Digest Journal of Nanomaterials and Biostructures Vol. 13, No. 1, January - March 2018, p. 245 - 252

# COMBINED CEFTRIAXONE SODIUM WITH ALPHA LIPOIC ACID NANOLIPOSOMES FOR MORE STABLE, AND LESS NEPHROTOXIC FORMULA IN PEDIATRICS

U. A. FAHMY*, H. ALDAWSARI
*Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia*

Serious and acute renal failure could be induced in pediatric patients as a result of treatment with ceftriaxone sodium (CTX). CTX may be crystalized in the urine and adhere to surface of renal tubular cells, causing the generation of reactive oxygen species and finally resulting in kidney damage. Our study aims to protect renal tubular cells and prevent calcium oxalate precipitation by combining CTX with alpha lipoic acid (ALA) liposomes. A thin-film hydration method was used for formulation, and the prepared liposomes were imaged with transmission electron microscopy (TEM). Particle size, entrapment efficiency, and drug release were measured, as was the antimicrobial activity of raw CTX and the prepared formula, to assess the effect of ALA on CTX antimicrobial activity. An in vivo study was carried out on 3 groups of rabbits to measure kidney function parameters, such as creatinine, urea, sodium calcium, and potassium. The study was conducted over 10 days of treatment to assess the degree of kidney protection. Results showed that small spherical liposomes measuring 60.4 ± 4.4 nm released CTX gradually, to about 100% after 8 hours. ALA has no effect on the antimicrobial activity of CTX. Data on kidney function showed an insignificant change in kidney function parameters, whereas raw CTX showed a partial difference after 5 days and a significant difference after 10 days. These findings demonstrate that ALA protects renal tubular cells from damage, which makes the combination a candidate for safe antimicrobial therapy.

(Received December 19, 2017; Accepted February 20, 2018)

*Keywords*: Cephalosporin, Infection, kidney, nephritis, nanoparticles

## 1. Introduction

CTX is a third-generation cephalosporin which used to treat many types of infections in pediatric patients. Approximately 30% to 65% of CTX is excreted unmetabolized in the urine, and traces amounts are excreted via biliary elimination.[1,2],another advantage for using CTX is a long half-life which reach to 8.7 h [3]. It was previously reported than on clinical studies, CTX can cause nephrolithiasis, bladder sludge, and biliary pseudolithiasis, especially in pediatric patients.[4,5] Ceftriaxone at therapeutic doses can lead to crystallization in the urine. These crystals adhere to the surface of renal tubular cells. Severe nephrolithiasis can cause post-renal acute renal failure.[6]

The FDA recommends not using CTX more than 14 days after reconstitution because of stability problems. CTX is less stable in its hydrated form,[7] so combination with the antioxidant ALA could play a role in CTX stability. ALA is an octanoic acid derivative that is essential for aerobic metabolism.[8] ALA is an antioxidant made by the body, and several studies suggest that ALA has many medical uses, such as lowering blood glucose levels. ALA is able to interact with free radicals, which decreases the risk of diabetic complications,[9] including symptoms like tingling, pain, burning, itching, and numbness in arms and legs as a result of nerve damage ending in peripheral neuropathy. Other preliminary studies have reported that lipoic acid also helps in the treatment of glaucoma and plays a role in the treatment of other disorders like erectile dysfunction

* Corresponding author: usamafahmy@hotmail.com

and cancer.[10,11] Our aim in this study is to combine CTX with a soluble form of ALA as liposomes that can protect CTX from fast degradation after reconstitution and can synergize its antimicrobial activity and decrease nephrotoxicity, depending on the antioxidant properties of lipoic acid.

## 2. Experimental

### Materials and methods

CTX was gifted from Jamjoom company for pharmaceutical industries (Jeddah, Saudi Arabia), ALA sodium was obtained from Sigma-Aldrich (St. Louis, MO), l-α-phosphatidylcholine was purchased from Avantilipids (Birmingham, AL, USA),cholesterol was purchased from Sigma-Aldrich (Detsenhofen, Germany), and Tween 20 was purchased from Merck (Darmstadt, Germany), All bacteria strains were gifted from Dr.Mahmoud ElFeky Natural product department, Faculty of pharmacy, King Abdulaziz university.

### Methods

#### *Preparation of CTX– ALA liposomes*

CTX–ALA liposomes were prepared by the thin-film hydration method[12] followed by extrusion. This method involves making a thin lipid film in a round-bottom flask by removing the organic solvent using a rotary evaporator (Rotavapor, BUCHI R-114, Switzerland). The lipid layer is composed of 200 mg cholesterol and 100 mg phosphatidylcholine dissolved in 10 mL ethanol at 40°C. Upon addition and agitation of the dispersion medium, composed of 200 mg CTX, 100 mg ALA, and 100 mg Tween 20, heterogeneous liposomes are formed. Finally, after extrusion through polycarbonate membranes, small homogeneous liposomes are obtained. The liposomes were stirred overnight at room temperature to evaporate the residual ethanol. Finally, CTX–ALA liposomes were lyophilized at –50°C using the Christ lyophilizer (Germany).

### Characterization

#### *Evaluation of CTX–ALA liposomes morphology by transmission electron microscopy (TEM)*

A few drops of the prepared CTX–ALA liposomes were mounted on a carbon-coated grid and left for 10 minutes to allow better adsorption on the carbon. Excess liquid was removed. Next, 1 drop of phosphotungstic acid (1%) was added. The tested nanoparticles then were investigated by TEM (Model JEM-1230, JOEL, Tokyo, Japan).

### Particle size and zeta potential

CTX–ALA liposome suspension was diluted (1:10) with distilled water. An instrument-acceptable concentration was confirmed by the green bar within the software and then was measured by Microtrac Dynamic Light Scattering Analyzer Zetatrac instrument (Microtrac, Inc., Montgomeryville, PA).

### CTX entrapment efficiency percent

To measure CTX and ALA entrapment efficiency percentage (EE%), the specified weight of the lyophilized CTX–ALA liposomes was accurately measured and dissolved in water. It was sonicated for 5 min, then filtered and directly injected into high-performance liquid chromatography (HPLC, Agilent 1200 series equipped with diode array detector, Germany) with a reversed phase column C18 (Hypersil, BDS, Thermo Fischer, USA) of 25 cm in length. The column effluent was monitored using a diode array detector at 260 nm, and the eluent flow was adjusted to 1 mL/min. The HPLC method for CTX determination was used previously by Karen[13] and was modified here by adding sodium hexane sulphonate to determine the ALA concentrations. The mobile phase was acetonitrile (0.05 M potassium hydrogen phosphate buffer of pH 3.5 [40:60 v/v]). CTX and ALA retention times were 3.5 and 6.5 minutes, respectively. All experiments were performed in triplicate (n = 3).

EE% was calculated as in Equation 1:

$$EE\% = (Fs / Ts) \times 100 \quad (1)$$

where Fs is the soluble free drug and Ts is the initial amount of drug added during the preparation of the CTX–ALA liposomes.

**CTX release study**

One milligram of raw CTX and CTX–ALA liposome suspension equivalent to 1 mg/mL of CTX was prepared in 0.1 mM phosphate buffer (pH 5.5). Samples were sited into the donor compartment of Franz diffusion cells (Hanson research, Microette Plus, Chatsworth, CA). The receptor chamber volume was 7 mL, and the prepared liposomes were allowed to diffuse through a cellulose ester dialysis membrane with a 20 kDa molecular weight cut-off. The samples were dialyzed against 0.1 mM phosphate buffer (pH 5.5) to maintain sink conditions (acceptor) at 37°C and a stirring rate of 300 rpm. The experiment was conducted for 24 h, 0.2-mL aliquots were withdrawn from the acceptor media and analyzed for CTX release percentage by the same chromatographic method described in the CTX EE% section. This experiment was compared with 1 mg/mL CTX solution (control) to determine its intrinsic diffusion across a cellulose ester membrane.

**CTX–ALA liposome stability study.**

The prepared CTX–ALA liposomes were subjected to a stability study using 3 cycles of freezing at –20 C and thawing at room temperature for 1, 2, and 4 weeks to determine the EE% in comparison with raw CTX.

**Antimicrobial Evaluation**

Two gram-negative bacteria ( *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 35218) strains while *Bacillus subtilis*ATCC ATCC 27853 and *Staphylococcus aureus* ATCC 29213 strains were represented the gram- positive bacteria in testing The antimicrobial activity of CTX–ALA liposomes and raw CTX, the detailed method of agar diffusion method was mentioned before by Uwingabiye et al. 2016, in summery, (1mm) gaps were made in agar plates, each petri dish of agar plate (150 mm) was filled with 25 mL of Muller-Hinton agar which contains I ml of bacterial culture (1 × 106 colony-forming units/mL). 200 µL of tested solutions in concentrations 0.5 mg/ml of raw CTX and CTX–ALA liposomes were filled the gaps, all diches were incubated at 37$^{o}$C for 24 h, a caliper was used to measure the inhibitory zone. All samples were done in triplicate, and results are expressed as mean ± SD.

**Experimental animals**

Thirty-six white Japanese rabbits weighing 3 ± 0.2 kg were acquired from the animal house of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. Every exploratory convention was approved by the Animal Ethics Committee of the Faculty of Pharmacy and complied with the Declaration of Helsinki, the Guiding Principle in Care and Use of Animals (DHEW production NIH 80-23), and the Standards of Laboratory Animal Care (NIH distribution #85-23, reconsidered in 1985).

The rabbits were habituated to the environment for no less than 14 days in naturally controlled enclosures (20°C ± 1°C and 12/12-hour dark/light cycle) with free access to standard feed and water. Rabbits were separated into 2 groups, each comprising 12 rabbits: the control group (gp A),the second raw-CTX group (gp B) and the third was CTX–ALA liposome group (gpC). Rabbits (gp G) received an intramuscular injection of raw CTX at a dose of 180 mg/kg,gp C received a dosage comparable to 180 mg/kg CTX from CTX–ALA liposomes. Doses were administered once a day for 10 days. Blood samples were collected at days 1, 5, and 10. Blood tests were gathered from the medial canthus of the eye by mean of capillary tubes for the three groups. Creatinine, urea, sodium, potassium, and calcium were measured as indicators for nephrotoxicity. The collected serum was kept at –80°C for quantitative determination of these parameters the previous parameters. The method is discussed in detail by Mossad.[15] Data are expressed as mean ± S.E and were statistically analyzed using analysis of variance to determine the differences between the parameters before, during, and at the end of the experiment.

## 3. Results

### Characterization of CTX–ALA liposomes

ALA has been combined with other drugs in the form of liposomes for many purposes, including enhancing stability, bioavailability, and cytotoxicity. Regarding CTX–ALA morphology, TEM images show the outer phospholipid layer and inner structure of the vesicles. The inner layer is composed of nanostructured dense lipid vesicles with a semisolid core and measuring approximately 50 nm, as depicted in Fig. 1.

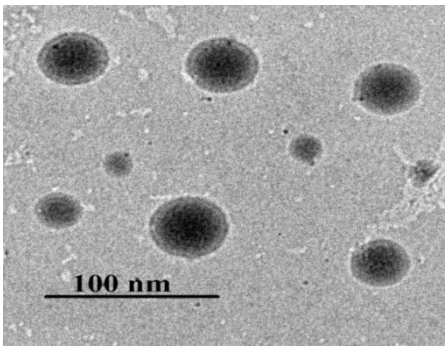


*Fig. 1. TEM image of CTX-ALA liposomes*

In this study, some trials were carried out to reach the smallest particle size with the highest EE%. The EE% CTX of the prepared CTX–ALA liposomes was 44.6 ± 3.1%, ALA content was 85.2 ± 5.1%, particle size was 60.4 ± 4.4 nm, and zeta potential was –14.6 ± 2.4 mV with a polydispersity index of 0.43 ± 0.12. Regarding the drug release study, the importance of this subject is measuring the CTX release rate from both CTX–ALA liposomes and the raw CTX. Comparative release profiles of raw CTX and CTX–ALA liposomes are depicted in Figure 2, which shows a dramatic, rapid release of raw CTX (~85%) after 1 hour whereas the release from CTX–ALA liposomes did not exceed 50% after 1 hour. After 2 hours, 95.3 ± 3.4% of raw CTX was released; however, the CTX-ALA liposomes had released 65.4 ± 2.4% after 2 hours.

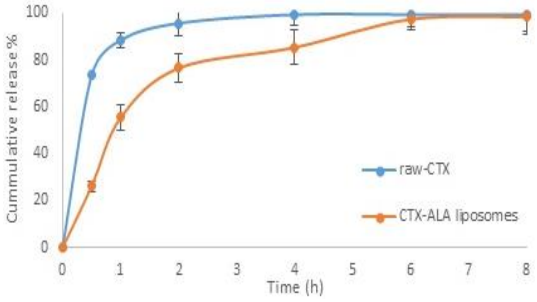


*Fig. 2.CTX cumulative release of raw CTX and CTX-ALA liposomes in potassium dihydrogen orthophosphate buffer pH (5.5)*

For stability data, there was no significant change in CTX–ALA liposome size or EE% after 3 cycles of freezing and thawing at 4 weeks after starting the study. However, the raw CTX had degraded to 67.43 ± 4.54% after 2 weeks. Antimicrobial activity was examined to evaluate the enhancement of CTX activity after incorporation with ALA. Data in Table 1 reveal that CTX–ALA liposomes have no advantage over raw CTX, and there was no significant difference in efficacy between the 2 treatments against all bacteria types.

*Table1: Antibacterial activities of raw-CTX and CTX-ALA liposomes.*

| | Zone diameter (mm) | | | |
|---|---|---|---|---|
| | Gram-positive bacteria | | Gram-negative bacteria | |
| | *Staphylococcus aureus* | *Bacillus subtilis* | *Escherichia coli* | *Pseudomonas aeruginosa* |
| Raw _CTX | 45 ± 2.1 | 41± 2.3 | 49.4± 2.1 | 48± 3.2 |
| CTX-ALA liposomes | 43± 1.3 | 40± 1.2 | 48.6± 1.1 | 48± 1.1 |

For the in vivo study, data in Table 2 reveal a significant increase in creatinine level from days 5 to 10 in the raw CTX group in comparison with the creatinine level at the start of the experiment, but an insignificant increase in the CTX–ALA liposome group. Other parameters—urea, potassium, and sodium levels—increased significantly after days 5 to 10, whereas the change was insignificant in the CTX–ALA liposome group. In the case of calcium and total proteins, there was a significant decrease in the raw CTX group compared with the CTX–ALA liposome group.

*Table 2: Kidney function parameters measured in rabbit plasma*

| | Parameters | Creatinine | Urea | Sodium | potassium | Calcium |
|---|---|---|---|---|---|---|
| | Unit | (mg/dl) | (mg/dl) | (mmol/L) | (mmol/L) | (mg/dl) |
| Control gp | Day 1st | 2.3± 0.4 | 111.2±2.2 | 55.6±2.3 | 20.2±0.12 | 5.43±0.7 |
| | Day 5th | 2.5± 0.3 | 110.1±1.3 | 56.3±2.3 | 22.1 ±2.6 | 5.3±1.5 |
| | Day 10th | 1.9± 0.6 | 109.2±2.5 | 56.5±3.4 | 20.3±1.2 | 5.2±1.1 |
| raw- CTX gp | Day st | 2.1± 0.3 | 110.2±2.4 | 55.6±2.3 | 20.2±0.12 | 5.4±0.7 |
| | Day 5th | 5.4±1.0* | 108.4±3.3 | 56.3±2.2 | 22.1 ±2.1* | 3.2±1.2* |
| | Day 10th | 6.3±1.6* | 88.43±2.4* | 66.5±1.2* | 23.3±1.2* | 2.21±1.1* |
| CTX-ALA liposomes gp | Day st | 2.1± 0.4 | 112.1±2.9 | 57.9±2.9 | 21.6±0.2 | 5.4±0.7 |
| | Day 5th | 1.8±0.9 | 110 ±3.2 | 54.7±3.4 | 20.2±1.5 | 5.3±1.72 |
| | Day 10 th | 2.2± 0.22 | 109 ±4.2 | 56. 2±2.1 | 21.3±1.11 | 5.5±0.72 |

(*)P-values were <0.05.

## 4. Discussion

CTX is in the cephalosporin family, which is used to treat bacterial infection. It is proven to be broad spectrum antibiotic and easy to use[16]. Cephalosporins are the most common antibiotics used as initial empiric therapy for treating serious infections.[17] According to TEM images, their circular edges could be attributed to the hydrophilic head (hydroxyl group) and hydrophobic tail (hydrocarbon chain) of the cholesterol, which is hydrophilic during formulation. Cholesterol is consolidated in the bilayer layer. The hydrophilic head makes a beeline for the watery stage, and the aliphatic tail lies parallel to the hydrocarbon chains. Cholesterol is known to expand the chained cationic lipids and reinforce the nonpolar tail of the nonionic surfactant. Therefore, cholesterol is responsible for the close density of the surfactant monomers and the diminished size of liposomal vesicles.[18] An increase in EE% of ALA compared with CTX is logical as a result of CTX solubility. Because CTX is freely soluble in water, it easily escapes in water through filtration. ALA is insoluble in water, so it could be sharing in the formation of the lipid layer of the liposome. In the case of CTX–ALA liposomes, the delay in CTX release is due to CTX being entrapped in the lipid core of the liposomes. However, the raw drug dissolves immediately in the diffusion medium.(Ahmed et al. 2017). Another unique property for CTX that its ability to bind irreversibly with calcium ions furthermore, liberates bilirubin from albumin so, lead to lithiasis and sludge of the biliary in pediatrics [20,21]. ALA could prevent calcium oxalate

crystals precipitation in the renal tubular cells in some mammals.[22] ALA has a significant influence on carbohydrate metabolism enzymes and is reported to have inhibitory effects on some enzymes that metabolize carbohydrate. In previous studies, ALA was found to prevent calcium oxalate formation after induction of crystal formation using glycollate oxalate. Furthermore, ALA decreases expression of glucose-6-phosphatase and fructose-1, 6 diphosphatase enzymes, which play a role in the formation of calcium oxalate crystals. Many reports suggest an inhibitory role of ALA in preventing hemolysis. In vitro supplementation of ALA, known to have potent antioxidant properties, can decrease oxidation, increase glutathione synthesis, and inhibit red blood cell sickling, thus protecting against peroxyl radical-induced hemolysis. Urothelium is caused by calcium oxalate precipitation, which initiates free radicals and finally leads to induced membrane damage .Many published reports have revealed that potent antioxidants can protect the kidneys from calcium oxalate crystal deposition and retention.[23–25]

The amount of excreted creatinine and urea is an indicatorof glomerular filtration rate and kidney function in this study. Creatinine clearance was significantly increased [26] in Group A, which might be attributed to damage of the renal tubules. In Group B, no significant change was observed in kidney function or glomerular filtration rate.[27,28] Urea is produced as a result 8za of protein catabolism in the liver. Most urea is excreted via the kidneys, so increasing urea concentration in the blood means a decrease in glomerular filtration rate and vice versa. Our data revealed increasing serum urea, which could be attributed to impaired kidney function because of damage of renal tubules. In contrast, in the CTX–ALA liposome group, there was no significant change in serum urea level.[29]. Sodium and potassium levels in serum were significantly increased in the raw CTX group, whereas no significant change was found in the CTX–ALA group. The obtained results agree with previous studies,[30,31] which found a decrease in sodium and potassium excretion after administration of CTX in experimental animals. Another report confirms these data and recorded that cefprozil decreased urinary excretion of electrolytes in rats, depending on the dose.[32] Calcium serum concentrations decreased significantly in Group A as a result of kidney failure due to renal lesions. Another reason for this hypocalcemia is a decrease in serum proteins. It was found previously that calcium binds to plasma proteins, so hypoprotenimia leads to hypocalciemia. In a previous study, some cephalosporins as ( cefpirome sulphate and cefazoline sodium) were injected intravenous in male Sprague-Dawley rats, decrease in serum protein level and proteinuria were observed as a result of protein excretion in the urine after kidney damage .[33]. ALA was studied previously as a protective agent against the nephrotoxicity induced by many drugs, like some aminoglycosides, cisplatin, and cyclosporine. This property of ALA is due to the prevention of lipid peroxidation by increasing the inhibited activities of enzymatic antioxidants and decreasing the level of non-enzymatic antioxidants. ALA role confirmed by glutathione and glutathione-related enzymes, which act as a reductant that converts hydrogen peroxide and lipid hydroperoxides directly to H2O, thus protecting cells from reactive oxygen species; this reaction is catalyzed by GSH-Px. Glutathione and GSH-Px could be depleted from the cells after continued oxidative stress, resulting in DNA damage and cell death. This sequence could be stopped by increasing cysteine uptake, thus raising glutathione levels inside the cell. ALA inhibits glutathioneoxidation by the reactive oxygen species also, augments glutathione-related enzymes activity in the kidney and in the liver[34,35].

## 5. Conclusions

The study confirms the protective effect of ALA on CTX nephrotoxicity. Moreover, the prepared CTX–ALA liposomes achieved relatively acceptable EE%, with a relatively extended release profile and a good, stable formula with no effect on CTX antimicrobial activity. Both creatinine and urea levels confirmed the protective effect of ALA on renal tubular cells. Finally, loading CTX and ALA together may provide a novel combination for protection of the kidney in pediatric patients receiving treatment with CTX.

**Acknowledgements**

This work was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. G-188-166-38. The authors therefore gratefully acknowledge with thanks DSR for technical and financial support.

## References

[1] D.M. Youssef, L.M. Sherief, H.S. Sherbiny, M.Y. Elattar, A.R.M. El Sheikh, F.M. Fawzy, T. Adham, Nephrology. **21** (2016). doi:10.1111/nep.12625.
[2] R.C. Moellering, J.R. Graybill, J.E. McGowan, L. Corey, American Journal of Infection Control. **35** (2007). doi:10.1016/j.ajic.2007.08.001.
[3] J. Wedgwood-krucko, patients, causing, (1988).
[4] N. Li, X. Zhou, J. Yuan, G. Chen, H. Jiang, W. Zhang, Pediatrics. **133** (2014). doi:10.1542/peds.2013-2103.
[5] X. Cong, X. Gu, X. Sun, B. Ning, L. Shen, Urology. **83** (2014). doi:10.1016/j.urology.2013.09.029.
[6] S. Chutipongtanate, V. Thongboonkerd, Biochemical and Biophysical Research Communications. **406** (2011). doi:10.1016/j.bbrc.2011.02.053.
[7] K. Nidhi, S. Indrajeet, M. Khushboo, K. Gauri, D.J. Sen, International Journal of Drug Development and Research. **3** (2011) .doi:10.1002/jps.
[8] K.E. Reedlt, J.E. Cronan, sequencing and functional characterization of Lipoic Acid Metabolism in Escherichia coli : Sequencing and Functional Characterization of the hpA and lpB Genes, **175** (1993).
[9] L. Shinto, J. Quinn, T. Montine, H.H. Dodge, W. Woodward, S. Baldauf-Wagner, D. Waichunas, L. Bumgarner, D. Bourdette, L. Silbert, J. Kaye, Journal of Alzheimer's Disease. **38** (2014) .doi:10.3233/JAD-130722.
[10] T. Zee, N. Bose, J. Zee, J.N. Beck, S. Yang, J. Parihar, M. Yang, S. Damodar, D. Hall, M.N. O'Leary, A. Ramanathan, R.R. Gerona, D.W. Killilea, T. Chi, J. Tischfield, A. Sahota, A. Kahn, M.L. Stoller, P. Kapahi, Nature Medicine. **23** (2017). doi:10.1038/nm.4280.
[11] M.B. Gomes, C.A. Negrato, Diabetology & Metabolic Syndrome. **6** (2014). doi:10.1186/1758-5996-6-80.
[12] S. Ghanbarzadeh, H. Valizadeh, P. Zakeri-Milani, BioImpacts. **3** (2013). doi:10.5681/bi.2013.016.
[13] K. Gaudin, A. Acta, In Vitro Ceftriaxone Stability at New-borns â€™ rectal PH Assessed by UV and HPLC Methods Pharmaceutica In Vitro Ceftriaxone Stability at New-borns ' Rectal PH Assessed by UV and HPLC Methods, **(2015)**. doi:10.4172/21532435.1000393.
[14] J. Uwingabiye, M. Iken, A.G. Zohoun, L. Boumhil, Z. Lemkhente, H. Naoui, M. Bouchrik, B. Lmimouni, Journal de Mycologie Medicale. **26** (2016) doi:10.1016/j.mycmed.2015.09.002.
[15] V. Medicine, W. Address, V. Medicine, M.H. Aboubakr, No Title, (n.d.).
[16] D.M. Livermore, N. Woodford, The ??-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter, Trends in Microbiology. **14** (2006). doi:10.1016/j.tim.2006.07.008.
[17] M. Tumbarello, M. Sanguinetti, E. Montuori, E.M. Trecarichi, B. Posteraro, B. Fiori, R. Citton, T. D'Inzeo, G. Fadda, R. Cauda, T. Spanu, Antimicrobial Agents and Chemotherapy. **51** (2007). doi:10.1128/AAC.01509-06.
[18] dddt-9-6129 (1).pdf.crdownload, (n.d.).
[19] O.A.A. Ahmed, U.A. Fahmy, A.S. Al-Ghamdi, B.M. Aljaeid, H. Aldawsari, O. Fahmy, H.A. Sarhan, M.G.K. Asri, Journal of Bioactive and Compatible Polymers. **1** (2017) 883911517694397. doi:10.1177/0883911517694397.
[20] M.H. Wilcox, J.D. Chalmers, C.E. Nord, J. Freeman, E. Bouza, Journal of Antimicrobial Chemotherapy. **72** (2017) . doi:10.1093/jac/dkw385.
[21] R.W. Lucek, W.A. Colburn, Clinical Pharmacokinetics of the Retinoids, Clinical Pharmacokinetics. **10** (1985). doi:10.2165/00003088-198510010-00002.

[22] B. Grohe, A. Taller, P.L. Vincent, L.D. Tieu, K.A. Rogers, A. Heiss, E.S. Sorensen, S. Mittler,
H.A. Goldberg, G.K. Hunter, Langmuir. **25** (2009). doi:10.1021/la901145d.
[23] A.B. Shukla, D.R. Mandavia, M.J. Barvaliya, S.N. Baxi, C.R. Tripathi, M. Jasmatbhai, Avicenna J Phytomed. **4** (2014). http://www.ncbi.nlm.nih.gov/pubmed/25050313%5Cn http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4104626.
[24] K. Venkateswarlu, J.K. Preethi, K.B. Chandrasekhar, J.N. Technological, J.N. Technological, J.N. Technological, K. Venkateswarlu, Antiurolithiatic Activity of Ethanolic Extract of Taxillus Tomentosus Plant on Ethylene Glycol and Ammonium Chloride Induced Urolithiasis in Wistar, **27** (2016) 66–73. doi:10.14499/indonesianjpharm27iss2pp66.
[25] journal.pone.0093056.PDF.crdownload, (n.d.).
[26] 3097808citation, (n.d.).
[27] L. De Nicola, F.B. Gabbai, M.E. Liberti, A. Sagliocca, G. Conte, R. Minutolo, American Journal of Kidney Diseases. **64** (2014) 16–24. doi:10.1053/j.ajkd.2014.02.010.
[28] K. Vallabhaneni, H. Haller, Page 1 of 23 1, (n.d.) 1–23.
[29] L. Zhang, Z. Xiong, Y. Chen, L. Peng, B. Yu, X. Gao, R. Zhang, L. Zhang, W. Zhang, RSC Adv. **6** (2016). doi:10.1039/C6RA00326E.
[30] M.E.T. McMurdo, D. Sumukadas, P.T. Donnan, V. Cvoro, P. Rauchhaus, I. Argo, H. Waldie, R. Littleford, A.D. Struthers, M.D. Witham, Arthritis Care and Research. **68** (2016). doi:10.1002/acr.22724.
[31] A.D. DiVasta, H.A. Feldman, C.T. Rubin, J.S. Gallagher, N. Stokes, D.P. Kiel, B.D. Snyder, C.M. Gordon, Osteoporosis International. **28** (2017) doi:10.1007/s00198-016-3851-9.
[32] M.G. Elsayed, A.A. Elkomy, M. Gaballah, M. Elbadawy, Journal of Pharmacology and Pharmacotherapeutics. **5** (2014) . doi:10.4103/0976-500X.124419.
[33] X. Li, Z. Wang, W. Liu, Y. Wang, B. Li, Z. Fei, Biological Rhythm Research. **44** (2013). doi:10.1080/09291016.2011.652862.
[34] M. Nematbakhsh, Z. Pezeshki, F.E. Jazi, B. Mazaheri, M. Moeini, T. Safari, F. Azarkish, F. Moslemi, M. Maleki, A. Rezaei, S. Saberi, A. Dehghani, M. Malek, A. Mansouri, M. Ghasemi, F. Zeinali, Z. Zamani, M. Navidi, S. Jilanchi, S. Shirdavani, F. Ashrafi, Asian Pacific Journal of Cancer Prevention. **18** (2017). doi:10.22034/APJCP.2017.18.2.295.
[35] H. Asci, M. Saygin, F.N. Cankara, D. Bayram, S. Yesilot, I.A. Candan, I. Ilhan, Renal Failure. **37** (2015). doi:10.3109/0886022X.2014.967645.