Chapter 1

INTRODUCTION

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder which is identified by fasting and postprandial hyperglycaemia, and hyperlipidaemia, resulting from a deformity in carbohydrate, fat and protein metabolism [1, 2]. DM is a disorder that produces its effect on the people of all age groups in the whole world. Type-1 diabetes (T1D) is caused by a decline in the release of insulin from the pancreatic β-cells [3], while type-2 diabetes (T2D) is recognised as decreased insulin sensitivity and gradual dysfunction of β-cells, and it accounts for more than 90% of all diabetic cases. Insulin resistance development leads to a decrease in response of tissues to the circulating insulin. Initially, there is a rise in the insulin secretion to normalise blood glucose level that ultimately causes chronic overstimulation and destruction of β-cells which results in an insufficiency of insulin secretion and remarkable hyperglycaemic condition. Therefore, alleviation of insulin resistance is very essential in the prevention and treatment of T2D [4, 5]. The T2D induction by a high-fat diet (HFD) and the low-dose streptozotocin (STZ) combination in rats produces the condition identical to the late-stage T2D in humans [6]. Diabetic nephropathy (DN) is a leading complication of DM, also known as diabetic kidney disease, and it is the most predominant factor responsible for the end-stage renal failure [7, 8]. DN is usually recognised by renal hypertrophy, mesangial cells proliferation, mesangial matrix accumulation, glomerulosclerosis and proteinuria [9]. Whereas, the specific molecular mechanisms mainly involved in the pathogenesis of DN are not well established.

The treatment of T2D is a very challenging task still as the presently available conventional drugs like sulfonylureas, biguanides, thiazolidinediones, α-glucosidase inhibitors and dipeptidyl peptidase-4 (DPP-4) inhibitors are associated with various side effects such as hypoglycaemia, nausea, weight gain, diarrhoea, hypersensitivity,

gastrointestinal discomfort, heart and liver failure [10]. Above established medication, few scientists reported that products obtained from the medicinal plants have anti-diabetic potential with minor toxicity and side-effects [11]. Presently, in developing countries, naturopathy is broadly appreciated as there the resources are insufficient for proper medication, and the price of regular medicines is also too high [12, 13]. Therefore, the compounds derived from natural sources may be a better therapeutic alternative over conventional agents for the treatment of diabetes. One of the predominant complications of diabetes is DN, and there are various factors involved in the pathophysiology of this complication. There is growing evidence that oxidative stress plays a major role in the pathogenesis of DN [14]. The renal apoptosis is also responsible for the development of DN, and the tubular epithelium apoptosis is a reasonable aspect of this complication. The hyperglycemic condition aggravates the free radicals generation leading to oxidative stress in the cells of the nephron tubule [15]. Therefore, in the prevention and treatment of DN, the amelioration of apoptosis and oxidative stress is essential [16, 17] and the agents having anti-apoptotic, anti-hyperglycemic and anti-oxidative properties may prove to be a better agent for DN treatment.

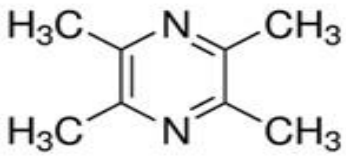

Figure 1.1: Chemical structure of Tetramethylpyrazine.

A compound tetramethylpyrazine (TMP; $C_8H_{12}N_2$) (**Figure 1.1**) is chiefly isolated from the rhizomes of the well-known traditional medicinal plant *Ligusticum chuanxiong*. TMP, sedanoic acid, chrysophanol, Ferulic acid, alkaloid and essential oils like ligustilide and butylphthalide are the active constituents of *Ligusticum chuanxiong*

[18]. TMP is the *Ligusticum chuanxiong's* vital active ingredient [19, 20]. Presently, TMP is well accepted for the stimulation of neurogenesis in the rat brain after focal ischemia and has also been employed in the cardiovascular disorder and stroke treatment from a long time in Oriental medicine [21]. One of the earlier experiments has shown that TMP induces differentiation in neurons as an outcome of TopoIIβ up-regulation through the PI3K/Akt/Sp1 signalling pathway [22]. The literature survey also shows that TMP could protect endothelial cells from the damage induced by elevated blood glucose level, through an increase in NO generation, reduction of reactive oxygen species production and up-regulation of Akt / eNOS phosphorylation [23].

The binding of insulin with insulin receptor on the cell surface stimulates the receptor's tyrosine kinase activity, that leads to the tyrosine phosphorylation of insulin receptor substrates (IRS-1 & 2), which in turn produces the activation of downstream effectors of this signalling pathway phosphatidylinositol-3-kinase (PI3K), PI3K-dependent kinase (PDK1) and later protein kinase B (Akt). Akt stimulated phosphorylation of 160 kDa Akt substrate (AS160) leads to the glucose transporter-4 (GLUT-4) translocation from the cytoplasm to the surface of the cell. The GLUT-4 in the plasma membrane boosts extracellular glucose uptake by the cell, which results in the fall of blood glucose level and improvement of insulin resistance induced diabetic symptoms [24, 25]. In skeletal muscle, glucose uptake is mainly produced by an insulin signalling pathway through PI3K and Akt activation [26]. In humans, greater than 70% serum glucose removal occurs through glucose uptake by skeletal muscle [27]. Since the TMP is having PI3K activating potential and the activation of PI3K/Akt signalling is responsible for GLUT-4 translocation on the plasma membrane resulting in the reduction of blood glucose level and alleviation of diabetic complications, thus TMP should exert its therapeutic effect in amelioration of T2D through the stimulation of

PI3K/Akt signalling. However, till now no study was carried out on the evaluation of the role of PI3K/Akt pathway in TMP mediated T2D treatment thus, the present study was conceptualised.

Akt pathway is a predominant signalling pathway that restrains apoptosis and stimulates cell proliferation [28]. Obstruction in Akt expression is essential for DN progression, and various signalling pathways have been involved in reducing the expression of Akt in DN. Therefore, the Akt signalling activation may be a main therapeutic goal for the cure of DN [29]. Hyperglycemia is also a well-recognised factor involved in chronic complications of diabetes mellitus such as DN. It produces excessive free radicals and can inhibit antioxidative machinery [30]. The outcomes of many experiments show that in the progression and development of DN, oxidative stress plays a major role and abnormal rise in reactive oxygen species level takes part in the DN pathogenesis [31, 32]. Thus, a compound showing both hypoglycemic and antioxidant activity might be recognised as a therapeutic agent against DN. It has also been identified that TMP might reduce the kidney damage caused by diabetes by down-regulating renal vascular endothelial growth factor (VEGF) expression [33]. Since TMP produces the activation of Akt signalling, reduces reactive oxygen species production and ameliorates hyperglycemic condition probably through PI3K/Akt/GLUT-4 signalling and the stimulation of Akt pathway through its anti-apoptotic action, reduction of oxidative stress and inhibition of hyperglycemia-induced free radical generation produces beneficial therapeutic effect in DN, therefore TMP should strive its therapeutic effect in DN through these signalling pathways. But, till now no experiment was carried out suggesting the role of Akt signalling, anti-hyperglycemic action and oxidative stress quenching in TMP mediated treatment of DN thus, the present experiment was speculated.

Based on this, we have hypothesised that TMP may produce an anti-diabetic effect by decreasing insulin resistance through the up-regulation of the expression of p-PI3Kp85/p-Akt/GLUT4. These factors also led to the hypothesis that TMP can ameliorate DN through the apoptotic inhibition by the activation of the Akt signal pathway and the reduction of oxidative stress by restricting hyperglycemia-induced free radicals generation and via direct obstruction of reactive oxygen species production. Thus, the current study was designed with an aim to investigate the anti-diabetic potential of TMP, using HFD – STZ - induced T2D rat model, and to explore the role of PI3K/Akt pathway in the anti-diabetic mechanism of TMP. Along with that, the present experiment was also conceptualised to identify the protective effect of TMP on DN using STZ-nicotinamide (NCT)-induced T2D rat model and to determine the competence of Akt signalling pathway and oxidative stress in providing a potential therapeutic target for DN treatment.