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Research Article

Lithium Potentiate Oxidative Burden and Reduced Antioxidant Status in Different Rat Organs System

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ABSTRACT

Long term lithium (Li) therapy has extensively used in the treatment of bipolar disorder. Therefore, awareness of the numerous side effects and pathogenesis of this lightest alkali metal is needed for such treatments. To date, information on the interaction of Lithium (Li) with oxidative markers and systemic toxicity organs is limited. The present study was designed to investigate the systemic effects of Li on rat organs (kidney, Liver, brain and testies). 100 mg /kg b.w. of Lithium Chloride ($LiCl_3$) was orally administered to rats for 21 days. The lipid peroxide levels (LPO), superoxide dismutase (SOD) and catalase (CAT), glutathione peroxidase and glutathione reduced along with the liver and kidney function test were evaluated. In the organs (kidney, liver, brain, testies) of the group, oral administration of Li increases LPO, PC and decreases SOD and CAT enzyme significantly ($p<0.01$) in comparison to control. The changes in liver function and kidney function indicates impaired function of the xenobiotic metabolism.These findings suggest that oral administration of Li may produce pro-oxidant effect in rats and could be of interest for understanding the controversial role of Li in treatment of bipolar disorders. However, the precise mechanism of lithium toxicity is still incompletely understood.

Key words: Lithium; Kidney; Liver; Brain; testies; mood disorders; oxidative stress

INTRODUCTION

Lithium (Li) is a 27th most abundant ubiquitous element present in the earth's crust to the extent of about 0.006 percent (Habashi, 1997). Li has been used as a medication treatment for individuals suffering from manic and bipolar disorders. Li in medicine can readily cross the placental barriers and produce teratogenic effects and toxicity. Li readily absorbed through the gastrointestinal tract (GIT). In early pregnancy, lithium therapy elicited to be associated with a several fold increase in the incidence of cardiovascular anomalies in newborn, including tricuspid valve abnormalities. There are several clinical and experimental studies reported that detrimental effects of excessive lithium on testicular and gametogenic activity [2, 3, 4]. Li inhibits concentration of sex hormones i.e. follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone and Some case reports were also demonstrated that male sexual dysfunction associated with lithium therapy [5, 6]. Li has also been induced kidney failure in treatment of mental illness [7]. It has been dispute about Li to linked chronic kidney disease and renal failure, and its negative relationship with renal disease has apparent implications for the long term use of Li [8] and the Long term $LiCl_3$ administration caused neuronal apoptosis in rat brain [9] and chronic exposure to atypical antipsychotics produced oxidative brain damage [10, 11]. The nervous system is the primary target organ of lithium toxicity. Neurologic effects occurring during prolonged Li therapy of include cognitive decline and neuromuscular disorders [12]. Movement disorders (myoclonus, choreoathetosis), proximal muscle weakness, fasciculation, gait disturbances, incontinence, corticospinal tract signs, and a Parkinsonian syndrome (cogwheel rigidity, tremor) have been also reported [13].

On the other hand, many studies regarding its numerable adverse effects have been reported The reactive oxygen species (ROS) are induced in metabolic processes as a consequence of incomplete reduction of the oxygen molecule Numerous investigations have revealed that they cause damage to the organism and One of the oxidative processes is lipid peroxidation (LPO), succeeded by the increase of malondialdehyde (MDA) concentration. Have been observe that the wide medical application of lithium and the importance of balance between anti- and prooxidative processes, we have try to evaluate the influence of the oral administration of longterm Li doses on the elements of antioxidant status in male rats. Our study has included activities of the main antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD), as well as the concentration of MDA – the marker of lipid peroxidation. In the present study has been planned to investigate the comparative effect of lithium on different body organs (brain, liver, kidney and testes) and associated neuromuscular changes. Behavioural, biochemical and morphological changes were carried out followed by the long term dose 100mg /kg b w of $LiCl_3$ for 21 days to rats.

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Table-1. Body and organ weight of control and experimental rats.

	Control	Li treated
Body Weight	174.4 ± 2.7	158.6 ± 2.2*
Liver Weight	26.7 ± 0.62	24.7 ± 0.58^{ns}
Kidney Weight	3.87 ± 0.05	3.04 ± 0.04*
Brain Weight	1.28 ± 0.01	1.03 ± 0.01*
Testes Weight	0.39 ± 0.01	0.30 ± 0.01^{ns}

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Statistical significance was determined by Mann-Whitney p-test. Probability, (p<0.05) was considered statistically significant control and treated groups on different intervals.

Table-2. Effect of lithium on ALP, SGOT and SGPT in control and experimental rats

	07 days		14 days		21 days	
	Control	Exp	Control	Exp	Control	Exp
ALP	29.3 ± 3.9	34.8 ± 3.9*	26.4 ± 4.9	35.5 ± 7.8*	28.5 ± 4.1	57.1 ± 4.5*
SGOT	17.5 ± 2.7	20.4 ± 1.9^{ns}	16.7 ± 3.5	23.4 ± 5.6*	17.6 ± 2.3	36.4 ± 3.8*
SGPT	13.8 ± 2.8	15.3 ±11.4^{ns}	14.2 ± 2.8	21.5 ±11.7*	14.0 ± 3.2	31.1 ± 11.6*

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Alkaline phosphate (ALP; KA), serum oxaloacetic acid transaminase (SGOT; U/ml) and serum glutamine pyruvate transaminase (SGPT; U/ml) activity in control and experimental group. Statistical significance was determined by Mann-Whitney p-test. Probability, p-value less than 0.05 were considered statistically significant control and treated group.

Table-3. Effect of lithium on Urea, Creatinin and glucose in control and experimental rats

	07 days		14 days		21 days	
	Control	Exp	Control	Exp	Control	Exp
Urea	34.2 ± 4.8	37.3 ± 5.8^{ns}	33.8 ± 5.1	45.7 ±13.1*	35.2 ± 5.8	65.2 ± 15.5*
Creatinin	0.68 ± 0.6	0.95 ± 0.8*	0.72 ± 0.6	1.12 ± 0.7*	0.73 ± 0.6	1.72 ± 0.6*
Glucose	146.5 ± 23.3	141.7 ± 29.2^{ns}	147 ± 27.5	134 ± 30.4^{ns}	145.2 ± 23.3	118.5 ± 29.5*

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Statistical significance was determined by Mann-Whitney p-test. Probability, (p<0.05) was considered statistically significant control and treated groups on different intervals.

METHODOLOGY

Chemicals: Nitroblue tetrazolium Cat N-5514 (NBT), thiobarbituric acid Cat T-5500 (TBA), phenazinemetho sulphate Cat N-9625 (PMS), nicotinamide adenine dinucleotide Cat N-6754 (NADH), 5.5'-dithio *bis* 2-nitrobenzoic acid Cat D-5420 (DTNB), nicotinamide adenine dinucleotide phosphate Cat N- 7785 (NADPH) tricholoroacetic acid Cat T- 8657 (TCA) and reduced glutathione Cat G-4251 (GSH) were purchased from Sigma Chemical Co., St. Louis, MO, USA, All other reag ents used were of high quality and analytical grade.

Animals:Total number of sixteen male albino rats (Weight- 147 ±1.7 grams, n=12) were taken for this study. The animals were housed separately in polypr opylene cages in a room (temperature; 22±2 °C, relative humidity; 50±10%) 12 h light dark cycles in the animal house of the Nims University. They were fed on Hindustan Lever Food Pallets diet and water *ad libitum.* All the experimental procedure was approved by institutional animal ethical committee.

Dose: All the animals were randomly divided into two groups (N=8) namely normal saline treated control and $LiCl_3$ treated experimental rats for 21 days. The dose was given orally by gavage method which was directly introduced into the rat pharynx via a feeding cannula (The sharp edge of the tip of a hypodermic needle no. 16 was blunted by grinding on a stone and thereafter bent to 120° so that the curved needle could easily be introduced into the rat pharynx via oral cavity without the pointed tip lacerating the passage) to experimental group and an equivalent volume of physiological saline was given to control groups.

Blood collection*:* After 07, 14 and 21 days of treatment, the blood was collected from the tail vein of the rats in plain vial for serum separation. After the blood clotting serum was separated out and keep in -40^{o}C until use.

Serum liver and kidney function test: Serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatine and glucose were assayed using commercially available Qualigens kits (Mumbai, India) on the day of 07, 14 and 21.

Tissue Homogenate*:* After the experimental period rat were sacrificed by cervical dislocation and liver, kidney, brain and testes was removed for biochemical investigations. Ten percent (w/v) homogenate of the different body organs were prepared using York's homogenizer fitted with Teflon plunger in 0.1 M phosphate buffer (pH 7.2). The whole homogenate was first centrifuged at 2500 x g for 10 minutes in a refrigerated centrifuge. The pellet consisting of nuclear fraction and cell debris was discarded. The supernatant

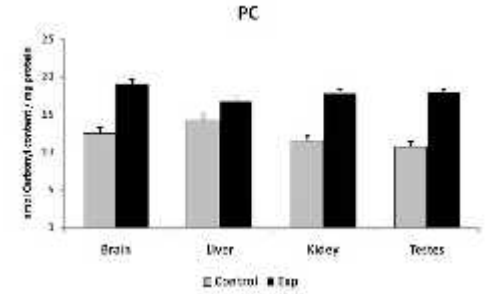

Fig.1 *Concentration of protein in control and Li treated group of liver, kidney, brain and testes. The results are expressed as Mean ± SEM in six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control and treated group*

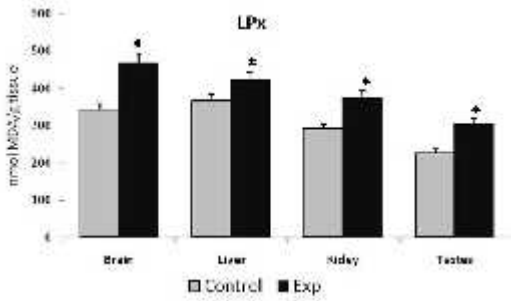

Fig.2 *Lipid peroxide level in control and Li treated group of liver, kidney, brain and testes. The results are expressed as Mean ± SEM in six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control and treated group*

was further centrifuged at 11,000 x g for 15 minutes and mitochondrial fraction was separated. The clear supernatant was further centrifuged at 105,000 x g for 90 minutes and the resultant supernatant was used for determining enzyme activities.

Protein and Protein oxidation: The protein oxidation content was measured by the method of [14] using bovine serum albumin (BSA) as standard. The protein oxidation was measured by estimating the protein carbonyl levels by the method of [15]. Protein carbonyl content was determined in the samples by measuring the DNPH adducts at 375 nm. Carbonyl contents were calculated by using a molar extinction coefficient (*e*) of 22,000 M^{-1} cm^{-1}. Data were expressed as nmoles carbonyl /mg.

Lipid peroxidation: The lipid peroxide (LPx) levels were measured by the method of [16]. The thiobarbituric acid reacting substances (TBARS) of the sample were estimated spectrophotometrically at 532 nm and expressed as n mole of MDA/gm tissue.

Superoxide dismutase: The superoxide dismutase (SOD EC 1: 15.1.1) activity was determined from its ability to inhibit the reduction of NBT in presence of PMS according to the method of [17].The reaction was monitored spectrophotometrically at 560nm. The SOD activity was expressed as U/mg protein (1 unit is the amount of enzyme that inhibit the reduction of NBT by one half in above reaction mixture).

Catalase: Catalase (CAT, EC 1.11.1.6) activity was assayed as per the method of [18] using hydrogen peroxide as substrate; the decomposition of H_2O_2 was followed at 240nm on spectrophotometer. The CAT activity was expressed as U/mg protein.

Glutathione peroxidase: The glutathione peroxidase (GSHPx, EC 1.11.1.0) was assayed by the method of [19] and its activity of GSHPx was expressed as n moles of NADPH oxidized / min / mg protein.

Reduced glutathione: Reduced glutathione was measured using Ellman reagent (5, 5' dithiobis (2-nitro benzoic acid) [20]. The optical density of the pale colour was measured on the spectrophotometer on 412 nm and its level was expressed as µg / g tissue.

RESULTS

Body and organ weight: The body, kidney and brain weight were significantly (P< 0.05) reduced in lithium treated rats when compared with their respective control rats, while liver and testes were insignificantly changed when comparisons with controls.

Serum Biochemistry*:* The biochemistry test is shown in table-1. ALP was found to be gradually increased after Li treatment on 7^{th}, 14^{th} and 21^{st} day as compared with their controls. SGOT and SGPT were found to be significantly increased on the day 14^{th} and 21^{st} in Li treated rats when compared with controls. The concentration of urea was found to be increased significantly on 7^{th}, 14^{th} and 21^{st} day and creatinine was increased on 14^{th} and 21^{st} day of Li treatment. The concentration of glucose was markedly changed on 21^{st} day as compare with the controls.

Biochemical study: The concentration of protein carbonyl content was found to be significantly increased in brain, kidney and testes of Li treated rats when compared with

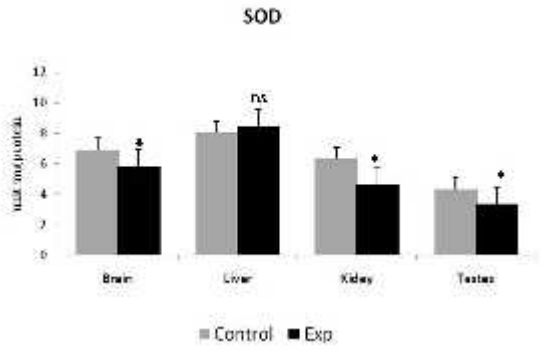

Fig.3 *Activity of superoxide dismutase (SOD) in control and Li treated group of liver, kidney, brain and testes. The results are expressed as Mean ± SEM in six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control and treated group*

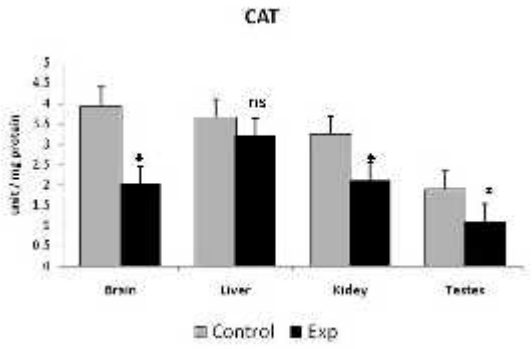

Fig.4 *Activity of catalase (CAT) in control and Li treated group of liver, kidney, brain and testes. The results are expressed as Mean ± SEM in six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control and treated group*

the controls (Fig-1). The maximum increament was observed in testes. While, PC were insignificant changed in liver. The concentration of lipid peroxidation product i.e., melanodialdehyde was increased significantly in all the organs as compared to controls. The maximum increment was observed in brains. The activity of antioxidant enzymes namely SOD, CAT and GPx were found to be significantly altered in brain, kidney and testes. While, liver exhibited insignificantly change followed by Li treatment. The maximum reduction of SOD was observed in kidney. Significantly catalase activity was observed in brain, kidney and testes. While, there is no changes was seen in liver. The glutathione peroxidase activity reduced significantly reduced in brain, kidney and testes in Li treated rats when compared with the controls. Moreover, the glutathione content was found to be significantly reduced in kidney, brain and testes when compared with controls. While the liver exhibited insignificant change in Li treated rats.

DISCUSSION

We examined the chronic effects of daily Li treatment for 21 days on male albino rats. Our results indicate that body weight was significantly reduced. The effect of Li on body weight gain, food intake and feed efficiency was progressively increased during the experimental period of both the groups. The final body weight of intoxicated rats with Li was significantly lower than that of the health normal group. These results clearly indicate that Li toxicity is significant decrease in body weight. And adverse effect of Li on the body weight gain was increased paralleled with increasing time of exposure. Moreover, the amount of food intake of both groups was unchanged. It is suggestive that the value of food intake was not paralleled to the rate of growth and feed efficiency. Earlier, It has been reported that, long term Li dose may be gradually increase the serum lithium levels as was also observed by [21].

In present study, we observe that ALP, SGOT and SGPT was significantly increased in serum of lithium treated rats, which might be due to the liver dysfunction resulting into disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membranes. ALP is important enzymes in biological processes are responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions.

On the other hand, increasing concentration of urea and creatinine in the serum provide information from cellular damage of kidney. Creatinine is a known marker of renal functioning and its concentration in the serum increases as renal function decreases. The elevated creatinine levels observed in serum of Li treated rats may be the reflection of reduced glomerular filtration capacity. Urea is also increased in Li exposed rats as compared with their control. In fact, urea is the first acute renal marker which

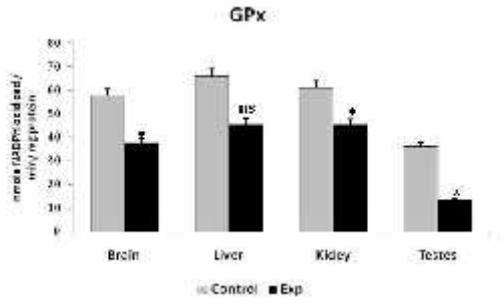

Fig.6 Activity of Glutathione peroxidase (GPx) in control and Li treated group of liver, kidney, brain and testes. The results are expressed as Mean ± SEM in six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control and treated group

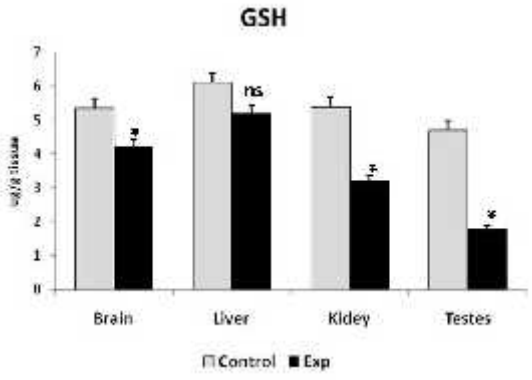

Fig.7 Activity of reduced glutathione (GSH) in control and Li treated group of liver, kidney, brain and testes. The results are expressed as Mean ± SEM in six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control and treated group

increases when the kidney suffers any kind of injury otherwise, creatinine is the most trustable renal marker but this increase only occurs when the majority of renal function is lost. The reduction in glucose concentration in Li treated rats, which may be due to the liver being unable to release glucose from glycogen in [22]. The reduction of glucose is strongly linked with insufficient ATP generation via glycolysis and TCA cycle in Li treated rats. Brain requires a disproportionately large amount of energy to sustain the translocation of ions to maintain action potential and for its high biosynthetic activity. It derives most of its energy from glucose. Therefore, reduced glucose and insufficient energy may be responsible for the various physiological changes in the brain.

In our study, the administration of lithium raises lipid peroxidation products. During physiological states, SOD metabolizes superoxide anion ($O2^-$), producing hydrogen peroxide (H_2O_2), which can react with iron to generate highly reactant hydroxyl radicals via the Fenton reaction. CAT is the most important peroxidase enzyme in detoxifying excess hydrogen peroxide to prevent hydroxyl production. Thus decrement in SOD or CAT levels indicate increased oxidative burden, whereas an imbalance between SOD and CAT activities could lead to an excessive generation of free radicals. The antioxidative defense system like SOD and CAT showed lower activities in liver and kidney during toxicity condition. This study suggests that possible mechanism of lithium induced changes may be due to free radical-toxicity.

CONCLUSION

On the basis of results it may conclude that 100mg /kg body weight of $LiCl_3$ administration for 21 days elevate lipid peroxide levels and delineate antioxidant enzymes that may be correlates with increment of oxidative stress. The study recommends antioxidant therapy along with lithium treatment in mood disorders. In addition, further study is required for the increment of depth knowledge regarding the mechanism of action of lithium and liver. However, further in depth studies in other models are required to unravel the role of Li in the pathophysiology of mood disorder. Li treated rats reflects burden of excessive deposition of Li in the brain, kidney and testes. It may be via the generation of reactive oxygen species.

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