

RESEARCH ARTICLE

Biochanin A Has Protective and Therapeutic Effects against Li₂CO₃-induced Nephrotoxicity in Rats ModelZaenah Zuhair Alamri^{1,*}, Soliman Mohammed Alfaifi²¹Department of Biological Sciences, College of Science, University of Jeddah, P.O. Box 80327, Jeddah 21589, Saudi Arabia²Department of Pharmacy, Tamer Healthcare Tamerceutical Company, 'An Nakhil District, Jeddah 23242, Saudi Arabia

*Corresponding author. Email: zzalamri@uj.edu.sa

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Abstract

BACKGROUND: Long-term usage of lithium carbonate (Li₂CO₃) as treatment of bipolar disorder might cause nephrotoxicity. Li₂CO₃-induced nephrotoxicity usually treated by renal dialysis, which is quite expensive. Usage of natural products that possess anti-inflammatory and antioxidant properties as biochanin A (BCA) may be useful against Li₂CO₃ nephrotoxicity. Therefore, this study was conducted to evaluate Li₂CO₃ nephrotoxic effects and BCA therapeutic and protective effects against it.

METHODS: Twenty-eight rats divided into 4 groups: negative control (NC), Li₂CO₃, Li₂CO₃ + BCA therapeutic, and BCA + Li₂CO₃ protective group. Complete blood picture, serum kidney function tests (urea, creatinine, and cystatin C) and kidney tissue homogenate oxidative stress index as malonaldehyde (MDA), superoxide dismutase (SOD), glutathione S-transferase (GST) and catalase (CAT) were measured using enzyme linked immunosorbent assay (ELISA) methods. While, kidney histopathological changes were evaluated under light microscope.

RESULTS: Li₂CO₃ administration elicited significant changes in hematological parameters as hypochromic anemia, decreased platelets and leukocytosis. Serum urea, creatinine and cystatin C were elevated. Renal homogenate analysis revealed significant elevation of MDA and decline in GST, CAT, SOD levels. Kidney histopathology analysis revealed hemorrhage between renal tubules, glomerular capillaries congestion, Bowman's space dilatation, structural alterations in renal tubules cells include loss of brush border, deeply stained nuclei and elevated in size and number of cytoplasmic vacuoles. Therapeutic and protective groups of BCA showed amelioration of hematological alteration, kidney functions, oxidative stress markers and restoration of kidney tissues.

CONCLUSION: BCA has both protective and therapeutic effects against Li₂CO₃-induced nephrotoxicity possibly through the reduction of oxidative stress.

KEYWORDS: biochanin A, hematological changes, kidney, lithium carbonate salt, oxidative stress, protective effects, therapeutic effects

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Introduction

The preferred medication for treating bipolar illness, which affects around 1% of the population in the world, is lithium carbonate (Li₂CO₃). (1) A few applications of Li₂CO₃ treatment have been included in defense against

dementia, cerebrovascular illnesses, and suicidal thoughts. (2) Even though Li₂CO₃ is a very important medication, a number of side effects have been documented, including nausea, vomiting, diarrhea, trembling in the hands, mental confusion, hallucinations, convulsions, nephrotoxicity and endocrine manifestations, especially to thyroid and parathyroid glands. (3)

Li_2CO_3 primarily targets the kidneys and prolonged lithium medication may result in progressive nephrotoxicity. There are three types of renal reactions to lithium: acute lithium intoxication, chronic kidney disease, and nephrogenic diabetes insipidus (NDI). (4) Li_2CO_3 toxicity has often been linked to disruptions in cells metabolism. (3) Urine concentration deficit linked to Li_2CO_3 toxicity is mostly caused by decreased in aquaporin-2 (AQP2) protein in cells of collecting duct. (5) Alterations in the epithelial sodium channels (ENaCs) of principle cells of collecting duct that are crucial for sodium reabsorption are another mechanism implicated in Li_2CO_3 -induced nephrotoxicity. (6) The entrance of Li_2CO_3 inside cell by ENaCs is a crucial stage in NDI development, since it has been demonstrated that blocking ENaCs functioning in collecting ducts also inhibits predicted polyuria caused by Li_2CO_3 . (7) Li_2CO_3 's harmful effects on the kidney are also caused by its toxicity to blood vessel endothelium. Furthermore, through disruption of the mitochondrial respiratory system, which increases reactive oxygen species (ROS) formation by lipid peroxidation of cell membrane, oxidative stress hypothesized as alternate mechanism for Li_2CO_3 renal toxicity. Moreover, another mechanism for Li_2CO_3 renal toxicity has been proposed, which is through oxidative stress. This occurs when the mitochondrial respiratory system is disrupted, increasing ROS generation through cell membrane lipid peroxidation. (8) It was reported that small dosages of Li_2CO_3 were reported to generate oxidative stress in the liver, kidney, and erythrocyte tissues in a rat study. (9) The most used treatment of Li_2CO_3 -induced nephrotoxicity is by renal dialysis (10), which is quite expensive.

Therefore, giving antioxidants such as N-acetyl cysteine and the caffeic acid phenethyl ester, which is a part of the honeybee propolis, astaxanthin during Li_2CO_3 treatment, significantly has protective effect against Li_2CO_3 -induced renal failure in rats. (6,11,12) Plant-based foods such as fruits, grains, chocolate, vegetables, and legumes can provide bioactive components called polyphenols. Strong immunostimulating, anti-inflammatory, and antioxidant properties of polyphenols support human health. Furthermore, it has been demonstrated that polyphenols are beneficial in reducing the fibrosis and inflammation linked to diabetic nephropathy. (13) A dietary isoflavonoid-type polyphenol that has notable pharmacological advantages, biochanin A (BCA) or 5,7-Dihydroxy-4'-methoxy-isoflavone, is widely found in alfalfa, cabbage, soybean, chickpea, and red clover sprouts. Its antidiabetic, cardioprotective, antioxidative, renoprotective, neuroprotective, and anti-inflammatory properties are encouraging. (14) By inhibiting nucleotide-

binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasomes, BCA was reported to reduce renal fibrosis linked to obstructive nephropathy. (15) Oral BCA administration had been shown to have therapeutic potential in treating rats with diabetic nephropathy induced by streptozotocin (STZ) (16), via its anti-oxidant activity. However, only a few studies examined the effects of BCA against toxic effects of medications. These beneficial anti-oxidative properties prompted the recommendation of BCA as therapeutic and protective drug for rat nephrotoxicity tests against Li_2CO_3 . This study was conducted to investigate the therapeutic and protective actions of BCA administration against the renal adverse effects of long-term usage of Li_2CO_3 in rats.

Methods

Animals Handling

The twenty-eight mature male Wister Albino rats, weighed between 180-250 g were purchased from the Faculty of Pharmacy's Animal House, King Abdul-Aziz University of Jeddah. Rats were kept in broad cable-based polypropylene cages prior to the start of the study in order to minimize coprophagy. Each cage held seven rats, and the rats were given a week to adjust to the laboratory's temperature (ranged from 20–23°C), humidity (ranged from 55–65%), and light/dark (12/12 hours). Animals had unrestricted access to rodent laboratory pellets and water. The handling of experimental animals was conducted according to the ARRIVE guidelines for care and use of laboratory animals. This study protocol was approved by Faculty of Pharmacy's Ethical Committee, The King Abdulaziz University of Jeddah (Reference # TEMPORARY-10, date: 31/1/2024).

Animal Intervention

Minimum sample size of animals involved was calculated according to the following equation: $E = \frac{\text{total number of animals} - \text{total number of groups}}{\text{total number of groups}}$. The "E" value should be between 10 and 20 to be considered as adequate. Following this method, 24 rats were required. After adding 4 more rats as reserve in case of sudden death, thus, the final number of subjects was 28. Experimental animals were then randomly sorted into 4 groups, with 7 rats in each group.

The animals were distributed to cages based on the assigned group. Negative control group (NC) consisted of rats received 1 mL/kgBW normal saline solution (1:9, DMSO: PBS) orally every alternate day for 10 weeks. Positive control, or the Li_2CO_3 group, consisted of rats that

received 24 mg/kg Li_2CO_3 by oral gavage every alternate day for 7 weeks to induce renal injury. (17) Li_2CO_3 and BCA therapeutic group (Li_2CO_3 + BCA) consisted of rats received 24 mg/kg Li_2CO_3 orally every alternate day for 7 weeks, then receive 50 mg/kg BCA orally daily for 3 weeks (18). BCA and Li_2CO_3 protective group (BCA+ Li_2CO_3) consisted of rats received 50 mg/kg BCA orally at same time with 24 mg/kg Li_2CO_3 every alternate day for 7 weeks. (18) Li_2CO_3 (99.99% purity) (Cat. No. CAS #554-13-2; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water before used. Meanwhile, BCA powder reagent ($\text{C}_{16}\text{H}_{12}\text{O}_4$, >98% purity) (Cat. No. CAS #207-744-7; Sigma-Aldrich) was mixed in dimethyl sulfoxide (DMSO) to form 200 mM stock solution, then 0.7 mL of stock solution was combined with 6.3 mL of phosphate-buffered saline (1:9, DMSO: PBS) to create a newly created working solution.

Total body weights of rats estimated at beginning and end of experimental using digital scale. At 7th weeks, followed 12 hours of experimental end, rats anesthetized by inhalation of diethyl ether. A digital scale was used to record rats' body weights at the beginning and end of the experiment. The final total body weight was subtracted from beginning total body weight to determine weight increase, which then divided by the initial total body weight and multiplied by 100 to determine the percentage changes in body weight.

The retro-orbital venous plexus, belly, and thoracic cavity were opened in order to collect blood. The kidneys underwent isolation, cleaning, and distal water washing. After weighing the kidneys, the kidney indices were computed by dividing the kidney weight by final total body weight and multiplying the result by 100. Right kidney was then divided sagittal in two, using one half for histological analysis and the other for tissue biochemistry. Ultimately, the rats were sacrificed by cervical decapitation to assess the therapeutic and preventive effect of BCA against the renal damage caused by Li_2CO_3 therapy.

Collection of Blood Samples and Estimation of Kidney Function Test

Samples of blood were collected into two varieties of tubes. Five mL of the blood was collected in the first tubes that contained EDTA, which was used in conjunction by an automated hematology analyzer (BC-2800) to estimate the hemoglobin contents (HGB), platelets, white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HBG), hematocrit values (HCT), and mean corpuscular volume (MCV). To allow for coagulation, 5 mL of blood samples in the other plain tubes were left for a short while. The sera

were obtained by centrifuging at 600 g for 10 minutes. Afterward, the sera were separated and kept at refrigerator with temperature -80°C till required. The acquired serum was utilized to compute kidney function tests, including urea, creatinine, and cystatin C, using enzyme linked immunosorbent assay (ELISA) methods.

Preparation of Kidney Tissue Homogenate and Estimation of Oxidative Stress Markers

Sections of the right kidney's tissue homogenized in a potassium phosphate buffer with a 50 mM pH (7.4) to measure the amount of protein and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione S transferase (GST). To estimate malondialdehyde (MDA), other portions of the right kidney homogenized in a potassium phosphate buffer at a pH of 10 mM (7.4). The crude kidney homogenate was centrifuged utilized Mixer Mill MM 400 (Retsch, Haan, Germany) at 600 g for 15 minutes at 4°C . Supernatant was extracted, kept at -80°C , and utilized to measure oxidative stress indicators. As a reference, the amount of protein in tissue quantified utilizing bovine serum albumin.

The measurements of MDA, SOD, CAT, and GST activities activity in kidney tissue homogenate supernatants was performed using Rat ELISA kits for MDA (Cat. No. MBS268427; MyBioSource, San Diego, CA, USA), SOD (Cat. No. MBS036924; MyBioSource), CAT (Cat. No. MBS2600683; MyBioSource), and GST (Cat. No. MBS202088; MyBioSource) based on the manufacturer's manual.

Histological Examinations of the Kidney

The midsections of left kidney were prepared for light microscopy to examine the kidney histopathological. This technique included embedding the specimen in paraffin, slicing sections 5 mm thick, staining with hematoxylin and eosin, fixing specimen in a 5% neutral formalin solution, and gradually dehydrating specimen in 50–100% ethanol before clearing it in xylene and microscopically examining it. The examination was performed by a blinded-to-study histopathologist in the pathology lab.

Statistical Analysis

Data expressed as mean \pm standard deviation (SD). SPSS version 22 (IBM Corporation, Armonk, NY, USA) was utilized to analyze values. Shapiro-Wilk test was utilized to determine whether value distributions were normal, One-Way ANOVA was utilized to examine the data, and then Tukey's test was utilized to compare between groups as data

were distributed normally. A $p < 0.05$ considered statistically significant.

Results

In this study, 24 mg/kg Li_2CO_3 was administered orally every alternate day for 7 weeks to induce a model of kidney injury as assessed by the measurement of serum levels of urea, creatinine and cystatin C as well as histological examination of kidney tissues. Elevated levels of urea, creatinine and cystatin C were observed in the Li_2CO_3 . A crucial indicator of Li_2CO_3 -induced toxicity was also shown by the changes in the body weight of rats as well as their relative kidney weights.

Li_2CO_3 and BCA Effects on Body Weights and Kidney Weights

There were insignificant changes in the initial body weights between different studied groups. The final total body weights were significantly decreased in Li_2CO_3 , $\text{Li}_2\text{CO}_3 + \text{BCA}$ therapeutic, and $\text{BCA} + \text{Li}_2\text{CO}_3$ protective groups compared to the NC group ($p < 0.0001$, $p < 0.010$ and $p < 0.050$, respectively). Percentage changes in body weight were significantly declined in Li_2CO_3 group versus NC, $\text{Li}_2\text{CO}_3 + \text{BCA}$ therapeutic, and $\text{BCA} + \text{Li}_2\text{CO}_3$ protective groups ($p < 0.0001$ for all) indicating that Li_2CO_3 induced toxicity.

Meanwhile, kidney weights were significantly increased in the Li_2CO_3 group versus $\text{Li}_2\text{CO}_3 + \text{BCA}$ therapeutic and $\text{BCA} + \text{Li}_2\text{CO}_3$ protective groups ($p < 0.050$ for both). Kidney indices were significantly decreased in Li_2CO_3 group versus NC, $\text{Li}_2\text{CO}_3 + \text{BCA}$ and $\text{BCA} + \text{Li}_2\text{CO}_3$ groups ($p < 0.0001$ for all) (Table 1).

Li_2CO_3 and BCA Actions on Hematological Parameters

WBCs counts were significantly increased in Li_2CO_3 group versus NC, $\text{Li}_2\text{CO}_3 + \text{BCA}$ and $\text{BCA} + \text{Li}_2\text{CO}_3$ groups ($p < 0.0001$ for all) and in $\text{Li}_2\text{CO}_3 + \text{BCA}$ versus NC ($p = 0.010$) indicating that Li_2CO_3 causes inflammation. Meanwhile, RBCs, HBG, HCT, MCV and platelets counts were significantly decreased in Li_2CO_3 group versus NC, $\text{Li}_2\text{CO}_3 + \text{BCA}$ and $\text{BCA} + \text{Li}_2\text{CO}_3$ groups ($p < 0.0001$ for all) indicating that Li_2CO_3 toxicity induced suppression of RBCs and platelets formation (Table 1).

Li_2CO_3 and BCA Effects on Kidney Function Tests

Serum urea, creatinine and Cystatin C were significantly increased in Li_2CO_3 group versus NC, $\text{Li}_2\text{CO}_3 + \text{BCA}$ and $\text{BCA} + \text{Li}_2\text{CO}_3$ groups ($p < 0.0001$ for all). Also, serum creatinine level still significantly elevated in $\text{Li}_2\text{CO}_3 + \text{BCA}$ therapeutic group versus NC group ($p = 0.010$) (Table 2).

Li_2CO_3 and BCA Effects on Renal Homogenates of Oxidative Stress Markers

Kidney homogenate levels of MDA were significantly increased in Li_2CO_3 group versus NC, $\text{Li}_2\text{CO}_3 + \text{BCA}$ and $\text{BCA} + \text{Li}_2\text{CO}_3$ groups ($p < 0.0001$ for all). Kidney homogenate values of MDA still significantly increased in $\text{Li}_2\text{CO}_3 + \text{BCA}$ therapeutic group versus NC group ($p = 0.010$). Kidney homogenate values of GST, SOD and CAT significantly declined in Li_2CO_3 group versus NC, $\text{Li}_2\text{CO}_3 + \text{BCA}$ and $\text{BCA} + \text{Li}_2\text{CO}_3$ groups ($p < 0.0001$ for all) (Table 2).

Li_2CO_3 and BCA on Kidney Structure

Renal cortex of NC group was constituted of renal tubules and renal corpuscles, according to an examination of

Table 1. Comparison of body weights, kidney weights and hematological alterations in various studied groups.

Parameters	NC	Li_2CO_3	$\text{Li}_2\text{CO}_3 + \text{BCA}$	$\text{BCA} + \text{Li}_2\text{CO}_3$
Initial body weights (grams)	213.14±1.87	212.29±2.39	218.71±7.74	213.71±4.03
Final body weights (grams)	253.43±10.60	228.57±4.83 ^{***}	240.43±8.20 [*]	236.00±10.23 ^{**}
Body weight changes (%)	15.79±3.54	7.12±2.20 ^{***}	9.03±1.46 ^{###}	9.44±2.40 ^{###}
Kidney weights (grams)	0.75±0.04	0.81±0.07	0.72±0.06 [#]	0.71±0.03 [#]
Kidney index (%)	0.30±0.02	0.35±0.03 ^{***}	0.30±0.02 ^{###}	0.30±0.01 ^{###}
WBCs ($10^3/\mu\text{L}$)	27.68±2.28	66.31±9.44 ^{***}	40.38±7.21 ^{**###}	31.35±4.04 ^{###}
RBCs ($10^6/\mu\text{L}$)	9.25±0.52	6.16±1.36 ^{***}	9.13±0.67 ^{###}	8.77±0.79 ^{###}
HBG (g/dL)	16.37±0.68	7.35±3.06 ^{***}	15.20±1.65 ^{###}	15.45±2.03 ^{###}
HCT (%)	62.81±9.13	41.80±4.35 ^{***}	55.2±4.12 ^{###}	56.74±2.67 ^{###}
MCV (fL)	58.74±5.17	41.16±1.63 ^{***}	54.66±3.58 ^{###}	54.47±3.83 ^{###}
Platelets ($10^3/\mu\text{L}$)	950.57±33.81	492.29±63.23 ^{***}	929.71±68.20 ^{###}	911.29±63.57 ^{###}

*significant compared to NC; #significant compared to Li_2CO_3 . * or #: $p < 0.050$; ** or ##: $p < 0.010$; *** or ###: $p < 0.001$.

Table 2. Comparison of kidney function tests in serum and oxidative stress biomarkers in kidney homogenate in various groups.

Parameters	NC	Li ₂ CO ₃	Li ₂ CO ₃ + BCA	BCA + Li ₂ CO ₃
Urea (mg/dL)	14.16±1.22	55.14±8.07 ^{***}	17.94±2.81 ^{###}	15.27±2.60 ^{###}
Creatinine (mg/dL)	0.62±0.10	1.76±0.24 ^{***}	0.93±0.16 ^{*,###}	0.70±0.13 ^{###}
Cystatin C (mg/dL)	0.46±0.03	2.15±0.39 ^{***}	0.66±0.10 ^{###}	0.52±0.06 ^{###}
MDA (nmol/mg protein)	0.37±0.05	1.50±0.34 ^{***}	0.76±0.09 ^{*,###}	0.52±0.05 ^{###}
GST (ng/mg protein)	17.60±1.08	3.43±0.77 ^{***}	16.34±0.90 ^{###}	17.31±1.23 ^{###}
SOD (U/mg protein)	180.29±7.76	79.29±1.98 ^{***}	172.00±5.74 ^{###}	172.29±11.44 ^{###}
CAT (Mu/mg protein)	118.43±4.65	59.86±5.46 ^{***}	121.00±1.53 ^{###}	118.14±3.53 ^{###}

*significant compared to NC; #significant compared to Li₂CO₃. * or #: $p < 0.050$; ** or ##: $p < 0.010$; *** or ###: $p < 0.001$.

hematoxylin and eosin (H&E)-stained sections of renal cortex. The parietal layer of the glomerular tuft of capillaries, which surrounded the renal corpuscles, was bordered by a layer of simple squamous epithelium. Bowman's capsule surrounded this structure. The glomerulus and the parietal layer of Bowman's capsule were divided by Bowman's gap. The proximal-convoluted tubules had brush border, small lumen, and cuboidal cells layer with rounded basal nuclei and granular acidophilic cytoplasm. Wider lumen without brush boundary, pale cuboidal cells, and central rounded nuclei were seen in the distal convoluted tubules (Figure 1A, Figure 1B).

In the Li₂CO₃ group, distortion in the histological structure of renal cortex was observed. Hemorrhage between renal tubules and congestion in glomerular capillaries was observed. In addition, dilatation in Bowman's space was seen (Figure 2A, Figure 2B). The structural alterations in cells of proximal and distal convoluted tubules include loss of brush border and vacuoles of various sizes appeared and deeply stained pyknotic nuclei. Additional apical surface loss and cell detachment from the basement membrane were seen, together with a virtually total loss

of the brush boundary and an increase in the quantity and size of cytoplasmic vacuoles. While some proximal and distal convoluted tubules had partial desquamation or necrosis of their lining cells, others displayed the total loss of their epithelial lining. These necrotic cells' nuclei showed indications of apoptosis. There was infiltration of mononuclear cells between the renal tubules in several places (Figure 2B, Figure 2C).

The majority of the renal tubules' histological structure was restored and the renal corpuscles were preserved in the lithium and BCA co-treated treatment group (Li₂CO₃ + BCA) (Figure 3A). There were normal renal corpuscles with an entire regular Bowman's capsule, a glomerular tuft, and a urinary gap. With acidophilic cytoplasm and an apical brush boundary in proximal convoluted tubules, most of proximal and distal convoluted tubules seemed normal, but some tubules had degenerative alterations (Figure 3B). Furthermore, a few hemorrhages between renal tubules and congestion in glomerular capillaries were seen in certain specimens (Figure 3C).

The kidney's histological structure improved in the kidney from the Li₂CO₃ and BCA concurrently

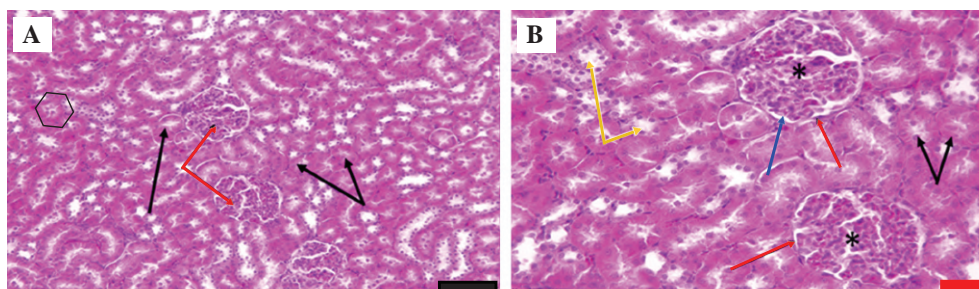


Figure 1. A photomicrograph in negative control (NC) rat kidney stained with H&E showing normal renal structure. A: Renal cortex displays normal renal corpuscles (red arrow) and renal tubules (black arrow). Black bar: 50 μ m. **B:** Renal corpuscles with a glomerular tuft of capillaries (*) and urinary space (blue arrow) and intact regular Bowman's capsule (red arrow) lined with simple squamous epithelium. Proximal convoluted tubules (black arrow) lined with cuboidal cells having rounded basophilic nuclei, acidophilic cytoplasm, and apical brush borders. Distal convoluted tubules lined with low cuboidal cells and wide lumen (yellow arrow). Red bar: 30 μ m.

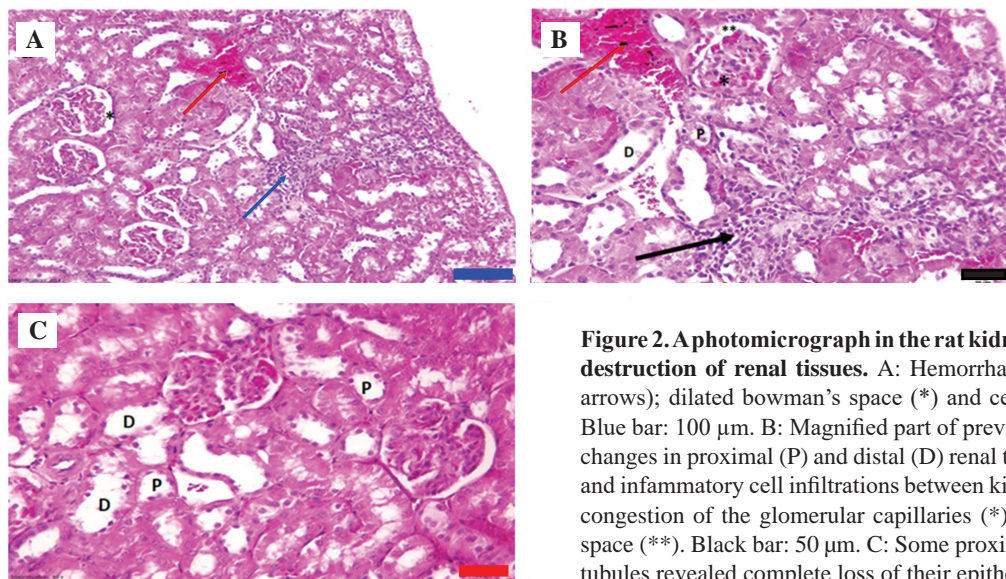


Figure 2. A photomicrograph in the rat kidney treated with Li_2CO_3 showing destruction of renal tissues. A: Hemorrhage between kidney tubules (red arrows); dilated bowman's space (*) and cellular infiltrations (blue arrow). Blue bar: 100 μm . B: Magnified part of previous section showed degenerative changes in proximal (P) and distal (D) renal tubules. Hemorrhage (red arrow) and inflammatory cell infiltrations between kidney tubules (black arrow). Note: congestion of the glomerular capillaries (*) and dilatation in the Bowman's space (**). Black bar: 50 μm . C: Some proximal (P) and distal (D) convoluted tubules revealed complete loss of their epithelial lining. Red bar: 30 μm .

treated protective group (BCA + Li_2CO_3), according to histopathological analysis (Figure 4A). Most renal tubules and corpuscles had structures that resembled those of a normal kidney. The normal renal corpuscles with an undamaged regular Bowman's capsule and a glomerular tuft were seen. In most areas, proximal convoluted tubules and distal convoluted tubules seemed normal, but in other areas, a small percentage of them had degeneration (Figure 4B). Renal corpuscles in certain specimens showed typical histological structure, with the exception of a few glomerular capillary congestions. Furthermore, there were indications of degeneration and expansion of the glomerular tuft of capillaries in additional locations inside the Bowman's space (Figure 4C).

Discussion

It is well recognized that prolonged Li_2CO_3 usage can result in irreversible renal injury.(6,8) Three ways exist via which Li_2CO_3 damages the kidneys. The first is a reduction in the kidney's AQP2 levels (5), the second is a change in collecting tubules' ENaCs (17), and the third is oxidative stress brought on by damage to kidney cells' mitochondria (8). A crucial indicator of Li_2CO_3 -induced toxicity was supplied by changes in the body weight of rats as well as their relative kidney weights. In this research, the final body weight, percentage changes in body weights and kidney indices were significantly decreased; meanwhile, kidney weights

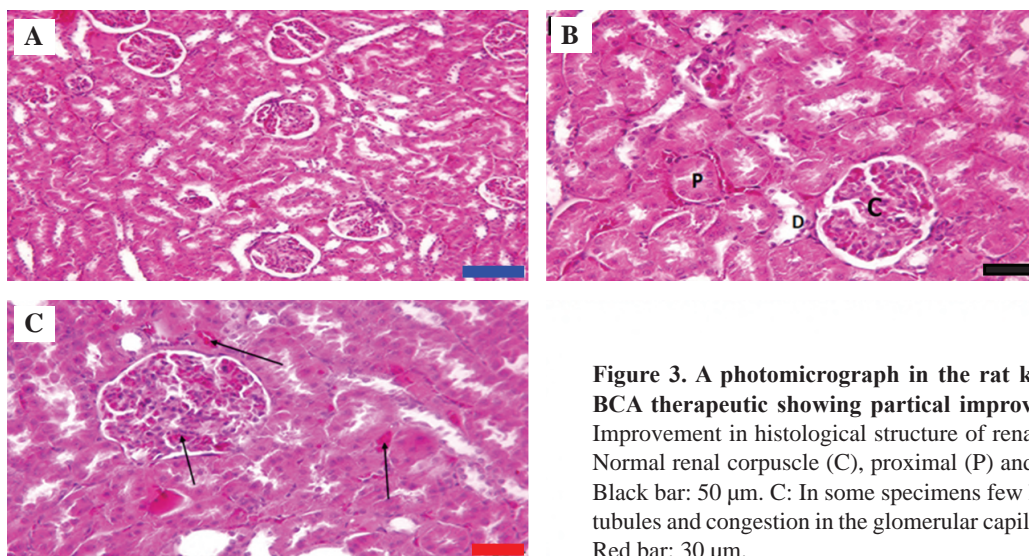


Figure 3. A photomicrograph in the rat kidney treated with Li_2CO_3 + BCA therapeutic showing partial improvement of renal structure. A: Improvement in histological structure of renal cortex. Blue bar: 100 μm . B: Normal renal corpuscle (C), proximal (P) and distal (D) convoluted tubules. Black bar: 50 μm . C: In some specimens few hemorrhages between the renal tubules and congestion in the glomerular capillaries were seen (black arrows). Red bar: 30 μm .

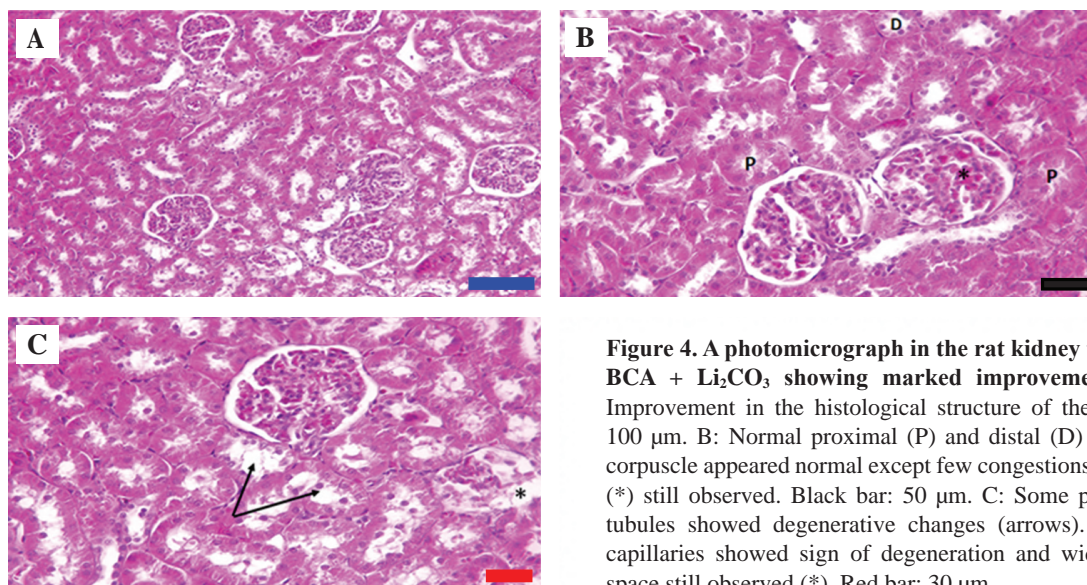


Figure 4. A photomicrograph in the rat kidney treated with protective BCA + Li_2CO_3 showing marked improvement of renal tissue. A: Improvement in the histological structure of the renal cortex. Blue bar: 100 μm . B: Normal proximal (P) and distal (D) renal tubules. The renal corpuscle appeared normal except few congestions in glomerular capillaries (*) still observed. Black bar: 50 μm . C: Some proximal and distal renal tubules showed degenerative changes (arrows). The glomerular tuft of capillaries showed sign of degeneration and widening in the bowman's space still observed (*). Red bar: 30 μm .

were significantly increased in Li_2CO_3 versus negative control group may be due to inflammation of kidney in Li_2CO_3 treated groups. Increased body weight implies that anabolic rather than catabolic processes predominate. (19) In this respect, one research reported that rats given a 30-day dose of lithium experienced a slight decline in body weight and decline in relative kidney weights. (8) Another study reported decline in total body weight in rats received lithium chloride orally for 12 weeks versus negative control. (20) Also, experimental study reported that intra-peritoneal injection of lithium chloride for 6 weeks led to significant decrease in body weight gain versus negative control. (21) This could be attributed to lithium direct cytotoxic actions on somatic cells as well as its indirect effects through the central nervous system, which regulates the endocrine system, feed and water intake, and appetite, all of which lead to decreased absorption of nutrients from the gut and decreased appetite as well as anorexia and polydipsia. (21) Increased in kidney weights in Li_2CO_3 group indicates kidney damage. Regarding the decline in the relative weights of the kidney after exposure to Li_2CO_3 may be explained by connection between elevated kidney weight and toxicological consequences like fibrosis, or by experimental animals' slower rate of body weight gain. (6) Meanwhile, this study revealed that final body weights were significantly decreased in Li_2CO_3 , Li_2CO_3 + BCA therapeutic and BCA + Li_2CO_3 protective groups versus NC group which indicated that BCA did not ameliorate toxic effect of Li_2CO_3 on total body weight. While, percentage changes in total body weight significantly increased in Li_2CO_3 + BCA therapeutic and BCA + Li_2CO_3 protective groups versus Li_2CO_3 group.

Also, kidney weights were significantly decreased, while kidney indices were significantly increased in Li_2CO_3 + BCA and BCA + Li_2CO_3 groups versus Li_2CO_3 group indicating improvement of renal damage caused by Li_2CO_3 . In this respect, a study reported that BCA administration for two months to thioacetamide induced liver cirrhosis in rats led to significant elevation in body weight compared to thioacetamide induced liver cirrhosis group. (22) Also, others found that in contrast to diabetic nephropathy group, oral BCA administration at dose for 8 weeks considerably reduced weight loss versus diabetic group. These effects may be contributed to the antioxidant effects of BCA. (23)

Hematological markers have been used to assess the toxicity of medications and environmental pollutants. (24) In this study, WBCs counts were significantly increased, while RBCs, HBG, HCT, MCV and platelets were significantly decreased in Li_2CO_3 group versus negative control group. Administering lithium therapy alters the membrane dynamics of RBCs, as well as increasing the number of WBCs counts. (25) RBCs reduction may be due to direct injurious toxin effect on animals. (17) Leukocytosis induction is thought to be immunologically significant in response to the unfavorable environment created by entrance of foreign bodies, which is the lithium, into blood. It could also be for elimination of toxin-damaged tissue waste. (17) In this study, supplementation of BCA concomitant or after Li_2CO_3 administration in both therapeutic and protective groups mitigated action of above mentioned variables, so decreasing hematotoxicity in Li_2CO_3 -intoxicated rats. BCA for 6 weeks have protective actions versus arsenic-induced hepatotoxicity in rats, including improvements in white

blood cell count compared to normal control rats may be through its anti-inflammatory and anti-oxidant effects.(26)

The results of this research revealed significant elevation in urea, creatinine, and cystatin C serum levels in Li_2CO_3 group versus negative control group that related to the observed kidney damage. Serum creatinine and cystatin C levels that change with Li_2CO_3 are often interpreted as signs of aberrant glomerular function.(27) The destruction of the proximal tubules, the main location of lithium accumulation in rats, may be linked to the rise in renal MDA levels after lithium treatment. A study reported that oral intake of Li_2CO_3 for 7 weeks led to elevation of serum urea and induced kidney damage.(9) Another experimental study results revealed also that lithium-induced renal injury manifested by the alteration of creatinine, urea, and uric acid levels.(8) Others reported significant increase in serum creatinine levels followed lithium administration for 4 weeks compared with control.(28) Results of this research showed significant decline in serum levels of urea, creatinine, and cystatin C in Li_2CO_3 + BCA therapeutic and BCA + Li_2CO_3 protective groups compared to Li_2CO_3 group. Moreover, serum creatinine level was still significantly increased in Li_2CO_3 + BCA therapeutic group versus negative control group indicating that the protective was better than therapeutic effect in ameliorating kidney toxicity in rats. Reduced oxidative stress and renal tissue regeneration may be the cause.(29)

In this respect, others found that BCA administration for 6 weeks protected kidney against arsenic intoxication as revealed by restoration of plasma creatinine and urea values.(30) Also, another study found in contrast to diabetic nephropathy group, the oral BCA intervention for 8 weeks considerably enhanced glomerular filtration as revealed by amelioration of creatinine.(23)

Results of this research revealed that kidney homogenate levels of MDA significantly increased; while antioxidant defense systems values of GST, SOD and CAT significantly decline in Li_2CO_3 group versus negative control group. MDA is widely utilized as indicators of cellular redox state that noticed to increase in rat's kidney took Li_2CO_3 .(7) Rats that took lithium + cyclosporine A (CyA) demonstrated increased renal damage and oxidative stress in comparison to rats treated with CyA alone. This result unequivocally shows that lithium, even at safe dosages, may considerably worsen CyA-induced nephrotoxicity and renal oxidative destruction in rats.(31) Others reported that lithium exposure for 2 weeks raised the total oxidative status levels in both plasma and kidney tissue homogenate. Total antioxidant status values decreased, and oxidative stress

index value increased, but not in a statistically significant way.(7) Li_2CO_3 was observed to lower kidney thyroid stimulating hormone (TSH) values and antioxidant enzyme activity, including CAT, SOD and glutathione peroxidase (GPx). This study revealed that BCA intake to Li_2CO_3 -induced toxicity in rats significantly elevated activities of measured antioxidant enzymes especially in protective and therapeutic groups. Moreover, removal of MDA was more in protective than therapeutic group. Rats exposed to arsenic BCA might prevent oxidative damage to their kidneys.(30) It's possible that BCA's capacity to scavenge free radicals from its distribution in lipid membranes and strong lipotropic profile, which protects against oxidative stress. BCA has antioxidant action when transition metal ions are present.(32) Antioxidants found in kidney tissue may work by trapping the free radicals or by breaking down peroxide. Hydrogen peroxide is converted to water by both CAT and GPx together.(6) Oxidative stress in renal tissue may be connected to the reduced antioxidant enzyme activity in kidney tissue following lithium injection.(6) The reduced SOD and CAT activities in the renal tissue of the Li_2CO_3 -treated rats in this study might indicate an excess of these enzymes linked to elevated oxidative stress. Furthermore, in rats treated with Li_2CO_3 + BCA, the elevated antioxidant enzyme activities could represent an adaptive and mitigating response to the renal impairment. Thus, the elevated endogenous H_2O_2 and reduced enzyme activity in the renal tissue point to oxidative stress significant level.(6)

Present results revealed that treatment with Li_2CO_3 showed distortion in the histological structure of renal cortex, hemorrhage between renal tubules and in glomerular capillaries and dilatation in Bowman's space. Structural alterations in mainly in proximal convoluted tubules include loss of brush border and vacuoles of various sizes appeared and deeply stained pyknotic nuclei and also in the distal convoluted tubules. Renal tubules are known to be the main sites of cell damage and death during lithium-induced renal impairment. Although apoptosis happens in both tubular segments, proximal tubules experience the bulk of it. These results are in accord with results of others.(7,17,28,33) The direct toxic actions of lithium on the renal tissue included glomerulosclerosis and renal vascular congestion.(33) During lithium medication, cortical and medullary tubular cysts or dilatation linked to chronic tubulointerstitial nephropathy.(17,34) Hydropic degeneration may be the consequence of sodium pump disruption, which causes vacuolation of the renal tubules. Glomerulosclerosis and renal vascular congestion were two of direct negative actions of lithium on the renal tissue.(35) Kidneys of rats given

lithium treatment for four weeks had obvious abnormalities in their renal morphology, including vacuolation, renal tubule degeneration, hyaline casts in collecting duct lumen, and loss of periodic acid–Schiff (PAS)-positive brush border of proximal convoluted tubules.(28) Lithium is absorbed completely by gastrointestinal tract, completely filtered by renal glomeruli, and then reabsorbed, mainly in proximal convoluted tubule. It is likely that little if any reabsorption happened in loop of Henle.(36) The kidneys' cortical section of the collecting tubule is unable to produce cyclic adenosine monophosphate in response to stimulation by antidiuretic hormones when lithium is present.(37) Moreover, it has been demonstrated that the direct toxic impact of lithium on renal blood capillaries results in congestion and dilatation, both of which harm the vessels.(38) Attenuated tubular degeneration was seen in both the therapeutic and protective groups' proximal convoluted tubules and distal convoluted tubules after treatment with lithium and BCA. The majority of the renal tubules' histological structure was restored, and Bowman's gap was preserved. Nevertheless, a few vacuolated tubules were seen. These findings was in line with previous study that stated the renal histoarchitecture of the rats treated with Li_2CO_3 showed significant necrotic alterations, tubule degeneration, and damaged glomeruli.(8) BCA administration reduced histological changes provoked by Li_2CO_3 was noticeable. Kidneys from the cisplatin and BCA groups had a mostly normal tubular structure with a little amount of degraded cells.(39) BCA impact on kidney tissues is ascribed to its chelating and antioxidant qualities, which dramatically decreased the oxidative danger and restored normal physiological functioning while also reducing pathological alterations. According to the current findings, there were no discernible changes between the protective and treatment groups' histological structures.

This study had some limitations as urine output were not collected to estimate glomerular filtration rate (eGFR) and also electron microscopic examinations of the kidney tissue were not made to see histological alteration of kidney tissue. Further studies are needed that studies toxic effects of different doses and duration of lithium administration on kidney structure and functions. It is also necessary focus on separating the bioactive components of BCA in order to identify possible pharmacological agents.

Conclusion

Thus, the results of this research revealed that administration of BCA appeared to have therapeutic and protective effects

on kidney structure and functions due to the presence of high antioxidant phytochemicals and male rats' properties from oxidative stress induced by Li_2CO_3 by enhancing enzymatic and nonenzymatic antioxidants activities and reducing the intensity of lipid peroxidation. The preventive benefits are far superior to the curative ones.

Authors Contribution

ZZA was involved in the conceptualization, data curation, resources collection, original draft writing, as well as reviewing and editing the manuscript. SMA was involved in the conceptualization, preparatipn of methodology, data curation, resources collection, as well as reviewing and editing the manuscript.

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