3. Objectives and plan of work

3.1. Objectives

Objectives of the present study are as follow:

I. To investigate the anti-diabetic potential of TMP, using HFD-STZ-induced T2D rat model, and to examine the role of PI3K/Akt pathway in the anti-diabetic mechanism of TMP.

II. To explore the protective effect of TMP on DN, using STZ-NCT-induced T2D rat model, and to identify the role of Akt signalling pathway and oxidative stress in providing good therapeutic resolution for DN treatment.

3.2. Detailed research plan

Study I

1. Firstly the research problem was identified then the study was hypothesized, objectives were determined and all the drugs, chemicals and animals were arranged.
2. Induction of T2D through HFD and STZ in rats.
3. Treatment of diabetic rats with TMP (100, 150 and 200 mg/kg/day) for 28 days through intragastric gavage.
4. Evaluation of Biochemical parameters:
    a. Body weight
    b. Fasting blood glucose (FBG)
    c. Fasting serum insulin (FSI)
    d. Homeostatic model assessment-Insulin resistance (HOMA-IR)
    e. Homeostatic model assessment-β-cell function (HOMA-B%)
    f. Oral glucose tolerance test (OGTT)

g. Insulin tolerance test (ITT)

h. Glycosylated haemoglobin (HbA1c)

i. Lipid profile

5. Estimation of pro-inflammatory cytokines: Interleukin-6 (IL-6) and C-reactive protein (CRP).
6. Evaluation of molecular mechanism of tetramethylpyrazine in the treatment of T2D through western blot analysis and real-time polymerase chain reaction (RT-PCR).

Study II

1. Firstly the research problem was evaluated then the study was hypothesized, objectives were determined and all the drugs, chemicals and animals were procured.
2. Induction of T2D through STZ-NCT in rats.
3. Treatment of diabetic rats with TMP (100, 150 and 200 mg/kg/day; p.o.) for 56 days.
4. Evaluation of biochemical parameters:

   a. Body weight

   b. FBG

   c. FSI

   d. OGTT

   e. HbA1c

   f. Lipid profile

5. Estimation of oxidative stress parameters:

   Superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px).

6. Evaluation of renal biochemical markers:

   Blood urea nitrogen (BUN), serum creatinine (SCR) and 24 h urinary protein.

7. Assessment of renal morphology through histopathological analysis.

8. Evaluation of molecular mechanism of TMP in DN treatment through western blot.