Abstract
Mushrooms have become attractive as a functional food and an important source of physiologically beneficial medicines and nutraceuticals. They are ubiquitous in nature and produce various classes of biologically active primary and secondary metabolites. Among them, mushrooms in the genus *Phellinus* have received great attention recently due to its long history of use in oriental countries and its medicinal values for the treatment of many diseases such as stomachaches, inflammation, arthritis, gastroenteric dis-orders, tumors and lymphatic disorders. *Phellinus rimosus* is a parasitic host specific polypore mushroom often found growing on jack fruit tree trunks in Kerala. It is a less extensively studied species of the genus *Phellinus*. No study has been carried out on its antidiabetic and hypolipidemic effect of this mushroom. Thus, in the current study, the aqueous ethanol (70%) extract of *P. rimosus* was evaluated for their antidiabetic and hypolipidemic effects. Again, the *in vitro* antioxidant activities, toxicity studies, and phytochemical analysis of the extract were also examined.

The increasing prevalence of diabetes mellitus worldwide is an issue of major socio-economic concern. Diabetes mellitus is a complex and a multifactorial group of disorders that disturbs the metabolism of carbohydrates, fat and protein. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs. Diabetic complications arise partly from glycosylation damage to structural and functional proteins and reflect chronic failure to maintain blood glucose homeostasis. Other complications such as retinopathy, neuropathy and cardiomyopathy prevail as a result of hyperglycemia.

The present study was aimed to investigate the effects of aqueous ethanolic extract of *P. rimosus* on streptozotocin (STZ) and alloxan induced diabetes in rats by measuring fasting blood glucose, serum insulin, lipid profiles (atherogenic index), lipid peroxidation (LPO) and activities of both non-enzymatic and enzymatic antioxidants and histopathological changes of kidney, liver and pancreas. The activities of mitochondrial dehydrogenases as well as on respiratory chain complexes and ATP level in the renal mitochondria of streptozotocin induced diabetic rats were also investigated. Diabetes was induced by single intraperitoneal injection of STZ (45 mg/kg) in streptozotocin model and alloxan (140 mg/kg) in alloxan model to Wistar rats.
An increased reactive oxygen species and insufficient antioxidant activity is associated with diabetes mellitus, which is mainly responsible for diabetic pathogenesis. The role of extract on antioxidant markers of blood, pancreas, liver and kidney were estimated. The diabetic rats exhibited lower activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and reduced glutathione (GSH) content in pancreas, hepatic and renal tissues as compared with normal rats. The activities of SOD, CAT, GPx and GSH were found to be increased in *P. rimosus* treated diabetic rats. The increased level of lipid peroxidation (thiobarbituric acid reactive substances and hydroperoxide) in diabetic rats was also found to be reverted back to near normal status in extract treated groups. *P. rimosus* ameliorated the pathological changes in the kidney, liver and tissues. Glibenclamide was used as reference and showed similar antidiabetic effect.

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* at doses of 250 and 50 mg/kg for 30 days in streptozotocin model and 10 days for alloxan model resulted in a significant reduction in fasting blood glucose, TC, TG, LDL-C, VLDL-C and tissue LPO levels coupled with elevation of HDL-C.

Mitochondrial electron transport chain (ETC) is considered as a major intracellular source of ROS. Mitochondrial membrane lipid peroxidation results in irreversible loss of mitochondrial functions such as mitochondrial respiration, oxidative phosphorylation and ion transport. Early stages of diabetes induced alterations in respiratory chain complexes, 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 and SDH activities and respiratory chain complexes I, III and IV thereby restoring the mitochondrial functional status in diabetic rats. *P. rimosus* treatment also elevated the mitochondrial ATP level by enhancing the Krebs cycle enzyme activity as well as the activity of complexes I and IV. Finally, strategies to limit the extent of renal mitochondrial damage during hyperglycemia (by therapeutic agents that will specifically modulate mitochondrial function) might prevent or inhibit the development of nephropathy in the diabetic population.

Liver plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes. Liver and kidney participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. *P. rimosus* treatment decreased the activities of SGOT, SGPT, ALP and levels of serum urea and creatinine compared to diabetic control rats, indicate the protective role against liver and kidney damage. The results suggest that *P. rimosus* could be used, as a dietary supplement, in the treatment of chronic diseases characterized by atherogenous lipoprotein profile, aggravated antioxidant status and impaired glucose metabolism and also in their prevention.

Cardiovascular disease is a leading cause of global mortality, accounting for almost 17 million deaths annually. Atherosclerosis, in particular, is the main contributor for the pathogenesis of myocardial and cerebral infarction. Elevated levels of plasma low-density lipoprotein cholesterol (LDL) and triglycerides, accompanied by reduced high-density lipoprotein (HDL) levels, is often associated with an increased risk of coronary heart disease. The effect of *P. rimosus* on hyperlipidemia induced by triton WR 1339 (Tyloxapol: a nonionic detergent, oxyethylated tertiary octyl phenol formaldehyde polymer) and high cholesterol diet has not yet been studied. In triton model to study the hypolipidemic drugs, triton WR 1339 is administered (300 mg/kg i.p) in rats to produce hypercholesterolemia by accelerating hepatic cholesterol synthesis. While in second model, hyperlipidemia is produced by feeding high cholesterol diet.

Triton WR-1339 has been widely used to produce acute hyperlipidaemia in animal models in order to screen natural or chemical drugs and to study cholesterol and triacylglycerol metabolism. The accumulation of plasma lipids by this detergent appears to
be especially due to the inhibition of lipoprotein lipase activity. Serum was analysed 24 hr after triton administration for lipid profile and the activity were compared to the cholesterol-lowering drug, atorvastatin (2.5 mg/kg). Lipid profile was reverted back to near normal values after *P. rimosus* treatment or standard drug atorvastatin. Lipid peroxidation was found to be decreased.

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. The hypolipidemic and antioxidant effects of the *P. rimosus* were evaluated in rats that were fed 4 weeks of high-fat diet.

Administration of *P. rimosus* to rats with high-fat diet 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 addition, the heart and liver content of thiobarbituric acid related substances significantly decreased, while the superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) activities and glutathione (GSH) levels were significantly increased.

Furthermore *P. rimosus* extract on both models showed a significant ameliorative action on elevated atherogenic index (AI) and LDL/HDL-C ratios. *P. rimosus* treatment also ameliorated the pathological changes in the liver tissue. These results suggest that *P. rimosus* consumption can improve lipid profiles, inhibit peroxidation, and increase the activity of antioxidant enzymes, and is thereby likely to reduce the risk of coronary heart disease associated with hyperlipidemia and oxidative stress.

The *in vitro* antioxidant activity was evaluated by different methods such as FRAP assay, DPPH assay, lipid peroxidation inhibition assay, hydroxyl radical scavenging assay, superoxide radical scavenging assay, nitric oxide radical scavenging activity and ABTS radical scavenging assay. The results showed that the aqueous ethanol extract of *P.*
*Phellinus rimosus* possess significant and dose dependent antioxidant activity and radical scavenging ability. BHA, the synthetic antioxidant was used as the positive standard.

The acute and sub acute toxicity studies of aqueous ethanol extract of *P. rimosus* have been performed. The extract upto a dose of 2500 mg/kg body weight 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 changes in the hematological or biochemical parameters compared to the normal group of animals. Results of these toxicity studies clearly indicated the non toxic nature of these extract. The histopathological examination of the liver and kidney tissues of the treated animals also supports this.

Further, the phytochemical evaluation of the aqueous ethanol extract of *P. rimosus* showed the presence of polysaccharides, phenolics, saponins, quinones flavonoids, terpenoids and proteins. The extract contains 13-15% carbohydrate and 35% protein contents. TLC and HPTLC analysis indicated the presence of several components in the extract. The HPTLC analysis showed that the extract contains 9 compounds corresponding to the 9 peaks.

The study concluded 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 antioxidant property. The results of the investigations indicate the promising therapeutic value of these mushroom.

**Key Words:** *Phellinus rimosus*, Mushroom, Antidiabetic, Streptozotocin, Alloxan, Mitochondria, Antioxidants, Oxidative stress, Atherosclerosis, Triton WR-1339 Hyperlipidemia, LDL oxidation, Lipid profile.