cdot -}$). Inhibition of SOD results in the generation of partially reduced oxygen species. When SOD activity is decreased, neurons are more vulnerable to oxyradical injury (33). CAT primarily causes the decomposition of H_2O_2 to water at a much faster rate, in association with GPx. Thus, a decrease in the activities of both CAT and GPx leads to an accumulation of H_2O_2. Also, a decrease in the activity of GSSH is due to the depletion of thiol, leading to related pathologies (34). Significant reduction in the above mentioned enzymatic antioxidants were observed in the ethanol-treated rats. Co-administration of the PEF, CF and EAF fractions of *C. grandis* and vitamin E increased the activities of enzymatic antioxidants in ethanol-treated rat brain, thus exerting a beneficial action against superoxide anion and hydroxyl radicals.

GSH is a major non-protein thiol in living organisms and protects the cellular defence system against the toxic effects of lipid peroxidation. Due to oxidative stress, the brain is unable to maintain the GSH redox state and oxidized glutathione (GSSG) is concentrated in some regions of the brain (35). Also, the cellular levels of the active forms of vitamin C and α-Tocopherol are maintained by GSH by neutralizing the free radicals. A decrease in GSH level leads to a decrease in cellular levels of vitamin C and α-Tocopherol (36). In our study, a significant decrease in the levels of non-enzymatic antioxidants like GSH and α-Tocopherol were observed in ethanol-treated rats. This could be due to an increased utilization of GSH to scavenge the toxic products formed from ethanol. Rats treated with the fractions of *C. grandis* and vitamin E showed a significant restoration of GSH and α-Tocopherol contents in the brain suggesting the involvement of GSH-dependent detoxification of free radicals. Rats fed with ethanol produced gliosis in brain tissue. Treatment with the pet-ether, choloform and ethyl acetate fractions of *C. grandis* reduced gliosis in brain tissues. These observations reveal that the fractions confer protection against ethanol toxicity presumably by enhancing the antioxidant potential.

Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals (37, 38). Their scavenging ability is mainly due to the presence of hydroxyl groups and are potent antioxidants. Our previous study shows that the presence of high phenolic and flavonoid content in the fractions has contributed directly to the antioxidant activity by neutralising the free radicals (12). Supplementation with antioxidant such as vitamin E has been reported to be useful in the prevention of alcohol-induced neural disorders (39). The chloroform fraction of *C. grandis* exhibited highest antioxidant activity and significantly inhibited lipid peroxidation and the activity is almost comparable to the effect of the standard drug vitamin E.

In conclusion, the results obtained from the present study provide a rationale for the use of the leaves of *Coccinia grandis* for the treatment of alcohol-related disorders. Studies are presently undertaken in our laboratory to characterize the active ingredients.

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