J Lasers Med Sci 2023;14:e18
10.34172/jlms.2023.18

Superparamagnetic iron oxide (Fe3O4) nanoparticles and curcumin loading

Nano particles was provided by Dr. Behnaz Ashtari. Curcumin was integrated in the modified nano particles (NP)s by several series thermo-sensitive protocols, at the elevated temperature. Briefly, curcumin (0.01 g) was melted in acetone (10 ml), and gradually released into the precooled modified NPs solution in distilled water (DW) (4°C). Subsequently, the temperature of the solution was elevated up to 37ºC to encapsulate curcumin in the NPs. The volume of integrated curcumin was defined by calculating the absorbance of the supernatant, using a UV-Vis spectrophotometer at 416 nm, after separating SPIONs by magnetic field and centrifugation. The drug loading efficiency of nanoparticles was 75%, calculated by the following equation **:**

$$\text{Loading efficiency } (\%) = \frac{\text{Total amount of Curcumin} - \text{Free Curcumin}}{\text{Total Curcumin}} \times 100$$

For determining the release of curcumin, drug loaded nano particles solution (1 mg/mL) were transfered into a dialysis container, to be dialyzed against 150 ml DW (pH 7.4), containing Tween-80 (0.5% w/w) at 37ºC and 45ºC, with continuous stirring. Fe_3O_4 Mag-curcumin nanoparticles were suspended in DW. The drug release of curcumin profile was measured for 60 hours at 37°C and 45°C. Transmission electron microscope (TEM) examination was performed on curcumin nanoparticles. At regular time intervals, the incubation medium was substituted with new media. The volume of the released medication, in the incubation medium was

measured by UV-Vis, using a spectrophotometer, and the remedy release percentage was computed, by the following equation(1, 2, 3):

$$\text{Release (\%)} = \frac{\text{released curcumin}}{\text{total curcumin}} \times 100$$

Biodistribution of CUR

In four rats with skin wounds, 0.0054 mg of curcumin nanoparticles were administrated, similar to the rats of groups 4, and 5. Another four rats received the same amount of curcumin nanoparticles without any wounds. Skin samples were harvested after 24, and 72 hours exposure with curcumin nanoparticles and digested. Briefly, 0.05 g of each skin sample was digested, using a 10 mg/mL elemental analysis. The sample was digested again in ultrapure nitric acid. Then, H_2O_2 was added and the mixture was heated in a high-pressure condition, until sample was completely digested. Finally, nitric acid was added. Data are expressed as nanograms per gram of fresh tissue.

References

1. P. Naserzadeh, A.A. Hafez, M. Abdorahim, M.A. Abdollahifar, R. Shabani, H. Peirovi, A. Simchi, K. Ashtari, Curcumin loading potentiates the neuroprotective efficacy of Fe(3)O(4) magnetic nanoparticles in cerebellum cells of schizophrenic rats, Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 108 (2018) 1244-1252.

2. A. Moradi, Y. Kheirollahkhani, P. Fatahi, M.A. Abdollahifar, A. Amini, P. Naserzadeh, K. Ashtari, S.K. Ghoreishi, S. Chien, F. Rezaei, M. Fridoni, M.

Bagheri, S. Taheri, M. Bayat, An improvement in acute wound healing in mice by the combined application of photobiomodulation and curcumin-loaded iron particles, Lasers in medical science, 34 (2019) 779-791.
3. M. Mahmoudi, H. Hosseinkhani, M. Hosseinkhani, S. Boutry, A. Simchi, W.S. Journeay, K. Subramani, S. Laurent, Magnetic resonance imaging tracking of stem cells in vivo using iron oxide nanoparticles as a tool for the advancement of clinical regenerative medicine, Chemical reviews, 111 (2011) 253-280.

In vivo toxic studies

20 Wistar rats that weighed ~ 270 g were enrolled in this section. Rats were haphazardly distributed into four groups: 1) healthy control group, 2) healthy curcumin-loaded superparamagnetic iron oxide nanoparticles (CUR) injected group, 3) TIDM control rats, and TIDM, CUR injected group. The induction of TIDM was mentioned in section 2.2. Next, each group was subdivided into control and CUR groups. Each rat was intraperitoneally injected with 0.216 mg/400 µL curcumin-loaded iron nanoparticles (CUR) using an insulin syringe having a needle size of 28. Rats in the control groups received 0.9% normal saline. Twenty-four hours after the CUR injection, a 1-mL blood sample was obtained from the heart of each rat through the transcardial route and transferred to a test tube. The sample was submitted to liver and kidney function tests including serum levels of alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and creatinine (CRE) using Clinical Chemistry Analyzers and Assays Beckman Coulter (Selectra Pro XL) clinical system. Besides histological samples of livers and kidneys of the studied groups were subjected to the light microscopic study and were stained with hematoxylin and eosin (H&E) method, and were descriptively examined by an expert pathologist who was blinded to the nature of the groups.

Findings of toxic evaluations

The results of functional tests of liver and kidney which were shown in table two did not show any significant differences between subgroups of healthy and diabetic classifications, while a huge increase in the serum levels of the studied parameters were observed in diabetic groups. In addition as Fig. 7 shows that in histopathologic findings there were no changes on morphology of the healthy rats' kidneys and livers. Besides in the diabetic rats, there were red blood cells in the tubules of the kidneys, and lymphocytes in the central veins of the livers.

PARAMETERS →		**ALT(IU/I)**	**AST(IU/I)**	**BUN(mg/dl)**	**CRE(mg/dl)**
GROUPS↓	**SUB GROUPS AND NUMBER OF RATS↓**				
HEALTHY	**CONTROL(5)**	40±5	80±10	12±1	0.33±0.12
	INJECTED CUR(5)	41±4.1	78±8.1	12.4±0.9	0.34±0.10
DIABETIC	**CONTROL(5)**	240±5	880±21	54±1.2	0.7±0.01
	INJECTED CUR(5)	244±6	887±18	55±1.1	0.72±0.13

Table 1. Mean±SD of functional tests in the liver and kidney of rats including serum alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and creatinine (CRE) of studied groups: control healthy, experimental healthy plus curcumin-loaded iron nanoparticles (CUR), control diabetic, and experimental diabetic plus CUR, compared by t-test.