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Original article

Ameliorative effect of lithium chloride against D-galactose induced behavioral and memory impairment, oxidative stress and alteration in serotonin function in rats

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ABSTRACT

Background: Aging is a phenomenon that all living organisms surely face. D-galactose (D-gal) has been used to develop an aging model of brain. Lithium (Li) has been proposed to have neuroprotective properties in relation to several neurological disorders. The goal of the current studyis to evaluate the effect of Lithium Chloride (LiCl) on D-gal induced neurological disorders and oxidative stress.
Methods: Rats were treated with D-gal at a dose of 300 mg/ml/kg and various doses of LiCl (20, 40 and 80 mg/ml/kg) for 14 days. After that behavioral analysis (Elevated plus maze (EPM); Light dark box test (LDT); Morris water maze (MWM); Forced swim test (FST)) were performed. Animals were decapitated after behavioral tests and brain samples were collected for biochemical (malondialdehyde (MDA); superoxide dismutase (SOD); catalase (CAT); glutathione peroxidase (GPx); acetylcholiesterase (AChE)) and neurochemical analysis (5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA)).
Results: The results showed that administration of LiCl at all doses ameliorates D-gal induced, decreased time spent in the open arm and light box in EPM and LDT respectively, increased immobility in FST, increased latency escape in MWM, increased MDA levels, decreased antioxidant enzyme, increased AChE activity and decreased 5-HT metabolism.
Conclusions: In conclusion, the present study indicated that D-gal induced anxiety/depression like symptoms and memory impairment were ameliorated by LiCl (at all doses) possibly *via* its antioxidant effects and normalizing 5-HT function.

Introduction

Aging is an extremely complex and slow biological phenomenon leading to progressive, physiological dysfunction [1]. The result of age-related physiological disorders is a lot of disarrays such as homeostatic imbalance [2] and a range of pathological conditions such as memory impairment, anxiety and depression-like behavior etc. [3]. Extensive studies indicated that oxidative stress occurred by an inequity between free radical production and antioxidant defence system in the aging progression [4–6]. Furthermore, for long life span it is necessary to reduce oxidative stress and maintain homeostasis [7,8]. Thus, the study of age dependent pathophysiology due to impaired redox status is a great challenge for medical gerontology. Therefore, reductions in oxidative stress is perhaps a valuable curative approach to avert aging progression and associated ailments.

D-galactose (D-gal) is an aldohexose (monosaccharide) that is naturally found in the body [9]. It is also present in many foods such as dairy food, non-dairy foods and vegetables [10]. It is a reducing sugar, which accelerates aging by altering antioxidant defence system and deteriorates the cellular and molecular functions [11]. D-gal may also alter the configuration of protein and peptide and make combinations with their amine groups that cause an increase of advanced glycation end (AGE) product in absence of enzymatic glycation [12]. Oxidation of D-gal causes free radical generations which inhibit cellular defence mechanism and elevate lipid peroxidation (LPO) which may cause cellular damage and dysfunctioning of the central nervous system [3]. Long-term treatment with D-gal mimics natural aging consequence in various tissues of preclinical animal model [13]. It is demonstrated that D-gal intoxication cause behavioral and neurochemical alterations [3,14]. Pathological and physiological symptoms in D-gal

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intoxicated rats were alike to those evaluated in normal aged rats [15]. Abnormal production of free radicals by prolonged treatment with D-gal could be liable for neurotoxicity which may lead to impairment of cognitive function, alteration in the levels of biogenic amines [3] and other behavioral alterations [14]. Previously it has been reported that D-gal induced anxiety-like behavior was attenuated by metformin [16], atorvastatin [17] and quercetin [18] while depressive symptoms were overturned by n-3 polyunsaturated fatty acids [19] and metformin [16].

Lithium (Li) salt was approved and considered as an effective therapeutic agent in various depressive disorders [20] and commonly recognized as mood stabilizing agent [21]. It is a probable substitute and a novel remedy for various neurodegenerative disorders [22–24]. The neuroprotective role of Li was due to various mechanisms of neuronal homeostasis in preclinical and clinical studies, the mechanisms include activation of neurotrophic responses [25], modulation of oxidative stress [26], inflammatory cascades [27], up-regulation of mitochondrial function [28], and other specific biological effects implicated in the pathogenesis of neurodegenerative diseases. The effect of Li on serotonin metabolism is well documented [29,30]. It is reported that Li increases levels of tryptophan, serotonin and its metabolites [31]. It also increases serotonin (5-HT) release without affecting 5-HT uptake in primary serotonergic raphe neurons [32].

Apart from antidepressant effect of Li in previous studies [20], antioxidative effect of Li is also well documented [33–35]. Here, we hypothesized that as a potential antioxidant, *intraperitoneal (ip)* administration of LiCl could be ameliorating D-gal-induced behavioral deficits and cognitive impairment possibly *via* reduction of oxidative stress and normalization of serotonin metabolism in adult rats. Various doses of LiCl (20, 40 and 80 mg/ml/kg) were used in the present study to find out effective dose for prevention of D-gal-induced neurological diseases in an aging rat model.

Materials and methods

Animals

The study was conducted on 40 adult male rats weighing 180–220 g. Animals were set aside at 26 ± 1 °C on 12-h day/night cycle with free availability to food and water. All experiments were carried out in a balanced design to avoid the influence of order and time. All experiments were approved by Departmental Bioethical Committee (D/1891/2018/Biochem; Dated 10/04/2018).

Chemicals

D-gal, LiCl, Acetylthiocholine, Sodium azide, EDTA, Thiobarbituric acid (TBA), H_2O_2 stock (35%) solution, Nitrobluetetrazolium (NBT), Trichloroacetic acid (TCA) and Dithio-bisnitrobenzoic acid (DTNB) and all other chemicals were purchased from Sigma Chemicals Co. (St. Louis, USA).

Experimental protocol

Randomly male Sprague-Dawley rats were alienated into eight groups (n = 5/group); (I) Control (II) LiCl; 20 mg/ml/kg (III) LiCl; 40 mg/ml/kg (IV) LiCl; 80 mg/ml/kg (V) D-gal (VI) D-gal + LiCl (20 mg/m/kgl) (VII) D-gal + LiCl (40 mg/ml/kg) (VIII) D-gal + LiCl (80 mg/ml/kg). Control group received 0.9% saline (1 ml/kg), while D-gal was injected at a dose of 300 mg/ml/kg. All drugs dissolved in saline solution, were administered *ip* once daily for 14 days. After that behavioral tests were conducted that include; light dark activity test (for anxiety), elevated plus maze (for anxiety), Morris water maze (for long term and short term memory), forced swim test (for depression). Rats were sacrificed after the behavioral test to collect brain samples and then immediately stored at -40 °C for biochemical and neurochemical assays (Fig. 1).

Behavioral assessment

To evaluate anxiety profile light dark activity and elevated plus maze test were performed as described previously [36,37]. Time spent in a light box and open arm were recorded to examine anxiety-like behavior. Forced swim test which is pharmacologically recognized to evaluate the depression-like system was conducted as reported earlier [36,37], immobility time was recorded to assess the depression like symptoms. Morris water maze test which is recognized to evaluate spatial memory was performed by the earlier reported method [37]. The test is comprised of three sessions acquisition; short term (conducted after 4-h of acquisition) and long term (performed after 24-h of acquisition) memory. Time spent to reach the platform was recorded to assess the learning and memory.

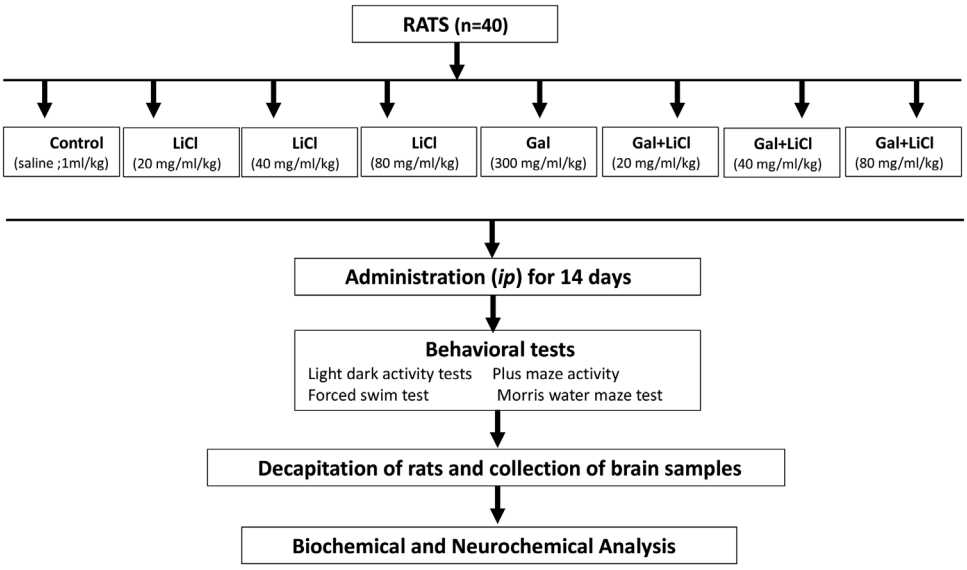

Fig. 1. Schematic representation of experimental protocol.

Biochemical analysis

The whole brain was washed with 0.9% saline solution and weighted. A 10% (w/v) brain homogenate was prepared with 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000×*g* for 10 min at 4°C. The supernatant was used to calculate the quantity of oxidative stress marker, malondialdehyde (MDA) [38] and activity of antioxidant enzyme include superoxide dismutase (SOD) [37], catalase (CAT) [39] and glutathione peroxidase (GPx) [40]. The method of Ellman et al. [41] was used to find out the activity of acetylcholinesterase (AChE) in the brain of rat. Acetylthiocholine was used as a substrate to estimate the activity of AChE.

Neurochemical analysis

For estimation of brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels, the brain was homogenized in an extraction medium using electrical homogenizer (Polytron; Kinematica). The neurochemical analysis was done to assess concentrations of 5-HT, and their metabolites 5-HIAA in brain as described by Samad and Haleem [42].

Statistical analysis

The results are presented as mean ± SD for five animals in each group. The statistically significant differences were evaluated by Tukey's test following two-way ANOVA using SPSS version 20. Value of $p < 0.05$ was considered as a significant difference

Results

Effect of LiCl on D-gal induced anxiety-like symptoms

Fig. 2 shows the effects of LiCl on anxiety profile in vehicle and D-gal treated rats observed in EPM and LDA. Data for time spent in open arm (1 A, EPM) analyzed by two-way ANOVA revealed that the significant effect of D-gal [$F_{1,32} = 45.93$, $p < 0.01$], LiCl [$F_{3,32} = 214.65$, $p < 0.01$], and interaction of D-gal x LiCl [$F_{3,32} = 6.82$, $p < 0.01$]. Tukey's test showed that D-gal significantly reduced the time spent in open arm than control animals. The time spent in open arm significantly increased in LiCl treated (at all doses) saline and D-gal treated rats. The increase of the time spent was smaller at 20 mg/ml/kg dose of LiCl in D-gal than saline treated animals

Data for time spent in light box (1B, LDA) analyzed by Two-way ANOVA showed significant effect of D-gal [$F_{1,32} = 42.37$, $p < 0.01$], LiCl [$F_{3,32} = 137.29$, $p < 0.01$], and D-gal x LiCl [$F_{3,32} = 6.64$, $p < 0.01$]. Tukey's test showed that D-gal significantly reduced the time spent in light box than control animals. The time spent in light box significantly increased in LiCl (at all doses) and LiCl+ D-gal treated rats.

Effect of LiCl on D-gal induced depression-like behavior

Effect of LiCl administration on immobility in FST following treatment with D-gal is shown in Fig. 3. Two-way ANOVA showed significant effect of D-gal [$F_{1,32} = 208.72$, $p < 0.01$], LiCl [$F_{3,32} = 196.35$, $p < 0.01$] and D-gal x LiCl interaction [$F_{3,32} = 55.58$, $p < 0.01$] in FST. Tukey's test showed that D-gal significantly increased immobility time in FST than control animals. Immobility time was significantly reduced at all doses of saline+LiCl and D-gal+LiCl treated rats. The decreases of immobility time were smaller at low and high doses of LiCl+D-gal than saline+LiCl injected rats.

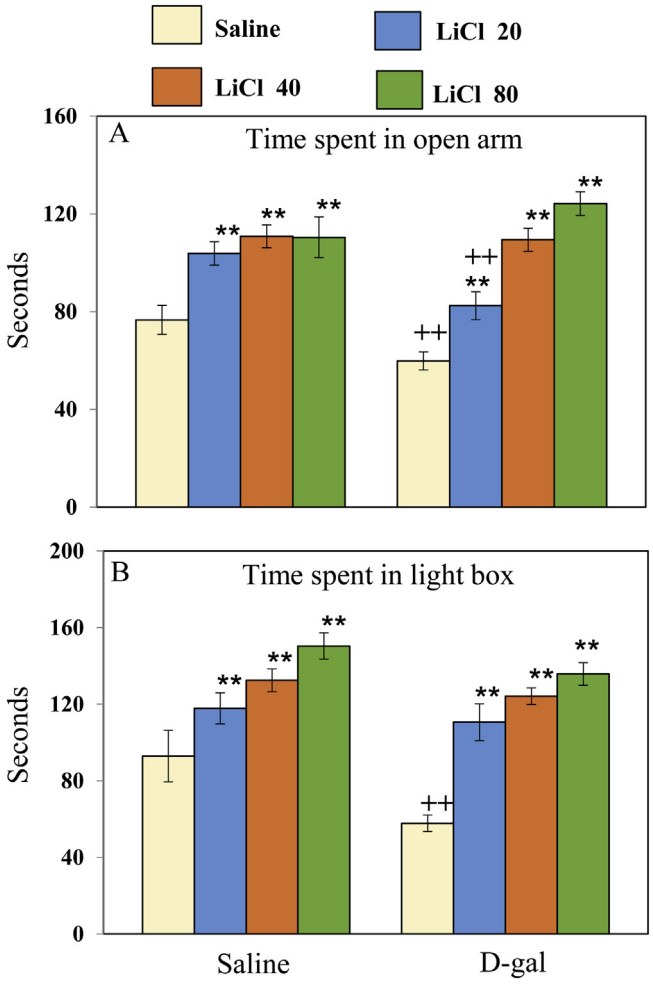

Fig. 2. Time spent in open arm (A) and light box (B) in elevated plus maze and light dark activity test respectively for the saline + saline, saline + LiCl, D-gal + saline and D-gal + LiCl treated rats. Values are mean ± SD (n = 5). Statistical analysis was done by Tukey's test following two-way ANOVA. Statistical difference is represented as $^{**}p < 0.01$ *versus* respective control and $^{++}p < 0.05$ *versus* saline + saline and saline + LiCl treated animals.

Effect of LiCl on D-gal induced impaired memory function

MWM was conducted to evaluate cognitive function in saline + LiCl and D-gal + LiCl treated rats (Fig. 4). Latency escape in MWM activity is conducted immediately after training (acquisition, Fig. 4A), 4-h (short term memory, Fig. 4B) and 24-h (long term memory, Fig. 4C). Two-way ANOVA (on acquisition) showed significant effect of D-gal [$F_{1,32} = 108.35$, $p < 0.01$], LiCl [$F_{3,32} = 109.62$, $p < 0.01$] and D-gal x LiCl [$F_{3,32} = 52.74$, $p < 0.01$]. Two-way ANOVA for short term memory showed that significant effects of D-gal [$F_{1,32} = 220.28$, $p < 0.01$], LiCl [$F_{3,32} = 257.57$, $p < 0.01$] and D-gal x LiCl [$F_{3,32} = 70.26$, $p < 0.01$]. Two-way ANOVA for long term memory showed that significant effects of D-gal [$F_{1,32} = 61.23$, $p < 0.01$], LiCl [$F_{3,32} = 249.02$, $p < 0.01$] and interaction between D-gal x LiCl [$F_{3,32} = 75.10$, $p < 0.01$]. Tukey's test revealed that administration of D-gal significantly decreased latency escape. Administration of LiCl significantly increased latency escape at low and medium doses in D-gal treated rats while at high doses in both saline and D-gal treated rats. Results of short term memory and long term memory revealed that D-gal administration increased latency escape. Administration of LiCl at all doses decreased the latency escape in both saline and D-gal treated animals. The increase of latency escape (short term

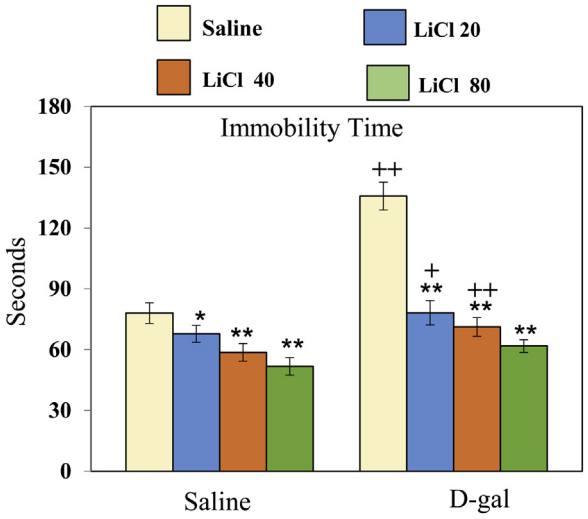

Fig. 3. Immobility time observed in forced swim test to evaluate depression like behavior in saline + saline, saline + LiCl, D-gal + saline and D-gal + LiCl treated animals. Values are mean ± SD (n = 5). Statistical analysis was done by Tukey's test following two-way ANOVA. Statistical difference is represented as $^{**}p < 0.01$, $^{*}p < 0.05$ *versus* respective control and $^{++}p < 0.01$ $^{+}p < 0.05$ *versus* saline + saline and saline + LiCl treated animals.

memory) was greater at 40 mg/ml/kg dose of LiCl in D-gal than saline treated animals.

Effect of LiCl on D-gal induced oxidative stress

Effects of administration of LiCl on MDA levels in D-gal and saline treated rats is shown in Fig. 5. Two-way ANOVA revealed significant effects of D-gal [$F_{1,32} = 127.32$, $p < 0.01$], LiCl [$F_{3,32} = 111.97$, $p < 0.01$] and interaction between D-gal x LiCl [$F_{3,32} = 18.88$, $p < 0.01$]. Tukey's test showed that administration of D-gal increased MDA levels. The levels of MDA were reduced at all doses of LiCl + saline and LiCl + D-gal treated rats. The levels of MDA were greater at 40 mg/ml/kg and 80 mg/ml/kg of LiCl in D-gal than saline treated animals.

Effect of LiCl on D-gal induced alteration of brain antioxidant enzymes activity

Effect of administration of LiCl on antioxidant enzymes in D-gal and saline treated animals are shown in Fig. 6. Data for activity of SOD (Fig. 6A) analyzed by two-way ANOVA revealed significant effects of D-gal [$F_{1,32} = 65.74$, $p < 0.01$], LiCl [$F_{3,32} = 265.59$, $p < 0.01$] and interaction between D-gal x LiCl [$F_{3,32} = 35.90$, $p < 0.01$]. Tukey's test showed that administration of D-gal decreased the activity of SOD. Administration of LiCl at all doses increased the activity of SOD in saline and D-gal treated animals.

Data for activity of CAT (Fig. 6B) analyzed by two-way ANOVA showed significant effects of D-gal [$F_{1,32} = 180.79$, $p < 0.01$], LiCl [$F_{3,32} = 210.70$, $p < 0.01$] and D-gal x LiCl [$F_{3,32} = 37.80$, $p < 0.01$]. Tukey's test showed that administration of D-gal declined the activity of CAT. Administration of LiCl at all doses increased the activity of CAT in saline and D-gal treated rats. The increase of CAT at 20 mg/ml/kg dose of LiCl was smaller in D-gal than saline treated rats.

Data for activity of GPx (Fig. 6C) analyzed by two-way ANOVA showed significant effects of D-gal [$F_{1,32} = 66.74$, $p < 0.01$] and LiCl [$F_{3,32} = 57.34$, $p < 0.01$]. Interaction between D-gal x LiCl [$F_{3,32} = 1.68$, $p > 0.05$] was not significant. Tukey's test showed that administration of D-gal reduced the activity of GPx. Administration of LiCl at all dose increased the activity of GPx in saline and D-gal treated rats. The increase of GPx at 20 mg/ml/kg dose of LiCl was smaller in D-gal than saline treated rats.

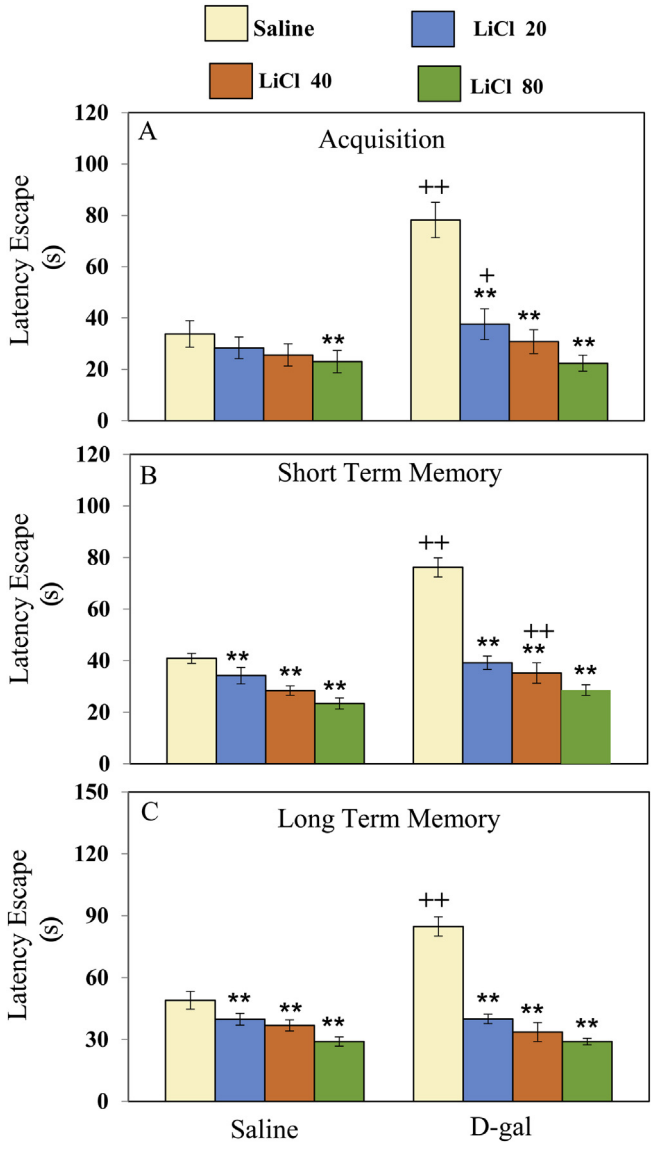

Fig. 4. Effect of LiCl on D-gal induced impaired acquisition (A) short term memory (B) and long term memory (C) in terms of escape latency (s) assessed by Morris water maze. Values are mean ± SD (n = 5). Statistical analysis was done by Tukey's test following two-way ANOVA. Statistical difference is represented as $^{**}p < 0.01$, *versus* respective control and $^{++}p < 0.01$ $^{+}p < 0.05$ *versus* saline + saline and saline + LiCl treated animals.

Effect of LiCl on D-gal induced altered brain Acetylcholinesterase (AChE) activity

Effect of LiCl on brain AChE activity in D-gal and saline treated animals are shown in Fig. 7. Two way ANOVA showed significant effects of D-gal [$F_{1,32} = 189.49$, $p < 0.01$], LiCl [$F_{3,32} = 190.73$, $p < 0.01$] and D-gal x LiCl [$F_{3,32} = 50.71$, $p < 0.01$]. Tukey's test showed that administration of D-gal increased AChE activity. Administration of LiCl at all doses decreased the activity of AChE in saline and D-gal treated animals. Administration of LiCl at 20 mg/ml/kg exhibited smaller decrease of AChE activity in D-gal than saline treated rats.

Effect of LiCl on D-gal induced alteration in 5-HT metabolism

Fig. 8 shows the effects of LiCl on brain 5-HT (A) and 5-HIAA (B) levels in saline and D-gal treated animals. Data on 5-HT levels analyzed by two-way ANOVA exhibited significant effects of D-gal [$F_{1,32} = 244.74$, $p < 0.01$], LiCl [$F_{3,32} = 155.77$, $p < 0.01$] and

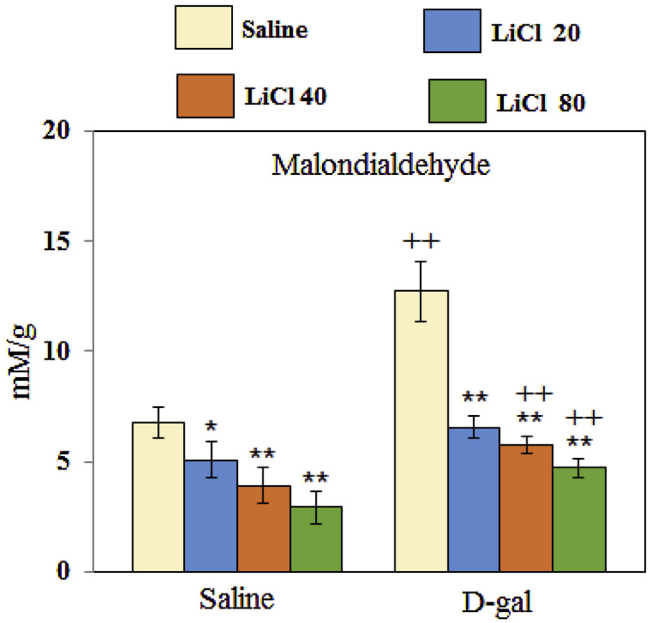

Fig. 5. Effect of LiCl on D-gal induced oxidative stress. Values are mean ± SD (n = 5). Statistical analysis was doneby Tukey's test following two-way ANOVA. Statistical difference is represented as $^{**}p < 0.01$, $^{*}p < 0.05$ *versus* respective control and $^{++}p < 0.05$ *versus* saline + saline and saline + LiCl treated animals.

interaction between the two factors [$F_{3,32} = 8.39$, $p < 0.01$]. Tukey's test showed that administration of D-gal decreased 5-HT levels. Administration of LiCl increased the 5-HT levels at all doses in saline and D-gal treated animals. The levels of 5-HT were smaller at all doses of LiCl in D-gal than saline treated animals.

Data on 5-HIAA levels was analyzed by two-way ANOVA revealed significant effects of D-gal [$F_{1,32} = 48.07$, $p < 0.01$], LiCl [$F_{3,32} = 68.97$, $p < 0.01$] and interaction between D-gal x LiCl [$F_{3,32} = 3.09$, $p < 0.05$]. Tukey's test showed that administration of D-gal decreased 5-HIAA levels. Administration of LiCl increased 5-HIAA levels at all doses in saline and D-gal treated animals. The levels of 5-HIAA were smaller at 20 and 40 mg/ml/kg doses of LiCl in D-gal than saline treated animals.

Discussion

Administration of D-gal is well recognized to produce an aging model [3]. In the present study repeated administration of D-gal (300 mg/ml/kg/day) produced aging related changes including behavioral impairment (anxiety and depression-like symptoms), altered learning and memory functions, increased in AChE activity, and enhanced oxidative stress followed by a reduction in antioxidant enzymes and decrease in serotonin metabolism. However, LiCl prevented these harmful effects of D-gal at all, low (20 mg/ml/kg/day), medium (40 mg/ml/kg/day) and high (80 mg/ml/kg/day) doses.

Neurotoxicity induced by D-gal aging model has been widely used for anti-aging therapeutic research [43,44]. It is well documented that repeated intake of D-gal produces behavioral changes and impairs cognitive function in rats [3,44]. It has been reported that aging can cause neurodegeneration [45], alteration in neurotransmission in various brain areas [24] and induce oxidative stress [4]. The findings of the present study show that D-gal increased the immobility time in FST indicating a depression-like symptom (Fig. 3) with decreased levels of 5-HT and 5-HIAA (Fig. 8). On the other hand, the time spent in light box and open arm was also decreased in the aging model (Fig. 2) that could not be justified with decreased serotonin metabolism. Though, in anxiety,

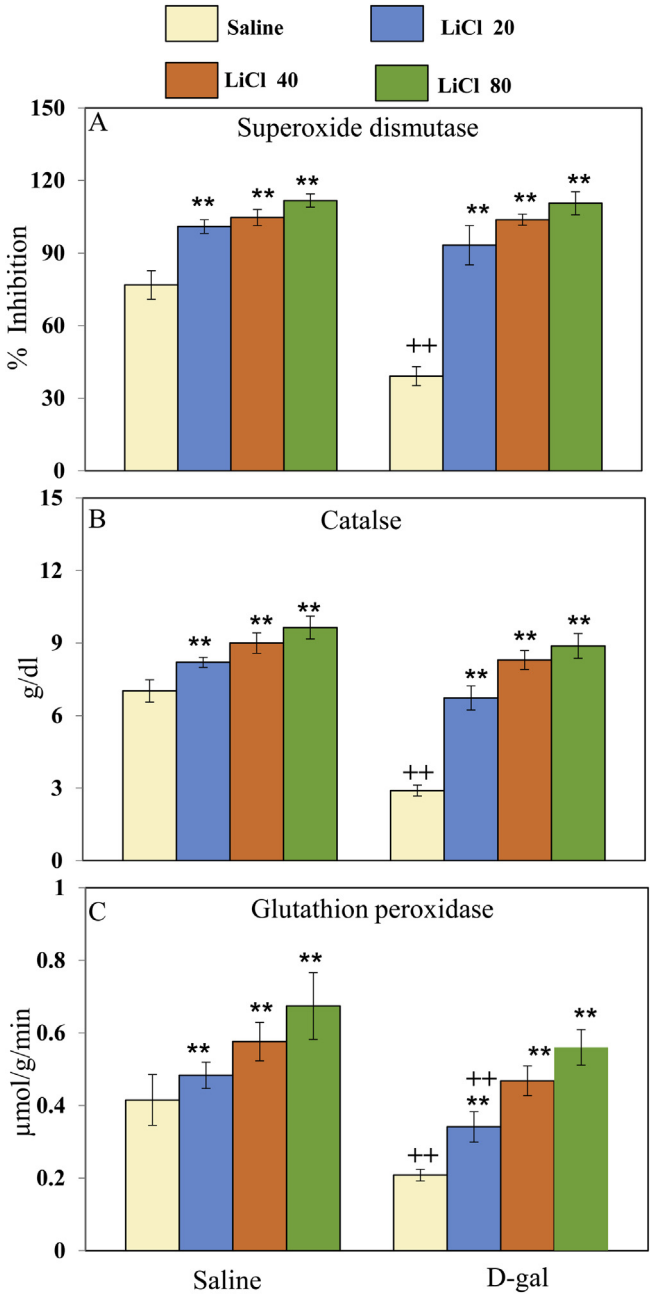

Fig. 6. Effect of LiCl on D-gal induced altered brain antioxidant enzyme activity such as SOD (A), CAT (B) and GPx (C) activity. Values are mean ± SD (n = 5). Statistical analysis was done by Tukey's test following two-way ANOVA. Statistical difference is represented as $^{**}p < 0.01$, *versus*respective control and $^{++}p < 0.05$ *versus* saline + saline and saline + LiCl treated animals.

levels of 5-HT become increased, it could be possible that increased levels of 5-HT in the brain cause an upregulation of 5-HT2C receptor which is involved in anxiogenesis. Another reason which could be behind to anxiety- like symptoms is that D-gal may also lead oxidative stress which probably may result into anxiety like behavior. Our results also showed that LiCl (20, 40 and 80 mg/ml/kg) produced anxiolytic (Fig. 2) and antidepressant (Fig. 3) effectsin control and D-gal treated rats.

Li is a well-known mood stabilizer and has neuroprotective role in animal [44] and in human [46] as well. It was observed in the present study that administration of Li increased 5-HT levels and produced antidepressant effects in control and D-gal treated animals. Administration of Li increased the 5-HT turnover rate [47]

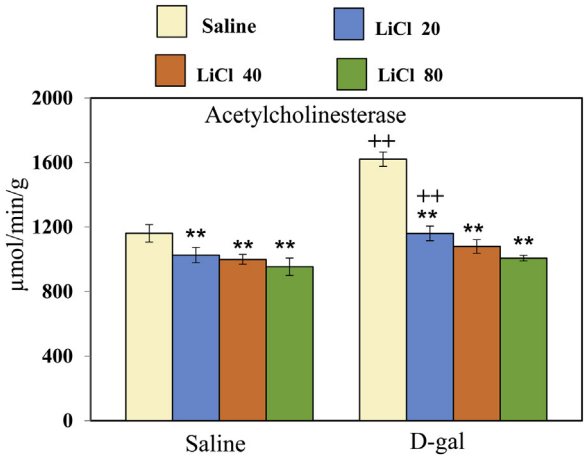

Fig. 7. Effect of LiCl on D-gal induced impaired brain acetylcholinesterase activity. Values are mean ± SD (n = 5). Statistical analysis was done by Tukey's test following two-way ANOVA. Statistical difference is represented as $^{**}p < 0.01$ *versus* respective control and $^{++}p < 0.05$ *versus* saline + saline and saline + LiCl treated animals.

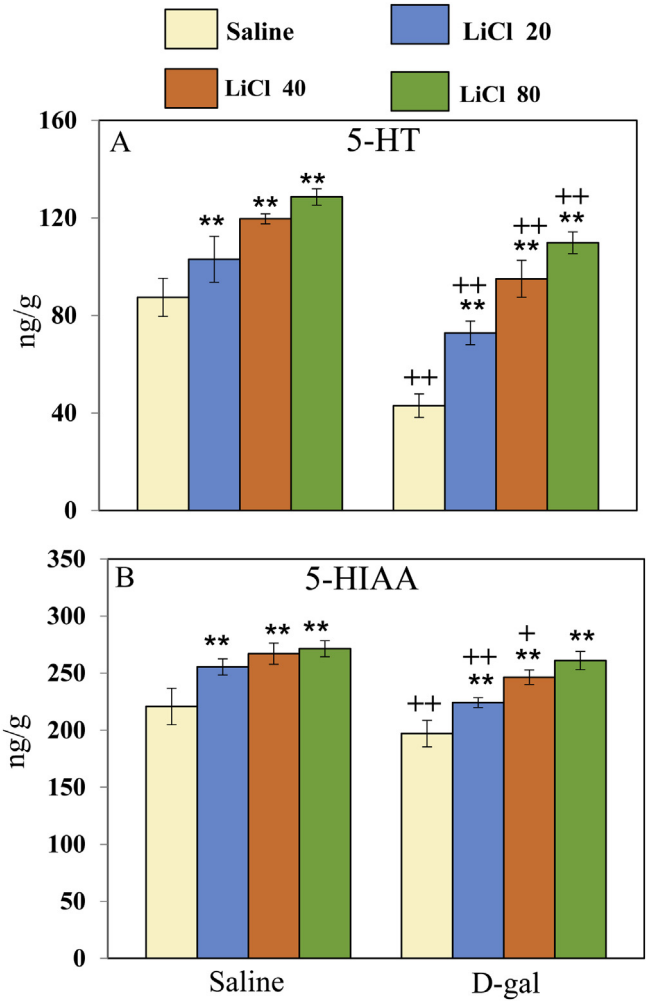

Fig. 8. Effect of LiCl on D-gal induced changes in 5-HT (A) and 5-HIAA (B) levels. Values are mean ± SD (n = 5). Statistical analysis was done by Tukey's test following two-way ANOVA. Statistical difference is represented as $^{**}p < 0.01$ *versus* respective control and $^{++}p < 0.01$ $^{+}p < 0.05$ *versus* saline + saline and saline + LiCl treated animals.

and the levels of 5-HT, 5-HIAA as well as tryptophan (precursor of 5-HT) in the brain [48]. However, effect of repeated treatment of Li on 5-HT may be presynaptical, which stimulates serotonin synthesis [41] and 5-HT release in raphe neurons [49]. This may stimulate the regulationof 5-HT release via presynaptic 5-HT autoreceptors in rat hippocampus causing the decreasein releaseof 5-HT. Li is also reported as an antioxidant [34,35] and ameliorates D-gal induced oxidative stress and may serve to correct abnormalities of 5-HT function.

An important feature of aging and aging related neurological disorders is an alteration in cognitive functions [50]. Abnormalities in antioxidant defence mechanism play a crucial role in aging related alteration in memory [51]. In the present work D-gal induced memory impairment in rats (Fig. 4). It was observed that short term and long term memory was altered in D-gal treated animals which were observed in MWM. One of the important neurotransmitters is acetylcholine, which is concerned in memory function. For the determination of cholinergic function, one of the most essential markers is the determination of AChE activity [52,53]. Previous research suggested a link between AChE activity and memory function but a definite uniform pattern for this relationship is not defined. Previously it has been reported that with the aging process the activity of AChE become increased that lead memory impairment [54]. The present study also revealed that D-gal enhanced the activity of AChE which may be the possible cause of memory impairment. However, the decline in the 5-HT release may be a factor of cognitive impairment. LiCl increased the acquisition (Fig. 4A), short term memory (Fig. 4B) and long term memory (Fig. 4C) which may be attributed to the decreased AChE activity (Fig. 7) in control and D-gal treated animals. In view of previous report, we assume that Li improves the neuronal plasticity [55], which in turn affects the brain neurotransmitter levels. Hence, an increase in the brain acetylcholine levels, the substrate for the enzyme, could be the cause of decreased AChE activity that was observed in the present study (Fig. 7). However, it is also suggested that increased levels of 5-HT (Fig. 8) may also have a role in the improvement of cognitive function because increased serotonin function is also involved in improvement of memory function [37].

Oxidative stress plays a significant role in the pathogenesis of many neurological and psychiatric diseases such as anxiety, depression, dementia and Alzheimer's disease [36]. Extensive studies have reported that repeated administration of D-gal induced oxidative stress [56]. Oxidative stress enhanced peroxidation of cell membrane lipid followed by decreased activity of antioxidant enzymes [36,37,57]. Under the condition of oxidative stress SOD works like the first line of defence by converting superoxide radicals into H_2O_2 which further neutralized by CAT and GPx into molecular oxygen and water. Reduction in the activity of antioxidant enzymes increased the production of free radicals which then injurious for tissues and enhanced lipid peroxidation and cause oxidative stress. In agreement with the previous studies, present finding also revealed that D-gal increased the levels of MDA (oxidative stress biomarker; Fig. 5) and decreased SOD, CAT and GPx activities (Fig. 6) in the brain of rats than control. Li has been reported to improve the ROS levels in an animal model of mania [26,58] and reduce oxidative stress markers such as thiobarbituric acid, MDA in animal model [26] and in un-medicated manic patients [59]. The present study revealed that D-gal induced oxidative stress was prevented by treatment of low (20 mg/ml/kg), medium (40 mg/ml/kg) and high (80 mg/ml/kg) doses of LiCl (Figs. 5 and 6) and underscoring LiCl neuroprotective aspects. The aforementioned results related to anti-anxiety, antidepressant and memory enhancing effects of LiCl could also be explained by its antioxidant potential.

In conclusion, our findings show that all doses of LiCl have the ability to ameliorate D-gal induced neurological diseases. The present study also confirms the neuroprotective role of LiCl *via*its antioxidant potential which produces anxiolytic, antidepressant and memory improving effects and also regulates 5-HT functions.

Conflict of interest

The authors report no conflict of interest.

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