Research Article

Neuroprotective action of 4-hydroxyisophthalic acid against ethanol-induced oxidative stress in the rat brain

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Abstract

Objective: 4-Hydroxyisophthalic acid (4-HIPA) is a novel bioactive molecule isolated in our laboratory from the edible roots of *Decalepis hamiltonii*. Earlier we have shown free radical scavenging activity of 4-HIPA *in vitro* and cytoprotective action. Here we report *in vivo* neuroprotective potential of 4-HIPA against ethanol-induced oxidative stress in the brain of laboratory rat. **Materials and Methods:** Rats were treated with ethanol (5g/kg bw) in single and multiple doses with/without pre-treatment with 4-HIPA (200mg/kg bw). **Results:** Ethanol induced oxidative stress in both single and multiple dose treatment showed regional differences. Oxidative stress in the cerebral cortex and cerebellum being higher in the latter as indicated by the increased reactive oxygen species, lipid peroxidation, protein carbonyls and glutathione depletion. Pre-treatment with 4-HIPA significantly restored the alterations induced by ethanol. 4-HIPA protected against ethanol-induced oxidative stress by boosting the antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase. **Conclusion:** 4-HIPA is a potential neuroprotective molecule of natural origin and shows the promise natural bioactive compound derived from edible source against alcohol toxicity. Therefore, 4-HIPA has health implications as a nutraceutical against alcohol abuse.

Keywords: *Decalepis hamiltonii*, 4-HIPA, neuroprotection, ethanol, GSH, ROS, antioxidant enzymes

Introduction

Oxidative stress is profoundly observed in brain among other organs of human body, due to its high metabolic activity aided by presence of numerious mitochondria, increased oxygen uptake andabundance of PUFA and catecholamines. Also, it has inefficient oxidative defense and DNA repair mechanisms making it susceptible to free radical mediated cellular damage (Shirpoor et al., 2009; Mecocci et al., 1993; Barja 2004). Superoxide radicals produced predominantly in mitochondria damage cellular components. Peroxisomal oxidation of fatty acids and microbial debris clearance involving phagocytes induce oxidative outburst in brain (Balaban et al., 2005; Reddy et al., 2013).

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Ethanol, a neurotoxic substance has been well documented for its oxidative stress effects on the nervous system. Being soluble in both water and lipids ethanol easily diffuses through the cell membrane and oxidizes rapidly, which results in multiorgan toxicity (Harlan et al., 1991; Montolie et al., 2002; Altomore et al., 1996; Albano et al., 2012; Liang et al., 2012). Although, the mechanism of neurotoxicity of ethanol is not well understood, experimental evidences indicate that ethanol induce damage by free radical intermediates (Nordmann et al., 1992; Sherman et al., 2002). The pathogenesis of ethanol-induced damage involving oxidative stress has been well established (Tseckamole et al., 1985). Metabolism of ethanol by alcohol dehydrogenase and Cytochrome P450, produce free radicals and its oxidation leads to concomitant changes in $NADH/NAD^+$ redox ratio resulting in ROS generation (Montoliu et al., 2002; Bailey et al., 2002; Cederbaum 1992). Further, alcohol intoxication is attributed to ROS-mediated blood brain barrier permeability, tubulin disruption, impaired mitochondrial respiration, and

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inflammation are responsible for pathophysilogical changes (Blanco et al., 2005). Ethanol-induced ROS/RNS generation alter mitochondrial membrane properties via altered membrane fluidity, ion permeability, disrupted electron transport chain and reduced oxidative phosphorylation, which ultimately lead to lipid peroxidation, increased membrane fluidity, disturbed intracellular calcium homeostasis and mitochondrial dysfunction (Nordmann 1994). Although chronic alcohol effects on the brain are known, acute and sub-acute effects on the brain have not been clearly elucidated. Since oxidative stress is implicated in neurotoxicity and neurological disorders, bioactive molecules from edible plant source with potent antioxidant activity are considered to be promising therapeutic agents. Several natural antioxidant compounds such as resveratrol from grapes (Sun et al., 1997; Sinha et al., 2002; Gilgun-Sherki et al., 2001; Antonio et al., 2008; Kumar et al., 2011), curcumin from *Curcuma longa* (Cole et al., 2007), quercetin from *Opuntiaficus-indicavarsaboten* (Dok-go et al., 2003), epigallocatechin-3-gallate found in green tea (Antonia et al., 2008) have been shown to possess neuroprotective potential in reversing oxidative changes in neurodegenerative disorders (Sun et al., 2008). Therefore, antioxidants from natural origin are being subjected to scientific scrutiny for their ameliorating potential targeting xenobiotic-induced neurotoxicity.

Decalepis hamiltonii (family: Asclepiadaceae), is an endemic and endangered climbing shrub found in the forests of peninsular India. Roots of *D. hamiltonii* are used in folk medicine and consumed as pickles and a health drink for its alleged health benefits (Reddy and Murthy, 2013). Tuberous roots of *D. hamiltonii* possess potent antioxidant properties, which could be responsible for its health implications (Srivastava et al., 2006a). We have earlier shown that the aqueous extract of the roots of *D. hamiltonii* protects against CCl_4 and ethanol hepatotoxicity which is attributed to the novel antioxidant compounds present in it (Srivastava and Shivanandappa, 2006, Srivastava et al., 2006a, Srivastava et al., 2006b; Srivastava and Shivanandappa, 2010). Further, neuroprotective effect of *D. hamiltonii* root extract against ethanol neurotoxicity in rats and *Drosophila* were reported earlier (Srivastava and Shivanandappa, 2010; Jahromi et al., 2015). 4-Hydroxyisophthalic acid (4-HIPA), a novel antioxidant, isolated from the aqueous extract of *D. hamiltonii* is a potent scavenger of free radicals and an inhibitor of lipid peroxidation *in vitro* (Srivastava et al., 2006b; Srivastava et al., 2007). 4-HIPA has shown cytoprotective potential *in vitro* (Srivastava et al., 2012). Although *D. hamiltonii* root extract has shown to exhibit neuroprotective potential, the active principles in the extract responsible for neuroprotective effect have not been investigated (Srivastava and Shivanandappa, 2009, 2010). Here we report the neuroprotective potential of 4-HIPA against ethanol-induced toxicity in the rat brain.

Materials and methods

Chemicals

Nicotinamide adenine dinucleotide reduced (NADH), 1-chloro-2,4-dinitrobenzene (CDNB), thiobarbituric acid (TBA), glutathione (GSH), 2,4-dinitrophenylhydrazine (DNPH), 2′, 7′-dichlorofluorescein diacetate (DCF-DA), and pyrogallol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), 5,5'dithiobis (2-nitrobenzoic acid) (DTNB), hydrogen peroxide (H_2O_2), were purchased from Sisco Research Laboratories, Mumbai, India. Diethyl ether, ethylene diamine tetra acetic acid (EDTA), sodium dodecyl sulfate, ethanol was redistilled before use.

Isolation of 4-hydroxyisophthalic acid

4-Hydroxyisophthalic acid (4-HIPA) from *D. hamiltonii* extract was isolated and characterized as described earlier (Srivastava et al., 2006b; Srivastava et al., 2012).

Animals and treatments

Twelve week old adult male Wistar rats (200 – 230 g) were divided into four groups of 6 animals each. The animal experiments were approved by institutional animal ethics committee. Animals were maintained at room temperature with automatically controlled cycle of 12hrs dark and 12hrs light and provided standard laboratory animal feed and water ad libitum.

Single dose treatment

In single dose pre-treatment experiment, 4-HIPA (200mg/kg bw/day) was orally administered which was followed by administration of ethanol 5gm/kg bw/day (20% v/v in water, after 1hrs 4-HIPA pre-treatment) through gavage.

Group I – Control (Saline); Group II - Ethanol (5gm/kg bw/day); Group III - 4-HIPA (200mg/kg bw/day) + ethanol (5gm/kg bw/day); Group IV - 4-HIPA (200mg/kg bw/day).

Multiple dose treatment

4-HIPA (200mg/kg bw/day; pre-treatment) was administered orally for 7 consecutive days followed by ethanol at 5gm/kg bw/day (20% v/v in water; after 1hrs of 4-HIPA pre-treatment) through gavage for 7 days.

Group I – Control (Saline); Group II - Ethanol (5gm/kg bw/day, for 7 days); Group III - 4-HIPA (200mg/kg bw/day, for 7 days) + ethanol (5gm/kg bw/day, for 7 days); Group IV - 4-HIPA (200mg/kg bw/day, for 7 days). The dosage of 4-HIPA was selected based on our earlier results.

Animals were sacrificed by ether anesthesia 16hrs after

ethanol administration, the brain (cerebral cortex and cerebellum) dissected on ice, perfused with saline and processed for biochemical assays.

Reactive oxygen species determination

Reactive oxygen species (ROS) were measured by adding the reagent DCF-DA, to the tissue homogenate (10% in 50 mM Tris buffer, pH 7.4) and the volume was made up to 3 ml with the buffer (50 mM, pH 7.4) and incubated for 15 min at room temperature. Fluorescence was measured at an excitation wavelength of 488 nm and the emission wavelength, 525 nm (Cathcart et al., 1983).

Glutathione determination

Reduced glutathione (GSH) content was estimated by Ellman's reagent (Ellman, 1959). Tissue homogenate (10% TCA+10mM EDTA) was centrifuged at 5000 g for 15 min and 200 µl supernatant (GSH) was mixed with DTNB (10 mM) in phosphate buffer (0.2 M, pH 8.0) and incubated for 10 min at room temperature. The color was read at 412 nm and GSH was calculated by a standard curve and expressed as µg GSH/mg protein.

Lipid peroxidation determination

1 ml of tissue homogenate (10% w/v in 0.25 M sucrose) was mixed with 1.5 ml 20% TCA and 1.5 ml 0.6% TBA and boiled for 30 min, cooled, followed by the addition of butanol and centrifuged at 2000 g for 5 min. The upper layer was read at 535 nm in a spectrophotometer. Thiobarbituric acid reactive species (TBARS) were expressed as n moles of as malondialdehyde (MDA) formed/mg protein (Ohkawa et al., 1979).

Protein carbonyls determination

Tissue was homogenized (10% w/v) in Tris HCl buffer (20 mM, pH 7.4), centrifuged at 3000 g for 20 min at 4^0C, the supernatant was precipitated with 20% TCA and centrifuged. The pellet was suspended in DNPH (10 mM) and allowed to stand at room temperature for 60 min with occasional vertexing. 1 ml 20% TCA was added to the reaction mixture with the pellet was washed three times with ethanol: ethylacetate (1:1) and 2% SDS (20 mM Tris HCl, 0.1 M NaCl, pH 7.4) was added to solubilise the pellet and incubated at 37^0C over night. The absorbance was read at 360 nm in spectrophotometer and the protein carbonyl content was calculated using a molar extinction coefficient of 22,000 $M^{-1}cm^{-1}$ (Levine et al., 1990).

Antioxidant enzymes determination

Brain tissue was homogenized (10% w/v) in ice cold phosphate buffer (50 mM, pH 7.4), centrifuged at 10,000 g for 20 min at 4^0C and the supernatant was used for the enzyme assays.

Superoxide dismutase (SOD) activity was measured by inhibition of pyrogallol (2 mM) autooxidation (Marklund and Marklund, 1974). The reaction mixture contained 0.1 M Tris buffer (pH 8.2) and 10% tissue homogenate. The reaction was started by adding 2 mM pyrogallol. The absorbance was read at 420 nm for 2 min at an interval of 30 sec. The enzyme activity was expressed as µg/mg protein.

Catalase (CAT) activity was measured using H_2O_2 (3%) as the substrate in phosphate buffer. The reaction was started by the addition of 100 µl of enzyme and the change in absorbance was read at 240 nm for 2 min at 30 sec interval. The activity was expressed as µ mole H_2O_2/min/mg protein (Aebi, 1983).

Glutathione peroxidase (GPx) activity was assayed by the indirect method using glutathione reductase (Mannervik, 1985). Cumene hydroperoxide (1 mM) and glutathione (0.25mM) were used as substrates and coupled oxidation of NADPH by glutathione reductase (0.25U) in tris buffer (50mM, pH 7.6) was monitored at 340nm.

Glutathione-S-transferase (GST) activity was assayed by the method of Warholm (Warholm et al., 1985). The reaction mixture contained 20 mM GSH and the 20 µl brain enzyme supernatant in 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA. The reaction was started by adding 30 mM CDNB and change in the absorbance was measured at 344 nm. The enzyme activity was expressed as n mole CDNB conjugate/min/mg protein.

Glutathione reductase (GR) activity was measured by the method of Calberg and Mannervick (Calberg and Mannervik, 1985). The reaction mixture contained 200 mM phosphate buffer (pH 7.4), 20mM GSSG and 2 mM NADPH. The reaction was started by adding the enzyme and the absorbance change was measured at 340 nm for 3 min.

Determination of protein content

Protein content was determined by the method of Lowry using bovine serum albumin as the standard (Lowry et al., 1951).

Statistical analysis

The data were expressed as means ± S.E (n=5) and significant differences (p<0.05) were determined using a one-way analysis of variance (ANOVA) followed by a Neuman-Keul's test for multiple comparisons (SPSS version 14).

Results

Ethanol administration resulted in increased production of ROS both in single and multiple dose treatment. ROS induction in cerebellum was found to be much higher than that of cerebral cortex. 4-HIPA pre-treatment significantly

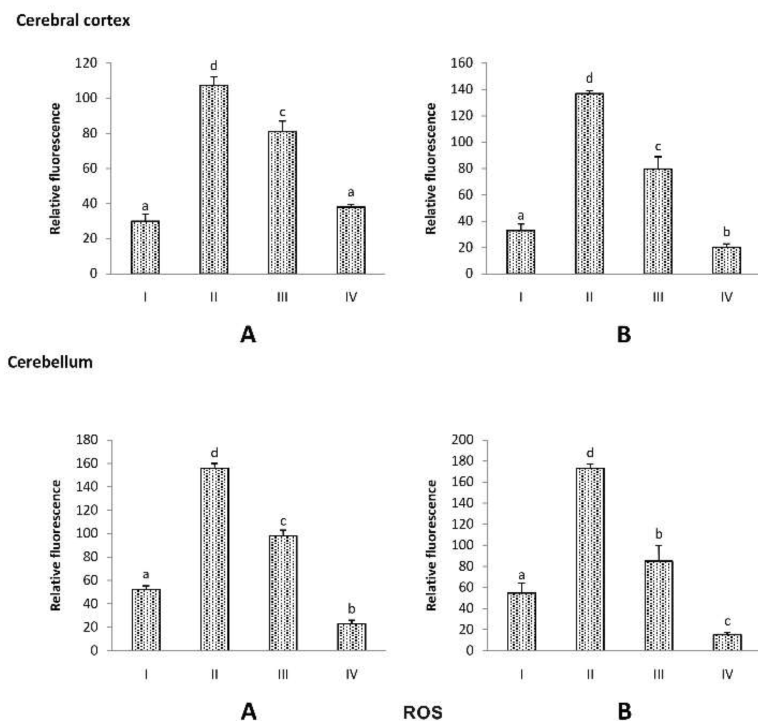

Figure 1. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **ROS;** Reactive oxygen species. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

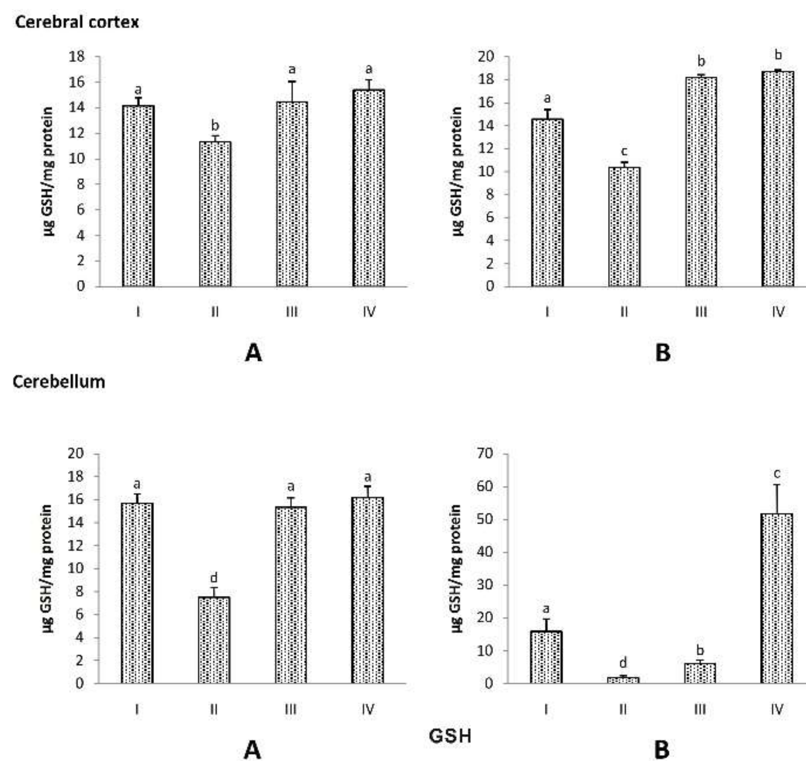

Figure 2. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **GSH;** Glutathione. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

lowered the ethanol-induced ROS production in both brain regions. Interestingly 4-HIPA alone significantly reduced the ROS generation which is much lower than that of normal control group. However, ROS level in ethanol 4-HIPA fed rats were comparable to control group (Figure 1).

GSH content in the cerebral cortex and cerebellum showed significant depletion in rats treated with ethanol. Cerebellum showed higher GSH reduction compared to cerebral cortex in both single and multiple dose study. In group 3, GSH levels were restored to normal values by 4-HIPA pre-treatment.

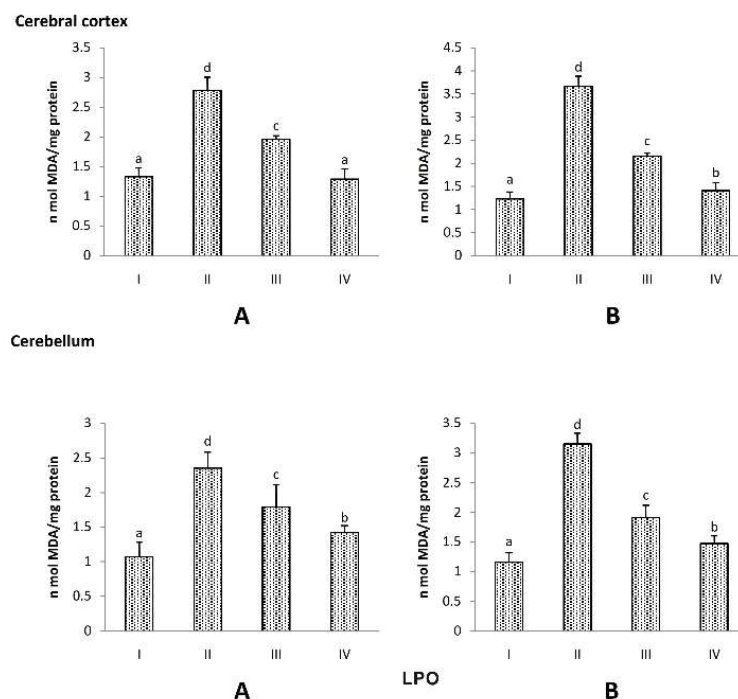

Figure 3. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **LPO;** Lipid peroxidation. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

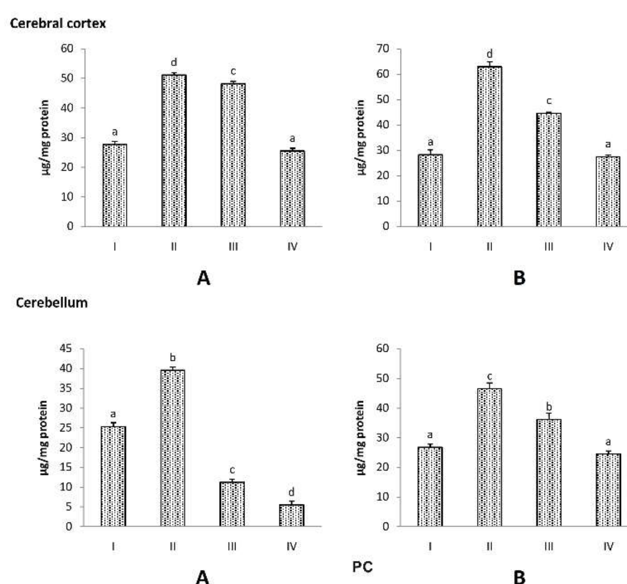

Figure 4. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **PC;** Protein carbonyls. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days) + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

Further, 4-HIPA alone treatment maintained GSH levels which were comparable to control group (Figure 2).

A significant increase in LPO was observed in ethanol treated group. Compared with ethanol treatment group TBARS levels were significantly reduced by 4-HIPA pre-treatment. Group received 4-HIPA alone showed TBARS levels comparable with control group. Rats pre-treated with 4-HIPA were significantly protected from the LPO induced by ethanol (Figure 3).

Protein carbonyl levels were elevated in both brain regions of ethanol treated group. Pre-treatment with 4-HIPA has significantly reduced these levels. Whereas, the administration of 4-HIPA alone significantly decreased the pc levels which were almost similar to control group. Interestingly cerebellum showed much lower levels of pc both in group III and IV in a single dose study (Figure 4).

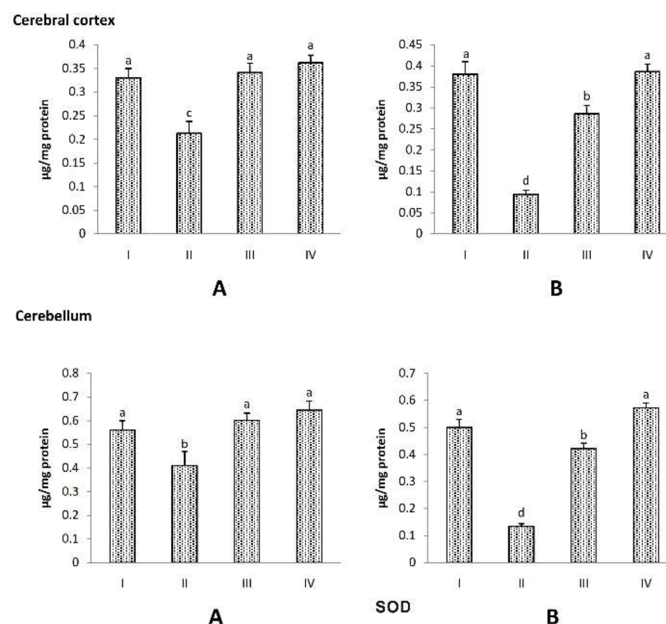

Figure 5. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **SOD;** Superoxide dismutase. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

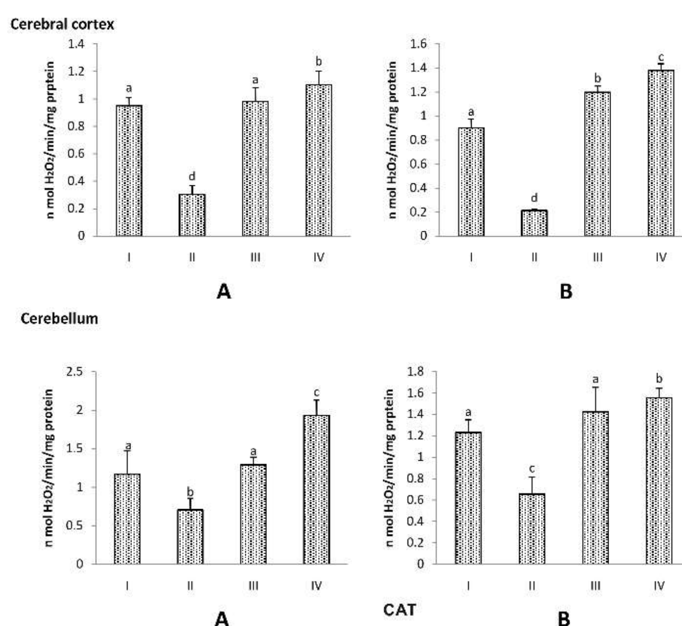

Figure 6. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **CAT;** Catalase. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

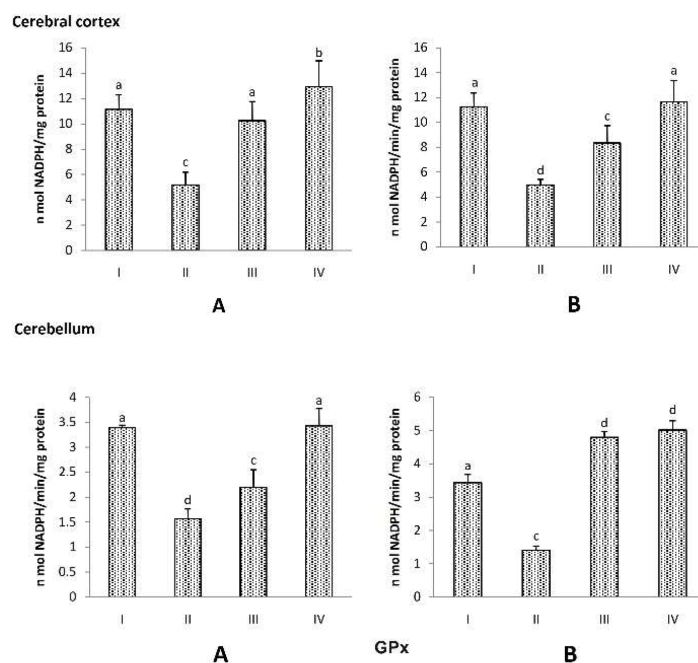

Figure 7. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **GPx;** Glutathione peroxidase. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

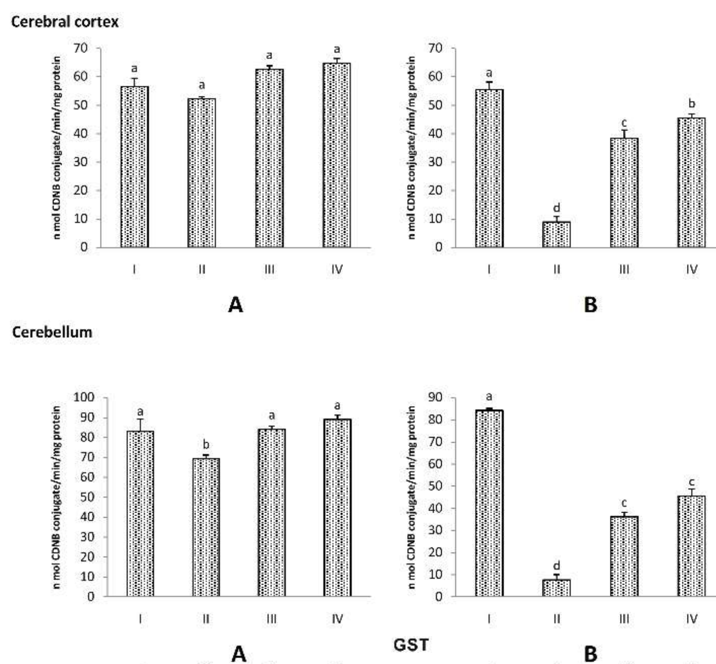

Figure 8. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **GST;** Glutathione-S-transferase.**A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

Similarly, ethanol group showed significant reduction in antioxidant enzyme activities such as SOD, CAT, GPx, GST and GR (Figure 6 to 9). However, GST and GR showed lesser reduction in ethanol treated group. Rats pre-treated with 4-HIPA were able to significantly prevent the decrease of enzyme activities. Further, animals which received 4-HIPA alone maintained enzyme activities to the similar levels to the control animals.

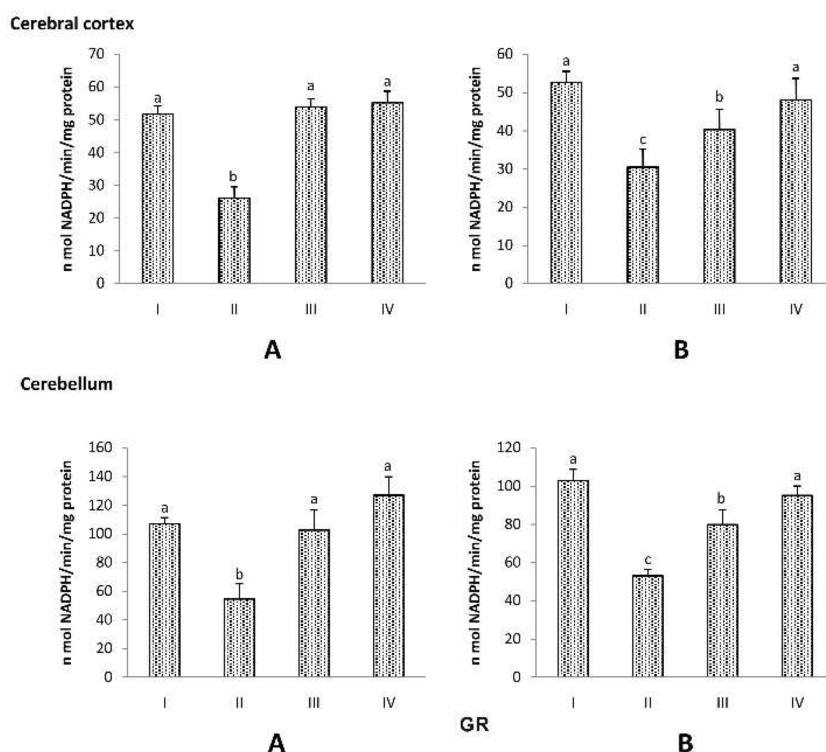

Figure 9. Amelioration of ethanol-induced neurotoxicity by 4-HIPA. **GR;** Glutathione reductase. **A;** *Single dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw/day); **III**; Ethanol (5g/kgbw/day) + 4-HIPA (200mg/kgbw/day): **IV**; 4-HIPA (200mg/kgbw/day). **B;** *Multiple dose study*: **I**; Control (saline): **II**; Ethanol (5g/kgbw for 7 days); **III**; Ethanol (5g/kgbw for 7 days + 4-HIPA (200mg/kgbw for 7 days): **IV**; 4-HIPA (200mg/kgbw for 7 days). Each bar represents the mean ± S.E, (n = 6) bars with different alphabets differ significantly at $p < 0.05$ level (Neuman-Keuls Test).

Discussion

Brain, being a highly metabolically active organ, is also susceptible to oxidative damage by excessive production of reactive oxygen species through oxidative stress (Wu and Cederbaum, 2003). Biochemical and molecular action of ethanol in the brain rather complex. Primary mechanism of action of ethanol intoxication involving ROS production, reduced antioxidant defenses, altered metal ion homeostasis leads to neuronal dysfunction. Ethanol metabolism and subsequent ROS generation is thought to induce oxidative stress which is implicated in neurotoxicity (Ponnappa et al., 2000; Heinz et al., 2003). Further, ethanol neurotoxicity is attributed to excessive neurotransmitter release, altered membrane potential, disruption in receptors and ion channels by complex neurochemical modifications.

Our study clearly demonstrated regional differences in ethanol-induced oxidative stress the brain as evident from ROS induction, lipid peroxidation and antioxidant enzymes. Ethanol treatment resulted in induction of ROS, a primary event which further contribute in the cascade of cellular damage through multiple mechanisms. Higher oxidative stress in the brain of rats in subacute ethanol trated group could be due to the stimulation of FWH reaction via CYP2E1 activation and/or altered antioxidants consequently exacerbate neuronal damage.

GSH a critical cellular antioxidant play a vital role in preventing oxidative stress induced changes mainly in mitochondria. Its depletion, impaired synthesis and defective transport to mitochondria make it unprotected via radicals mediated cellular damage. Consequently, the toxic effects of ethanol oxidants uproot primary defense systems and diminish cell survival. Endogenous glutathione peroxidase and glutathione reductase are important antioxidant enzymes, which maintain GSH levels and protect against free radical mediated oxidative stress (Brind et al., 2004). In this study, GSH depletion was observed in both single and multiple doses of ethanol treated group. GSH depletion may be due to the increased degradation via the gamma glutamyle cycle (Morton and Mitchell, 1985).In the single dose study, 4-HIPA pre-treatment maintained the GSH levels to its normal values in both brain regions. Pre-treatment with 4-HIPA alone enhanced GSH levels compared to that of control group in multiple dose treatment. 4-HIPA reversed the ethanol-elicited oxidative changes in the rat brain. 4-HIPA pre-treatment significantly suppressed the ROS generation and, 4-HIPA alone, reduction in ROS levels both in cerebral cortex and cerebellum suggesting that the neuroprotetive effect involves reduction in oxidative stress in the brain.

4-HIPA pre-treatment suppressed the ethanol-induced lipid

peroxidation in the brain which indicates it's *in vivo* antioxidant action. Increased protein carbonyl formation, wherein proteins are oxidatively modified, is one of the indicators of oxidative damage and 4-HIPA significantly prevented the formation of protein carbonyls in the rat brain (Altomare et al., 1996).

Ethanol is known to reduce the activity of the antioxidant enzymes, CAT, SOD, GPx, GST, and GR, in the brain (Shirpoora et al., 2009). Our results are consistent with earlier reports and clearly show the differences in the effect of single and multiple dose of ethanol on the brain regions. Brain shows distinct variation in the regional distribution of the antioxidant defenses which could be due to differences in metabolic activity that could be responsible for differential oxidative damage in brain regions (Lieber, 2005; Hussain et al., 1995). It is suggested that vulnerability to oxidative stress in the brain is region specific (Baek et al., 1999; Srivastava and Shivanandappa, 2005). 4-HIPA significantly prevented the ethanol-induced reduction of antioxidant enzyme activities and 4-HIPA alone markedly raised the enzyme activities in the brain. These observations suggest the potential of 4-HIPA in boosting antioxidant defenses against ethanol-induced oxidative stress. The mechanisms by which 4-HIPA enhance the antioxidant enzyme levels is not clear. It is suggested that, 4-HIPA may induce phase II enzymes and corresponding genes through antioxidant response elements. Our study suggests that, 4-HIPA could protect ethanol mediated neurotoxic effects in cerebral cortex and cerebellum possibly via antioxidative action. This study provides evidence for the neuroprotetive potential of 4-HIPA, a novel antioxidant molecule from edible plant source, against ethanol-induced oxidative stress in the brain which has implications for development of therapeutics in preventive medicine for neurodegenerative diseases.

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Conflicts of interest: Not declared.

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