2.3.7. Evaluation of hepatic first pass metabolism of insulin by *In situ* liver perfusion method

Male Sprague-Dawley rats weighing 200-250 gm were fasted for 24 h before use. *In situ* liver perfusion experiment was conducted on normal and diabetic rats. The apparatus of perfusion were as previously described. Rats were anesthetized with 2ml halothane using a small animal anesthesia system; the rats were restrained in a supine position on aboard which was kept at surface temperature of 37 °C.

A small midline incision was made in the abdomen. The hepatic portal vein was exposed by carefully moving the abdominal contents to the animal's right. Loose ligatures will be placed around the portal vein ensuring exclusion of the hepatic artery and the second ligature around the inferior vena cava (IVC) beside the left kidney (Figure 2.4). The portal vein will be cannulated with 16 GA catheter and perfusion start at a flow rate of 13 ml/min, using peristaltic pump.

(Figure 2.4): Ligation of the portal vein and the inferior vena cava (IVC) beside the left kidney.