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Research Article

Codelivery of Doxycycline and Hydroxychloroquine to Treatment of Brucellosis: An Animal Study

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Evaluation of various biochemical and immunological parameters in infectious diseases is one of the best indicators for a diagnosis and treatment process. The main goal of this project is to determine the effect of hydroxychloroquine and doxycycline loading into solid-lipid-nanoparticles (DOX-HCQ-SLN) on the both acute and chronic phases of brucellosis. In addition, evaluate some biochemical factors, trace elements, and inflammatory elements. Blood serum levels of Zn, Fe, Na, and K and hepatic biochemical parameters (AST, ALT, ALP, and TBil) were remarkably different between infected and healthy rats. Vitamin D was decreased, and CRP was increased in chronic and acute brucellosis. Quantitative evaluation of these mentioned parameters can be useful to diagnose brucellosis in advance. Due to the good effect of the synchronized use of hydroxychloroquine and doxycycline in the form of nanoparticles, the manipulation of these nanoparticles can help for better treatment and also reduction in brucellosis reinfection.

1. Introduction

Brucellosis is a zoonotic infection caused by four genus of *Brucella*, including *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*. The disease has nonspecific symptoms, multiple complications, and involvement of various organs [1, 2]. These intracellular pathogens, in the cell, can be safe in the extracellular conditions, and also the intracellular environment enables them to be resistant to extracellular antibiotics [3, 4].

As bacteria reside within macrophages and prevent apoptosis, the capability of bacteria to adapt the new conditions, bacterial growth in the macrophage cell, the inability of the host and treatment failure, and bacterial eradication are difficult and therefore increase chronicity and disease recurrence [5, 6]. Successful treatment depends on its duration and also the patient's cooperation. Recurrences in the first six months of treatment happen milder than the early stages of the disease. Consequently, new methods are designed to decrease the chronic problems of infection [7, 8]. The most promising strategy is to use nanocarriers. Precise formulation of nanocarriers leads to higher stability and can speed up dissolution and their therapeutic effect and upgrade their bioavailability [9–11]. Trace elements are very important in biochemistry, nutrition, and enzyme structure [11]. So identifying the exact role of these trace elements particularly in puberty, their function in activity and body defense can be a useful as well as helpful method in cell biology and genetics [11]. Trace elements have a critical role in the structure and

function of immune cells [12, 13]. Cytochrome A, B, and C, NADH, and succinate dehydrogenase (SDH) needs iron, and copper are essential for mitochondrial electron transport chain (cytochrome C oxidase function) [14]. Moreover, trace elements are vital for the survival and proper activity of enzymes which has a critical role in the cell defense process [15]. Magnesium is involved in the stability of the structure and proper function of some enzymes involved in the metabolism of different macromolecules in the host. [11].

Vitamin D has a significant role in both innate and acquired immune systems because it is involved in calcium homeostasis [16]. Resistance and susceptibility to some infectious diseases correlated to this vitamin. The correlation between serum level of vitamin D and the risk of involving infection has been well confirmed. Furthermore, vitamin D can improve the macrophage function and increases the construction of the antimicrobial peptide like cathelicidin and killing of intracellular bacteria [17]. The antimicrobial pathway of INF1-dependent in macrophages is connected to the serum level of vitamin D [17]. Brucellosis can interfere the function of some vital organs of the body like the spleen, liver, and joints; as a result, some proteins and enzymes in the body decrease or increase. Some studies have shown that serum protein levels change during infectious diseases. Reactor C proteins (CRP) are acute phase reactants. Serum CRP is typically less than 1 mg/L in healthy adults. In most infectious diseases, a rapid increase in CRP is seen. Brucellosis causes the destruction of liver and upsets the balance of some liver enzymes, for example, ALT, TBil, ALP, and AST. Recently, SLNs have been utilized as a drug delivery for different aims [18, 19]. Many drugs have been successfully included in SLN. These carrier systems control the release of drugs and upgrade their chemical stability [18, 20, 21]. The main idea of the present survey is to evaluate the therapeutic effect of cadmium telluride-labeled SLNs containing doxycycline and hydroxychloroquine on the both chronic and acute brucellosis and, furthermore, evaluate its effect on CRP, vitamin D, liver organ, and also kidney enzymes in *in vivo*.

2. Material and Methods

2.1. Synthesis of DOX-HCQ-SLN. The synthesis of SLNs was reported earlier. Briefly, in the first step, stearic acid or palm oil warmed to 70°C (5°C above the melting point) using Ben Marie, at which time poloxamer/lecithin and the antibiotics doxycycline or hydroxychloroquine were added to the melted oil and placed on magnetic stirring and mixed. Then, the heated distilled water was added to the mixture and homogenized followed by sonication at 45°C for 60 s to obtain the initial emulsion. Secondly, heated tween-80 was added to the initial emulsion and mixed. To obtain the second emulsion, homogenize by ultrasonic device at 45°C in a rotation for 1 min. After that, the mixture was gently added to 4°C distilled water containing quantum dot cadmium telluride on a magnetic stirrer and dispersed in a solution by the magnet for 5 minutes to stabilize the synthesized nanoparticles. Moreover, similar steps were performed to the prepare Free SLN (drug-free solid lipid nanoparticles). Finally, the synthesized nanoparticles were centrifuged (35,000 rounds for 20 minutes) and washed 3 times with distilled water [9].

2.2. Nanoparticle Properties. Some physiochemical properties of synthesized nanoparticles such as PDI, zeta potential, and mean particle size of DOX-HCQ-SLN were estimated. The maximum wavelength (lambda max (λ max)) of each drug was determined. Then, they were diluted and determined by a spectrophotometer at their maximum OD wavelength, and then based on the obtained data, to each drug, the standard curve was drawn. To evaluate the encapsulation and drug loading of synthesized nanoparticles, direct and indirect methods were used. The thermal behavior of the final formulation and its components were assessed using Differential Scanning Calorimetry (DSC) (Mettler Toledo, Hong Kong) device. For spectroscopy test of the samples, the prepared nanoparticle lyophilized powders were mixed with some potassium bromide (kb) simultaneously and converted into compact discs by a hydraulic compressor. The disks were then recorded in the path of infrared light in the middle IR range (400-400 cm) using an FTIR spectroscope (PerkinElmer, spectrum400, America). Drug release from the synthesized nanoparticle matrix was done utilizing dialysis bag method (molecular weight of 14000 to 12000 Daltons and pores of 2.5 nm). To ensure the stability of the optimal formula both in terms of appearance and physicochemical properties, the suspension was monitored in short term once a week and monthly, respectively. The suspension was also evaluated in long term (6-12 months) in terms of physicochemical properties and appearance. A field emission scanning electronic microscope (FeSEM) was used to examine the morphology of the nanoparticle [9, 22].

2.3. Animal Testing: Assessing the Treatment of Infection in In Vivo. Instructions to work on laboratory animal were approved by Hamadan University of Medical Sciences ethics committee (No: IRUMSHA. REC1399-736).

Male Wistar rats weighing $250 \pm (30\,\text{g})$ and aged 6 to 8 weeks were obtained from the animal house of Hamadan University of Medical Sciences. The rats were injected the intraperitoneal penetration of 1.5×10^6 CFU *B. melitensis* strain m16. Later, by passing acute stage of infection (10 days after injection) and after 6 weeks of injection (chronic stage of infection), rats were divided into eight groups.

In the acute stage, an infected group without treatment was considered as the positive control, and one group was kept as healthy rats (without bacterial injection) and the other 6 groups (5 rats in each group) with free doxycycline (2.5 mg/kg), free hydroxychloroquine (2.6 mg/kg), free doxycycline and hydroxychloroquine, DOX-SLN (2.5 mg/kg), HCQ-SLN (2.6 mg/kg), and DOX-HCQ-SLN were treated on 11, 13, and 15 days after infection.

In the chronic phase, 5 weeks after *B. melitensis* strain m16 injection, rats were studied. An infected group was without treatment as the positive control, and one group was kept as healthy rats (without bacterial injection) and the other 6 groups (10 rats in each group) with free doxycycline (2.5 mg/kg), free doxycycline and hydroxychloroquine, free hydroxychloroquine (2.6 mg/kg), HCQ-SLN (2.6 mg/kg), DOX-SLN (2.5 mg/kg), and DOX-HCQ-SLN were treated for ten days (once daily). One day after the last dose, ketamine/xylazine (87/13 mg/kg) were used to anesthetize the rats and blood samples were collected from their hearts [18].

2.4. Serum Level of Trace Elements. Blood serum levels of trace elements (Zn, Cu, Fe, Ca, Pho, and Mg) were analyzed by the audit kit (Ireland) with the photometry method and automatic analyzer (Hitachi 902, Japan). By using Convergys® ISE (Germany), serum levels of Na and K were also measured [11].

2.5. Blood Serum Levels of Biochemical Parameters. By a photometry method, the serum levels of AST, ALT, creatinine, TBil, and urea were evaluated using the audit kit (Ireland) and automatic analyzer (Hitachi 902, Japan) [11].

2.6. Blood Serum Levels of CRP. NycoCard kits (Norway) were utilized to evaluate the CRP level of rats in different groups. To evaluate CRP in the serum of rats, the immunochemical method was used.

2.7. Blood Serum Levels of Vitamin D. 25-OH-Vitamin D Elisa kit (Monokit-IRAN) was used for the analysis of serum level of vitamin D according to the kit protocol [16]. In summary, 25 microliters of serum sample from each standard, negative, and positive control was added to each ELISA microplate well. 100-microliter releasing agent was added to each well and kept at room temperature for 30 min; after this, the wells were washed three times (using the kit wash solution). In the following, 100 microliter enzyme conjugate solution was added to each well and kept at room temperature for 30 min. Again, the wells were washed three times. 100 microliter substrate solution was added to each well and left in the dark for fifteen min; next, 50 microliters of stop solution was added. Finally, the samples were read at a wavelength of 450 nm with reference wavelength of 620 nm in the dark environment.

2.8. Statistical Analysis. The mean and standard deviation of serum levels of CRP, vitamin D, liver, and kidney enzymes were determined, in both acute and chronic phases of disease. Duncan's multiple range and analysis of variance (ANOVA) tests were used to compare the positive control group with the experimental group. Pearson's correlation analysis was utilized to obtain the relationships between the studied parameters in the groups. The confidence level and p value were 95% ($p < 0.05$), respectively, which was considered statistically significant.

3. Results

3.1. Nanoparticle Specifications. In the nanoparticle formulation, the means of PDI, particle size, zeta potential, drug encapsulation, and loading efficiency were 0.385 ± 0.022, 214 ± 25 nm, -18.7 ± 2.3 mV, $94.15 \pm 2.6\%$, and $17.7 \pm 1.5\%$, respectively. The amount of drug loaded and encapsulated in DOX-HCQ-SLN was $19.1\% \pm 1.3$ and $95.8\% \pm 3.2$ for doxycycline and $92.5\% \pm 2.7$ and $16.3\% \pm 1.1$ for hydroxychloroquine, respectively. The nanoparticle size was almost constant until the eighth month, and there was no remarkable size difference ($p > 0.05$). After 12 months, the nanoparticle size showed a 10.1% increase in diameter (from 214 to 235 nm). The results of morphological analysis of DOX-HCQ-SLN by FE-SEM microscope showed that most of the particles were spherical and had a smooth surface with homogeneous dispersion (Figure 1). The FTIR results

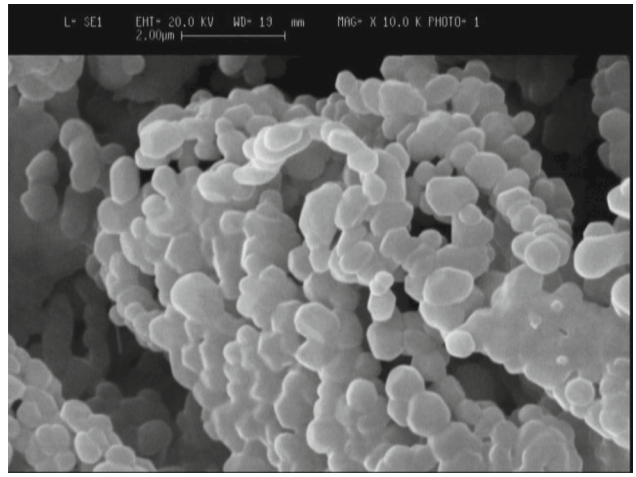

Figure 1: Field emission scanning electronic microscope image of DOX-SLN.

showed that in the process of nanoparticle synthesis, there was no chemical reaction that led to the formation of a new chemical compound. According to the findings of DSC test, the drugs are molecularly located in the lipid matrix and are not crystalline and free. It seems 75 hours is needing to 80% of the drug be released from the synthesized nanoparticles.

3.2. Determination of Blood Serum Levels of K, Na, Ca, and Pho. Based on the results of this study, the amount of Na in positive control was lower than healthy rats ($p < 0.05$). Na levels in rats treated with DOX-HCQ-SLN were approximately equal to the positive control group. The important point in the obtained results is the serum Na level in rats treated with DOX-HCQ-SLN, which significantly brought the Na level closer to its serum level in healthy rats. Serum K levels were similar to Na. The treatment with DOX-HCQ-SLN was fully effective for recovery of positive control group and increased the amount of K in the serum of rats treated with it as healthy rats. According to the results, brucellosis has little effect on the amount of Ca and Pho in the body. There was no statistically notable difference in serum Ca and Pho levels between healthy rat and positive control groups ($p > 0.05$). In general, in both acute and chronic phases, the amount of trace elements did not differ in different groups (Figure 2).

3.3. Determination of Blood Serum Levels of Cu, Zn, Fe, and Mg. As shown in Figure 3, there is remarkable difference in serum levels of Zn in different rat groups. In the positive control group, the concentration of Zn decreased significantly compared to the healthy rat group ($p < 0.05$). Rats treated with DOX-HCQ-SLN in acute and chronic brucellosis had increase Zn levels. The amount of copper and magnesium of the infected groups in the acute and chronic phases decreased in comparison with the positive control group. However, this reduction was low and were not considered statistically remarkable ($p > 0.05$). But the serum level of Fe was significantly reduced in acute and chronic stages of brucellosis. The treatment with synthesized nanoparticles and free drugs in this study, the level of trace

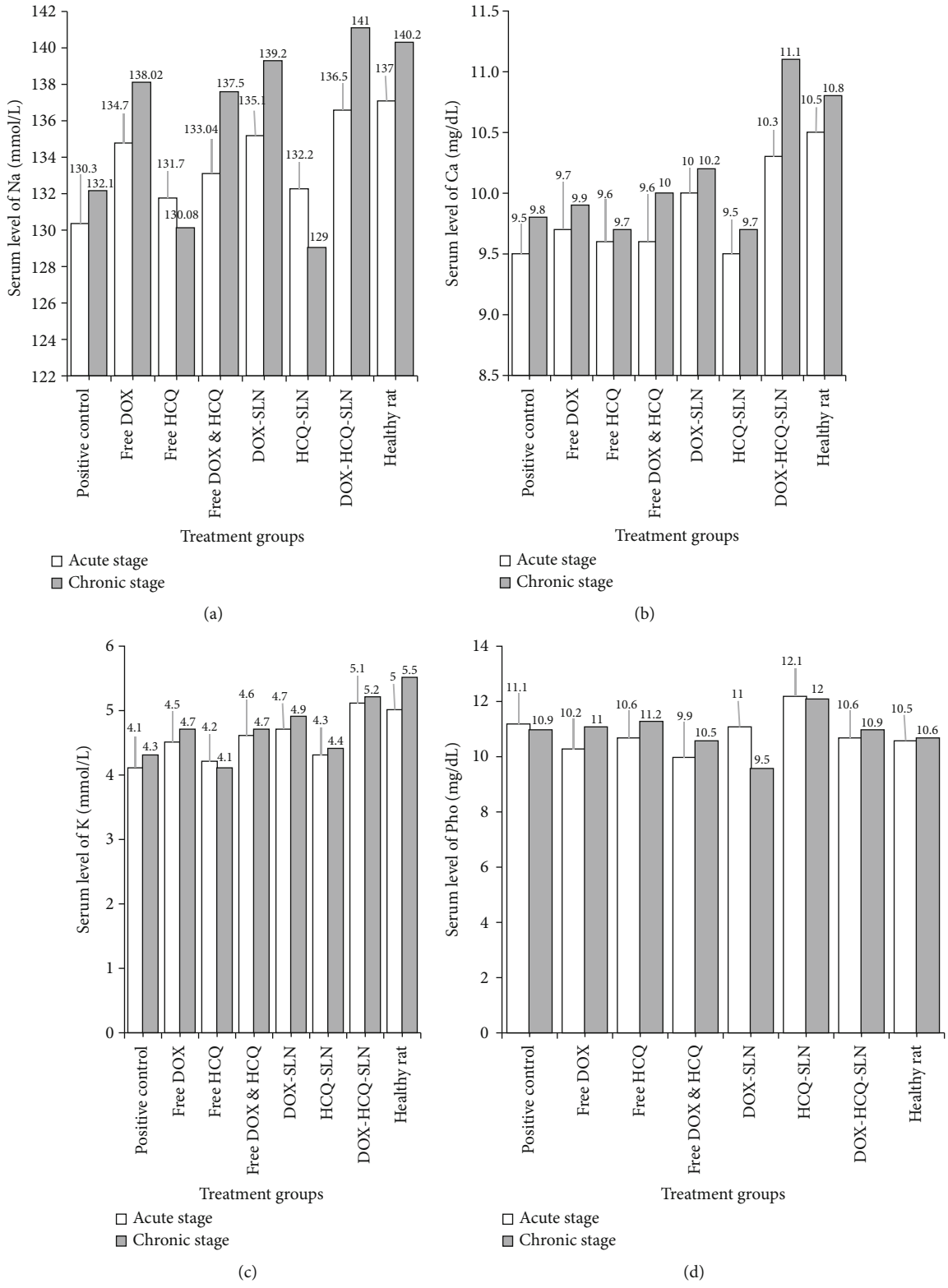

Figure 2: Serum level of trace elements in the acute and chronic stage of brucellosis. Concentration of Na (a), Ca (b), K (c), and Pho (d).

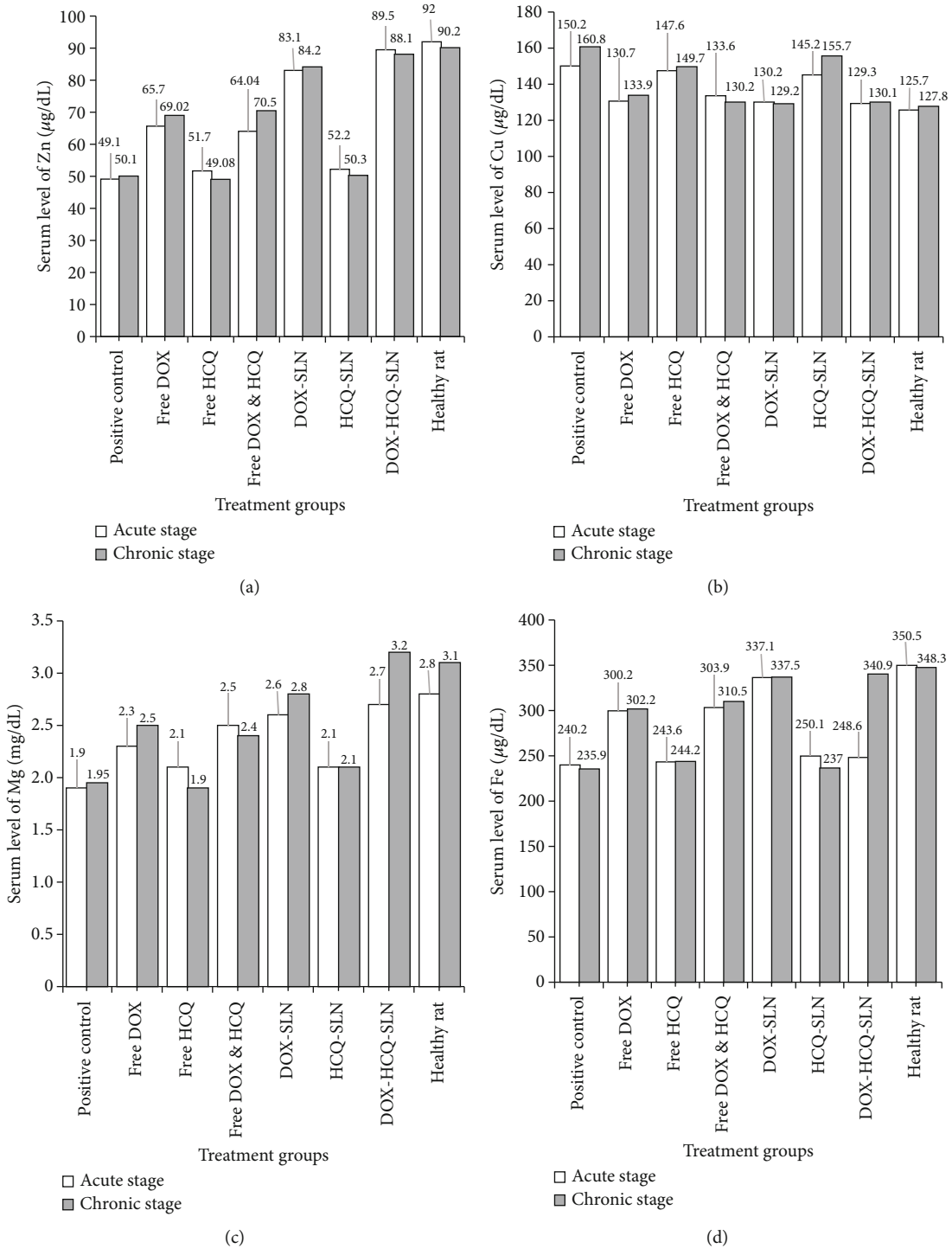

Figure 3: Serum level of trace elements in the acute and chronic stage of brucellosis. Concentration of Zn (a), Cu (b), Mg (c), and Fe (d).

elements were normalized and in general DOX-HCQ-SLN had the great effect on the recovery of rats compared to DOX-SLN and HCQ-SLN.

3.4. Results of Liver and Kidney Enzyme Analysis. The results of analysis of serum levels of liver enzymes showed that enzymes are significantly different in positive control and healthy rats. AST in the positive control group increased by 65% compared to healthy rats. Rats treated with DOX-HCQ-SLN also significantly decreased serum AST ($p < 0.05$). Treatment with Free DOX also decreased serum AST levels, but this decrease in AST levels was not statistically notable compared to healthy rats in the group ($p > 0.05$). The results also indicated that serum ALT level had less changes in different groups and serum levels of TBil and ALP such as serum AST levels in groups treated with DOX-HCQ-SLN, DOX-SLN, and Free

DOX compared to the positive control group were found. No statistically remarkable differences were observed in serum levels of Urea and Cr ($p < 0.05$) (Table 1).

3.5. Serum Levels of Vitamin D and CRP. Serum levels of vitamin D in the acute phase of positive control rats were lower than those of healthy rats, and this difference was statistically notable ($p < 0.05$). According to Figure 4, blood serum levels of vitamin D in the chronic stage of brucellosis had a greater decrease than those in the acute phase. Treatment of rats with DOX-HCQ-SLN was able to increase the amount of vitamin D in healthy rats at the end of treatment. CRP enhanced in both chronic and acute phases of the positive control group ($p = 0.001$). Rats treated with DOX-HCQ-SLN, DOX-SLN, and Free DOX in both acute and chronic phases had decreased in CRP compared to the positive control group, although this reduction was not statistically significant (Figure 5).

4. Discussion

Trace elements are vital components of the human immune system. Enzymes and hormones need a minimal amount of trace elements for their function as small fluctuations in trace element levels cause a negative impact on their function [12, 23]. This makes the body weak against microorganisms, and as a result, the immune system's ability to fight disease is weakened. One of the most important organs in the reticuloendothelial system that plays an important role in the immune system and is damaged by a variety of disorders is the liver [24]. Brucellosis is a multisystem illness that, when present in hepatic macrophages, can cause liver cell destruction and an increase in liver enzymes. The acidic condition within phagosomes and the restriction of access to antibiotics in the intracellular components and antibiotic action may vary, and exposure to the last antibiotic during infections may not occur [25]. As a result, nanoparticles containing antibiotics are required, which can be transformed in these environments and stay for a longer time while gently and continually targeting the drug delivery organs and microorganisms has happened. Serum levels of various trace elements, liver and kidney enzymes, vitamin D, and CRP were measured in infected rats in this study [26]. In this study, the levels of these components were investigated across different groups.

According to the findings of this investigation, liver enzymes in rats increased during both the acute and chronic phases of infection with *B. melitensis*. In this study, in the acute phase, ALT AST, TBil, and ALP increased to 33%, 50%, 75%, and 63%, respectively, which is valuable ($p < 0.05$). These results are similar to outcomes of Bozukluhan et al., which reported 42% and 61% increases in AST and ALT after infection. [27]. In Singh et al.'s study, brucellosis-infected cows showed a 20% increase in ALT levels and a 15% increase in AST levels [28], which is consistent with the outcomes of our experiment. Another study also reported an increase in the liver enzymes of level in the both chronic and stages of brucellosis [11, 16]. According to the findings of this study and the previous investigations, it can be concluded that testing the level of liver enzymes can be one method of brucellosis diagnosing. In this study, the use of DOX-HCQ-SLN and DOX-SLN in infected rats significantly reduced levels of liver enzyme compared to the positive control ($p < 0.05$). The reduction in the number of enzymes in the chronic phase is greater than that in the acute phase, indicating that brucellosis requires more time to treat. According to the findings, hydroxychloroquine can improve the efficiency and effectiveness of doxycycline against Brucella and speed up the healing process of brucellosis. In the current investigation, serum vitamin D levels in the acute and chronic stages were lower in the positive control group than in the healthy rat group. Hosseini et al. conducted a comprehensive study on the effect of rifampin as a nanoparticle against *Brucella melitensis*, which showed a decrease in serum levels of vitamin D in rats with brucellosis and an increase after treatment [16]. Beltrân et al.'s study resulted that in patients with brucellosis, serum vitamin D levels were 9.6 ng lower than in healthy individuals [29]. Another study comparing serum vitamin D levels in tuberculosis patients with healthy individuals indicated that the serum vitamin D levels in the group were 15 ng/dL lower than those in the healthy group. Serum vitamin D levels in the control group (healthy individuals) were 17 ng/dL, which is agreeable with our study [30]. Also, Ataei et al. evaluated vitamin D serum levels in people with hepatitis C [31] and implemented that the vitamin D serum levels in sick individuals and healthy were 26.23 ng/dL and 29.6 ng/dL, respectively, which is the same as the results of this survey.

Vitamin D serum levels in most patients with chronic liver disease and cirrhosis have been inadequate [29]. In our study, an increment in serum levels of vitamin D was noticed after treatment with DOX-HCQ-SLN in the acute and chronic stages of the disease that can offer a good therapeutic effect of DOX-HCQ-SLN compared to the free drug. Nanoparticles release the drug at the bacterial site continually and boost vitamin D levels in mouse serum through enhancing hepatic tissue following DOX-HCQ-SLN treatment.

Trace elements play an indispensable part in the body's resistance to many viral infections [32]. In the current investigation, serum zinc levels were lowered by nearly 80% in both the acute and chronic stages of brucellosis. An increase in serum levels of zinc was witnessed in the groups treated with free DOX, free DOX-SLN, DOX-HCQ, and DOX-HCQ-SLN. In general, treatment of rats in the acute and chronic stages of brucellosis with hydroxychloroquine alone or together had little effect on the treatment of positive control group, and therefore, serum Na levels in rats treated with free HCQ and HCQ-SLN were not significantly different from positive control rats. However, rats treated with free DOX and DOX-SLN were more effective in treatment. Some interleukins released by phagocytes and leukocytes are activated by transporting zinc from the bloodstream to the liver, thereby alleviating zinc shortage in viral illnesses [33]. Comparing serum levels of Na in different treatment groups, the group receiving DOX-HCQ-SLN had the best results. In our study, serum K levels in rats decreased significantly after the development of brucellosis. Following treatment with several formulations, it was discovered that DOX-HCQ-SLN had the best effect in the treatment of positive group. Serum Cu levels in acute and chronic disease circumstances were lower in the current investigation than in the

Table 1: Comparison of biochemical parameter between different treatment groups in acute and chronic brucellosis.

Group/parameter	Stage of disease	AST[1] (U/L)	ALT[2] (U/L)	ALP[3] (U/L)	TBil[4] (mg/dL)	Urea (mg/dL)	Cr[5] (mg/dL)
Positive control	Acute	162 ± 5.01	75 ± 6.01	1100 ± 25.1	0.4 ± 0.01	55.2 ± 1.5	0.45 ± 0.05
	Chronic	142 ± 4.03	64 ± 3.02	900 ± 15.3	0.42 ± 0.02	45.2 ± 0.5	0.55 ± 0.02
Free DOX	Acute	120 ± 6.02	70 ± 6.20	850 ± 11.02	0.2 ± 0.009	50.2 ± 1.7	0.47 ± 0.06
	Chronic	134 ± 6.08	59 ± 5.04	810 ± 35.60	0.21 ± 0.01	48.2 ± 2.1	0.49 ± 0.01
Free HCQ	Acute	151 ± 3.4	73 ± 2.9	1110 ± 32.6	0.39 ± 0.02	45.9 ± 2.6	0.49 ± 0.01
	Chronic	144 ± 6.01	65 ± 7.01	897 ± 19.32	0.4 ± 0.01	44.5 ± 1.9	0.51 ± 0.03
Free DOX & HCQ	Acute	118 ± 8.8	71 ± 6.80	840 ± 18.8	0.24 ± 0.01	49.3 ± 3.1	0.48 ± 0.08
	Chronic	132 ± 3.90	58 ± 4.23	845 ± 45.56	0.21 ± 0.01	46.4 ± 2.5	0.48 ± 0.07
DOX-SLN	Acute	115 ± 6.06*	68 ± 3.02*	800 ± 26.6*	0.15 ± 0.00*	59.2 ± 2.8	0.5 ± 0.02
	Chronic	92 ± 3.01*	57 ± 5.11*	500 ± 18.7*	0.14 ± 0.02*	49.2 ± 2.1	0.51 ± 0.05
HCQ-SLN	Acute	149 ± 5.6	72 ± 9.0	990 ± 25.3	0.38 ± 0.02	48.7 ± 3.9	0.49 ± 0.03
	Chronic	137 ± 7.22	63 ± 6.01	879 ± 29.75	0.35 ± 0.04	48.2 ± 2.5	0.47 ± 0.02
DOX-SLN-HCQ	Acute	107 ± 3.2**	67 ± 3.1*	540 ± 31.2**	0.11 ± 0.03**	52.6 ± 2.6	0.46 ± 0.04
	Chronic	84.1 ± 2.73**	53 ± 5.01*	456 ± 15.1**	0.12 ± 0.01**	49.1 ± 1.7	0.49 ± 0.11
Healthy rat	Acute	98 ± 3.0**	66 ± 4.8*	450 ± 13.5**	0.09 ± 0.01**	54.9 ± 1.8	0.40 ± 0.01
	Chronic	80.2 ± 3.04**	50 ± 3.12*	403 ± 21.61**	0.1 ± 0.02**	47.3 ± 2.3	0.44 ± 0.12

1: aspartate transaminase; 2: alanine transaminase; 3: alkaline phosphatase; 4: total bilirubin; 5: creatinine. Results are represented as the mean ± SD. *$p < 0.05$ and **$p < 0.001$, significantly compared to the positive control.

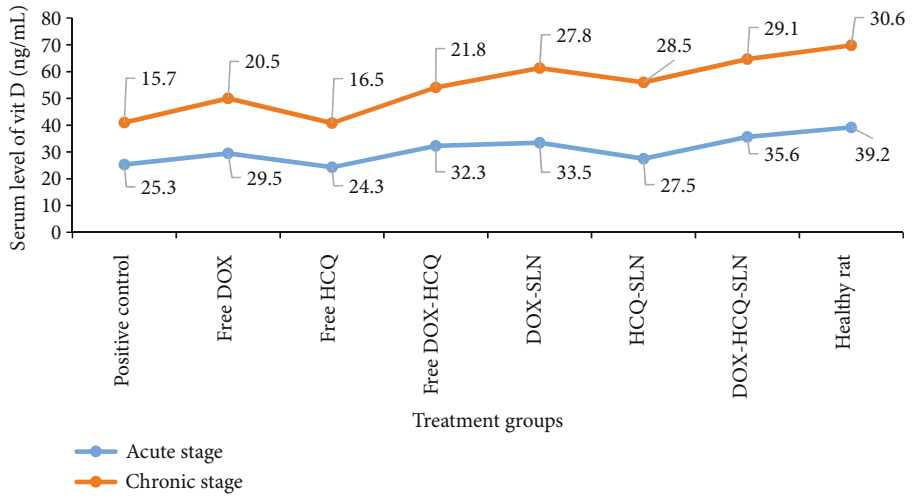

Figure 4: Serum level of Vitamin D in the acute and chronic stage of brucellosis.

healthy rat group, but the difference was not statistically valuable ($p > 0.05$).

The outcome of this study implemented that the serum level of iron in chronic and acute conditions of brucellosis had decreased, which was statistically remarkable compared to the group of healthy rats ($p < 0.05$). After treatment with DOX-HCQ-SLN in the chronic stage of the disease, the impressive increase in serum iron levels was witnessed. Bozukohan et al. discerned a 50% reduction in serum levels of iron in cows with brucellosis compared to healthy [27], which is in deal with the results of this project. Because iron plays an essential role in biosynthesis, low iron levels can be ascribed to liver damage and malfunction, as well as increased iron absorption by microbes [32]. Serum levels of calcium and phosphorus had not significant differences in various groups in both chronic and acute phases.

In this experiment, the amount of CRP in the chronic and acute stages decreased in the control (healthy rat) compared to the positive control group. Serum levels of CRP were assessed before and after treatment [34]. Demirdag et al. investigated serum levels of cytokines and CRP in brucellosis infection, and according to their finding demonstrated in acute brucellosis, the serum levels of CRP were 52.4 mg/L, which after treatment decreased to 11 mg/L. Their results indicated there is a

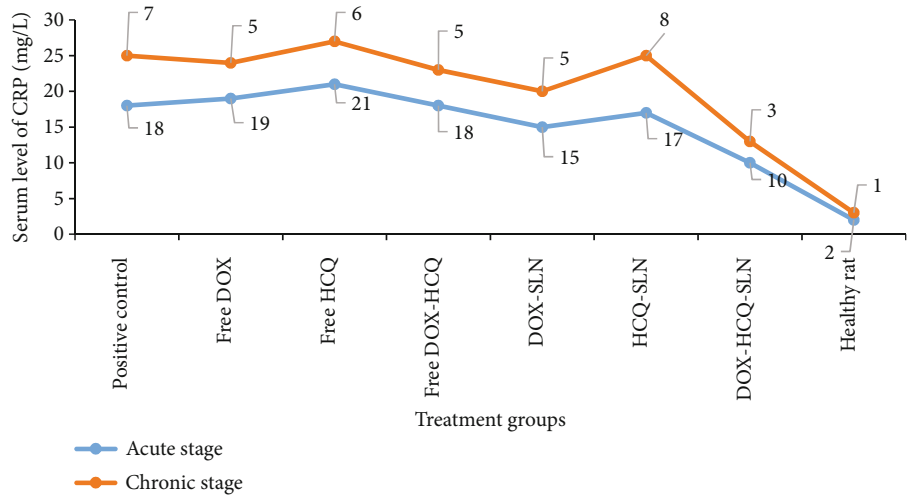

Figure 5: Serum level of CRP in the acute and chronic stage of brucellosis.

relationship between serum levels of TNF-α and INF-1 with serum levels of CRP. CRP is a protein produced in the liver through acute inflammation of disease [35].

5. Conclusion

Today, solid lipid nanoparticles are the basis for medical applications in various fields used by biologists, chemists, and physicians as well as industrial and academic groups. Based on the outturns of this survey, serum levels of trace elements and also biochemical parameters, CRP is used beside other diagnostic methods such as serological tests and culture to diagnose brucellosis. Due to good effect of the concurrent use of hydroxychloroquine and doxycycline in the form of nanoparticles, extensive use of nanoparticles in various forms can lead to better treatment and reduction in brucellosis reinfection.

Data Availability

The data can be accessible to the interested researchers by the corresponding author on reasonable request.

Ethical Approval

Animal protocols were approved by the ethics committee of Hamadan University of Medical Sciences (No.: IRUMSHA.REC1399-736). All experiments involving animals were performed according to the guidelines for maintenance, surveillance, and usage of laboratory animals published by the National Institute of Health United State (NIH publication No. 85-23, revised 1985).

Disclosure

This study has been adapted from a research project at Hamadan University of Medical Sciences (Project No. 9910167133).

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

SMH and MA designed the study. SMH contributed in the experimental studies, and drafting the work. MT and MYA performed the analysis of the data. SA and SS contributed in the cell culture. SMH and AF designed the nanoparticles. All authors read and approved the final manuscript.

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