Original Article

Anatomy

Histochemical and Serological Parameters of Hepatic Lithium Toxicity Ameliorated by L-Arginine Supplementation

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ABSTRACT

Objective: The purpose of this study was to judge the ability of L-arginine to restore the carbohydrate, protein and fat metabolism particularly in the liver against lithium carbonate induced hepatotoxicity.

Study Design: This study is randomized, interventional, prospective, morphometric and Histochemical study.

Place and Duration of Study: This study was conducted in the department of anatomy, BMSI, JPMC, Karachi. Animals were obtained from the animal house of BMSI, JPMC, Karachi. The duration of this study comprises of two, six and twelve weeks.

Materials and Methods: Sixty albino adult rats 90 – 120 days of age weighing about 200 – 300 grams were used for this study. Animals were divided into four different groups 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. 4 um thick sections of rat liver were stained with PAS haematoxylin, Gomori's calcium phosphate and oil red O. Serum analysis of ALT and ALP was done.

Results: Lithium treated group on PAS staining displayed depletion of glycogen content of hepatocytes. Gomori's calcium phosphate staining revealed diminution of the intracellular enzyme contents of hepatocytes especially alkaline phosphatase (ALP) and concomitant rise in serum hepatic enzymes like Alanine aminotransferase (ALT) and ALP. Oil red O stained sections of the liver depicted microvesicular fatty infiltration in liver cells in lithium treated group B animals.

Conclusion: This study revealed that the toxicity of lithium is manifested histochemically by disturbances of in the glycogen, lipid and enzyme metabolism in the liver simultaneously exhibiting the restoration of the same metabolism by L-arginine as a best adjuvant in the treatment of bipolar disorder with lithium carbonate.

Key Words: L-arginine, lithium carbonate, rat liver, Histochemical changes.

INTRODUCTION

One of the anti-psychotic drugs used is lithium carbonate (Li_2CO_3). Lithium causes multi system toxicity[1]. 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)[1,2,3].

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[4,5]. It impairs the DNA synthesis and DNA repair[6].

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[5].

Involvement of ROS(reactive oxygen species) causes oxidative damage of lipid membrane, protein and DNA fragmentation causing apoptosis and cell death in liver[7].

L-arginine, a nitric oxide donor plays very important protective role in attenuating the adverse toxic effects of anti-psychotic drug lithium carbonate[8].

Nitric oxide has several mechanisms to affect the biology of the cell. These include oxidation of iron containing proteins (Ribonucleotide reductase and aconitase). Nitric oxide causes activation of soluble guanylate cyclase, ADP ribosylation of proteins, activation of protein sulfhydryl nitrosylation and iron regulatory factor activation[9,10,11].

MATERIALS AND METHODS

Sixty 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 four major groups A,B, C & D 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. Group C was co-administered L-arginine along with lithium. Group D was fed on diet containing L-arginine alone. Lithium was used in the dosage of 20 mg/kg body weight/day in water[12]. L-arginine was given in dosage of 300 mg/kg body weight/day in feed[13,14]. 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 lithium carbonate + L-arginine treated rats from the control rats were evaluated by the student T-test[15]

RESULTS

The results of this study were based on microscopic examination.

Group B animals treated with lithium over the periods of 2 –6 –12 weeks displayed significant diminution in glycogen content in all sub-groups B1, B2 and B3. In group B3 animals the depletion of glycogen was markedly exhibited by the hepatic lobular cyto-architecture in all the three zones of the lobule.

The glycogen content in group C was evenly distributed in all hepatic lobular zones after co-treatment with lithium plus L-arginine for variable time interval. Thus the reduction of glycogen content in group C was not as marked as in B group animals.

The histochemical examination of the lipid content of the hepatocytes by Oil Red O stained 10µm thick frozen section of the liver tissue of B group animals revealed marked and significant changes. As the duration of treatment with lithium increased, so increased the fat content of the hepatocytes as shown in table 2.

In sub-groups B2 and B3 there was marked infiltration of fat-deposits in the hepatocytes in the form of fat gloubules.

There was significantly reduced deposition of fat in hepatocytes in group C animals as depicted in Table No 2.

To observe the activity of alkaline phosphatase (ALP) in hepatocytes the study of Gomoris calcium phosphate stained section of group B animals showed decreased amount of brownish black deposits of cobalt sulfide, in different hepatic zones . It meant alkaline phosphatase activity was decreased in all the three zones in all subgroups B1, B2 and B3. Lithium plus L-arginine treated group C animals depicted normal ALP activity around the portal triad areas though there was mild depletion of these deposits in sub group C2 and C3 as shown in Table 3.

The alkaline phosphatase (ALP) deposits in hepatic tissue were correlated with serum hepatic enzyme ALP. In group B animals decreased ALP in liver tissue was associated with simultaneous increase in the serum hepatic ALP and the level of serum ALT(SGPT) was also markedly raised.

The levels of serum ALT and ALP were significantly reduced in group C animals with L-arginine.

All the above mentioned features can be observed in the figures 1-2 and Photomicrographs 1-3.

Table No.1: Glycogen Content of Hepatocytes in Different Groups of Albino Rats at Variable Time Interval

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				+++
C (n=15)	C1	Lithium carbonate + L-arginine	N+		
	C2			N+	
	C3				+

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

Table No.2: Grading of Fat Content in Hepatocytes in Different Groups of Albino Rats at Variable Time Interval

Groups	Sub Groups	Treatment Given	Fat 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				+++
C (n=15)	C1	Lithium carbonate + L-arginine	N+		
	C2			N+	
	C3				+

Normal N+
Mild + }
Moderate ++ } Deposition
Marked +++ }

Table No.3: Distribution of the Activity of Alkaline Phosphatase in Hepatic Lobules of Different Groups of Albino Rats at Variable Time Interval

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				+++
C (n=15)	C1	Lithium carbonate + L-arginine	N+		
	C2			N+	
	C3				+

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

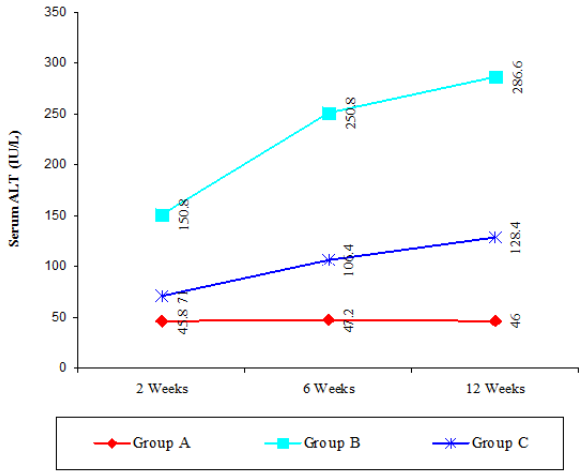


Figure No.1: Mean Values of Serum ALT (Iu/L) in Different Groups of Albino Rats at Variable Time Interval

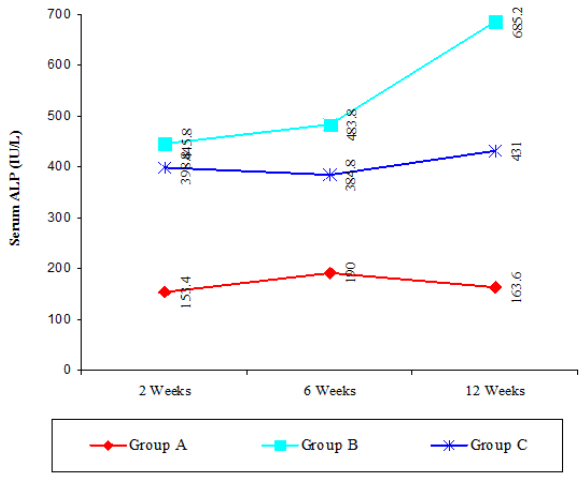
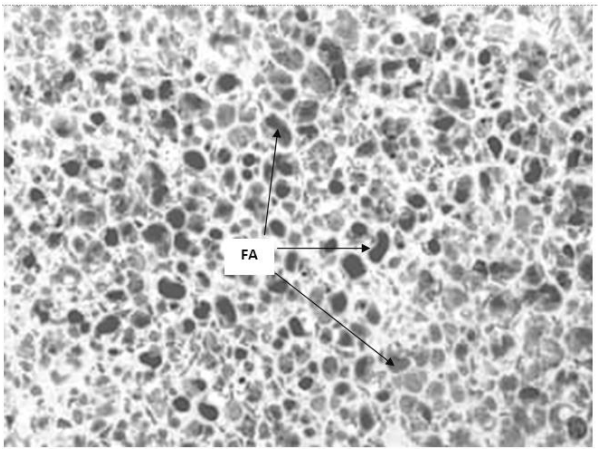
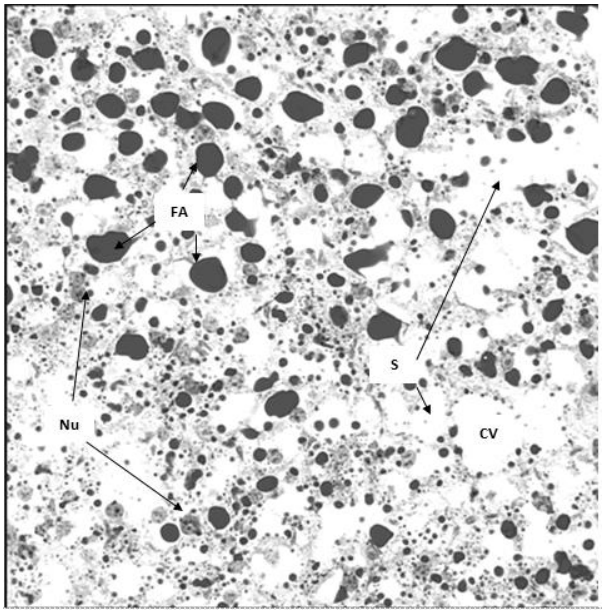


Figure No.2: Mean Values of Serum ALP (Iu/L) in Different Groups of Albino Rats at Variable Time Interval



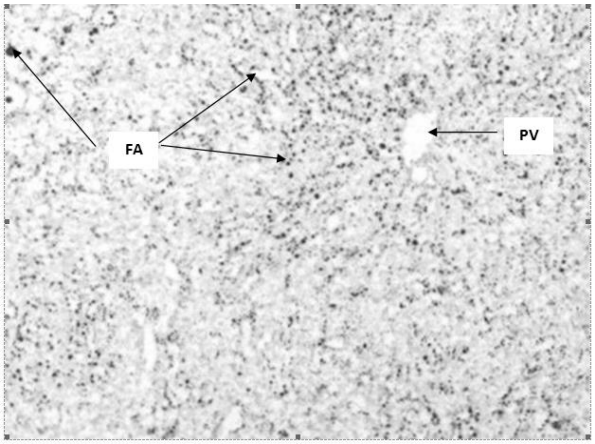
Photomicrograph 1: Photomicrograph of oil red O stained, 10µm thick frozen section of liver exhibiting densely packed fat globules (FA) in the hepatocytes of the lobular architecture in zone-II and zone-III in albino rats after 6 weeks of lithium carbonate treatment X 400



Photomicrograph 2: 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.

DISCUSSION

Group B animals treated with lithium exhibited significant depletion in glycogen content in hepatocytes which was chiefly attributed by the lithium hepatotoxicity. Lithium causes disturbance of the glucose metabolism in vivo. This observation is in agreement with the findings of Sharma and Iqbal[3] who did their experiment on 24 male albino wistar rats to examine the lithium induced toxicity in rats. It is also in agreement with the observations of Rodriguez-gill et al[2] who studied the effect of lithium on rat glucose metabolism in vivo. It also correlated with the observations of Ebaid et al[16] who reported similar type of depletion in glycogen content in hepatocytes treated with piroxicam in experimental mice.



Photomicrograph 3: Photomicrograph of Oil red O stained 10µm thick frozen section of liver showing mild to moderate degree of deposition of fat globules (FA) in the hepatocytes of the lobular architecture exhibiting portal vein (PV) on the side in the albino rats after 6 weeks of lithium carbonate plus L-arginine treatment X 400.

The glycogen content depletion in group C was not as marked as in B group animals. This was due to the protective and anabolic effect of L-arginine on hepatocyte biology. Glycogen content was increased due to restoration of glucose and protein metabolism. This corresponds with the finding of Kennedy et al[17] who studied the effect of L-arginine on modulation of body weight and immunity in rats having obstructive jaundice as shown in table 1.

This study was also in agreement with the works of Witte and Barbal[18] who studied the effect of L-arginine on hepatocyte energy metabolism in rat with obstructive jaundice. Gunther et al[19] is also in agreement with the same observation in his study: zinc causes restoration of glycogen content in salicylate induced hepatic injury in experimental albino rats.

The histochemical examination of the lipid content of the hepatocytes in B group animals revealed significant changes. As the duration of treatment of lithium increased, so increased the fat content of the hepatocytes in the form of fat globules. The fat globules caused space occupying lesion and thereby led to inflammation, swelling apoptosis and necrosis. This study is in agreement with the studies of Vijaimohan et al[5] and Kolachi[8], who examined the lithium liver toxicity and its attenuation with sobatum and lithium nephrotoxicity and its attenuation with L-arginine

respectively.It is also in agreement with the study of El-Beshbishy[20] on hepatoprotective effect of green tea (Camellia sinesis) extract against tamoxifen induced liver injury in rats.

Significant reduction in the deposition of fat in hepatocytes in group C animals was due to protection by L-arginine which inhibits LPO, reduces serum lipids, diminishes liver lipid content. It also agreed with the studies of Mahmoud et al[14] who studied the protective effect of L-arginine against nephrotoxicity induced by cyclosporine in rats.

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 as described by Kumar et al[21]. These observations match with the observations of Sharma and Iqbal[3] and Chadha et al[7] who have examined liver and kidney toxicity.

Lithium plus L-arginine treated group C depicted increased (ALP) activity in hepatocytes around the portal triad areas and the same was correlated with the serum hepatic ALP.

In group B animals decreased ALP in liver tissue was associated with simultaneous increase in the serum hepatic ALP. This was in accordance with the study of Sharma and Iqbal[3] and Chadha et al[7].The above observation also conformed with the study of Hussein et al[22] who also reported an increase in serum level of ALP in ethanol induced hepatotoxicity.

In the group B animals, the level of ALT (SGPT) was markedly increased in serum. This was due to hepatocyte destruction resulting in increased membrane permeability and leakage of enzyme into sinusoids and then into circulation as suggested by Kumar et al[21] and Sharma and Iqbal[3].

The serum ALT level in group C animals was restored but the reversal was not complete. This restoration was executed by L-arginine as was observed by Chadha et al[7], Burden et al[23] and Das et al[24].

In the light of the above back ground, the results of present study suggest that lithium causes direct as well as indirect toxic insult of liver tissue and co-administration of L-arginine with lithium resulted in normalizing the hepatic lobular cytoarchitecture quite appreciably.

CONCLUSION

This study demonstrates that L-arginine supplementation in patients undergoing lithium therapy deters the worsening of Histochemical and serological parameters of liver.

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