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RESEARCH ARTICLE

EFFECTS OF LITHIUM AND ETHANOL ON OXIDATIVE STRESS INDUCED BY METHYLPHENIDATE AND CORTICOSTERONE IN MALE MICE BRAIN

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ABSTRACT

The present study was designed to explore the effects of lithium and ethanol in the markers of lipid peroxidation, antioxidant enzymes and reduced glutathione activities on oxidative stress induced by methylphenidate (MPD) and corticosterone (CORT) in the brain tissues. The studies were conducted on the brain of adult male mice for 21 days. The concentrations of MPD 2.0 mg/kg BW and CORT 20.0mg/Kg BW were administered via intraperitoneally. However, LiCl 50.0 mg/kg BW and EtOH 2.0 g/kg BW was dissolved in water and administered via oral gavage. We observed thiobarbituric acid reactive substances (TBARS) levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx) enzymes and reduced glutathione (GSH) activities by the spectrophotometer. Our results demonstrated that MPD and CORT treatment triggers oxidative stress in brain as revealed by an increased level of lipid peroxidation, and decreased levels of glutathione peroxidase and reduced glutathione respectively. We also noticed that the SOD and CAT activities were significantly higher in the CORT groups. Histopathological evaluation demonstrated that lithium did not show visible improvement in the MPD and CORT treatment groups. In addition, long-term ethanol treatment increased reactive oxygen species level in the brain. From these results it is possible to conclude that concomitant administration of LiCl with EtOH may enhance the toxicity in the brain tissues. Studies with larger samples are warranted to further clarify this issue.

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INTRODUCTION

Oxidative stress is a consequence of either increased generation of reactive oxygen species or impaired enzymatic or non-enzymatic defence against it. When compared to other organs, the brain is more susceptible to the generation of reactive oxygen species (ROS). It possesses the highest oxygen metabolic rate of any organ in the body as its cells utilize 20% of the oxygen consumed by the entire organism (Sokoloff, 1999), thus having the potential to generate a high quality of ROS during oxidative phosphorylation. ROS produced in excess, injure tissues through peroxidation of membrane lipids, breakage DNA strands, alteration of amino acids, and disruption of cellular metabolism. ROS have been implicated in a variety of acute and chronic neuropsychiatric conditions (Klamt et al., 2001). A series of antioxidant defence systems normally eliminates ROS production and limit tissue damage. Indeed, thiobarbituric acid reactive substances (TBARS) levels are considered as a direct index of

cell lipid peroxidation and the primary antioxidant system involves coordinated effects induced by superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Reddy et al., 1991; Wang et al., 2003). Superoxide dismutase (SOD) and catalase (CAT) are the most important antioxidant enzymes and have been widely studied in psychiatric disorders (Kapczinski et al., 2008). Similarly, GSH is among the most abundant soluble antioxidant molecule in the brain, which plays an important role in the detoxification of ROS (Dringen, 2000). GSH reacts directly with radicals in non-enzymatic reactions and *in vitro* evidences have shown that the depletion of GSH in brain cells damages mitochondria and increases lipid peroxidation (Sen, 1997). Studies have been shown that methylphenidate can influence on production of superoxide anion in some brain areas (Gomes et al., 2009) and enhance oxidative stress after chronic treatment in young rat brain (Martins et al., 2006; Gomes et al., 2008). However, chronic exposure to exogenous corticosterone induces atrophy of hippocampal neurons and decrease neurogenesis in the hippocampus (Mirescu et al., 2006). Elevation of corticosterone in hippocampal tissue increased in ROS and lipid peroxidation with depletion of intracellular antioxidants (Baitharu et al., 2012).

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Regarding pharmacological treatment, lithium has shown to exert antioxidant and neuroprotective effects by increasing tolerance to oxidative stress (Schäfer *et al.*, 2004; Yuan *et al.*, 2004). Similarly, showed lithium treatment prevents excitotoxicity by inhibition of oxidative stress (Shao *et al.*, 2005). Examining neurochemistry associated with oxidative stress is an area of interest given ethanol's demonstrated propensity to stimulate the formation of ROS, which in turn depletes antioxidant defence in the brain (Nordmann *et al.*, 1990). Thus, the purpose of the present study is to assess the effects of lithium and ethanol in the reduced glutathione (GSH) level, SOD, CAT, GPx and TBARS activities on oxidative stress induced by methylphenidate and corticosterone in the brain tissues as compared to the healthy control subjects.

MATERIALS AND METHODS

Animals

In the present study, we used adult male Swiss albino mice (*Mus musculus*) purchased from Rajah Muthiah Medical College, Annamalai University. Male Swiss albino mice (n=72) weighing from 25-30g were housed in the experiment room in groups of six per cage (size: 41×34×16 cm) at an ambient temperature of 23±2°C and relative humidity of 55±10%. Animals were maintained on a 12:12 h light (07.00-19.00) /dark (19.00-07.00) cycle with ad libitum, food pellets and water. Wood chip bedding was changed once in a week.

Ethics

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Rajah Muthiah Medical College and Hospital, Annamalai University (Reg. No. 160/1999/CPCSEA). All efforts were made to minimize the number of animals used and their suffering.

Drugs

Methylphenidate hydrochloride (MPD; Inspiral, Ipca pharmaceutical company) was dissolved in physiologic saline (0.9% NaCl), at a dose of 2.0 mg/kg BW. Corticosterone (CORT, Sigma-Aldrich, India) was first dissolved in 0.1% v/v ethanol at a dose of 20 mg/kg BW before being diluted to the mentioned concentrations in saline. This concentration of ethanol was also present in the drinking solutions of the CORT groups. The concentrations of methylphenidate and corticosterone were adjusted for each mouse to ensure a similar dose in all animals (2.0 mg/kg BW for MPD and 20 mg/Kg BW for CORT). Methylphenidate and corticosterone treatments were injected intraperitoneally. Ethanol solutions (20% v/v) were prepared from 96% ethanol dissolved in water and administered via oral gavage (dose of 2.0 g/Kg BW). Likewise LiCl 50 mg/kg BW was also dissolved in water and administered via oral gavage. All the drug administration were of equal volume (0.5 ml) and given between 12:00 h and 14:00 h. The dosage of the drugs was based on previous work (Rajeshwaran *et al.*, 2013).

Experimental groups

The mice were divided randomly into twelve groups (6 mice in each group) and treated for 21 days as follows. 1) Vehicle treated control group; 2) Vehicle + LiCl (50 mg/kg) group; 3) Vehicle + EtOH (2.0 g/kg) group; 4) Vehicle + LiCl (50 mg/kg) + EtOH (2.0 g/kg) group; 5) MPD (2.0 mg/kg) group; 6) MPD (2.0 mg/kg) + LiCl (50 mg/kg) group; 7) MPD (2.0 mg/kg) + EtOH (2.0 g/kg) group; 8) MPD (2.0 mg/kg) + LiCl (50 mg/kg) + EtOH (2.0 g/kg) group; 9) CORT (20 mg/Kg) group; 10) CORT (20 mg/Kg) + LiCl (50 mg/kg) group; 11) CORT (20 mg/Kg) + EtOH (2 g/kg) group; 12) CORT (20 mg/Kg) + LiCl (50 mg/kg) + EtOH (2 g/kg) group. On day 1 all the groups received normal saline (0.9% NaCl, i.p.) injections. Second day onwards drug treatments were started for all the groups except vehicle treated control. The groups were housed in separated cages during the study.

BIOCHEMICAL TESTS

Tissue preparation

All animals were sacrificed by decapitation 4h after final treatments. The brains were immediately removed; weighed and washed using the chilled saline solution. Tissues were minced and homogenized (10% w/v), separately, in ice cold sodium potassium phosphate buffer (0.01M, P^H 7.4) containing 1.15% KCl in potter-Elvehjem type homogenizer. The homogenate was centrifuged at 10,000xg for 20 min at 4°C, and the resultant supernatant was used for the determination of antioxidant enzyme assays, TBARS and GSH content (Yousef *et al.*, 2010).

Oxidative damage assays in brain tissue

Measurement of thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation

According the method of (Esterbauer and Cheeseman, 1990), the extent of lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formulation was measured. Brain tissue supernatant was mixed with 1ml TCA (20%), 2ml TBA (0.67%) and heated for 1h at 100°C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535nm using a blank containing all the reagents except the sample. As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction coefficient of MDA, which is $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. The results were expressed as nanomoles of malondialdehyde per milligram of protein.

Enzyme activities in brain tissue

Determination of superoxide dismutase (EC 1.15.1.1, SOD)

Superoxide dismutase was assayed according to (Misra and Fridovich 1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant and the change in extinction

coefficient was followed at 480 nm in a Spectrophotometer. Data were expressed as units (U) of enzyme/mg of tissue protein. One unit of enzyme activity was defined as the quantity of SOD required to cause a 50% inhibition of the absorbance change per min of the blank reaction (diluent rate).

Determination of catalase (EC 1.11.1.6, CAT)

The activity of CAT was assayed by the method of (Sinha 1972). Briefly, the assay mixture contained 0.5 ml of 0.2 M H₂O₂, 1 ml sodium phosphate buffer, and 0.4 ml distilled water. Subsequently, 0.1 ml tissue extract was added to initiate the reaction. Then, 2 ml dichromate-acetic acid reagent was added after 15, 30, 45, and 60 s, to arrest the reaction. To the control tube, the enzyme was added after the addition of the dichromate-acetic acid reagent. The tubes were then heated for 10 min, allowed to cool, and the green color that developed was read at 590 nm against blank containing all components except the enzyme in a spectrophotometer. The enzyme activity was expressed as units (U) of enzyme/mg of tissue protein. One unit of catalase activity is defined as the amount of catalase to degrade 1mmol of hydrogen peroxide in 1min.

Determination of glutathione peroxidase (EC 1.11.1.9, GPx)

The activity of GPx was determined by the method of (Rotruck *et al.* 1973). Briefly, the assay mixture containing 0.5 ml sodium phosphate buffer, 0.1 ml of 10 mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H₂O₂, and 0.5 ml of 1:10 tissue extract was taken and the total volume was made up to 2.0 ml with distilled water. The tubes were incubated at 37°C for 3 min and the reaction was terminated by the addition of 0.5 ml of 10% TCA. To determine the residual glutathione content, the supernatant was removed after centrifugation, and to this 4.0 ml disodium hydrogen phosphate (0.3 M) solution and 1 ml dithio-bis-nitrobenzoic acid (DTNB) reagents were added. The color that developed was read at 412 nm against a reagent blank containing only phosphate solution and DTNB reagent in a spectrophotometer. Suitable aliquots of the standard were also treated similarly. The enzyme activity was expressed as units per mg of tissue protein (1 U is the amount of enzyme that converts 1 mmol reduced glutathione (GSH) to GSSG in the presence of H₂O₂/min).

Antioxidant molecules assays in brain tissue

Determination of reduced glutathione

Reduced glutathione was estimated according to the method described by (Ellman 1959). Supernatant (1ml) was precipitated with 1ml of 4% sulfosalicylic acid and cold digested at 4°C for 1 h. The sample was centrifuged at 1200g for 15 min at 4°C. To 1ml of this supernatant, 2.7 ml of phosphate buffer (0.1M, pH 8) and 0.2 ml of 5, 5-dithiobis (2-nitrobenzoic acid) were added. The yellow color developed was read immediately at 412 nm using the Shimadzu Spectrophotometer. The results were calculated using a molar extinction coefficient of chromophore (1.36 x 10⁴ M⁻¹ cm⁻¹) and expressed as nmol/mg protein.

Histopathology of brain tissue

Following decapitation, the whole brain was dissected out from the skull and stored immediately in 10% formalin. The brain was then washed with 0.1 M PBS (pH 7.4) for 1 h, dehydrated in alcohol, and then embedded in paraffin wax. Serial coronal sections (5µm thickness) of whole brain were then obtained. The paraffin sections of the whole brain (thickness 5µm) were dewaxed and rehydrated with alcohol for hematoxylin-eosin (H&E) staining and mounted on neutral distyrene-dibutyl phthalate-xylene (DPX) medium and examined by light microscope for histopathological changes (Senthilkumar *et al.*, 2003)

Statistical analysis

All data were expressed as mean (±S.D.) for each treatment group, differences between groups means were assessed by one way analysis of variance (ANOVA) followed by post-hoc analysis using Duncan's multiple range test (DMRT). A value of P<0.05 was considered statistically significant for analysis.

RESULTS

Thiobarbituric acid reactive substances activity

As shown in Fig 1, TBARS levels were significantly increased in the brain of the vehicle+EtOH treated mice group, when compared to vehicle treated control mice. However, vehicle+LiCl+EtOH treated group significantly decreased TBARS levels as compared to vehicle+EtOH treated group (P<0.05).

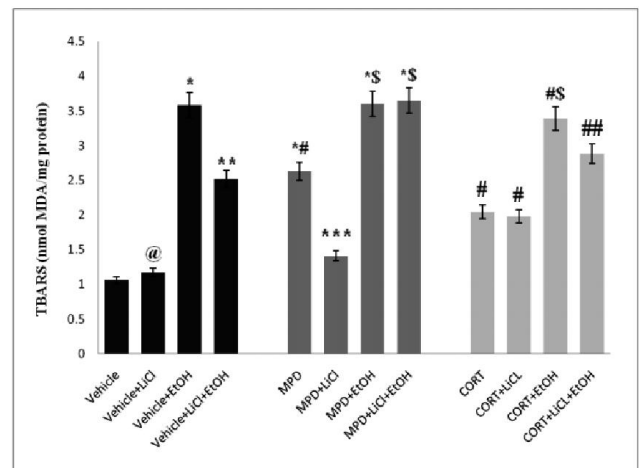


Fig 1. Changes in the levels of MDA in the brain tissue in various groups of treated mice

Values are expressed as mean (±S.D.) with n=6 in each group; one way ANOVA followed by Duncan's multiple range test (DMRT). No significant difference was found between MPD+EtOH and MPD+LiCl+EtOH treated groups; CORT+LiCl and CORT treated groups. Significant differences: @, *, **, # p<0.05 compared with the vehicle treated control groups. ** P<0.05 compared with vehicle+EtOH treated groups. *** P<0.05 compared with MPD treated groups. *\$ P<0.05 compared with MPD+LiCl treated groups. #S P<0.05 compared with CORT treated groups. ## P<0.05 compared with CORT+EtOH treated groups.

The administration of LiCl alone did not affect the TBARS activity in the vehicle control group. Administration of MPD 2mg/kg increased lipid peroxidation in all brains, elevated as indicated by increased levels of TBARS, compared to vehicle-treated control group mice. Treatments with MPD+LiCl (50mg/kg, p.o.) significantly attenuate the increase of TBARS levels in the brain, when compared to MPD alone treated group. A combination of MPD+LiCl+EtOH treatment reversed the neuroprotective effect of LiCl (50mg/kg, p.o.) significantly increased TBARS levels as compared to MPD+LiCl treated group ($p < 0.05$). However, no significant difference was observed between MPD+EtOH and MPD+LiCl+EtOH treated groups. Analysis of the data showed significant increase in malondialdehyde formation in CORT alone treated group, as compared to the vehicle treated control group. However, CORT+LiCl administration, mice did not produce any significant effect on TBARS levels. Also, CORT+EtOH treated mice exhibited a significant ($P < 0.05$) increase in TBARS activities in the brain when compared to CORT treated group. Additionally, results showed a significant ($P < 0.05$) decrease in oxidative lipid peroxidation (TBARS) levels in CORT+LiCl+EtOH treated groups, as compared to the CORT+EtOH treated mice.

Superoxide dismutase activity

As can be observed in Fig 2, Vehicle+EtOH treated group displayed increased SOD activity ($P < 0.05$) when compared with vehicle treated control group.

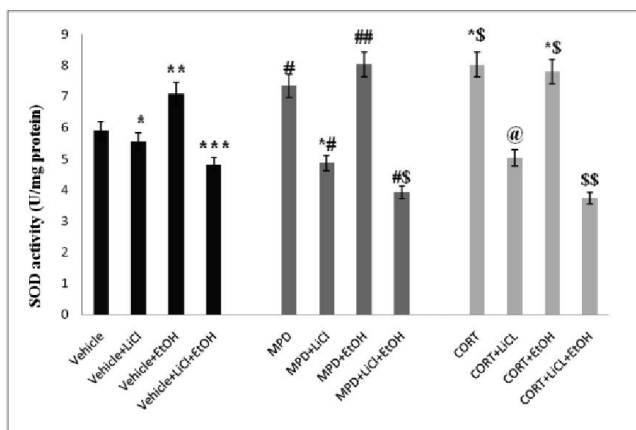


Fig 2. Changes in the brain superoxide dismutase activity in various groups of treated mice

Values are expressed as mean (\pm S.D.) with $n=6$ in each group; one way ANOVA followed by Duncan's multiple range test (DMRT). No significant difference was found between CORT+EtOH and CORT treated groups. Significant differences: **, #, #S $p < 0.05$ compared with the vehicle treated control groups. *** $p < 0.05$ compared with the vehicle+LiCl treated groups. ##, # $p < 0.05$ compared with the MPD treated groups. #S $p < 0.05$ compared with the MPD+LiCl treated groups. @ $p < 0.05$ compared with the CORT treated groups. \$\$ $p < 0.05$ compared with the CORT+EtOH treated groups.

However, combination of vehicle+LiCl+EtOH treatment mice significantly decreased SOD activity ($P < 0.05$) as relative to their vehicle+LiCl group. Analysis of the data showed that MPD treated group had the highest response to SOD activity, as compared to the value measured in the vehicle-control

group ($P < 0.05$). During treatment with LiCl (50mg/kg, p.o.) significantly decreased the SOD activity in the brain, when compared to MPD alone treated group. Although, significant increase in SOD ($P < 0.05$) was observed in the brain of MPD+EtOH treated group as compared to the MPD treated group. However, the combination of MPD+LiCl+EtOH caused a significant reduction in SOD formation in the brain compared to MPD+LiCl treated group ($P < 0.05$). The amount of SOD level was higher in CORT treated group compared to vehicle treated control group ($P < 0.05$). Fig 2 illustrates, CORT+LiCl treatment resulted in a decrease of SOD activities compared to CORT treated mice. The treatment with CORT+EtOH treated group did not alter SOD activities in the elevated structures relative to their CORT treated group. However, combination of CORT+LiCl+EtOH treatment significantly decreased SOD activities in the brain as compared to the CORT+LiCl treated mice.

Catalase activity

Fig 3 shows that treatment with LiCl (50mg/kg, p.o.) significantly decreased the CAT activity in the brain, when compared to vehicle treated control group.

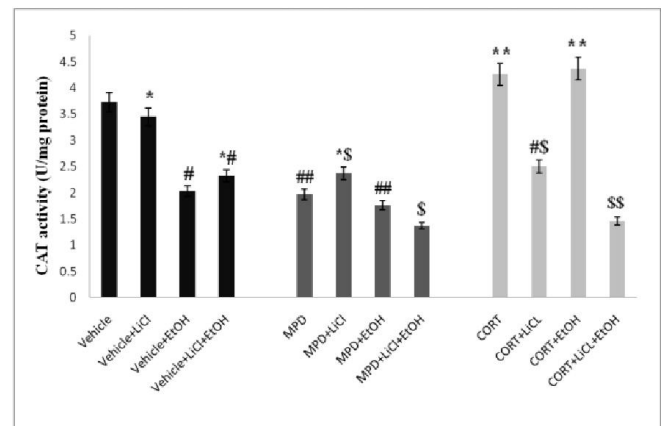


Fig 3. Changes in the brain catalase activity in various groups of treated mice

Values are expressed as mean (\pm S.D.) with $n=6$ in each group; one way ANOVA followed by Duncan's multiple range test (DMRT). No significant difference was found between MPD+EtOH and MPD treated groups; CORT+EtOH and CORT treated groups. Significant differences: *, #, ##, ** $p < 0.05$ compared with the vehicle treated control groups. # $p < 0.05$ compared with the Vehicle+EtOH treated groups. #S $p < 0.05$ compared with the MPD treated groups. ^S $p < 0.05$ compared with the MPD+LiCl treated groups. #S $p < 0.05$ compared with the CORT treated groups. \$\$ $p < 0.05$ compared with the CORT+LiCl treated groups.

Repeated treatment with ethanol produced a significant reduction in CAT levels ($P < 0.05$) in vehicle+EtOH mice as compared to vehicle treated control mice. Conversely, vehicle+LiCl+EtOH treatment significantly increased CAT levels in the brain of mice as compared to vehicle+EtOH treated group. CAT activity was significantly decreased in the brain of MPD treatment mice as compared to vehicle treated control mice. However MPD+LiCl treatment reversed the CAT activity in the brain, but the treatment with MPD+EtOH

did not produce any significant effect in CAT activity compared to MPD treated group. Also, the repeated treatment with MPD+LiCl+EtOH produced a significant decrease ($P<0.05$) in CAT activity compared to MPD+LiCl treated mice. Repeated CORT treatment significantly increased CAT activity in the mice compared with vehicle treated control group. However, CORT+LiCl treatment reduced the CAT activity in a significant manner relative to their CORT, but CORT+EtOH administration did not produce significant alteration in CAT activity relative to their CORT group. In contrast, we observed that CORT+LiCl+EtOH produced significant decrease ($P<0.05$) in CAT activity in the brain compared with CORT+LiCl group.

Glutathione peroxidase activity

As can be seen in Fig 4, GPx presented lower activity in the vehicle+EtOH treatment group as compared to vehicle treated control mice. We also found that vehicle+LiCl+EtOH treatment significantly decreased ($P<0.05$) GPx activity as compared to the vehicle+LiCl treated group. We verified also that administration of MPD (2mg/kg) significantly decreased ($P<0.05$) GPx activity in the brain, as compared to vehicle treated control group. However, MPD+LiCl treatment did not produce significant effects in GPx activity as compared to MPD alone treated group. Conversely, the MPD+LiCl+EtOH treatment group was significantly higher ($P<0.05$) than in the MPD+EtOH treatment group. CORT alone when injected at a dose of 20mg/kg significantly decreased the glutathione peroxidase level in the brain as compared to the vehicle treated control group. But CORT+LiCl administration significantly ($P<0.05$) increased the GPx level than in the CORT treated group. However, CORT+EtOH treatment further decreased the GPx levels in the brain as compared with CORT treated group. In addition, CORT+LiCl+EtOH treatment reversed the GPx activity in the brain, as compared to CORT+EtOH treated group.

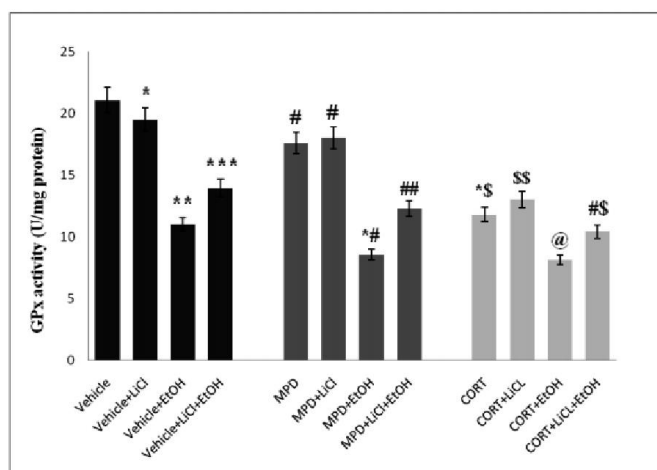


Fig 4. Changes in the brain glutathione peroxidase activity in various groups of treated mice

Values are expressed as mean (\pm S.D.) with $n=6$ in each group; one way ANOVA followed by Duncan's multiple range test (DMRT). No significant difference was found between MPD+LiCl and MPD treated groups. Significant differences: **, #, \$\$\$ $p<0.05$ compared with the vehicle treated control groups. *** $p<0.05$ compared with the

Vehicle+LiCl treated groups. ## $p<0.05$ compared with the MPD+EtOH treated groups. \$\$\$, @ $p<0.05$ compared with the CORT treated groups. \$\$\$ $p<0.05$ compared with the CORT+EtOH treated groups.

Reduced Glutathione activity

As shown in Fig 5, vehicle+LiCl treatment significantly decreased the GSH level ($P<0.05$) in the brain as compared with the vehicle treated control group. But the levels of reduced glutathione were significantly increased by the lithium chloride administration in vehicle+LiCl+EtOH treated group as compared to the value measured in the vehicle+EtOH treated group. The administration of MPD (2mg/kg) significantly decreased glutathione level in the brain, as compared to the vehicle treated control group. However, the MPD+LiCl treatment group significantly increased the GSH level ($P<0.05$) as compared to MPD alone treated group. No significant difference was observed between MPD+EtOH and MPD+LiCl+EtOH treated groups. Analysis of data showed that repeated CORT treatment significantly decreased glutathione levels as compared to the vehicle treated control group. However, CORT+LiCl restored glutathione activity as compared to CORT treated group ($P<0.05$). Unlike lithium, the administration of CORT+EtOH caused a significant reduction in glutathione formation in the brain compared to CORT alone treated group ($P<0.05$). No significant difference was observed between CORT+LiCl+EtOH and CORT treated groups.

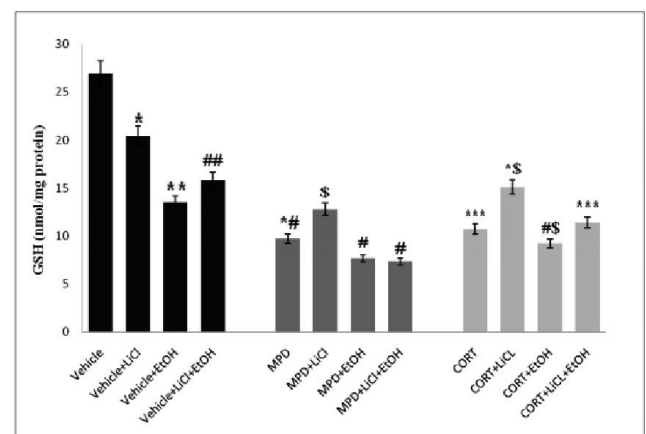


Fig 5. Changes in the reduced glutathione levels in the brain tissue in various groups of treated mice

Values are expressed as mean (\pm S.D.) with $n=6$ in each group; one way ANOVA followed by Duncan's multiple range test (DMRT). No significant difference was found between MPD+LiCl+EtOH and MPD+EtOH treated groups; CORT+LiCl+EtOH and CORT treated groups. Significant differences: *, **, *** $p<0.05$ compared with the vehicle treated control groups. ## $p<0.05$ compared with the Vehicle+EtOH treated groups. \$\$\$ $p<0.05$ compared with the MPD treated groups. \$\$\$, # $p<0.05$ compared with the CORT treated groups.

Histopathological results

Conventional light microscopic examination

Histopathological examination of brain sections revealed striking differences among the twelve treatment groups, the brain tissue of control-treated group showed normal

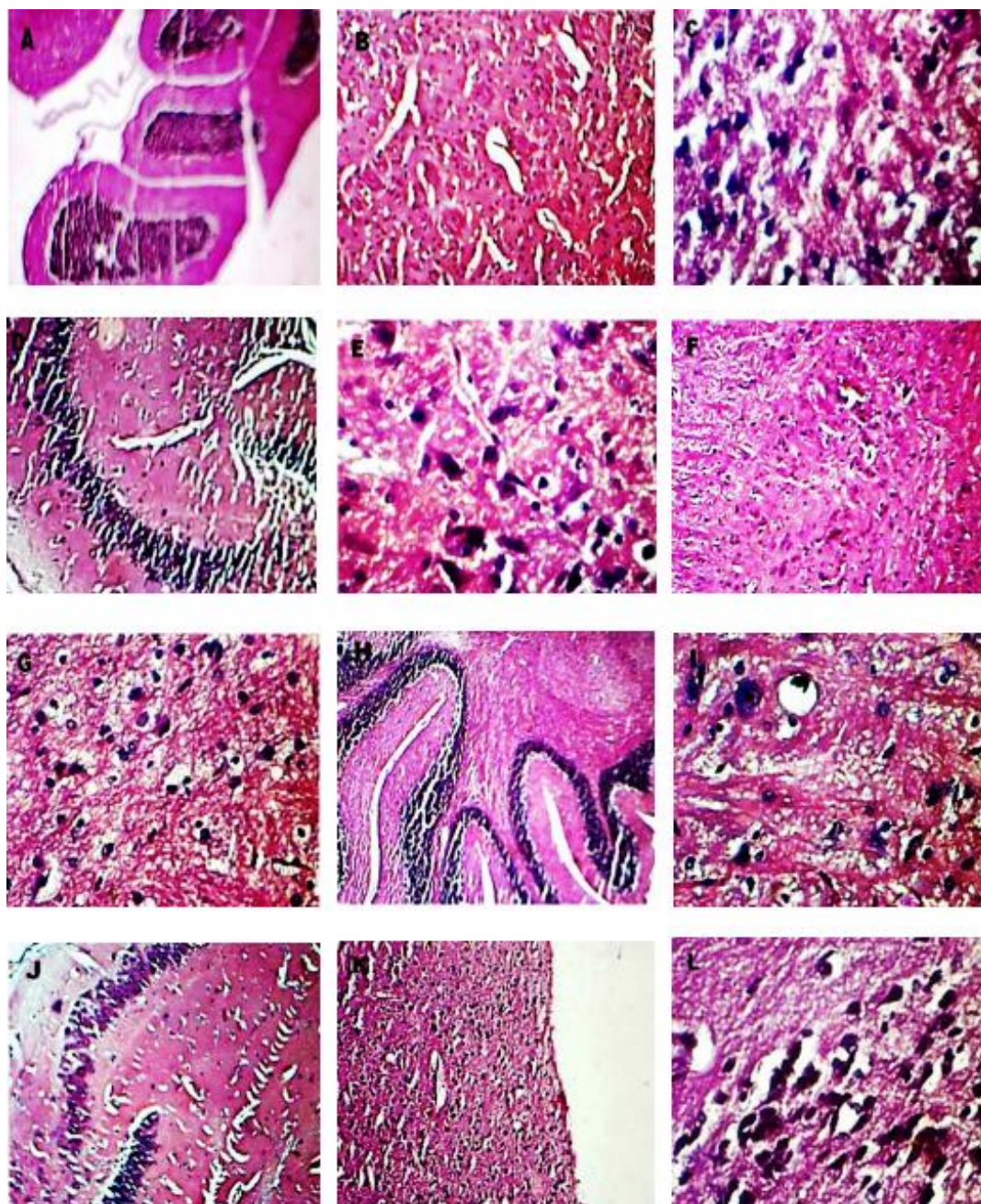


Fig 6. Representative photomicrographs of mice brain sections stained with hematoxylin and eosin

(H&E, A: Vehicle treated control shows normal pattern of cerebellum; B: Vehicle + LiCl group shows cerebral edema and congestion; C: Vehicle + EtOH group shows necrosis; D: Vehicle + LiCl + EtOH group shows cerebellar edema; E: MPD group shows pyknotic nuclei and clustering of nuclei; F: MPD + LiCl group shows cerebral edema; G: MPD + EtOH group shows cerebral necrosis; H: MPD + LiCl + EtOH group shows cerebellar edema; I: CORT group shows pyknotic nuclei and clustering of cells; J: CORT + LiCl group shows cerebellum with mild congestion; K: CORT + EtOH group shows cerebral edema with congestion and perivascular cuffing by lymphocytes; L: CORT + LiCl + EtOH group shows clustering of cells. (Magnifications were A, C-E, G-I, L: 450 X; B, F, J, K: 100 X)

appearance of the cerebellum (Fig 6A), while LiCl alone treated group showed cerebral edema and congestion (Fig 6B). The brain tissue of ethanol alone treated group showed some degree of necrosis as seen in (Fig 6C). On the other hand, LiCl+EtOH treated mice exhibited cerebellar edema (Fig 6D). There were similar morphological alteration was observed in MPD and CORT treated mice showing pyknotic nuclei and clustering of cells probably due to severe process of

degeneration (Fig 6E & 6I). Animals administered with MPD+LiCl showed edema in the cerebral region of the brain (Fig 6F). Exposure to MPD+EtOH treated group showed cerebral necrosis (Fig 6G). However, MPD+LiCl+EtOH treated animals showed cerebellar edema as seen in (Fig 6H). CORT+LiCl treated mice showed mild congestion in the cerebellum region (Fig 6J). However, CORT+EtOH treated group showed cerebral edema with congestion and

perivascular cuffing by lymphocytes (Fig 6K). Additionally, CORT+LiCl+EtOH treated mice showed clustering of cells in the brain section (Fig 6L).

DISCUSSION

We report in accordance with the results MPD and MPD+EtOH treated groups caused a significant elevation in TBARS levels and SOD activity by simultaneous inhibition in the activities of antioxidant enzymes; CAT and GPx in the brain. A decrease activity of CAT is associated with a large amount of H₂O₂ available to react with transition metals and to generate the radical hydroxyl (the most harmful radical), resulting in increased lipid peroxidation and as a consequence, neuronal damage (Matés and Sánchez-Jiménez, 1999). Our histopathological results also showed cerebral necrotic changes in the brain. Oxidative stress, which is defined as a disturbance in the balance between the production of ROS and antioxidant defence systems, may contribute to neuronal injury induced by corticosterone (Schmidt *et al.*, 2002; Zafir and Banu, 2009). Consistent with these findings, animal studies have demonstrated that SOD and CAT activity significantly increased in the CORT (Mao *et al.*, 2011) and CORT+EtOH treated groups, but GPx and GSH activities significantly decreased in the brain. The increasing of antioxidant levels was reported to be associated with reflecting a preceding cellular oxidative stress or a compensatory mechanism (Dakhale *et al.*, 2004; Kunz *et al.*, 2008). SOD and CAT are antioxidant enzymes that act together. Superoxide dismutase (SOD) is a protective enzyme that can selectively scavenge the superoxide anion radical (O₂⁻) by catalyzing its dismutation to hydrogen peroxide (H₂O₂), and CAT metabolizes the excess of H₂O₂ producing O₂+H₂O. We have also found that the level of MDA, a good indicator of oxidative stress, was significantly higher in the CORT treated group than the vehicle treated control group. Previous studies found that an active GPx system is essential for maintaining CAT activity. It was demonstrated that CAT cannot compensate for a complete loss of GPx-mediated clearance of hydrogen peroxide, although GPx can fully compensate for a loss of CAT function in astroglia-rich primary cultures (Sokolova *et al.*, 2001). These results are further evidenced through histopathological studies which show the presence of a large number of edema and perivascular cuffing by lymphocytes. However, we also observed that consistent treatment with MPD and CORT decreased GSH levels significantly in mice brain. Previous study reports that GSH level significantly decreased to 42% of the control value, suggesting that CORT may induce oxidative stress (Mao *et al.*, 2011). Glutathione is a ubiquitous tripeptide present in all cell types in millimolar concentrations. The major roles of GSH are to maintain the intracellular redox balance and to eliminate xenobiotics and ROS (Myhrstad *et al.*, 2002). Therefore, it can be assumed that decrease in GSH concentration might cause the effectiveness of GPx activity to be restricted, as evident by the intensification of lipid peroxidation (Czczot *et al.*, 2006). In the present study, a similar pattern of morphological alteration was observed in MPD and CORT treated mice, showing pyknotic nuclei and clustering of cells. The major findings in the current studies were the chronic treatment with LiCl at therapeutically relevant concentrations did not show noticeable improvements

in the MPD and CORT treatment groups. This result is consistent with current evidence that TBARS and antioxidant enzyme levels are not improved in the mice brain. The concomitant administration of LiCl with EtOH may enhance the toxicity of the brain. However, the brain of LiCl (50 mg/kg) treated animals displayed edema and congestion in the cerebral region. Our data clearly showed that ethanol administration (2g/kg) significantly increased TBARS (Sohda *et al.*, 1993) and decreased the antioxidant enzymes in the brain (D'Almeida *et al.*, 1994; Pushpakiran *et al.*, 2005). Accordingly, glutathione (GSH) levels of the brain are decreased after ethanol treatment in animals (Agar *et al.*, 1999). Based on our findings suggest that decreased GSH appears to be directly related to ethanol use. Previous data also reported that significant pathomorphological alterations in the brain were observed in ethanol treated mice. These changes can alter the properties of a cell (Senthilkumar *et al.*, 2003). We have correlated histological data with biochemical markers will probably supports the findings.

Conclusion

In conclusion, our data postulated that prolonged exposure to MPD and CORT substance evoked deleterious effects of ROS-mediated damage in the brain, finally leading to neuronal dysfunction. Studies on the brain tissues confirm previous findings that oxidative damage increases during ethanol administration. This appears to be due to both an increased generation of oxidants and increase in susceptibility to oxidative damage. Finally we suggest that the administration of LiCl with EtOH did not prevent oxidative stress in the brain. Further studies are needed to expand a better understanding of the consequences and mechanisms associated with the drugs.

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