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STUDIES ON THE EFFECT OF ARTIFICIALLY CULTIVATED GANODERMA LUCIDUM ON STREPTOZOTOCIN INDUCED DIABETIC RATS

Chapter-6
Discussion
6. DISCUSSION

The practice of traditional medicine are based on hundreds of years of belief and observation, which predate the development of the modern medicine remains as an integral part of the formal health system and exists on an equal footing with the current therapy. The method of practices of traditional medicine may appear to be numerous and dissimilar, but all represent variations of three basic activities, viz., faith healing, hygienic measures and drug therapy. Traditional medicine of India has been handed down from the forefathers by oral tradition; however, these are disappearing from our modern society. Chinese traditional medicines had used *Ganoderma lucidum* in curing and preventing many diseases. That mushroom *Ganoderma lucidum* used in the current study was collected from Kollihills that is located at the west of Pachaimalais, in Namakkal District of Tamil Nadu, India. It comprises a compact block of hills with total area of 490 square kilometre and altitude ranging from 1000 to 1300m above MSL. The average rainfall is about 1200 mm per annum. The district as the whole is characterized by warm climate with low humidity owing to altitude and vegetation (Anand et al., 2005).

The present study relates to a method of artificially cultivating *Ganoderma lucidum* (Fr.) Karst. collected from the Kolli hills in an artificial solid medium. In more detail, the study relates to a method of cultivating *Ganoderma lucidum* (Fr.) Karst. to obtain large amount of fruit bodies in a short cultivation period and the fruit bodies can be used for the treatment of Diabetic mellitus by all sorts of people themselves.

6.1 Artificial Cultivation

*Ganoderma lucidum* strain collected purely from the natural world was aseptically plate cultured in an agar culture medium and stored at a low temperature because it was preferable to preserve the strain at a low temperature to retain the activity of the hyphae (mycelia) for a long time. When ever needed mycelial growth of *Ganoderma lucidum* was carried out on potato Dextrose Agar medium by tissue culture method. The fungus mycelium grew well at 25-27°C (Tiwari et al., 2004). It has been noted that under uniform environmental conditions, there was a high degree of radial symmetry in fungal colonies, with all portions of the mycelia front extending
at the same rate (Edelstein and Asegal, 1983) with no acceleration of growth over time (Brancato and Golding, 1953). Hence, it can be assured that the growth rate of fungi is constant (Trinci, 1971).

The seed culture used in the study was prepared in an artificial solid medium containing *Sorgum vulgare* grains mixed with calcium carbonate in plastic bottles and plugging the opening of the bottle with cotton wool, the bottles were subjected to sterilization for the preparation of the solid medium in the bottle. Then, the mycelia proliferated on the agar culture medium was aseptically inoculated to the solid medium together with the agar piece. The inoculated culture medium was cultivated for about 20 days at about 25° C.

A carbon source such as glucose, maltose and the like, a nitrogen source such as yeast extract, pepton and the like, a pH-adjusting agent such as calcium carbonate, vitamins, inorganic salts as well as growth-promoting agents are compulsorily needed for the better growth of mycelia. For the same purpose *Sorgum vulgare* grains and calcium carbonate was used for preparing seed culture.

According to Wada *et al.*, (1984) mixture of 2 to 6 parts by weight of sawdust and 1 part by weight of rice bran was a preferable base material containing various nutrient components in suitable amounts and ratios. As the sawdust, those derived from a broad-leaved tree such as beech, oak, walnut and the like are preferably used. In the present study 2:1 ratio of saw dust and rice bran was used and various plants materials like *Pithecolobium dulce*, Benth., *Eichhornia crassipes*, Solms., *Acacia arabica*, Willd., *Delonix regia* (Borj.ex.Hook) Raf., *Morniga oleifera*, Lam. were also used. Additionally rice bran, saw dust, agro waste and paddy straw were used separately.

Among the ten substrates used as the bed material for the cultivation of *Ganoderma lucidum* the percentage of Food consumption was very high in *Delonix regia* (49.6%) substrate. The mass obtained by the *Ganoderma* in this bed material was also very high (76gm) when compared to other materials. Other materials used for cultivation were also not so bad and it indicates that the substrates which are available plenty in the cultivation area can be used for cultivation depending upon the agro resource.

*Studies on the Effect of Artificially Cultivated Ganoderma lucidum on Streptozotocin induced Diabetic rats.*
Chinese medical texts traditionally call for using 1.5 to 9 grams of dry mushroom per day which approximates to 150 to 900 mg of concentrated Reishi extract (Carlson, 1996; Kenneth, 1992). It was taken as a powder in hot water or in whisky, or by boiling the fruiting body and drinking the Ganoderma “tea” (Quimio, 1986). Since the work was carried out in albino rats the aqueous extract concentration ranged from 10mg to 30mg was selected considering the average body weight.

6.2 Induction of Diabetes

Among animal models of diabetes, Streptozotocin rat was considered especially as the precious tool to study both pathological mechanisms of diabetes mellitus and hypoglycaemic activity of medicinal plants (Kedar and Chakrabani, 1982; Obatomi et al., 1994; El Fiky et al., 1996; Jouad et al., 2000). Streptozotocin was recognizes as a toxic agent for β-cells in islets of Langerhans (Gunnarsson, 1975; Agarwal, 1980) and widely used for the induction of diabetes with concomitant insulin deficiency (Anderson et al., 1974; Serrada et al., 1989).

The STZ dosage used to induce diabetes was 55 mg/kg b.w. because the range of the STZ dose is not as narrow as in the case of alloxan. The frequently used single intravenous dose in adult rats to induce IDDM was between 40 and 60 mg/kg b.w. (Ganda et al., 1976), but higher doses were also used. STZ was also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40mg/kg b.w. might be ineffective (Katsumata et al., 1992).

6.2.1 Changes in body weight

Induction of diabetes with STZ was associated with the characteristic loss of body weight, which was due to increased muscle wasting (Swanston-Flat et al., 1990) and due to loss of tissue proteins (Chatterjea and Shinde, 2002). Diabetic rats treated with the Ganoderma lucidum aqueous extract showed an increase in body weight as compared to the diabetic control, which may due to its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also due to an improvement in insulin secretion and glycemic control.
6.2.2 Effect on Glucose

The present investigation showed that treatment with *Ganoderma lucidum* reduces the blood sugar level and it may be due to stimulating effect on insulin release from regenerated beta cells of the panaces or may be due to increased cellularity of the islet tissues and regeneration of the beta cells. The aqueous extract might be reducing its hypoglycaemic effect by an extra-pancreatic action (Dabis *et al.*, 1984), e.g. possibly by stimulating glucose utilization in peripheral tissues (Naik *et al.*, 1991; Obatomi *et al.*, 1994). Also, it could be the result on an increase in glycolytic (Steiner and Williams, 1959) and/or glycogenic enzymes activity in peripheral tissues (Naik *et al.*, 1991). It might be also possible that the aqueous extract may decrease the secretion of the counter regulatory hormones (glucagons, corisols and growth hormones) (Roman-Ramos *et al.*, 1995). In classical forms of Diabetes, elevation of blood glucose is held to be the consequence of increased hepatic glucose output in concert with reduced peripheral glucose utilization (Consoli *et al.*, 1989).

Diabetic rats induced by STZ shows an increased sensitivity to oxygen free radicals and hydrogen peroxide, the breakdown products of liver, which impose oxidative stress in diabetes and would damage inner endothelial tissue; this would eventually be directly responsible for high blood glucose (Reddi and Bollineni, 2001). Although the mechanism involved with suppressing blood glucose levels by *Ganoderma lucidum* aqueous extract was not clearly demonstrated, at least three possibilities can be suggested: 1. Modulation of glucose transport (Yamasaki *et al.*, 1993), 2. Glucose disposal (Yokozawa *et al.*, 1984) or 3. Insulin secretion (Waki *et al.*, 1982).

6.2.3 Effect on Plasma insulin

The STZ-induced diabetic control rats showed decreased level of insulin in the plasma than the normal control rats. The treatment with *Ganoderma lucidum* aqueous extract had increased the insulin level to near normal level. The elevation of plasma insulin in the *Ganoderma lucidum* treated STZ diabetic rats could be due to the insulinotropic substances present in the extract, which induce the intact functional β-cells of the langerhans islet to produce insulin (Jeong-Sook, 2006). Insulin deficiency is clearly associated with change in hepatic metabolism (Consoli *et al.*, 1989).
6.3 Oxidative stress

Oxidative stress results from an imbalance between ROS (e.g. superoxide anion, hydroxyl radicals, peroxynitrite, hydrogen peroxide) and antioxidants such as superoxide dismutase (SOD), catalase, glutathione, vitamin C, vitamin E and α-lipoic acid. Hypothetically, since oxidative stress plays a pathogenic role in diabetes, supplementation with antioxidants should attenuate oxidative stress and improve oxidative stress-mediated damage in diabetes. However, many interventional studies to evaluate the effect of antioxidant supplementation in diabetes have provided inconsistent results (Shelton et al., 2005). Therefore, there is an urgent need to identify effective antioxidants with therapeutic potential to ameliorate diabetes induced complications (Singh et al., 2009).

6.3.1 Effect on Antioxidant enzymes

6.3.1.1 Super oxide Dismutase and Catalase

Hyperglycemia increases oxidative stress through the overproduction of reactive oxygen species which results in an imbalance between free radicals and the antioxidant defense systems of the cell, such as antioxidants and antioxidant enzymes. Endogenous antioxidant enzymes (SOD, CAT, and GPx) are responsible for the detoxification of the deleterious oxygen species (Ugochukwu and Cobourne, 2003).

SOD and CAT are two major scavenging enzymes that remove the toxic-free radical in vivo. The enzyme SOD scavenges superoxide radicals (\(\cdot O_2^-\)) by catalysing the conversion of two of these radicals into hydrogen peroxide and molecular oxygen. The hydrogen peroxide formed by SOD and other processes is scavenged by CAT, a ubiquitous haeme protein that catalyses the dismutation of hydrogen peroxide into water and molecular oxygen. Reduced activities of SOD in erythrocytes and CAT in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Santhakumari et al., 2003). From the present study it was found that the activities of SOD and CAT in untreated diabetic control animals were significantly lower than the normal control animals. This could be attributed to higher levels of superoxide radicals and hydrogen peroxide as indicated by increased ROS levels in those rats, which reduced the antioxidant enzymes activities. However, compounds
possessing antioxidant activity are shown to protect hepatic and nephritic tissues by attenuating the increased antioxidant enzymes due to their compensatory elevation of antioxidant defence mechanism (White, 2006).

In the present investigation *Ganoderma lucidum* aqueous extract decreased the levels of antioxidant enzymes in pancreas, kidney and liver tissues of diabetic rats by reducing the oxidative stress due to its potential antioxidant activity and these results agree with the previous studies on antioxidants (Anuradha & Selvam, 1993). The result of increased activities of SOD and CAT suggest that *Ganoderma lucidum* aqueous extract contains a free radical scavenging activity, which could exert a beneficial effect against pathological alterations caused by the presence of ·O₂⁻ and ·OH⁻. Administration of *Ganoderma lucidum* aqueous extract may prevent or attenuate decrease in tissue antioxidant enzymes in animal models of oxidative stress to provide cellular protection against reactive oxygen species as like the report of Lakshmi *et al.*, (2006) and Zhang *et al.*, (2003).

6.3.1.2 GSH,GPx and GST

Glutathione (GSH) is a metabolic regulator and putative indicator of health. GSH has a multifaced role in antioxidant defence (Soon and Tan, 2002). GSH functions as free radical scavenger and in the repair of free radical caused biological damage. Loen *et al.*, (1986) have suggested that the decrease in hepatic GSH could be the result of decreased synthesis, or increased degradation of GSH by oxidative stress in diabetes. Consequently, tissue antioxidant status is suggested to be an important factor in the development of diabetic complications (Venkateswaran and Pari, 2002). GSH is required for the recycling of vitamin C and acts as a substrate for GPx and GST that are involved in preventing the deleterious effect of oxygen radicals. GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions. Indeed GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects, for example, a decrease in the rate of gluconeogenesis or an increase in glycogenolysis (Yu, 1994). Reduced level of GSH in the circulation during diabetes represents its increased utilization due to oxidative stress.
Glutathione S-transferase (GST) and Glutathione peroxidise (GPx) are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates which have electrophilic functional groups. They play an important role in the detoxification and metabolism of many xenobiotic and endobiotic compounds (Ji et al., 1992). The increment in the activity of Glutathione S-transferase (GST) is in consistent with the induction in the generation of free radicals (El-Demerdash et al., 2005). Increased GST activity might be one of the defence mechanisms in these animals to detoxify or neutralize the toxic metabolites, e.g., ketone bodies, generated in liver by the diabetes. The effects of the antioxidant may be useful in delaying the complicated effects of diabetes as retinopathy, nephropathy and neuropathy due to imbalance between free radicals and antioxidant systems.

GPx and GST catalyze the reduction of hydrogen peroxide and hydroperoxides to nontoxic metabolites. Due to decrease in the concentration of GSH, the substrate for GPx, the activity of GPx and GST are reduced in diabetes. The increased levels of GSH in tissues are utilized by GPx (Shanthi and Ramakrishnan, 1994). In the present study lower level of plasma, liver and kidney GSH and GST was observed in STZ induced diabetic rats. GPx level was also decreased in plasma, liver, kidney and pancreas. It appeared that increased levels of glucose improved utilization of GSH, as reported earlier (Garg et al., 1996; Mitra et al., 1995; Prakasam et al., 2003) and is responsible for the reduction of GSH, GST and GPx levels.

Concerning to the changes in lipid peroxidation, the diabetic tissue showed decreased activity of the key antioxidants SOD, CAT, glutathione, GPx and glutathione S-transferase, which plays an important role in scavenging the toxic intermediate of incomplete oxidation. The decrease in the activity of these antioxidants can lead to an excess availability of the superoxide anion (·O$_2^-$) and hydrogen peroxide in biological systems, which in turn generate hydroxyl radicals resulting in initiation and propagation of lipid peroxidation (Kumuhekar and Katyane, 1992). The induction of SOD activity by *Ganoderma lucidum* aqueous extract may be attributed to inhibition of the generation of active oxygen species from autooxidation of glucose generation from the action of STZ. The increased activity of
SOD accelerated dismutation of superoxide radicals to $H_2O_2$, which is removed by CAT, GST and GPx. This indicates that the *Ganoderma lucidum* aqueous extract treatment have altered the SOD, CAT, GSH, GST and GPx activities and reduced oxidative stress in the diabetic rats, resulting in a lower TBARS concentration.

### 6.3.1.3 Lipid peroxidation markers (TBARS and Hydroperoxides)

Since high blood glucose is susceptible to oxidation, hyperglycemia causes high ROS production and in turn, leads to high TBARS in tissues (Wolff and Dean 1987, Das *et al.*, 2000) and membrane damage (Hunt *et al.*, 1988). In the current study, the concentration of hepatic TBARS was significantly increased after treatment of STZ in the rats. The increased concentration of TBARS suggests that an increase in oxygen free radicals could be due to either their increased production or decreased destruction (Kakkar *et al.*, 1995).

Several studies have shown increased lipid peroxidation in clinical and experimental diabetes (Sundaram *et al.*, 1996; Kakkar *et al.*, 1998). According to Ivorra *et al.*, (1989) STZ has been shown to produce oxygen free radicals. Lipid peroxide mediated tissue damages have been observed in the development of type I and type II mellitus. Previous studies have reported that lipid peroxidation in liver, kidney and brain of diabetic rats was increased (Latha and Pari, 2003; Venkateswaran and Pari, 2002).

GPx and GST catalyze the reduction of hydrogen peroxide and hydroperoxides to nontoxic metabolites. Due to decrease in the concentration of GSH, the substrate for GPx, the activity of GPx and GST are reduced in diabetes. And so the hydroperoxides level in Diabetic control rats were higher. The level of hepatic, renal and pancreatic TBARS and hydroperoxides in *Ganoderma lucidum* aqueous extract treated rats showed a significant reduction, which indicates a decreased lipid peroxidation.

### 6.3.2 Effect on non enzymatic antioxidants

Vitamins C and E are interrelated by recycling process. Recycling of tocopheroxyl radicals to tocopherol is achieved with vitamin C, which is a powerful water-soluble antioxidant and present in the cytosolic compartment of the cell. Vitamin C serves as an electron donor for vitamin E radicals generated in the cell.
membrane during oxidative stress. Vitamin E neutralizes the free radicals, preventing the chain reaction that contributes to oxidative damage (Sun et al., 1999).

*In vitro* studies have demonstrated that antioxidant vitamins can prevent lipid peroxidation and that vitamins C and E have synergistic effects, but supporting in vivo evidence is sparse (Packer et al., 1997).

### 6.3.2.1 Vitamin C

Vitamin C is an excellent water-soluble antioxidant that primarily scavenges oxygen radicals. Vitamin C has been reported to contribute to up to 24% of the total peroxyl radical trapping antioxidant activity (Atanasiu et al., 1998). A decreased level of plasma vitamin C was observed in the diabetic rats. The decreased level could be due to increased utilization of vitamin C in deactivation of the increased levels of reactive oxygen or due to the decrease in the GSH level, since the GSH is required for the recycling of vitamin C (Chatterjee and Nandi, 1991; Infers and Sies, 1988). The treatment to STZ-induced diabetic rats with aqueous extract of *Ganoderma lucidum* has increased the Vitamin C level in the plasma, liver and kidney. It was observed that GSH had also increased by the treatment and it may also be the reason for the increase in Vitamin C level.

### 6.3.2.2 Vitamin E

Alpha tocopherol can prevent glomerular hyperfiltration and increased albuminuria in streptozotocin-induced diabetic rats (Koya et al., 1997; Koya et al., 2003). Esqueda et al., (2005) reported that vitamin E reduced the occurrence of glomerular membrane thickening, podocyte flattening, as well as loss of fenestration in the endothelial layer in STZ-induced diabetic rat kidneys. It was suggested that such an effect of vitamin E is mediated through decreased diacylglycerol levels and prevention of the activation of PKC. In contrast, others (Mustata et al., 2005) reported only marginal beneficial effects of vitamin E and C supplementation on DM-induced nephropathy in rats. Researchers (Haidara, et al., 2004) also reported that vitamin E administration to diabetic rats reduced plasma levels of cholesterol, triglycerides, and low density lipoprotein cholesterol and deterioration of renal functions in diabetics (Hsu et al., 2001; Sun, Halaihel et al., 2002). In the present study also have revealed that the STZ-induced diabetic rats had reduced level of Vitamin E and the treatment
with *Ganoderma lucidum* aqueous extract augmented the vitamin E level in the plasma, kidney and liver of diabetic induced rats. It may be also due to increased GSH level and Vitamin C level.

**6.4 Antihyperlipidemic effect**

Oxidative stress caused by free radicals damages the endothelial cells in the blood vessels, increases blood cholesterol levels, promotes lipid peroxidation (LPO) and plays a central role in pathogenesis of secondary complications of DM.

Diabetes is associated with profound alterations in the plasma lipid, triglycerides and lipoprotein profile and with an increased risk of coronary heart disease (Huang *et al.*, 1988; Fontbonne *et al.*, 1989). High level of total cholesterol is one of the major factors for coronary heart diseases and it is well known that hyperlipidemia and incidence of atherosclerosis is increased in diabetes (Lewis, 1978). The liver and some tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoprotein. Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications (Betteridge, 1997). Diabetes is also known to be associated with an increase in the synthesis of cholesterol, which may be due to the increased activity of HMG CoA reductase. (Goodman *et al.*, 1982; Glasgow *et al.*, 1981).

Oral administration of *Ganoderma lucidum* aqueous extract normalized these effects, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. In addition, *Ganoderma lucidum* aqueous extract may improve hypercholesterolemia by modifying lipoprotein metabolism; enhanced uptake of LDL by increasing LDL receptors (Slater *et al.*, 1980) and/or by increasing the lecithin-cholesterol acyl transferase (LCAT) activity (Khanna *et al.*, 2002) which may contribute to the regulation of blood lipids. LCAT plays a key role in incorporating free cholesterol into HDL and transferring back to VLDL or IDL which is taken back by the liver cells (Rajlakshmi and Sharma, 2004). *Ganoderma lucidum* aqueous extract may facilitate rapid catabolism of LDL. The observed hypotriglyceridemic effect may be due to a decrease of fatty acids synthesis.
(Bopanna et al., 1997), enhanced catabolism of LDL, activation of LCAT and tissues lipases (Khanna et al., 2002) and/or inhibition of acetyl-CoA carboxylase and production of triglycerides precursors such as acetyl-CoA and glycerol phosphate. The mechanism by which *Ganoderma lucidum* aqueous extract exerts its cholesterol lowering effect may also by a decrease in cholesterol adsorption from the intestine and bile acids excretion (Eddouks et al., 2005).

Many studies have shown that the increase in fatty acid delivery to the liver leads to increased triglyceride synthesis (Richards et al., 1968; Heimberg and Wilcox, 1972; Jones et al., 1967). It is an important factor in controlling the hepatic triglyceride secretion, and inhibition of protein synthesis has been shown to reduce triglyceride secretion. Insulin resistance is associated with increased triglyceride secretion. (Sparks et al., 1986; Durrington et al., 1982; Forget et al., 1974). The present studies reveal that treatment of diabetes with *Ganoderma lucidum* aqueous extract served to lower plasma triglyceride levels by returning lipoprotein levels to normal. The restoration of triglycerides level following *Ganoderma lucidum* aqueous extract treatment is supported by above reports.

Accumulation of fatty acids results in higher levels of their metabolites such as acyl-carnitine and long chain acyl-CoA and this may be a cause for diabetic complications associated with renal tissue may be partly due to abnormalities in lipid metabolism. Fatty acids undergo changes during the process of injury, repair and cell growth (Cameron and Cotter, 1997). The increase in the serum lipids on the diabetic subject is mainly due to the increased mobilization of free fatty acids from peripheral deposits, since insulin inhibited the hormone sensitive lipase (Al-Shamaony et al., 1994). On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat deposits (Goodman and Gilman, 1985). Segneur et al., (1994) have reported that there is a significant alteration in the fatty acid composition of serum and variety of tissues in experimental diabetes. Similarly in the present study the free fatty acid level was increased in STZ-induced diabetic rats and the treatment with *Ganoderma lucidum* aqueous extract exerted fatty acid lowering effect.
The elevated serum phospholipid levels are a consequence of elevated lipoproteins. Jain et al., (2000) have suggested that the levels of glycemic control and elevated levels of HDL cholesterol and decreased levels of triglycerides in the blood are significantly correlated with the phospholipid levels. The serum cholesterol/phospholipid ratio is one of the important markers of development of atherosclerosis. The restoration of phospholipids using *Ganoderma lucidum* extract may be by controlled mobilization of serum triglycerides, controlling the tissue metabolism and improving the level of insulin secretion and action presumably mediated cholesterol and phospholipids.

An increase in the total lipids of liver and kidney in STZ induced diabetic rats may indicate an increased synthesis of lipids and storage capacity, which might have caused an increase in serum triglycerides and phospholipids. Rajalingam et al., (1993) have reported that variety of derangements in metabolic and regulatory mechanisms in diabetic rats was due to insulin deficiency and responsible for the observed accumulation of lipids. The increase in total lipids observed in diabetic rats was due to the impairment of insulin secretion, which resulted in enhanced mobilization of lipid from the adipose tissue to the plasma.

The possible underlying mechanism by which *Ganoderma lucidum* aqueous extract can exert its lipid lowering activities is not elucidated. At this stage of the study, several fundamental mechanisms could be proposed to explain the results. Previous studies have reported a decrease in cholesterol adsorption from the increasing faecales bile acids excretion to explain the hypolipidemic properties of *Momordica charantia*. The same mechanism may be appropriate to explain the observed triglycerides lowering activity of *Ganoderma lucidum* aqueous extract. On the other hand *Ganoderma lucidum* aqueous extract can also act by decreasing the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-Co A reductase)activity (Key enzyme of cholesterol biosynthesis) (Kedr andChakrabarti,1982; Sharma et al., 2003) and/or by reducing the NADPH required for fatty acids and cholesterol synthesis (Chi,1982). The hypolipidemic effect mediated by *Ganoderma lucidum* may be anticipated to have biological significance and provide a scientific rationale for the use of...
Ganoderma lucidum aqueous extract as an anti-diabetic agent. Further study is required to confirm the route in which the antihyperglycaemia is shown by the Ganoderma lucidum aqueous extract. Additionally, precise molecular mechanism and active substance(s) need to be determined. Such active principle(s) could be precious in atherosclerosis and cardiac diseases therapy and control.

6.5 Key enzymes of carbohydrate metabolism

6.5.1 Glucose-6-phosphatase

Insulin decreases gluconeogenesis by decreasing the activities of key enzymes such as glucose-6-phosphatase, fructose 1,6-bisphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxykinase (Murray et al., 2000).

The liver and skeletal muscle are the major organs for glucose disposal. Glucose-6-phosphatase, a key enzyme in the homeostatic regulation of blood glucose concentration, is expressed mainly in the liver and kidney and is critical in providing glucose to other organs during diabetes, prolonged fasting or starvation (Bouché et al., 2004). It catalyzes the dephosphorylation of glucose-6-phosphate to free glucose as the terminal step in gluconeogenesis and glycogenolysis. This reaction occurs in the lumen of the endoplasmic reticulum and the enzyme complex is composed of a glucose-6-phosphate transporter that transports glucose-6-phosphate from the cytoplasm into the lumen of the endoplasmic reticulum and a glucose-6-phosphatase catalytic subunit that hydrolyzes the glucose-6-phosphate to glucose and phosphate (Chou et al., 2002). Glucose is transported out of the liver to increase blood glucose concentration. STZ increases the expression of G6Pase (Massillon et al., 1996; Liu et al., 1994). In contrast, insulin and metformin inhibit the hepatic glucose production by suppressing G6Pase activity (Chen et al., 2000; Wiernsperger and Bailey, 1999). In the present study treatment of Ganoderma lucidum aqueous extract enhanced the reversal of high G6Pase activity in diabetic rats. The reduction of G6Pase can lead to a decrease in gluconeogenesis and blood glucose concentration.

The observed increase in activity of Glucose-6-phosphate dehydrogenase may be geared towards the production of NADPH for the regeneration of the glutathione-GSH (Chance et al., 1979) and the prevention of abnormal reactions of the fatty acids.
in the cell membrane with oxygen (Lehninger, 1982). It in part explains the reported decrease in lipid peroxidation (McAnuff et al., 2003)

The activity of glucose-6-phosphatase is stimulated by cAMP and repressed by insulin. Insulin deficiency achieved by experimental diabetic rats increases glucose-6-phosphatase activity. In 1998, Trinh et al., have demonstrated that glucose-6-phosphatase activity impairs hepatic glucose utilization, while simultaneously enhancing hepatic glucose production (Trinh et al., 1998). It is concluded that enhanced hepatic glucose output in streptozotocin induced diabetic rats probably involves dysregulation of both the liver and kidney glucose-6-phosphatase and the activity is close to normal in the diabetic rats treated with *Ganoderma lucidum* aqueous extract for 45 days

6.5.2 Fructose-1,6-bisphosphatase

Fructose-1,6-bisphosphatase is a highly regulated, rate-limiting enzyme that catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate, the second to last step in the gluconeogenic pathway (Pilkis and Claus, 1991). It is abundant in the liver and kidneys but is scantily expressed in the pancreatic \( \beta \)-cells under normal conditions. An increase in the activity of fructose-1,6-bisphosphatase has been suggested as a possible mechanism for the production of increased endogenous glucose after it was shown that diabetics have an increase in gluconeogenesis from glycerol (Nurjhan et al., 1992), a substrate that enters into the gluconeogenic pathway immediately before fructose-1,6-bisphosphatase activity. The increased activity of fructose-1,6-bisphosphatase has been observed in animal models of diabetes, insulin resistance and obesity and suggests a principal role for fructose-1,6-bisphosphatase in the flux of gluconeogenesis and endogenous glucose production (Andrikopoulos et al., 1993). The decreased activity of fructose-1,6-bisphosphatase in the diabetic animals treated with *Ganoderma lucidum* aqueous extract may be by the inhibition of gluconeogenesis from all gluconeogenic substrates while avoiding direct effects on glycogenolysis, glycolysis and the tricarboxylic acid cycle and there by maintains the blood glucose homeostasis.
6.5.3 Hexokinase

Hexokinase (HK) is an isoenzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate thus playing a crucial function in tissue intermediary metabolism. There are four isoforms of mammalian hexokinases involved in the oxidation of glucose (Wilson, 1995). Hexokinases I–III have a high affinity for glucose and are feedback-inhibited by physiologic concentrations of glucose-6-phosphate, whereas, glucokinase (HK-IV or GK), the major glucose-phosphorylating enzyme, has a lower affinity for glucose and its abundance is regulated transcriptionally by insulin and glucagon and post translationally by the GK regulatory protein (GKRP) (Collier and Scott, 2004). Among four isoforms of hexokinases, HK-I and GK are expressed in the liver. Reports on animal models and isolated hepatocytes established that hepatic hexokinase exerts a strong impact on glucose utilization and glycogen synthesis (Postic et al., 2001) and their levels are very low in both human and rodent diabetes; insulin administration rapidly reinstates hexokinase activity to the hepatocytes (Ferre et al., 1996). Because of these observations, restoration of hepatic hexokinase activity provides a possible therapeutic strategy for diabetes treatment. The markedly decreased level of insulin in the streptozotocin-induced diabetic animals ultimately leads to the impairment in the activity of hexokinase, since insulin deficiency is a hallmark of diabetes (Postic et al., 2001). However, the modest increase in the activity of hexokinase as observed in the diabetic animals administered with *Ganoderma lucidum* aqueous extract protects the hepatic and extrahepatic tissues against streptozotocin-induced diabetes by stimulating insulin from the remnant \( \beta \) -cells, since streptozotocin selectively destroys pancreatic \( \beta \) -cells. This study also demonstrated that a modest augmentation of hexokinase activity in the liver and kidney enhances glucose metabolism and promotes overall glucose homeostasis similar to the studies of Palsamy and Subramanian, (2009).

6.6 Total protein

Reduction in plasma total protein was observed in diabetic rats. This is in agreement with the results obtained by Bakris (Bakris, 1997 and Tuvemo et al., 1997). Distinct metabolic renal alterations are demonstrable in experimental diabetes, leading
to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Bhavapriya et al., 2001). Improvement of plasma protein was observed after oral administration Ganoderma lucidum aqueous extract to diabetic rats.

6.7 Total Hb and Glycosylated haemoglobin

Reduction in plasma total protein was observed in diabetic rats. In diabetes, protein synthesis is decreased in all tissues due to relative insulin deficiency and thus the synthesis of haemoglobin is also suppressed (Chatterjee and Shinde, 1994). Increased glycation of protein has been found to be a consequence of diabetic complications. A number of proteins, including haemoglobin, are glycated to a greater degree in diabetes (Alberti, 1982). The increase in glycosylated haemoglobin is directly proportional to the fasting blood glucose level (Jackson et al., 1997).

During oxidative stress, hydrogen peroxide (H2O2) and hydroperoxides are known to induce iron release from haemoglobin and glycosylated haemoglobin (GlyHb), which promotes the iron-mediated free radical reactions. This could be lead to structural, conformational change and functional modifications in erythrocyte as a result of destruction and decrease of haemoglobin level in diabetic condition, which correlates with our results. GlyHb concentration is proportionately increased in diabetes with ambient hyperglycemia and reflects the extent as well as management of diabetic condition (Wolffenbuttel et al., 1996). H2O2- mediated increased level of iron release from GlyHb may, thus, be a source of oxidative stress and cellular injuries, which may be caused by Fenton reaction in uncontrolled diabetes mellitus, where GlyHb level is significantly elevated (Sena et al., 2005).

Glycosylated hemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level (Bunn et al., 1981; Guoyan et al., 1992) Glycosylated hemoglobin had been found to increase in patients with diabetes mellitus (Baskaran et al., 1990) and in diabetic animals and this increase was found directly proportional to the fasting blood glucose level (Koenigetal.1976). Treatment with Ganoderma lucidum aqueous extract to STZ- induced diabetic rats for 45 days reduced the HbA1c level.
Glycated haemoglobin levels were found to be increased in the untreated diabetic control group. Increased non-enzymatic and autooxidative glycosylation is one of the possible mechanisms linking hyperglycemia and the vascular complications of diabetes (Hall et al., 1984). Diabetic rats showed higher levels of glycated haemoglobin and decreased total haemoglobin indicating their poor glycemic control. Treatment with *Ganoderma lucidum* aqueous extract showed a significant decrease in the glycated haemoglobin levels and increased haemoglobin level which could also be due to an improvement in insulin secretion.

6.8 Hepatic marker enzymes

6.8.1 AST, ALT and ACP

Possible explanation for the differential effects of *Ganoderma lucidum* aqueous extract on the activities of AST, ALT and ACP in plasma is that it may inhibit the liver damage induced by STZ and appears to contribute in alleviating the adverse effect of diabetes mellitus by enhancing lipid metabolism as well as the hepatic antioxidant defense system. The level of Serum enzymes including AST, ALT, LDH, ALP, and ACP show increased activities and thereby reflecting active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels (Foreston et al., 1985; Hultcrantz et al., 1996). Ohaeri, (2001) has also found that liver was necrotized in STZ-induced diabetic rats. Therefore an increase in the activities of AST, ALT, LDH, ALP and ACP in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993).

6.9 Bilirubin

Diabetes mellitus leads to a series of metabolic disturbances; they are found not only in the metabolism of carbohydrates, lipids and proteins but also in drug metabolism. Among the phase I drug-metabolizing enzymes both experimentally induced insulin-dependent diabetes and genetically developed insulin dependent diabetes are associated with increases in the activities of cytochrome P-450 (P450) 1, 2B and 2E (Thomas, et al., 1987; Ioannides et al., 1988). The regulation of the steady-state concentration of P450 2E1 in diabetes is an extremely complex process involving different mechanisms (Koop and Tierney, 1990; Koop and Casazza, 1985).
Among phase II reactions of biotransformation, glucuronidation is arguably the most important detoxification pathway in all vertebrates, quantitatively (Clarke and Burchell, 1994). UDP-glucuronosyl transferases (UGTs) are a family of closely related membrane-bound microsomal enzymes with an intravesicular active site (Clarke and Burchell, 1994). UGTs convert a wide range of xenobiotics and endobiotics to biologically inactive glucuronides that are readily eliminated. Among the endogenous compounds the end product of haem catabolism, bilirubin, is the most extensively studied substrate of UGTs (Clarke and Burchell, 1994). The requirement for the body to remove bilirubin is paramount, because at high concentrations it causes brain and kidney damage (Clarke and Burchell, 1994). Several reports have indicated that ethanol enhances the clearance of bilirubin and hepatic haem turnover, resulting in beneficial or preventive effects both in Gilbert's disease and neonatal hyperbilirubinaemia (Samson et al., 1976; Waltman et al., 1969; Dioguardi et al., 1970).

The increase in plasma bilirubin (hyper-bilirubinemia) may be resulted from the decrease of liver uptake, conjugation or increase bilirubin production from hemolysis. Also, the elevation in plasma bilirubin indicates liver damage as confirmed by the changes in the activities of plasma and liver enzymes. *Ganoderma lucidum* aqueous extract improved the level of plasma bilirubin in STZ induced diabetic rats indicating the hepatoprotective effect of the extract.

6.10 Glycoproteins

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principle components of animal cells. Hexoses, hexosamine and sialic acid are the basic components of the glycosaminoglycans and glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to cell surface and the secretion and absorption of macromolecules (Mittal et al., 1996).

The levels of glycoproteins are reported to be significantly increased in diabetes mellitus and cardiovascular disease (Gandhi and Roy, 1979; Lindberg, 1991) and an increase in plasma glycoprotein components has been reported to relate to the duration, severity and existence of degenerative vascular diseases (Mathew et al.,

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The present study clearly shows that an increase in the glycoprotein components namely hexoses, hexosamine, fucose, sialic acid in STZ induced diabetic rats. One possible explanation could be secretion (or) shedding of glycoproteins from cell membrane into the circulation due to peroxidative damage of membrane proteins. Increase in sialic acid may be due to shedding of sialic acid from erythrocytes, since it has been reported that the level of erythrocyte sialic acid is decreased in human diabetics (Gandhi and Roy, 1979). It has been shown that an increased sialic acid concentration is a risk factor for cardiovascular mortality (Lindberg, 1991). Increase in glycoprotein level could also be due to increased synthesis to repair the damaged membrane structure by peroxidation (Kaviarasan et al., 2005). The glycoprotein levels were significantly reduced by oral administration of *Ganoderma lucidum* aqueous extract treatment for diabetes induced rats. It indicates that the peroxidative damage is reduced by *Ganoderma lucidum* aqueous extract.

6.11 Glycogen

Glycogen, a branched polymer of glucose residues synthesized by the enzyme glycogen synthase, is the primary intracellular storable form of glucose and its quantity in various tissues is a direct manifestation of insulin activity as insulin supports intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Pederson et al., 2005). Glycogen synthase, a crucial and rate-limiting enzyme in tissues nonoxidative glucose disposal, catalyzes the transfer of glucose from UDP-glucose to glycogen in animal cells. There are two mammalian isoforms of glycogen synthase. One appears to be expressed only in liver while a second is expressed in skeletal and cardiac muscle as well as adipose tissue, kidney and brain. The activity of glycogen synthase is regulated by decreased cellular glycogen content, hormone signaling, subcellular localization, targeting of phosphatase and allosteric activation by glucose-6-phosphate (Parker et al., 2004). Glycogen phosphorylase, a rate-limiting enzyme of glycogenolysis, cleaves $\beta (1\rightarrow 4)$ linkages to remove glucose molecules from the glycogen. This enzyme exists as a dimer with each subunit linked to the essential cofactor pyridoxal phosphate, which donates the phosphate as an electron donor for release of glucose-1-phosphate (Greenberg et al., 2006). Its activity is regulated by phosphorylation and by allosteric
binding of AMP, ATP, glucose-6-phosphate and glucose (Bollen, 1998). Since streptozotocin causes selective destruction of pancreatic β-cells resulting in apparent decline in insulin levels, it is responsible for the decreased glycogen levels in major storage tissues such as liver and skeletal muscle as they depend on insulin for entry of glucose (Whitton and Hems, 1975; Golden et al., 1979; Bishop, 1970). During diabetic conditions, the glycogen levels, glycogen synthase activity and responsiveness to insulin signalling are diminished and glycogen phosphorylase activity is significantly increased (Parker et al., 2004). The oral administration of *Ganoderma lucidum* aqueous extract to diabetic rats regulated the activity of glycogen metabolizing enzymes by stimulating the remnant β-cells to secrete more insulin which in turn reactivate the glycogen synthetase system thereby normalized the altered glycogen content (Palsamy and Subramanian, 2009; Lolitkar and Rao, 1966).

Glycogen levels in various tissues especially skeletal muscle are direct reflection of insulin activity. Insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZ selectively damages β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin influx of glucose (Whitton and Hems, 1975). Moreover, this alteration in muscle and hepatic glycogen was normalized by insulin treatment (Weber et al., 1966). The previous study (Hikino et al., 1989) showed that hepatic and skeletal muscle glycogen content reduced drastically in diabetic rabbit. The entry of renal glucose is not dependent on action of insulin and, therefore, in the event of hyperglycemia there is an increase in the entry of glucose (Belfiore et al., 1986). This has been postulated to cause increased intra-renal glycogen deposition, which leads to glycosylation of basement membrane collagen in the kidney (Anderson and Stowring, 1973). From the present study too it is clear that STZ induced diabetic rats had increased glycogen level in kidney and decreased level in Liver and skeletal muscle. Treatment with *Ganoderma lucidum* aqueous extract had reverted it by decreasing the renal glycogen and increasing the hepatic and skeletal muscle glycogen. It may be assumed by the above explained reasons.
6.12 Histopathology

6.12.1 Hepatic

Liver is the key organ and the principle site where the metabolism of carbohydrates, lipids and proteins take place (Bhavapriya et al., 2001). Activation of NADPH oxidase and G6PD, by hyperglycemia and/or hyperinsulinemia, could be a signal to either trigger or sustain hepatic cell growth in the genetic model of type 2 diabetes. In this regard, there is suggestive evidence that reactive oxygen species may play a role in the initiation of liver regeneration/growth, because of increased electron transport, resulting from the high metabolic load imposed on hepatocytes after partial hepatectomy of rat and mouse liver (Lee et al., 1999; Nakatani et al., 1997). Alternatively, elevated oxidants in conjunction with up-regulation of G6PD activity could increase fatty acid synthesis and play a potential role in evoking either liver dysfunction or damage (determined by increase in the serum γ-GT, ALP, and ALT levels) in fa/fa rats. Glucose metabolism through the glycolysis and hexosamine pathway has been shown to be altered in type 2 diabetes (Rakhee et al., 2009). The portal lobule is the functional unit which can be used in connection with liver physiology and pathology (Freeman and Bracegirdle, 1973). In the present study the specimens revealed fatty degeneration of hepatocytes. Sinusoids and vein were filled with fatty vacuoles and it gains the support of the above findings.

In the liver biopsy of *Ganoderma lucidum* aqueous extract treated diabetic rat all the intoxication caused by STZ were reduced. A similar hepatoprotective effect was observed in the stem of *Entilago leicocarpa* (Lin et al., 1995).

6.12.2 kidney

Diabetic nephropathy is generally considered to be a nonimmune disease. However, examination of human biopsies and animal models has shown accumulation of macrophages and overexpression of leukocyte adhesion molecules and chemokines in diabetic kidneys (Sassy-Prigent et al., 2000; Young et al., 1995; Furuta et al., 1993). Macrophages that migrate into renal tissue could cause structural damage through the release of profibrotic cytokines as well as reactive oxygen species (ROS) (Chow et al., 2004; Van Goor et al., 1994).

The pathological changes caused by STZ such as fatty changes, ballooning degeneration and inflammatory infiltration of lymphocytes and kupfer cells around
the renal area are minimized by *Ganoderma lucidum* aqueous extract treatment. STZ treatment caused congestion in cortical region along with prominent tubular necrosis, degeneration of tubules in the medullary region. *Ganoderma lucidum* aqueous extract treatment had a protective effect in kidney as evidenced by moderate degree of necrosis and restoration of size of tubules in the medullary region. The present study correlates with previous findings of Khandelwal *et al.*, (2001). The expansion of capsular space is probably caused by enhanced angiotensin II level leading to increased intraglomerular pressure and filtration rate (Wolf *et al.*, 1996)

### 6.12.3 Pancrease

Streptozotocin is a selective β-cell genotoxicant and when administered in a single high dose it induces rapid onset of diabetes by generating sufficient levels of DNA adducts to cause overactivation of poly (ADP) ribose synthetase in the base excision repair (BER) pathway (Burns and Gold, 2007). The extensive poly (ADP) ribose synthetase activation results in rapid depletion of cellular NAD+ that leads to β-cell death through necrosis.

In the present study, almost all most of the insulin-positive β-cells were degenerated or necrosed in the STZ treated rats leading to decrease in insulin secretion and an increase in blood glucose concentration. STZ induced a significant decrease in the area of insulin synthesising β-cells. *Ganoderma lucidum* aqueous extract treatment protected majority of cells of Langerhans islets. Nevertheless, light hydropic degeneration, degranulation and necrosis were observed in the remaining cells. *Ganoderma lucidum* aqueous extract treatment partially prevented degeneration of β-cells. It increased the area of insulin synthesising β-cells significantly.

All the above, ultimate result from the present study reveals that the *Ganoderma lucidum* aqueous extract can act as effective antioxidant, hypoglycaemic agent, cholesterol lowering source, hepato and renal protective agent and overall it can serve as a potential hypoglycaemic and hyperinsulinaemic source. This clearly gives the information that the aqueous extract obtained from the artificially cultivated fruit bodies of *Ganoderma lucidum* collected from Kolli Hills can serve as an effective Anti-Diabetic agent. More attractive report is that *Ganoderma lucidum* aqueous extract from the above said artificially cultured mushrooms was effective even with comparatively low dose (30mg/kg).