

**Core-Shell Structured 5-FU@ZIF-90@ZnO as a Biodegradable Nanoplatfrom for
Synergistic Cancer Therapy**

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Experimental Section

Drug loading and release in vitro:

Different concentrations of 5-FU/PBS solutions were accurately prepared. Then, the solution was scanned with UV-vis spectrophotometer, and the maximum absorption wavelength on 262 nm was monitored. A series of concentrations of 5-FU in PBS were formulated to establish a standard curve for absorbance (A) and concentration (C) at this wavelength. The standard curves of 5-FU were used for calculating the drug loading and release contents. The 5-FU loading efficiency (LE) can be calculated by the equation:

$$\text{LE (\%)} = [\text{m}_{\text{original 5-FU}} - \text{m}_{\text{5-FU in supernatant}}] / \text{m}_{\text{FZZ}} \times 100 \%. \quad (1)$$

For the release test of 5-FU from FZZ nanoparticles, FZZ nanocomposites were dispersed in 10 mL PBS (pH = 7.4, 6.5 and 5.5) with gentle shaking at 37 °C for predetermine time. At predetermined time intervals, the PBS solution was centrifuged and the supernatant was removed and replaced with an equal volume of fresh PBS. The released amount of 5-FU in the supernatant solutions was quantified by UV-vis spectrophotometer at 262 nm.

Cell lines:

L929 (mouse fibroblast cell line) and HeLa (human cervical cancer cell line) were chosen for cell tests. L929 and HeLa cells were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. L929 cells were cultured in MEM supplemented with 1% (v/v) penicillin, 1% (v/v) streptomycin, and 10% (v/v) heat-inactivated (FBS, GIBCO) at 37 °C in an atmosphere of 5% CO₂. HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% (v/v) penicillin, 1% (v/v) streptomycin, and 10% (v/v) fetal bovine serum at 37 °C in an atmosphere of 5% CO₂.

Quantification of the internalization of FZZ nanoparticles by HeLa cells:

HeLa cells were cultured in 6-well plates at a density of 4.0×10^5 per well. Then, the cells were incubated with FZZ nanoparticles (100 $\mu\text{g/mL}$) for different time points (0.5, 1, 2, 4, 8, 12, 24 h), and the cells were washed three times with PBS buffer to remove any particles immobilized on the cell surface. The cells were counted and centrifuged at 1000 rpm for 5 minutes and then the cells were treated with cell lysis buffer. The content of the Zn^{2+} in HeLa cells was determined by ICP.

Cell compatibility:

To evaluate the biocompatibility of ZZ nanocomposites, the L929 mouse fibroblast cells were chosen as the normal cell to evaluate the biocompatibility of ZZ nanoparticles by using the standard MTT cell assay. The L929 cells were seeded into 96-well plates at a density of 8000 cells per well in 100 μL and incubated at 37 $^{\circ}\text{C}$ under 5% CO_2 overnight. Then different concentrations of ZZ (200, 100, 50, 25, 12.5, 0 $\mu\text{g mL}^{-1}$) were added to the medium, and the cells were incubated in 5% CO_2 at 37 $^{\circ}\text{C}$ for 24 h. After 24 h incubation, 20 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution (diluted in a culture medium with a final concentration of 0.5 mg mL^{-1}) was added to each well and incubated for another 4 h. The supernatant in each well was aspirated. 150 μL of dimethyl sulfoxide (DMSO) was added to each well before the plate was examined using a microplate reader (Thersmo Multiskan MK 3) at the wavelength of 490 nm. Cell viability (%) was calculated based on equation (2):

$$\text{Cell viability (\%)} = X_{\text{sample}} / X_{\text{control}} \times 100\% \quad (2)$$

where, X_{sample} and X_{control} represent the average absorbance of the sample and control

wells, respectively.

In vitro cytotoxicity of ZZ NPs was assayed against HeLa cells. The cells were seeded in 96-well plates at a density of 8000 cells per well and grown in 5% CO₂ at 37 °C overnight. Then different concentrations of free 5-FU, ZZ and FZZ ranging from 0 to 200 µg mL⁻¹ were added to the medium, and the cells were incubated in 5% CO₂ at 37 °C for 24 h. After 24 h incubation, the medium containing nanoparticles was removed and 10 µL of MTT solution was added into each well and cultured for another 4 h. Finally, the supernatant was replaced with 150 µL of dimethyl sulfoxide (DMSO) each well. The plate was shaken for 10 min and examined using a microplate reader (Therom Multiskan MK 3) at the wavelength of 490 nm. The results were expressed as the percentage of cell viability.

The live/dead cell experiment was also used to verify the anti-cancer properties of FZZ. HeLa cells were seeded in 96-well plates at a density of 8000 cells per well and cultured overnight at 37 °C in 5% CO₂. And then, cells were treated under different conditions: (1) control, (2) ZZ, (3) 5-FU, (4) FZ, (5) FZZ group. After different treatments, the cell culture medium was discarded. In addition, fresh medium containing calcein-AM (10 µg mL⁻¹) and PI (5 µg mL⁻¹) was then added. After 20 min, the cells were washed with PBS, and the cells were observed under an inverted fluorescence microscope. Flow cytometry was used to detect apoptosis analysis. The HeLa cells were seeded in a 6-well plate at a density of 10⁵ cells/well and cultured overnight at 37 °C under 5% CO₂. The adherent cells were subjected to live/dead treatment as described above, and after 12 hours, the cells were collected by centrifugation, and then the cells were washed with PBS. Next, 500 µL of binding buffer was added to redisperse the cancer cells. Annexin V (5 µL) and PI (5 µL) were used to stain the cells. Finally,

flow cytometry was used to detect apoptosis after treatment.

Western blot:

In the presence of ZZ, HeLa cells (5×10^5 cells/mL) were incubated for 12 h, then washed with ice-cold phosphate-buffered saline and lysed in buffer, with saline as a control. Cell lysates were subsequently incubated at 4 °C for 30 min and then centrifuged at 12,000 rpm for 15 min, and the supernatant was transferred to a new centrifuge tube. The protein concentration of the supernatants was determined using a protein assay kit (Beyotime Biotechnology, China). An appropriate amount of 5 × SDS-PAGE gel was added to the collected protein samples and heated at 100 °C to completely denature the protein. After the sample was cooled to room temperature, the protein sample was transferred by electroblotting to a PVDF membrane (Beyotime Biotechnology, China). After blocking with 5% nonfat dry milk in Tris-buffered saline (TBS), the membranes were incubated with the primary antibodies at an appropriate dilution overnight at 4 °C, and then washed three times in TBST buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween 20) for ten minutes each time at room temperature. And then, the membranes were incubated with diluted secondary antibodies (1:5000) for 1 h and washed three times in TBST. The blots were visualized with ECL (Beyotime Biotechnology, China). All the antibodies were purchased from Beyotime Biotechnology.

Animal Experiments:

Female Balb/c mice (six weeks old, 19-28 g) were purchased from the Center for Experimental Animals, Jilin University (Changchun, China). All animal studies were conducted in accordance with the guidelines of the National Regulation of China for Care and Use of Laboratory Animals. The H22 tumor models were successfully established by

subcutaneous injection of 4×10^6 cells suspended in 100 μ L PBS into the right and left axilla of each mouse. The mice were treated when the tumor volumes approached 60-70 mm³.

In vivo Biocompatibility of FZZ Nanoparticles:

To evaluate the biocompatibility of nanomedicine in Balb/c mice, healthy Balb/c mice weighing approximately 25 g were divided into five groups, saline solution, 5-FU, FZ, ZZ and FZZ group, five mice per group, and the concentration of 5-FU contained was 4 mg/kg. The nanoparticle solution was injected twice through the tail vein on day 0 and day 2, respectively, and the tumor volume and body weight of the mice was monitored every other day. The mice were then euthanized 15 days after the injection. The main organs such as heart, liver, spleen, lung and kidney were analyzed by hematoxylin and eosin (HE) staining, and the blood of the experimental group and the control group were collected. Serum biochemical assays were then performed. The liver function indexes including alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST), as well as the kidney function indicator, blood urea nitrogen (BUN) and creatine (CRE) were all measured.

The Tumor size was calculated as follow:

$$\text{Tumor volume (V)} = \text{width}^2 \times \text{length} / 2 \quad (3)$$

$$\text{Relative tumor volume (R)} = \text{Tumor volume after treatment (V)} / \text{Tumor volume before treatment (V}_0\text{)} \quad (4)$$

In Vivo Biodistribution of FZZ

To evaluate the biodistribution in Balb/c mice, intravenous FZZ was used. Mice were then sacrificed at different time points (2, 4, 8, 12 h, 1, 3, 5 D) (n = 3). The main organs and tumors such as heart, liver, spleen, lung and kidney were taken and placed in a flask and weighed. All

organs were treated with H_2O_2 and HNO_3 (v/v = 1:2) heated ($60\text{ }^\circ\text{C}$) for 10 h until the solution became clear. Finally, after dilution with deionized water, the zinc content in the solution was determined by inductively coupled plasma mass spectrometry (ICP-MS), and the concentration of zinc in each organ was calculated. The body metabolism was studied by intravenous injection of FZZ (50 mg kg^{-1}), and then urine and feces were collected at predetermined times (1, 3, 5, 7, 9, 11, 13, 15 D), and finally ICP-MS was used to analyze the Zn content in urine and feces.

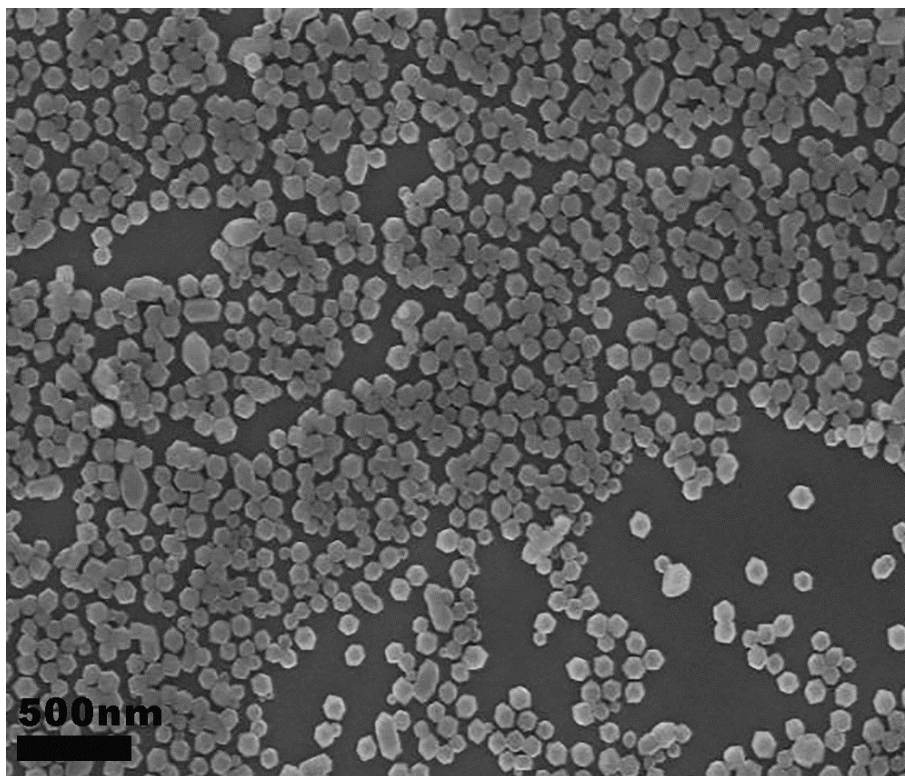


Figure S1. The SEM image of ZIF-90 nanoparticles.

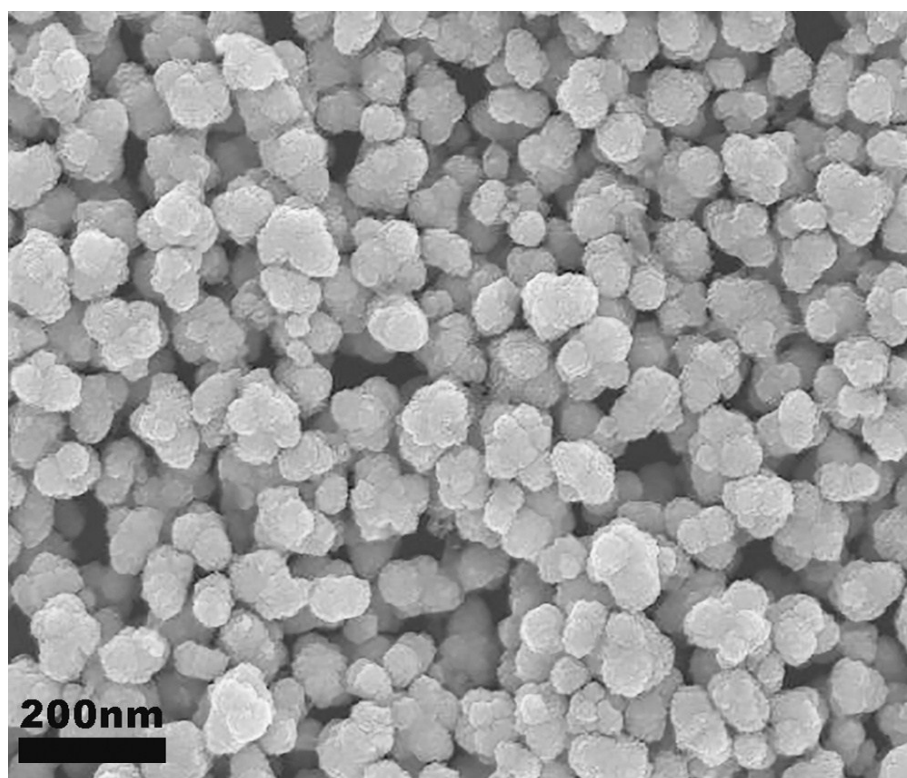


Figure S2. The SEM image of ZZ nanoparticles.

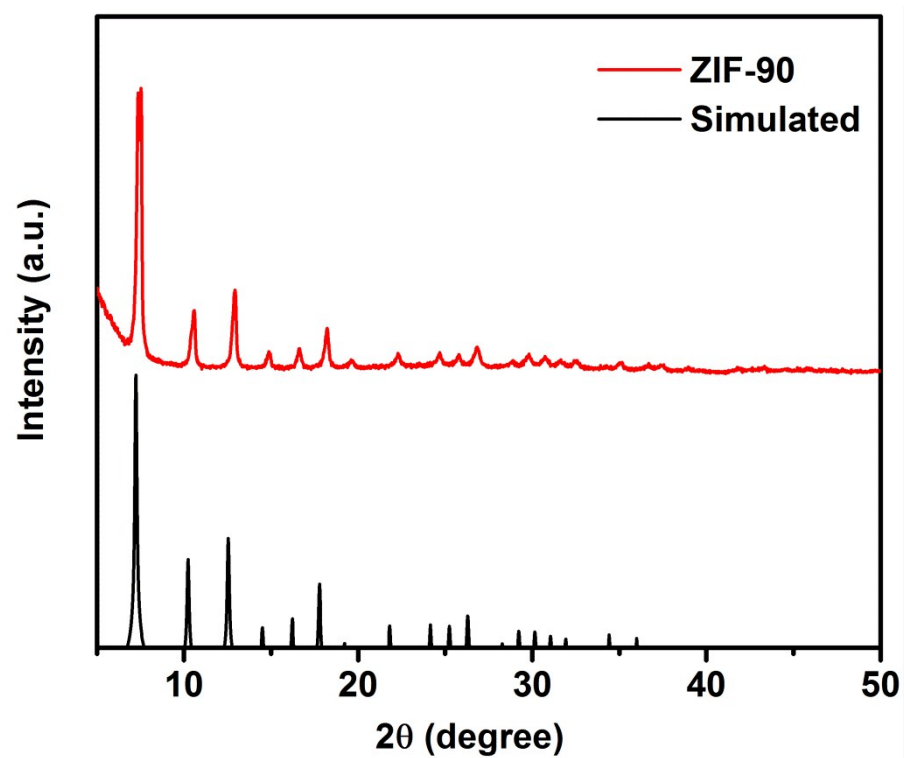


Figure S3. XRD analysis of the as-prepared ZIF-90 nanoparticles and the simulated pattern.

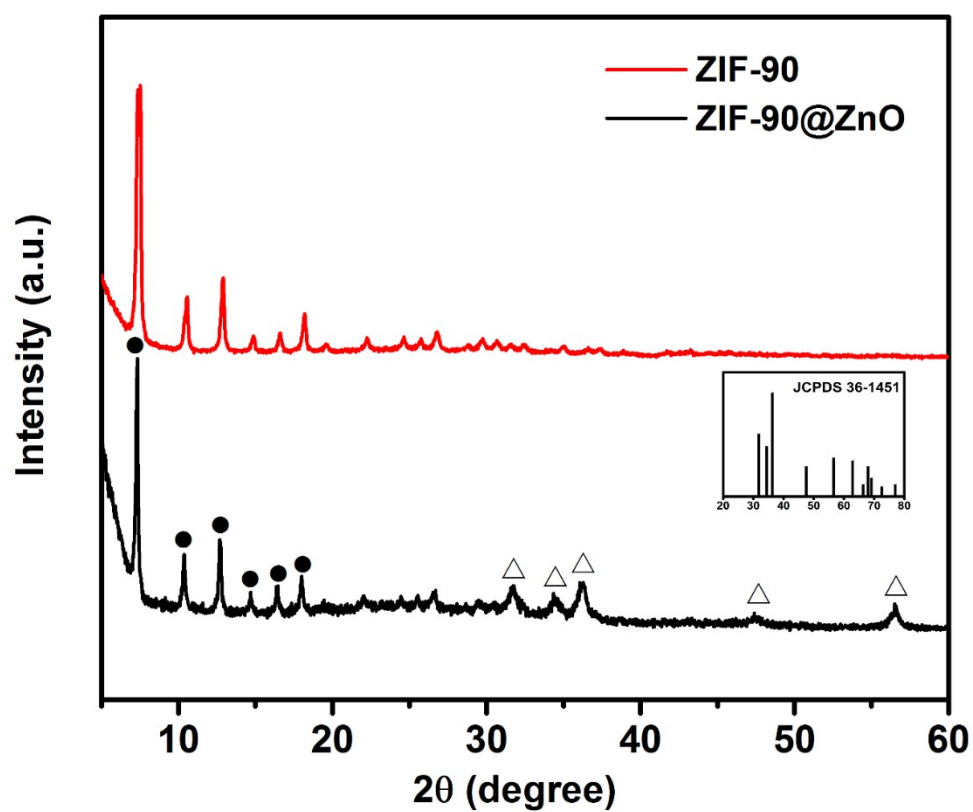


Figure S4. XRD analysis of the ZIF-90 and ZZ and ZnO (JCPDS 36-1451). The peaks of ZIF-90, and ZnO are marked with circle and triangle, respectively.

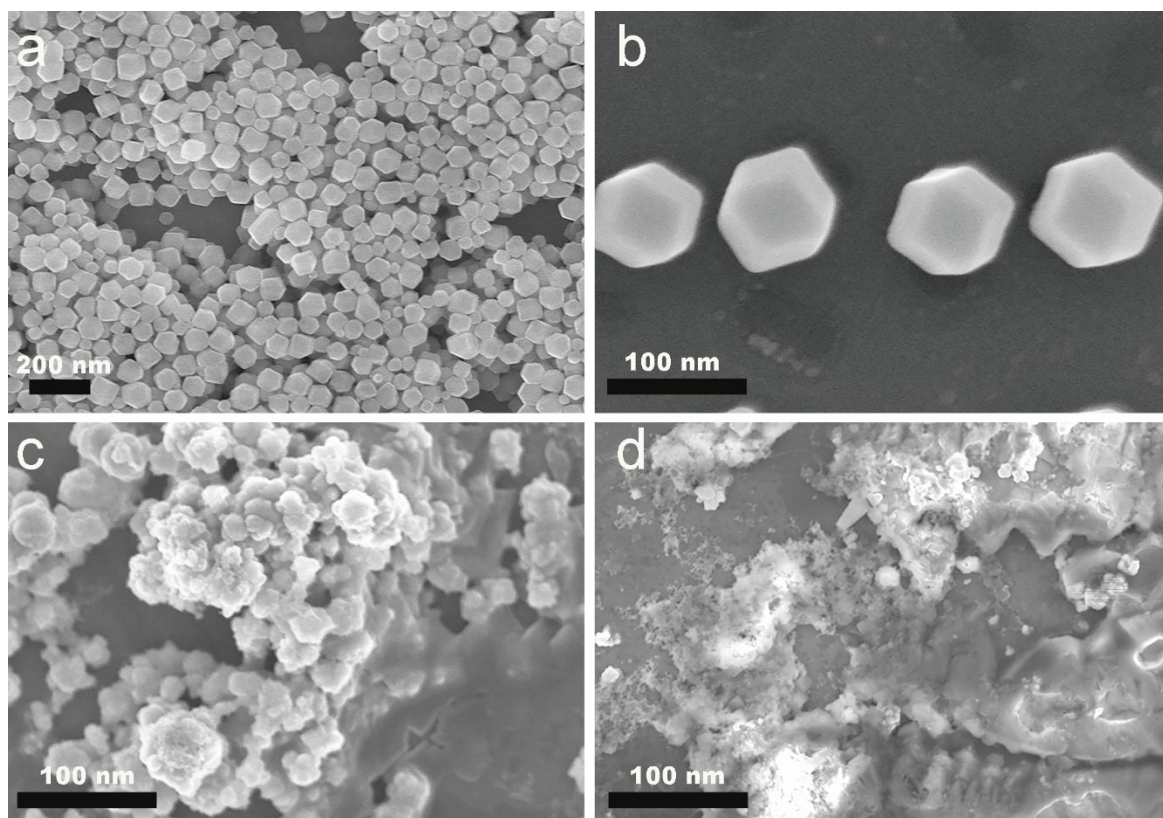


Figure S5. SEM images of ZIF-90 crystal after soaking in PBS buffer with (a), (b) pH = 7.4, (c) pH = 5.5 and (d) pH = 2.0 for 10 min, respectively. The concentration of ZIF-90 was 10 mg/mL.

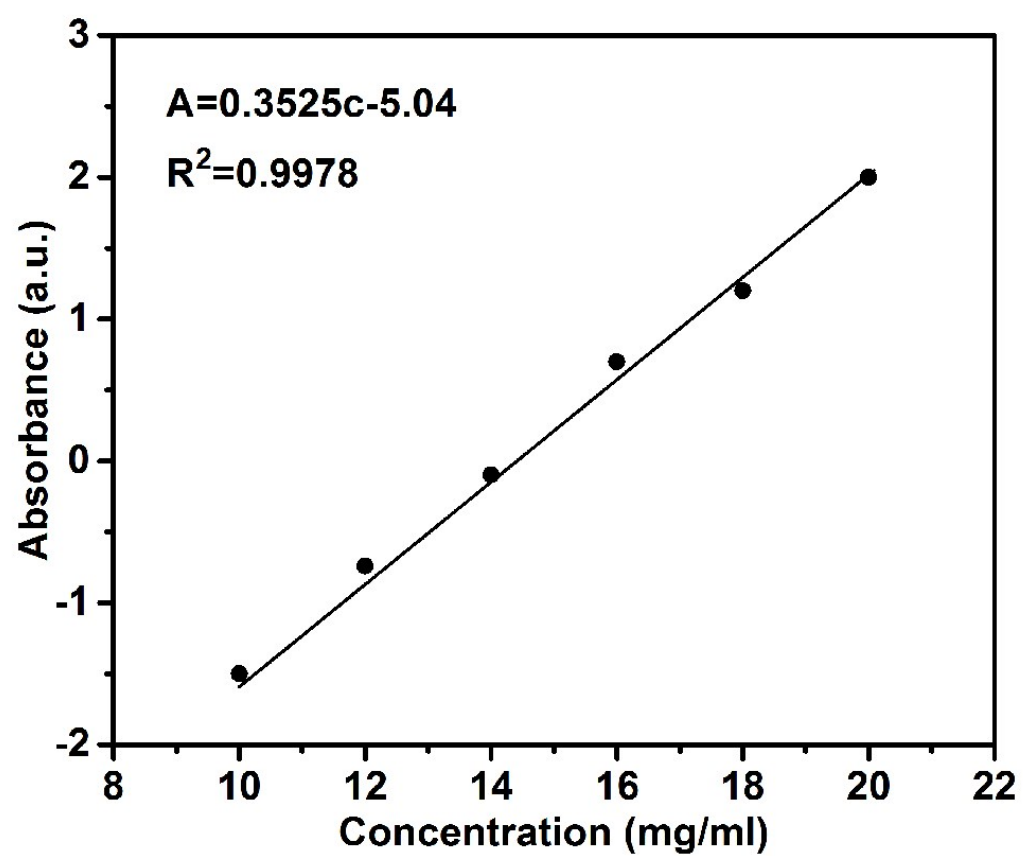


Figure S6. Standard curve of 5-FU determined by UV-vis spectra.

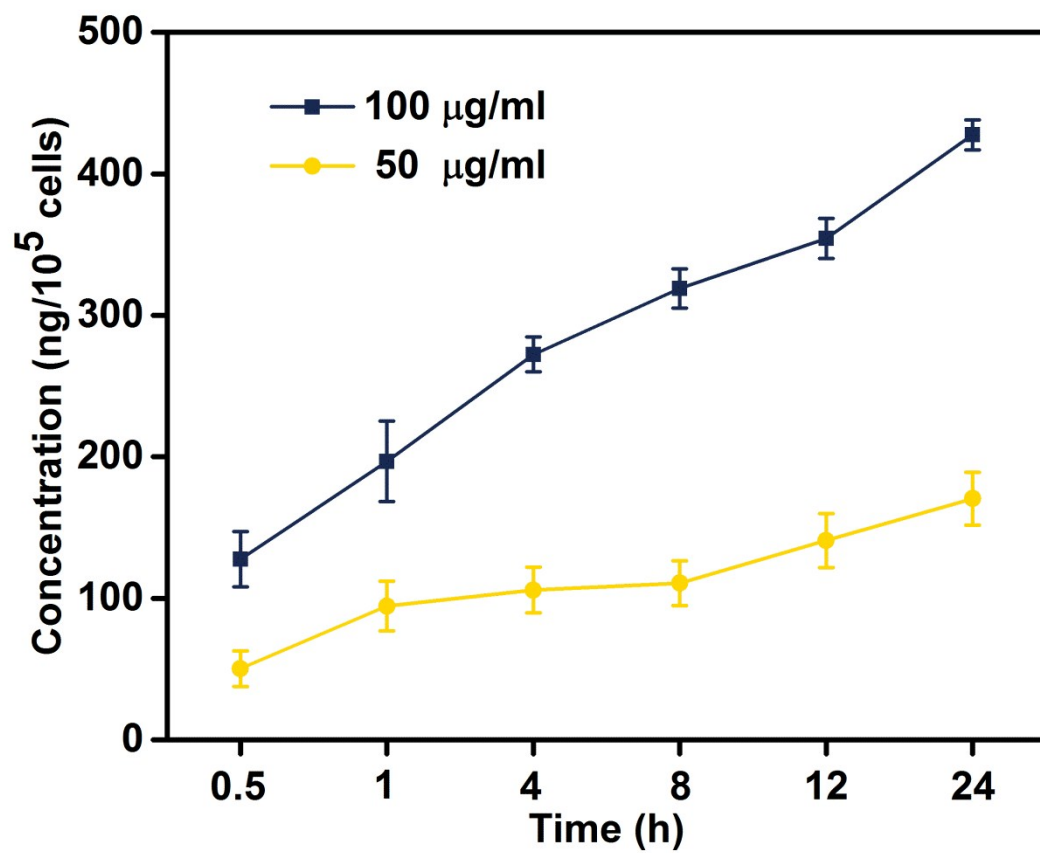


Figure S7. Mass of Zn²⁺ ions internalized by HeLa cells after incubation with different concentration of FZZ (100 µg/mL and 50 µg/mL) nanoparticles at different times (0.5, 1, 4, 8, 12 and 24 h).

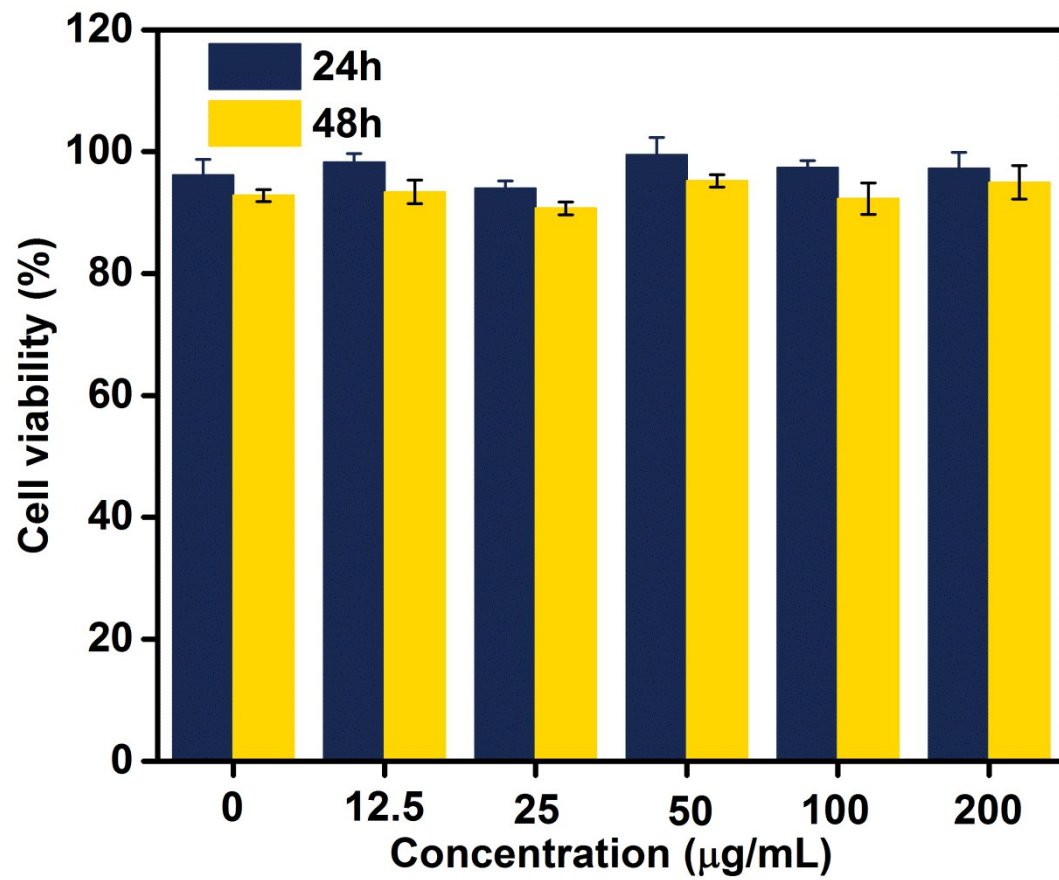


Figure S8. Cell viability of L929 cells incubated with ZZ for 24 h and 48 h at different concentrations.

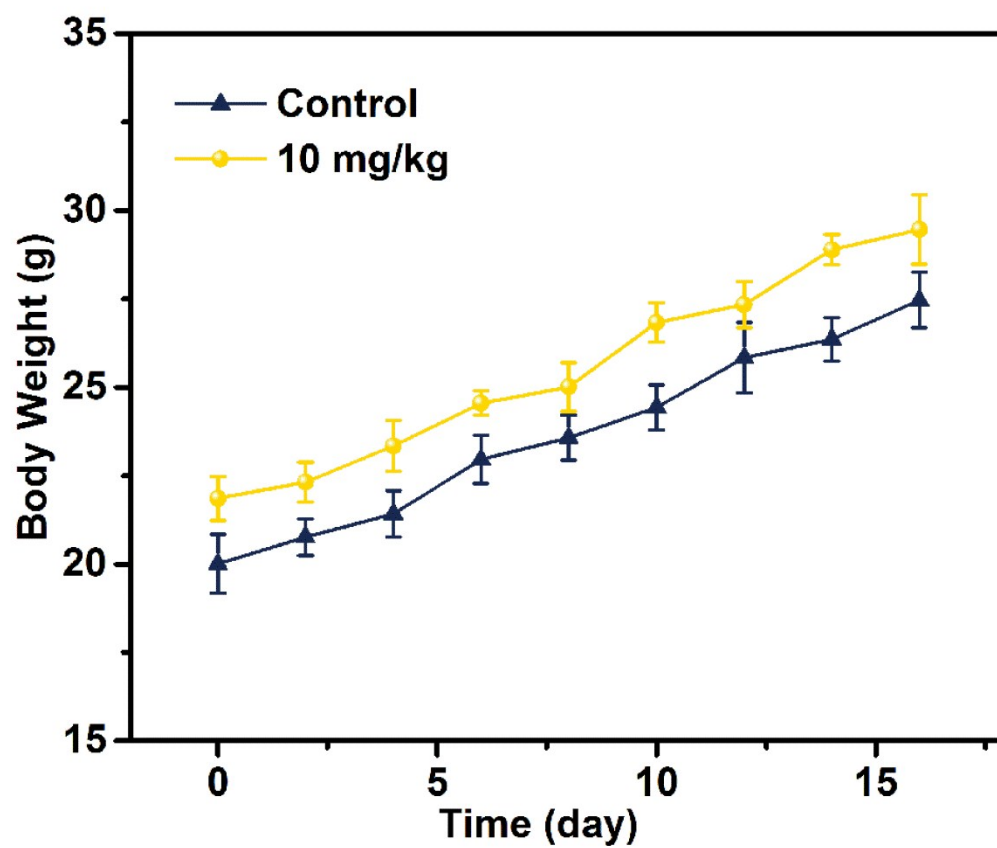


Figure S9. Body weight of mice treatment with PBS and FZZ doses of 10 mg kg⁻¹.

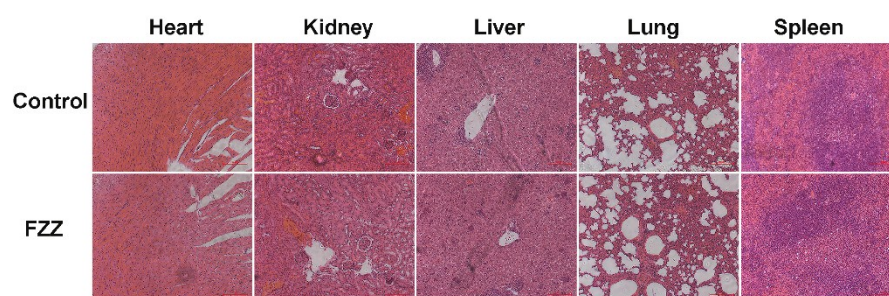


Figure S10. HE stained images of main organs of normal mice at 15 days after intravenous injection of PBS and FZZ (10 mg kg⁻¹).

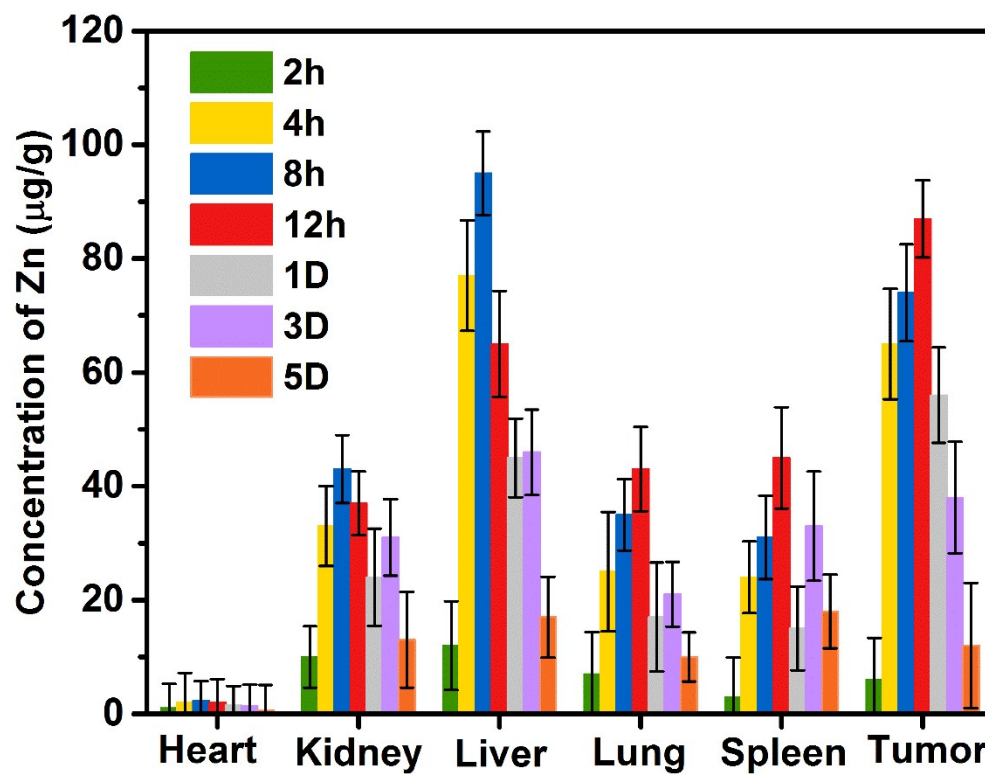


Figure S11. The in vivo biodistribution of Zn after injected with FZZ at different time.

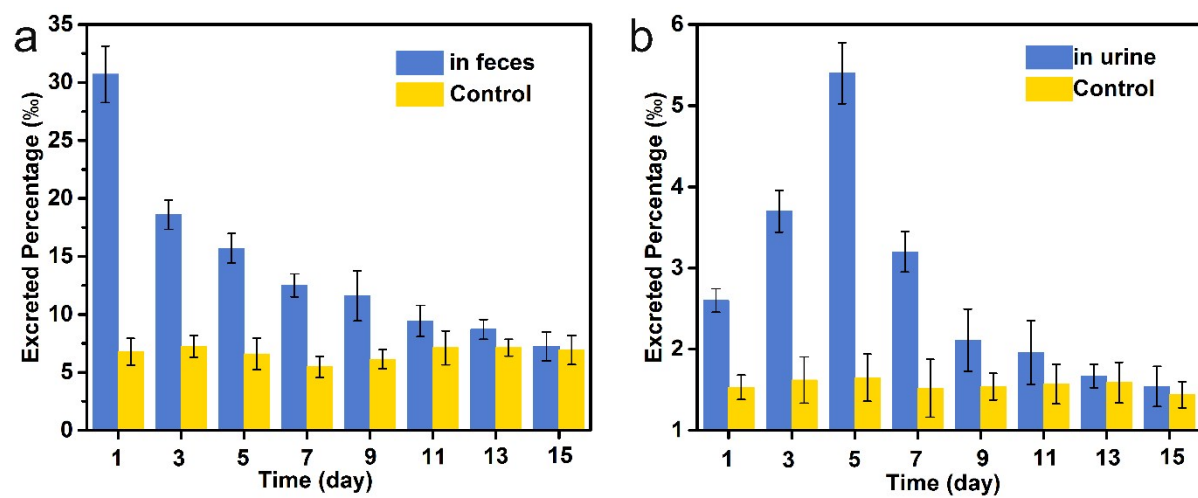


Figure S12. Accumulated Zn excretion out of the body after the intravenous injection of FZZ for different periods with normal saline as control: (a) in feces (b) in urine.

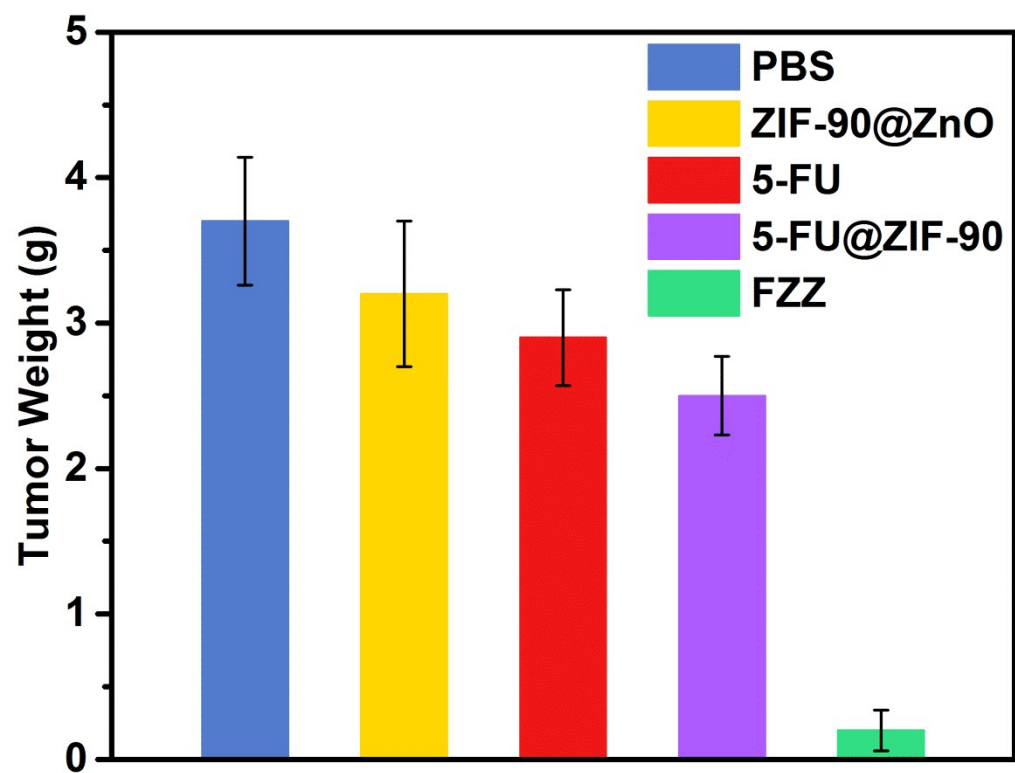


Figure S13. Tumor weights harvested by mice at the end of various treatments.

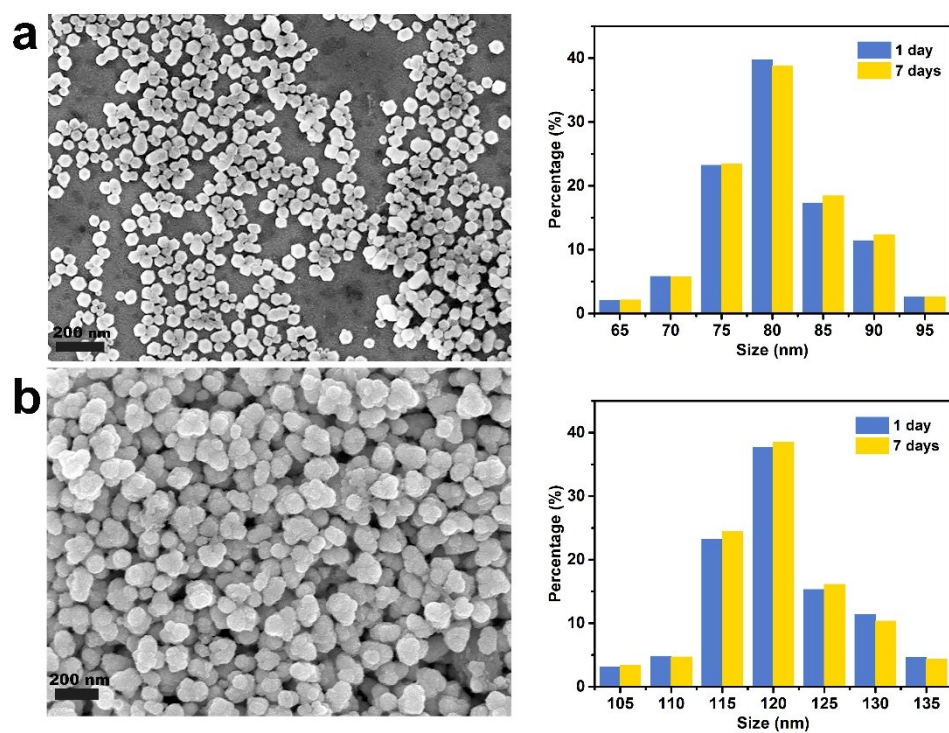


Figure S14. The size distribution (1 day and 7 days) and SEM images (7 days) of (a) ZIF-90 and (b) FZZ nanoparticles dispersed in PBS solution for different days.

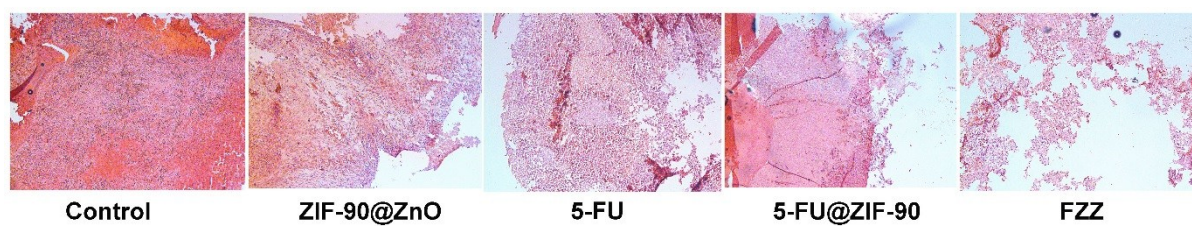


Figure S15. HE staining in tumor site in each group after different therapy.

Table S1. The total pore volume (V_{total}) and Brunauer-Emmett-Teller (BET) surface area (S_{BET}) of ZIF-90, FZ and FZZ nanoparticles.

Sample	S_{BET} (m^2g^{-1})	V_{total} (cm^3g^{-1})
ZIF-90	1082.2	0.54
FZ	32.71	0.07
FZZ	81.3	0.27

Table S2. Inductively coupled plasma (ICP) quantitative study of the internalization process of FZZ (100µg/mL) nanoparticles for three times. The results are expressed as the mass of Zn²⁺ ions per 10⁵ cells.

Time (h)	Zn ²⁺ uptake (ng/10 ⁵ cells)		
0.5	93.35	127.74	84.53
1	182.48	196.84	148.68
4	253.56	272.39	252.70
8	304.68	318.95	307.38
12	369.14	354.42	376.94
24	386.67	427.55	406.85

Table S3. Blood test parameters of mice of PBS control group and the FZZ treatment group (1 day, 7 days, 14 days). Including liver function indicators include alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST), as well as renal function indicators, blood urea nitrogen (BUN) and creatine (CRE).

	Reference Range	PBS Control	FZZ		
			1 day	7 day	14 day
BUN (mmol/L)	20±13	21.06±11. 43	22.78±8.94	19.46±10. 01	24.68±7. 83
CRE (mmol/L)	31±13	33.86±3.4 6	39.63±4.67	28.73±4.3 5	38.47±5. 62
ALT (IU)	63±20	64.37±5.8 2	70.60±21.36	73.47±14. 78	69.04±5. 56
AST (IU)	154±87	121.54±1 5.82	143.58±22.3 1	150.74±2. 76	149.47±4 .84
ALP (IU)	187±78	210.92±4. 56	190.45±17.8 5	194.57±6. 91	225.48±6 .74