

Ampicillin-Loaded Solid Lipid Nanoparticles as Drug Delivery System for Treatment of UTI Caused by *E. Coli* in Rat

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Abstract

Solid lipid nanoparticles (SLNs) have been studied as a drug-delivery system for the controlling of drug release. Ampicillin as a β -lactam antibiotic was studied to load on SLNs for control of drug release to increase administration intervals and decrease dose of drug to increase patient compliance and decrease antibiotic resistance. The AMP-SLNs formulation showed significantly higher ($p < 0.05$) antibacterial activities against the tested bacteria. The signs and symptoms of UTI appeared rapidly after 48 h. After inducing infection animals, were suffering from anorexia, dehydration, fever and the frequency of urination increased gradually from the first day after infection. After finishing the treatment course for 120 h, the values of the bacterial count varied between the different groups, the 2nd group that was treated with standard AMP achieved a significant decrease ($P < 0.05$) in the bacterial count which is reached $6.3 \times 10^4 \pm 0.66$ cfu/ml, but it didn't return to its normal values before the inducing of infection, while the 1st group recorded the best results between all tested groups as the bacterial count returned to its normal values before the inducing infection ($6.3 \times 10^2 \pm 0.22$ cfu/ml). The results of the bacterial count continued at the normal values in the samples of the animals of the 1st group after 24 hours of the end treatment with AMP-SLNs. while the 2nd group that was treated with standard AMP recorded a continuous increase in the bacterial count, which means the continuation of the infection and the need for a longer treatment period using AMP antibiotic. In both groups 3rd and 4th, the infection persisted, as they recorded the highest values of bacterial counts during the study. In general, from all the above, we can conclude that the use of the AMP-SLNs for the treatment of experimentally induced *E. coli* in laboratory rats, gave better results compared to using the antibiotic standard AMP alone without loading it on the lipid nanocarrier.

Keywords: solid lipid nanoparticles, *E. Coli*, UTI, Rat

1. Introduction

Solid lipid nanoparticles (SLNs) as colloidal carrier systems have the advantages of traditional systems but avoid some of their major disadvantages [1]. Some advantages of SLNs are possibility of the incorporation of lipophilic and hydrophilic drugs, possibility of controlling drug release and drug targeting, increased drug stability, high drug payload, lack of biotoxicity of the carrier, no problems with respect to large-scale production, sterilization possibility, and good tolerability [2]. The objective of study focuses on the isolation and characterization of *E. coli* isolated from UTIs in women and study in the vitro and vivo antibiotic of the standard Ampicillin and Ampicillin loaded solid lipid nanoparticles formulation against *e. coli* isolated.

2. Materials and Methods

176 of urine samples were collected from women, from different hospitals around wasit province (Al – kut Hospital for Gynecology and obstetrics and pediatrics) from 15 October for 15 desumber. The identification of the isolate included morphological characteristics and biochemical testes which carried out depending on Bergy's Manual of

Systematic Bacteriology, 2nd edition [3]; Epi20E, Vitek 2 system.

Experimental Animals

Twenty male Wister albino rats about three months of age and with body weight ranged between 250-300 g. were used to perform two experiments of the present study. Rats were housed in plastic cages 20 × 50 × 75 cm dimensions, placed in a special housing room belongs to the Department of Biology/College of Science for two weeks for adaptation. Standard rodent diet (Commercial feed pellets) and tap water were freely available. Housing conditions were maintained at 20-25 °C in air-conditioned room, the air of the room was changed continuously by using ventilation vacuum, while the light/dark cycle was 14/10 in housing place. The litter of the cages was changed weekly

Bacterial spp. of Study

Pathogenic bacterial isolates of *E. Coli* were used from the samples that collected from women suffering from symptoms of urinary tract infections (UTIs) from different hospitals around Wasit Province (Al-Kut Hospital for Gynecology and obstetrics and pediatrics).

Preparation of Standard Bacterial Suspension

The average number of viable, *E. coli* organisms

per ml of the stock suspensions was determined by means of the Standard McFarland solution No.0.5. By taking 1 ml from overnight culture (nutrient agar) of bacterial suspension washing with 9 ml of pepton water, then taking 1 ml of this suspension and making serial ten-fold dilution. Standard McFarland solution No.0.5 was prepared as follows:

Solution (A) this was prepared by dissolving 1.175 gm of barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in 100 ml distilled water.

Solution (B) this was prepared by adding 1 ml of concentrated sulfuric acid (H_2SO_4) in 100 ml distilled water. The two solutions were mixed by the addition of 0.5 ml from solution A to 99.5 ml from solution B. The prepared solution was used to compare the turbidity of two bacterial suspensions to obtain an approximate cell density of 1.5×10^8 cell/ml [4].

In-vitro antibacterial study of ampicillin and AMP-SLNs

The agar well diffusion method was adopted according to [5], for assessing the antibacterial activity of the prepared extract. 0.2 ml of standardized bacterial stock suspensions (1.5×10^8) cell/ml of *E. coli* was thoroughly mixed to each 20 ml of sterile nutrient agar. This 20 ml of the inoculated nutrient agar was distributed into sterile petri dishes. The agar was left to set and in each of these plates 4 well, 8 mm in diameter were cut using a sterile corn borer and the agar discs were removed, after that wells were filled with 0.1 ml of each concentration of 1, 2, 4, 8, 16, 32, 64, 125, 250, 500 and 1000 $\mu\text{g}/\text{ml}$ of ampicillin, for both, standard ampicillin and ampicillin-SLNs by using microtiter-pipette that allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. Three replicates were carried out for each concentration and the activity of tested form agents was

determined by measuring the diameter of inhibition zone around each well by millimeter against each of the tested organism. Simultaneously, addition blank SLNs formulation and D.W were used as positive and negative control, respectively. The results and standard errors mean values were tabulated.

Determination of MIC and MBC

To determine MIC and MBC of AMP-SLNs and standard AMP against *E. coli*, broth dilution method was used. AMP stock solutions of 1000 $\mu\text{g}/\text{ml}$ for different AMP-SLNs formulation and standard AMP were prepared, and then further diluted to yield concentration range of 1 to 1000 $\mu\text{g}/\text{mL}$ in 4mL of Muller–Hinton broth. Final concentration of bacteria in individual tubes adjusted to about 5×10^6 CFU/mL by adding 50 μL of bacterial inoculums. The blank SLNs formulation and normal saline solution were also used as controls. To test MIC, after 24 h incubation at 37°C , the test tubes were examined for possible bacterial turbidity, to detect the lowest drug concentration that could inhibit visible bacterial growth; the MBC was measured by sub-

culturing from MIC broth tubes onto fresh agar plates. The *in-vitro* MBC value was the lowest concentration of the drug that results no growth of the bacteria that being tested [6].

Experimental Design

Thirty rats were divided equally into four groups, five rats in each group (Treatment begin after 24 hrs. after inducing infection):

1-Group (1): infected and treated orally with 25 mg/kg B.W of AMP-SLNs for five days (using a gavage stomach tube).

2-Group (2): infected and treated orally with 25 mg/kg B.W of Standard AMP five days (using a gavage stomach tube).

3-Group (5): +ve control- infected group and treated orally with SLNs, for five days (using a gavage stomach tube).

4-Group (6): -ve control-not infected group which given only D.W orally for five days (using a gavage stomach tube).

The volume of oral dosing for all groups were 0.2 ml/100 gm. of rat B.W.

Inducing Infection (UTI)

Inducing bacterial of UT with *E. Coli* isolates is done according to [7] and [4].

Preparing of the tested rats

The area around the external bladder opening was shaved and sterilized.

Cleaning and sterilizing the external urethral opening, clipping and shaving the hair around the opening area, and applying a local anesthetic (lidocaine ointment) around the area.

Rats were anesthetized by intramuscular injection (0.1 ml) of 1:9 ketamine + xylazine per 100 g BW [7].

Preparation (of the inducing bacterial inoculum

The infecting bacterial inoculum is prepared using McFarland 0.5 tubes method by taking 1 ml of *E. coli* growth cultured overnight in the medium of NB and adding to it 9 ml of peptone water, then 1 ml of this solution is taken and a one/ten dilution is performed for it and the recognized tubes are compared with a 0.5 McFarland tube, the bacteria count in the turbidity-like tube to 0.5 McFarland is equals 1.5×10^8 cfu/ml, of which 100 μL is injected to infect rats for inducing UTI with *E. Coli*.

Inducing UTI

Before the inducing infection, the bladder content is evacuated through a massage to the abdominal area to stimulate the rats to urinate.

1- Use the yellow catheter for children after getting rid of the metal needle inside for inducing bacterial infection.

2- The catheter is inserted through the external urethral opening (it is recommended to put a local anesthetic or any lubricating substance around the catheter) to allow it to be easily entered.

3- The catheter is slowly pushed into the opening towards the back of the animal until it goes beyond

the vaginal opening and then changes to the frontal direction of the animal, and the animal is lying on its back and under control with the dimensions of the tail back and the abdomen pulled forward, allowing access to the area of the urination opening.

After the catheter reaches the inside of the bladder, the bacterial inoculum is ejected inside it [8].

Clinical signs of infection

Elevation of temperature (by checking a rectal temperature). Low feed consumption. Increased water consumption.

Clinical signs

Change in the weight of the rats.

Bloody urine (in some cases).

Urine sample collection

After 24 h of inducing infection, urine samples were collected from all four tested groups by using the capillary tubes (used in blood tests) by inserting them at the beginning of the external urine opening, then adding the drawn urine sample to peptone water (10 ml) and incubating for 24 h, then a loop full is taken from it and cultured on MacConkey agar, Mannitol agar and on Blood agar (this method was performed before the inducing infection to ensure that there was no previous infection in rats with *E. Coli* [9].

Bacterial count

Rectal bacterial count was done according to pour plate method [10]. compared between many methods to enumerate TTI of *E. coli* and found that this method and spread plate method on MacConky agar were easier and more convenient to be carried out, it was less labor intensive and less expensive, and give results for fecal *E. coli* that were available within one day.

E. coli bacterial count in urine samples were performed and compared before 24 h of infection, 2 days after infection and at 24, 48, 72, 96, and 120 of treatment with AMP-SLNs, standard AMP, SLNs and D.W, then at the end of 120 h of treatment (after 48 of end treatment). Bacterial count is done by taking 1 ml of peptone tube

(containing the urine sample) and diluting one/ten dilution method: 1/10, 1/100, 1/1000, 1/10000, 1/100000. 0.1 mL of each dilution was grown on MacConkey agar medium (20 mL) before it solidified at room temperature (to avoid high temperature killing of bacteria). Then the MacConkey agar media were incubated for 24 hours at 37 °C. Counting the regular bacterial colonies on MacConkey agar media was carried out using manual colony counting (marker dot for each continuous). on the following equation: Number of bacteria (CFUs)/Dilution × amount plated = amount of bacteria/ml [4];[6].

Statistical Analysis

The data of the present study was analyzed statistically by statistic package for social science (SPSS) version 27 program using chi-square test (X²) and two-way ANOVA and Least significant differences (LSD). The level of significance was set to 5%. P0.05 was considered as non-significant

3. Results and Discussions

In-vitro Antibacterial Activity

The antibacterial activity of AMP-SLNs formulation and standard AMP against UTI isolates of *E. coli* is shown in table 3. It was found that *E. coli* was sensitive to the tested formulations. the AMP-SLNs formulation showed significantly higher ($p < 0.05$) antibacterial activities against the tested bacteria in terms of zone of inhibition, the first result of inhibition was achieved at 4 µg/ml, while the first zone of inhibition for standard AMP was recorded at 64 µg/ml. At 1000 µg/ml AMP-SLNs given higher antibacterial activity in comparing with standard AMP (30 and 20mm respectively).

In general, both formulations showed antibacterial activity with increasing their concentration of ampicillin, thereafter the bacterial growth inhibited, suggesting that antibacterial activity is related drug encapsulation and type of tested formulation.

SLNs was used as +ve control, it was not giving any noticed zone of inhibition against tested bacterial [12].

Table: - *In-vitro* antibacterial activity of AMP-SLNs formulation and standard AMP against UTI isolates of *E. coli* bacteria expressed as zone of inhibition (mm).

| Concentration µg/ml | AMP-SLNs (Inhibition zone-mm) | AMP (Inhibition zone-mm) |
|---|-------------------------------|--------------------------|
| | Mean ±SE | Mean ±SE |
| 1 | 0.00±0.00 Ga | 0.00±0.00Fa |
| 2 | 0.00±0.00Ga | 0.00±0.00Fa |
| 4 | 12.00 ±0.57 la | 0.00±0.00Fb |
| 8 | 14.33 ±0.33Ha | 0.00±0.00Fb |
| 16 | 16.00±0.57 Ga | 0.00±0.00Fb |
| 32 | 18.33±0.33 Fa | 0.00±0.00Fb |
| 64 | 20.66±0.37Ea | 10.00±0.00 Eb |
| 125 | 22.33±0.66 Da | 12.00±0.00 Db |
| 250 | 24.37±0.43 Ca | 15.00±0.57 Cb |
| 500 | 26.75±0.40 Ba | 16.00±0.66 Bb |
| 1000 | 30.23±0.45 Aa | 21.00±0.33 Ab |
| +ve control (SLNs) | 0.00±0.00 Ja | 0.00±0.00Fa |
| -ve control (DW) | 0.00±0.00 Ja | 0.00±0.00Fa |
| LSD(P<0.05) | 0.54 | |
| Values represent mean ±S. E | | |
| Different capital letters mean significant (P<0.05) results between different concentrations. | | |
| Different small letters mean significant (P< 0.05) results between different tested formulation | | |

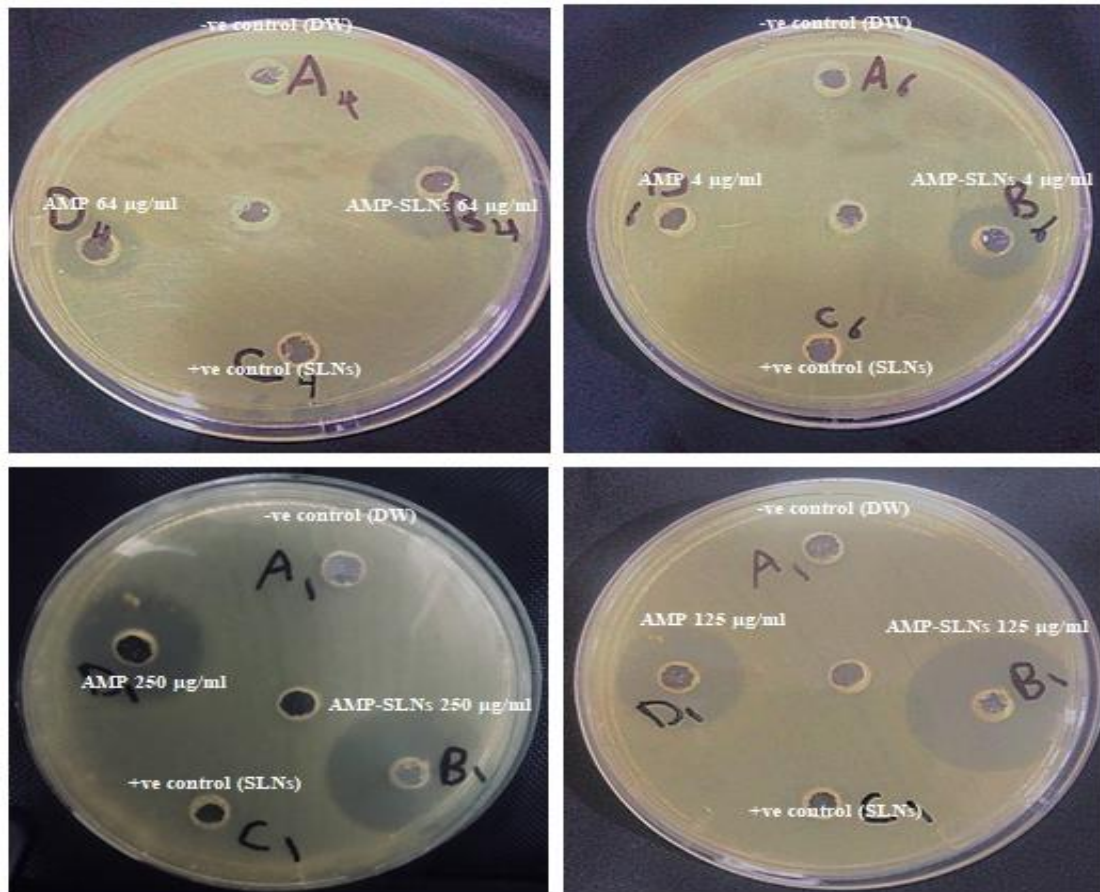


Figure:- In-vitro antibacterial activities of different concentrations of AMP-SLN, standard AMP, +ve control, and -ve control DW, respectively against *E. coli* bacteria.

These results indicated that AMP-SLNs could inhibit the growth of bacteria for a longer time period than free AMP. This indicated that AMP-SLNs have superior antibacterial activity related to free AMP. The reasons for these results could be due to the lipophilic nature of tested formulation that improve the cellular entering of AMP into the bacterial membrane and the small size of the particles [13]. It has been noted that using of nanoparticles to entrap antibacterial agents may improve their activity due to their sustained release and higher ability to penetrate the bacterial cell wall due to their small MD and their hydrophobic structure which is similar to gram negative bacterial cell wall. They may also effectively decrease the action of P-gp efflux pumps. The blank control formulations had no antibacterial activity, so the enhanced antibacterial efficacy is due to the ability of these formulations to deliver the AMP molecules efficiently to the site of action [4],[6] Enhanced antibacterial properties of drug loaded SLNs was reported by (Ali hosseini et al.,2015).

Clinical signs of UTI

The signs and symptoms of UTI appeared rapidly after 48 h. in all infected rats in all groups. There was no difference in the severity of infection. Three patients in each group failed to complete the study. In the SLNs treated group, there was two deaths from UTI.

In all infected animals treated with AMP-SLNs and standard AMP, the bacteriologic result of treatment

was classified as a cure. No instance of bacterial persistence or superinfection was observed in either group. There were two probable instances of relapse in the group treated with standard AMP. In both groups, all *E. coli* isolates were susceptible to the antibiotic in use, but there was appreciable difference between the susceptibility of *E. coli* to AMP-SLNs and standard AMP according to their formulation.

After inducing infection animals, were suffering from anorexia, dehydration, fever and the frequency of urination increased gradually from the first day after infection. All the animals exhibited clinical signs of fever, low feed consumption, increased water consumption, change in the weight of the rats and bloody urine (in some cases). The presence of bacteria in the urine, known as bacteriuria, it does not always occur in conjunction with symptoms (asymptomatic bacteriuria), but when it does (symptomatic bacteriuria), this typically represents urothelial inflammation due to urinary tract infection. The clinical syndrome of cystitis is defined by the presence of lower urinary tract symptoms such as frequency, urgency, and/or dysuria. Acute pyelonephritis, another clinical syndrome, is characterized by fever, chills, and flank pain [15].

The ascending route is responsible for introduction of pathogenic organisms into the urinary tract in nearly all cases of uncomplicated UTI. Adherence of pathogens to the urothelial surface of the urethra allows for migration into the bladder where infection can be established. Rapid proliferation occurs within the host cell, but several toxins released by the

invading bacteria cause destruction of the urothelium, providing greater access to nutrients and promoting deeper penetration while at the same time creating a proinflammatory environment [8].

UT bacterial count:

UT bacterial count was done according to pour plate method. 8(Grabow et al.,1992) compared between many methods to enumerate TTI of E. coli and found that this method and spread plate method on

MacConky agar were easier and more convenient to be carried out, it was less labor intensive and less expensive, and give results for fecal E. coli that were available within one day.

The count of E. coli bacteria for the four groups were done with cell forming unit /ml (cfu/ml) for groups 1-4 respectively, as showed in table 4.... There were no significant differences ($P < 0.05$) between all groups.

| Period Group | Before 24 h of inducing infection | After 48 h of inducing infection | After 24 h of end treatment | After 120 h of treatment |
|---|-----------------------------------|----------------------------------|------------------------------|------------------------------|
| G ₁ : AMP-SLNs 25 mg/Kg | $6.5 \times 10^2 \pm 1.76Ab$ | $6.4 \times 10^6 \pm 0.89Aa$ | $6.5 \times 10^2 \pm 0.87Cb$ | $6.3 \times 10^2 \pm 0.22Cb$ |
| G ₂ : Standard AMP 25mg/Kg | $6.6 \times 10^2 \pm 1.62Ad$ | $6.2 \times 10^6 \pm 0.96Aa$ | $6.3 \times 10^5 \pm 0.35Bb$ | $6.3 \times 10^4 \pm 0.66Bc$ |
| G ₃ : +ve control (SLNs) | $6.5 \times 10^2 \pm 1.07Ad$ | $6.3 \times 10^6 \pm 0.92Ac$ | $7.5 \times 10^7 \pm 0.45Aa$ | $6.5 \times 10^7 \pm 0.27Ab$ |
| G ₄ : -ve control (DW) | $6.0 \times 10^2 \pm 1.53Ad$ | $6.2 \times 10^6 \pm 0.88Ac$ | $7.6 \times 10^7 \pm 0.56Aa$ | $6.6 \times 10^7 \pm 0.43Ab$ |
| | | | LSD($P < 0.05$) | 0.18×10^1 |
| Values represent mean \pm S. E, Group rat no.= 5 | | | | |
| Different capital letters mean significant ($P < 0.05$) results between groups. | | | | |
| Different small letters mean significant ($P < 0.05$) results between periods. | | | | |

The results of bacterial counting of the four groups before inducing infection showed that the number of E. Coli bacteria isolated from the UT samples were within normal values (about 6.5×10^2 cfu/ml), which do not indicate the presence of a prior infection in the urinary tract of the tested rats. After 48 of the inducing the infection in the UT tested rats, the bacterial count of E. coli increased significantly ($P < 0.05$) in all infected groups and reached more than 100000 bacteria cells/ml of urine, and this indicates the occurrence of a pathological infection in the UT, While after 48 of the Inducing infection, the bacterial count of E. coli increased significantly ($P < 0.05$) in all infected groups and recorded more than 100000 bacteria cells/ml of urine, and this indicates the occurrence of a pathological infection in the UT. After finishing the treatment course for 120 h, the values of the bacterial count varied between the different groups, the results showed an increase in the bacterial count of the 3rd and 4th groups that did not receive any antibacterial treatment, while the 1st and 2nd groups recorded a difference in values, where the 2nd group that was treated with standard AMP achieved a significant decrease ($P < 0.05$) in the bacterial count reached $6.3 \times 10^4 \pm 0.66$ cfu/ml, but it didn't return to its normal values before the inducing of infection, while the 1st group recorded the best results between all tested groups as the bacterial count returned to its normal values before the inducing infection ($6.3 \times 10^2 \pm 0.22$ cfu/ml). The results of the bacterial count continued at the normal values in the samples of the animals of the 1st group after 24 hours of the end treatment with AMP-SLNs, while the 2nd group that was treated with standard AMP recorded a continuous increase in the bacterial count, which means the continuation of the infection and the need for a longer treatment period using AMP antibiotic. In both groups 3rd and 4th, the infection persisted, as they recorded the highest

values of bacterial counts during the study (after 6 days of infection) [12]. UT bacterial count was done according to pour plate method compared between many methods to enumerate UTI of E. coli and found that this method and spread plate method on MacConky agar were easier and more convenient to be carried out, it was less labor intensive and less expensive, and give results for fecal E. coli that were available within one day. Antimicrobial resistance (AMR) is a global problem. AMR has posed new challenges in treatment of infectious diseases [11].

4. Conclusions

These findings suggest that Ampicillin-loaded LNPs could be having good potential for the Sustained delivery of antibiotics to urinary tract infections.

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