2.2.7 *In Situ* Single-Pass Intestinal Perfusion (SPIP)

2.2.7.1 Washing and perfusion solution preparation

All drugs used in SPIP experiment (GlcN, cimetidine, and rifampin) were dissolved in normal saline solution (NaCl, 9 mg/ml). Washing solution was prepared by dissolving GlcN, cimetidine or rifampin (according to the experiment) in saline solution to obtain a concentration of 10, 1, and 2 mg/ml, respectively. Perfusion solution was prepared by dissolving PRN in saline solution to give 1 mg/ml concentration together with GlcN or cimetidine or rifampin to obtain a final concentration of 10, 1, and 2 mg/ml, respectively.

2.2.7.2 Intestinal perfusion

A fasted female non pregnant rat was anaesthetized and its intestine was exposed surgically as described in **(section 2.2.6)**. A semi-circular incision was made and an inlet silicon tube (0.3 cm diameter) was inserted into the duodenum 4 cm away from the pylorus. A second incision was made in the ileocecal end and an outflow silicon tube was fitted. Both tube ends were tied securely using surgical suture (Atramat®, Mexico City, Mexico) and linked with suitable tube fittings and inserted in the peristaltic pump at a flow rate preset to 3.6 ml/min. Initially, intestinal segments were rinsed with pre-warmed (37 °C) saline washing solution for 20 min until the outlet solution was clear then perfusion solution was switched. A pre-sample drug was drawn from perfusion solution as well as zero-time blood sample was drawn from rat's tail before perfusion solution was switched. Perfusion solution was perfused for 60 min and blood samples were quantitatively pooled from rat's tail at different time intervals namely; at 10, 20, 30 and 60 min. All blood samples were left to clot, centrifuged for 10 min at 14000 rpm, then serum was separated,