Environment and Pollution; Vol. 4, No. 1; 2015
ISSN 1927-0909 E-ISSN 1927-0917
Published by Canadian Center of Science and Education

The Protective Effects of Vitamin E and Zinc Supplementation Against Lithium-Induced Brain Toxicity of Male Albino Rats

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Received: October 2, 2014 Accepted: October 20, 2014 Online Published: December 2, 2014

doi:10.5539/ep.v4n1p9 URL: http://dx.doi.org/10.5539/ep.v4n1p9

Abstract

Lithium (Li) therapy has widely used in the treatment of bipolar disorder. Consequently, consciousness of the side effects and pathogenesis of this metal is needed for such treatments. Recently, information on the interaction of Li with oxidative markers and organs toxicity attend the researchers over the world. In the present study we have tried to evaluate the influence of oral administration of LiCl for 4 weeks on the oxidative stress marker and histological structure of brain in male rats. Fifty adult male albino rats weighing 135±15 gm was categorized into 5 groups (10 rats each). Group I worked as negative control, group II administrated with LiCl (0.20 mg/kg bw) in drinking water, group III, IV and V were administrated with Zn (10 mg/kg bw), VE (100 mg/kg bw) and their combination twice a week besides the daily administration of LiCl for 4 weeks, respectively. Rats after anesthesia with ether killed for collocation of brain for histopathological and biochemical analysis. Data obtained showed a significant increase in LPO, NO, GSH and Li content and the activities of SOD, CAT and AChE with demylination of the nerve fibers and degeneration of neurons in brain of LiCl treated rats. Co-treatment of rats with Zn or VE results in a significant decrease in LPO, NO, GSH content in the activities of SOD, CAT and AChE with less or normal structure of the brain. However, co-treatment with combination of Zn and VE caused a significant increase in SOD, CAT and AChE activities with normal histological structure.In conclusion, the data from the present study show that Zn and VE and their interaction are effective in protection against Li-induced brain toxicity in rat with priority for the combination.

Keywords: Lithium, vitamin E, brain, rats, oxidative stress, acetylcholinestrase (AChE)

1. Introduction

Lithium is a toxic alkaline metal that occur in the environment as industrial pollution and a therapeutic use. Moreover, it accumulated in algae, marine animals, vegetables, rock and tobacoo leaves (Schrauzer, 2002). Lithium is not essential element it used in therapeutic psychiatric diseases, in particular bipolar disorders like depression (Schou, 2001; Aral & Vecchio-Sadus, 2008). For therapeutic use, the dose of Li carbonate usually varies from 7-25 mg/kg per day (Allaguri *et al*., 2006). For it is slowly movement from extracellular compartment to intracellular space it may require 6-10-days to reach steady blood concentration that desired for therapeutic responses (Groleau, 1994).

Clinical trial suggests that Li stops the progression of amyotrophic lateral sclerosis and inhibits a number of kinases and phosphatases that in turn affects many systems including inflammation, metabolism, receptor sensitivity, and adenyl cyclase (Young, 2009). However, prolonged Li therapy causes neuromuscular disorders (Sansone, 1985) and neuronal apoptosis in rat brain that effect on acetylcholine esterase (ACE) (Martins *et al*., 2008). ACE is enzymes that terminate the neurotransmission at cholinergic synapses by splitting the neurotransmitter ACh to chloline and acetate (Tripathi & Srivastava, 2008). Acetylcholine plays an important role in sending signals from one neuron to the next when it is released from vesicles in the axon terminus, across the synapse, and onto receptors in the dendrites of the next neuron (Habila *et al*., 2012).

Oxidative stress is one of the important mechanisms of toxic effects of Li (Oktem *et al*., 2005). In fact, part of the adverse effects of Li seems to result from excessive formation of ROS andinhibition of antioxidant enzyme activities (Oktem *et al*., 2005; Allagui *et al*., 2007). Vitamin E (VE) as antioxidant is the primary membrane

bound lipid-soluble, chain-breaking antioxidant that protects cell membranes against oxidative stress (Soylu *et al*., 2006). VE prevents formaldehyde-induced tissue damage in rats (Gulec *et al*., 2006) and endotoxin-induced oxidative stress in rat tissues (Kheir-Eldin *et al*., 2001).

The role of zinc (Zn) is very important in antioxidant defense mechanism as well as in regeneration of damaged cells (Nuzhat & Mahboob, 2012). It is an essential trace mineral with important anti-inflammatory function (An *et al*., 2005), antiapoptotic (Powell *et al*., 2000), and antioxidant (Holland *et al*., 1995). The role of Zn in antioxidant defense mechanism includes the protection due to redox active transition metals such as copper and iron, and the protection of -SH groups of protein from oxidative damage. The chronic antioxidant effects of Zn result in the induction of metellothionein synthesis that act as scavengers of toxic metals (Chvapil *et al*., 1976), protection against VE depletion(Parsad *et al*., 1988), induction of cell-proliferation and inhibition of NADPH oxidases (Oteiza *et al*., 2000).

According to the aforementioned findings, the present work was aimed to study the protective effect of Zn, VE or combination of Zn and VE against Li induced brain toxicity in rats through measurement of oxidative stress markers and observation of histopathological changes.

2. Material and Methods

Chemicals: Lithium chloride (LiCl), zinc sulphate, VE (α-tochopherol), N, N diphenyl-p-phenylenediamine, superoxide dismutase, epinephrine, thiobarbituric acid (TBA), naphthylethylenediaminedihydrochloride, 5,5 dithiobis (2-nitrobenzoic acid (DTNB), triton-X100, sulfanilamide and acetylthiocholine (ATC), were obtained from Sigma Chemical Co. (St. Louis, MO, USA. All other chemicals and reagents were of the highest purity commercially available**.**

Animals: Fifty adult male albino rats (135±15 gm) were purchased from the Animal House of the Faculty of Medicine, Assuit University, Assuit, Egypt. Rats were housed in cages and were kept in a room temperature ($30^{\pm}3^{o}$C) with normal 12 h light/12 h dark cycle. They were allowed to acclimatize for one week before the experiments.

Animal groups and treatment:

Rats were divided into 5 groups of 10 rats each.

Group I: served as a control group.

Group II: received a dose of LiCl (0.20 mg/Kg bw) daily for 4 weeks in drinking water.

Group III: received a dose of LiCl (0.20 mg/Kg bw) and zinc sulphate (10 mg/kg bw) daily for 4 weeks in drinking water.

Group IV: received a dose of LiCl (0.20 mg/Kg bw) daily with VE (100 mg/kg bw) injected intraperitoneally twice a week for 4 weeks.

Group V: received a dose of LiCl (0.20 mg/Kg bw) with zinc sulphate (10 mg/kg bw) daily for 4 weeks, in drinking water with VE (100 mg/kg bw) injected intraperitoneally twice a week for 4 weeks.

Collection and preparation brain cytosol:

Animals of the different groups were killed after anesthesia with ether. The brain quickly removed and washed in (0.1 M) phosphate buffer (pH 7.4) and then stored at -20 °C for biochemical studies. Pieces of brain were fixed immediately in 10% neutral buffered formalin for histological studies. All experiments followed protocols approved by the Institutional Animal Care and Life Committee, Assiut University. 10% homogenate of brain was prepared by homogenization of 0.25 gm of tissue in 2.5 ml (0.1 M) phosphate buffer (pH 7.4) using homogenizer (IKA Yellow line DI 18 Disperser, Germany). The homogenates were centrifuged at 6,000 rpm for 1 hour at 4 °C and the supernatant cytosols were kept frozen at -20 °C for the subsequent biochemical assays.

Biochemical measurements:

Total protein concentration was determined by the method of Lowry *et al*. (1951)**.** LPO products as TBARS were determined according to the method of Ohkawa *et al.* (1979)**.** Nitric oxide (NO) was measured as nitrite concentration colorimetrically using the method of Ding *et al.* (1988). GSH was determined using the method of Beutler *et al. (*1963).Activity of SOD was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of Misra and Fridovich (1972). Activity of CAT was determined by the procedure of Luck (1963), basing on its ability to decompose hydrogen peroxide. Activity of AChE was estimated by method of Ellman *et al*. (1961).

Lithium, zinc and copperconcentrations in the samples were determined by ICP-MS (Thermo Fisher Scientific

(Bremen) GmbH) in central lab of Faculty of Science in New Valley. Standard solutions of multi-elements were prepared from commercial stock standard solutions at concentrations of 100 mg/L double deionised water. Working standard solutions were prepared by dilution of stock standard solution with the addition of hydrochloric acid, so that the acid concentration in working standard solutions matched the acid concentration in digested solutions.

For the histological part fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin (H & E) according to Drury and Wallington (1980).

Statistical analysis: Data were subjected to mean ± STD Err. The differences between means were done by using The Tukey-HSD test. Range test was used as a post-hoc test to compare between means at $p<0.05$. These analyses were carried out using Statistical Package for Social Sciences (SPSS) for windows, version 16.

3. Results

Figs (1a, b) show the level of LPO as TBARS and NO as nitrite in brain tissue. As compared to control rats, TBARS and nitrite level were significantly elevated in brain of Li group. In the same figure, data revealed that Zn, VE and the combination recovered TBARS and nitrite level significantly in brain tissue in comparison with Li group. **Fig (1 c, d)** showed the level of SOD and CAT activities in the brain tissue. Both enzyme activities were elevated in the brain of Li treated rats. Co-treatment of rats with Zn or VE alone caused reduction in these activities in comparison with Li treated rats, however the combination of Zn and VE not shown any effects. **Fig (1e)** show the level of GSH in brain tissue, as compared to control rats, GSH level elevated in brain of Li and Li, Zn treated groups. Also, the same fig, revealed that the treatment with VE and the combination have no significant effect in brain tissue. **Fig (1f)** showed the level of AChE in brain tissue, as compared to control rats, AChE showed no significant change in Li, Li, Zn and Li, VE, but it was highly significant increase in Li, Zn and VE treated groups.

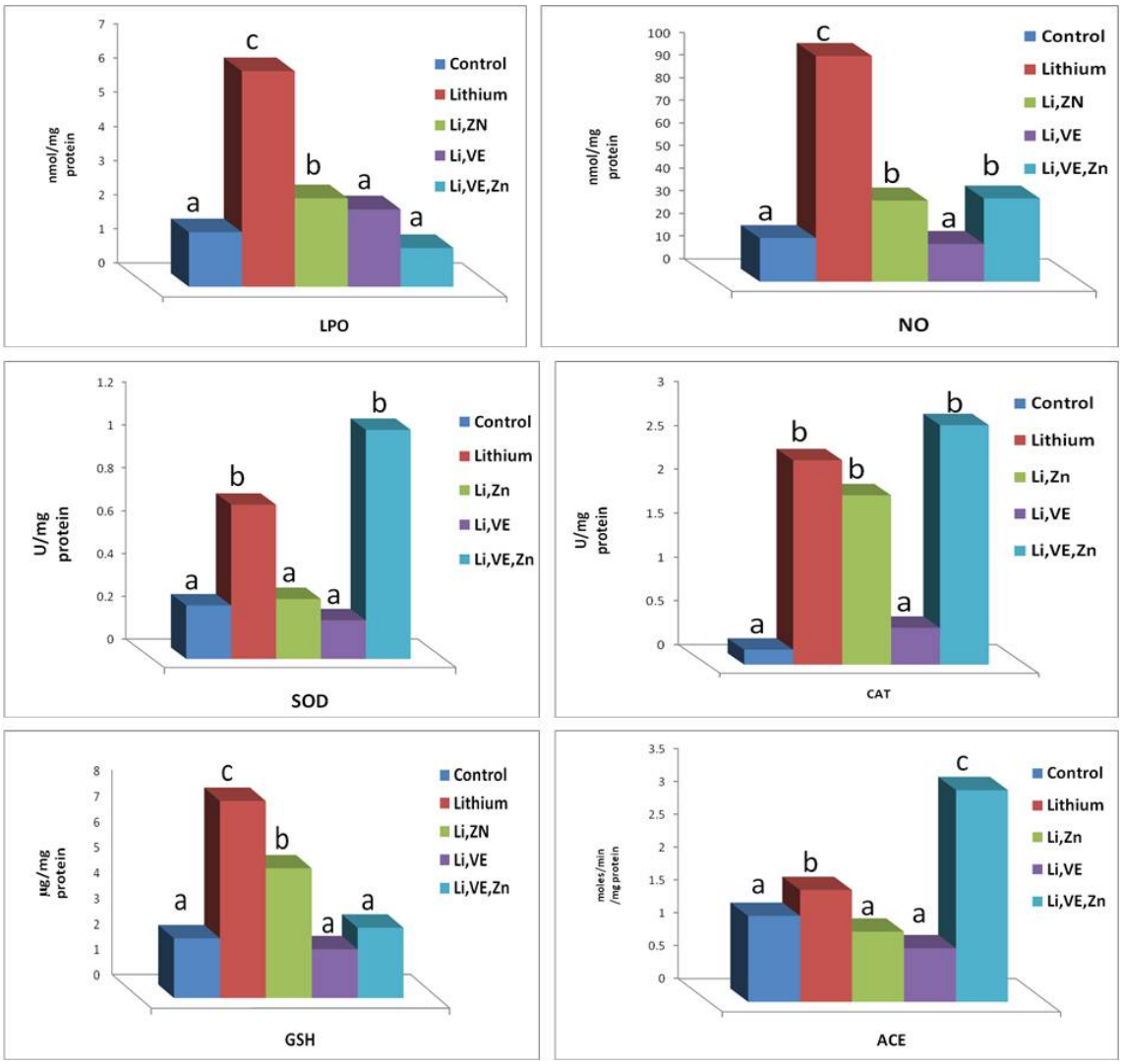

Figure 1. ShowLPO level (A), NO level (B), SOD activity (C), CAT activity (D), GSH level (E) and ACE level (F). Results presented as mean ± SE, different letter means significant different at $p<0.05$ between different group, where n=6

Analysis of some trace elements

The values of Li, Zn and Cu as ppm in brain tissue in normal and different treated rats are presented in Fig (2). As compared to control rats, Li level was significantly increased in Li treated group. Co-treatment with combination of Zn & VE caused decrease in Li level in comparison to Li treated group and gives better result than Zn or VE alone.Level of Zn was significantly increased in Zn and Zn and VE treated groups, but still normal in Li and VE treated group. Cu level was significantly increased in interaction of Li, Zn and VE treated group, but still normal in Li, VE and Zn treated group

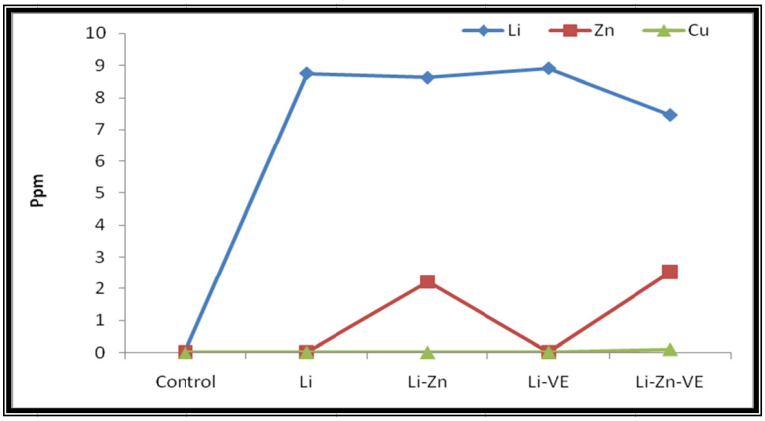

Figure 2. Show the some trace elements analysis in brain of normal and different treated rats

Fig. 3: Photomicrographs of brain sections: **A** control rat showing normal histological appearance of neurons in the white matter, **B** control rat showing normal histological appearance of neurons in the white matter, **C** brain or rat exposed to Li showing marked demylination in the nerve fibers **(arrows), D** rat exposed to Li showing degeneration of the neurons **(arrow), E** rat exposed to Li and treated with Zn showing perivascular edema **(arrow)** and perineural edema **(quadrate), F** rat exposed to Li and treated with Zn showing mild degeneration changes in the neuronal cell body, **G** rat exposed to Li andtreated with VE showing perivascular edema **(arrow),** perineural edema **(quadrate)** and mild degeneration changes in the neurons**, H** rat exposed to Li andco-treated with VE showing mild degeneration changes in the neurons, **I** rat exposed to Liand co-treated with combination of Zn and VE showing perivascular edema **(arrow) and J** rat exposed to Li and co-treated with combination of Zn and VE showing perivascular edema **(arrow) (H&E) (400X)**.

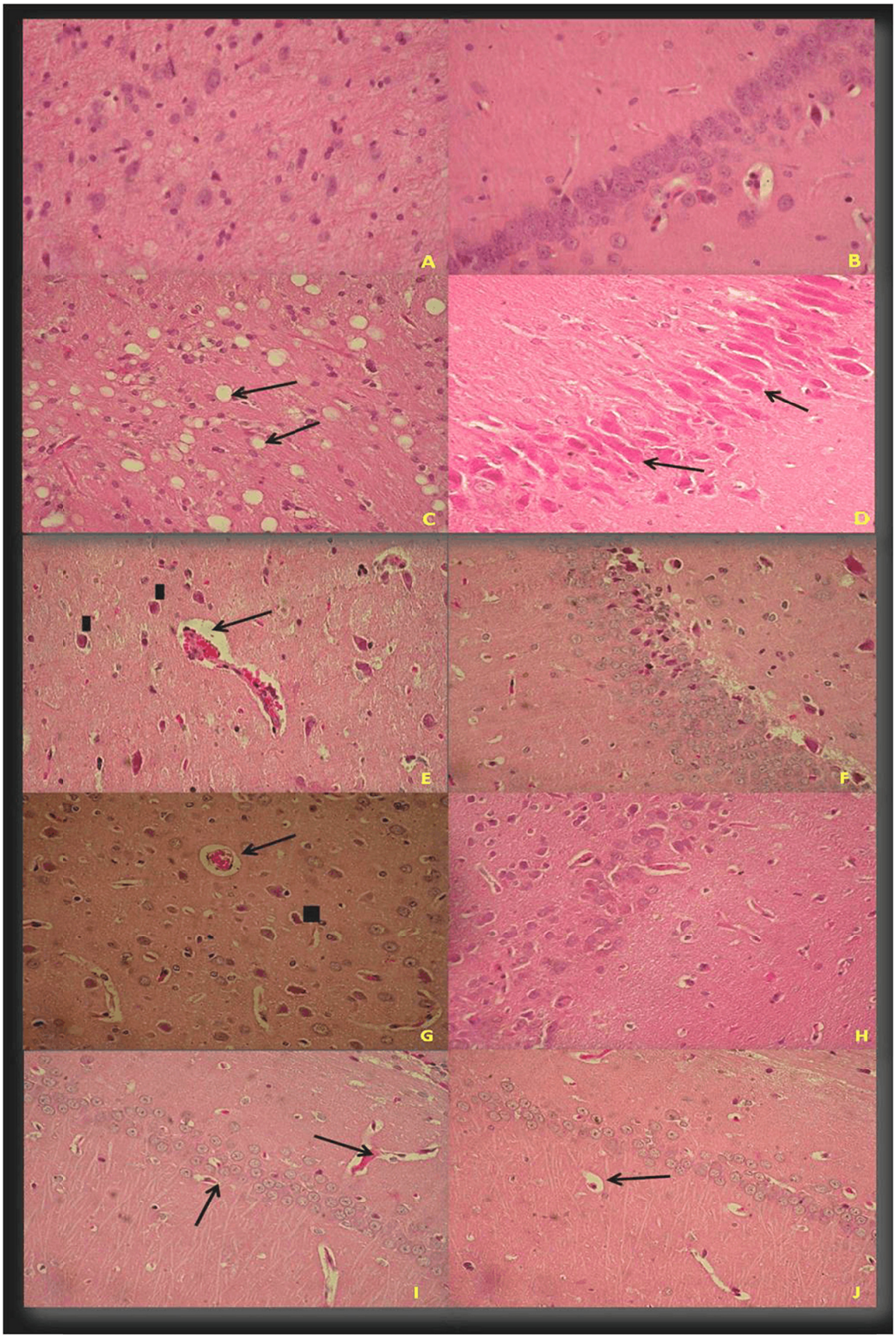

Figure 3.

4. Discussion

The present study showed a significant increase in LPO level in brain tissue of Li treated rats. Similar result was obtained by Buhalla *et al*. (2007) who found a significant increase in LPO level in brain tissue after Li treatment. Peroxidation of polyunsaturated fatty acids leads to degradation of phospholipids and cellular deterioration (Abou-Donia, 1981). The present result showed that Zn, VE and their interaction countered the LPO produced by Li. Similar result was observed by Buhalla *et al*. (2007) in the brain of rats treated with Li carbonate added to diet for two months. In this aspect, Chan *et al.* (1998) have demonstrated that Zn is involved in destruction of free radicals through Zn-metallothioneins which may serve as an efficient antagonist in inhibiting LPO in the brain tissue. Moreover, Zn causes inhibition of endogenous LPO to stabilize biomembranes (Dhawan & Goel, 1995).

NO is a messenger molecule with different functions in the body including long term potentiation, learning and memory (Floyd and Hensley, 2002). Inducible nitric oxide synthatase (iNOS) produces high amount of NO as a major contributor in the toxicity and disease pathway (Gouda *et al.,* 2010).In the present study, Li treatment elevated NO levels and caused degenerative changes in brain. Similarly, Harvey *et al*. (1994)found a significant increase in NO level in brain tissue after Li treatment. Also, strong positive immunoreactions for iNOS in cerebellar cortex with degenerated neurons and dilated congested capillaries of Li treated rats were observed by Bashandy (2013). Moreover, a significant increase in expression of iNOS in rat cerebellum under stress was detected by Gouda *et al.* (2010).In the current study, NO levels of rats treated with Zn, VE or their combination significantly decreased with improvement in the histological structure as compared with Li treated rats. In comparison, Bashandy (2013) found that neurons in the cerebellar medulla retained their normal appearance but still some degenerated neurons and slightly congested capillary in brain of rats treated Li and selenium. This could be attributed to the properties of Zn like selenium has antioxidant properties which provide protection from ROS induce cell damage (Chen and Berry, 2003).The protective effect Zn and VE may be due to it is role in regulation of redox status under physiological conditions (Reddy *et al.,* 2009) and reduction of LPO and NO (Savaskan *et al.,* 2003).

The present data showed that Li–induced the activities of SOD and CAT in the brain tissues of rats treated with Li. This altered of the two antioxidant balance in the brain by administration of Li may perturb the brain cell normal functioning, because balance between SOD and CAT are relevant for cell function (Savolainen, 1978). Co-treatment of rats with Zn along Li results in decline in the activities of SOD and CAT in comparison with Li treated alone. Several studies on the antioxidant property of Zn were reported (Sidhu, *et al.,* 2005 and 2006; Buhalla *et al*., 2007),due toZn plays an important role as structural element of non-mitochondrial form of SOD (Choi, 1993). GSH is the most abundant low molecular weight thiol involved in antioxidant defense in animal cells. In the present study, the level of GSH in the brain tissue was increased by Li treatment. Similar results was obtained by Nanda *et al.* (1996) and Cui *et al*. (2007) who found a significant increase in GSH level in the brain of Li treated rat. However, Joshi *et al.* (2013) found a significant decreased in GSH level in different organs of rat treated with Li carbonate for 21 days. The increased levels of GSH in Li-treated rats may be due to increased detoxification capacity of the brain, most of the GSH in the brain is localized in glial cells rather than neurons, suggesting that Li affects the glial cells **(**Meister, 1984). Moreover, in brain, astrocytes play a central role in the metabolism of GSH (Takuma *et al*., 2004). Co-treatment of rats with Zn or VE results in decline the level of GSH in the brain. This effect of Zn or VE could be returned to the antioxidative properties of Zn and VE as evident by decreasing the LPO levels and SOD and CAT activities in the present study.

Activity of AChE in brain homogenate showed a significant increase in comparison with control group and co-treatment of rats with Zn, VE or combination of Zn and VE elevated the reduction in activity of AChE. Jope (1979) found that LiCl treatment stimulates cholinergic activity in certain brain regions which may play a role in the therapeutic effect of LiCl in neuropsychiatric disorders. Also, Zn may act as a neuromodulator of excitatory or inhibitory processes (Vera-Gil *et al.*, 2003). Short-term orally supplementation of *Sonchusasper*, is traditionally used as a folk medicine to treat mental disorders, elevated brain antioxidant enzymes and inhibited ACE activity (Kumar *et al*., 1994).

Generally, the levels of elements reflected dietary concentrations of these elements (Reinstein *et al*., 1984). In the present study, concentration of Zn and Cu was increased in brain of rats treated with Zn or combination of Zn and VE. Autopsy studies of adults revealed that the cerebellum retains more Li than other organs, followed by the cerebrum and the kidneys (Schrauzer, 2002). Onosaka and Cherian (1982) returned this increase due in part to increase binding of Cu by metallothionein, which increases when the concentration of Zn increases. Also, in the present study, Li level was not detected in tissues of normal rats, however, it increased in tissues of rats treated with Li alone and in the rats co-treated with Zn, VE or combination of Zn and VE.

In conclusion, the data from the present study showed that Zn and VE and their interaction are effective in protection against Li- induced brain toxicity in rat. The effect of Zn may be attributed to formation Zn-metallothionein. In addition, Zn metallothionein and VE are free radical scavenger.

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