Supplementary information

Injectable Leonurine Nanocrystals-loaded Microspheres for Long-term Hyperlipidemia Management

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Supplementary experimental

Similarity factor (f_2) analysis: Comparison among the dissolution profiles of selected formulations was performed by calculating the similarity factor (f_2):

$$f_2 = 50\log\left\{\left(1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right)^{-0.5} \times 100\right\}\#$$

where n is the sampling number, T_t and R_t are the percentages of release for the test and reference group at each time point t. f_2 factor is 100 when the test and reference profiles are identical, and approaches 0 as the dissimilarity increases.

Drug release mechanism exploration: The weight fraction of drugs released with time follows a power law relationship. For all groups, % cumulative drug release (% M) was fitted to the following kinetic equations: (i) Ritger-Peppas: plotted as log of % M versus log time, (ii) Zero order: plotted as% M versus time, (iii) First order: plotted as log % M retained versus time, and (iv) Higuchi, plotted as % M versus square root of time, the corresponding equation was listed as follows:

Ritger-Peppas	$\dfrac{M_\infty}{M_t} = at^n$	(ii)
First order	$M_\infty = M_t \mathbb{e}^{-k_1 t}$	(iii)
Zero order	$M_t = K_0 t$	(iv)
Higuchi	$M_t = k_H\sqrt{t}$	(v)

where M_t is the cumulative amount of drug released at time t, M_∞ is the total amount of drug in the matrix, k_0 is the zero-order rate constant, k_1 is the first-order release constant, and k_H is the Higuchi model-based release constant. The regression coefficient (R^2) values obtained in various models were compared to get the most fitted kinetic model. The results were listed in **Table S1**.
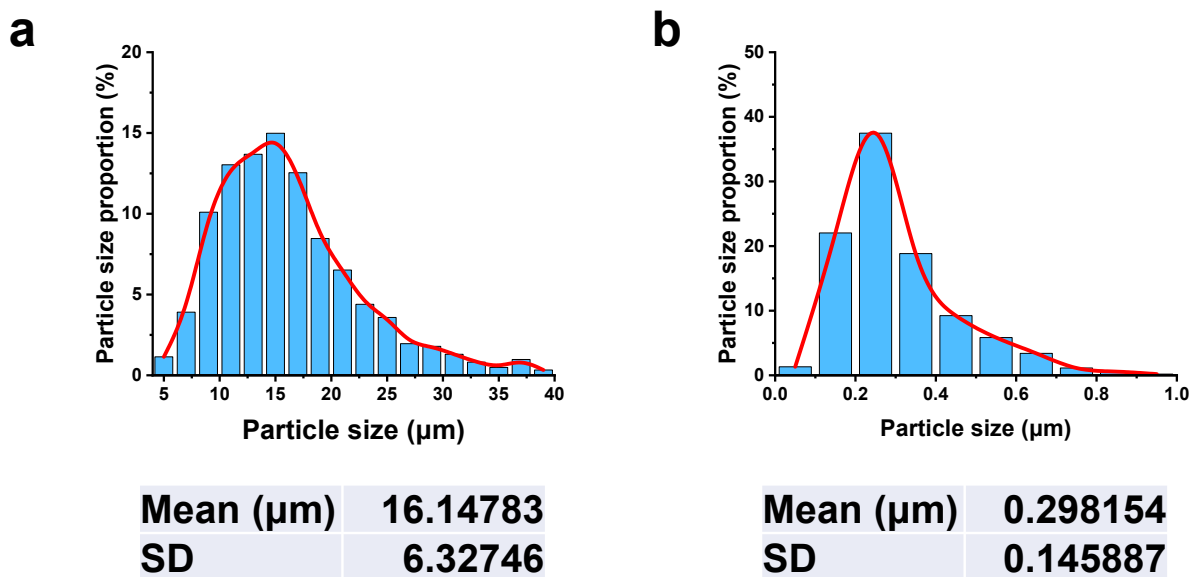
| Mean (µm) | 16.14783 |
| SD | 6.32746 |

| Mean (µm) | 0.298154 |
| SD | 0.145887 |

Fig. S1. Size distribution of Leo-micro and Leo-nano quantified by Image J.
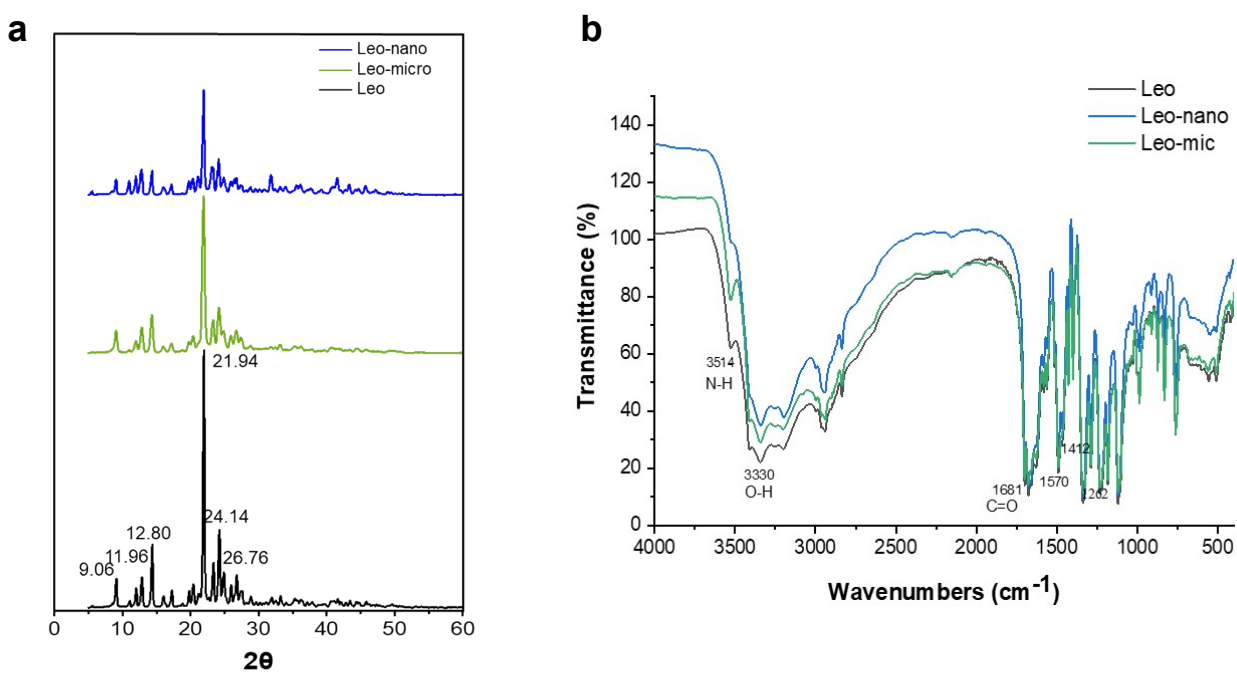

Fig. S2. (a) XRD and (b) FT-IR spectra of Leo, Leo-micro, and Leo-nano.
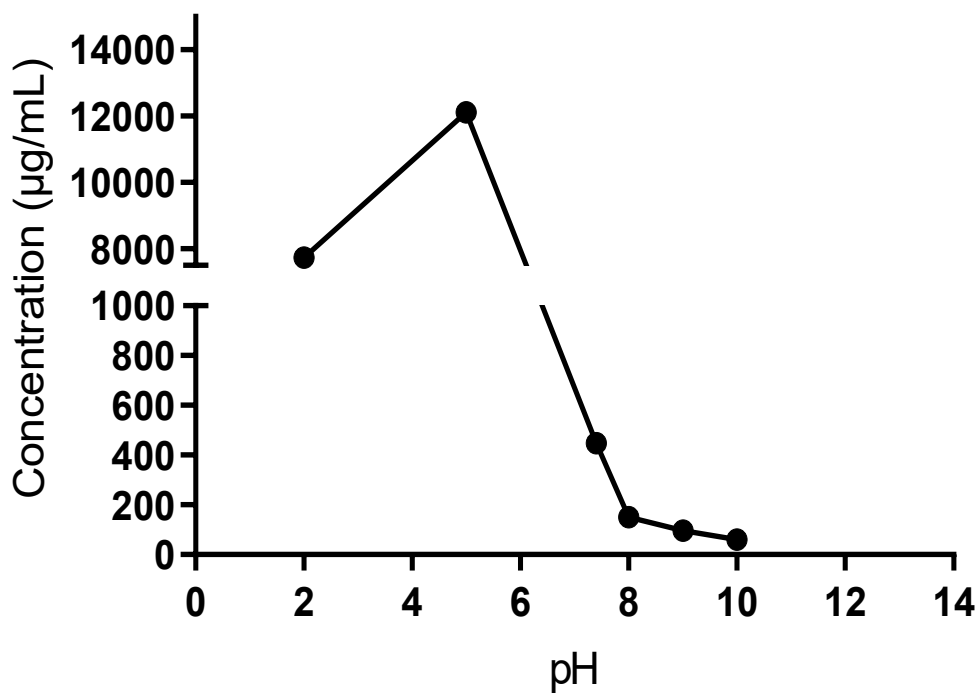
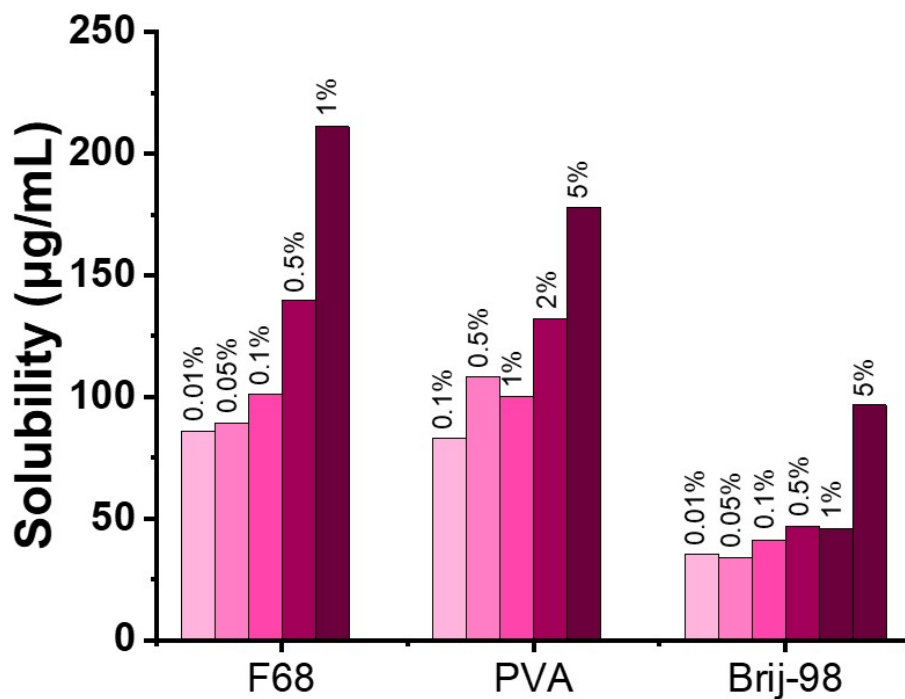**Fig. S3.** pH dependent-solubility of Leo in aqueous media.

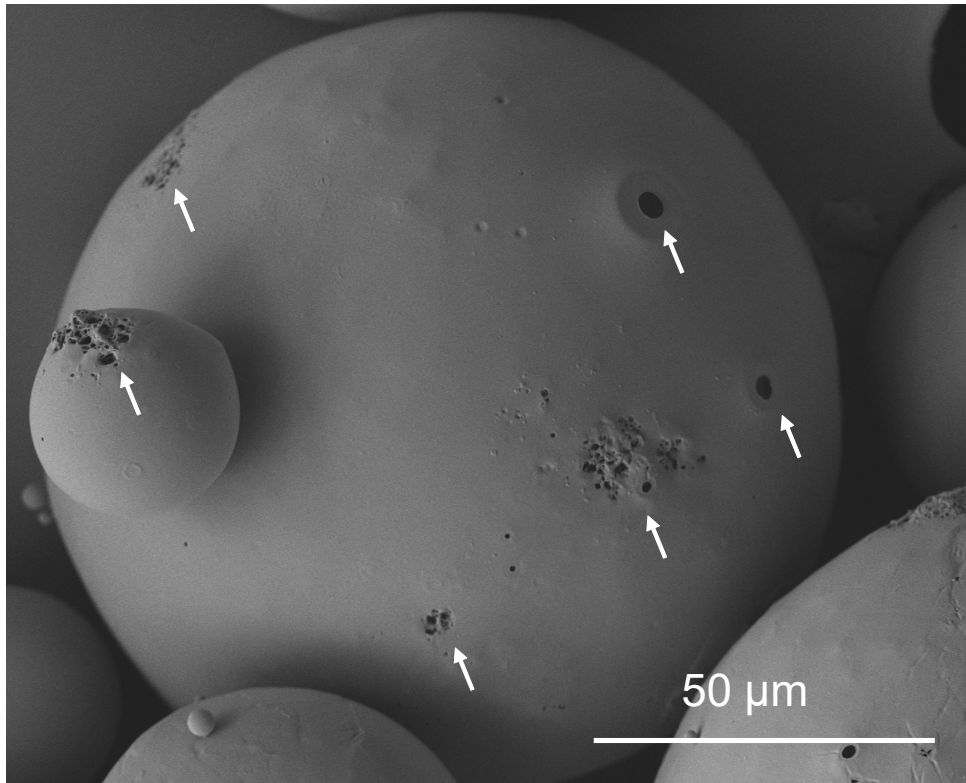**Fig. S4.** Impact of surfactant type and concentration on the solubility of Leo in aqueous media.

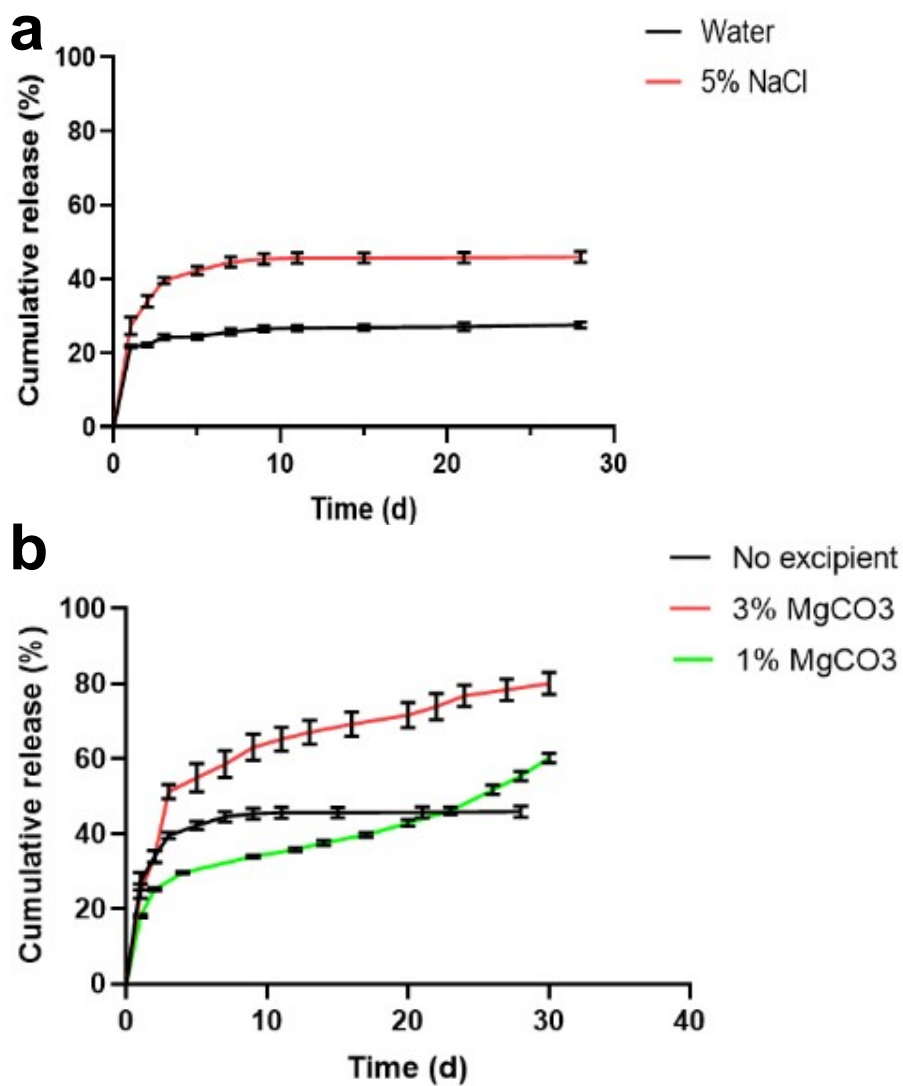**Fig. S5.** SEM image of Leo-nano loaded microspheres prepared without additives.

Fig. S6. Preparation of Leo-micro loaded microspheres. Impact of (a) osmotic pressure and (b) $MgCO_3$ on drug release kinetics. Data were presented as mean ± SD, n = 3.
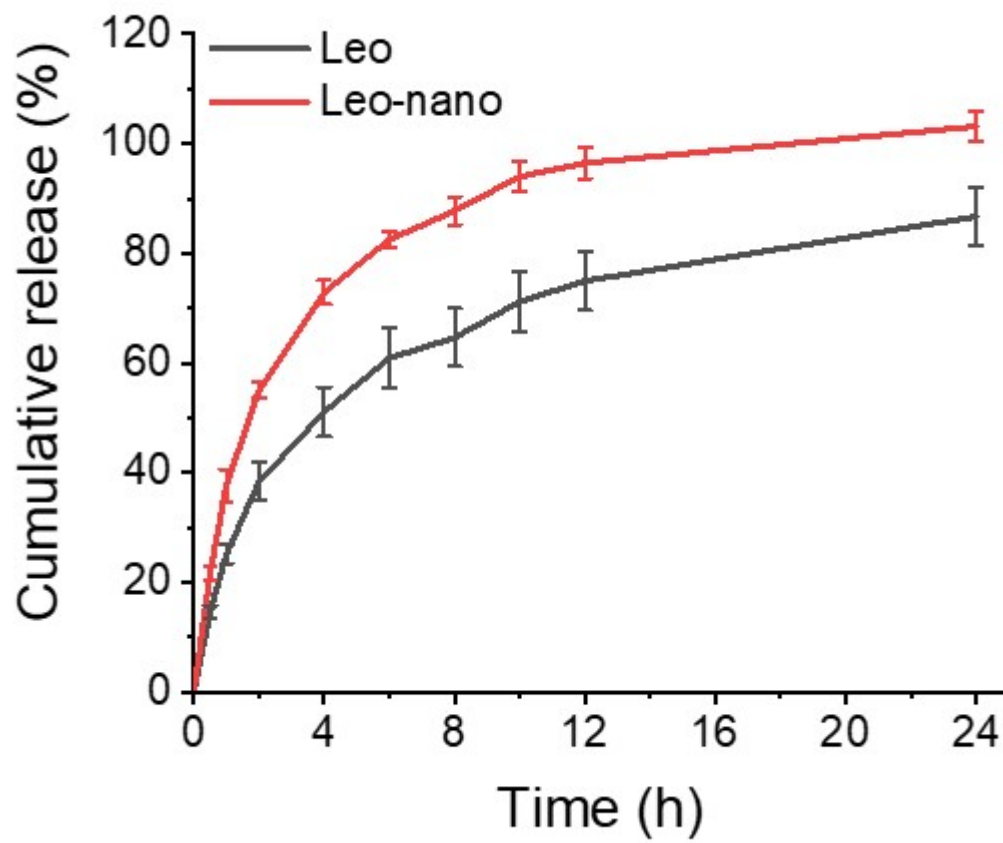
Fig. S7. Drug release profile of Leo and Leo-nano in PBS (10 mM, pH = 7.4). Data were presented as mean ± SD, n = 3.
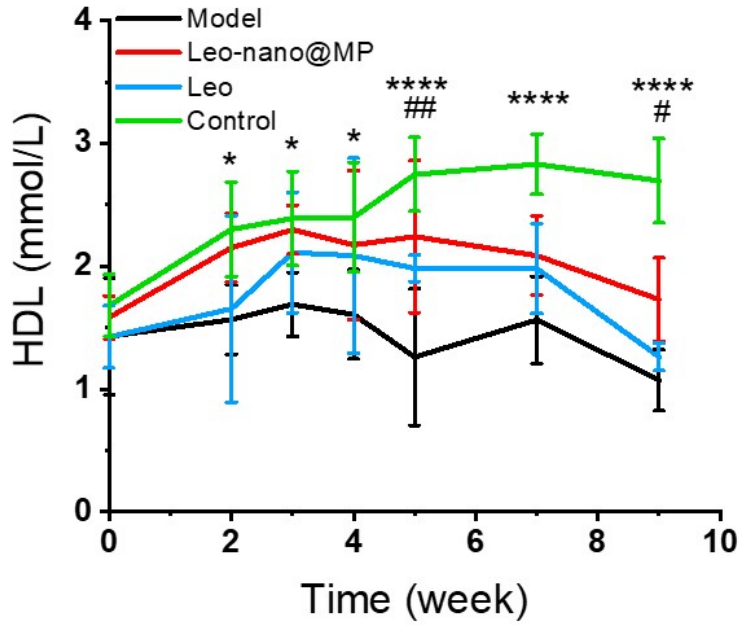
Fig. S8. HDL levels of HFD-fed rats with different treatments. Data were presented as mean ± SD, n = 6. The variance between model and model (*) and Leo-nano@MP (#) group was determined by one-way ANOVA with Dunnett's post-hoc test (*p <0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; #p <0.05, ##p < 0.01).
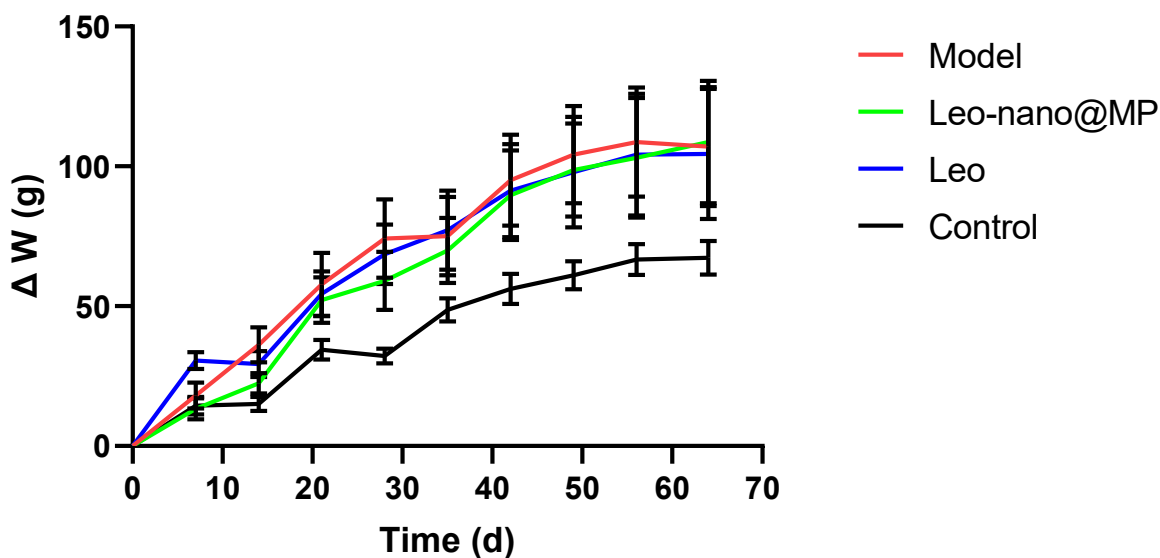
Fig. S9. Weight changes (ΔW) of rats after different treatments. Data were presented as mean ± SD, n = 6.

Table S1. Drug content after milling (n = 3)

Group	Drug content (%)
Leo-nano	100.1 ± 1.2
Leo-micro	99.8 ± 0.7

Table S2. Model Fitting of the drug release profile

Group	Equation	R^2	n
Leo-nano@MP (NO additives)	$Y=6.11X^{0.38}$	0.9745	0.38
Leo-nano	$Y=99.27X^{0.45}$	0.9502	0.45