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A renoprotective role of chitosan against lithium-induced renal toxicity in rats

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Abstract

Background: Lithium (Li) is considered as the first therapeutic line for treatment of bipolar affective disorders and manic-depressive illness. Renal toxicity can be categorized as the most common undesirable side effect of Li therapy. Therefore, the present study aimed to reveal efficiency of chitosan (CS) as a natural chelator against renal toxicity induced as a result of Li injection. During current experiment, the renal functions (urea, creatinine, blood urea nitrogen (BUN), uric acid and total protein) were determined in serum samples. The urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) was assayed through measuring its level in urine samples. Moreover, markers of the oxidative stress (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and lipid peroxidation product (LPO)) were assessed in renal tissues. In addition, histopathological and ultrastructural examinations were carried out in that tissue.

Results: It was found that Li injection caused significant ($P < 0.05$) elevation in serum urea, creatinin and BUN levels associated with decline in uric acid and T. protein. Moreover, Li increased the urinary excretion of NAG significantly ($P < 0.05$). Administration of CS orally restored levels of these measurements to normalcy in CS pre-treated group through lowering urea, creatinin, BNU levels and urinary excretion of NAG with enhancing uric acid and T. protein levels.
On the other hand, it was found that Li caused significant ($P < 0.05$) elevation in the LPO level associated with decline in activity of the antioxidant system in the renal tissues. Likewise, it caused severe alterations in the renal levels at histopathological and ultrastructural levels. The treatment with CS reduced the renal LPO associated with stimulating the antioxidant system through increasing levels of SOD, CAT, GPx and GSH in addition to its beneficial role against the histopathological abnormalities.

Conclusions: The CS exhibited promising role in the protection against the oxidative stress and renal toxicity induced as a result of Li injection.

Keywords: Lithium therapy, Renal toxicity, Chitosan, Antioxidants, Ultrastructure

Background

Lithium (Li) is a toxic monovalent alkaline metal. It occurs naturally in all soils, surface and drinking water. Moreover, it accumulates in marine animals, algae and vegetables which represent the primary dietary Li sources and may contribute from 66% to 90% of the total Li intake (Curran and Ravindran 2014; McCartney et al. 2014). Lithium carbonate has been used as an invaluable drug to cure bipolar disorders and manic-depressive illness for more than 60 years (Grunze et al. 2010; Calkin and Alda 2012; Vieta and Valenti 2013). Moreover, it was reported that this drug used as chronic treatment to prevent development of Alzheimer's disease (Forlenza et al. 2011) and for treatment of refractory depression (Edwards et al. 2013; Cleare et al. 2015). During treatment of bipolar disorders, the prolonged exposure to Li exhibits toxic effects on several biological systems (McCartney et al. 2014; Albert et al. 2014). The gastrointestinal, renal, neurological and endocrine disorders belong to the numerous undesirable side effects occurred as a result of Li therapy (Adityanjee and Munshi 2005; Kalantari et al. 2015).

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As suggested recently by Close et al. (2014) and supported by Bocchetta et al. (2015), absorption of Li occurs completely in the gastrointestinal tract and filtered through the glomeruli then reabsorbed in the proximal tubule which represents the primary site at which the reabsorption process occurs. It accumulates in the collecting tubule and interferes with capacity of the collecting tubule (especially the cortical portion) in both kidneys to generate cyclic adenosine monophosphate as response of stimulation of antidiuretic hormone (Erden et al. 2013; Oruch et al. 2014; Clos et al. 2015).

Tubulointerstitial nephropathy is the most common deleterious side effect of Li on renal functions; however, the renal ischaemia occurred by pathogenetic mechanism through damage of the mitochondria and endoplasmic reticulum (Markowitz et al. 2000). It was stated that the mechanism by which Li induced nephrotoxicity is still poorly understood (Oktem et al. 2005). There were no procedures suitable to be established for the early diagnosis of Li nephrotoxicity (Ida et al. 2001). Moreover, renal impairment may not be recovered even after interruption of Li therapy (Markowitz et al. 2000).

It is well known that urinary N-acetyl-β-D-glucosaminidase (NAG) is used as sensitive marker of renal tubular injury and elevation of its level has been associated with Li-induced renal tubular damage (Chmielnicka and Nasiadek 2003). It has been suggested that oxidative stress belongs to the most important mechanisms by which Li exhibit its adverse effects (Oktem et al. 2005). It increased generation of the reactive oxygen species (ROS) such as superoxide and nitric oxide (NO). These reactive species exhibit their adverse role and contribute to the pathophysiology of Li-induced renal impairment through increasing the lipid peroxidation in kidney tissue and hence tissue injury (Sawas et al. 1986). These continuously produced ROS are scavenged by antioxidative system which can be divided into enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) and non-enzymatic (reduced glutathione (GSH)) (Draper and Hadley 1990).

It is worth to mention that antioxidants have attracted much attention as useful alternative medicines for treating or preventing disorders related to the abnormal lifestyle (Rajkapoor et al. 2008). The synthetic antioxidants have restriction for medicinal uses, as they are suspected to be carcinogenic (ElFar and Taie 2009). The recent studies directed to search in the nature to select one of them to be suitable for the therapeutic purposes as antioxidants in reducing free radical-induced tissue injury (Erdemoglu et al. 2006). In 2005, Efrati et al. (2004) suggested N-Acetylcysteine to ameliorate renal failure induced by Li in rats. Also, it was showed that caffeic acid phenethyl ester exhibits a protective effect against damages of the renal tubules occurred as a result of oxidative stress in Li-treated rats (Oktem et al. 2005).

Chitosan (CS), a linear and semi-crystalline polysaccharide, is naturally occurring non-toxic polymeric compounds derived from chitin which is the major component of the crustaceans' shells (e.g., crab, shrimp, prawn, lobster, and crawfish) (Xia et al. 2011). It is consisting to (1 → 4)-2-acetamido-2-deoxy-β-D-glucan (N-acetyl D-glucosamine) and (1 → 4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) which randomly distributed. The CS grade depends in its structure on content of the acetylated component (Rinaudo 2006; Dong et al. 2010). Due to its special structure and biochemical property, it has received various applications in biomedicine, agriculture and environmental field (Park et al. 2003). Physiologically, CS is considered a dietary fiber because it cannot be degraded by digestive enzymes (Harish Prashanth and Tharanathan 2007). Furthermore, it is characterized by its excellent biological activity such as high biocompatibility, biodegradability, immunogenicity and low toxicity. Therefore, it promises for being a perfect biological material and pharmaceutical formulation (Xia et al. 2011; Dong et al. 2010). In recent years, CS has attracted much attention because of various biological activities related to the antioxidant activity of it and its hydrolyzed products. It exhibits hepatoprotective effects (Jeon et al. 2003), hypocholesterolemic, antiosteoarthritic (Samarasinghe et al. 2014), antitumor (Qi and Xu 2006), antimetastatic (Shen et al. 2009), antiulcer, immunostimulatory (Neimert-Andersson et al. 2011) and antibacterial activities (No et al. 2002). The physicochemical properties of the CS structure may refer to presence of the reactive functional groups that correlate with their chelation and flocculation efficiency (Gamage and Shahidi 2007; Renault et al. 2009). It has been extensively studied for its cation-binding ability (Bravo-Osuna et al. 2007).

CS exerted its high hydrophilic ability to attract the cations. The high hydrophilicity may refer to large number of hydroxyl groups in its active sites, enabling it to attract those cations (Crini and Badot 2008). For this reason, the present study aimed to reveal the CS efficiency as a natural chelator to resist the renal toxicity induced by Li injection for therapeutic purposes.

Materials and methods

Materials: chemicals, reagents and kits

All chemicals and reagents were of analytical grade and of highest purity. Chitosan, Lithium carbonate (Li_2CO_3) and Tris buffer were purchased from Sigma-Aldrich. Hematoxylin and Eosin stains were obtained from SRL, India. 2-Thio-barbituric Acid

(TBA) was procured from Sigma Chemicals Company (London, UK). Kits for urea, creatinine and total protein were obtained from Spectrum Diagnostics Egyptian Company for Biotechnology (Cairo, Egypt).

Animals

Forty-eight healthy adult male Wistar albino rats (mean weight 230 ± 30 g) were housed in six polypropylene cages (eight per cage) under hygienic conditions. The animals were maintained at normal room temperature at 25 ± 2 °C. A commercially balanced diet and tap water were provided ad libitum for 1 week before start of the experiment.

Experimental design

The rats were randomly divided into six experimental groups. The first group (control): rats were allowed to feed with normal diet as ad libitum and received distilled water for 30 days. The second group (CS-treated group): rats were fed with normal diet associated with oral gavage treatment with CS at a dose of 200 mg/kg b.w./day for 14 days (Jeon et al. 2003). The third group (Lithium--treated): rats were injected intraperitoneally (i.p.) with Li_2CO_3solution (25 mg/kg Li_2CO_3 solution in saline) twice daily for 30 days (Leschiner et al. 2000). The fourth group (CS pre-treated): rats were treated with CS orally for 14 days followed be injection with Li_2CO_3 solution i.p. twice daily for another 30 days. The fifth group (CS simultaneous-treated): rats were injected with Li_2CO_3 solution i.p. twice daily for 30 days in coincide to treatment with CS orally for 14 days. The sixth group (CS post-treated): rats were injected with Li_2CO_3 solution i.p. twice daily for 30 days followed by CS treatment orally for another 14 days.

Specimens collection and preparation

Urine samples were accumulated during the experimental period from each group by metabolic cages then centrifuged for 10 min at 1500 rpm. At end of the experiment (after 24 h of the last injection), rats were fasted for 16–18 h then anesthetized through slight exposure to diethyl ether. Before sacrificing, blood samples were drawn from retro-orbital plexus and allowed for clotting at room temperature and then centrifuged at 3000 rpm for 15 min; the serum was separated and kept in clean stoppered vials at − 20 °C until the biochemical assay. After sacrificing the animals by cervical dislocation, the kidney tissues were dissected and washed in ice-cold saline. Portion of renal tissues was taken and immediately fixed in 10% formal saline for histopathological examination. Another portion was immediately fixed in glutaraldehyde for ultrastrcucture examination by electron microscope. 0.2 g of kidney tissue was homogenized in 3 mL pH 7.4 Tris-HCl buffers that contained 0.25 M sucrose by a motor-driven tissue homogenizer. The tissue homogenates were centrifuged at 2000 rpm for 10 min for settling down the cell debris and unbroken cells. The supernatants were pipetted into eppendorff tubes and stored at − 80 °C till to be assayed.

Biochemical analysis

The Li levels were quantified in serum samples by inductively coupled plasma-optical emission spectrometry (Varian Co). Serum urea, uric acid, creatinine, total protein and blood urea nitrogen (BUN) were measured in all experimental animals by standard spectrophotometric methods using commercially available kits obtained from Spectrum Diagnostics Egyptian Company for Biotechnology (Cairo, Egypt).

The urinary NAG level was measured in the clear supernatants of urine samples spectrophotometrically at 580 nm as 3-cresol sulfonphthalein released from 3-cresol sulfonphthaleinyl β-D-glucosaminide according to the method described by Yakata et al. (1983). Furthermore, creatinine was measured in urine using standard spectrophotometric methods and hence urinary NAG/creatinine ratio was calculated.

Markers of the oxidative stress

The lipid peroxidation product (LPO) was determined in the renal tissue homogenates spectrophotometrically at wavelength 535 nm using a UV-vis spectrophotometer (Shimadzu uv-2401 pc) according to method suggested by Ohkawa et al. (1979).

The total (Cu–Zn and Mn) superoxide dismutase (SOD) activity was assayed and expressed as units per gram protein (U/g) in renal tissue homogenates based on method suggested by Sun et al. (1988) and a slightly modified by Durak et al. (1993). Catalase (CAT) activity was measured spectrophotometrically in the presence of hydrogen peroxide (H_2O_2) as substrate according to method Aebi (1984) who suggested that activities of the enzymes were expressed as k (rate constant) per gram (k/g) protein. The glutathione peroxidase (GPx) activity was measured based on the method described by Paglia and Valentine (1967). The enzymatic reaction was consisting of reduced nicotinamide adenine dinucleotide phosphate, reduced glutathione, sodium azide and glutathione reductase. It was initiated by addition of H_2O_2 as substrate and the change in absorbance was monitored spectrophotometerically at wavelength 340 nm. The enzyme activity was given in units per gram protein (U/g). Furthermore, the reduced glutathione (GSH) content was assayed in tissue homogenates through the reaction with 5.5′-dithio-bis-2-nitrobenzoic acid (DTNB) to form product measured

spectrophotometrically at 412 nm. The result was expressed as μmol of GSH per mg of protein (μmol GSH/mg protein) (Ellman 1959).

Histopathological examination

The renal specimens were prepared according to method described by Banchroft et al. (1996). The specimens were washed in tap water then dehydrated in serial dilutions of alcohol solutions. Tissue fragments were cleared in xylene and embedded in paraffin. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μm thickness using sledge microtome. The tissue sections were collected on glass slides and stained by hematoxylin & eosin (H&E) then studied under the light electric microscope.

Scanning microscopic examination

The ultrastructural examination was carried out in specimens autopsied from renal tissues using scanning electron microscope (SEM). The tissues were preserved in gluteraldehyde for 24 h and processed according to method suggested by Tánaka (1989) and modified by Kiernan (2000) who reported that the specimen dehydrated through series of the alcoholic solutions. At end of the last dehydration step, the specimens were incubated at 37 °C for 15 min and then coated with the golden atoms to be ready for the examination by electron microscope.

Statistical analysis

Data for all animal groups were represented by mean ± standard error (SE) of eight individual values. Data of all groups were compared using one-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparison tests. Pearson correlation between the study parameters was calculated. The probability of $p < 0.05$ was considered significant.

Results

All the rats survived the experimental period until sacrifice. It was found that Li was completely absent in sera of control and CS-treated groups. The Li-treated and CS simultaneous and post-treated groups were noticed with high serum-Li concentrations (0.42 ± 0.01, 0.40 ± 0.02, and 0.41 ± 0.01 mmol/l, respectively). It was noticed that CS reduced the Li concentration significantly ($P < 0.05$) in serum of the CS pre-treated group (0.162 ± 0.002 mmol/l) when compared to other Li-treated groups.

Based on data depicted in Table 1, it was found that lithium caused significant ($P < 0.05$) elevation in serum urea, creatinin and BUN associated with significant ($P < 0.05$) decline in serum uric acid and T. protein. Moreover, it increased the urinary excretion of NAG significantly ($P < 0.05$) as illustrated in Fig. 1. Administration of CS restored levels of these measurements to normalcy in CS pre-treated group through lowering urea, creatinin and BNU levels significantly ($P < 0.05$) with respect to Li-treated group. Although CS could not restore these measurements to normal values in CS simultaneous and post-treated groups, it decreased their levels significantly ($P < 0.05$) as compared to Li-treated group. Furthermore, CS increased levels of T. protein and uric acid to be normal in CS pre-treated group. While in CS simultaneous and post-treated groups, CS could not restore these measurements to normal levels but it enhanced their levels significantly ($P < 0.05$) with respect to Li-treated group.

As illustrated in Fig. 2, it was found that the renal LPO level increased significantly ($P < 0.05$). As revealed in Table 2, levels of the antioxidant measurements (SOD, CAT, GPx, and GSH) decreased significantly ($P < 0.05$) in renal tissue of the Li-treated group in comparison with control group.

The treatment with CS reduced the renal LPO significantly ($P < 0.05$) associated with stimulating the antioxidant system by increasing levels of SOD, CAT, GPx, and GSH significantly ($P < 0.05$) in the renal tissue as compared to Li-treated group. It was emphasized that CS restored levels of the antioxidants in the pre-treated group to normal values. While in CS simultaneous and post-treated groups, CS could not restore their values to normalcy, but it exhibited improvement through enhancing their values (except LPO and GSH levels) with respect to Li-treated group.

Table 1 Prophylactic and therapeutic effect of chitosan on renal functions against Li_2CO_3 induced renal toxicity in rats

	C	CS	Li	Pre-treated	Sim-treated	Post-treated
Urea (mg/dl)	26.96 ± 0.30	26.47 ± 0.21	49.10 ± 0.16[a]	27.22 ± 0.41[b]	38.48 ± 0.25[ab]	39.39 ± 0.36[ab]
Creatinin (mg/dl)	0.73 ± 0.01	0.75 ± 0.01	2.22 ± 0.02[a]	0.78 ± 0.01[b]	1.71 ± 0.02[ab]	1.85 ± 0.02[ab]
BUN (mg/dl)	8.17 ± 0.09	8.02 ± 0.06	14.88 ± 0.05[a]	8.25 ± 0.12[b]	11.66 ± 0.08[ab]	11.94 ± 0.11[ab]
T. protein (g/dl)	8.19 ± 0.03	8.09 ± 0.04	4.01 ± 0.08[a]	7.89 ± 0.10[b]	5.28 ± 0.02[ab]	5.89 ± 0.06[ab]
Uric acid (mg/dl)	4.22 ± 0.03	4.29 ± 0.06	0.80 ± 0.01[a]	4.31 ± 0.04[b]	1.61 ± 0.01[ab]	1.48 ± 0.01[ab]

Values are expressed as mean ± SE for 8 rats per group
[a]Statistically difference in comparison with control group (significant at $P < 0.05$)
[b]Statistically difference in comparison with Li-treated group (significant at $P < 0.05$)

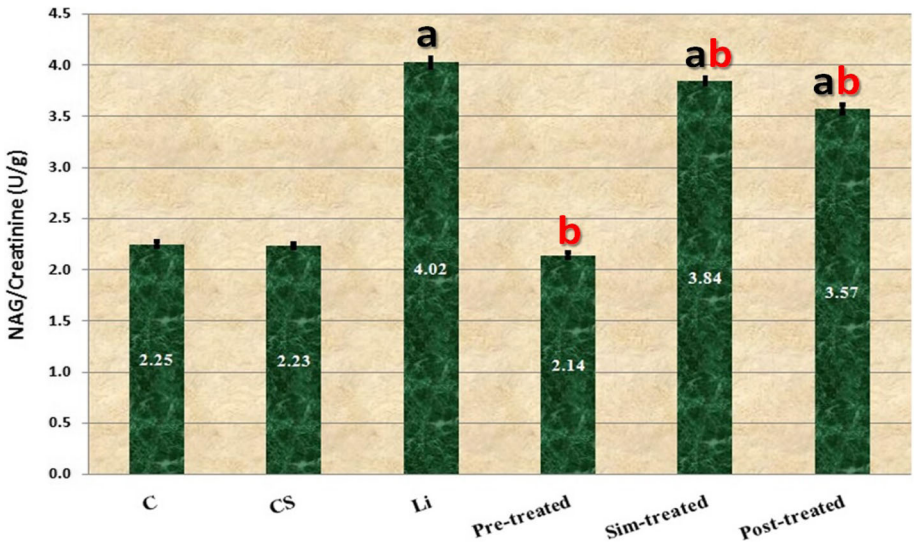

Fig. 1 Prophylactic and therapeutic effect of chitosan on urinary NAG/creatinine (N-acetyl-β-D-glucosaminidase/creatinine) ratio against Li_2CO_3-induced renal toxicity in rats. Bars represent the group means ± SE. **a** Significant difference from control group at $P < 0.05$. **b** Significant difference from Li-treated group at $P < 0.05$

It was revealed that there were no histopathological alterations in kidney of control rats. As presented in Fig. 3a, the glomeruli and tubules were recorded at the cortex with normal histological structure. In CS-treated group, there was no histopathological alteration and no deviation from control group (Fig. 3b). While in Li-treated group, there was vacuolization in the endothelial cells lining tufts of the glomeruli with degeneration in the surrounding tubules (Fig. 3c). In contrast, in CS pre-treated group, there was no histological alteration of the glomeruli and tubules (Fig. 3d). In CS simultaneous-treated group, congestion was detected in the cortical blood vessels (Fig. 3e). Also, in CS post-treated group, there was congestion in the cortical blood vessels (Fig. 3f).

As presented in the electron micrograph (Fig. 4a), the renal ultrastructure of control and CS-treated groups showed normal architecture. Administration of CS alone

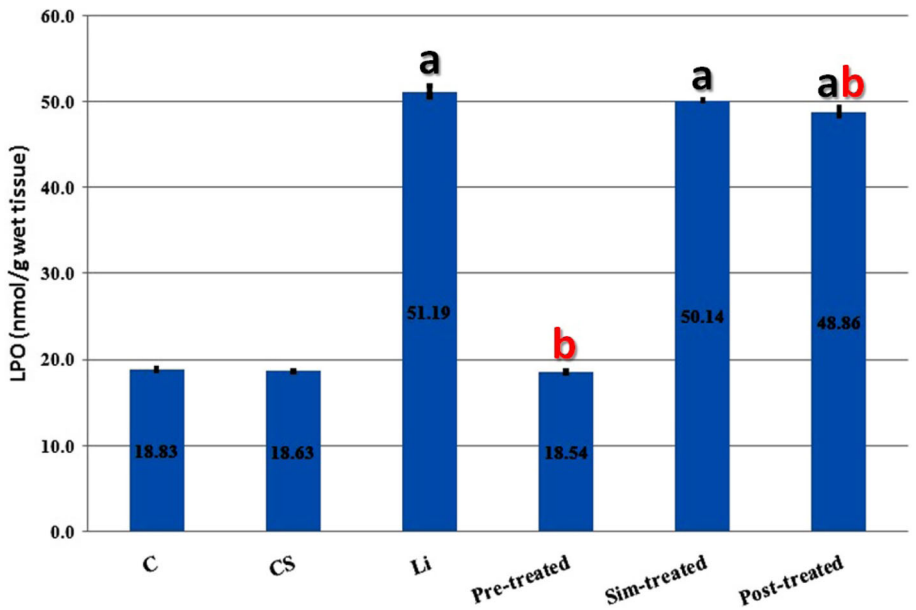

Fig. 2 Prophylactic and therapeutic effect of chitosan on renal LPO (lipid peroxidation product) level against Li_2CO_3-induced renal toxicity in rats. Bars represent the group means ± SE. **a** Significant difference from control group at $P < 0.05$. **b** Significant difference from Li-treated group at $P < 0.05$

Table 2 Prophylactic and therapeutic effect of chitosan on activities of the antioxidants against Li_2CO_3-induced renal toxicity in rats

	C	CS	Li	Pre-treated	Sim-treated	Post-treated
SOD (U/g protein)	0.86 ± 0.01	0.82 ± 0.01	0.48 ± 0.01[a]	0.80 ± 0.01[b]	4.20 ± 0.02[ab]	4.12 ± 0.05[ab]
CAT (*k*/g protein)	556.28 ± 1.77	551.03 ± 1.41	348.17 ± 3.36[a]	550.47 ± 0.73[b]	370.47 ± 1.94[ab]	379.82 ± 2.83[ab]
Gpx (U/g protein)	569.48 ± 1.12	572.33 ± 0.96	335.21 ± 1.44[a]	567.30 ± 1.22[b]	325.50 ± 1.07[ab]	329.89 ± 1.04[ab]
GSH (μmol/mg protein)	3.93 ± 0.06	3.83 ± 0.03	1.88 ± 0.02[a]	3.78 ± 0.05[b]	1.87 ± 0.06[a]	2.21 ± 0.05[ab]

Values are expressed as mean ± SE for 8 rats per group
[a]Statistically difference in comparison with control group (significant at $P < 0.05$)
[b]Statistically difference in comparison with Li-treated group (significant at $P < 0.05$)

displayed no alterations in the tissue surface with respect to control (Fig. 4b). Obvious lesions were noticed in kidney of Li-treated group and represented by deep irregular cracking (red arrow) with cellular bridging (Fig. 4c).

In the CS pre-treated group, CS exhibited beneficial effect against the necrotic effect induced by Li and improved the kidney architecture where the renal tissue appeared unchanged and smooth in this group with some blood aggregates (yellow arrow) (Fig. 4d). In the CS simultaneous and post-treated groups (Fig. 4e, f), there were severe ultrastructural alterations represented by deep irregular cracking (red arrow) associated with cellular bridging (black arrow) and cellular lose.

Discussion

Lithium is considered as the first therapeutic line in patients with bipolar affective disorders (Boton et al. 1987). It is well known that nephrotoxicity that is represented by impairment of the concentrating ability and renal tubular damage can be categorized as the most common adverse Li effect (Markowitz et al. 2000; Prense et al. 2003).

In the present study, it was found that the Li concentration increased in sera of Li-treated rats. This was in accordance to Zhang et al. (2009) who reported that the Li doses resulted in serum levels of < 0.9 mmol/L, which is the lower normal therapeutic serum level for bipolar disorder. CS showed an effective role in decreasing the Li concentration especially in sera of the CS pre-treated group. This was in agreement with Toz and Değer (2018) who showed that CS was able to in remove the bivalent metals from the circulatory system effectively and enforced the defense (antioxidant) system against free radicals attack. Furthermore, Wang et al. (2016) revealed that efficacy of

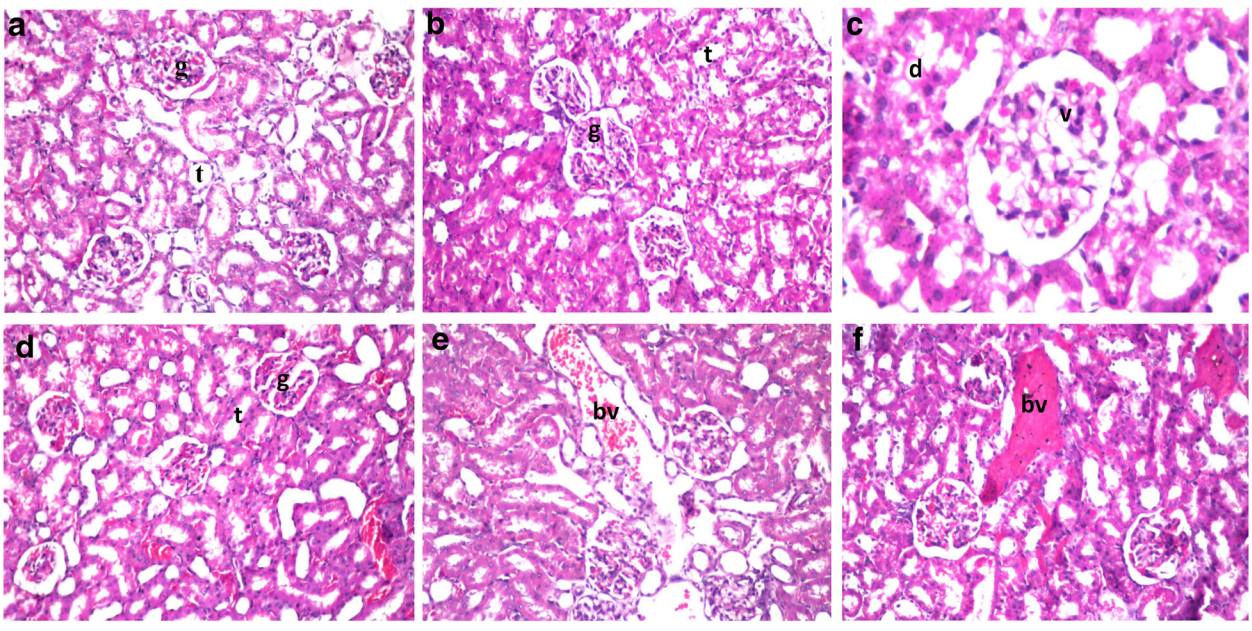

Fig. 3 Kidney tissue showing **a** control group showing glomeruli (g) and tubules (t) with normal histological structure at the coretx (H&E, × 40), **b** CS-treated group without deviation from control group (H&E, × 40), **c** Li-treated group showing vacuolization (v) in endothelial cells lining the tufts of the glomeruli with degeneration (d) in the surrounding tubules (H&E, × 80), **d** CS pre-treated group without alterations or deviation from control group (H&E, × 40), **e** CS simultaneous-treated group with congestion in the cortical blood vessels (bv) (H&E, × 40), and **f** CS post-treated group with congestion in the cortical blood vessels (bv) (H&E, × 40)

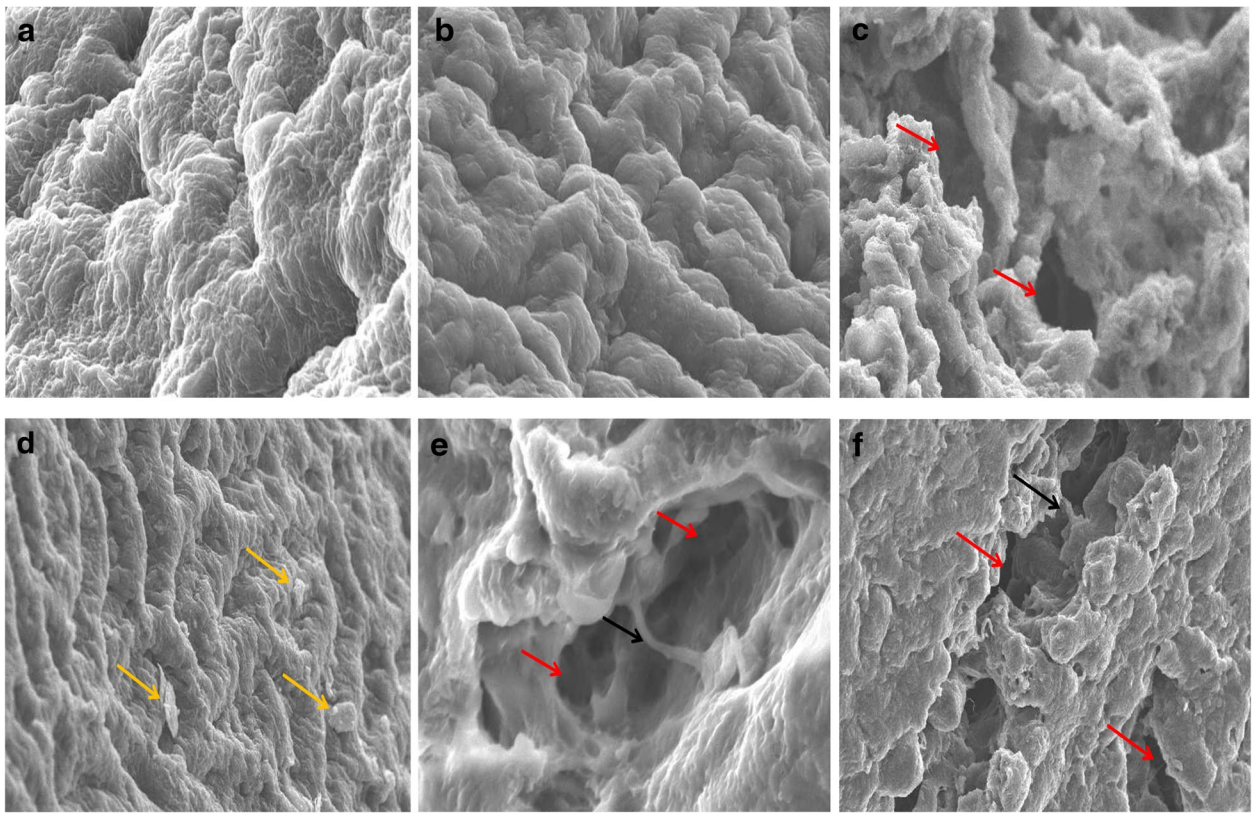

Fig. 4 Scanning electromicrograph in renal tissue showing **a** control group with normal ultrastructure (× 1500), **b** CS-treated group without deviation from control group (× 1300), **c** Li-treated group with obvious lesions represented by deep irregular cracking with cellular bridging (× 1300), **d** CS pre-treated group without alterations or deviation from control group (× 1000), **e** CS simultaneous-treated group with severe ultrastructural alterations represented by deep irregular cracking with cellular bridging (× 1700), and **f** CS post-treated group with ultrastructural variations represented by irregular cracking with cellular bridging in addition to cellular lose (× 1000)

CS might be increased when taken in conjunction with a suitable chelator for eliminating metal from the circulation.

Lithium affects both kidneys at different levels and this effect is expressed by a series of physiologic changes (Mwaheb et al. 2016). It was found that presence of Li in the carbonate form increases the selective permeability of lithium and sodium. This might be attributable to the ability of carbonate to produce ion pairs with them (Ullah and Khan 2014). Polyuria is considered as the most adverse side effects of Li. This might refer to ability of Li to replace sodium or potassium in the cell. Consequently, this leads to alteration in salt, water and electrolyte handling (Bedford et al. 2008; Mwaheb et al. 2016).

It was found that Li caused significant increase in measurements of the renal functions (urea, creatinine and BUN). This was in accordance with Ahmed et al. (2011) and supported recently by Mwaheb et al. (2016) who notify that Li is able to stimulate the toxicity at physiological and histopathological grades in renal tissues of rats. Moreover, the severe elevation in renal functions related to renal tissues lesions (Cherpakov et al. 2015). The CS administration resulted in a significant decrease in levels of these measurements as compared with Li-treated group. This was in accordance with the study carried out by Anraku et al. (2012) who postulated that CS resulted in a significant reduction in levels of the pro-oxidants thereby inhibiting the subsequent development of oxidative stress.

As suggested by Toplan et al. (2013) and supported by Weiner et al. (2014), it was emphasized that the proximal tubule represents the primary site through which Li is reabsorbed and accumulates in the distal parts of the nephron through the epithelial sodium channel. Therefore, it is considered as one of the reasons which lead to reducing levels of serum uric acid and T. protein associated with elevation of urinary excretion of NAG and renal LPO level. Moreover, these biochemical abnormalities in renal tissues may occur as a result of effect of the oxidative stress which represents one of the important deleterious mechanisms of Li. Consequently, this leads to generation of the reactive oxygen species (ROS) and hence induces renal impairment (Oliveira et al. 2010). In

addition, the urinary excretion of NAG might be elevated as a result of the deleterious effect of Li at early stage in the renal tubuli (Jeon et al. 2003; Bosomworth et al. 1999). CS showed effective role in lowering the urinary excretion of NAG and the renal LPO. This might refer to the effective role of CS against attack of hydroxyl radicals and hence inhibiting peroxidation of phosphatidylcholine and linoleate liposomes (Jeon et al. 2003; Ozcelik et al. 2014).

In the renal tissues, there were various antioxidants to prevent the lesions occurred as a result of attack the excessive oxygen metabolites. They act either by decomposing peroxide or trapping the free radicals. Both CAT and GPx together play the same action through stimulating H_2O_2 to water. SOD is a specific antioxidant enzyme that dismutates O^{-2}, forming H_2O_2, which is scavenged by peroxisomal CAT or GPx. In addition, it protects the cell against the deleterious effect of superoxide radicals. The extracellular SOD distributes in vessel walls of the renal tissue to detoxify the superoxide anions produced in sera of oxidative stress-exposed rats (Fadillioglu et al. 2003; Yilmaz et al. 2004).

GPx plays a vital role in removing H_2O_2 and lipid hydroperoxides through using reduced glutathione (GSH) as a hydrogen donor. During this reaction, GPx converts glutathione from the reduced (GSH) to its oxidized form (GSSG). In return, the cells utilize glutathione reductase to stimulate recycling GSSG to GSH (Zasadowski et al. 2004; Ilhan et al. 2004). As a result of lowering GPx activity, the H_2O_2 increased and subsequently leads to elevation of LPO and hence increasing the urinary NAG excretion.

As revealed during the present study, Li caused significant alterations in the antioxidant system in the renal tissue by lowering levels of enzymatic and non-enzymatic antioxidants. These results coincide with other study carried out by Oktem et al. (2005) who reported that these alterations might be attributed to the increased oxidative stress that leads to an over consumption of these antioxidants. Therefore, lowering the renal antioxidants indicates a high degree of oxidative stress leading to enhancement of the endogenous H_2O_2. As illustrated in results of the current study and supported by Mueller et al. (1997), the reduced LPO is accompanied by increased activities of CAT and GPx that are responsible for H_2O_2 elimination through its conversion into water and alcohol. Moreover, stimulation of the oxidative stress might refer to adverse effect of Li on the mitochondrial membranes which represent the major site for the mitochondrial respiratory chain and production of superoxide radicals (O^{-2}) (Nicholls and Budd 2000). This leads to generation of excess amounts of oxygen radicals and subsequently causes extreme use of SOD in rat kidneys (Toplan et al. 2016). The treatment with CS significantly increased SOD, CAT and GSH in the renal tissues as compared with Li-treated group. This was in accordance with Wang et al. (2016) who reported that CS exhibited efficacy against oxidative stress through reducing the LPO levels associated with elevation in activities of the antioxidant enzymes. Moreover, it elevated the GSH level although it does not have a known stimulating effect on GSH biosynthesis. CS exhibits beneficial effect on the antioxidant system by same mechanism of taurine through inhibiting the peroxidation reactions, thereby mitigating the GSH consumption and hence activating the antioxidant enzymes (Toz and Değer 2018).

Based on the histopathological investigation carried out at end of the experiment, it was noticed that Li caused various deformative alterations represented by damage of the epithelium lining of the glomeruli in renal tissue without signs of cellular proliferation as illustrated in the histopathological picture of the renal tissues. This was in accordance with Sharma and Iqbal (2005) and supported recently by Mwaheb et al. (2016) who suggested that lesions of the renal tissue might refer to the central role of oxidative stress in the pathogenesis process induced by ROS attack.

The SEM has become a powerful and suitable tool to study the morphological and ultrastructural alterations in the kidney tissue (Vodenicharov 2007). The renal ultrastructure examined by SEM has demonstrated severe lesions represented by deep irregular cracking with cellular bridging in surfaces of the renal tissues, implicating nephrotoxicity as one of the most important deleterious mechanisms of Li. Furthermore, in case of discontinuation of Li therapy, the renal deteriorate may continue as insidious and progressive (Markowitz et al. 2000). This might be due to effect of nitric oxide (NO) generated as a result of inducible nitric oxide synthase and responsible for the tissue damage. Consequently, peroxynitrite anions generated in the tubular epithelium as a result of the interaction of NO with superoxide anions (O_2) has the potential to alter the adhesion properties of tubular cells (Hierholzer et al. 1998; EMcKim et al. 2003). It was noticed that CS exhibited a renoprotective effects against renal lesions through reducing the free radicals in addition to its role in reducing deleterious effect of carbonyl groups. Moreover, Chou et al. (2015) postulated that CS showed a positive antagonistic effect by mechanism similar to that of metformin (standard drug) and may thus be a promising new treatment to protect against gentamicin-induced nephrotoxicity.

Conclusions

The current study provides a clear experimental evidence for promising role of CS in the protection against the oxidative stress and renal toxicity induced by Li. This is attributed to presence of polymeric biologically active compounds derived from chitin and exhibit antioxidant activities. Thus, CS was able to show its efficiency to improve renal toxicity when it can be used alone as a preventive agent without conjunction with chelating agents.

Abbreviations

ANOVA: One-way analysis of variance; BUN: Blood urea nitrogen; CAT: Catalase; CS: Chitosan; DTNB: 5.5′-dithio-bis-2-nitrobenzoic acid; GPx: Glutathione peroxidase; GSH: Reduced glutathione; H&E: Hematoxylin and eosin; H_2O_2: Hydrogen peroxide; Li: Lithium; Li_2CO_3: Lithium carbonate; LPO: Lipid peroxidation product; NAG: N-acetyl-β-D-glucosaminidase; NO: Nitric oxide; ROS: Reactive oxygen species; SE: Standard error; SEM: Scanning microscopic examination; SOD: Superoxide dismutase

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Availability of data and materials

The authors have ready to send data and the detailed scientific materials if it is useful for readings.

Authors' contributions

Both authors carried out literature review and draft the manuscript. Wael M. Aboulthana carried out the experimental work. Noha E. Ibrahim participated in the collection of data and arranged in tabular form. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was carried out on the experimental animals. They were handled according to the experimental protocol which was approved by Institutional Animal Ethics Committee of National Research Centre, Dokki, Giza, Egypt and were conducted in accordance with guidelines as per "Guide for the care and use of laboratory animal" and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals.

Consent for publication

Not applicable

Competing interests

The present study aimed to optimize CS as a promising protector against oxidative stress and renal toxicity induced by Li in the hope that this compound may be further explored as novel antioxidative protector. The authors declare that they have no competing interests.

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