

EFFECT OF LYCOPENE ON LITHIUM INDUCED NEPHROTOXICITY IN RATS

Somsubhra Pal and Shivalinge Gowda KP*

Department of Pharmacology, PES College of Pharmacy, Bangalore-560050, Karnataka,
India.

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*Correspondence for

Author

Dr. Shivalinge Gowda KP

Department of Pharmacology,
PES College of Pharmacy,
Bangalore-560050, Karnataka,
India.

ABSTRACT

The present study was carried out for the investigating the protective effects of lycopene on lithium induced nephrotoxicity in rats. Nephrotoxicity was induced by lithium carbonate (25 mg/kg lithium carbonate solution in 0.9% NaCl twice daily for 4 weeks). Lycopene was given in low, medium and high doses (10mg/kg, 30mg/kg and 50mg/kg respectively by oral gavaging for 4 weeks). Serum sodium, potassium, creatinine, blood urea nitrogen (BUN) were assessed after 4 weeks. Glutathione peroxidase, superoxide dismutase (SOD) and catalase content in kidney tissue were assessed and kidney tissue histopathology was performed. Lycopene in doses of 10, 30 and 50 mg/kg resulted in a decrease in serum sodium, potassium, creatinine

and BUN. Lycopene, being a potent antioxidant resulted in an increase in glutathione, SOD and catalase level in kidney tissue. Histopathological evaluation of kidney tissue revealed that the group treated with lithium carbonate only showed dilatation of blood vessels. Some renal tubules displayed casts. Glomerulus showed hypocellularity with increased Bowman's space. The group treated with low dose of lycopene showed normal cellularity. Some tubular epithelial cells showed hydropic changes. However groups treated with higher doses showed less damage to kidney. Lycopene seemed to offer effective protection against lithium induced nephrotoxicity probably due to its antioxidant status.

KEY WORDS: Lycopene, lithium carbonate, glutathione, catalase, creatinine.

INTRODUCTION

Nephrotoxic injury occurs due to damage to one or both of the kidneys from exposure to a toxic material. Nephrotoxicity can lead to acute renal failure, in which the kidneys lose their

ability to function, or chronic renal failure, in which there is slow deterioration of kidney function. If untreated, renal failure can cause death. Chronic exposure to drugs, occupational hazards, or environmental toxins can be causative of chronic interstitial renal diseases¹. Lithium is being used therapeutically for nearly 150 years. In the nineteenth century lithium salts were used for the treatment of gout and uric acid nephrolithiasis. The calming effect of lithium was observed on guinea pigs by Cade. Lithium showed encouraging results in ten manic patients in 1949. However, in the same year, the drug was withdrawn from U.S. market by the Food and Drug Administration (FDA) due to the death of several patients due to lithium intoxication. The studies on the effects of lithium as a mood stabilizer progressed slowly and only in 1970 FDA approved its use in the treatment of mania².

Lithium is also highly nephrotoxic. It causes impairment of tubular function (particularly the development of nephrogenic diabetes insipidus and renal tubular acidosis) and progressive chronic kidney disease³. Chronic lithium therapy can also cause hyperparathyroidism and hypercalcemia. The mechanisms of the chronic nephrotoxic effects of lithium, are poorly understood. As well as inhibiting GSK-3 β , lithium inhibits the activity of inositol monophosphatase, which results in depleted inositol level and inhibition of cell-cycle progression⁴. Accumulation of lithium in cells of the distal nephron and early collecting duct via ENaC could account for the chronic nephrotoxic effects.

The mechanisms underlying lithium-induced water diuresis and natriuresis are also not well established. The postulated mechanisms include inhibition of adenylyl cyclase, decreased AVP receptors density, and reduced expression of AQP2⁵. Administration of lithium for 25 days induced a reduced AQP2 expression in rat kidney medulla.

Lycopene is the most abundant carotenoid in human tissues (21-43% of all carotenoids). It is also present in human blood. Lycopene is found in tomatoes, water melons, pink grapefruits, pink guavas etc. Studies have revealed that Lycopene has potent anti-oxidant action. Lycopene quenches singlet oxygen. Research suggests that Lycopene may be beneficial in cardiovascular diseases, cancer, diabetes, osteoporosis and male infertility. As per the literature survey, no research work has been done on the protective actions of Lycopene on Lithium induced nephrotoxicity. Hence the present study has been undertaken to determine the protective effects of Lycopene on Lithium induced nephrotoxicity.

MATERIALS AND METHODS

Lithium carbonate tablets (300 mg) were procured from local medicine shop. Lycopene was procured from Avyukt Pharmaceuticals, Bangalore. Male albino wistar rats weighing 150-200 grams were procured from Sri Raghavendra Enterprises, Bangalore. The animals were housed under conditions of controlled temperature and 12 h day-night cycle, $25\pm 3^{\circ}\text{C}$ and 35%-60% humidity. The animals were fed standard chow diet and water ad libitum. The study has been referred to the ethical committee of PES College of Pharmacy and clearance has been obtained vide **Ref No- PESCP/IAEC/09/2012-13 and CPCSEA Reg No- 600/PO/c/02/CPCSEA dated-17-1-2012.**

Induction of nephrotoxicity and treatment protocol

Nephrotoxicity was induced with Lithium carbonate (25 mg/kg lithium carbonate solution in 0.9% NaCl twice daily)⁶ for 4 weeks. Lycopene was administered in three doses: low, medium and high doses respectively (10mg/kg, 30 mg/kg and 50 mg/kg)⁷ via oral gavage.

Sample collection

Blood was collected by intra-cardiac blood collection technique under general anaesthesia induced by Xylazine+Ketamine in eppendorf tubes. The collected blood was centrifuged at 5000 rpm for 10 minutes and blood serum was collected and used for the different estimations.

Biochemical parameters

Plasma sodium, potassium, BUN and creatinine were measured using commercial kits (ERBA diagnostic kits). Antioxidant parameters like Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and catalase were studied.

Estimation of glutathione peroxidase (GPx) in kidney

The reactive mixture consisted of 0.02 ml of 0.8 mM EDTA, 0.1 ml of 10 mM sodium azide, 0.1 ml of 2.5 mM H₂O₂, 0.2 ml of homogenate and was arrested by adding 0.5 ml of 10% TCA. The tubes were centrifuged at 2000 r.p.m. for 15 min. 3.0 ml of 0.3 mM of Disodium hydrogen phosphate and 1.0 ml of 0.04% DTNB [5,5-dithiobis (2-nitrobenzoic acid)] were added to the supernatant and develop color which was detected at 420 nm immediately. GPx activity was expressed as μ mole of the oxidized glutathione/min/mg protein⁸.

Estimation of SOD in kidney

The total SOD assay volume (3.0 ml) consisted of 1.5 ml of 59 Mm Tris-Cacodylate (Tris and Sodium cacodylate) buffer pH 8.2 (pH adjusted with 0.1 N Hcl), 0.3 ml of nitro blue tetrazolium (NBT) (1mM in water), 0.3 ml of Triton X-100 (0.01%), 0.8 ml water, 0.1 ml of sample and 0.01 ml of pyrogallol (60mM in water). A blank was run simultaneously consisting of 0.1 ml water instead of 0.1 ml of sample. Enzyme kinetic activity was recorded at 540 nm for three minutes and change in O.D/min (Δ O.D) was used to calculate % auto-oxidation inhibition to derive SOD units (U). One unit of SOD was defined as 50% inhibition of the auto-oxidation caused by a certain value of the enzyme. The results of SOD activity have been expressed as U/mg of protein⁹.

$$\text{SOD} = \frac{C \times \text{Total Volume} \times 1000}{50 \times \text{Sample Volume} \times \text{mg protein per ml}}$$

Unit: Units/ mg protein

Estimation of catalase in kidney

Catalase activity in rat kidney homogenate was measured colorimetrically at 570 nm. Briefly, the homogenized tissues were centrifuged at $569.5 \times g$ for 20 mins and supernatant diluted $\times 50$. A 1 ml of appropriately diluted enzyme preparation was rapidly mixed with reaction mixture containing 4 ml of H_2O_2 and 5 ml of phosphate buffer in a conical flask at RT. A 1 ml portion of reaction mixture was withdrawn and blown into 2 ml dichromate/acetic reagent at 60 sec. Interval and absorbance were read at 570 nm. Protein content was estimated using Biuret method as described by Gornal et al. (1949)¹⁰.

Statistical Analysis

Statistical analysis was performed using one way ANOVA and Tukey's post test using Graphpad Prism 6.0.

RESULTS

Effect of lycopene on serum sodium, potassium, BUN and creatinine

Serum sodium, potassium, BUN and creatinine had increased in the lithium treated groups. However treatment with lycopene in various doses (10mg/kg, 30 mg/kg and 50 mg/kg) decreased these values.

Effect of lycopene on kidney tissue glutathione peroxidase

Glutathione peroxidase (GPx) detoxifies free hydrogen peroxide, which is responsible for significant oxidative stress. In the lithium only treated group GPx showed decreased levels. In the groups treated with the various doses of lycopene, GPx levels showed increased value.

Effect of lycopene on kidney tissue SOD

SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Hence, they act as antioxidant defence in all cells exposed to oxidative stress. In the lithium only treated group decreased levels of SOD were observed. In the groups treated with the various doses of lycopene, SOD levels showed increased value.

Effect of lycopene on kidney tissue Catalase

In the lithium only treated group decreased levels of catalase were observed. In the groups treated with the various doses of lycopene, catalase levels had increased.

Table 1: Effect of vehicle, lithium and lycopene on serum sodium, potassium, creatinine and BUN in lithium induced nephrotoxicity in albino wistar rats

Group (n=6)	Treatment		Sodium (mEq/L)	Potassium (mmol/L)	BUN (mg/dL)	Creatinine (mg/dL)
	Dose and Route	Duration				
I	Rat feed and water	4 weeks	130.06±6.88	4.28±0.94	14.49±1.39	0.52±0.05
II	Li ₂ CO ₃	4 weeks	217.33±17.13 ^{#####}	16.33±2.36 ^{#####}	59.73±5.25 ^{#####}	1.87±0.08 ^{#####}
III	Li ₂ CO ₃ + Lycopene (10mg/kg)	4 weeks	186.76±17.14 ^{**}	12.10±2.31 ^{**}	49.88±3.48 ^{***}	0.80±0.06 ^{****}
IV	Li ₂ CO ₃ + Lycopene (30mg/kg)	4 weeks	158.19±13.51 ^{****}	9.05±0.97 ^{****}	35.46±3.16 ^{****}	0.65±0.03 ^{****}
V	Li ₂ CO ₃ + Lycopene (50mg/kg)	4 weeks	131.14±11.43 ^{****}	5.97±1.24 ^{****}	18.54±2.15 ^{****}	0.580.04 ^{****}

Lithium carbonate= Li₂CO₃ Lithium carbonate dose: 25 mg/kg Li₂CO₃ solution in 0.9% NaCl twice daily, Lycopene was given by oral gavaging.

Mean±SD (n=06). ^{****}P<0.0001, ^{***}P<0.001, ^{**}P<0.01, ^{*}P<0.05, ns=non-significant when compared to Lithium alone treated rats. ^{#####}P<0.0001 when compared to normal control rats. One-way ANOVA followed by Tukey's Post Test.

Table 2. Effect of vehicle, lithium and lycopene on kidney tissue catalase, SOD and Glutathione peroxidase in lithium induced nephrotoxicity in albino wistar rats.

Group (n=6)	Treatment		Catalase ($\mu\text{mole of H}_2\text{O}_2/\text{Sec}/\text{mg protein per ml}$)	SOD (Units/ mg protein)	GPx ($\mu\text{ mole of the oxidized GSH}/\text{min}\times\text{mg protein}$)
	Dose and Route	Duration			
I	Rat feed and water	4 weeks	15.65 \pm 1.78	21.21 \pm 3.19	37.37 \pm 4.16
II	Li ₂ CO ₃	4 weeks	6.36 \pm 1.35 ^{####}	5.83 \pm 1.81 ^{####}	15.53 \pm 2.41 ^{####}
III	Li ₂ CO ₃ + Lycopene (10mg/kg)	4 weeks	7.92 \pm 1.05 ^{ns}	13.10 \pm 3.47 ^{***}	22.12 \pm 3.34 [*]
IV	Li ₂ CO ₃ + Lycopene (30mg/kg)	4 weeks	9.56 \pm 0.85 ^{**}	15.89 \pm 2.69 ^{****}	25.92 \pm 2.59 ^{***}
V	Li ₂ CO ₃ + Lycopene (50mg/kg)	4 weeks	12.64 \pm 1.03 ^{****}	18.18 \pm 2.13 ^{****}	30.40 \pm 3.65 ^{****}

Mean \pm SD (n=06). ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns=non-significant when compared to Lithium alone treated rats. ####P<0.0001 when compared to normal control rats. One-way ANOVA followed by Tukey's Post Test.

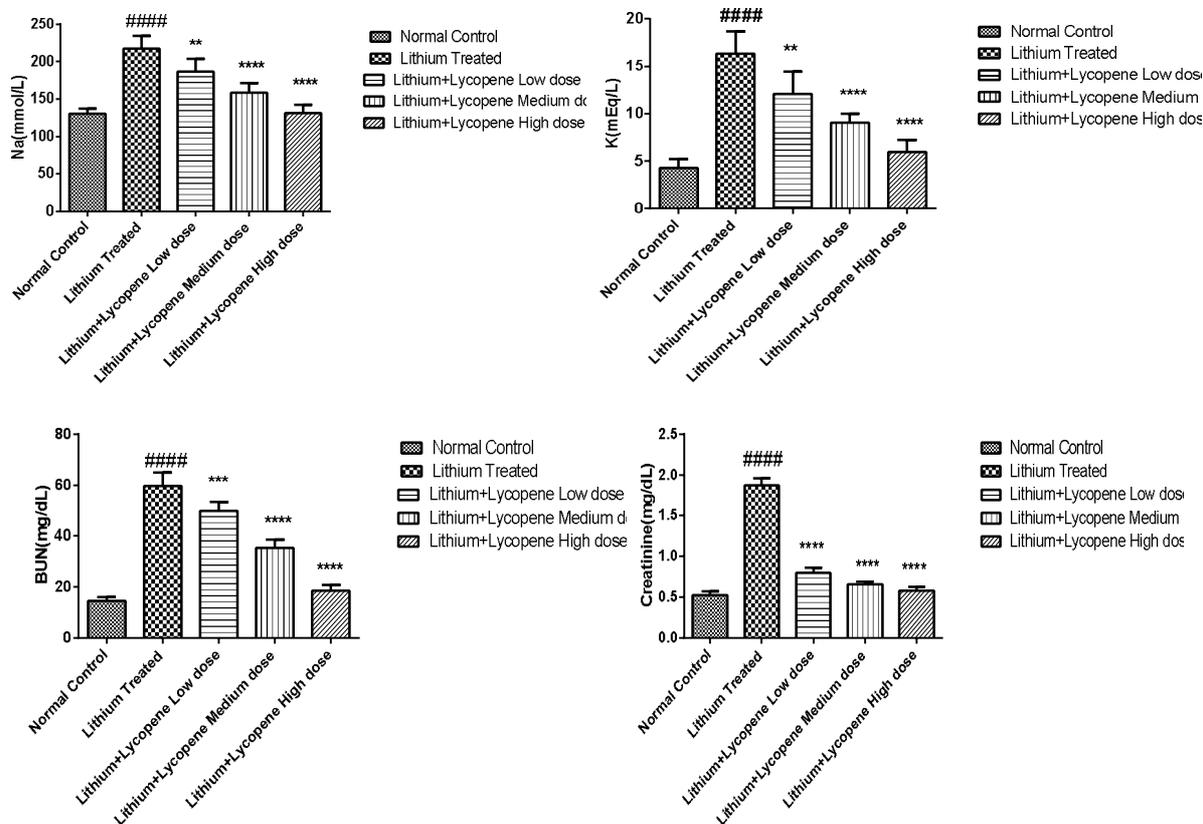


Figure 1: Effect of lycopene on biochemical and tissue parameters

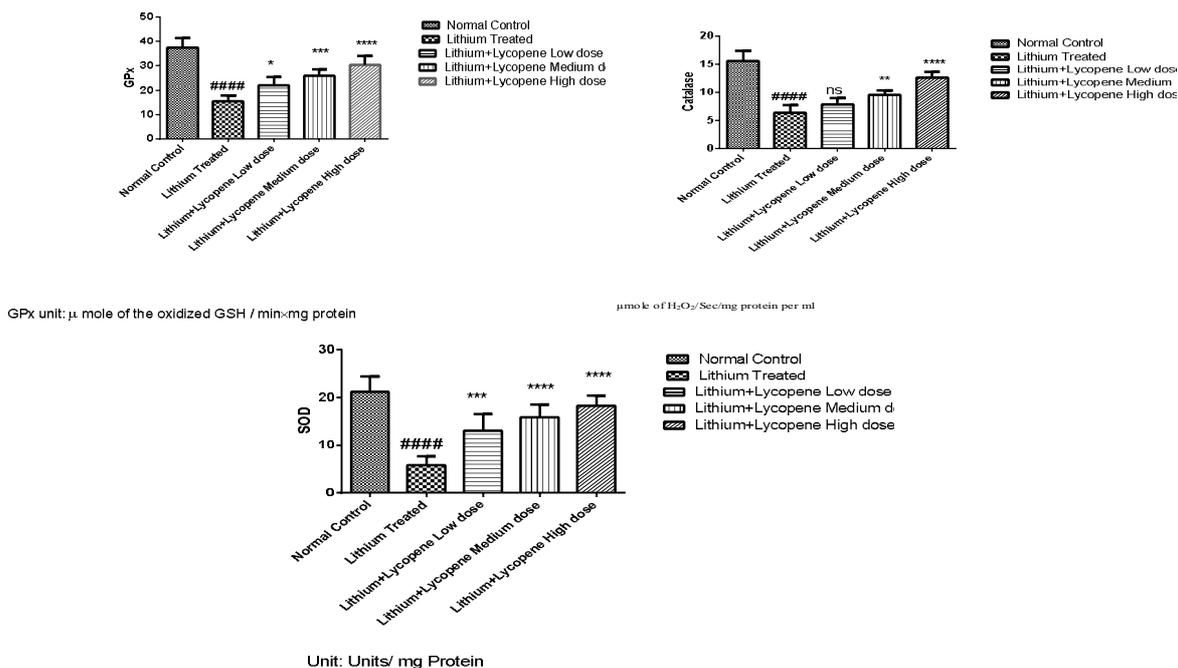
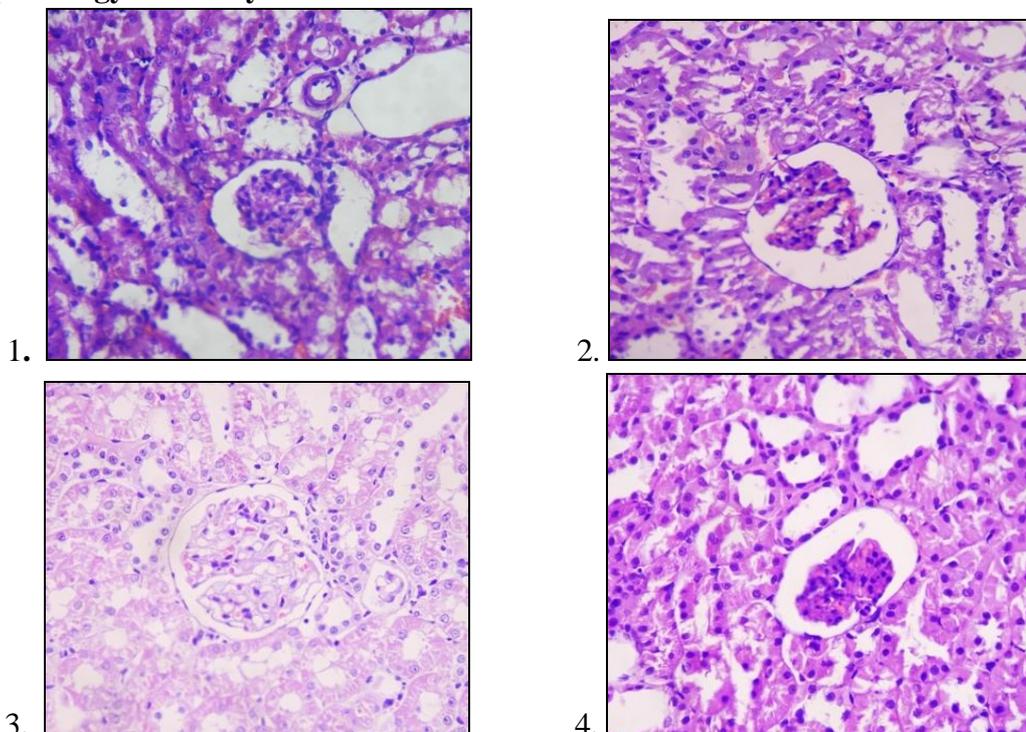
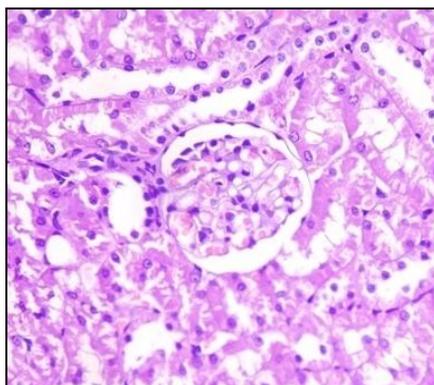


Fig 1. Effect of Lycopene on serum sodium, potassium, BUN, creatinine and tissue glutathione peroxidase, SOD and catalase in lithium induced nephrotoxicity. Each bar represents Mean \pm SD (n=06). ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns=non-significant when compared to Lithium alone treated rats. #####P<0.0001 when compared to normal control rats. One-way ANOVA followed by Tukey’s Post Test.

Histopathology of kidney tissue





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Figure 2. Photomicrographs of kidney tissue (400X)

Figure 2. Photomicrographs of kidney tissue

1. Histopathology of normal rat kidney showing intact architecture, normal cellularity of glomerulus, normal renal tubule and interstitium, 2. Histopathology of lithium control group showing kidney damage characterized by hypocellularity of glomerulus with increased Bowman's space, dilated blood vessels and tubular casts, 3. Histopathology of lycopene low dose (10mg/kg) treated group showing normal cellularity of glomerulus but dilated and congested blood vessels and inflammatory infiltrations in interstitium, 4. Histopathology of lycopene medium dose (30mg/kg) treated group showing hypocellularity of glomerulus with increased Bowman's space. Some tubular epithelial cells show hydropic changes, 5. Histopathology of lycopene high dose (50mg/kg) treated group showing normal cellularity, some tubular epithelial cells show hydropic changes.

DISCUSSION

Lithium chloride and Lithium carbonate are used to treat manic-depressive disorder (bipolar disorder). It works to stabilize the mood and reduce extremes in behavior by restoring the balance of neurotransmitters in the brain. But it is highly nephrotoxic. Most of the nephrotoxic drugs produce renal toxicity due to generation of free radicals. Thus generated free radicals destroy the inbuilt protective mechanism, resulting in the nephrotic damage and necrosis.

In this study, the rats (group II) administered with lithium chloride in nephrotoxicity model has shown significant increase in the serum sodium and potassium compared to normal control group. Lithium is responsible for tubular cell necrosis, which is largely confined to the proximal convoluted tubule and pars recta. Several functional changes in the tubular cell membrane occur with lithium exposure. Expression of sodium transporters in proximal tubules (PT) and Na^+K^+ -ATPase were altered, which may be due to interference in ATP

production. Further, there is toxic damage to the brush border membrane (BBM) present in renal proximal tubule, which is responsible for reabsorption of Na^+ and K^+ ions. Thus, there is reduced reabsorption of Na^+ and K^+ ions leading to increased excretion in urine¹¹.

The significant increase in serum creatinine levels in lithium induced rats when compared to normal rats may be due to structural damage of the podocytes, pedicels of the visceral layer of the Bowman's capsule and the endothelium of the glomerular capillaries of the nephron. This disturbs the glomerular filtration of creatinine. Hence creatinine excretion gets decreased and it remains in the blood. Hence, there is an increase in serum creatinine level. The rats treated with lycopene and lithium have shown significant fall in the serum creatinine level when compared to lithium only treated rats. This protective effect of lycopene may be due the prevention of the damage of the glomerular filtration apparatus and the normal filtration of creatinine across the glomerulus.

The rats treated with lithium have shown significant increase in the serum BUN concentration when compared to normal rats. This is due to kidney damage caused by lithium. The nephron loses its capacity to excrete urea. This is due to structural damage of the glomerular filter leading to the accumulation of urea in serum. Decreased serum BUN levels in lycopene treated rats indicate nephroprotection.

Glutathione peroxidase (GPx), an enzyme dependent on the micronutrient selenium (Se), plays a critical role in the reduction of lipid and hydrogen peroxides. If GPx activity is decreased, more hydrogen peroxide is present, which leads to direct tissue damage and activation of nuclear factor- κB -related inflammatory pathways. The antioxidant system was deranged after treatment with Lithium. Oxidative stress and subsequently cellular damage due to lithium could decrease glutathione level which was seen in Lithium only treated group. In the lycopene treated groups the glutathione levels had increased indicating probable nephroprotection due to the antioxidant status of lycopene. Furthermore, histopathological evaluation of rat kidney confirmed that lycopene conferred considerable nephroprotection. In rats treated with lithium a decreased level of SOD was seen as compared to normal rats. This is because lithium induced nephrotoxic effect on kidney causes reduction in SOD levels, which act as free radical scavengers. This shows redox imbalance and is due to excessive consumption of SOD by the enormous amount of free radicals that are produced due to nephrotoxic action of lithium, thus depleting the SOD concentration. Rats treated with lycopene and lithium showed a significant increase in SOD levels as compared to

nephrotoxic rats. This may be due to the anti oxidant activity of lycopene, which helps in scavenging the free radicals. Further, there was a significant reduction in catalase levels in rats treated with lithium as compared to normal rats. Catalase is an anti oxidant enzyme which catalyzes the breakdown of hydrogen peroxide into hydrogen and water. In lithium induced nephrotoxicity the catalase levels are depleted due to excessive consumption of catalase as well as due to mitochondrial damage. In rats treated with lycopene and lithium, the catalase levels increased significantly, showing nephroprotective action of lycopene. Lycopene seemed to be effective in nephrotoxicity, which is probably due to its antioxidant status. Further research is warranted to understand the molecular mechanism of lycopene in lithium induced nephrotoxicity.

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