Diabetes is a metabolic disorder typified by persistent hyperglycemia and impaired glycemic control. Diabetes (both T1DM and T2DM) markedly alters the cardiac gene expression patterns of several metabolic, structural, signal transductions, stress response proteins, leading to the development of cardiac pathologies. Diabetic cardiomyopathy is characterized by alterations in the morphology and functions of the diabetic heart without hypertension or coronary heart disease (Rubler et al., 1972; Aneja et al., 2008). Perivascular and interstitial fibrosis is a distinctive feature of diabetic cardiomyopathy, and the heart weight correlates with the extent of fibrosis (Rubler et al., 1972; van Hoeven et al., 1990). Apart from the increased deposition of collagen, diabetes enhances the cross-linking of collagen fibers and leads to diminished ventricular compliance (Goldin et al., 2006). Hearts of diabetic patients demonstrated enhanced interstitial fibrosis and cardiomyocyte size compared with hearts of non-diabetic patients when biopsied at the time of coronary bypass surgery (Fischer et al., 1984). T1DM and T2DM animal models shown an enhanced cross-sectional area of cardiomyocytes with or without interstitial fibrosis (Li et al., 2010; Sakata et al., 2007). These alterations accelerate the susceptibility of the heart muscle to myocardial ischemia-reperfusion (IR) injury (Kandula et al., 2016; Li et al., 2013b), resulting in reduced clinical prognosis after myocardial infarction (Kim et al., 2013). Moreover, diabetic patients have a higher risk of developing myocardial IR than non-diabetic population (Hur et al., 2016). Although clinical trials reported that diabetic patients treated with reperfusion therapy demonstrated enlargement of infarct size (Alegria et al., 2007), preclinical experiments had shown the differential impact on infarct size due to diabetes induction. The discrepancy in results may due to multiple differences in the protocols employed; different plasma insulin levels (i.e. T1DM vs T2DM) and duration of diabetes. Two other experiments also supported that diabetic hearts demonstrated

increased resistance against ischemia/reperfusion injury during initial stages of diabetes; however, resistance was disappeared during later stages of diabetes (Ma et al., 2006; Xu et al., 2004). Marfella et al. (2002) demonstrated that cardiac infarct size after ischemia (25 minutes) followed by reperfusion (2 hours) was greater in streptozotocin-induced diabetic rats than that in non-diabetic rats (Marfella et al., 2002). A recent study showed that streptozotocin-induced diabetic rats exhibited enhanced cardiac oxidative stress and decreased antioxidant capacity, which rendered the diabetic heart more susceptible to IR injury (Li et al., 2013a).

Diabetes causes the deregulation of survival signalling pathways for myocardial protection. The substantial evidence demonstrated that phenomena like ischemic preconditioning or postconditioning lost their cardioprotective nature in diabetic condition (Bouhidel et al., 2008; Przyklenk et al., 2011). Furthermore, multiple survival signalling mechanisms have been deregulated in diabetic myocardium. For example, T2DM rats demonstrated suppressed janus kinase 2/phosphatidylinositol-3-kinase/Akt signalling via enhanced calcineurin activity and dysfunction of extracellular signal-regulated kinase (ERK)/glycogen synthase kinase 3β signalling through increased endoplasmic reticulum stress (Hotta et al., 2010; Miki et al., 2009). There is a limited data on whether glycemic control normalizes the deficient cardioprotective signalling pathways in diabetic hearts. Dextrose-induced acute hyperglycemia prevented the cardiac infarct size limitation by anaesthetic agents, ischemic preconditioning and mitochondrial K+ATP channel opener (Amour et al., 2010; Raphael et al., 2010), suggesting a promising role of hyperglycemia in diminishing survival pathways.

Adenosine monophosphate-activated protein kinase (AMPK) has stood out as a master regulator of metabolic energy; and is a cellular adaptive mechanism activated during the

times of metabolic stress to boost energy production and salvage the failing myocardium (Russell et al., 2004c). Of note, AMPK activation has been verified to confer cardioprotection against diabetic myocardial IR injury by limiting cardiac apoptosis through attenuation of endoplasmic reticulum stress (Russell et al., 2004c) and has a significant role in the improvement of mitochondrial biogenesis (Zong et al., 2002). Interestingly, long-term high fructose diet reduces AMPK activation in several tissues including skeletal muscles and adipocytes (Bonnard et al., 2008). Liver-specific stimulation of AMPK results in suppression of lipogenesis and hepatic triglyceride accumulation in mice fed with high fructose diet (Woods et al., 2017b). Furthermore, diminished cardiac AMPK activity is known to enhance the vulnerability of hearts to ischemia-reperfusion insult in fructose-fat fed rats (Axelsen et al., 2010). Recent studies reported that AMPK could suppress oxidative stress through stimulation of nuclear factor erythroid 2–related factor 2 (Nrf2)-dependent upregulation of heme-oxygenase (HO-1), and the significant crosstalk has been observed in *Caenorhabditis elegans* (Onken & Driscoll, 2010), mammalian inflammatory systems (Mo et al., 2014) and human endothelium (Liu et al., 2011). Moreover, AMPK activation improved cognitive deficit by enhancing peroxisome proliferator-activated receptor gamma coactivator (PGC-1α)-regulated mitochondrial biogenesis and Nrf2-induced downstream antioxidant defence to suppress oxidative stress in prenatal restraint-stressed rats (Cao et al., 2014). Thus, it is hypothesised that dysfunction of AMPK and its downstream signalling molecules (Nrf2 and HO-1) might contribute a significant role in the diabetes-induced cardiac pathologies.

Intriguingly, several AMPK activators such as metformin (Calvert et al., 2008), trimetazidine (Liu et al., 2016b), rosiglitazone (Morrison et al., 2011) have been demonstrated to dampen diabetic myocardial ischemic-reperfusion injury via AMPK

stimulation. However, the appearance of serious adverse effects like hypoglycemia, lactic acidosis and gastrointestinal disturbances associated with these drugs puzzled their therapeutic utility in diabetic patients (Li et al., 2004). Thus, there is a pressing need for the discovery of novel dietary functional ingredients for the treatment of diabetic cardiomyopathy and ischemic heart diseases by combating glycemic control alterations. Amongst all such agents, polyphenolic compounds have attracted the interest of the scientific community and had been thoroughly investigated for this purpose in recent years (Zhang & Tsao, 2016). Pterostilbene (PT) ($C_{16}H_{16}O_3$; Table 1), a polyphenolic stilbene derivative, a major bioactive constituent of blueberries, has become increasingly popular because of its promising health benefits, including antidiabetic (Elango et al., 2016; Gomez-Zorita et al., 2015), anti-inflammatory (Yu et al., 2017) and cardioprotective actions (Yu et al., 2017). PT was reported to improve glucose homoeostasis in streptozotocin-induced diabetic rats (Elango et al., 2016) and obesogenic fed insulin resistant rats (Gomez-Zorita et al., 2015). Although PT ameliorated glucose homeostasis in obesogenic (high fat) diet-induced insulin resistant rats by increasing GLUT4 expression, glucokinase activity and phosphorylated-Akt/total Akt ratio (Gomez-Zorita et al., 2015), however, its effects on other metabolic complications associated with insulin resistance and oxidative stress have not been well studied in the fructose-fed type2 diabetic model.

In a recent clinical trial, PT at the daily doses of 100 mg to 250 mg for 6-8 weeks did not produce any significant adverse drug events in hyperlipidemic patients (Riche et al., 2014b). Moreover, daily administration of 450 mg dose of *Pterocarpus marsupium* extract in healthy volunteers resulted in detectable PT serum levels up to two weeks after administration and did not produce any signs of toxicity (Hougee et al., 2005a). Thus, the ultra-high safety profile of PT, coupled with its broad spectrum activities has

stimulated the interest to consider it as an attractive therapeutic candidate for diabetes and associated cardiac complications. A few studies have demonstrated the cardioprotective effect of PT by assessing the markers of oxidative stress and inflammatory response in the hearts of diabetic rats (Yu et al., 2017). Furthermore, cardioprotective effects of PT centres around its suppressive effects on oxidative stress, apoptosis, and inflammation (Remsberg et al., 2008), which improves myocardial IR injury in non-diabetic rats. The biological actions of PT are thought to obtain from its antioxidant potential of stimulating Nrf2 and HO-1 regulated antioxidant defence in animal models (Elango et al., 2016). Besides, PT induces mitochondriogenesis and enhances mitochondrial oxidative capacity in gastrocnemius muscle of insulin-resistant rats by increasing carnitine palmitoyltransferase-1B, mitochondrial transcription factor, citrate synthase, cytochrome c oxidase II activities (Gomez-Zorita et al., 2015). Interestingly, PT was demonstrated to reduce lipogenesis & fat accumulation, promotes macroautophagy and inhibits apoptosis in non-myocardial tissues like adipocytes and vascular endothelial cells, respectively (Gomez-Zorita et al., 2014; Zhang et al., 2013a). Also, resveratrol (a metabolite of PT) has been extensively studied for its beneficial effects against diabetic myocardial IR through AMPK signalling mechanism (Yang et al., 2016a). A study of AMPK-knockout mice confirmed that AMPK is the central target for metabolic effects of resveratrol (Um et al., 2010). Considering that PT is a parent compound of resveratrol with superior bioavailability and stronger potency (Lin et al., 2009), high safety profile (Riche et al., 2014b), it is reasonable to hypothesize that PT can also exert a protective effect against diabetic cardiomyopathy via AMPK stimulation. However, no studies have been performed to reveal the therapeutic efficacy and underlying mechanisms of PT against diabetic cardiomyopathy either in type 1 diabetic (streptozotocin-induced) and type 2 diabetic (fructose-fed) rat models till the

conceptualization of the study. Deciphering how PT exerts myocardial benefits on diabetes-associated cardiovascular complications is decisive for the design of rational therapy of diabetic cardiomyopathy.

Dietary fructose induces metabolic syndrome due to its unique metabolism (Segal et al., 2007). Fructose is taken up into liver by the GLUT5 carrier induces marked glucose oxidation and lipogenesis (Segal et al., 2007). Further, fructose is metabolized in liver via fructolysis, and the primary metabolites include glucose, free fatty acid (FFA), triglycerides (TG), lactate, methylglyoxal and uric acid are overproduced and secreted into blood, impairing tissue energy homeostasis directly. In addition, rapid fructolysis also leads to a high level of metabolic stress via ATP depletion (Abdelmalek et al., 2012; Zhang et al., 2017), enhancing AMP break down to increase hepatic uric acid levels, and subsequently leading to elevation of blood uric acid levels. Moreover, ATP degradation stimulates oxidative stress and inflammatory response to alter the organ function, resulting in abnormal production of insulin, adiponectin, leptin and inflammatory cytokines (Abdelmalek et al., 2012; Zhang et al., 2017). These indirect dangerous factors are released into systemic circulation, further aggravating metabolic burden in tissues and organs, leading to insulin resistance, dyslipidemia and hypertension.

Fructose stimulates aldolase B enzyme to enhance methylglyoxal synthesis in liver, releasing into systemic circulation (Wang et al., 2008), which in turn inhibits AMPK activation by blocking allosteric binding of AMP to AMPK. AMPK is a key energy sensor to regulate glucose and lipid metabolism in various tissues and organs, including liver, adipose, heart and skeletal muscle. AMPK activation inhibits acetyl CoA carboxylase to decrease malonyl CoA, a substrate for FFA synthesis (Muoio et al.,

1999). Therefore, the impairment of AMP-sensing capacity of AMPK by methylglyoxal promotes *de novo lipogenesis*, leading to the development of insulin resistance under high fructose diet (Gugliucci, 2009). Meanwhile, AMPK suppression gives rise to gluconeogenesis and glucose output, all of which may promote fructose-induced metabolic syndrome (Gugliucci, 2009). Recent study demonstrated that AMPK activation in liver completely prevented hepatic triglyceride accumulation and de novo lipogenesis in high-fructose diet-fed mice (Woods et al., 2017a). This finding may have implications for the therapeutic potential of targeting AMPK in high-fructose-fed type 2 diabetic model.

In the present investigation, both type 1 (streptozotocin-induced) and type 2 diabetic (fructose-fed) models were employed to explore the therapeutic efficacy of PT against diabetes-associated cardiac complications. It is hypothesized to investigate the therapeutic potency of PT on insulin resistance and underlying signalling mechanism of PT's myocardial benefits on long-term high fructose diet-induced myocardial oxidative stress, inflammation and mitochondrial impairment, with a particular attention on the AMPK/Nrf2/HO-1 signalling. Also, envisaged to whether PT attenuates myocardial IR injury in streptozotocin-induced diabetic rats and if so, to investigate whether PT protects diabetic myocardium against IR injury by stimulating AMPK signalling pathway.
