Summary & Conclusion
Diabetes mellitus is an endocrine and metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Despite the fact that it has worldwide a high prevalence, morbidity and mortality it is regarded as a non-curable but controllable disease. Pharmacotherapy involves insulin injection and oral hypoglycemic drugs, but their use is complemented with side effects including haematological effects, coma and disturbances of liver and kidney functions.

Streptozotocin and alloxan are the most frequently used drugs for pharmacological induction of diabetes and these models has been useful for the study of multiple aspects of the disease. The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species, but both drugs differ in their mechanism of action (Federiuk et al., 2004; Lei et al., 2005). Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreatic β-cells (Szudelski, 2001). The range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic and may cause the loss of many animals. Streptozotocin enters the pancreatic β-cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore, STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic β-cells are destroyed by necrosis (Mythili et al., 2004).

Increased blood glucose level was observed in alloxan and streptozotocin and induced diabetic rats when compared to normal. Alloxan and streptozotocin induced massive reduction in insulin release is mediated by the destruction of the β cells of the islet of Langerhans. Treatment with the *P. rimosus* and glibenclamide showed a significant reduction in the glucose level in diabetic animals.

Serum insulin level was found to be significantly reduced in streptozotocin treated rats when compared to that of normal. This decreased insulin was reverted to normal by the administration of *P. rimosus* and glibenclamide, which suggests the antihyperglycemic role of the *P. rimosus* extract. The possible mechanism by which the herbal formulation mediates its antidiabetic action may be by potentiation of pancreatic secretion of insulin.
from existing β cells of islets or due to enhanced transport of blood glucose to peripheral tissues.

As an index of liver and kidney damage produced by streptozotocin, significant rise in concentration of serum SGOT, SGPT, ALP, urea and creatinine was observed in STZ induced diabetic rats. Treatment with *P. rimosus* normalised the levels of all biochemical constituents comparable to that of glibenclamide, these indicated that the *P. rimosus* prevents the progression of hepatic and renal damage in diabetic rats. There was a significant decrease in liver glycogen levels in liver of rats exposed to alloxan and streptozotocin. Glycogen levels were restored to normal by treatment with the *P. rimosus* extract.

The potentially imprudent derivatives of oxygen, endorsed as ROS such as O$_2^-$, H$_2$O$_2$ and ‘OH radical are incessantly generated within the human body as a consequence of revelation to a superfluity of exogenous chemicals in our ambient milieu and/or a number of endogenous metabolic processes linking redox enzymes and bioenergetic electron transmit. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA. This oxidative damage is a decisive etiological factor concerned in quite a number of chronic human diseases such as cancer, atherosclerosis, arthritis, diabetes mellitus, and neurodegenerative diseases and also in the ageing process (Hogg, 1998). Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the expediency of antioxidants in protection against these diseases is defensible.

Oxygen-derived free radicals may potentially play an important role in the pathophysiology of vascular complications associated with diabetes. It is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to heart and kidney. Glucose level increase the production of free radicals, cell damage markers such as malondialdehyde and conjugated dienes. Alterations of metabolic processes in diabetes also influence enzymatic defences and these changes may be associated with late complications of diabetes.
Alloxan and streptozotocin induced diabetic rats showed a significant increase of TBARS. Oxidative damage induced by alloxan and streptozotocin resulted in the formation of highly reactive hydroxy radical, which stimulates the lipid peroxidation that causes destruction and damage to the cell membrane. Treatment with the *P. rimosus* extract reduced the level of lipid peroxides indicating the effective antioxidant property of the *P. rimosus* in the moderation of tissue damage.

There is clear cut evidence to show the role of free radicals in diabetes and studies indicate that tissue injury in diabetes may be due to free radicals. Antioxidative enzymes form the first line of defense against ROS in the organism. The diabetogenic action of streptozotocin and alloxan can be prevented by antioxidant enzymes, such as SOD, CAT and GPx. In addition to these enzymes, Glutathione reductase (GR) and Glutathione S-transferase (GST) provide glutathione and help to neutralize toxic electrophiles, respectively. We have also observed the decrease in GSH levels and SOD, CAT and GPx, activities in liver, kidney, pancreas and plasma in diabetic rats. Similarly mitochondrial SOD, GPx and GSH levels in kidney was also found to be decreased in the kidney of streptozotocin induced diabetic rats. This could be attributed to higher levels of superoxide radicals and hydrogen peroxide as indicated by increased ROS levels in these rats, which reduced the antioxidant enzymes activities. Following the treatment with *P. rimosus* extract the activities of SOD, CAT and GSH level was augmented in blood, pancreas, liver and kidney of diabetic rats. Similarly SOD and GSH level in kidney mitochondria was also improved. This could be attributed to the strong antioxidant property of the *P. rimosus* extract.

In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis, resulting in hyperlipidemia. Significant change in lipid metabolism and structure occur in diabetes. The structural changes are clearly oxidative in nature and are associated with development of vascular disease in diabetes. The increase in blood glucose level, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL), LPO level with decrease in high density lipoprotein cholesterol (HDL-C) were the salient features observed in diabetic rats. On the other hand, oral administration of *P. rimosus* resulted in a significant reduction in fasting blood
Mitochondrial electron transport chain (ETC) is considered as a major intracellular source of ROS (Boveris and Chance, 1973). Mitochondrial membrane lipid peroxidation results in irreversible loss of mitochondrial functions such as mitochondrial respiration, oxidative phosphorylation and ion transport (Bacon and Britton, 1990). The mitochondrial enzymes (ICDH, SDH, MDH and α-KGDH) catalyse the oxidation of several substrates through the tricarboxylic acid (TCA) cycle, yielding reducing equivalents which are channeled through the respiratory chain for the synthesis of adenosine triphosphate (ATP) by oxidative phosphorylation. Inhibition of these enzymes by ROS may affect the mitochondrial substrate oxidation, resulting in reduced oxidation of substrates, reduced rate of transfer of reducing equivalents to molecular oxygen and depletion of cellular energy (Capetenaki, 2002). Earlier studies in diabetic rats (Hu et al., 2009) have observed decreased expression and activity of complexes II and IV in cardiac myocytes exposed to high glucose. Renal Complex-III appears to be an early and specific mitochondrial target during experimental type-1 diabetes (Shankar et al., 2009). Complex-III is centrally located in the electron transfer process and has been implicated as one of the major sites for superoxide generation in the mitochondria during diabetes. The impairment of complex I and III activities may increase the electron leakage from the ETC, generating more superoxide radical and perpetuating a cycle of oxygen radical-induced damage to mitochondrial membrane constituents (Paradies et al., 2002).

Early stages of diabetes induced alterations in respiratory chain complexes, increased ATP synthase activity, and renal dysfunction. These diabetes-induced alterations in activities of mitochondrial complexes and energy status could contribute to the underlying role of oxidative stress in the pathogenesis of diabetic nephropathy. The administration of P. rimosus had significantly protected the renal mitochondria by enhancing the renal antioxidant status and also showed considerable increase of TCA enzymes such as ICDH, α-KGDH, MDH, SDH activities and respiratory chain complexes I, II and IV thereby restoring the mitochondrial functional status in diabetic rats. Finally, strategies to limit the extent of renal mitochondrial damage during hyperglycemia (by glucose, TC, TG, LDL-C, VLDL-C and tissue LPO levels coupled with elevation of HDLC.

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therapeutic agents that will specifically modulate mitochondrial function) might prevent or inhibit the development of nephropathy in the diabetic population.

Histopathological evaluation reveals that alloxan and streptozotocin caused extensive damage in pancreatic β-cells, such as a decrease of islet cells’ number, cell necrosis. In fact, the cytotoxic action of this diabetogenic agent is mediated by the formation of ROS (Heikkila et al., 1976) with a simultaneous massive in cytosolic calcium concentration (Kim et al., 1994; Park et al., 1995). Administration of *P. rimosus* extract to alloxan and streptozotocin induced diabetic rats restored partially the normal pancreatic islets. Histopathological analysis of the Liver and kidney tissues also showed the protective effect of the extract against diabetes induced tissue damage.

The *in vitro* free radical scavenging studies of the aqueous ethanol extract of *P. rimosus* showed that the extract efficiently scavenged the free radical generated in the *in vitro* system. The extract showed both first and second line of defense against free radicals. It efficiently carried out the reduction, removal as well as suppression of free radical formation. The extract showed significant superoxide radical (IC$_{50}$ 96.56 ± 30.41 µg/ml), hydroxyl radical (IC$_{50}$ 300.99 ± 25 µg/ml) and nitric oxide radical (IC$_{50}$ 310.29 ± 19.43 µg/ml) scavenging activities. The FRAP assay indicted the non enzymatic antioxidant activity of the extract. In the second line of defense, the antioxidants will suppress chain initiation and / or break chain propagation reaction. In the DPPH assay the extract significantly removed the DPPH radical from the system by suppressing chain initiation (IC$_{50}$ 40.54 ± 6.71 µg/ml). The ABTS radicals generated *in vitro* in the system by the reaction between ABTS and ammonium persulphate were efficiently removed by the extract (IC$_{50}$ 14.27 ± 6.3).

Lipid peroxidation, being a chain reaction, is one of the most important organic expressions of oxidative stress. Following peroxidation of ω - 6 and ω -3 polyunsaturated fatty acids (PUFAs), relatively unstable fatty acids hydroperoxides may be converted into more stable carbonyls such as malondialdehyde (MDA). In fact, MDA formation, often assayed with thiobarbituric acid (TBA) assay, is the most widely used index of lipid peroxidation in human and animal studies. The *in vitro* lipid peroxidation induced by Fe$^{2+}$-
ascorbate system in rat liver was found to be reduced significantly by the extract (IC\textsubscript{50} 405.75 ± 33.19).

Experimental and epidemiological studies have shown that the plasma hypercholesterolemic state could contribute to the development of atherosclerosis and related cardiovascular system diseases (CVD) which are the most common cause of death in both western and eastern societies. Indeed, clinical trials have demonstrated that the increase in plasma low density lipoprotein cholesterol (LDL-C) levels is implicated in the early development and progression of atherosclerosis. However, high density lipoprotein cholesterol (HDL-C) is an anti-atherogenic fraction. Triglycerides (TGs) may also be a risk factor, especially in individuals with diabetes (West et al., 1983).

Triton WR-1339, a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to produce acute hyperlipidaemia in animal models in order to screen natural or chemical drugs (Schurr et al., 1972) and to study cholesterol and triacylglycerol metabolism (Zeniya and Reuben, 1988). The accumulation of plasma lipids by this detergent appears to be especially due to the inhibition of lipoprotein lipase activity (Hayashi et al., 1981).

Epidemiological and experimental studies have suggested that high dietary fat intake of rats is associated with many physically degenerative diseases. Since oxidative stress and abnormal lipid metabolism have been speculated to be critical mechanisms underlying degenerative diseases, we hypothesized that a high-fat (HF) diet might induce oxidative stress or lipid oxidation and subsequently contribute to the high risk of some diseases such as cardiovascular and cerebrovascular ones. Administration of \textit{P. rimosus} to rats resulted in a significant decline in levels of serum triglyceride, total cholesterol, serum low-density lipoprotein cholesterol, while the serum high-density lipoprotein cholesterol was significantly increased in HCD and Triton induced hyperlipidemic rats. In addition, the heart and liver content of thiobarbituric acid related substances, a lipid peroxidation product, significantly decreased, while the superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (Gpx) activities and glutathione (GSH) levels were significantly increased in HCD induced hyperlipidemic rats.
3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA) is a key rate limiting enzyme involved in the cholesterol biosynthetic pathway (Goldstein and Brown, 1990). Therefore, inhibition of HMG-CoA reductase decreases intracellular cholesterol biosynthesis (Steinberg et al., 1989). HMG CoA reductase inhibitors are the most commonly prescribed class of lipid lowering drug. HMG CoA reductase inhibitors have a favorable profile in the reduction of lipoprotein concentrations. In the present study, hepatic HMG-CoA reductase activity was significantly decreased after treatment with \textit{P. rimosus} in HCD and triton induced hyperlipidemic rats compared to normal. The decrease has been ascribed to inhibition of HMG-CoA reductase activity by exogenous cholesterol, resulting in up regulation in order to supply other essential products of synthetic pathways downstream of the enzyme. From the results, it is evident that cholesterol- lowering effect of \textit{P. rimosus} and atorvastatin are due to the decreased cholesterol biosynthesis by inhibiting the activity of HMG CoA reductase.

The acute and sub acute toxicity studies of aqueous ethanol extract of \textit{P. rimosus} have been performed. The extract up to a dose of 2500 mg/kg body weight orally did not produce any external symptoms of toxicity or mortality. In subacute toxicity studies, treatment with the different concentration of the extract (50 and 250 mg/kg) were not able to produce any statistically significant change in the hematological or biochemical parameters compared to the normal group of animals. Doses selected for subacute toxicity were based on the biological effects produced by these selected doses. Results of the acute toxicity clearly indicated the non toxic nature of these extract; compounds. The histopathological examination of the liver and kidney tissues of the treated animals also supports the non toxic nature of the extract, protein bound polysaccharides and total triterpenes.

In the present study, an attempt has also been made to identify the major classes of phytochemicals present in \textit{P. rimosus} extract. Preliminary phytochemical analysis revealed the presence of terpenoids, saponins, quinones, phenolics and flavonoids. While the tests for alkaloids, steroids and coumarins were found to be negative with the extracts. Further, the phytochemical evaluation of the aqueous ethanol extract of \textit{P. rimosus} showed the presence of polysaccharides and proteins. The extract contains 13-15% carbohydrate and
35% protein contents. The HPTLC analysis showed that the extract contains 9 compounds corresponding to the 9 peaks.

The results of the investigations indicate that that aqueous ethanol extract of *P. rimosus* possesses profound antioxidant, antidiabetic and hypolipidemic activity. The study also showed that *P. rimosus* is capable of protecting the renal mitochondria against oxidative stress and by maintaining the mitochondrial antioxidant status, mitochondrial ATP levels, activities of mitochondrial enzymes such as TCA and ETC complexes. Most of the activities elicited by the extract can be attributed to its profound antioxidant property. The results of the investigations indicate the promising therapeutic value of these mushroom.