150 µl of Rh-insulin solution (F.C. = 2 IU/ml) was injected as a single dose through the perfusion buffer. The total effluent from the liver will be collected by fraction collector at 5 sec intervals, the sample collecting will start at the same time of insulin dose injection. Then samples will be frozen and prepared for measuring the insulin levels and its hypoglycemic effects (Sahin & Rowland, 2000).

2.3.8. Evaluation the effect of different flow rates on insulin metabolism in liver

In order to study the effect of using different flow rates on insulin metabolism, *in situ* liver perfusion technique on normal and diabetic livers was performed. The experiment was carried out with two different flow rates, namely 5 ml/min and 1 ml/min on the same liver. The surgical procedure was the same as that described previously (section 2.3.7.). Briefly, after induction of anesthesia, cannulation and perfusion Krebs-bicarbonate buffer (pH=7.2) for 10 min, the liver was perfused in a single-pass mode with the first 25 ml of Rh-insulin solution (80 mU/ml) at 5 ml/min flow rate. The total outflow was collected via tubing in the first bottle. After washing the liver with perfusion Krebs-bicarbonate buffer for 10 min, the second perfusion of 25 ml insulin solution (80 mU/ml) was started at a flow rate of 1 ml/min. The effluent was pooled in the second bottle to freeze-dry. All operative procedures were completed within 40 min without interruption of flow to the liver.

To obtain a pharmacological action of outflow perfused insulin, the two bottles were freeze-dried and stored at 4°C until used. The freeze-dried insulin powder from each bottle was dissolved in 2 ml distilled water and subcutaneously injected to number of normal rats (n=8). Blood glucose level was measured from tail vein by glucometer at 0, 15, 30, 60, 90 and 120 min.