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Research Article
Utilization of Nanotechnology and Thioctic Acid Against the Lithium Carbonate Toxicity in the Management of Schizophrenia

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Abstract

Background and Objective: Although lithium carbonate (LiC) has a wide scope in the treatment of schizophrenia, its poor solubility and associated nephrotoxicity have limited its use. The present study aimed to enhance LiC solubility and reduce its renal toxicity in combination with thioctic acid (ThA) antioxidant in a nanostructured lipid carriers ((NSLCs). **Materials and Methods:** The NSLCs containing LiC and ThA were prepared using hot emulsification combined with ultrasonication method. The size and morphology of the prepared nanocarrier were assessed. The *in vitro* stability and entrapment efficiency were determined and the selected optimum formulation was subjected to *in vivo* nephrotoxicity studies on rabbits. **Results:** The resulting nanoparticles of 31 nm size had 16.2 mV surface charges. The nanocarriers were spherical in shape with the percentage entrapment of 83.5±7.5 and 47.2±4.4% for lithium and ThA, respectively. The *in vitro* release indicated the prolonged release of lithium from nanocarriers with a minimal burst in comparison to pure powder. The kidney treated with ThA based lithium nanocarriers and the group treated with pure lithium did not differ significantly from the control group in the functional parameters (urea, calcium, sodium and serum creatinine). **Conclusion:** The study concluded that ThA-based nanostructured lipid carriers can enhance the *in vitro* solubility and reduce the *in vivo* nephrotoxicity of lithium.

Key words: Lithium carbonate, schizophrenia, thioctic acid, nanostructured lipid carriers, nephrotoxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Numerous advancements have been made in the fields of medicine and pharmaceuticals with nanotechnologies. Nanotechnology has improved the target-based drug design[1]. Nanostructured lipid carriers (NSLC) are vesicular systems with improved efficacy and safety. Lipids have been used in NSLC for targeted and prolonged delivery of an intended drug or medicinal moieties[2]. Innovative drug delivery, like NSLC, is always in demand and a great help to patients because of its ability to reduce unwanted side effects in the treatment of disease relapse as well[3].

Schizophrenia is a complex chronic brain disorder with seriously impaired behavioral, speech and cognitive ability. A deep heterogeneity of schizophrenia is due to poor diagnostic criteria, pathophysiology and treatment options available till date. The pharmacological approaches involving antipsychotics have failed as a treatment option[4]. Relapse of schizophrenia in certain individuals deteriorates the prognosis. Co-therapy strategy with adjunctive medication has a profound influence on stabilizing the mental conditions[5]. Lithium or few anticonvulsant combinations have been found to improve the condition, but their use is limited due to their uncommon effects on vital organs such as kidneys[6].

Thus, their safer use in available treatment options should be further explored. Lithium carbonate is an alkali metal and traditionally tried for affective psychoses. Lithium has been used in the management and treatment of mania and even in the prevention of relapse in affective psychoses (bipolar)[7]. Its use is finite due to the narrow therapeutic window and rapid elimination that requires high dosing frequency leading to nephrotoxicity. This potential harm is due to lithium altering the phospholipid metabolism of distal convoluted tubular cells[8].

Lithium-induced nephrotoxicity is caused mainly by elevated reactive oxygen radical species (ROS). Thioctic acid, as a strong antioxidant can have a positive protective effect by scavenging these ROS[9]. Thioctic acid has been previously reported to minimize drug-induced neuro[10] and hepatic/nephrotoxicities[11]. It has been also used in the treatment of diabetes[12], glaucoma[13] and even cancer[14]. Incorporating thioctic acid in NSLC to reduce the unwanted effects of lithium is a novel idea. Considering the positive outcomes of lithium in the treatment and management of psychosis, strategies to minimize nephrotoxicity and to implement it for clinical use must be framed, which is an important objective of the present study. The present study aimed to optimize the preparation of thioctic acid-based lithium NSLC, characterize its formulation for physiochemical properties and evaluate it for *in vitro* release, pharmacodynamic efficacy like renal function and oxidative stress markers along with stability studies.

MATERIALS AND METHODS

Lithium carbonate (LiC) and thioctic acid (ThA) powder were purchased from Xian Sunny Biochemical Technology (Shaanxi, China). Lecithin was a kind gift from Nikko Chemicals Co., Ltd. (Japan). Castor oil was purchased from Sigma (USA). Glyceryl dibehenate, glyceryl distearate, suppocire, lauryl macrogol-32 glyceride and labrafac were gifted from Gattefosse (France).

Methodology

Preparation of LiC-ThA NSLCs: The NSLCs containing LiC and ThA were prepared using hot emulsification combined ultrasonication method[15]. Both LiC and ThA exhibited better solubility in glyceryl distearate in comparison to suppocire and glyceryl distearate; therefore, glyceryl distearate was selected as lipid matrix for the formulations. Phospholipid (5%) and glyceryl distearate (30%) were dissolved in 30 mL of chloroform:methanol (1:1). The LiC (30%) and ThA (10%) were dissolved in labrafac oil (20%) along with the remaining NSLC components. After removing the organic solvents by a rotary evaporator (ROTEVA, India), the left lipid layer was melted at 80°C. About 5% of Lauryl macrogol-32 glyceride was dissolved in 20 mL of double distilled water and heated up to the lipid temperature. Both mixtures were combined at same temperatures and homogenized at 15,000 rpm IKA Ultra-Turrax T8 homogenizer (IKA, Wilmington, NC, USA) for 50 min followed by probe sonication (Sonics VC750 (Newtown, CT, USA)). The hot nanoemulsion was cooled to room temperature in order to obtain the LiC-ThA NSLCs.

Size and surface charge analysis: Both particle size and surface charge of the prepared LiC-ThA NSLCs were determined using Zetasizer (Zetatrac, Montgomeryville, PA, USA). The formulation was diluted in 1:25 of distilled water and analysis was performed in triplicate.

Surface morphology studies of LiC-ThA NSLCs: Surface morphology of the prepared formulation was examined using transmission electron microscopy (TEM) (Philips XL30, Eindhoven, Netherlands). The sample was prepared similarly to the previously reported method to determine both structure and size[16].

Determination of percentage entrapment efficiency (EE %) of LiC-ThA NSLCs: Approximately 2 mg of prepared lyophilized LiC-ThA NSLCs were dissolved in chloroform and 2 mL of water was added followed by vortex for 5 min. The LiC and ThA were extracted from water and chloroform layers, respectively and quantified individually using previously reported high-performance liquid chromatography (HPLC) method[17-19].

LiC-ThA NSLCs stability studies: To understand the physical stability of the LiC-ThA NSLCs formulation, the samples were exposed to 3 cycles of freezing at -20°C followed by thawing. The parameters, such as; size, surface charge and EE (%) were evaluated[20].

***In vitro* release study of LiC-ThA NSLCs:** Franz cell diffusion set up was employed to determine the release of LiC from the prepared formulation. Approximately 30 mg of LiC-ThA NSLCs was placed in the donor cell and LiC was allowed to diffuse into the receptor chamber across the 0.2 µm silicon membrane. A phosphate buffer solution of pH 5.5 was used as a release medium and sampling was performed at 1, 2, 4, 6, 8 and 12 h intervals. The quantification of LiC was performed using earlier reported HPLC method[18].

***In vivo* nephrotoxicity studies of LiC-ThA NSLCs:** Eighteen rabbits (average weight of 2.5±0.8 kg) were grouped into three groups of 6 animals each. The control group was injected with normal saline as opposed to the other two groups that received LiC (30 mg kg^{-1}) dissolved in saline for every 12 h up to 10 days. The first group and the other two groups were given pure LiC and LiC-ThA NSLCs (30 mg kg^{-1}) intramuscularly. All animals were procured from the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. All procedures were approved by the Animal Ethics Committee of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia and they complied with the Declaration of Helsinki, the Guiding Principle in Care and Use of Animals (DHEW production NIH 80-23) as well as the "Standards of Laboratory Animal Care" (NIH distribution #85-23, reconsidered in 1985). The animals were maintained at 20±1°C and 12/12 h dark/light cycle in natural controlled enclosures with standard feed and water up to 14 days. Blood samples were collected from the eye (medial canthus) on 1st, 5th and 10th days. The isolated serums were maintained at -80°C and were examined for nephrotoxicity indicators, such as; urea, potassium, sodium, calcium and creatinine levels using a previously reported method[19].

Statistical analysis: All the data were analyzed using one-way ANOVA and differences between the parameters were expressed as mean±SE.

RESULTS

Lipids are important components in formulating ideal NSLCs. Solubility studies of Li and ThA in various solid and liquid lipids were determined. The solubility of Li in glyceryl distearate was found to be highest at 40% (w/w) in comparison to and glyceryl dibehenate at 15% (w/w) and suppocire at 26% (w/w). The ThA had also the highest solubility in glyceryl distearate at 16% (w/w) as well in comparison to other two lipids. Therefore, glyceryl distearate was selected for the formulation of optimized LiC-ThA NSLCs (Table 1).

Size and surface charge analysis: The size and charge of the optimized LiC-ThA NSLCs formulation were about 31±5 nm and 16.2±4 mV, respectively.

Surface morphology studies: The optimized nanostructured formulation indicated spherically shaped vesicles in the TEM images. The lipid structures were evident with an inner semisolid core surrounded by outer phospholipids as depicted in Fig. 1.

Table 1: Solubility of LiC and ThA in various lipids

Lipid components	LiC solubility (%) (w/w)	ThA solubility (%) (w/w)
Suppocire	26	7
Glyceryl dibehenate	15	12
Glyceryl distearate	40	16

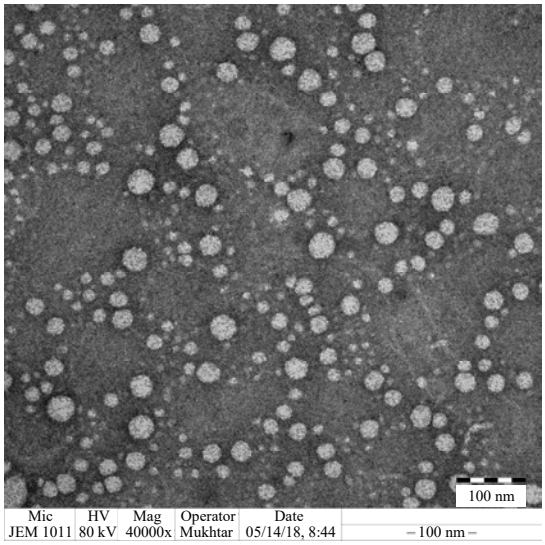

Fig. 1: Transmission electron microscopic (TEM) image of optimized LiC-ThA NSLCs formulation

Table 2: Stability studies data for the optimized LiC-ThA NSLCs formulation

Measured parameters for optimized formulation	Particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)	
			LiC (%)	ThA (%)
At time zero	31±5	16.2±4	83.5±7.5	47.2±4.4
After Cycle 1	31±8	17.3±3	82.5±1.5	45.3±5.2
After Cycle 2	33±3	16.1±2	84.5±2.5	46.6±3.3
After Cycle 3	36±6	13.6±7	81.5±3.4	45.2±2.1

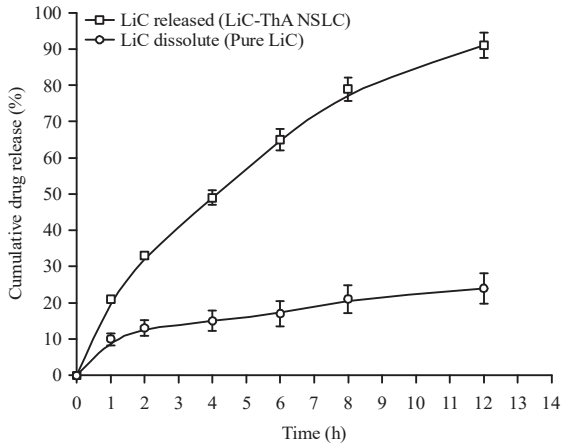

Fig. 2: Comparative *in vitro* release study pure vs. NSLCs LiC ThA formulations

Determination of percentage entrapment efficiency (EE %): Drug loading capacity is an important parameter to determine the lipid suitability to be used to formulate NSLCs and the results indicated that the prepared NSLCs entrapped about 83.5±7.5% of LiC and about 47.2±4.4% of ThA (Table 2).

Stability studies: After 3 cycles, no changes in particle size, zeta potential and EE (%) were found as reported in Table 2. This could be due to the stability of glyceryl distearate lipid used in the formulation of NSLCs for temperature changes.

***In vitro* release study:** Figure 2 showed the *in vitro* release of LiC from NSLCs compared to pure LiC powder. The results showed that 91% of LiC was released at 12 h in case of NSLCs compared to pure LiC, which showed only 24% dissolution. Even though the NSLCs indicated burst release (33%) at 2 h, it greatly enhanced the LiC solubility in comparison to the pure LiC. This prolonged release of LiC from NSLCs can be beneficial in reducing the associated toxicity as well.

***In vivo* nephrotoxicity studies:** Neuroprotective effect of ThA in LiC induced nephrotoxicity was evident by evaluating various kidney function parameters. The results are illustrated in Fig. 3. No significant change in kidney parameters was observed after administering LiC-ThA NSLCs to the control group, which indicated that the kidney function parameters were unaltered. On day 10, the group treated with LiC only showed a significant decrease in glucose, urea and calcium levels and a significant increase in creatinine, sodium and potassium levels compared to the control group.

DISCUSSION

Although LiC is being widely explored for its use in the treatment of schizophrenia, doctors emphasize the associated side effects limiting its use. The LiC is being repurposed to minimize leukopenia and neutropenia side effects arising from antipsychotic drugs as well. A drug like lithium that has a narrow therapeutic index needs a safe delivery system that can essentially maintain therapeutic levels within a maximum tolerable dose. The NSLCs can satisfy the requirement[20]. To determine the suitability of lipids, ThA NSLCs as blank was synthesized and then LiC was loaded. Selected lipids have a profound effect on the particle size and EE (%) of the LiC. Glyceryl distearate as solid lipid has shown improved stability without any crystallization or drug expulsion during storage[21]. Moreover, both LiC (40%) (w/w) and ThA (16%) (w/w) have indicated the enhance solubility in comparison to other lipids.

The surface charge is an indication of potential generated around the particle due to lipid used. Stability of the NSLCs depends on surface charge aiding in providing sufficient repulsion preventing agglomeration[22]. Both the solid lipid and surfactant combination had potential greater than 16 mV, indicating better physical stability with minimal size[23,24].

The TEM images revealed spherical shaped nanoparticles. The phospholipid had expanded the cationic nature with the bilayer structure due to good homogeneity and uniformity, which is consistent with earlier reported studies[25].

High EE of LiC and ThA were observed as a result of the formation of liquid nanocompartments formed by phospholipid and Labrafac oil due to structural and spatial arrangement difference entrapped within the solid lipids. Unlike previously studies reported that, the study did not observe an increase in the EE (%) with an increase in solid lipid concentration[25].

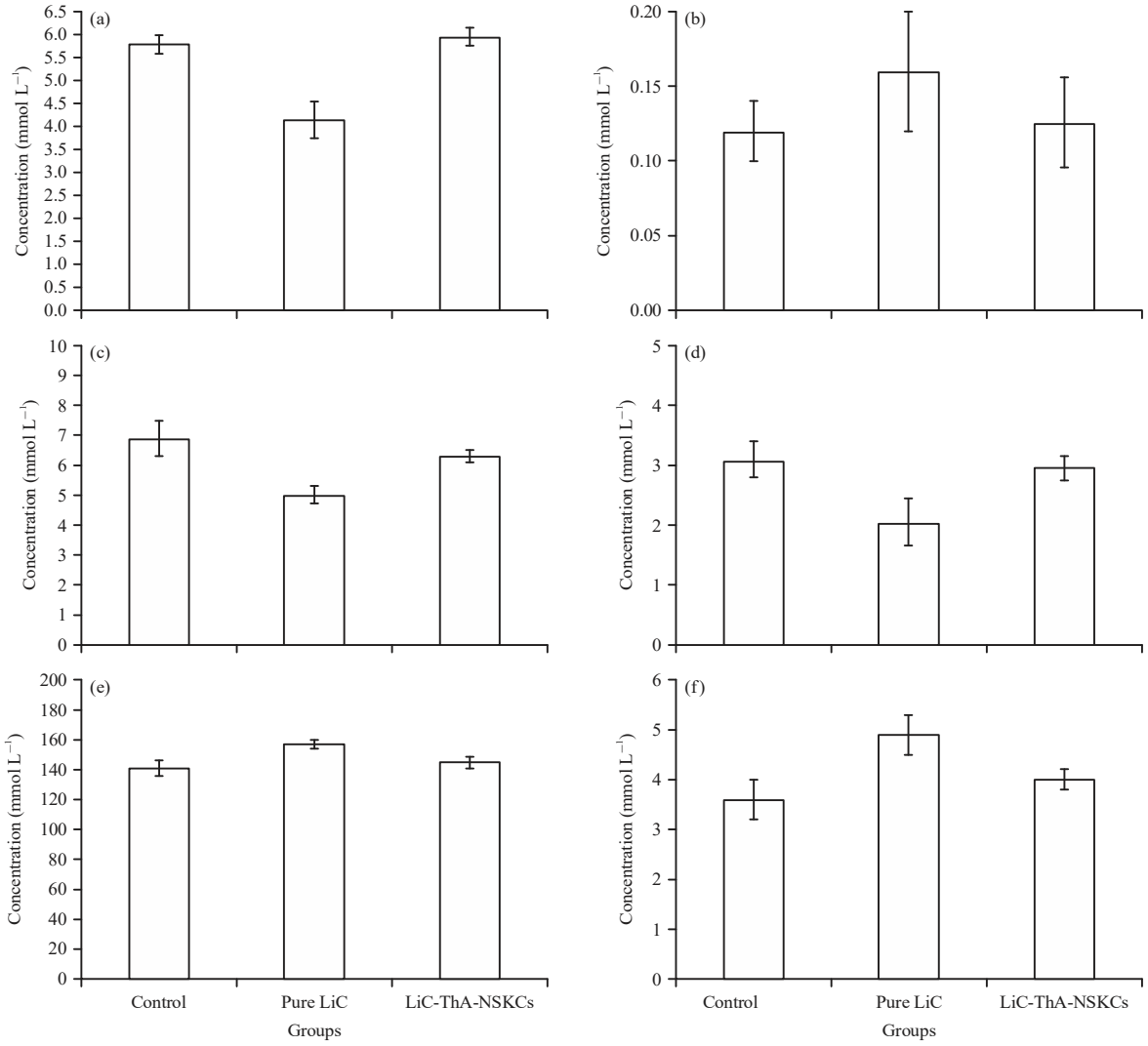

Fig. 3(a-f): Kidney function parameters levels (a) Glucose, (b) Creatinine, (c) Urea, (d) Calcium, (e) Sodium and (f) Potassium (mmol L^{-1}), measured in rabbit plasma after 10 days of treatment

From the *in vitro* release data, the release of LiC from NSLCs was slow and time-dependent in comparison to pure drug at 12 h, which is in line with previous reports. This was due to the fact that LiC got entrapped deep within the lipid core of NSLCs and the drug was able to be released through erosion or degradation of liquid core that can be beneficial for minimizing the LiC nephrotoxicity[26]. The nephrotoxicity reduction was indicated in the key kidney parameters in the group treated with LiC-ThA NSLCs in comparison to control group. Glucose as a component, is rapidly reabsorbed within renal tubules and any alteration would affect this process and result in higher elimination in the urine[27]. The LiC contributed to glycosuria (decrease in serum glucose) and elevated serum creatinine levels that can be due to the renal tubule damage. The end product of protein catabolism in the liver is urea. About 20-30% of urea is reabsorbed back from the kidneys. Urea clearance is greatly reduced by the LiC, since glomerular filtration is affected due to renal tubule damage[28]. The decrease in tubular active secretion will decrease the glomerular filtration of electrolytes; therefore, increasing their elimination in the urine. The LiC group was not significantly different from the control group in terms of reduction of calcium parameters. Oxidative stress in the animal body is due to the imbalance between antioxidant defense and free radical production system. ThA promoted and metabolized free oxygen radicals generated in the body. It also helps prevent further formation of free radicals, tissue damage and repairs. Total antioxidant status (TAS) levels increased in the LiC ThA NSLCs group in comparison to LiC treated group. The results of the current study strongly

supported the protective effect of ThA in NSLCs in reducing the LiC nephrotoxicity, in contrast to previously reported studies[29,30]. Obtained data implicated that evaluating the LiC- ThA NSLC in sub-acute dose-limiting toxicity studies can be valuable, as it can facilitate the progression to human clinical trials and rationalize its use in the pharmacotherapy of schizophrenia.

CONCLUSION

The ThA based LiC NSLCs was successfully prepared using hot emulsification combined with ultrasonication method. Optimal size and charge were obtained for the LiC-ThA NSLCs with enhanced entrapment efficiency. The *in vitro* release demonstrated the prolonged release of LiC from the NSLCs. *In vivo* studies evaluated ThA for their potential in minimizing the LiC induced nephrotoxicity. ThA-NSLCs have significantly contributed to safeguarding kidneys from LiC. Further clinical developments can enable and extend the use of LiC NSLCs in the treatment of schizophrenia.

SIGNIFICANCE STATEMENT

This study examined the use of ThA in minimizing the nephrotoxicity of LiC, which can be beneficial in the treatment of schizophrenia. This study will help the researcher uncover the critical areas in the use of both LiC (alkali metal) and ThA (antioxidant) as combinatorial therapy for psychological disorders that many researchers were not able to explore. Thus, a new theory on schizophrenia treatment strategy may be generated.

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