2.3.3. Preparation of Krebs- bicarbonate buffer

A simulated physiological solution, Krebs-bicarbonate buffer was prepared by the mixing the following volume (in ml) of 1 M: NaCl 118, KCl 4.5, $MgSO_4$ 1.6, $NaHCO_3$ 25, KH_2PO_4 1.2, Glucose 5.5 and $CaCL_2$ 2.5, complete the volume to 1 L by distilled water. The buffer was oxygenated with carbon gas (95% O_2 + 5% CO_2). However, $CaCl_2$ was added after oxygenation to prevent $CaCO_3$ formation. The pH was adjusted to 7.2 using 1M NaOH.

2.3.4. Evaluation of pharmacological activity of insulin-loaded nanoparticle preparation

STZ-diabetic and normal rats were used to evaluate the hypoglycemic effects of, freshly formulated, oral insulin-loaded nanoparticles. The rats were considered to be diabetic when the fasted glucose levels exceeded 220 mg/dL after STZ treatment. Rats were fasted for 12 h prior to the experiment with free access to water. The total duration of the experiment after dosing was 18 h. The animals were fasted from food, but had free access to water during the first 9 h, while in the second 9 h the animals had free access to both water and food. Diabetic rats were separated randomly and divided into three groups for the antidiabetic study. Also the normal rats were separated into two groups.

Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
(Diabetic rats n= 10 rats)	**(Diabetic rats n=10 rats)**	**(Diabetic rats n=10 rats)**	**(Normal rats n= 7 rats)**	**(Normal rats n= 7 rats)**
Oral insulin in a dose (50 IU/Kg)	**SC insulin in a dose (1 IU/Kg)**	**Oily placebo**	**Oral insulin in a dose (50 IU/Kg)**	**SC insulin in a (dose 1 IU/Kg)**