Original Article

Tissue Toxicity Threatens the Gold Standard Image of Lithium as a Mood Stabilizer Drug

Lithium is a Toxic Agent to Tissues

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ABSTRACT

Objective: To demonstrate that the lithium is generally a toxic agent to tissues of both animals and humanbeings but especially to liver and kidney.

Study Design: It is prospective, interventional, morphometric and theoritico-emperical study.

Place and Duration of Study: This study was conducted at the Basic Medical Sciences Institute, Jinnah Post Graduate Medical Centre Karachi University, Karachi, Sindh from October 2012 to March 2013.

Materials and Methods: 30 Albino rats were nursed under all necessary parameters and under the study plan were exposed to lithium carbonate with 20mg per Kg body weight per day. Under the study plan, rats were divided into two groups A and B, each group comprising of 15 rats. Group A is control group while group B is Experimental group exposed to lithium dose. Animals were killed after duration of two, six and twelve week's exposure to lithium dosage as each group was divided into three sub groups each comprising of five animals.

Results: Results of this study highlighted the fact that Lithium as the toxic metal displays significant toxic liver manifestations and disturbs the cytoarchitecture significantly along with the disturbances in the carbohydrates, protein, and fat metabolism. Alkaline phosphatase enzyme displays significant disruption therefore needs extensive evaluation for not only the hepatotoxicity but also biotoxicity and ecotoxicity.

Conclusion: conclusively inference can be made that the lithium has lost its reputation as gold-standard mood stabilizer drug but can be employed as an investigative tool in Physiology. Biochemistry,Genetics and Pharmacology but particularly in Psycho-neurology.

Key Words: Lithium, hepatotoxicity, Genotoxicity, biotoxicity, etc.

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INTRODUCTION

Human toxicity: Long-term effects on organ systems

The three organ systems that may be negatively affected by lithium are the thyroid gland, kidneys and parathyroid glands.

Lithium and the kidneys: The polyuria reflects lithium's effect on the renal tubular system. Concerns that this might reflect structural irreversible damage, as opposed to simply reversibly interfering with tubular function, began with the first reports of biopsy-proven interstitial nephritis in lithium-treated patients almost 40 years ago [1]. all studies examining renal morphology in lithium-treated patients have consistently found the same results: focal nephron atrophy, and interstitial fibrosis with relative preservation of glomeruli [2].

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This is consistent with the clinical features of lithium-associated nephropathy—obligate polyuria—but without marked decrease in filtering capacity of the kidneys as measured by eGFR and secondarily by serum creatinine. (The latter measure is less accurate than eGFR since it also reflects muscle mass which decreases with age. Thus, an older person may have substantially diminished eGFR but a relatively normal serum creatinine.) Polyuria correlates only weakly with reduced kidney function with the former rather common and the latter unusual [3].

Although lithium-treated patients have, in general, a lower eGFR than those not treated, the eGFR does not correlate with time on lithium suggesting that it is not progressive within groups. However, a subgroup of lithium-treated patients does show progressive renal insufficiency. This is manifested by "creeping creatinine" [4] with a gradual rise in serum creatinine and a decrease in creatinine clearance over years. This phenomenon occurs in approximately 20% of lithium-treated patients[5]. In one study, approximately 1/3 of lithium-treated patients had an eGFR <60 ml/min while 5% showed an eGFR of <30 ml/min[6,7]. An even smaller subgroup of lithium-treated patients progresses towards end-stage renal disease (ESRD) and ultimately dialysis and/or renal transportation. The prevalence of ESRD associated with lithium is difficult to estimate. One

study found the risk to be almost eightfold compared to the general population[6,7]. In contrast, another study found risk for renal insufficiency but not ESRD[8]. While a third study found no differences in the rate of eGFR declines in lithium-treated patients vs. those treated with other psychotropic agents [9]. In the most recent study, compared to patients treated with other mood stabilizers such as valproate, olanzapine or quetiapine, lithium was associated with higher rates of chronic renal disease (eGFR <60 ml/min) but not more severe renal disease, Since lithium-associated ESRD is virtually exclusively seen in patients treated for a very long term—in one study, the average time on lithium for those with ESRD was 27 years [6,7,10], studies of only 10–15 years may not show the increase in ESRD. Some recent studies have suggested both lower rates of ESRD in lithium-treated patients over the last thirty years when mean therapeutic lithium levels are lower than before [6,7] and less effect on renal function in general with lower levels [11].

However, the largest study of unselected: patients continued to find significant rates of renal damage and ESRD in a lithium-treated population [6,7,10].

Risk factors for lithium-induced nephropathy are length of treatment, age, and prior episodes of lithium toxicity [6,12,9]. Whether the lithium regimen—once-daily vs. multiple doses—predicts differential rates of ESRD is unclear. Some evidence exists that progressive renal impairment continues even after lithium discontinuation, [12,13]. Since the progression of renal damage is slow, if discontinuing lithium is deemed necessary, the second mood stabilizer should be added, titrated to full dose and only then should the lithium be tapered and discontinued gradually over 4–8 weeks.

Toxicity in Animals: Lithium causes multi system toxicity[14]. Oral administration of lithium carbonate to healthy rats strongly decreased liver glycogen content despite the simultaneous activation of glycogen synthase and the inactivation of glycogen phosphorylase. The effect seemed to be related to a decrease in glucose 6 phosphate concentration. Lithium inhibits the enzymes of glucose metabolism e.g. glucose kinase, pyruvate kinase and super oxide dismutase (SOD)[14,15,16].

Lithium carbonate in the dose of 150 mg /kg body weight when administered in drinking water for 30 days induces lipid peroxidation (LPO) to a significant extent that was accompanied by a marked reduction in reduced glutathione, SOD, catalase, Glutathione S-transferase (GST) and Glutathione peroxidase (GPX) activities and parallel decline in Adenosine triphosphate (ATP) in tissues. Toxicity resulted in abnormal elevation of lipids such as cholesterol, triglycerides, phospholipids and fatty acids in liver tissues[17,18]. It impairs the DNA synthesis and DNA repair[19].

The process of LPO and DNA oxidation leads to disruption of the ultra structure of the cell machinery, diminution in the quantity of cellular ATP, reduction in the anti-oxidant systems resulting in programmed cellular

Death and necrosis: The inflammation is very wide.[16,17] Echo toxicity and biotoxicity are also prevalent.

MATERIALS AND METHODS

Thirty albino adult rats of 90 – 120 days of age weighing about 200 – 300 grams were used for this study. Animals were obtained from the animal house of BMSI, JPMC, Karachi. These were divided into two major groups A and B each comprising 15 rats. Each major group was sub-divided into three sub-groups 1, 2 & 3 on the basis of 02 weeks, 6 weeks and 12 weeks duration of treatment respectively. Group A was control and fed on lab diet. Group B was treated with lithium in drinking water. Lithium was used in the dosage of 20 mg/kg body weight/day in water[17]. Each sub-group was sacrificed at the end of their corresponding duration of treatment under ether anesthesia, dissected and blood was collected through intra-cardiac puncture for serum ALP and ALT analysis. Each liver was cut into two halves. One half was fixed in buffered neutral formalin. Paraffin embedding of tissues were done after processing of the fixed tissues. 4 um thick sections were cut using rotary microtome for H&E, PAS & Gomori's calcium phosphate staining. Representative sections from the second half of liver were immediately frozen using cryostat and 10 um thick sections were cut and subjected to Oil Red O staining.

The statistical significance of the differences of various Histochemical as well as serological changes between lithium carbonate and diet treated rats from the control group were evaluated by the student T-test.

RESULTS

Results are based upon both gross and microscopic examination, the absolute and relative weights of livers were recorded and present study demonstrated that Lithium induces hepatotoxicity in animals. The dose of Lithium used in this study is similar to that used by Kolachi[6].

Observations on absolute and relative liver weight disclosed moderately significant to significant increase in weight of liver when B group was compared to A group.

The morphological examination of haematoxylin and eosin (H&E) stained 4 um thick sections displayed dilatation and congestion of central and portal veins along with the congestion of sinusoids. There was a distortion of the wall of the central veins, plates or cords of hepatocytes seemed to be irregular and exhibited distortion. The hepatocytes were scattered in the lobules revealing vacuolation both in pericentral and periportal areas. Cytoplasm revealed mild granularity. Hepatocytes showed pyknosis of nuclei and

disintegration thereof. Kupffer cells were prominent and hypertrophied. Binucleate hepatocytes were also seen revealing proliferation of the parenchyma. **(Tables 1-4, Fig 1-4)**

TableNo.1: Mean values of absolute liver weights (G).

Groups	Sub Groups	Treatment Given	Final weights at variable time intervals		
			2 weeks	6 weeks	12 weeks
A (n=15)	A1	Control (Normal Lab Diet)	8.20 ± 0.8		
	A2			8.40 ± 0.51	8.80 ± 0.37
	A3				
B (n=15)	B1	Lithium carbonate treated	12.00 ± 0.44		
	B2			14.00 ± 0.44	
	B3				15.00 ± 0.70

Statistical Analysis of mean absolute liver weight between different groups

Statistical Comparison	P Value
A1 VS B1	P<0.05**
A2 VS B2	P<0.01***
A3 VS B3	P<0.01***

Key: Non Significant*
Significant**
Moderately significant***
Highly significant****

Table No.2: Mean values of relative liver weights (G).

Groups	Sub-groups	Treatment	Final weights at variable time intervals		
		Given	2 weeks	6 weeks	12 weeks
	A1	Control	3.30±0.32		
A	A2	(Normal Lab		3.31±0.18	
(n=15)	A3	Diet)			3.56±0.13
	B1	Lithium	4.56±0.15		
B	B2	Carbonate		5.30±0.17	
(n=15)	B3	Treated			5.66±0.27

Statistical analysis of mean relative liver weight between A and B groups.

Statistical comparison	P value
A1 VS B1	P<0.05**
A2 VS B2	P<0.01***
A3 VS B3	P<0.01***

Key: Non Significant*
Significant**
Moderately significant***
Highly significant****

Table No.3: Glycogen Content of Hepatocytes.

Groups	Sub Groups	Treatment Given	Glycogen content in Hypatocytes at variable Time interval		
			2 weeks	6 weeks	12 weeks
A (n=15)	A1	Control (Normal Lab Diet)	**N+**		
	A2			**N+**	
	A3				**N+**
B (n=15)	B1	Lithium carbonate treated	+		
	B2			++	
	B3				+++

Table No.4: Distribution of the activity of alkaline phosphatase in hepatic lobules.

Groups	Sub Groups	Treatment Given	Contents of crystals (Cobalt sulphide) in Hepatic lobule at variable Time interval		
			2 weeks	6 weeks	12 weeks
A (n=15)	A1	Control (Normal Lab Diet)	**N+**		
	A2			**N+**	
	A3				**N+**
B (n=15)	B1	Lithium carbonate treated	+		
	B2			++	
	B3				+++

Normal N−
Mild +
Moderate ++ } Depletion
Marked +++

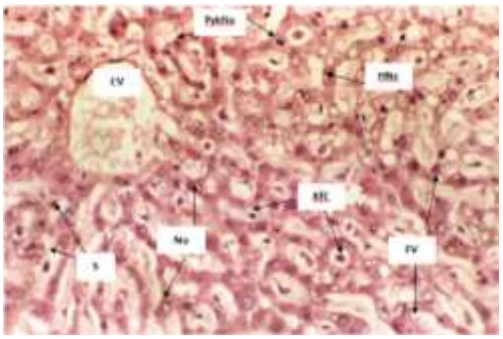

Figure No.1: Photomicrograph of H&E stained 4µm thick section of liver showing enlarged hepatocytes with large nuclei (Nu) and highly congested and dilated sinusoids (S) with disruption and distortion of lobular architecture with pyknosis of nuclei (PykNu) and cytoplasm displaying small vacuoles of microvesicular fatty change (FV), prominent Kupffer cells (KfC) in the lining of sinusoids and necrosed hepatocytes (HNc) in albino rats, after 6 weeks of lithium carbonate treatment X 1000.

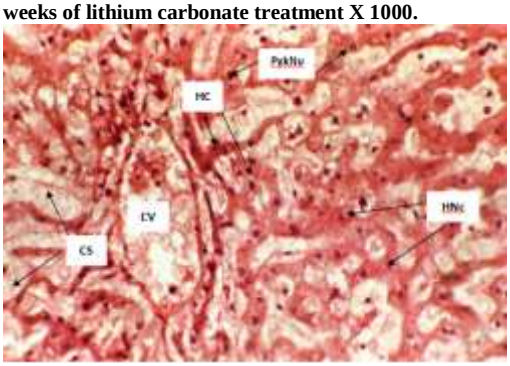

Figure No.2: Photomicrograph of H&E stained 4µm thick section of liver showing hepatic lobular architecture displaying central vein (CV) around which is depicted pyknosis of nuclei (PykNu), dilated and congested sinusoids (S) and distorted hepatic cords (HC) in the albino rats after 12 weeks of lithium carbonate treatment X 1000.

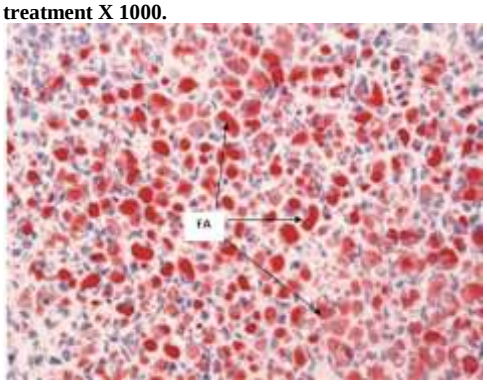

Figure No.3: Photomicrograph of oil red O stained 10µm thick frozen section of liver exhibiting densely packed fat globules (FA) in the hepatocytes of the lobular architecture in zone-II and zone-III in albino rats after 6 weeks of lithium carbonate treatment X 400.

DISCUSSION

Liver gross examination of group B animals showed significant hislogical alteration due to lithium toxicity. This observation correlates with the work of Sharma and[14,16.] The augmentation in absolute and relative liver weight was due to cellular hypertrophy, hyperplasia, swelling, hydropic degeneration, increased mononuc ear cell infiltration, accumulation and hypertrophy of kupffer cells, enhancement in fatty infiltration in the form of microvesicular fatty globules and dilatation and congestion of portal and central veins which was in agreement with the suggestions of [19,20,16].

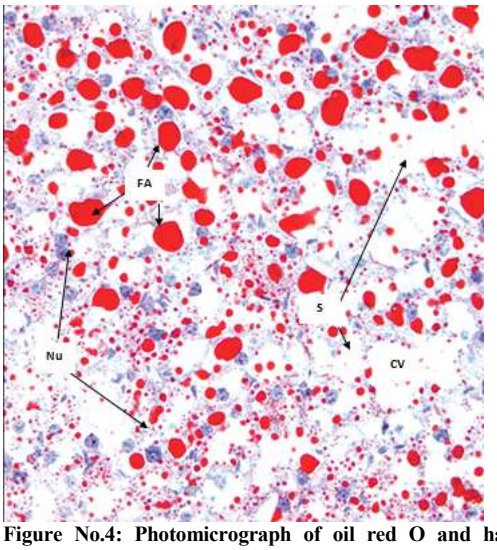

Figure No.4: Photomicrograph of oil red O and haematoxylin stained, 10µm thick frozen section of liver showing hepatic lobular cytoarchitecture displaying central vein (CV), sinusoids (S) and blue stained nuclei (Nu) of hepatocytes. It also depicts densely packed fat globules (FA) in the liver cells in all three zones in albino rats after 12 weeks of lithium carbonate treatment X 1000.

Also Group B animals demonstrated significant depletion in glycogen content in hepatocytes which was chiefly attributed by the lithium hepatotoxicity. Lithium causes disturbance of the glucose metabolism in vivo. It is also in agreement with the observations of[21], who studied the effect of lithium on rat glucose metabolism in vivo. It also correlated with the observations of[22] who reported similar type of depletion in glycogen content in hepatocytes treated with piroxicam in experimental mice.

The histochemical study of Gomoris calcium phosphate stained section of group B animals showed decreased amount of brownish black deposits of cobalt sulfide meant thereby reduced alkaline phosphatase (ALP) activity. This was due to hepatic injury which increased the permeability of cell membrane with resultant leakage of enzymes from cytoplasm to sinusoids and then into circulation with simultaneous increase in the serum hepatic ALP as described by[19,23]. These observations match with the observations of [14,16] who have examined liver and kidney toxicity.

CONCLUSION

Empirical outcome reveals that The Lithium is an excellent mood-stabilizer and gold-standard antisuicidal drug with very narrow therapeutic margin.But unfortunately Lithium is also widely toxic metal for all living organisms and Biosphere of planet Earth. It is toxic to both animal and humanbeings but predominantly to Kidney, Liver and Heart organs. It has very diverse Pharmacodynamics at various levels of biomolecular cascade systems of cellular machinery but yet its mechanisms of actions are not fully known

despite very intensive as well as extensive research over the last 70 years. Therefore conclusively inference can be made that the lithium has lost its reputation as gold-standard mood stabilizer drug but can be employed as an investigative tool in Physiology. Biochemistry, Genetics and Pharmacology but particularly in Psycho-neurology.

Author's Contribution:

Concept & Design of Study:	Saleem Ahmed Bhutto
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Data Analysis:	Mir Khater Ali, Muhammad Aslam Channa
Revisiting Critically:	Saleem Ahmed Bhutto, Muqeem Mangi
Final Approval of version:	Saleem Ahmed Bhutto

Conflict of Interest: The study has no conflict of interest to declare by any author.

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