Original Article

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L-Arginine as a Protective Adjuvant in the Treatment of Bipolar Disorders with Hepatotoxic Agent Lithium

1. Saleem Ahmed Bhutto 2. Pirbho Mal Makhija 3. Abid Hussain Chang 4. Anjum Naqvi

1. M Phil Scholar, BMSI, JPMC, Karachi 2. Asstt. Prof. Medical Unit 2, GMMC, Sukkur

3. Asstt. Prof. LUM&HS, Jamshoro 4. Assoc. Prof. of Anatomy, BMSI, JPMC, Karachi

ABSTRACT

Objective: The purpose of this randomized experimental study was to explore the beneficial effects of L-arginine on lithium carbonate induced liver toxicity.

Study Design: This study is randomized, interventional, prospective and experimental in nature.

Place and Duration of Study: This study was conducted in the department of anatomy, Basic medical sciences institute, Jinnah post graduate medical centre, Karachi. Animals were obtained from the animal house of BMSI, JPMC, Karachi. The duration of this study comprises of two to twelve weeks.

Materials and Methods: Sixty albino adult rats of 90 – 120 days of age weighing about 200 – 300 grams were used for this study. 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. 4 um thick sections of rat liver were cut using rotary microtome for H&E. The statistical significance of the differences of various quantitative changes between lithium carbonate and lithium carbonate + L-arginine treated rats from the control rats were evaluated by the student T-test**.**

Results: Lithium treated group exhibited significant augmentation in absolute and relative liver weight. Histopathological findings of liver revealed dilatation of central and portal veins, congestion of sinusoids, increment in mononuclear cell infiltration, microvesicular fatty change, swelling and hydropic degeneration of hepatocytes leading to pyknosis of nuclei, disintegration of organelles consequently leading to cell apoptosis and necrosis. Rats fed on co-administration of lithium plus L-arginine displayed significant improvement in the altered histology of liver lobules.

Conclusion: This study revealed that concomitant administration of L-arginine with lithium considerably reduces lithium's adverse effects.

Key Words: L-arginine, lithium carbonate, rat liver, bipolar disorder, hepatotoxicity.

INTRODUCTION

One of the anti-psychotic drugs used is lithium carbonate (Li_2CO_3). The cyto-toxic effects of lithium that effect the liver tissue are manifested by the disturbances of nitric oxide (NO) metabolism, a key mediator of signaling events linked to apoptotic cell death in liver[1]. Lithium leads to liver damage. The toxicity of lithium is due to the production of reactive oxygen species (ROS), lipid per oxidation (LPO) of cellular and organellar membranes leading to damage of mitochondria, endoplasmic reticulum, microsomes, peroxisomes and other ultra structures[2].

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[3,4].

Involvement of ROS causes oxidative damage, DNA fragmentation, apoptosis and cell death in liver[5]. L-arginine, one of the biologically important nitric oxide donors plays very important protective role in attenuating the adverse toxic effects of anti-psychotic drug lithium carbonate[6].

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[7,8,9].

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[10]. L-arginine was given in dosage of 300 mg/kg body weight/day in feed[11,12]. Each sub-group was sacrificed at the end of their corresponding duration of treatment. Each animal was sacrificed under ether anesthesia and dissected. The livers were taken out and were subjected to gross examination. Absolute and relative liver weights were recorded. 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. The statistical significance of the differences of various quantitative changes between lithium carbonate and lithium carbonate + L-arginine treated rats from the control rats were evaluated by the student T-test[13].

RESULTS

During this experimental study the observations and results of the animals were based on gross and microscopic examination.

The livers on gross examination in control group displayed normal features. The liver in lithium treated group B looked normal except dark brown color as compared to reddish brown in control animals. The surface was smooth but the consistency was firm as compared to control group. The liver looked moist and swollen. A few livers also exhibited mild hemorrhagic spots with pallor intervening areas. While in group C animals the liver displayed almost normal gross features except a little heavier than the normal. Observations on absolute liver weight in group B disclosed moderately significant increase when compared to control as shown in table 1 and figure 1. Conversely group C animals depicted moderately significant decrease in absolute liver weights in comparison to group B animals as shown in table 1.

Observations on relative liver weight in group B animals exhibited from significant to moderately significant increase as compared to control group as depicted in table 2 and figure 1. Group C animals displayed moderately significant difference in relative liver weight when compared to lithium treated group B animals as shown in figure 1.

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 vacoulation both in pericentral and periportal areas. Cytoplasm depicted 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. Mononuclear cells were increased in number in the periportal and pericentral areas. Central and portal veins were markedly dilated and filled with blood and revealed great distortion of its wall. Sinusoidal obliteration was depicted with distortion of lobular architecture. Liver cells showed marked fatty change in the form of microvesicles of fat globules. There was disruption of the cell membrane with leakage of their contents into the sinusoidal space. The number of necrosed cells and fragmented nuclei were also increased in group B animals. Number of kupfffer cells also increased. These observations are depicted in figures 2-5.

The group C animals on coadministration of L-arginine + lithium disclosed dramatic results. The liver displayed more or less normal features on gross examination.

Table No.1: Mean values of absolute liver weights (g) in different groups of albino rats at variable time interval

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
C (n=15)	C1	Lithium carbonate + L-arginine	9.60 ± 0.51		
	C2			9.60 ± 0.51	
	C3				9.20 ±0.37

Statistical analysis of mean absolute liver weight between different groups

Statistical Comparison	P Value
A1 VS B1	P<0.05**
A1 VS C1	P>0.05*
B1 VS C1	P<0.05**
A2 VS B2	P<0.01***
A2 VS C2	P>0.05*
B2 VS C2	P<0.01***
A3 VS B3	P<0.01***
A3 VS C3	P>0.05**
B3 VS C3	P<0.01***

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

Table No.2: Mean values of relative liver weights (g) in different groups of albino rats at variable time interval

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)	3.30±0.32		
	A2			3.31±0.18	
	A3				3.56±0.13
B (n=15)	B1	Lithium carbonate treated	4.56±0.15		
	B2			5.30±0.17	
	B3				5.66±0.27
C (n=15)	C1	Lithium carbonate + L-arginine	3.94±0.20		
	C2			3.81±0.20	
	C3				3.63±0.16

Statistical analysis of mean relative liver weight between different groups

Statistical Comparison	P Value
A1 VS B1	P<0.05**
A1 VS C1	P>0.05*
B1 VS C1	P<0.05**
A2 VS B2	P<0.01***
A2 VS C2	P>0.05*
B2 VS C2	P<0.01***
A3 VS B3	P<0.01***
A3 VS C3	P>0.05**
B3 VS C3	P<0.01***

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

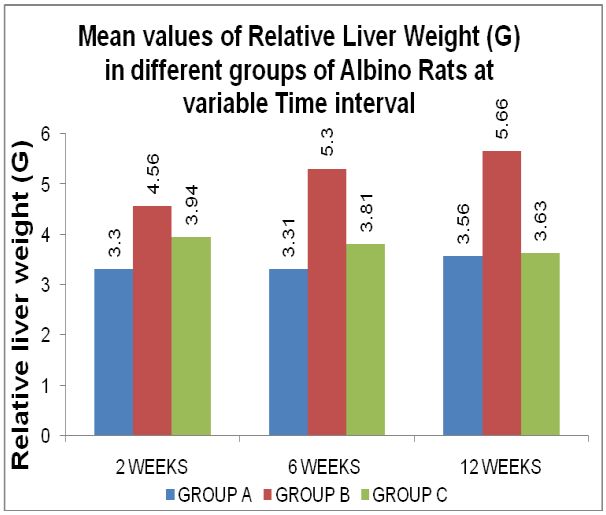

Figure No.1: Mean values of relative liver weight (g) in different groups of albino rats at variable time interval

The morphological examination of this group by H&E stained sections demonstrated nearly normal hepatic lobular architecture as compared to control except mild dilatation of central and portal veins. Hepatic cords showed regular arrangement and the width of the sinusoids was increased in comparison to lithium carbonate treated group.

Though the hepatocytes were still swollen but it was negligible. These displayed clear cell boundaries and the cytoplasm exhibited fine granularity. The nuclear membrane was intact and the nuclei distinct. Kupffer cells were prominent with inflammatory infiltrate but was comparable to that of control. All the pathological features almost disappeared. Histology of the liver cytoarchitecture came to touch normal.

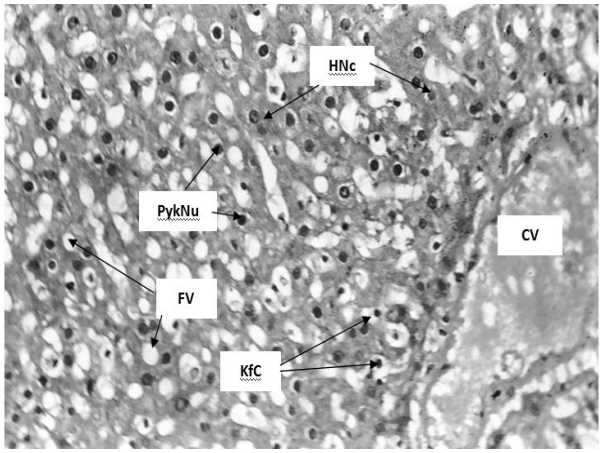

Figure No.2: Photomicrograph of H&E stained 4µm thick section of liver showing central vein (CV) with enlarged hepatocytes with large nuclei and clumping of chromatin, and cytoplasm showing small vacuoles of microvesicular fatty change (FV), prominent Kupffer cells (KfC) in the lining of sinusoids, pyknosis of nuclei (PykNu) and necrosed hepatocytes (HNc) around the central vein (CV) after two weeks of lithium carbonate treatment X 400

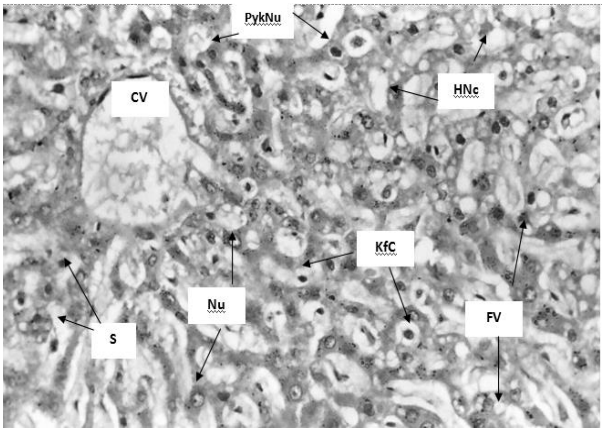

Figure No. 3: Photomicrograph of H&E stained 4µm thick section of liver showing enlarged hepatocytes with large nuclei (Nu) and highly congested and dilated sinusoids (S) with disruption and distortion of lobular architecture with pyknosis of nuclei (PykNu) and cytoplasm displaying small vacuoles of 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.

DISCUSSION

Before recording the morphometric findings on liver the effects of lithium and L-arginine on absolute and

relative weights of liver were recorded. The observations and results of the present study demonstrated that lithium produces hepatic damage and L-arginine reduces that damage in experimental rats. The dose of lithium used in this study was similar to that used on the kidney by Kolachi[6].

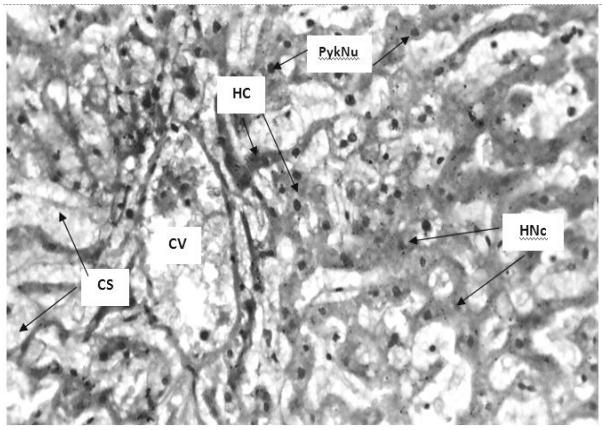

Figure No.4: Photomicrograph of H&E stained 4µm thick section of liver showing hepatic lobular architecture displaying central vein (CV) around which is depicted pyknosis of nuclei (PykNu), dilated and congested sinusoids (S) and distorted hepatic cords (HC) in the albino rats after 12 weeks of lithium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

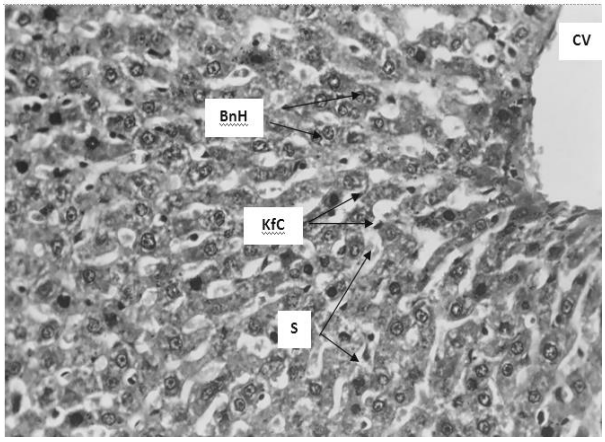

Figure No.5: Photomicrograph of H&E stained 4µm thick section of liver of albino rat showing reduced hepatocyte swelling, binucleate hepatocytes (BnH) and Kupffer cells (KfC) lying along the walls of sinusoids (S) in the lobular architecture showing central vein (CV) after 12 weeks of lithium carbonate and L-arginine treatment X 1000.

Liver gross examination of group B animals displayed significant alteration due to lithium toxicity. This observation correlates with the work of Sharma and Iqbal[14] and Chadha et al[5]. Group C animals exhibited on the other hand nearly normal results. The augmentation in absolute and relative liver weight was due to cellular hypertrophy, hyperplasia, swelling, hydropic degeneration, increased mononuclear cell iinfiltration, 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 Kumar et al[15], Nakayushi and Uemitse[16] and Chadha et al[5]. Group C animals displayed significant improvement as compared to group B. This could be due to significant diminution in the inflammatory changes, decreased cellular hyperplasia and hypertrophy, less fatty globules in liver parenchyma, less sinusoidal congestion and these changes were similar with the observations of Kubes et al[17], Lin et al[18] and Mahmoud et al[12].

The protective features of L-arginine displayed in group C treatment are also in conformity with the observations of Chattopadhyay et al.[19] who studied protective effect of L-arginine against necrosis and apoptosis induced by experimental ischemicon and reperfusion injury in rat liver. The above findings are also matching with the findings of Ozsoy et al.[20] who studied the effects L-arginine on liver damaged in experimental acute cholestasis: an immunohisto-chemical study.

CONCLUSION

This experimental and randomized study highlights the need to the orientation of an anti-psycotic drug lithium toxicity in the treatment of bipolar disorder and demonstrates that L-arginine should be added as an adjuvant along with lithium but still it needs further substantiation of the idea in the human trials.

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Address for Corresponding Author:
Dr. Saleem Ahmed Bhutto,
M Phil Scholar, BMSI, JPMC,
Karachi