

Supplementary information

HPLC analysis of serum and tissue samples

Mice were sacrificed at 8,24,48 and 72h after administration of single dose of free and hydroxycitrate exoformulation and tissues including liver, lungs, kidney, spleen, and heart were removed. The tissues were harvested, followed by quick washing with cold saline and homogenized followed by centrifugation at 15000g for 30 min at 4°C. For preparation of protein free filtrates an equal volume of 10% trichloroacetic acid was added to the serum/homogenized tissue supernatants. This mixture was then centrifuged at 20,000 g for 20min at 4°C to obtain protein free supernatant. The clear supernatant was used for drug analysis by HPLC. The pharmacokinetic parameters like Area under the curve (AUC), Maximum serum drug concentration (C_{max}), Half-life ($T_{1/2}$) and Mean retention time (MRT) were determined from the mean drug concentration time profile curve. C_{max} , T_{max} values were estimated from the concentration-time profile curve and area under the concentration- time curve (AUC) was calculated by the trapezoidal rule.

Immunohistochemistry

Sections were mounted on albumin coated slides and dewaxed in xylene and dehydration was done by incubating for 5mins in graded alcohol solutions. Pretreatment or antigen retrieval was done according to the manufacturer's protocol. Blocking was done with 5% skim milk in tris buffered saline for 60mins to prevent non-specific binding of antibodies, followed by three washings with TBS at room temperature and then the primary antibody (TTF-1) incubation was done for 1h at room temperature. Afterwards, sections were incubated with secondary antibody for 30 mins and then Di-amino benzidine (DAB) solution was added for 1-5min (till brown color developed) followed by several washings with distilled water. Next, Mayer's hematoxylin

was added as a counter stain followed by few washings with water and after dehydration with alcohol, the sections were mounted with DPX for visualization under light microscope (30).

Histopathological analysis of mice tissues

In addition to the lung tissue, other vital organs such as liver and kidney were also examined for histological changes (figure S1). In the free potassium hydroxycitrate group, the regional portal vein obstruction caused sinusoidal dilatation and congestion in the liver tissue while interstitial inflammation was observed in the kidney tissue, although it had normal glomeruli and tubular architecture. These effects were not seen in the exoformulation treated group.

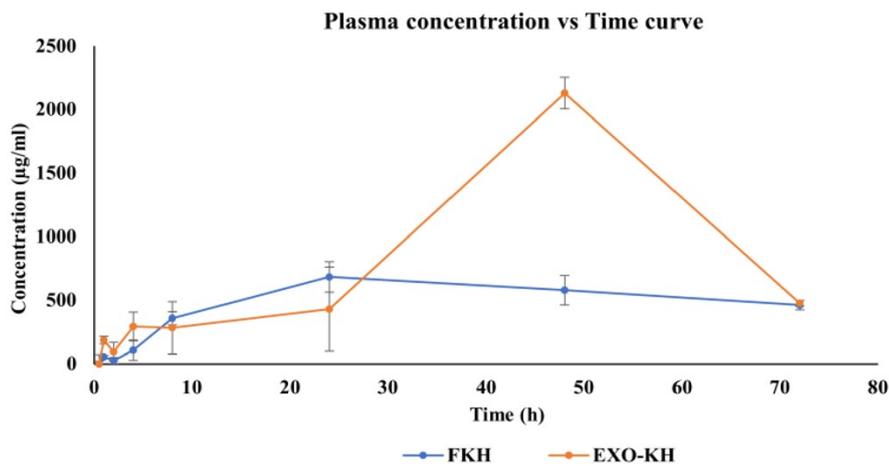


Figure S1: Plasma vs time curve of free potassium hydroxycitrate and its exoformulation.

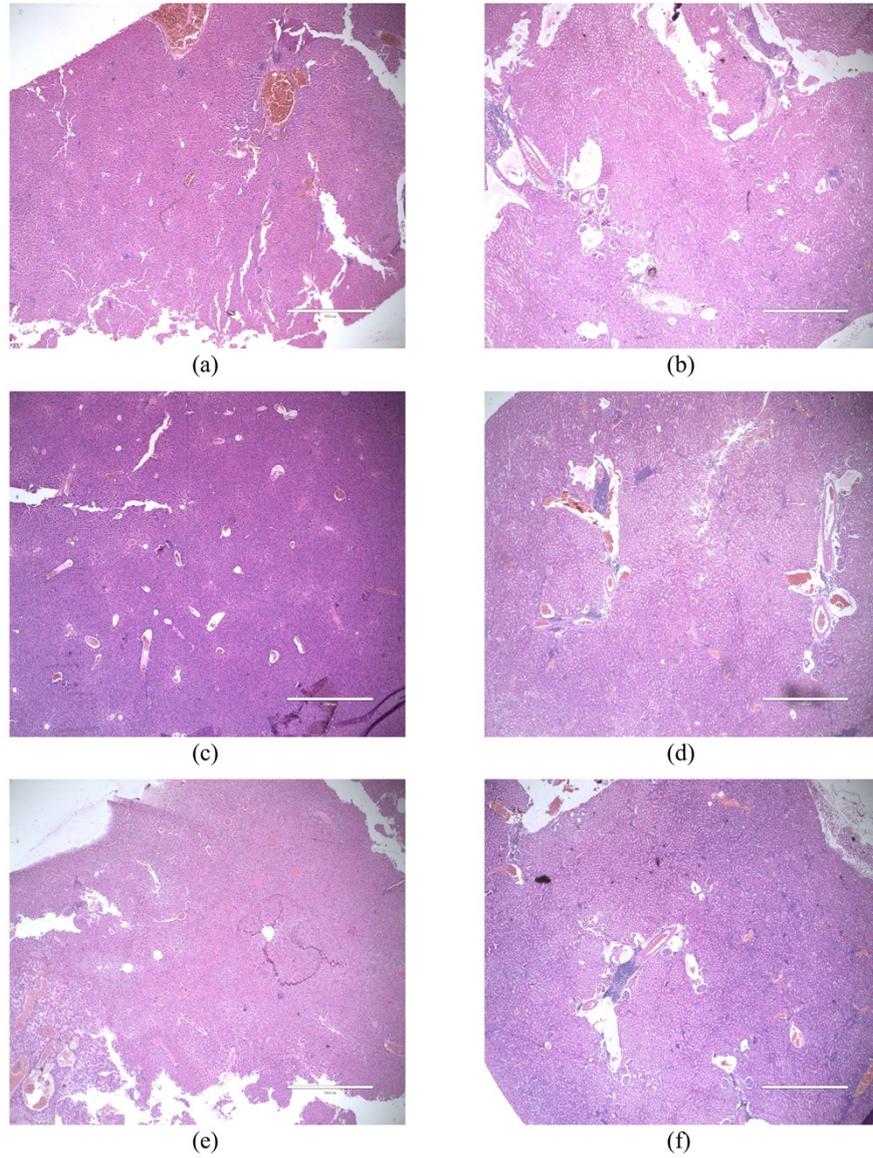


Figure S2: Representative H&E images of (a) untreated liver (b) untreated kidney (c) FKH liver (d) FKH kidney (e) EKH liver and (f) EKH kidney.

Western blot images for Exosome marker (Alix)

I. Alix (90-100kda)

