Role of Pycnogenol® in the treatment of diabetes mellitus in streptozotocin-induced rat model
INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder and a major worldwide health problem. Currently, there are over 150 million diabetics worldwide, and this is likely to increase to 300 million or more by the year 2025 due to increased sedentary lifestyle, consumption of energy-rich diet and obesity [Yajnik, 2001]. Type 2 diabetes mellitus (T2DM), one of the fastest-growing epidemics of our time, is a chronic metabolic disorder characterized by insulin resistance in the liver, muscle, and adipose tissue, as well as progressive β-cell dysfunction leading to hyperglycemia [Gastaldelli et al., 2004; Taniguchi et al., 2006]. It is characterized by chronic hyperglycemia resulting from a defect in insulin secretion or action, or both [Limaye et al., 2003], often accompanied by polyuria, polyphagia, polydipsia and weight loss [American Diabetes Association, 2005; Celik et al., 2002]. Hyperglycemia leads to long-term complications of diabetes, which are the major causes of morbidity and mortality in human populations [Palsamy and Subramanian, 2009]. Treatment of DM remains a challenging issue. During the last decade, there has been much interest in the development of anti-diabetic drugs [McNeill et al., 1992; Alice et al., 2005; Karmaker et al., 2006]. However, clinical trials have been disappointing as, so far, they all have failed due to therapeutic limitations. The search for a safe and effective drug which can reduce the many harmful effects of DM, including hyperglycemia, hyperlipidemia, oxidative stress, renal damage, inflammation, and atherosclerosis, among others, is thus urgently needed. Diabetes and its associated complications are still an important medical and social problem, in spite of the use of many oral hypoglycemic agents such as sulphonylureas and biguanides. Recently, diabetic healthcare professionals had a considerable interest in considering complementary and alternative approaches, including herbal medicines due to the fact that they are less toxic than hypoglycemic agents [Chattopadhyay, 1993].

Although the exact mechanisms which contribute to diabetic complications remain unclear, much attention has been paid to the role of oxidative stress, widely believed to contribute to the acceleration of diabetes and its complications [Domínguez et al., 1998, Palsamy and Subramanian, 2009; Vessby et al., 2002]. Under normal conditions, these oxidants (ROS) are quenched by the body's endogenous antioxidant systems. Oxidative stress is a component of molecular and cellular tissue damage that leads to an imbalance in oxidant/antioxidant systems and results in enhanced lipid peroxidation (LPO), depletion of antioxidant enzymes and impaired glutathione metabolism [Bagri et al., 2009]. Multiple factors contribute to increased oxidative stress in diabetes. Persistent hyperglycemia itself increases the production of ROS [e.g., superoxide radical (O$_2^-$), hydroxide radical (OH$^-)$, hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOO$^-$)] through glucose auto-oxidation and non-enzymatic protein glycation [Hunt et al., 1990; Wolff, 1993]. O$_2^-$ and H$_2$O$_2$ have been shown
to stimulate stress-signaling pathways such as NF-κB, p38-MAPK and STAT-JAK resulting in VSMC migration, proliferation and inflammation. ONOO− causes single-strand DNA breakage which in turn activates nuclear enzyme poly(ADP-ribose) polymerase (PARP) [Soriano et al., 2001], which is now viewed as an important effector of oxidative–nitrosative injury in diabetes [Orosova et al., 2004]. Additionally, PARP activation leads to the depletion of its substrate NAD+; energy failure; inhibition of G3PDH of glycolysis, and, in extreme cases, necrosis [Garcia et al., 2001]. Furthermore, hyperglycemia induced oxidative stress may lead to activation of several damaging pathways, all of which have been proven to be involved in the pathogenesis of diabetic complications [Ceriello et al., 1992; Johansen et al., 2005; Mullarkey et al., 1990].

Recently the use of antioxidants for diabetes has been advocated, as oxidative stress is known to play an important role in the onset as well as development of further secondary complications of diabetes. Many plant-derived antioxidants have received great attention as providing a potential therapeutic approach to prevent diabetic complications associated with oxidative stress [Zibadi et al., 2008; Islam et al., 2008; Daisy et al., 2009]. French maritime pine (Pinus maritima) bark extract, Pycnogenol® (PYC), a patented combination of bioflavonoids, has a high antioxidant potential [Packer et al., 1999; Rohdewald, 2002]. Major constituents of PYC are bioflavonoids, such as monomeric and oligomeric units of catechin, epicathechin, and taxifolin. PYC, by virtue of being a very potent scavenger of free radicals, has diverse beneficial effects on a wide range of medical conditions, including cancer, atherosclerosis, diabetes, inflammation, asthma, hypertension, atherosclerosis, immune disease, and others [Rohdewald 2002; Rohdewald 2005; Grimm et al., 2006]. Some earlier studies have evaluated the beneficial effects of PYC in diabetes such as its hypoglycemic and high anti-oxidant potential [Jankyova et al., 2009; Berryman et al., 2004; Liu et al., 2004a; Maritini et al., 2003; Zibadi et al., 2008]. Moreover, PYC supplementation has been demonstrated to lower low-density lipoprotein cholesterol (LDL-C) level and increase high-density lipoprotein cholesterol (HDL-C) levels [Devanaj et al., 2002]. PYC also has the ability to enhance the status of the endogenous antioxidant system, including vitamin-E, GSH and antioxidant enzymes [Wei et al., 1997; Packer et al., 1999, Siler-Marsiglio et al., 2004] and protect them from oxidative damage [Wei et al., 1997; Packer et al., 1999; Nelson et al., 1998]. The present study evaluated the role of PYC on hyperglycemia, hyperlipidemia, renal functions, inflammatory markers and oxidative damage in the liver and pancreas of diabetic rats. The effects produced by PYC were compared with the standard drug glibenclamide. Our results support the efficacy of PYC in lowering glucose and improving diabetic complications in a clinically relevant type 1 and type 2 diabetic models.
MATERIALS AND METHODS

Chemicals
As described in materials and methods, section-III.

Preparation of extract and phytochemical screening
Preparation of extract was done and subjected to qualitative phytochemical analysis as described in materials and methods, section-III.

Determination of total phenolic and flavonoid content
Total phenolic content and flavonoid content were estimated as described earlier in materials and methods, section-III.

IN VITRO ANTIOXIDANT ACTIVITY
Reducing power activity, hydrogen peroxide scavenging activity and DPPH scavenging capacity of the extract was determined as described earlier in materials and methods, section III.

EFFICACY OF PYC FOR THE TREATMENT OF TYPE 1 DIABETES

Experiment I: Evaluation of therapeutic dose of PYC against type I diabetes mellitus (T1DM) and check whether the required therapeutic dose is safe or produces some type of toxicity

Experimental procedure and induction of T1DM
Experiment was carried out to evaluate the therapeutic dose of PYC for the treatment of diabetes. Experimental setup and induction of T1DM was done according to procedures as described in materials and methods, section-III.

Estimation of fasting blood glucose
On the morning of the end of experiment, after overnight fasting, the rats were anesthetized by ether inhalation and blood was collected from the dorsal aorta. Serum was separated and blood glucose level was measured as described in materials and methods, section-III.

Experiment II: Effect of Pycnogenol (PYC) on biochemical, histological and immunohistochemical alterations in STZ-induced type 1 diabetic rats.

Experimental design and development of the STZ model of type 1 diabetes
As described in materials and methods, section-III. Thirty two rats were divided into four groups of eight animals each as described earlier.
Biochemical analyses
Metabolic markers (OGTT, FBG, HbA1c, insulin, amylase, hepatic glycogen, FFAs, lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C)), inflammatory markers (NO, IL-1β, TNF-α), and renal function markers (BUN, Scr, ALP) were performed as described in materials and methods, section-III. Oxidative damage parameters (TBARS, MDA, PC, GSH, GST and CAT) and protein content were determined as described in materials and methods, section-III.

Histological and immunohistochemical analyses
These were done in the same manner as described in materials and methods, section II.

Efficacy of PYC for the treatment of type 2 diabetes
Experiment I: Evaluation of therapeutic dose of PYC against type 2 diabetes mellitus (T2DM) and check whether the required therapeutic dose is safe or produces some type of toxicity

Experimental procedure and induction of T2DM
As described in materials and methods, section-III.

Estimation of fasting blood glucose
On the morning of the end of experiment, after overnight fasting, the rats were anesthetized by ether inhalation and blood was collected from the dorsal aorta. Serum was separated by centrifugation at 3000 rpm for 15 min and blood glucose level was measured as described in materials and methods, section-III.

Experiment II A: Effect of Pycnogenol (PYC) on biochemical and morphological alterations in HFD/STZ-induced type 2 diabetic rats.

Experiment II B: Effect of Pycnogenol (PYC) on pAkt expression and apoptotic β-cell death in HFD/STZ-induced type 2 diabetic rats

Experimental design and development of HFD/STZ model of type 2 diabetes
Experiment was carried out as described in materials and methods, section-III. Rats were divided described in materials and methods, section-III.

Biochemical analyses
Metabolic markers (OGTT, FBG, HbA1c, insulin, FFAs, hepatic glycogen, lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C)), inflammatory markers (NO, IL-1β, TNF-α), and renal function markers (BUN, Scr, ALP) are described in materials and methods, section-III. Oxidative damage parameters (TBARS, MDA, PC, GSH, GST, GPx, GR, SOD and CAT) and protein content were determined as described in materials and methods, section-III.
Histological examinations
As described in materials and methods, section-III.

Tunnel staining and Western blotting
As described in materials and methods, section-III

Statistical analysis
As described in materials and methods, section-III.

RESULTS

Phytochemical screening
A phytochemical screening of the PYC revealed the presence of phenolic compounds, broadly divided into monomers (catechin, epicatechin, and taxifolin) and condensed flavonoids (classified as procyanidins/proanthocyanidins because they release anthocyanins when heated in acidic conditions). The presence of flavonoids belonging to the family of procyanidins in PYC was detected by means of the porter assay.

Total phenolic and flavonoid content of PYC methanolic extract
Phenolics and flavonoids are the most wide-spread secondary metabolite in plant kingdom. Therefore, in the present study, total phenolic and flavonoid content present in extract were estimated using modified Folin- ciocalteau method.

IN VITRO ANTIOXIDANT ACTIVITY

Reducing power activity of PYC methanolic extract
For the measurements of the reducing ability, the Fe$^{3+}$—Fe$^{2+}$ transformation was investigated in the presence of PYC. Fig. 1A. depicts the reductive capabilities of PYC extract compared to ascorbic acid. The reducing power of extract of PYC was very potent and the power of the extract was increased with increasing dosage. The result shows that PYC could reduce the most Fe$^{3+}$ ions, which had a lesser reductive activity than the reference standard of ascorbic acid.

Hydrogen peroxide scavenging activity of PYC methanolic extract
As shown in Fig. 1B. PYC also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner with an IC$_{50}$ of 1.54 μg/ml. The IC$_{50}$ value of the extract was found to be comparable to reference standard ascorbic acid (IC$_{50}$ 2.24 μg/ml).

DPPH antioxidant activity of PYC methanolic extract
Comparison of the antioxidant activity of the extract and ascorbic acid is shown in Fig. 1C. The methanol extract of PYC exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC$_{50}$) at a concentration of 1.35 μg/ml. The IC$_{50}$ value of the extract was found to be comparable to reference standard ascorbic acid (IC$_{50}$ 3.7 μg/ml).
Table 1: Phytochemical screening of methanol extract of PYC

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Constituent</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Sterols</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>Proteins and amino acid</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Fat</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Sugars and carbohydrate</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Resin</td>
<td></td>
</tr>
</tbody>
</table>

Note: (-) shows the absence of constituent in the extract, (+) shows the present of constituent and (+++) shows the present of constituent in higher amount.

Table 2- Total phenolic and flavonoid content of PYC

<table>
<thead>
<tr>
<th>Part used</th>
<th>Total phenolic content (mg/100 mg extract)</th>
<th>Total flavonoid content (mg/100 mg extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.M</td>
<td>Mean ± S.E.M</td>
</tr>
<tr>
<td>Bark</td>
<td>24.08± 1.63</td>
<td>77.95± 1.71</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Mean ± S.E.M. The total phenolic content was expressed as gallic acid equivalent in mg/100mg of the extract. The total flavonoid content was expressed as rutin equivalents in mg/100mg of the extract.

[158]
Fig 1. (A) Reducing power of ascorbic acid and methanol extract of PYC. (B) H2O2 scavenging activity of ascorbic acid and methanol extract of PYC. (C) DPPH radical scavenging activity of ascorbic acid and methanol extract of PYC. Values are the average of triplicate experiments and represented as Mean ± S.E.M.
EFFICACY ON TYPE 1 DIABETES

Effect of PYC on hyperglycemia in the STZ group (Dose evaluation)

The STZ group (Fig. 2) showed significant (\( P < 0.05 \)) increase in FBG compared to the control group. In the PYC (10mg/kg) treated group, the levels of FBG was significantly (\( P < 0.05 \)) attenuated as compared to other doses of PYC (5mg/kg; 20mg/kg) in STZ-induced diabetic rats. GL also produced significant (\( P < 0.05 \)) reduction in FBG level. Only PYC (10mg/kg) treatment did not show any significant change in the FBG compared to the control group.

STUDY ON TYPE 1 DIABETES WITH SELECTED DOSE

PYC supplementation ameliorated OGTT in the STZ-induced rat model of type 1 diabetes

Blood glucose levels of the controls, the STZ, STZ + PYC and STZ + GL groups at different time points (0, 30, 60 and 120 min) after oral administration of glucose (2 gm/kg) shown in fig 3. In the STZ group, the peak increase in blood glucose level was observed after 60 min and remained high over next 60 min. PYC treatment in the STZ + PYC group showed significant (\( P < 0.05 \)) decrease in blood glucose level at 60 and 120 min when compared to the STZ group. Glibenclamide treatment also showed significant (\( P < 0.05 \)) reduction in peak of blood glucose values.

Effect of PYC on body weight in the STZ-induced rat model of diabetes

STZ rats had significantly increased body weight (\( P < 0.05 \)) compared to control. Administration of PYC and GL restored body weight significantly (\( P < 0.05 \)) when compared to the STZ group. There was no significant difference in body weight between control animals and only PYC-treated animals (Table 3).

PYC supplementation restored FBG level in the STZ-induced rat model of type 1 diabetes

Effect of PYC on FBG level in diabetic and control groups is shown in table 4. A significant (\( P < 0.05 \)) increase in blood glucose level was observed in the STZ group compared to the control rats. The level of FBG in diabetic rats treated with PYC was close to those in diabetic rats treated with glibenclamide. Only PYC treatment did not show any significant change in the blood glucose level when compared to the control rats. The four-week treatment with PYC resulted in significant (\( P < 0.05 \)) antihyperglycemic effect in the STZ group.
PYC supplementation augmented HbA1c in the STZ-induced rat model of type 1 diabetes

Effect of PYC on HbA1c level in the STZ is shown in table 4. A significant (P < 0.05) increase in HbA1c level was observed in the STZ group when compared to the control rats. PYC treatment in the STZ + PYC group significantly (P < 0.05) decreased the HbA1c level. Glibenclamide treatment improved HbA1c level significantly (P < 0.05) when compared to diabetic group. There was no significant change in HbA1c level in PYC treatment in the control + PYC-treated rats when compared to control rats.

PYC supplementation modulated amylase activity and insulin level in the STZ-induced rat model of type 1 diabetes

Effect of PYC on amylase activity and insulin level in diabetic and control groups is shown in table 4. A significant (P < 0.05) decrease in amylase activity and insulin level was observed in the STZ group compared to the control rats. Administration of PYC in the STZ + PYC group increased both significantly (P < 0.05) when compared to the STZ group. Diabetic rats given GL showed significant (P < 0.05) improvement in the parameters when compared to diabetes group. Only PYC treatment did not show any significant change in both amylase activity and insulin level when compared to the control rats.

PYC supplementation increased hepatic glycogen content in the STZ-induced rat model of type 1 diabetes

The STZ group showed the lowest levels of glycogen content when compared to the control STZ + PYC and STZ + GL groups. The glycogen levels were significantly (P < 0.05) increased in diabetic rats treated with PYC and GL when compared to the STZ group. The results obtained for the glycogen content indicates that there was no significant difference in glycogen content between the control and control + PYC group.
Fig 2. Different doses of PYC were used for evaluating the dose showing maximum antihyperglycemic activity and compared to GL. Values are expressed as mean ± S.E.M (n = 8).

Fig 3. Glucose tolerance was evaluated from the time course of serum glucose after administration of glucose solution (2 g/kg, orally) in OGTT. Values are expressed as mean ± S.E.M (n = 8).
Table 3. Effect of PYC supplementation on body weight in the STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Control (C)</td>
<td>181±6.0</td>
</tr>
<tr>
<td>C + PYC</td>
<td>185±7.5</td>
</tr>
<tr>
<td>STZ</td>
<td>200±8.0</td>
</tr>
<tr>
<td>STZ + PYC</td>
<td>196±7.0</td>
</tr>
<tr>
<td>STZ + GL</td>
<td>192±5.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). The body weight of all animals was measured initially and after the end of experiment. STZ group showed a significant decrease in body weight compared with the control group (P < 0.05 STZ vs. control group). PYC or GL treatment significantly augmented body weight in the STZ + PYC or STZ + GL group compared with the STZ group (P < 0.05 STZ vs. STZ + PYC or STZ + GL group).

Table 4. Effect of PYC supplementation on FBG, HbAlc, amylase, insulin and hepatic glycogen in the STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>FBG (mg/dl)</th>
<th>HbAlc (%)</th>
<th>Amylase (U/dl)</th>
<th>Insulin (ng/ml)</th>
<th>Hepatic glycogen (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>110.21±2.4</td>
<td>6.4±0.25</td>
<td>36.43±0.86</td>
<td>2.7±0.12</td>
<td>212.31±5.0</td>
</tr>
<tr>
<td>C + PYC</td>
<td>112.48±2.6</td>
<td>6.6±0.26</td>
<td>35.21±0.97</td>
<td>2.6±0.11</td>
<td>214.65±4.9</td>
</tr>
<tr>
<td>STZ</td>
<td>256.45±5.4</td>
<td>12.98±0.91</td>
<td>10.76±0.33</td>
<td>0.35±0.02</td>
<td>98.65±1.9</td>
</tr>
<tr>
<td>STZ + PYC</td>
<td>140.59±3.9b</td>
<td>8.59±0.42b</td>
<td>24.31±0.89b</td>
<td>1.7±0.05b</td>
<td>159.89±3.9b</td>
</tr>
<tr>
<td>STZ + GL</td>
<td>137.32±3.6b</td>
<td>8.49±0.38b</td>
<td>25.04±0.52b</td>
<td>1.9±0.04b</td>
<td>153.98±3.8b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). STZ group showed a significant increase in FBG and HbAlc with the significant decrease in amylase, insulin and hepatic glycogen in the STZ group compared with the control group (P < 0.05 STZ vs. control group). PYC and GL treatment significantly augmented these parameters in the STZ + PYC and STZ + GL group compared with the STZ group (P < 0.05 STZ vs. STZ + PYC or STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or STZ group.
PYC supplementation ameliorated lipid profile and FFAs parameters in the STZ-induced rat model of type 1 diabetes

The effects of PYC on lipid profile and FFAs parameters in the STZ rats shown in table 5. STZ group showed a significant ($P < 0.05$) increment in serum TC, TG, FFAs, LDL-C and VLDL-C level while a significant ($P < 0.05$) decrement in serum HDL-C compared to the control group. Treatment of PYC in the STZ + PYC group significantly ($P < 0.05$) restored all the changes in the lipid profile and FFAs. Following administration of GL diabetic rats also showed a significant reduction in the levels of TG, TC, LDL, VLDL and FFAs but less prominent compared to PYC. PYC treatment did not show any significant changes in the lipid profile when compared to the control group.

PYC supplementation modulated renal markers in the STZ-induced rat model of type 1 diabetes

Effects of PYC on renal markers (BUN, Scr and ALP) were measured to demonstrate renal function in serum of the STZ group. There were no significant changes in BUN, Scr, and ALP in the control + PYC group, while these factors were significantly ($P < 0.05$) increased in the STZ group compared with the control group. PYC or GL supplementation in the STZ + PYC or STZ + GL group resulted in a significantly ($P < 0.05$) decrease in these markers compared with the STZ group (table 6).

Effects on serum TNF-α, IL-β and NO levels

Significant ($P < 0.05$) increased in the levels of serum TNF-α, IL-β and NO was observed in STZ group compared to control group. PYC or GL administration in STZ + PYC or STZ + GL group, however decreased significantly ($P < 0.05$) the serum TNF-α, IL-β and NO levels when compared to STZ group. Only PYC treatment did not show any significant difference in the levels of TNF-α, IL-β and NO when compared to the control rats.

PYC supplementation decreased TBARS and MDA contents in the STZ-induced rat model of type 1 diabetes

The effects of PYC on TBARS and MDA contents were measured to demonstrate the rate of LPO and end products in pancreas of STZ group. There were no significant changes in TBARS and MDA contents in the control + PYC treated group compared to control group. These parameters were significantly ($P < 0.05$) increased in the STZ group compared to the control group. Levels of TBARS and MDA in the STZ group decreased significantly ($P < 0.05$) with PYC or GL supplementation in the STZ + PYC or STZ + GL group (Fig 5. A and B). PYC was more effective than glibenclamide.
Table 5. Effect of PYC supplementation on serum lipid profile and FFAs in STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Parameters /Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>FFA (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>135.37±3.2</td>
<td>120.31±3.9</td>
<td>35.65±1.9</td>
<td>75.65±2.7</td>
<td>24.06±1.2</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>C + PYC</td>
<td>137.56±3.1</td>
<td>122.41±4.3</td>
<td>34.11±1.8</td>
<td>78.96±2.8</td>
<td>24.48±0.99</td>
<td>0.43±0.03</td>
</tr>
<tr>
<td>STZ</td>
<td>270.31±5.1</td>
<td>227.61±4.8</td>
<td>16.73±2.0</td>
<td>208.05±4.8</td>
<td>45.52±1.4</td>
<td>0.75±0.08</td>
</tr>
<tr>
<td>STZ + PYC</td>
<td>187.42±3.9</td>
<td>158.52±2.9</td>
<td>28.35±2.4</td>
<td>127.36±3.9</td>
<td>31.70±1.3</td>
<td>0.58±0.06</td>
</tr>
<tr>
<td>STZ + GL</td>
<td>196.35±3.5</td>
<td>162.34±2.9</td>
<td>29.56±2.1</td>
<td>132.66±3.1</td>
<td>33.76±1.5</td>
<td>0.54±0.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. STZ group showed a significant increase in TC, TG, FFAs, LDL-C and VLDL-C with decrease in HDL-C in the serum of the STZ group compared to the control group (#P < 0.05 STZ vs. control group). PYC and GL treatment significantly ameliorated these parameters in the STZ + PYC group compared to the STZ (#P < 0.05 STZ vs. STZ + PYC or STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or STZ group.

Table 6. Effect of PYC supplementation on renal function markers (BUN, Scr and ALP) in serum of the STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Parameters /Groups</th>
<th>BUN (mg/dl)</th>
<th>Scr (mg/dl)</th>
<th>ALP (units/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>14.52±1.1</td>
<td>1.18±0.03</td>
<td>25.51±0.92</td>
</tr>
<tr>
<td>C + PYC</td>
<td>16.12±1.3</td>
<td>1.19±0.05</td>
<td>27.01±0.98</td>
</tr>
<tr>
<td>STZ</td>
<td>44.23±1.7a</td>
<td>3.99±0.04a</td>
<td>52.72±1.6a</td>
</tr>
<tr>
<td>STZ + PYC</td>
<td>30.43±1.5b</td>
<td>2.68±0.01b</td>
<td>19.92±0.71b</td>
</tr>
<tr>
<td>STZ + GL</td>
<td>27.33±1.4b</td>
<td>2.58±0.01b</td>
<td>19.46±1.2b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). STZ group showed a significant increase in renal function markers (BUN, Scr and ALP) in serum of the STZ group compared with the control group (#P < 0.05 STZ vs. control group). PYC and GL treatment significantly ameliorated these parameters compared with the STZ group (#P < 0.05 STZ vs. STZ + PYC or STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or STZ group.
Fig 4. STZ group showed significant increase in TNF-α, IL-1β and NO levels compared with the control group (*P < 0.05 STZ vs. control group). PYC and GL supplementation significantly decreased these parameters compared with the STZ induced diabetic group (*P < 0.05 STZ vs. STZ + PYC or STZ + GL group). Values are expressed as mean ± S.E.M (n = 8).
Fig 5. (A). STZ group showed significant increase in TBARS levels compared to the control group ($P < 0.05$ STZ vs. control group). PYC and GL supplementation significantly decreased TBARS levels compared to the STZ induced diabetic group ($P < 0.05$ STZ + PYC or STZ + GL vs. STZ group). (B). STZ group showed significant increase in MDA levels compared to the control group ($P < 0.05$ STZ vs. control group). PYC and GL supplementation significantly decreased MDA in the STZ + PYC group compared to the STZ induced diabetic group ($P < 0.05$ STZ + PYC or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M (n = 8).
PYC supplementation decreased PC in the STZ-induced rat model of type 1 diabetes

PC content did not change by PYC supplementation in the control + PYC group compared to the control group. PC content was significantly (P < 0.05) increased in the STZ group compared to control alone. PYC or GL supplementation significantly (P < 0.05) decreased PC content in the STZ + PYC or GL group compared to STZ group alone (Fig 6).

PYC supplementation restored GSH in the STZ-induced rat model of type 1 diabetes

The level of GSH did not affect by PYC supplementation in the control + PYC treated group compared to the control group. However, a significant (P < 0.05) depletion in GSH was observed in the STZ group compared to the control group. PYC or GL supplementation significantly (P < 0.05) augmented GSH level in the STZ + PYC or STZ + GL group compared to the STZ group (Fig 7). The effect of PYC was more prominent compared with GL.

PYC supplementation increased GST and CAT activity in the STZ group

The activity of GST and CAT in group the control + PYC did not change significantly compared to the control group (Fig. 8. A and B). On the other hand, the activities of these enzymes were depleted significantly (P < 0.05) in the STZ group compared to the control group. The PYC and GL significantly (P < 0.05) increased the activity of these enzymes in the STZ+PYC and STZ + GL group respectively, as compared to the STZ group. The effect was more pronounced in the group of rats treated with PYC.
Fig 6. STZ group showed significant increase in PC content compared to the control group (\(P < 0.05\) STZ vs. control group). PYC and GL supplementation significantly decreased PC content in treatment group compared to the STZ induced diabetic group (\(P < 0.05\) STZ + PYC or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M (n = 8).

Fig 7. STZ group showed significant decrease in GSH content compared to the control group (\(P < 0.05\) STZ vs. control group). PYC and GL supplementation significantly increased GSH content in the treatment group compared to the STZ induced diabetic group (\(P < 0.05\) STZ + PYC or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M (n = 8).
Fig 8. (A). STZ group showed significant decrease in GST activity compared to the control group (*P < 0.05 STZ vs. control group). PYC and GL supplementation significantly increased GST activity compared to the STZ induced diabetic group (*P < 0.05 STZ + PYC or STZ + GL vs. STZ group). (B). STZ group showed significant decrease in CAT activity compared to the control group (*P < 0.05 STZ vs. control group). PYC and GL supplementation significantly increased CAT activity compared to the STZ induced diabetic group (*P < 0.05 STZ + PYC or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M (n = 8).
HISTOPATHOLOGICAL FINDINGS

Effect of PYC supplementation on H and E staining in the STZ group

H and E staining is used to visualize and differentiate between tissue components in normal and pathological conditions. The histological examination of the H and E-stained control liver tissues showed normal architecture of hepatocytes (Fig 9). Liver section of STZ-induced diabetic group showed vacuolization of hepatocytes in the liver parenchyma with significant dilatation of sinusoidal spaces. The STZ + PYC group that received PYC treatment showed normal hepatic parenchyma except for non-specific periportal inflammatory cell collection. No vacuolation of hepatocytes or sinusoidal dilatation was seen. PYC supplementation did not show any remarkable effects in the group treated with PYC alone compared with the control group (data not shown). Photomicrograph from STZ + GL group animal that supplemented with standard GL, showing regularly arranged liver lobules along with portal triad with mild hyalinization of hepatic arterial wall. The histological examination of the H and E-stained control pancreatic section showed normal appearance of pancreatic tissue with a single Islet of Langerhans seen in the centre (Fig 10). Pancreatic section of STZ-induced diabetic group showed beta cells with reduction of cytoplasmic mass, reduction of Islet size and atrophy of beta cells with vacuolization. The beta cell cytoplasm was very scant and a few lymphocytes were also seen. The severity of degenerative changes was lessened by PYC supplementation in the STZ + PYC group compared with the STZ group, showed the islet cells a good number of beta cells with abundant basophilic cytoplasm. No lymphocytes were seen. PYC supplementation did not show any remarkable effects in the group treated with PYC alone compared with the vehicle control group (data not shown). Pancreas of STZ + GL group animal showing a small islet with normal exocrine glands surrounding it. Numerous beta cells with abundant basophilic cytoplasm were seen in GL treated group.

Immunohistochemical evaluation on pancreas

Figure 11 demonstrates the immunohistochemical results on pancreas tissues in the control and experimental rats. In control rats, the islets showed the normal structure with a large central core formed by insulin-secreting \( \beta \) cells. In the STZ group, a significant decrease in insulin immunoreactivity and the number of immunoreactive \( \beta \) cells was observed when compared to the control groups. STZ + PYC group, showed significant increase in insulin immunoreactivity and the number of immunoreactive \( \beta \) cells as compared to STZ rats.
Fig 9. Photograph from the control group at low power -100 x. showing normal liver architecture. PT = Portal Triad, CV = Central Vein, at high power-400 x. showing details with normal portal triad. PV = Portal Vein, BD = Bile Duct. STZ group at low power-100 x. showing mild sinusoidal dilatation in the centrizonal area, at high power-400 x. showing vacuolated hepatocytes and mild sinusoidal dilatation in the centrizonal area. PYC supplementation in the STZ + PYC group at low power-100 x. showing normal hepatic parenchyma with normal PT and CV, at high power-400 x. showing a portal triad with a few inflammatory cells and hepatocytes all around are within normal limits. PV = Portal Vein, BD = Bile Duct. GL supplementation in STZ + GL group at low power -100x showing regularly arranged liver lobules. PT=Portal Triad, CV= Central Vein, at high power -400x. showing a portal triad with mild hyalinization of Hepatic arterial wall. BD= Bile Duct, HA=Hepatic Artery.
Fig 10. Photograph from the control group at low power -100 x. showing normal appearance of pancreatic tissue with a single islet of Langerhans seen in the centre, at high power -400 x. showing details of cells in the islet. STZ group at low power- 100 x. showing pancreatic tissue with small sized islet of Langerhans seen. The exocrine tissue is within normal limits, IL=Islet of Langerhans, Ex=Exocrine tissue. at high power -400 x. showing beta cells with reduction of cytoplasmic mass and also vacuolar change and atrophy of the beta cells. The beta cell cytoplasm is very scant and a few lymphocytes are also seen. PYC supplementation in the STZ + PYC group at low power-100 x. showing a normal sized islet of Langerhans surrounded by exocrine pancreatic tissue, at high power -400 x. showing the islet cells a good number of beta cells with abundant basophilic cytoplasm. No lymphocytes are seen. GL supplementation in the STZ + GL group at low power-100 x. showing normal sized islet of Langerhans. There are dilated spaces seen in the islet, at high power-400 x. showing a fair number of residual beta cells. There is prominent vacuolation and fibrosis within the islet tissue.
Fig 11. Immunohistochemical results on pancreas in experimental and control rats. In control rats, the islets showed the normal structure with a large central core formed by insulin-secreting β cells. In the pancreatic islets of the STZ-induced diabetic group, a significant decrease in insulin immunoreactivity and the number of immunoreactive β cells was observed when compared to the control groups. STZ + PYC group, showed significant increase in insulin immunoreactivity and the number of immunoreactive β cells as compared to STZ diabetic rats.
EFFICACY ON TYPE 2 DIABETES

Effect of PYC on hyperglycemia in the HFD/STZ group (dose evaluation)
The HFD/STZ group showed significant (P < 0.05) increase in FBG compared to the control group. In the PYC (10mg/kg) treated group, the levels of FBG was significantly (P < 0.05) attenuated as compared to other doses of PYC (5mg/kg; 20mg/kg) in HFD/STZ-induced diabetic rats. GL treatment also showed the significant (P < 0.05) decreased in FBG level. Only PYC (10mg/kg) treatment did not show any significant change in the FBG compared to the control group.

STUDY ON TYPE 2 DIABETES WITH SELECTED DOSE

Effect of PYC treatment on OGTT in the HFD/STZ-induced rat model of diabetes
Fig13. Shows the blood glucose levels of the control, HFD/STZ group and the HFD/STZ + PYC groups after oral administration of glucose (2 gm/kg). In the HFD/STZ group, the peak increase in blood glucose level was observed after 60 min. Its level remained high over next 60 min. The HFD/STZ + PYC or HFD/STZ + GL group showed significant (P < 0.05) decrease in blood glucose level at 60 and 120 min compared to the HFD/STZ group.

Effect of PYC on body weight in the HFD/STZ-induced rat model of diabetes
HFD/STZ rats had significantly increased body weight (P< 0.05) compared to control. Administration of PYC and GL in the HFD/STZ + PYC or HFD/STZ + GL group respectively restored body weight significantly (P < 0.05) when compared to the HFD/STZ group. There was no significant difference in body weight between control animals and only PYC-treated animals (Table 7).

Effect of PYC on hyperglycemia in the HFD/STZ-induced diabetes in rats
In the HFD/STZ-induced diabetic rats, level of FBG was found to be significantly (P < 0.05) raised (315.92±5.85 mg/dl) compared to the control rats (104.75±3.11 mg/dl). In the PYC treated group, the levels of FBG was significantly (P < 0.05) attenuated (142.13±4.43 mg/dl) compared to the HFD/STZ-induced diabetic rats (Table 8). Only PYC treatment did not show any significant change (105.56±2.91 mg/dl) in the blood glucose level compared to the control group. The four week treatment with PYC resulted in significant (P < 0.05) hypoglycemic effect and was comparable to standard, glibenclamide.
PYC treatment restored HbA1c level in the HFD/STZ-induced rat model of diabetes

Table 8 shows the effect of PYC on HbA1c level. A significant (P < 0.05) increase in HbA1c level was observed in HFD/STZ group compared to the control. PYC or GL treatment significantly (P < 0.05) decreased the HbA1c level in the HFD/STZ + PYC or HFD/STZ + GL group. There was no significant change in the level of HbA1c in the control + PYC-treated group compared to the control.

Effect of PYC on serum insulin level in the HFD/STZ-induced diabetes in rats

In the HFD/STZ-induced diabetic rats, serum insulin level was found to be significantly (P < 0.05) decreased (0.65±0.04 ng/ml) compared to the control rats (3.5±0.16 ng/ml). In the PYC treated group, the levels of insulin was significantly (P < 0.05) attenuated (2.6±0.09 ng/ml) compared to the HFD/STZ-induced diabetic rats (Table 8). GL also produced significant (*P < 0.05) increased in insulin level. Only PYC treatment did not show any significant change (3.6±0.08 ng/ml) in the serum insulin level compared to the control group.

PYC treatment augmented hepatic glycogen content in the HFD/STZ-induced rat model of diabetes

Table 8 shows the effect of PYC treatment on the hepatic glycogen content in the HFD/STZ group. The HFD/STZ group showed a significantly (P < 0.05) decreased in glycogen content compared to the control. PYC and GL supplementation significantly ("P < 0.05) increased glycogen content in the HFD/STZ + PYC and HFD/STZ + GL group respectively compared to the HFD/STZ group. The results obtained for the glycogen content indicates that there was no significant difference in glycogen content between the control and the control + PYC group.
Fig 12. Different PYC doses were used for evaluating the dose showing maximum antihyperglycemic activity and was compared to GL. Values are expressed as mean ± S.E.M (n = 8).

Fig 13. Glucose tolerance was evaluated from the time course of serum glucose after administration of glucose solution (2 g/kg, orally) in OGTT. Values are expressed as mean ± S.E.M (n = 8).
Table 7. Effect of PYC treatment on body weight of control and experimental groups.

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>Body weight (g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Control (C)</td>
<td>183±8.5</td>
<td>279±9.0</td>
<td></td>
</tr>
<tr>
<td>C + PYC</td>
<td>200±6.5</td>
<td>265±11.0</td>
<td></td>
</tr>
<tr>
<td>HFD/STZ</td>
<td>195±9.5</td>
<td>185±10.0</td>
<td></td>
</tr>
<tr>
<td>HFD/STZ + PYC</td>
<td>196±5.5</td>
<td>259±8.0</td>
<td></td>
</tr>
<tr>
<td>HFD/STZ + GL</td>
<td>199±5.0</td>
<td>250±7.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). The body weight of all animals was taken initially and after the end of experiment. HFD/STZ group showed a significant decrease in body weight compared with the control group (*P < 0.05 HFD/STZ vs. control group). PYC and GL treatment significantly augmented body weight in the HFD/STZ + PYC or GL group compared with the HFD/STZ group (*P < 0.05 HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group).

Table 8. Effect of PYC treatment on FBG, GHbA1c, serum insulin and hepatic glycogen level of control and experimental groups.

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>FBG (mg/dl)</th>
<th>GHbA1c (%)</th>
<th>Insulin (ng/ml)</th>
<th>Hepatic glycogen (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>104.75±3.11</td>
<td>5.58±0.07</td>
<td>3.5±0.16</td>
<td>357.00±3.67</td>
</tr>
<tr>
<td>C + PYC</td>
<td>105.56±2.91</td>
<td>5.63±0.12</td>
<td>3.6±0.08</td>
<td>361.4±2.4</td>
</tr>
<tr>
<td>HFD/STZ</td>
<td>315.92±5.85a</td>
<td>10.90±0.24a</td>
<td>0.65±0.04a</td>
<td>173.10±2.0a</td>
</tr>
<tr>
<td>HFD/STZ + PYC</td>
<td>142.13±4.43b</td>
<td>8.88±0.27b</td>
<td>2.6±0.09b</td>
<td>241.19±2.4b</td>
</tr>
<tr>
<td>HFD/STZ + GL</td>
<td>140.28±4.06a</td>
<td>8.12±0.31a</td>
<td>2.7±0.08a</td>
<td>245.51±3.4a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. The HFD/STZ group showed a significant decrease in insulin and hepatic glycogen level with the significant increase in FBG and GHbA1c in the HFD/STZ group compared to the control group (*P < 0.05 HFD/STZ vs. control group). PYC and GL treatment significantly restored these parameters in the HFD/STZ + PYC or GL group compared to the HFD/STZ group (*P < 0.05 HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group).
Chapter-III

**Pycnogenol**

**Effect of PYC on lipid profile level in the HFD/STZ-induced diabetes in rats**

The treatment of PYC to the HFD/STZ + PYC group showed a significant ($P < 0.05$) reduction of serum TC (-48.24%), TG (-34.00%), LDL-C (-63.82%), VLDL-C (-33.99%), FFAs (-34.14%), and increment in serum HDL-C (+47.03%) compared to the HFD/STZ group alone (Table 9). Following administration of GL diabetic rats also showed a significant reduction in the levels of TG, TC, LDL, VLDL and FFAs but less effective compared to PYC. However, it was found to have no influence on lipid profile of control + PYC group compared to the control group.

**PYC treatment ameliorated renal function markers in the serum of the HFD/STZ-induced rat model of diabetes**

The effects of PYC on renal markers (BUN, Scr and ALP) were measured to demonstrate renal function in serum of the HFD/STZ group. There were no significant changes in BUN, Scr, and ALP in the control + PYC group, while these factors were significantly ($P < 0.05$) increased in the HFD/STZ group compared to the control group. PYC supplementation significantly ($P < 0.05$) decrease renal function markers in the HFD/STZ + PYC group compared to the HFD/STZ group (Table 10). Similar effects were found with GL treatment as of PYC to restore renal function markers.

**Effects on TNF-α, IL-β and NO levels in the HFD/STZ-induced rat model of diabetes**

Significant ($P < 0.05$) increased in the levels of serum TNF-α, IL-β and NO was observed in HFD/STZ group compared to control group. PYC or GL administration in HFD/STZ + PYC or HFD/STZ + GL group however decreased significantly ($P < 0.05$) the TNF-α, IL-β and NO levels when compared to HFD/STZ group. Only PYC treatment did not show any significant difference in the levels of TNF-α, IL-β and NO when compared to the control rats.

**Effect of PYC supplementation on TBARS and MDA contents in the HFD/STZ-induced diabetes in rats**

Effects of PYC on TBARS and MDA contents were measured to demonstrate the rate and end products of LPO in the pancreas of HFD/STZ group. TBARS and MDA did not significantly changes in control + PYC treated group compared to the control group. These parameters were significantly ($P < 0.05$) increased in the HFD/STZ group compared to the control group. Contents of TBARS and MDA in the HFD/STZ group decreased significantly ($P < 0.05$) with PYC or GL in the HFD/STZ + PYC or HFD/STZ + GL group (Fig. 15. A and B).
### Table 9. Effect of PYC treatment on serum lipid profile and FFAs of control and HFD/STZ-induced experimental diabetic groups.

<table>
<thead>
<tr>
<th>Parameters/groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>FFA (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>123.46±2.7</td>
<td>97.40±3.1</td>
<td>54.03±1.8</td>
<td>49.95±2.3</td>
<td>19.48±1.3</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>C + PYC</td>
<td>124.23±3.4</td>
<td>95.25±2.9</td>
<td>55.10±2.1</td>
<td>50.07±2.6</td>
<td>19.05±1.2</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>HFD/STZ</td>
<td>275.90±4.8</td>
<td>194.03±3.9</td>
<td>28.34±1.3</td>
<td>38.8±1.7</td>
<td>4.1±0.4</td>
<td>(+115.78%)</td>
</tr>
<tr>
<td>HFD/STZ + PYC</td>
<td>142.80±3.9</td>
<td>128.05±3.5</td>
<td>41.67±1.4</td>
<td>75.51±2.2</td>
<td>25.61±0.9</td>
<td>(+34.14%)</td>
</tr>
<tr>
<td>HFD/STZ + GL</td>
<td>172.32±2.9</td>
<td>153.12±2.8</td>
<td>38.54±1.9</td>
<td>103.15±2.1</td>
<td>30.62±1.1</td>
<td>(+29.26%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. The HFD/STZ group showed a significant decrease in HDL-C with the significant increase in TC, TG, FFAs, LDL-C and VLDL-C in the HFD/STZ group compared to the control group (P < 0.05 HFD/STZ vs. control group). PYC and GL treatment significantly ameliorated these parameters compared to the HFD/STZ group (P < 0.05 HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group).

### Table 10. Effect of PYC treatment on BUN, Scr and ALP level in the serum of control and HFD/STZ-induced experimental diabetic groups.

<table>
<thead>
<tr>
<th>Parameters/groups</th>
<th>BUN (mg/dL)</th>
<th>Scr (mg/dL)</th>
<th>ALP (units/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>18.70±0.26</td>
<td>1.05±0.009</td>
<td>24.87±0.07</td>
</tr>
<tr>
<td>C + PYC</td>
<td>20.00±0.34</td>
<td>1.10±0.02</td>
<td>26.05±0.04</td>
</tr>
<tr>
<td>HFD/STZ</td>
<td>50.75±1.32</td>
<td>2.67±0.009</td>
<td>42.22±1.22</td>
</tr>
<tr>
<td>HFD/STZ + PYC</td>
<td>29.80±0.13</td>
<td>1.89±0.05</td>
<td>31.21±1.09</td>
</tr>
<tr>
<td>HFD/STZ + GL</td>
<td>25.23±0.11</td>
<td>1.79±0.03</td>
<td>29.14±1.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). HFD/STZ group showed a significant increase in renal function markers (BUN, Scr and ALP) in the serum of the HFD/STZ group compared with the control group (P < 0.05 HFD vs. control group). PYC and GL treatment significantly ameliorated these parameters in the HFD/STZ + PYC or GL group compared with the HFD/STZ group (P < 0.05 HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group.
Fig 14. HFD/STZ group showed significant increase in TNF-α, IL-1β and NO levels compared with the control group (*P < 0.05 HFD/STZ vs. control group). PYC and GL supplementation significantly decreased these parameters compared with the HFD/STZ induced diabetic group (*P < 0.05 HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M (n = 8).
Fig. 15. (A). The HFD/STZ group showed significant increase in TBARS contents compared to the control group (P < 0.05 HFD/STZ vs. control group). PYC and GL significantly decreased TBARS contents in the HFD/STZ + PYC or GL group compared to the HFD/STZ induced diabetic group (P < 0.05 HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). (B). The HFD/STZ group showed significant increase in MDA contents compared to the control group (P < 0.05 HFD/STZ vs. control group). PYC and GL decreased MDA in the treatment groups compared to the HFD/STZ induced diabetic group (P < 0.05 HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M (n = 8).
Effect of PYC supplementation on PC in the HFD/STZ-induced diabetes in rats
PC content did not change by PYC in the control + PYC group compared to the control group (Fig. 16). PC content was significantly (P < 0.05) increased in the HFD/STZ group compared with control alone. PYC and GL significantly (P < 0.05) attenuated PC content in the HFD/STZ + PYC and HFD/STZ + GL group respectively, compared to the HFD/STZ group alone.

Effect of PYC supplementation on GSH in the HFD/STZ-induced diabetes in rats
The level of GSH did not affect by PYC in the control + PYC treated group compared to the control group. However, a significant (P < 0.05) depletion in GSH was observed in the HFD/STZ group compared to the control group. PYC or GL significantly (P < 0.05) increased GSH level in the treatment group compared to the HFD/STZ group (Fig. 17).

PYC treatment increased GST and CAT activity in the HFD/STZ-induced rat model of diabetes
Effects of PYC on the activity of GST and CAT in the HFD/STZ group and control groups (Fig. 18). The activity of GST and CAT in the control + PYC group was attenuated but the elevation was not significant compared to the control group. On the other hand, the activity of GST and CAT was depleted significantly (P < 0.05) in the HFD/STZ group compared to the control group. PYC and GL supplementation significantly (P < 0.05) increased the activity of these enzymes in the treatment groups compared to the HFD/STZ group.
Fig 16. The HFD/STZ group showed significant increase in PC content compared to the control group (\(P < 0.05\) HFD/STZ vs. control group). PYC and GL supplementation significantly decreased PC content in the treatment groups compared to the HFD/STZ group (\(P < 0.05\) HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M (n = 8).

Fig 17. The HFD/STZ group showed significant decrease in GSH content compared to the control group (\(P < 0.05\) HFD/STZ vs. control group). PYC and GL supplementation significantly increased GSH content in the treatment groups compared to the HFD/STZ group (\(P < 0.05\) HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M (n = 8).
Fig 18. (A). HFD/STZ group showed significant decrease in GST activity compared to the control group (\( \Delta P < 0.05 \) HFD/STZ vs. control group). PYC and GL supplementation significantly increased GST activity compared to the HFD/STZ induced diabetic group (\( \Delta P < 0.05 \) HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). (B). HFD/STZ group showed significant decrease in CAT activity compared to the control group (\( \Delta P < 0.05 \) HFD/STZ vs. control group). PYC and GL supplementation significantly increased CAT activity compared to the HFD/STZ induced diabetic group (\( \Delta P < 0.05 \) HFD/STZ + PYC or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M (n = 8).
HISTOPATHOLOGICAL FINDINGS

Effect of PYC supplementation on H and E staining in the HFD/STZ-induced diabetes in rats

The histological examination of the H and E-stained control liver section showed normal architecture of hepatocytes (Fig. 19). Liver section of HFD/STZ-induced diabetic group showed vacuolization of hepatocytes in the centrilobular area with variation of nuclear size and dilated sinusoidal spaces. The severity of degenerative changes was lessened by PYC supplementation in the HFD/STZ + PYC group compared to the HFD/STZ group. PYC supplementation did not show any remarkable effects in the group treated with PYC alone compared with the vehicle control group (data not shown). Photomicrograph from GL treated group showing regularly arranged liver lobules and portal triad with moderate hyalinization of hepatic arterial wall. The histological examination of the H and E-stained control pancreatic tissues showed normal architecture of islet cells (Fig. 20). Pancreatic section of the HFD/STZ group showed degenerated islet cells with vacuolization and lymphocytic infiltration into the islet and dilated sinusoidal spaces. The severity of degenerative changes was lessened by PYC in the HFD/STZ + PYC group compared to the HFD/STZ group. Pancreas of GL treated animal showing a small islet with normal exocrine glands surrounding it. A cluster of lymphocytes on the edge of the islet and numerous beta cells with abundant basophilic cytoplasm in the centre were seen in GL supplemented group. PYC supplementation did not show any remarkable effects in the group treated with PYC alone compared with the vehicle control group (data not shown).
Fig 19. Photograph from the control group at low power -100 x. showing normal liver architecture. PT = portal triad, CV = central vein, at high power-400 x. showing details with normal portal triad. PV = portal vein, BD = bile duct, HA = hepatic artery. The HFD/STZ group at low power-100 x. showing mild sinusoidal dilatation in the centrilobular area, at high power-400 x. showing vacuolated hepatocytes, variation in nuclear size and mild sinusoidal dilatation in the centrilobular area. PYC supplementation in the HFD/STZ + PYC group at low power-100 x. showing normal hepatic parenchyma with normal PT and CV, at high power-400 x. showing a normal PV and BD. Photograph from the HFD/STZ + GL group at low power -100 x. showing regularly arranged liver lobules. PT=Portal Triad, CV= Central Vein, at high power-400 x. showing a portal triad with moderate hyalinization of Hepatic arterial wall. BD= Bile Duct, HA=Hepatic Artery.
Fig 20. Photograph from the control group at low power -100 x. showing normal appearance of pancreatic tissue with a single islet of langerhans seen in the centre. The islet size is also within the normal range of 200 to 400 microns, at high power-400 x. showing details of cells in the islet. HFD/STZ group at low power- 100 x. showing changes in islet of langerhans. The exocrine tissue is within normal limits, at high power-400 x. degenerated islet cells with vacuolization is seen. There is also significant lymphocytic infiltration into the islet and the sinusoidal spaces are dilated. PYC in the HFD/STZ + PYC group at low power-100 x. showing pancreatic tissue with a small sized islet of langerhans seen, at high power-400 x. showing the islet cells appear normal. GL in the HFD/STZ + GL group at low power-100 x. showing a small islet with normal exocrine glands surrounding it. at high power-400 x. Islet showing a cluster of lymphocytes on the edge of the islet and numerous beta cells with abundant basophilic cytoplasm in the centre.
Protective effect of PYC in apoptotic-HFD/STZ produced damage

The apoptosis of cells in pancreas was studied by the TUNEL method and observed by fluorescence microscopy. As shown in Fig. 21, Control group showing the less number of apoptotic β-cell. HFD/STZ group caused higher percentage of β-cell apoptosis in the diabetic rats than the control animals. The increase in apoptotic β- cells was induced by HFD/STZ was reversed by the PYC treatment in HFD/STZ + PYC group, which reached similar to control animals.

Effect of PYC on the phosphorylation of Akt (pAkt) in the HFD/STZ rat

Akt is thought to be a downstream target of phosphatidylinositol (PI) 3-kinase. Defects in Akt activation may be, in part, secondary to factors characteristic for diabetic milieu. Immunoblot analysis showed (Fig. 22) that HFD/STZ- induced diabetic group caused a decrease in the expression of pAkt level as compared to control group. When HFD/STZ group was treated with PYC in HFD/STZ + PYC group, the pAkt expression increased significantly as compared to HFD/STZ animals. Thus PYC treatment for four weeks increased pAkt expression in liver significantly. Pyc showed the significant increased phosphorylated Akt (p-Akt)/total Akt (t Akt) ratio in HFD/STZ + PYC group compared to HFD/STZ only.
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Fig 21. Representative photomicrographs of TUNEL staining in the pancreas (×200). Control group showing the less number of pancreatic β-cell undergoing apoptosis. HFD/STZ group showing the large numbers of apoptotic β-cell. Treatment with PYC in the HFD/STZ + PYC group reduced the numbers of apoptotic β-cell.
Fig 22. Western blot analysis of p-Akt/Akt ratio from representative control, diabetic (HFD/STZ) and HFD/STZ + PYC rats. Values are mean ±SE (*p<0.05 HFD/STZ vs. Control; #p<0.05 HFD/STZ vs. HFD/STZ + PYC, n=5)
DISCUSSION

The present study demonstrated that diabetes in rats causes hyperglycemia accompanied by alterations in lipid profile, renal function, inflammatory markers and the presence of oxidative damage in the liver and pancreas. Moreover, treatment with PYC, by virtue of its anti-oxidant potential, significantly modulated diabetes-induced alterations and also morphological changes in the rat liver and pancreas. The therapeutic value of PYC possess a vast ethnomedical history, PYC has been proven to be used as protective agent in various disease conditions [Rohdewald, 2002; Rohdewald, 2005; Grimm et al., 2006]. PYC supplementation is associated with reducing platelet aggregation [Araghi-Nickman et al., 2000], lowering LDL-C, increasing HDL-C [Devaraj et al., 2002], and modifying hypertension [Hosseini et al., 2001]. Moreover, PYC has been shown to reduce serum glucose levels and to lower glycosylated hemoglobin (HbA1C) in patients with type 2 diabetes mellitus [Liu et al., 2004a, b]. Maritim et al. [2003] confirmed the reduction in glycemic levels and an increase in antioxidant enzymes with treatment of PYC in STZ-induced type 1 diabetic rats. Recently, we have also demonstrated the antioxidant potential of PYC on potassium dichromate-induced renal toxicity in rats [Parveen et al., 2009]. PYC is known to bind the proteins, thereby affecting both structural and functional characteristics of key enzymes and other proteins involved in metabolism [Kobuchii et al., 1999].

In the present study the antioxidant activity of the methanolic extract of PYC was investigated by using reducing power, $H_2O_2$ scavenging and DPPH scavenging assay of the extract. All the three methods have proven the effectiveness of the methanol extract compared to the reference standard antioxidant ascorbic acid (Fig. 1 a, b & c). The result showed that PYC consists of hydrophilic poly phenolic compounds that cause the greater reducing power. The decomposition of $H_2O_2$ by PYC may at least partly result from its antioxidant and free radical scavenging activity. The results obtained in the present study indicate that PYC exhibited free radical scavenging, reducing power and chelating activity.

During oxidative stress auto-oxidation and the presence of an excess of hydroxyl radical damaged to carbohydrates. This reaction is well evidenced by the production of thiobarbituric acid-reactive material, reactive carbonyl compound and malonaldehyde [Gutteridge, 1981; Morelli et al., 2003]. These effects are regarded as an important risk factor in the acceleration of chronic diseases including diabetes [Ceriello, 2000; Baynes and Thrope, 1999; Rosen, et al., 2001]. Overproduction of free radicals in diabetes could be due to due to a persistent chronic increase in blood glucose levels [Baynes and Thrope, 1999]. In the present study, the diabetic rats showed a significant increase in the blood glucose level at 60 min and was maintained for 120 min following glucose administration in OGTT. PYC
supplementation improved glucose tolerance at both time points. Because it significantly decreased blood glucose in glucose-loaded rats, we hypothesize that PYC may enhance glucose utilization by peripheral tissues, and increase the glycogen stores in the liver by restoring delayed insulin response. In our study, diabetic group exhibited a hyperglycemic condition while PYC treatment reduced the elevated blood glucose level thereby showing its antihyperglycemic activity. It is well known that during diabetes insulin secretion is impaired and β-cells fail to secrete sufficient insulin to correcting prevailing hyperglycemia. In our study serum insulin content decreased in diabetic group consistent with the previous studies [Kergoat and Portha, 1985; James et al., 2006; Mamdouh and Fatma, 2009]. Treatment with PYC and GL significantly restored insulin content after 4 weeks treatment. Elevated insulin production by PYC treatment may also be responsible for the improved glucose level. In diabetes, there is an increased glycosylation of a number of proteins, including hemoglobin and β-crystalline of the eye lens [Alberti and Press, 1982]. Estimation of HbA1c has been found to be particularly useful in monitoring the effectiveness of therapy in diabetes [Goldstein, 1995]. In our study, the HbA1c level was found to be significantly increased in the diabetic group. Agents with antioxidant or free radical scavenging properties may inhibit oxidative reactions associated with protein glycation [Elgawish et al., 1996]. Administration of PYC reduced the glycosylation of hemoglobin by means of its free radical scavenging property and thus decreased the level of HbA1c. A decrease in blood glucose level might also contribute to the decreased level of glycated hemoglobin in the PYC and GL-treated groups. The liver is primarily responsible for maintaining normal blood glucose concentrations via its ability to store glucose as glycogen and its ability to produce glucose from glycogen breakdown or from gluconeogenic precursors. It has been previously demonstrated that glycogen deposition from glucose is impaired in diabetic animals [Bollen et al., 1998]. In our study, the hepatic glycogen content in the diabetic group decreased compared to the PYC and GL treated groups. This antidiabetic potential of PYC is consistent with previous studies which showed hypoglycemic and glycosylated hemoglobin (HbA1C) lowering effects of PYC in the diabetic patients [Maritim et al., 2003; Liu et al., 2004a].

Clinical studies in diabetic patients have shown that the impairment of pancreatic exocrine functions is due to insulin deficiency during T1DM [Hardt et al., 2000; Lernmark, 2000]. Insulin promotes the synthesis of pancreatic amylase and, under certain circumstances, has been shown to control mRNA content for this enzyme [Korc et al., 1981]. In our study, STZ-induced diabetic group exhibited decreased amylase activity and insulin level, and PYC treatment (STZ plus PYC group) or GL treatment (STZ + GL) increased both of these thereby correcting insulin level is related to improvement in amylase activity.

[193]
Dyslipidemia is one of the major cardiovascular risk factors. It has been demonstrated that insulin deficiency in DM leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulation of lipids such as TC and TG in diabetic patients [Goldberg, 1981]. Our data were in line with this notion as the model of diabetes exhibited clear-cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TC, TG, LDL-C, VLDL-C, FFAs and reduction of HDL-C levels.

Treatment with PYC for four weeks was sufficient to reverse these parameters in diabetic rats and was more prominent than standard GL. As decreased insulin levels and/or insulin sensitivity of peripheral tissues is the principal cause dyslipidemia during diabetes [Shigeru and Nobuhiko, 2002; Burton and David, 2005]. Therefore, the observed reversal of dyslipidemia upon PYC treatment could be due to the increased insulin activity and/or sensitivity. Thus, our results indicate that, PYC has a lipid-lowering effect on diabetic rats.

Evidence suggests that DM cause loss of cell membrane permeability and there is loss of functional integrity in the kidney [Im and Kim, 1995; Stumvoll et al., 1997]. An increase in Scr and ALP levels is indicative of impairment of renal functions [Parveen et al., 2009, Braulich et al., 1997]. The data obtained from the present work clearly show increased levels of kidney functional markers in serum (BUN, Scr, and ALP) in the both diabetic groups. In contrast, PYC or GL supplementation group showed a significant reduction in these markers, thus showing its ability to protect against diabetes-induced kidney damage.

Nitric oxide (NO), is a potent vasodilator, produced in vascular endothelium [Leclercq et al., 2002], where it plays a central role in modulating endothelial function. However, during hyperglycemia an overproduction of both superoxide and nitric oxide favors the production of a cytotoxic product, the peroxynitrite anion (ONOO). Moreover, studies suggest that inflammatory parameters, including inflammatory cytokines TNF-α and IL-1β, are involved in the autoimmunity process leading to pancreatic β-cell damage and the induction of T1DM [Lechleitner et al., 1999; Mandrup-Poulsen et al., 1990]. In the present study, NO, IL-1β and TNF-α level increased significantly in the serum of the STZ group. Treatment with PYC and GL significantly ameliorated these parameters.

Lipids and proteins, the major structural and functional components of the cell membrane, are the target of oxidative modification by free radicals. There is extensive evidence that lipid peroxidation and protein oxidation lead to loss of membrane integrity, an important factor in acceleration of DM [Baynes and Thrope, 1999, Mariith et al., 2003; Im and Kim, 1995]. LPO is frequently used as an index of tissue oxidative stress, in which oxygen interacts with polyunsaturated fatty acids and leads to the formation of lipid products such as MDA and 4-
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HNE, and increase generation of free radicals, which then leads to damage to membrane components of the cell, cell necrosis and inflammation [Salminen and Vihko, 1983]. MDA is end product of lipid peroxidation and the increased MDA production plays an important role in the progression of diabetes [Ilhan et al., 2001] by altering the transbilayer fluidity gradient, which could hamper the activities of membrane-bound enzymes and receptors. Increased generation of free radicals can also lead to the formation of protein–protein crosslinkages, oxidation of protein backbones resulting in protein fragmentation, and modification of amino acid side chains, which includes oxidation of sulphhydryl moieties and formation of PC [Berlett and Stadtman, 1997]. LPO may be enhanced due to the depletion of GSH content, which is often considered the first line of defense as an endogenous antioxidant. The liver plays a major role in glutathione homeostasis and is the main export organ for glutathione [Anuradha and Balakrishnan, 1998]. Increased oxidative stress can change the redox potential of GSH [Irshad and Chaudhri, 2002] due, it has been proposed, to oxidation of its thiol group [Jill Startman and Lardy, 1988]. Decreased levels of GSH in the liver of diabetic rats may increase susceptibility to oxidative damage. The depletion of GSH content also may lower antioxidant enzyme GST activity, because GSH is required as a substrate for GST activity. CAT and SOD are the two major scavenging enzymes that remove radicals in vivo. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (•OH), resulting in initiation and propagation of lipid peroxidation. SOD can catalyze dismutation of O_2^- into H_2O_2, which is then deactivated to H_2O by CAT or glutathione peroxidase [Aebi, 1984; Kumuhnkar and Katyan, 1992]. GPx catalyzes the reaction of hydroperoxides with GSH to form glutathione disulphide. GPx uses GSH as a proton donor, converts H_2O_2 to water and molecular oxygen; in this process GSH is oxidized to GSSG, which is reconverted to GSH by the action of enzyme GR, thus maintaining the pool of GSH. A significant decrease in GPx and GR activity could suggest inactivation by reactive oxygen species, [Haung and Philbert, 1996], which are increased in diabetic rats. The decrease may also be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes [Jain, 1998; Ugochukwu et al., 2004]. It has been demonstrated that the activity of these antioxidant enzymes (GST, CAT, SOD, GPx and GR) decrease in diabetic rats [Anuradha and Selvam, 1993; Jin et al., 2008; Ibrahim and Rizk, 2008]. In the present study, we observed the increased levels of TBARS (as an active index of rate of LPO, MDA (a final product of LPO) and PC with a concomitant decrease in GSH and antioxidant enzyme (GST, CAT, SOD, GPx and GR) activity in the liver and pancreas of diabetic rats. Treatment with PYC significantly prevented lipid and protein from oxidation by enhancing the level of GSH and the status of antioxidant enzymes in the PYC treated group. Antioxidant efficacy of PYC was found to
be more pronounced than GL. These effects indicate that PYC may scavenge or inhibit free radical formation and participate in stabilizing the endogenous antioxidant network, including GSH, and concomitantly decrease LPO in various conditions caused by free radicals, consistent with previous studies [Maritim et al., 2003; Packer et al., 2004; Rohdewald, 2002; Pietta, 2000; Grimm et al., 2006; Liu et al., 2004a]. PYC has been shown to alter the expression of genes [Park et al., 2000], enzymes [Hasegawa, 2000] and other molecules [Peng et al., 2000] which are involved in antioxidant defenses.

Our findings are also supported by histopathological observations. Sections from the liver of the diabetic group showed vacuolization of hepatocytes in the centrilobular area, with variation of nuclear size and dilated sinusoidal spaces. All these degenerative changes were ameliorated with PYC supplementation, indicating hepatoprotective effect of PYC as a potent antioxidant. GL treatment brought degenerative changes back to normal appearance. Our results were also supported by histological examination of the pancreatic sections. Control pancreatic sections showed normal architecture of islet cells while diabetic groups showed vacuolization and more lymphocytic infiltration into the islet and dilated sinusoidal spaces. The severity of degenerative changes was decreased by PYC treatment compared with the diabetic groups, indicating a partial protective effect of PYC in diabetic animals. An increased number of islets were also observed in the pancreatic sections taken from diabetic rats supplemented with glibenclamide.

Our results were further supported with immunohistochemical analysis on pancreatic sections. The present study corroborated that pancreatic β-cells were destroyed by STZ [Murugan and Pari, 2006]. A significant decrease in insulin immunoreactivity and the number of immunoreactive β cells was observed as in the STZ group compared to control group. However, PYC supplementation improved the insulin immunoreactivity and increased the number of immunoreactive β cells in the treatment group. Thus, PYC may have the ability to enhance insulin secretion or regenerate the β cells in Type 1 diabetic rats. The protective effect of PYC could be due to its direct influence on the endocrine pancreatic function in diabetic animals like drug THC [Pari and Murugan, 2005]. From results it is obvious PYC stimulate the β-cells to secrete insulin in STZ-induced diabetic rats, resulting in the improvement of carbohydrate metabolism towards the re-establishment of normal blood glucose level. Thus, PYC supplementation may help in maintaining the blood glucose homeostasis.
In the present study, we demonstrated the protective effect of PYC in the apoptotic damage induced by HFD/STZ to pancreatic β-cells. Our data demonstrate that HFD/STZ produced apoptotic damage in pancreatic islets, producing higher apoptotic damage in diabetic rats. While treatment with PYC reverse these effect due to its antioxidant property.

Akt (also termed PKB or Rac) is a 60 kDa serine threonine kinase, which is stimulated by a wide range of receptor tyrosine kinase [Burgering and Coffer, 1995]. Akt is thought to be a downstream target of phosphatidylinositol (PI) 3-kinase [Datta et al., 1996]. Several lines of evidence suggest that PI 3-kinase is both necessary and sufficient for insulin activation of Akt activity [Burgering and Coffer, 1995]. Furthermore, Akt/protein kinase B (Akt) has been implicated in regulation of a number of cell functions including glucose uptake, glycogen synthesis, cell growth, survival, apoptosis, protein synthesis, and endothelial nitric oxide production [Shiojima and Walsh, 2002]. Recently, Akt activity has been reported to be essential for activation of glycogen synthase by insulin [Kitamura et al., 1998]. The data of the present study demonstrating that diabetic rats caused a decrease in Akt phosphorylation in liver, Since Akt activity is essential for activation of glycogen synthase, a reduction in Akt phosphorylation in liver of HFD/STZ diabetic rats may certainly contribute to insulin resistance observed in these animals. The phosphorylation status of Akt was increased significantly in the PYC treated rats when compared to HFD/STZ-induced diabetic group, we can suggest that the increase in expression of pAkt level may contribute to molecular mechanism by which PYC improves insulin action.

Other than its antioxidant effects, plant inhibited the expression of nitric oxide synthase and helped in correcting the secretary defects in diabetes [Fukuda et al., 2001; Gunawardana et al., 2008]. The one possible mechanism of action of PYC might be that it stimulated the beta islets to secrete insulin by inhibiting the expression of nitric oxide synthase [Kobuchi et al., 1999]. This prevents depletion of glycogen in the liver possibly due to stimulation of insulin release from β-cells [Lolitkar and Rao, 1966]. Elevated insulin production by PYC treatment may be responsible for the alteration in glycogen content of the liver. PYC also has been found to stimulate glucose transporter 4 (Glut4) expression by regulating the kinases (p38 MAPK and phosphoinositol 3-kinase) which induce insulin resistance by affecting expression of Glut4 and insulin signaling [Lee et al., 2008]. Conclusively PYC stimulate glucose uptake due to presence of insulin-like effects in insulin sensitive cells that could help to explain their anti-hyperglycemic effect in vivo. Furthermore studies have suggested hyperglycemia and oxidative stress is linked to nuclear factor kappa-B (NF-κB) activation [Schreck et al., 1992]. Furthermore NF-κB is involved in regulations of COX-2 and iNOS expressions [Mohamed et al., 1999], which also play a role in hyperglycemia, and oxidative
stress [Surh et al., 2001]. PYC administration can counteract cytokine-induced activation of NF-κB [Fujimoto et al., 2005]. The inhibition of platelet aggregation by PYC is another important finding for treatment of type 2 diabetes patients [Araghi-Nickman et al., 1999]. Other possible mechanisms of action by which PYC show glucose lowering effect is due to presence of its constituent oligomeric procyanidin, monomeric epicatechin and catechin. These forms act through different mechanisms: procyanidin inhibit α-glucosidase activity [Schafer and Høgger, 2007], epicatechin induces pancreatic β-cell regeneration [Kim et al., 2003], catechin inhibits intestinal glucose absorption [Shimizu et al., 2000].

In conclusion, the anti-hyperglycaemic effect of PYC was comparable with that of glibenclamide. However, the hypolipidemic and antioxidant effects of the PYC were found to be more potent than those of glibenclamide. Our results showed that PYC through its antioxidant properties enhanced endogenous GSH levels and thereby conferred protection against DM in rats. Further, study suggests that PYC is effective in preventing diabetic complications such as hyperglycemia, hyperlipidemia, renal function alteration and oxidative damage and provide pancreatic β-cell protection. Thus, PYC may be considered as a potential candidate in the armamentarium of drugs for prophylactic treatment in diabetic patients, pending further investigations to trace out the exact mechanistic pathways.