2.2.9.2 Rat perfusion for liver isolation

Male Sprague Dawley rat was anaesthetized **(section 2.2.6)** and the abdominal contents were displaced to the animal's left to expose the liver. Hepatic portal vein (HPV) was exposed and two loose ligatures were passed, one around the PV, while the other was around the inferior vena cava (IVC). A 18-gauge angiocath (Becton Dickinson, Mountain View, CA, USA) was inserted into the HPV, whereas the perfusate tubing was connected to the needle and the infusion was initiated with pre-warmed 37 °C Perfusion buffer I. Once the liver was blanched to a light-brown color and all lobes began to swell a cut at IVC was made to allow buffer efflux. Thereafter, rat's chest was cut to place a second cannula of 18-gauge connected to a soft tube into the vena cava above the liver in order to enable a recirculating system. All loose ligatures were tied securely.

Perfusion solution was switched to perfusion buffer II plus collagenase II, and the flow rate was increased to 25 ml/min and the liver became pale in color. The recirculating perfusion mode with collagenase solution lasted for 15 minutes. Later, the liver was dissected. Once the liver looked mushy, it was minced and placed in pre-chilled sterile beaker with 20 ml collagenase to be transferred to the tissue cell culture hood.

2.2.9.3 Hepatocyte cell isolation

Within the cell culture hood, liver cells were dispersed gently using cell scraper (Fisher Scientific Ltd., Loughborough, UK) in a sterile petri dish containing collagenase solution. Cell suspension was filtered and dispensed through a 100 µm cell strainer (Becton Dickinson, Mountain View, CA, USA) into a centrifugation tube to remove connective tissues and undigested tissue fragments. Later, cells were suspended in 40 ml