

The beaker was oxygenated with 95% O₂ and 5% CO₂ for 1 min. The sacs were then transferred into two beakers on water bath constant temperature oscillator (SHA-B, Everich Corporation, Miami, USA) at 37°C. The first beaker contains the oral insulin formula (F.C. = 7 IU/ml) and the other beaker contains Rh-insulin solution (F.C. = 1 IU/ml). The sampling can be done at different time intervals to measure insulin levels. Equal number of sacs was removed from the two beakers at 20, 40 and 60 min. These sacs were emptied and the serosal fluid from the sacs was used for the analysis and measurement of insulin level by Elecsys 2010 analyzer.

Everted gut sac experiment was performed on normal and diabetic rats.

2.3.6. Evaluation the intestinal absorption of insulin-loaded nanoparticles by *in situ* intestinal perfusion technique

After an overnight fasting, normal and diabetic male Sprague-Dawley rats were anesthetized with 2ml halothane using a small animal anesthesia system, then placed on a board under a surgical lamp to maintain body temperature. Upon verification of the loss of pain reflex, a midline abdomen incision of 3-4 cm was made and the small intestine was exposed. The intestine (length 80 ± 5 cm) was isolated and cannulated at both ends with silicon tubing (internal diameter 2 mm). Both cannulas were secured with surgical silk sutures (Figure 2.3). The isolated intestine was perfused with physiological saline (0.9% w/v NaCl) to clean any residual debris until the out flowing became colorless and drops of saline were added to the surgical area then covered with wet gauze to avoid loose of fluid. Care was taken to handle the small intestine gently in order to maintain an intact blood supply. The experiment was