

AMELIORATIVE EFFECT OF CHITOSAN AGAINST LITHIUM CARBONATE SUBACUTE TOXICITY ON KIDNEY AND PARATHYROID GLAND OF ADULT MALE ALBINO RATS

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ABSTRACT

Background: Lithium-containing drugs are the recommended treatment for bipolar disorder. **Aim of the work:** The current study aimed to assess the toxic effects of lithium carbonate on the kidneys and parathyroid glands and evaluate the possible protective effect of chitosan. **Material and methods:** Sixty adult male albino rats were divided into six groups of ten rats each. Group I (negative control group). Group II: 200 mg/kg/day of chitosan was administered orally. Group III: 25 mg/kg/day of lithium carbonate was administered orally. Group IV: Rats were given chitosan (200 mg/kg/day) and lithium carbonate (25 mg/kg/day) orally. Group V: 50 mg/kg/day of lithium carbonate was administered orally. Group VI: Rats were given chitosan (200 mg/kg/day) and lithium carbonate (50 mg/kg/day) orally. After 4 weeks, blood samples were collected then, the rats were sacrificed after being anaesthetized. Kidneys were dissected for histopathological and immunohistochemical studies. **Results:** Lithium toxicity induced a significant statistical increase in the serum levels of urea, creatinine, parathormone hormone, and calcium. Also, there was a significant statistical decrease in TAC. The co-administration of chitosan with lithium resulted in an improvement in the biochemical parameters. Some histopathological changes were observed in the kidneys of lithium-treated rats. These changes were less evident when chitosan was co-administered. There was high positive TNF α expression in the kidneys of lithium-treated rats. Less TNF α expression was observed when chitosan was co-administered. **Conclusion:** Lithium carbonate subacute toxicity induced renal damage, hyperparathyroidism and hypercalcemia. The use of chitosan can reduce the negative effects of lithium due to its antioxidant and anti-inflammatory properties.

Keywords: lithium Nephrotoxicity, Oxidative Stress, Parathormone, TNF α

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INTRODUCTION

Lithium carbonate is thought to be the first line of treatment for bipolar illness that is accompanied by mania and depression (Ge and Jakobsson, 2019). Lithium is also useful in treating herpes virus infection, seborrhoeic dermatitis, and neurological illnesses (Saad et al., 2017; Ge and Jakobsson, 2019). Additionally, some research looked into how lithium might affect the inhibition of tumor growth (Bgatova et al., 2014).

Lithium is readily absorbed from the digestive system and reaches its maximum therapeutic level in two to four hours. The volume of distribution of lithium varies from 0.6 to 0.9 L/kg. Lithium is water soluble and spreads throughout the body, although its distribution

to the thyroid, kidney, and bone is significantly higher. Lithium has weak plasma protein binding. Lithium is primarily excreted by the kidneys (Abdel Hamid et al., 2020).

The majority of lithium intoxications are related to its therapeutic applications because it has a narrow therapeutic index. The therapeutic plasma concentration of lithium is 0.6–1.2 mEq/L (Ufelle and Barchowsky, 2019). Long-term therapeutic doses of lithium have various harmful effects (Saad et al., 2017).

Neurotoxicity, urethral cell destruction, skin complications, hyper- and hypothyroidism are among the harmful effects of lithium. Also, diabetes insipidus and teratogenicity were reported (Gitlin, 2016).

Nephrotoxicity has been reported to be the most common adverse effect of lithium (*Jing et al., 2022*). Lithium poisoning can also result in parathyroid disease (*Pattan et al., 2021*).

It was suggested that oxidative stress is the mechanism by which lithium induces its deleterious effects (*Abdel Hamid et al., 2020*).

Consequently, to combat lithium oxidative damage, supplementation with natural, safe antioxidant compounds is required (*Saad et al., 2017*).

Therefore, several pharmacological and natural compounds have been studied for their potential to protect tissues from lithium carbonate toxicity as vitamin E (*Bondok et al., 2018*), selenium (*Badawy et al., 2022*), and green tea extract (*Zaki et al., 2022*).

Chitin, the parent compound of chitosan, is the second-most common polymer found in nature. It is a linear polysaccharide composed of poly- β -[1,4]-N-acetyl-D-glucosamine units (*Kou et al., 2022*).

Chitin is mostly found in the exoskeletons of crustaceans. Chitin can be extracted via many processes, such as deproteinization and demineralization (*Jiménez-Gómez and Cecilia, 2020*).

Chitosan is described as the N-deacetylated form of chitin. Moreover, it exists by nature in some fungi's cell walls. It consists of repetitive hydrophilic units (D-glucosamine units) and residual hydrophobic units (N-acetyl-D-glucosamine units) (*Yarnpakdee et al., 2022*).

Chitosan exhibits favorable biological properties, including antifungal, anticancer, bacteriostatic, and anti-inflammatory effects (*Aranaz et al., 2021; Ke et al., 2021*). It is believed that chitosan and its derivatives are potent and promising antioxidants (*El-Araby et al., 2024*).

Despite numerous studies on the toxicity of lithium on the body's organs, the precise mechanism of lithium-induced parathyroid illness remains unclear (*Mahmoodnia, 2023*). Additionally, this study is the first to look into chitosan's potential protection against parathyroid illness brought on by lithium toxicity.

THE AIM OF THE WORK

The present study aimed to assess the toxic effects of sub-acute exposure to lithium carbonate on the kidney and parathyroid glands by measuring serum levels of urea, creatinine, parathyroid hormone, calcium and total antioxidant capacity. The histopathological changes and immunohistochemical expression of TNF alpha in kidney tissues were evaluated.

Also, it aimed to investigate any potential protective benefits of chitosan on lithium-induced toxic effects on the kidney and parathyroid glands.

MATERIAL AND METHODS

Type of study: Experimental animal study.

Experimental animals:

In this study, sixty adult male albino rats weighing 200 ± 20 gm were used. They were supplied by the Animal Facility Centre of the Faculty of Medicine, Sohag University. The rats were kept in metal cages at a room temperature of 21 ± 3 °C and 12-hour light/dark cycles. Animals were fed with standard pellet food and water. The rats were given a week to adjust to the laboratory conditions before beginning the treatment protocol.

Ethical consideration:

The ethics and husbandry conditions of animal research were considered according to the guide of care and use of laboratory animals approved by the Medical Research Ethics Committee of the Faculty of Medicine, Sohag University. The protocol got approval from Sohag Institutional Animal Care and Use Committee at the Faculty of Medicine, Sohag University (Sohag-5-9-2/2024-01).

Drugs and chemicals:

Lithium carbonate and chitosan were purchased from Universal Fine Chemicals Company in powder form. They were dissolved in distilled water. Kits of urea, creatinine and calcium were purchased from Beckman Coulter Inc. Company. Kits of parathormone hormone (PTH) were purchased from Cobas Company. Kits of total antioxidant capacity (TAC) were purchased from Bio diagnostic company. Hematoxylin and eosin stains were purchased from Alpha Chemika.

Study design:

The rats were divided randomly into six groups of 10 animals each.

Group I (negative control group): The rats were given a basal diet and distilled water.

Group II (chitosan-treated group): the rats received 200 mg/kg/day of chitosan (CS) dissolved in distilled water (*Aboulthana and Ibrahim, 2018; Abou Zaid et al., 2019*).

Group III received 25 mg/kg/day of lithium carbonate (LC) dissolved in distilled water (*Topçu, 2020; Ommati et al., 2021*), which is approximately 1/20 of the LD₅₀ of oral LC in rats (525 mg/kg) (*Badawy et al., 2022*).

Group IV: rats received CS (200 mg/kg/day) and LC (25 mg/kg/day) after 1 hour. Group V: LC was given in a dose of 50 mg/kg/day (*Topçu, 2020; Ommati et al., 2021*), which is approximately 1/10 of the LD₅₀ of oral LC in rats (525 mg/kg) (*Badawy et al., 2022*).

Group VI: The rats received CS (200 mg/kg/day) and LC (50 mg/kg/day) one hour apart.

Chitosan and lithium carbonate were administered daily by oral gavage for 4 weeks. No mortality occurred in the study groups. The doses of CS and LC were prepared freshly every day and were modified every week to account for variations in body weight. At the end of the study, the rats were anaesthetized then sacrificed 24 hours after the last dose.

The rationale of dose selection

The doses of LC were used according to *Topçu, (2020)*, who studied the toxic effects of lithium carbonate on thyroid hormones, parathormon, calcium levels and thyroid tissue of rats. Also, *Ommati et al (2021)* used the same doses of LC to study lithium-induced nephrotoxicity. The dose of chitosan was selected in accordance with *Aboulthana and Ibrahim (2018)*, who studied the protective effect of chitosan against lithium-induced renal toxicity in rats. Also, *Abou Zaid et al. (2019)* used the same dose of chitosan when they studied the protective effects of chitosan against diethylnitrosamine-induced hepatotoxicity in rats.

Collection of samples**Blood samples**

Samples of blood were drawn into sterile, dry tubes. The serum was extracted by

centrifuging the sample for ten minutes at 4000 rpm. Part of the serum was put into sterile screw-capped polypropylene tubes and examined for parathyroid and kidney functions. The other part was kept frozen at -70 °C, until the serum TAC analysis.

Tissue samples

The left kidneys were dissected, washed with cold, isotonic saline and prepared for histopathological and immunohistochemical studies. Parathyroid glands could not be dissected due to the very small size (1 mm) and weight (1mg) (*Mense and Rosol, 2018*).

Biochemical analysis

The enzymatic colorimetric approach was used to measure the levels of serum urea, as directed by the manufacturer (*Marchenko et al., 2015*). The urea concentration in the sample is directly correlated with the rate of change in absorbance at 340 nm. It was expressed as mg/dl. The kinetic reaction method was used to measure serum creatinine following the manufacturer's instructions (*Pundir et al., 2019*). The creatinine concentration in the sample is directly correlated with the rate of change in absorbance at 520/800 nm. It was expressed as mg/dl. Urea and creatinine were measured by Beckman Coulter AU480 Clinical Chemistry System (fully automated, analytical principles: spectrophotometry and potentiometry) present in Clinical Pathology Department – Sohag University Hospitals.

The sandwich-type immunoassay approach was used to quantify parathyroid hormone (*Smit et al., 2019*). It was tested using the Cobas E411, a fully automated analyzer found at the Clinical Pathology Department of Sohag University Hospitals. It uses Electro-Chemiluminescence (ECL) technology for immunoassay. It was measured as pg/ml.

Serum calcium was measured according to the manufacturer's instructions (Beckman Coulter). The absorbance was measured bichromatically at 660/700 nm. It was measured as mg/dl. Beckman Coulter AU480 Clinical Chemistry System was used to measure serum calcium level.

Total antioxidant capacity was measured according to manufacturers' instructions using colorimetric method (*Ciuti and Liguri, 2017*). It was measured as mM/l. It was

measured by UV 2300 spectrophotometer (USA). It is a Split Beam Scanning UV-Vis spectrophotometer which has a wave length range of 190-1100 nm, present in Faculty of Science, Sohag University.

Histopathological and immunohistochemical examination

Histopathological examination

The renal specimens were prepared according to the method described by **Bancroft and Layton, (2018)**. Kidneys were fixed in 10% formalin for 48 hours, dehydrated using various dilutions of alcohol solutions then, embedded in paraffin blocks. Five μm sections were cut and stained with hematoxylin and eosin. The slides were examined by light microscope then photographed.

Immunohistochemistry Staining protocol:

For immunohistochemical staining, the inflammatory marker tumor necrosis factor α (TNF- α) was used. Paraffin sections from rats' kidneys were stained by immunohistochemistry according to **Jammal et al (2015)**. Sections were applied to positively charged slides. Mouse Anti- TNF alpha Monoclonal Antibody (Elabscience Cat# E-AB-22159, Dilution: 1:50) was used. The previously mentioned antibody was added to sections from each study group, then the reagents required for the ABC technique (Vector labs, Vectastain ABC-HRP kit) were added. Peroxidase was used to identify the marker expression, and Sigma created diaminobenzidine (DAB) for staining to define the antigen-antibody complex. Instead of primary or secondary antibodies, non-immune serum was used to integrate the negative controls. Immuno-stained sections were examined and photographed using various magnification powers by Leica microscope (CH9435 Hee56rbrugg) (Leica Microsystems, Switzerland).

STATISTICAL ANALYSIS

To ascertain if the data were normally distributed, the Kolmogorov-Smirnov test of normality was applied. The results of this test indicated that the data were normally distributed; thus, descriptive analysis, one-way ANOVA, and post-hoc testing were conducted to investigate the inter-group relationship. A p-value of less than 0.05

indicates significance. IBM SPSS Statistics 20 for Windows was used to analyze the data.

RESULTS

Biochemical results

As regards kidney functions, there was a significant increase in the serum levels of urea and creatinine in groups III and V in comparison to the control group. However, the rise was significantly higher in group V (LC 50 mg/kg) compared to group III (LC 25 mg/kg). Administration of chitosan restored the serum levels of urea and creatinine to normal in groups IV and VI (**Table 1**).

Concerning parathyroid hormone (PTH) and calcium, there was a significant increase in their levels in groups III and V in comparison to the control group. However, the rise was significantly higher in group V (LC 50 mg/kg) compared to group III (LC 25 mg/kg). Administration of chitosan restored the serum levels of PTH and calcium to normal in groups IV and VI (**Table 2**).

As regards total antioxidant capacity (TAC), there was a significant decrease in the serum levels of TAC in groups III and V in comparison to the control group. However, the level of TAC was lower in group V (LC 50 mg/kg) compared to group III (LC 25 mg/kg). Administration of chitosan restored the serum levels of TAC to normal in groups IV and VI (**Table 3**).

Histopathological and immunohistochemical results

In the current study, examination of H&E stained renal cortex sections of groups I and II revealed normal histological structure (**Figures 1a, 1b**).

Administration of lithium carbonate at a dose of 25 mg/kg/day (group III) induced histopathological changes in the renal cortex in the form of lobulated glomerulus and widening of urinary space, widened renal tubules with degenerated epithelial cells and pyknotic nuclei, and loss of the brush border of proximal tubules. There was congestion between the tubules (**Figure 1c**).

Co-administration of chitosan with lithium (group IV) produced fewer histopathological changes in the renal cortex. There was slight congestion in the glomeruli, proximal and distal tubules were less dilated in comparison to the previous group with few degenerated

epithelial cells and slight capillary congestion in between the tubules (**Figure 1d**).

More extensive changes were seen with the higher dose of lithium (group V). There was congestion of glomerular capillaries and a widening of the urinary space. The proximal and distal renal tubules were dilated with vacuolated epithelial cells and loss of the brush border of the proximal tubules. There was extensive congestion around the tubules (**Figure 1e**).

Less histopathological changes were observed when chitosan was co-administered with lithium in group VI. There was dilatation of the urinary space and less congestion of the glomeruli. The renal tubules were less dilated compared to the previous group with few degenerated and vacuolated epithelial cells. Some proximal tubules restored the brush border. There was less congestion between the tubules (**Figure 1f**).

Regarding TNF α reactivity in kidney tissue sections (Renal Cortex Area), there was scarce cytoplasmic reactivity along the glomeruli and the cells lining the renal tubules in group (I) and group (II) (**Figures 2a, 2b**).

There was high positive cytoplasmic TNF α expression along the glomerulus in addition to nuclear reactivity along cells lining renal tubules in group (III) (**Figure 2c**). While group IV showed few positive cytoplasmic TNF α reactivity along the glomerulus of renal corpuscle and renal tubules lining cells (**Figure 2d**).

Renal cortex sections from group (V) revealed the highest positive nuclear TNF α reaction to glomerulus and cells lining renal tubules (**Figure 2e**). However, renal cortex sections from group (VI) exhibited moderate positive cytoplasmic TNF α reactivity along the glomerulus of renal corpuscle alongside renal tubules lining cells (**Figure 2f**).

Table (1) Comparison of serum urea and creatinine levels between the studied groups.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
	Mean \pm SD*	Mean \pm SD*
Group I (control group)	31 \pm 6.15 ^{c e}	1.12 \pm 0.28 ^{c e}
Group II (CS)	32.4 \pm 3.72 ^{c e}	1.26 \pm 0.34 ^{c e}
Group III (LC25)	42.2 \pm 8.13 ^{abde}	1.97 \pm 0.46 ^{abde}
Group IV(LC25+ CS)	29.9 \pm 6.14 ^{c e}	1.34 \pm 0.36 ^{ce}
Group V (LC50)	50.7 \pm 4.85 ^{abcd f}	2.61 \pm 0.54 ^{abcd f}
Group VI (LC50 + CS)	35.1 \pm 4.61 ^e	1.64 \pm 0.64 ^e
p-value	< 0.001	< 0.001

*SD: standard deviation. #significant p-value < 0.05. ^a significant when compared to group I. ^b significant when compared to group II. ^c significant when compared to group III. ^d significant when compared to group IV. ^e significant when compared to group V. ^f significant when compared to group VI. CS: chitosan. LC25: lithium carbonate at a dose of 25 mg/kg/day. LC50: lithium carbonate at a dose of 50 mg/kg/day.

Table (2) Comparison of serum parathyroid hormone and serum calcium levels in the studied groups.

Groups	Parathyroid hormone (pg/ml)	Calcium (mg/dl)
	Mean \pm SD*	Mean \pm SD*
Group I (control group)	7.46 \pm 2.5 ^{ce}	10.71 \pm 1.57 ^{ce}
Group II(CS)	7.01 \pm 1.73 ^{ce}	9.94 \pm 1.55 ^{ce}
Group III (LC25)	13.27 \pm 2.40 ^{abdef}	14.6 \pm 3.24 ^{abdef}
Group IV (LC25+ CS)	6.59 \pm 1.75 ^{ce}	8.95 \pm 1.26 ^{ce}
Group V (LC50)	16.8 \pm 4.42 ^{abdef}	18.7 \pm 3.86 ^{abdef}
Group VI (LC50 + CS)	8.12 \pm 2.11 ^{ce}	10.26 \pm 1.83 ^{ce}
p-value	< 0.001	< 0.001

*SD: standard deviation. #significant p-value < 0.05. ^a significant when compared to group I. ^b significant when compared to group II. ^c significant when compared to group III. ^d significant when compared to group IV. ^e significant when compared to group V. ^f significant when compared to group VI. CS: chitosan. LC25: lithium carbonate at a dose of 25 mg/kg/day. LC50: lithium carbonate at a dose of 50 mg/kg/day.

Table (3) Comparison of total antioxidant capacity level in the studied groups.

Groups	Total antioxidant capacity (mM/l)
	Mean \pm SD*
Group I (control group)	0.045 \pm 0.009 ^{ce}
Group II (CS)	0.047 \pm 0.012 ^{ce}
Group III (LC25)	0.031 \pm 0.008 ^{abdef}
Group IV(LC25+ CS)	0.049 \pm 0.006 ^{ce}
Group V(LC50)	0.020 \pm 0.005 ^{abdef}
Group VI (LC50 + CS)	0.051 \pm 0.005 ^{ce}
p-value	< 0.001 [#]

*SD: standard deviation. #significant p-value < 0.05. ^a significant when compared to group I. ^b significant when compared to group II. ^c significant when compared to group III. ^d significant when compared to group IV. ^e significant when compared to group V. ^f significant when compared to group VI. CS: chitosan. LC25: lithium carbonate at a dose of 25 mg/kg/day. LC50: lithium carbonate at a dose of 50 mg/kg/day.

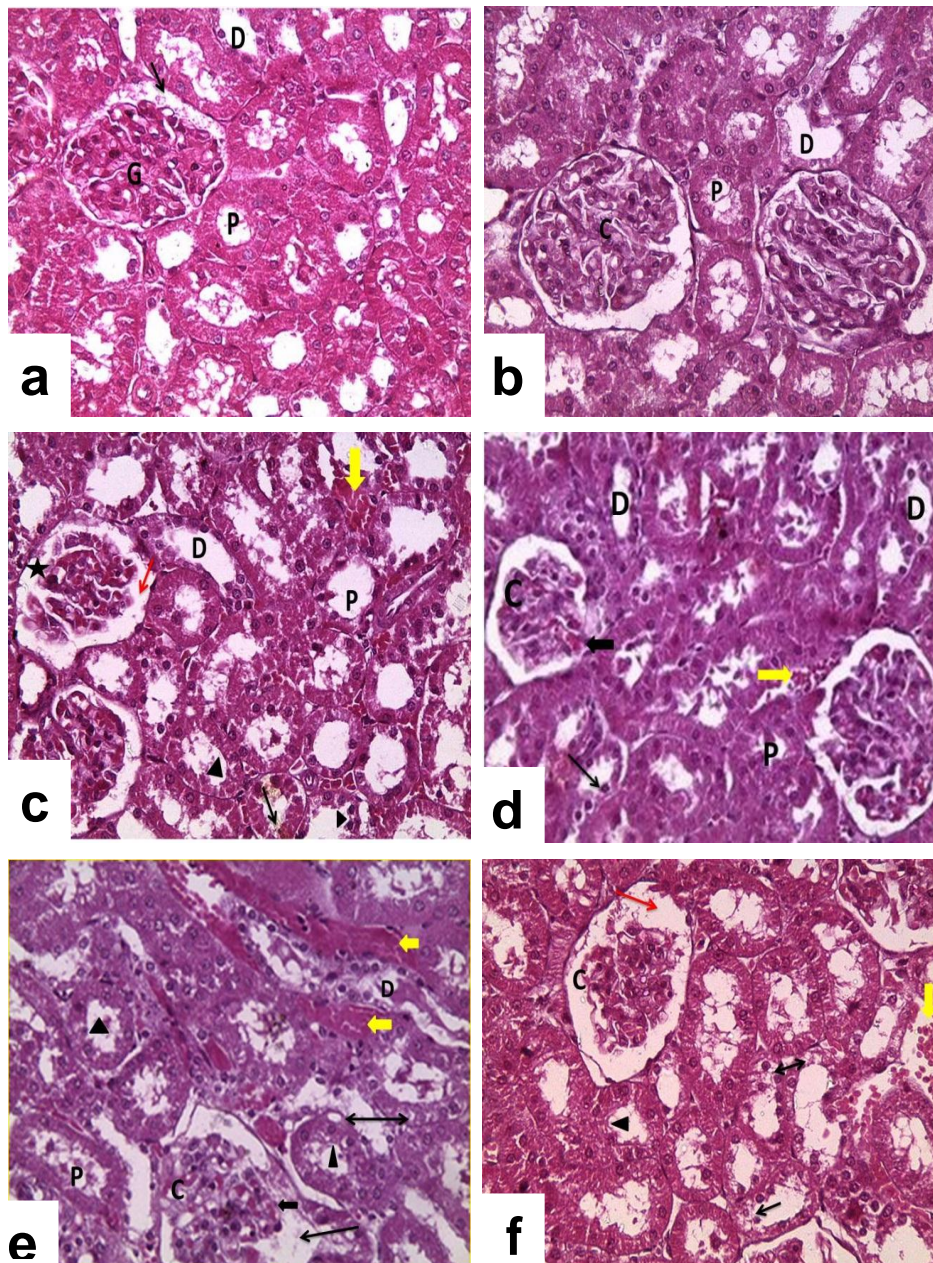


Figure (1): Photomicrographs presented hematoxylin and eosin stained kidney tissue sections (Renal Cortex Area), Magnification Power= $\times 400$. a: Renal cortex section of group I showing renal corpuscles with normal glomerular capillaries (G) and urinary space (arrow), proximal (P) and distal (D) convoluted tubules with normal histological structure. b: Renal cortex section of group II showing the renal corpuscle (C), proximal (P) and distal (D) convoluted tubules with normal histological structure. c: Renal cortex section of group III showing lobulated glomerulus (star) and widening of urinary space (red arrow), widened renal tubules (P, D) with degenerated epithelial cells and pyknotic nuclei (black thin arrow), loss of brush border of proximal tubules (arrow head). There is congestion between the tubules (yellow thick arrow). d: Renal cortex section of group IV showing the renal corpuscle (C) is more or less as control group with slight congestion in the glomeruli (black thick arrow). Proximal and distal tubules (P, D) are less dilated in comparison to the previous group with few degenerated epithelial cells (thin arrow) and slight capillary congestion in between the tubules (yellow thick arrow). e: Renal cortex section of group V showing the renal corpuscle (C) with congestion of glomerular capillaries (black thick arrow) and widening of the urinary space (red arrow). The proximal and distal renal tubules (P, D) are dilated with vacuolated epithelial cells (double headed arrow), and loss of brush border of the proximal tubules (arrow head). There is extensive congestion around the tubules (yellow thick arrow). f: Renal cortex section of group VI showing the renal corpuscle (C) with dilatation of the urinary space (red arrow), less congestion of the glomeruli, the renal tubules are less dilated compared to the previous group with few degenerated (black thin arrow) and vacuolated (double headed arrow) epithelial cells. Some proximal tubules restored the brush order (arrow head). There is less congestion between the tubules (yellow thick arrow).

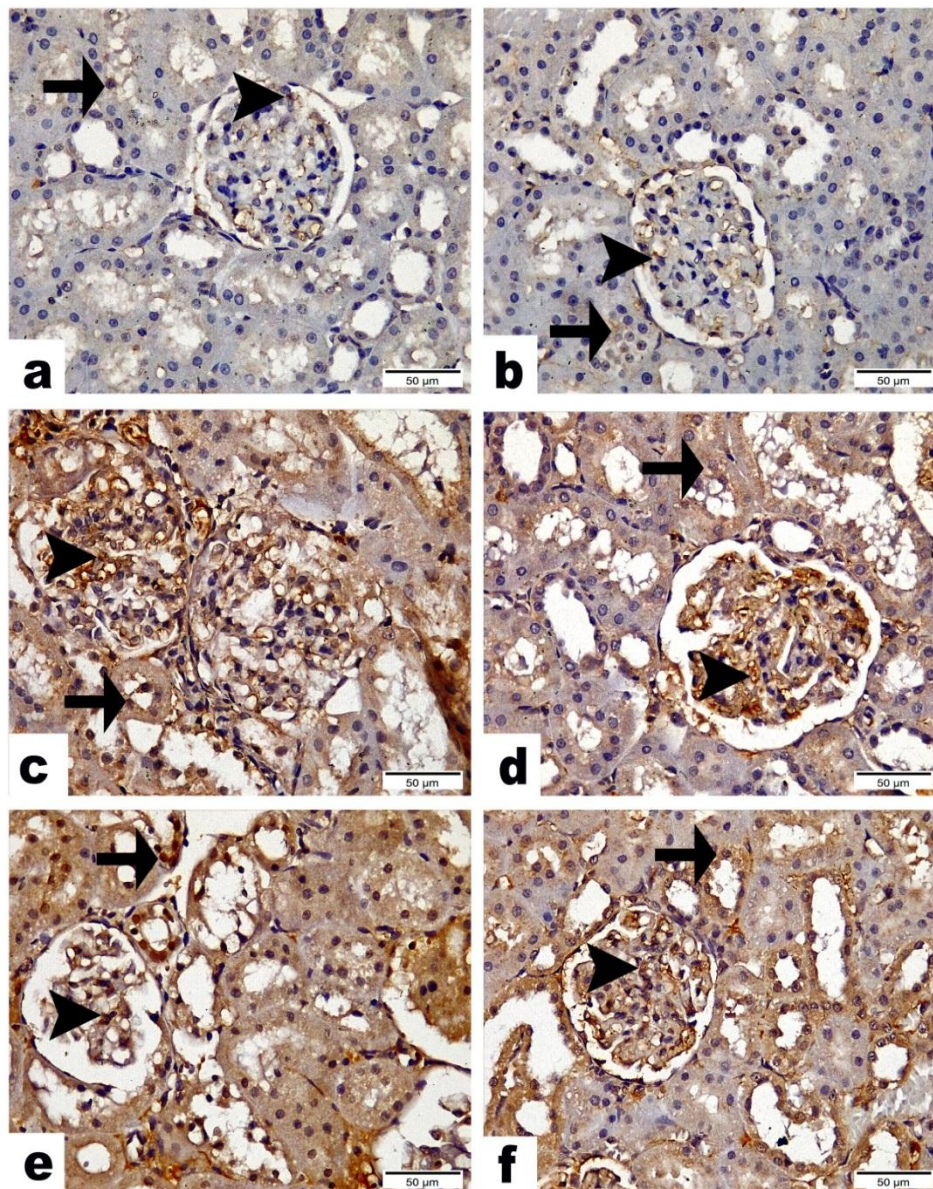


Figure (2): Photomicrographs presented the reactivity of TNF α in kidney tissue sections (Renal Cortex Area) between inspected groups (TNF α Antibody, Magnification Power= x400 & Scale Bar= 50 μ m):(a) Renal cortex section from group (I) signifying scarce cytoplasmic TNF α reactivity along glomerulus of renal corpuscle (arrow head) as well as renal tubules lining cells (arrow). (b) Renal cortex section from group (II) exhibiting expression identical to GP (I) as scarce cytoplasmic TNF α reactivity along glomerulus of renal corpuscle (arrow head) as well as renal tubules lining cells (arrow). (c) Renal cortex section from group (III) exposing high positive cytoplasmic TNF α expression along glomerulus (arrow head) in addition to nuclear reactivity along cells lining renal tubules (arrow). (d) Renal cortex section from group (IV) displaying few positive cytoplasmic TNF α reactivity along glomerulus of renal corpuscle (arrow head) besides renal tubules lining cells (arrow). (e) Renal cortex section from group (V) revealing the highest positive nuclear TNF α reaction to glomerulus (arrow head) and cells lining renal tubules (arrow). (f) Renal cortex section from group (VI) demonstrating moderate positive cytoplasmic TNF α reactivity along glomerulus of renal corpuscle (arrow head) alongside renal tubules lining cells (arrow).

DISCUSSION

Medications containing lithium, such as lithium carbonate (LC), are the gold-standard treatment for bipolar disorder (*Zaki et al., 2022*). The majority of lithium intoxications are associated with its therapeutic uses owing to its limited therapeutic index (*Ufelle and Barchowsky, 2019*). It was reported that the most frequent negative impact of lithium is nephrotoxicity, which is characterized by damage to the renal tubules and a reduction in concentrating ability (*Jing et al., 2022*). Lithium carbonate nephrotoxicity was confirmed in the current investigation, by the increase in serum urea and creatinine. On the other hand, renal function was restored when chitosan (CS) was administered synchronously.

The increased levels of urea and creatinine are warning signs of serious damage to the structural integrity of the nephron. Urea is one consequence of protein metabolism. Because the kidneys are the primary organ excreting urea, urea is a valuable marker of kidney function (*Salazar, 2014*). When the kidney is injured in any way, urea is the first renal marker to rise (*Mercantepe et al., 2018*).

The amino acid creatinine is produced when creatine in muscle tissue undergoes a non-enzymatic transition, followed by hepatic metabolism. It is not reabsorbed or processed by the kidney; rather, it passes through glomerular filtration. Therefore, the most prevalent marker to monitor glomerular filtration rate is creatinine clearance (*Mesallam et al., 2023*).

This was consistent with the findings of *Ommati et al. (2021)*, who found that treatment with lithium at doses of 25 and/or 50 mg/kg/day for 28 days caused a significant rise in serum biomarkers of renal damage.

Aboulthana and Ibrahim (2018) also found that administering CS along with LC reduced urea and creatinine considerably when compared to the group receiving LC only. CS was unable, however, to return these measures to baseline levels. The fact that lithium was given for 30 days whereas chitosan was only given for 15 days may account for the inadequate recovery.

In contrast to the current study, *Ossani et al. (2019)* found that there was no change in the

serum creatinine level following the administration of lithium for one month. The experimental groups received physiological solutions to preserve sodium balance and prevent lithium intoxication, which may account for the differing results.

One common adverse impact of lithium treatment is lithium-induced parathyroid disease (*Pattan et al., 2021*). However, compared to thyroid diseases, biochemical screening for parathyroid disorders caused by lithium is less frequent (*Bann et al., 2023*).

Hypoparathyroidism or hyperparathyroidism are two possible outcomes of lithium-induced parathyroid illness (*Pattan et al., 2021*). However, the most prevalent sign of parathyroid illness brought on by lithium toxicity is hyperparathyroidism. Thirty percent of cases show an increase in serum calcium and parathormone hormone (*Mahmoodnia, 2023*).

The current study revealed that administration of LC induced an increase in serum levels of parathormone hormone and calcium, with the effects being more pronounced at larger doses of LC. *Topçu (2020)* reported comparable outcomes.

LC directly affects the parathyroid glands by stimulating the proliferation of parathyroid cells. Also, it increases the threshold of calcium sensing receptor. Consequently, It reduces the serum calcium-induced inhibition of parathormone hormone (*Vantighem, 2023*). Additionally, LC has indirect effects via increasing intestinal absorption of calcium and decreasing urine calcium excretion, which is brought on by increased renal reabsorption as a result of elevated parathormone hormone (*Mifsud et al., 2020*). Adding of chitosan restored the levels of parathormone hormone and calcium toward normal levels as control group. As far as we know, no research had been done on the effects of chitosan on the parathyroid gland before the time of this study. Chitosan's beneficial effects on renal function may contribute to the improvement in parathyroid function by enhancing calcium excretion. Additionally, chitosan's antioxidant and anti-inflammatory properties may directly lessen the harmful effects of lithium on the parathyroid gland.

Total antioxidant capacity (TAC) is a measure of the extracellular system's ability to scavenge peroxy radicals. It is made up of proteins, urate, sulphhydryl groups, ascorbate, retinol, carotenoids and α -tocopherol. TAC indicates the remaining antioxidant capability after the depletion of reactive oxygen species (*Hewala and Elsoud, 2019*). TAC as a single measure could be more useful than measuring different antioxidants (*Wang et al., 2021*). A low level of TAC indicates an imbalance between ROS scavenging and production (*Asbaghi et al., 2021*).

In the current investigation, administration of LC reduced the serum TAC levels, which improved by the co-administration of chitosan.

Oxidative stress is one of the pathogenic pathways through which LC can promote organ dysfunction at the cellular level. Numerous studies have proved that oxidative stress plays significant role in the toxic effects induced by lithium on many organs, as the kidney, testes, and heart (*Ommati et al., 2021; Badawy et al., 2022*).

Chitosan has the ability to interrupt the oxidation chain reaction and thus protect the target tissues from oxidative stress-induced damage (*Muthu et al., 2021*). Chitosan monomers can scavenge free radicals because they have two hydroxyl groups (OH) and an amino group (NH₂) (*Aranaz et al., 2021*).

Research on the antioxidant characteristics of chitosan have primarily been conducted on experimental animals, including rats, cats, dogs, and prawns. Additionally, studies conducted in vitro have assessed the antioxidant activity of chitosan by measuring its reducing power and free radical scavenging ability (*Jagdale et al., 2023*).

After being completely filtered by the glomeruli, the proximal tubules reabsorb over 80% of lithium via the sodium/hydrogen exchanger, the same channel that mediates most of the luminal sodium uptake (*Alsady et al., 2016*).

Lithium induces most of its harmful effects in the collecting ducts and distal tubules. Lithium is taken up by the principal cells of the collecting ducts through the sodium channel present on the apical membrane. This sodium channel has a higher affinity for

lithium than sodium (*Kalita et al., 2018*). Afterwards, lithium accumulates in the principal cells because the Na/K ATPase pump has a lower affinity for lithium than for sodium (*Davis et al., 2018*).

According to the results of the histopathological and immunohistochemical examination performed in the present study, LC generated a variety of deformative alterations in the renal tubules and glomeruli, as well as congestion surrounding the tubules. Also, there were high cytoplasmic and nuclear TNF α reactivity. These modifications were more pronounced in the group that received the higher dose of LC.

The observed kidney damage in the LC groups is dependent on multiple factors. One of the pathogenic processes is oxidative stress. Numerous studies have demonstrated how oxidative stress contributes to the harmful effects of LC on a variety of organs (*Ossani et al., 2019*). Oxidative stress brought on by LC toxicity produces oxygen-free radicals, which interact with a variety of cell macromolecules to cause oxidative damage (*Birben et al., 2012*).

The second pathogenic mechanism of lithium-induced kidney injury is inflammation. Inflammation was evident by the high reactivity of the pro-inflammatory cytokines (TNF- α) in the renal tissues of lithium-treated rats. The growth, proliferation, differentiation, and viability of activated leukocytes are regulated by TNF- α . Moreover, TNF- α causes cells to secrete inflammatory mediators, chemokines, and cytokines (*Nair et al., 2006*). Furthermore, TNF- α plays a significant role in oxidative stress pathogenesis (*Kurt et al., 2015*).

Similar results were reported by *Jing et al. (2022)*, who observed that after toxic exposure to lithium, renal tissue showed signs of local necrosis, inflammatory cell infiltration, and a narrow tubular lumen. Also, an increased expression of TNF- α in the thyroid tissue's follicular cell nuclei was observed by *Zaki et al. (2022)* following LC administration to rats (30 mg/kg/day) for 8 weeks. Additionally, *Ommati et al. (2021)* reported that tubular degeneration, glomerular dilation, interstitial inflammation, and haemorrhage were shown to be the most

notable histopathological changes in animals treated with lithium.

Chitosan co-administration lessened the extent of kidney tissue injury and the cytoplasmic and nuclear reactivity to TNF α , as observed in groups IV and VI.

According to *Mahmoud et al. (2021)*, chitosan possesses antioxidant properties that prevent oxidative stress and kidney damage. Additionally, chitosan scavenges N-(carboxyethyl) lysine and methylglyoxal, which are the two primary contributors to the pathogenesis of nephropathy (*Chou et al., 2015*).

Numerous studies have investigated the anti-inflammatory and pro-inflammatory properties of chitosan. It was found that the usage of chitosan resulted in a considerable increase of anti-inflammatory cytokines and a decrease of pro-inflammatory cytokines, such as TNF- α , in vitro and in vivo models (*Kim, 2018; Li et al., 2018*). Similar results were reported by *Yin et al. (2023)*, who reported that oral chitosan treatment can preserve glomerular filtration and considerably reduce renal tubular damage. Also, *Aboulthana and Ibrahim (2018)* reported that congestion was observed in the renal cortical blood vessels in rats treated with lithium and chitosan simultaneously and in post-treated groups. However, there was no histopathological alteration in the tubules and glomeruli of the chitosan-pretreated group. This indicated that chitosan had a protective effect on kidney toxicity induced by LC.

CONCLUSION

The present study concluded that lithium carbonate subacute toxicity may lead to functional and structural renal damage, hyperparathyroidism and hypercalcemia. Oxidative stress and inflammation are the pathogenic mechanisms involved in lithium toxicity. Co-administration of chitosan with lithium exhibited ameliorative effect against these harmful effects of lithium. The beneficial effect of chitosan can be explained by its antioxidant and anti-inflammatory properties.

RECOMMENDATIONS

- Due to the limited therapeutic/toxic ratio of lithium salts, it is recommended that patients

taking these medications have their renal and parathyroid functions closely monitored.

- It is recommended to test the effectiveness of chitosan in improving the functions of the kidneys and parathyroid glands in patients of lithium poisoning.

- Further studies using other antioxidant and anti-inflammatory substances against lithium toxicity is recommended.

Limitations of the study: Dissection and histopathological examination of parathyroid glands could not be performed due to the small size and weight of them in rats.

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التأثير المحسن للشيتوزان ضد السمية تحت الحادة لكاربونات الليثيوم على الكلى والغدة الجاردرقية في ذكور الجرذان البيضاء البالغة

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الملخص العربي

المقدمة: الأدوية المحتوية على الليثيوم تستخدم لعلاج مرض الاضطراب ثنائي القطب.

الهدف من الدراسة: هدفت الدراسة الحالية إلى تقييم التأثير السمي تحت الحاد لكاربونات الليثيوم على الكلى والغدة الجاردرقية والتأثير الوقائي المحتمل للشيتوزان.

طريقة البحث: تم اجراء البحث علي عدد ٦٠ من ذكور الجرذان البيضاء البالغة. تم تقسيمهم إلى ست مجموعات، يتكون كل منها من عشرة جرذان. المجموعة الأولى (مجموعة ضابطة). المجموعة الثانية تم اعطائها الشيتوزان عن طرق الفم بجرعة ٢٠٠ ملجم/كجم/يوم. المجموعة الثالثة: تم اعطائها كاربونات الليثيوم عن طريق الفم بجرعة ٢٥ ملجم/كجم/يوم. المجموعة الرابعة: تم اعطائها الشيتوزان (٢٠٠ ملجم/كجم/يوم) مع كاربونات الليثيوم (٢٥ ملجم/كجم/يوم) عن طريق الفم. المجموعة الخامسة: تم اعطائها كاربونات الليثيوم عن طريق الفم بجرعة ٥٠ ملجم/كجم/يوم. المجموعة السادسة: تم اعطائها الشيتوزان (٢٠٠ ملجم/كجم/يوم) مع كاربونات الليثيوم (٥٠ ملجم/كجم/يوم) عن طريق الفم. بعد اربعة اسابيع تم ذبح الجرذان تحت التخدير الخفيف. تم جمع عينات الدم. تم تشريح جميع الحيوانات واستخراج الكلى لفحص الانسجة.

النتائج: تسمم الليثيوم ادي الي زيادة ذات دلالة احصائية في متوسط قيم اليوريا والكرياتينين وهرمون الباراثورمون والكالسيوم في الدم، وانخفاض ذو دلالة احصائية في TAC. أدي تناول الشيتوزان مع الليثيوم إلى تحسن في المعايير البيوكيميائية. لوحظ بعض التغيرات الباثولوجية في الكلى في المجموعات المعالجة بكاربونات الليثيوم. كانت هذه التغيرات اقل عند اعطاء الشيتوزان. كان هناك تعبير عالي الإيجابية ل TNF α في انسجة كليه الجرذان المعالجة بكاربونات الليثيوم. ولوحظ تعبير TNF α أقل عندما تم اعطاء الشيتوزان.

الاستنتاج: السمية تحت الحادة لكاربونات الليثيوم تسبب تلفاً في الكلى و زيادة نشاط الغدة الجاردرقية وزيادة الكالسيوم في الدم. استخدام الشيتوزان يمكن أن يقلل من الآثار السلبية لليثيوم بسبب خصائصه المضادة للأكسدة والمضادة للالتهابات.