The selection of scientific and systematic approach for the biological evaluation of plant products based on their use in the traditional systems of medicine forms the basis for an ideal approach in the development of new drugs from plants. Numbers of scientific and popular literatures has reported more than 1200 plants as hypoglycemic agents (Marles and Farnsworth 1995; Wang and Ng 1999), as plant drugs are frequently considered to be less toxic with lesser or rare side effects than those of synthetic ones (Pari and Umamaheswari 2000). The limitations of currently available pharmacological agents for control of blood glucose have encouraged the researcher to rethink the development of novel antidiabetic agents with different mechanism of action (Reddy et al., 2000). The study of such medicines might offer a natural key to unlock a diabetologist’s pharmacy for the future (Bakirel et al 2008).

Diabetes mellitus is a major heterogeneous endocrine and metabolic disorder, characterized by altered metabolisms of carbohydrate, lipid and protein, which not only lead to hyperglycaemia but also cause many complications, such as hyperlipidemia, hypertension and atherosclerosis (Bakirel et al 2008; Sepici et al., 2004; Luo et al., 2004). The increased glucose level tends to glucose auto oxidation and auto oxidative glycosylation of proteins, which leads to oxidative stress by increasing the reactive oxygen species (Baynes, 1991). The oxidative stress, caused by hyperglycemia induced free radicals, contributes to the development and progression of diabetes along with various secondary complications (Ceriello, 2003; Rahimi et al., 2005; Tang et al., 2006).

In chronic hyperglycemia, the potentially reactive oxygen species (ROS) such as $O_2^{•−}$, $H_2O_2$ and $•OH$, are continuously generated inside the human body as a consequences of exposure to a plethora of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer. Metabolism of oxygen by cells generates potentially deleterious reactive oxygen species including the superoxide ion ($O_2^{•−}$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($•OH$), and nitric oxide (NO) which act as intercellular and intracellular mediators of signal transduction process (Giugliano et al, 1996). These free radicals induce DNA fragmentation of the beta cells of islets of Langerhans, in diabetes mellitus, leading to the deficiency in insulin secretion. Increased oxidative stress due to chronic hyperglycemic conditions in diabetes mellitus plays a basic role in the genesis of endothelial dysfunction. Furthermore, oxygen free radicals are proinflammatory and stimulate monocyte migration and formation of oxidized low-density lipoproteins, which are toxic to vascular cells. However, under normal physiological conditions, the rate and magnitude of oxidant formation is balanced by the rate
of oxidant elimination. An imbalance between pro-oxidants and antioxidants in diabetes results in oxidative stress, which is the pathogenic outcome of overproduction of oxidants that overwhelms the cellular antioxidant capacity (Giugliano et al, 1996). Abnormally high levels of free radicals cause membrane damage due to lipid peroxidation and protein glycation and the simultaneous decline or disturbance of antioxidant defense mechanisms leads to cell and tissue damage (Tang et al., 2006). Moreover, antioxidants have been shown to prevent the destruction of β-cells (Slonim et al., 1983; Murthy et al., 1992) by inhibiting the peroxidation chain reaction and thus, may provide protection against the development of diabetes (Halliwell and Gutteridge, 1989; Gordon, 1996; Montonen, 2005).

As a new strategy for alleviating the oxidative damage in diabetes, interest has grown in the usage of natural antioxidants. It has been postulated that many of the negative effect of oxidative stress are diminished upon supplementation with certain dietary antioxidants such as vitamin E, C and other non-nutrient antioxidant such as phenolic compounds and flavonoids (Rahimi et al, 2005; Al-Azzawie and Alhamdani, 2006). Plants are a rich source of natural antioxidants such as phenolics and flavonoids that may occur in all parts such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990). Natural antioxidants, especially phenolics and flavonoids are safe and also bioactive than compared to that of the synthetic ones. Therefore, much attention has been focused on the use of natural antioxidants to inhibit Reactive oxygen species (ROS) production and protect from damage due to ROS in diabetes mellitus. The ROS readily attack and induce oxidative damage to various biomolecules including biomembranes, proteins, lipids, lipoproteins and DNA (Farber, 1994). These free radicals induce damage to the DNA of beta cells of islets of Langerhans in diabetes mellitus, leading to the deficiency in insulin secretion. This oxidative damage is a crucial etiological factor implicated in several chronic human diseases such as diabetes mellitus, and others diseases like cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process (Hogg, 1998; Pong, 2003; Halliwell, 1994; Aviram, 2000). Plants contain natural antioxidants (tannins, flavonoids, vitamins C and E, etc.) that can preserve β-cell function and prevent diabetes induced ROS formation (National Nutrition Council, 1999) and many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatment of DM (Abdel-Barry et al., 1997; Pushparaj et al., 2000). Despite this, few traditionally used antidiabetic plants have received proper scientific screening (Bakurel et al, 2008). The World Health Organization (WHO) has recommended that this area warrants further evaluation (WHO, 1980).
The present investigation demonstrate the antidiabetic and hypoglycemic effect with respect to the antioxidant properties (both in-vitro and in-vivo) of aqueous extract of leaves of *S. nigrum* (*ALSN*) and aqueous extract of aerial parts of *M. pentaphylla* (*AAMP*) on normal and alloxan induced diabetic rats. The experimented dose levels of both the extracts for systemic administration in animals were selected from the determination of LD_{50} in the acute toxicity study. The selected dose levels for the study for *ALSN* were 50 and 100 mg/kg body weight, and that for *AAMP* were 250 and 500 mg/kg body weight.

It has been reported that the presence of so many alkaloids, glycosides, flavonoids, phenols, coumarins, polysaccharides, terpenes, steroids and proteins in the plant extracts contribute to their various biological activity such as: antihyperglycemic, hypoglycemic, anti-hyperlipidemic / hypolipaemic and antioxidant property (Zhang and Xiao, 1993). The preliminary phytochemical investigation reports indicates that the aqueous extract of leaves of *S. nigrum* (*ALSN*) showed the presence of carbohydrates, polypeptides, saponins, tannins, alkaloids, flavonoids, coumarin, terpenoids, steroids and the aqueous extract of aerial parts of *M. pentaphylla* (*AAMP*) showed the presence of carbohydrates, glycosides, saponins, tannins, alkaloids, flavonoids, terpenoids and steroids as phytoconstituents. Therefore, keeping all these in mind, the present research work has been moved forward in the light of the presence of all the above said components in the aqueous extracts of both plants, for the evaluation of the antihyperglycemic, hypoglycemic and antioxidant potential.

The oral glucose tolerance test in the present study showed that both the extracts (*ALSN* & *AAMP*), at the tested dose levels, are having significantly good control over the blood glucose levels in the glucose loaded hyperglycemic animals. The results of the normoglycemic model showed that both the test extracts have shown dose dependent hypoglycemic effect in reducing the blood sugar level in normal rats, while in the alloxan induced diabetic models, both the extracts also registered significant effects (p<0.05 to p<0.001) in reducing the blood glucose levels in hyperglycemic animals, which again supported by one way analysis of variance (p<0.05 to p<0.01) within the groups. The conclusion derived from these data revealed a defined role of aqueous extracts in normoglycemic, glucose hyperglycemic and alloxan-induced diabetic rats, *ALSN* and *AAMP* found to possess dose dependent suppression of glucose level, with prolonged hypoglycemia at higher dose of 100mg/kg and 500 mg/kg respectively, which is almost same effect as of synthetic drug glibenclamide. However, *AAMP* was found to be significantly more potent in reducing the blood glucose levels than that of *ALSN*, in all of the above said experimented models. Moreover, it has been observed that, there was ameliorative changes occurred in the
urine sugar of the animals treated with the standard drug and the extracts when measured on 0\(^{th}\), 10\(^{th}\), 20\(^{th}\) and 30\(^{th}\) day of treatment. Both ALSN and AAMP could able to register marked reduction in the level of urine sugar progressively throughout the experiment.

The extracts also showed a significant (p<0.05 to p<0.001) increase in the peripheral glucose uptake as evidenced in glucose absorption, by isolated rat hemidiaphragm in all the tested dose levels either alone or in combination with insulin. Besides that, ALSN & AAMP at the tested dose levels, at the end of the 30\(^{th}\) day of experiment, significantly (p<0.05 to p<0.01) elevate the glycogen content of liver and kidney. In the study of estimation of plasma insulin levels, both the aqueous extract, exhibited the significant (p<0.01 to p<0.001) and consistent increase in the plasma insulin levels in the diabetic treated animals throughout the course of the experiment; and the extracts also registered good potency to produce degranulation & loss of immunostainable insulin content of islet of beta cells as evidenced in the β-cell degranulation score test. The degree of depletion was initiated in 5\(^{th}\) day post treatment and increased progressively with time exhibiting a maximum of 75% depletion of the beta cells in the ALSN & AAMP treated groups at the higher dose levels, at the end of study. Moreover, the Liver levels of other important metabolic enzymes like Glucose-6-phosphatase, HMG CoA reductase and Arginase activities in the diabetic treated rats has been significantly (p<0.05 to p<0.001) reduced by ALSN and AAMP at the tested dose levels, however a significant (p<0.05 to p<0.01) increase in the levels of Hexokinase were registered by the extracts at the same time.

Alloxan, a beta-cytotoxin, induces “chemical diabetes” by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation (Shankar et al., 2007). This damages a large number of β-cells, resulting in decrease in endogenous insulin release, which leads to decreased utilization of glucose by the tissue (Saravanan and Pari, 2005). It is generally accepted that alloxan treatment causes permanent destruction of β-cells and impairment of renal function; and sulfonylureas are known to lower the blood glucose level by stimulating β-cells to release insulin (Pari & Maheswari, 1999). However, the statistically significant anti-hyperglycemic as well as hypoglycemic activities shown by the aqueous extract of S. nigrum leaves and M. pentaphylla aerial parts in both single & multi dose treated normoglycaemic and hyperglycaemic models might suggest that the said effect be due to extra-pancreatic and extra-intestinal action of the test extract (Day et al, 1990) and may possibly due to the insulinotrop effect or beta cytotoxic effect at the islet beta cell level as evidenced by the increased plasma insulin levels and loss in granularity/ immunostainable
Analysis

insulin content of beta cells, respectively, as all these parameters are widely accepted as markers of insulinotropic / beta cytotropic effect (Saxena et al., 1996). Though there is no clear understanding on the mechanism of insulinotropic effect of sulfonyl ureas till yet, but thought to be due to its binding to pancreatic beta receptors (Krall, 1985) or ionophoretic (ATP sensitive K\textsuperscript{+} channel) modulation (Aguilar-Bryan et al 1995; Philipson and Steiner 1995) or facilitating effect of Ca\textsuperscript{2+} inflow into beta cells (Ribalet and Ciani 1987; Boyd 1988). All these hypoglycemic and antidiabetic effects of the extracts can be comparable with that of standard glibenclamide, which suggested that the extracts may act by reactivating/increasing the sensitivity of the β-cells in alloxan-induced diabetes (Ghosh & Suryawanshi, 2001). And the decreased activity in glucose level in OGTT might be, due to a decrease in the rate of initial glucose absorption in the intestinal tract when a plant fiber is given orally with glucose (Day et al, 1990).

In diabetic patients with insulin deficiency or insulin resistance or hyper-glucagonemia, there is an increase in hepatic glucose production, a decrease in peripheral glucose uptake, and a decrease in the conversion of glucose to glycogen in the liver (DeFronzo et al, 1992). Insulin lowers the concentration of glucose in blood by inhibiting hepatic glucose production and by stimulating the uptake and metabolism of glucose by muscle and adipose tissue (Davis and Granner, 2001). It has been reported that increase of glucose uptake may be due to extra pancreatic effect resemble to insulin (Chattopadhyay et al., 1992). The property of the extracts, towards increase of glucose utilization by isolated rat hemidiaphragm, supports the insulinotropic effect or direct insulin like activity. The improvement of the rate of glycogenesis is one of the probable ways of antidiabetic action (Maiti et al., 2004). Since in the present study of the estimation of liver and kidney glycogen, there is significant increase in the glycogen levels of the diabetic treated animals, hence it may be because of reactivation of the glycogen synthetase system.

Moreover, it is well known that, diabetes mellitus causes failure to use of glucose for energy which leads to increased utilization and decrease storage of protein, responsible for reduction of body weight, essentially by depletion of body proteins (Guyton and Hall, 2000). In the study of body weight variation test in diabetic animals under treatment of test extracts and standard drug, showed that, there is significant (p<0.05 to p<0.001) recovery of body weight when compared with solvent treated diabetic rats, at the end of the 30\textsuperscript{th} day of treatment. Both the extracts registered a significant (p<0.05 to p<0.001) and progressive reduction in the % loss in body weight through out the experimental period. However, AAMP registered more significant (p<0.001) potency, than that of ALSN in recovering the body
weight in the diabetic rats. This property of the test extracts to recover the body weight of animals suggesting the extra pancreatic action of the extract and might be contributed by increased utilization of glucose by the tissues.

Furthermore, the decrease in insulin secretion in diabetes mellitus results in lesser uptake of glucose by skeletal muscle and adipose tissues, which in turn increases the secretion of glucagon from the A cells of the pancreas and inhibits glycogen synthase and activate glycogen phosphorylase in liver, an enzyme which catalyzes glycogenolysis leading to formation of Glucose-6-phosphate. The resulting glucose 6-phosphate, in liver but not in muscle and adipose tissues, is hydrolyzed by the specific enzyme glucose-6-phosphatase, yielding glucose that is exported to the blood stream. So increase in this enzyme leads to increased formation of glucose leading to an increase in the blood glucose concentration (Mayes Peter & Bender David, 2003). Glucose 6-phosphate is the inhibitor of Hexokinase, an enzyme which is responsible to reverse the above reaction. Glucose enters glycolysis by phosphorylation to glucose 6-phosphate, catalyzed by hexokinase, using ATP as the phosphate donor. Under physiologic conditions, the phosphorylation of glucose to glucose 6-phosphate can be regarded as irreversible. Glucose 6-phosphate is an important compound at the junction of several metabolic pathways (glycolysis, gluconeogenesis, the pentose phosphate pathway, glycogenesis, and glycogenolysis). Hexokinase is inhibited allosterically by its product, glucose 6-phosphate (Mayes Peter & Bender David, 2003). So elevation in the levels of the enzyme hexokinase leads to the increased breakdown of glucose.

The results reported in the present study revealed that, both the test extracts registered significant (p<0.05 to p<0.001) activity in reducing the levels of Glucose-6-phosphatase and elevating the levels of Hexokinase activity in the Liver of the treated diabetic rats, thereby reduces the blood glucose level. The working mechanism of the extracts is again strengthened by the effective reduction of the urea cycle enzyme Arginase as well. Arginase catalyzes the conversion of arginine to ornithine and urea, completing the last step in the urea cycle. Ornithine is then transported back into the mitochondrion to begin another cycle. The urea is excreted. Arginase activity is a key diagnostic indicator. Increased levels of arginase activity in blood have been associated with liver damage (Moertel et al, 2008)). Hyperargininemia due to arginase deficiency is an inherited autosomal recessive disease (Pulichino et al, 2008).

Furthermore, it has been reported that the increase in glucose levels in alloxan-induced diabetic rats is associated with dyslipidaemia characterized by elevated serum triglycerides and total cholesterol levels. Alterations in secretion of insulin and glucagon also profoundly affect lipid, ketone, and protein metabolism. At concentrations below those
required to stimulate glucose uptake, insulin inhibits the hormone-sensitive lipase in adipose tissue and thus inhibits the hydrolysis of triglyceride stored in the adipocyte. This counteracts the lipolytic action of catecholamines, cortisol, and growth hormone and reduces the concentrations of glycerol (a substrate for gluconeogenesis) and free fatty acids (a substrate for production of ketone bodies and a necessary fuel for gluconeogenesis). These actions of insulin are deficient in the diabetic patient, leading to increased gluconeogenesis and ketogenesis. Insulin also enhances the transcription of lipoprotein lipase in the capillary endothelium. This enzyme hydrolyzes triglycerides present in very low density lipoproteins (VLDL) and chylomicrons, resulting in release of intermediate-density lipoprotein (IDL) particles. The IDL particles are converted by the liver to the more cholesterol-rich low-density lipoproteins (LDL). Thus, in the untreated or undertreated diabetic patient, hypertriglyceridemia and hypercholesterolemia often occur. In addition, deficiency of insulin may be associated with increased production of VLDL. In the adipose tissue of diabetic individuals, the effect of the decrease in insulin and increase in glucagon results in inhibition of lipogenesis and inactivation of lipoprotein lipase, and activation of hormone-sensitive lipase. This leads to release of increased amounts of glycerol (a substrate for gluconeogenesis in the liver) and free fatty acids, which are used by skeletal muscle and liver as their preferred metabolic fuels, so sparing glucose (Davis and Granner, 2001).

The improvement of blood glucose level induced by most hypoglycaemic treatments is associated with a reduction of serum triglycerides and total cholesterol (Dhanabal et al., 2006; Saravanan and Pari, 2006). In the present study, both the extracts significantly lower the level of Liver HMG CoA reductase, an enzyme which is responsible for the conversion of HMG CoA to Mevalonate in the presence of NADPH, the principal regulatory step in the pathway of cholesterol synthesis and is the site of action of the most effective class of cholesterol-lowering drugs (Mayes Peter & Botham Kathleen, 2003). Moreover, both the aqueous extracts, ALSN & AAMP, at the tested dose levels, registered significant reduction in the levels of LDL, VLDL, total cholesterol, triglycerides, free fatty acids, phospholipids & total lipids while increasing the levels of HDL in the diabetic rats after 30 days of treatment, which reveals that the extracts may affect the transcription of lipoprotein lipase similar to that of insulin, since in the untreated or under treated diabetes patients the level of triglycerides and cholesterol increases due to increased production of VLDL and unavailability of protein lipase which hydrolyses the triglycerides to VLDL because of insulin deficiency. The activity of the significant reduction in the levels of HMG CoA reductase (the rate controlling enzyme in the cholesterol synthesis) registered by the extracts, might be another reason for the
significant reduction in the total cholesterol, LDL & VLDL levels and an increase in HDL level in the diabetic rats.

As reported elsewhere, the presence of increased level of saturated free fatty acids in diabetes mellitus raises the serum cholesterol levels (Spady and Dietschy, 1988). The total cholesterol is reflected by its fractions like VLDL, LDL & HDL. Terpestra (1981) reported that the increase in serum total cholesterol was mainly reflected in the LDL & VLDL fractions, where as in the HDL fractions, there were only relatively mirror images. So being the herbal products containing glycosides, flavonoids, terpenes and phenols, both the plant extracts may be producing the above lipid lowering actions by (1) reducing the VLDL fractions which is a source of LDL fraction, by increasing the availability of lipoprotein lipase due to the increased level of insulin and there by blocking the hydrolysis of triglycerides to VLDL (2) increase in hepatic uptake of LDL through increase of LDL receptors, which usually reduced in case of diabetes. The HDL levels in the extract treated diabetic rats were also markedly increased, which facilitates transfer of non-esterified cholesterol from VLDL and LDL to hepatic tissue by HDL.

The Serum biochemical investigation report showing estimation of marker enzyme like ASAT, ALAT and ALP which are considered to be good indices of liver and kidney damage (Martin et al, 1981) are significantly reduced by the extracts and therefore it may be presumed that the extracts are having the potential to protect the cellular damage that occurs in chronic hyperglycemia. The other serum biochemical parameters like total bilirubin, direct bilirubin, Albumin, total protein, globulin were found insignificant different when compared with reference animal group. Moreover, the haematological parameters showed that the animals treated with Glibenclamide and test extracts registered normal values in RBC count, WBC count, Hb count, and clotting time. But AAMP at 500 mg/kg b.w showed a significant (p<0.05) increase in haemoglobin levels and all other parameters like neutrophil, eosinophil, basophil, lymphocyte and monocyte count appears to nearly equal with that of normal value in all extract treated groups. Therefore, it is suggested that the test ext. has no significant effect on the haematological parameters and is evident for the safety use for a longer duration of time.

Free radicals e.g. superoxide radical, hydroxyl radical, peroxy radical and singlet oxygen radicals have been implicated in many disease conditions. Free radicals refer to any chemical species (capable of independent existence) possessing one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. When paired in orbital, (the two electrons in an orbital have different spin directions), electrons are more stable.
Varying radicals and free radicals have been known to be generally less stable than non-radicals (Tappel, 1970). The potentially reactive oxygen species (ROS) such as $\text{O}_2^•−$, $\text{H}_2\text{O}_2$ and •OH, are continuously generated inside the human body as a consequence of exposure to a plethora of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer. Free radicals, especially the oxygen radical, superoxide, when formed could lead to the formation of other radicals. In fact, the toxicity of $\text{O}_2^•−$ in living organisms is due to its conversion into •OH and reactive radical metal complexes. Superoxide and hydrogen peroxide are converted into •OH and other reactive radical complexes through the iron catalyzed Haber-Weiss reaction or the superoxide driven Fenton reaction (Goldstein, 1993; Fenton, 1894; Koppenol, 1993). The uptake of one electron by molecular oxygen results in the formation of the superoxide anion radical. Superoxide anion radical is a strong base, a potent reducing agent, an oxidant and may initiate the oxidation of molecules like ascorbic acid or epinephrine following hydrogen abstraction due to its basicity. Hydrogen peroxide is a stable molecule, can act as both oxidizing and reducing agent and can generate hydroxyl radicals by an interaction with transition metal ions or a reaction with highly reactive oxidizing agents like NO and NO$_2$ (Maged, 1999).

As discussed earlier in this section, the oxidative damage is a crucial etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process (Hogg, 1998; Pong, 2003; Halliwell, 1994; Aviram, 2000). Under normal circumstances, the deleterious reactions triggered by these ROS are detoxified and controlled by a system of enzymic (superoxide dismutase, catalase and glutathione peroxidases) and non-enzymatic antioxidants which eliminate pro-oxidants and scavenge free radicals (Arouma, 1996) and there is equilibrium between the ROS generated and the antioxidants present. However, owing to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is hampered favouring the ROS upsurge that culminates in oxidative stress. Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is warranted. Epidemiological studies have found that the intake of antioxidants such as Vitamin C reduces the risk of coronary heart disease and cancer (Marchioli et al., 2001). The antioxidants may mediate their effect by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions (Robak and Marcinkiewicz, 1995). Several synthetic antioxidants, e.g., BHA and butylated hydroxytoluene are commercially available but are quite unsafe and their
toxicity like liver damage and mutagenesis is a problem of concern (Madhavi and Salunkhe, 1995; Grice, 1986), but the natural antioxidants, especially phenolics and flavonoids are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption.

Herbal drugs containing radical scavengers are gaining importance in treating such conditions. Many plants possess dynamic antioxidant properties owing to their phenolic and flavonoid contents (Larson 1988). Phenolic compounds such as tannins, flavonoids and phenolic acids are considered to be the major contributors to the antioxidant capacity of plants. All phenols and flavonoids particularly, are effective antioxidants because they donate electrons to radicals and break the radical chains. Phenolic compounds have been shown to exert a wide range of biological activities including scavenging various ROS (Frankel, 1999). Since the total phenols and total flavonoids contents of aqueous extract of leaves of S.nigrum are found to 33.83 µg of pyrocatechol equivalent /500mg and 5.86 mg equivalent of quercetin /gm and that of aqueous extract of aerial parts of M. pentaphylla are found as 75.16 µg of pyrocatechol equivalent /500mg and 9.58 mg equivalent of quercetin /gm respectively, which is quantitatively a greater value. It is well known that the presence of polyphenols and flavonoids in plants, mainly responsible for their dynamic antioxidant activity, the obtained amount of total phenolics & flavonoids in the extracts indicated that the extracts possess high antioxidant activity, so it may be presumed that, the antioxidant potential of the extract may plays a significant role for anti hyperglycemic potential of the plant extract.

In this present study, the antioxidant activity of the test extracts was also investigated by using total antioxidant activity and ferric reducing power (FRAP assay) of the extracts. Both methods have proven the effectiveness in showing profound antioxidant activity of the extracts when compared to the reference standard ascorbic acid. The total antioxidant activity of ALSN & AAMP was measured based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate / Mo (V) complex at acidic pH. The values were expressed in terms of ascorbic acid equivalent. On the other hand, the reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Mier et al., 1995) and generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants (i.e. antioxidants) in both the extracts causes the reduction of the Fe3+ /ferricyanide complex to the ferrous form. Therefore, the Fe2+ can be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. The reductive capabilities of the
ALSN & AAMP compared to ascorbic acid were found significantly very potent and the power of the extract was increased with quantity of sample which demonstrate the antioxidant potential of the extracts.

Besides that, in the in-vitro experiments, both the extracts significantly (p<0.05 to p<0.001) scavenged DPPH, Superoxide (O$_2^•$−), Hydrogen peroxide (H$_2$O$_2$) and Nitric oxide (NO) free radicals in a concentration dependent manner. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It has characteristic absorbance maxima at 517 nm, widely used to evaluate the free radical scavenging effect of natural antioxidants (Jao et al 2002). Many researchers have reported positive correlation between free radical scavenging activity and total phenolic content. DPPH radical scavenging activity increased with the increase of phenolic compound content (Oki et al 2002, Lu and Foo 2000, Siriwardhana 2003). The DPPH scavenging ability of the extract may be attributed to its hydrogen donating ability. The primary free radical in most biological systems is Superoxide (O$_2^•$−). Although O$_2^•$− itself is quite unreactive compared to the other radicals, but it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals (Dahl et al, 1978). The O$_2^•$− scavenging activity of the extracts were determined by Phenazine methosulphate/NADH-NBT system wherein O$_2^•$− derived from dissolved oxygen by Phenazine methosulphate/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anions in the reaction mixture. The spontaneous or catalytic dismutation of O$_2^•$− leads to the formation of H$_2$O$_2$, which in the presence of a transition metal ion like Fe$^{3+}$, decomposes into •OH radicals, a highly damaging species in free radical pathology (Pardini, 1995). Both the extract also scavenged H$_2$O$_2$ radical significantly (p<0.001) at 500 µg/ml. The extracts effectively and dose dependently quenched NO free radicals. Control experiments showed that even at high concentrations, the extract did not interfere with the reaction between nitrite and Griess reagent. ROS like O$_2^•$− may react with NO and give rise to various other reactive nitrogen species (RNS) such as NO$_2$, N$_2$O$_4$ and peroxynitrite etc. Both ROS and RNS together attack and damage various cellular molecules. Virtually all cellular components including lipids, proteins, nucleic acids, carbohydrates are susceptible to oxidative damage (Pacifici and Davies, 1991). ALSN & AAMP, owing to their free radical scavenging ability may provide protection against oxidative damage induced to the biomolecules: proteins and lipids. However, in all the above in-vitro antioxidant experimental models, AAMP was found to possess greater antioxidant activity, ferric reducing power and
free radical scavenging ability than that of ALSN, which might be due to the presence of more amounts of phenolic and flavonoid contents in AAMP than that of ALSN.

Moreover, it has been reported earlier about the role of oxidative stress which constitutes the key and common events in the pathogenesis of different diabetic complications. Hyperglycemia induces the generation of free radicals which can affect antioxidant defenses thus leading to the disruption of cellular functions, oxidative damage to membranes and increased susceptibility to lipid peroxidation (Giugliano et al., 1996). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation (Bakirel et al 2008). Reactions of oxygen free radicals with all biological substances especially with polyunsaturated fatty acids lead to increased lipid peroxidation (LPO) (Memisogullari and Bakan, 2004) resulting in impairment of membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Arulselvan and Subramanian, 2007).

In the *in-vivo* antioxidant experiments i.e. in the multi dose treated animals, both the aqueous extracts of *S. nigrum* (ALSN) and *M. pentaphylla* (AAMP), at the tested dose levels, registered very potent and significant activity (p<0.01 to p<0.001) in reducing the Liver lipid peroxidation products such as Thiobarbituric acid reactive substances (TBARS), Hydroperoxides (HP), Malondialdehyde (MDA) and Conjugated dienes (CD), and significantly (p<0.05 to p<0.001) elevating the levels of the Liver enzymatic antioxidant parameters like Reduced glutathione (GSH), Glutathione peroxidase (GSH-Px), Glutathione reductase (GR), Superoxide dismutase (SOD) and Catalase (CAT), in the diabetic rats, at the end of the 30th day of treatment. The diabetic rats showed significant increase in lipid peroxidation products such as TBARS, CD, MDA and HP content in liver, which suggest that peroxidative injury may be involved in the development of diabetic complications. Both the extracts showed significant reduction in liver lipid peroxidation products in diabetic rats which indicate that ALSN and AAMP are having potential to inhibit the oxidative damage of liver tissues. Moreover, GSH is mainly involved in the synthesis of important macromolecules and in the protection against reactive oxygen compounds (Manonmani et al., 2005). A marked decrease in liver GSH was observed in diabetic rats. The reduced GSH level contributes to the pathogenesis of complications associated with diabetic state. The significant increase in the content of GSH in the liver, showed by the test extracts, may be attributed to the presence of antioxidant compounds in *S. nigrum* leaves and *M. pentaphylla* aerial parts. Enzymatic antioxidant such as SOD and CAT are considered as primary enzymes
since they are involved in the direct elimination of ROS (Arulselvan and Subramanian, 2007). SOD is one of the most important enzymes and scavenges O$_2^-$ anion (which is the first product of O$_2$ radicals) to form H$_2$O$_2$ in the enzymatic antioxidant defence system and hence abolishes the toxic effects due to this radical or other free radicals derived from secondary reactions (Manonmani et al., 2005). The O$_2^-$ anion is reported to inactivate CAT and GSH-Px (Halliwell and Gutteridge, 1984). Catalase has been recognized as a major determinant of hepatic and cardiac antioxidant status (Wohaieb and Godin, 1987) and is known to be involved in detoxification of H$_2$O$_2$ concentrations (Yoshikawa et al., 1993), whereas GSH-Px is sensitive to lower concentrations of H$_2$O$_2$. In Diabetes, the alloxan-generated ROS causes non-enzymatic glycosylation and oxidation resulting in the inactivation and inhibition of antioxidant enzymes such as SOD and CAT (Al-Azzawie and Alhamdani, 2006). In the present long term treated study, it was observed that the extracts reverse the activities of these enzymatic antioxidants (i.e. increase in the SOD, CAT, GPx and GR activities), significantly, in the liver tissues of diabetic rats which might be due to decreased oxidative stress as evidenced by decreased LPO.

So from the antioxidant study, it could be revealed that both the plant extracts i.e. ALSN and AAMP are endowed with significant potential in scavenging free radicals *in-vitro* and in reducing the liver lipid peroxidation products along with elevating the enzymatic antioxidant parameters *in-vivo*. The results depicted in this study illustrated that the acute/sub-acute antidiabetic, hypoglycemic, insulinitropic and beta-cytotropic effects of ALSN and AAMP were similar to those of glibenclamide with regards to their effect on antioxidant status. Moreover, the possible mechanism, by which the plant extract mediates its antidiabetic action, is potentiation of pancreatic secretion of insulin from existing residual β-cell of islets and due to enhanced utilization of blood glucose by peripheral tissues as well.

**Histopathological studies of liver and kidney**

**Liver:**

The microscopic section of liver revealed consistent findings of degenerative and fatty changes in hepatocytes, Centrilobular necrosis & denucleation of hepatocytes and Very high mononuclear cell infiltration, congestion portal blood vessels and bile duct proliferation in the liver of the rats of diabetic control group. This showed that sustained hyperglycemia induced by alloxan can induce both toxic and inflammatory effects in the liver. The toxic effects are also substantiated by significant increase in the serum ASAT, ALAT & ALP enzymes. This was so due to necrosis in hepatocytes. These enzymes leaked from the liver due to increased permeability of liver cells. Moreover, hepatic necrosis may also be the
reason of increase in clotting time because it is the site where a number of clotting factors are synthesized. One interesting finding of liver was that the degenerative and necrotic changes were consistently present around the central vein while inflammatory changes were present in portal areas. This pattern of centrilobular necrosis suggested that alloxan had the direct effect on oxidative phosphorylation of the hepatocytes. So the necrotic changes were more pronounced in centrilobular zone (around the central vein), because around the central vein, the oxygen tension is less as compared to portal areas. However marked inflammatory reactions in the portal zone suggest the abnormal metabolic conversions in the hepatocytes. Moreover, a restraining inflammatory reaction and proliferative changes in the portal tract was also seen because of the fact that this zone is rich in vascular supply, which is an essential need of inflammatory process.

However, the liver of rats of group-3 received glibenclamide prevailed very mild congestion of sinusoids and portal veins and very mild mononuclear cell infiltration in portal tract. ALSN and AAMP appeared to ameliorate the effect of alloxan induced hyperglycemia in the livers of the treated rats because necrotic changes were milder in these groups than those of diabetic control group. Besides that, very mild mononuclear cell infiltrative and proliferative changes and very milder vacuolar degenerative changes in the hepatocytes were observed in these groups, which suggest the protecting effects of the extracts. This type of protective changes in the diabetic livers may be occurred due to the effects of the extracts in decreasing the levels of the marker enzymes and increasing the levels of antioxidant enzymes such as GSH, GSH-Px, GR, SOD and CAT in the liver as discussed earlier. The inflammatory changes were also milder in the groups treated with the higher dose levels of the extracts. The presence of some fatty changes in the extract treated groups suggests that there is some bearing of this on fat metabolism also, may be due to the insulinotropic effects of ALSN and AAMP.

Kidney:

Kidney tissues of the rats of diabetic control group also showed severe degenerative and desquamative granular as well as vacuolar changes in the proximal convoluted tubules (PCT) along with heavy congestion of blood vessels of glomeruli and interstitial spaces. This clearly shows the adverse effect of long term hyperglycemia on the epithelial cells of PCT. The PCT also associated with severe amyloid depositions in glomeruli, which resembled an eosinophilic acellular hyaline mass. This might be due to the accumulation and clumping of desquamated epithelial cells in the tubules, which might have occurred due to the long term hyperglycemia and altered metabolism of carbohydrates and proteins in the deficiency of
insulin associated with diabetes. Moreover, some inflammatory changes were also found in interstitial spaces. This may be due to toxic tubular nephrosis in PCT part or dilatation of the blood vessels resulting in leakage in interstitial spaces.

However, the groups treated with standard drug glibenclamide and the test extracts (ALSN and AAMP), showed very milder degenerative changes in the epithelial lining of DCT and PCT part, mild mononuclear cell infiltration in the interstitial spaces and very little or nil necrosis in the tubular epithelium. This might be due to effects of the extracts in stabilizing the abnormal metabolic effects associated with carbohydrate, lipid and proteins in diabetes, which may be due to the increase in the insulin secretion and significant reduction in the blood glucose level.

The histopathology study of liver and kidney summarized that the test extracts possess very good potential to protect the liver and kidney tissues of the diabetic animals.

The perusal of experimental results embodied in the thesis comes in a general view that the aqueous extracts from the leaves of S. nigrum and aerial parts of M. pentaphylla endowed with potential hypoglycaemic and antihyperglycaemic activity which could be attributed due to their possible multiple effects involving glucose and lipid metabolism at both pancreatic and extra-pancreatic site. On the other hand, the extracts exert very good potentials to scavenge toxic free radicals along with the inhibition of the liver lipid peroxidation products and activation of the enzymatic antioxidant defense mechanism in diabetic rats that might be due to the presence of high levels of phenolic and flavonoid contents, which could be responsible for the supporting properties of the extracts for their hypoglycaemic and antihyperglycaemic activity. Furthermore, the sub-acute and histopathology studies revealed the safetyness of the extracts in animals.

**Analysis of the compound isolated from AAMP**

Since the test report embodied in the thesis evidenced that AAMP shows comparatively better activity, hence it enforced us to isolate new compound present in the extract. The extract AAMP responded positively to the Salkowski’s test and Lieberman-Burchard test for steroids and triterpenes; so it is assumed to be a compound containing steroidal nucleus. The isolated phytochemical was found as a white crystalline compound with melting point of 322-324 °C. The elemental analysis (Elementar, Vario EL III) revealed that the compound contains 38.46% of Carbon, 61.54% of Hydrogen. The Nitrogen % was found to be nil.
The FTIR spectroscopic analysis exhibited characteristic broad peak centered at 3275 cm\(^{-1}\), that is characteristic of O-H stretching suggesting the presence of hydroxyl group which was substantiated by chemical identification tests; The absorption bands at 2944.78 cm\(^{-1}\) corresponds to CH\(_2\)- stretching, at 1564.36 cm\(^{-1}\) as a result of CH=CH stretching, absorption at 1391.31 cm\(^{-1}\) is due to aliphatic C-H stretching, and the band at 1062.73 cm\(^{-1}\) is due to the presence of C-O-C stretching.

The \(^{13}\)C-NMR spectrum revealed the presence of approximately 30-32 number of carbon atoms including seven methyls, ten methylenes, thirteen methane and seven quaternary carbons. The \(^{13}\)C-NMR has shown recognizable sharp signal at \(\delta\) 180.3 ppm, corresponds to the presence of –COOH group, which may be assigned to C-4a position. The absorption peaks at \(\delta\) 90.6 and 92.5 ppm that may correspond to angular C-O-C atoms at 2’ and 2” positions. The absorption peaks at \(\delta\) 63.1, 74.3, 90.9, 72.1, 65.0, 74.0 and 90.9 ppm illustrated the presence of multi-hydroxyl groups corresponding to the carbon positions of 3’, 4’, 5’, 3″, 4″, 5″ and 6″ respectively. The signals at \(\delta\) 23.9 and 37.8 ppm are assigned to the C-14 and C-14a double bonds respectively. The peaks at \(\delta\) 90.9 ppm corresponding to both the carbon positions at 6’ and 6″ are each of same value, which may be because of the presence of electron withdrawing groups like oxygen (C-O). The alkene carbons appeared at \(\delta\) 23.9 and 37.8 ppm (Agrawal et al, 1985; Pateh et al, 2009; Jamal et al, 2009).

The \(^1\)H-NMR spectrums displayed sharp signals for seven tertiary methyl groups, viz., at \(\delta\) 0.91 ppm that may be assigned to the methyl groups attached at the positions of H-2, H-2 and H-6b (i.e. proton containing carbon positions); a sharp peak at \(\delta\) 1.20 ppm which may be attributed to the two methyl groups attached at the positions of H-9 and H-9; and at \(\delta\) 1.22 ppm that may be assigned to the position of H-6a. Moreover, it also shown two singlet protons at \(\delta\) 1.98 and 5.04 ppm ascribable to the methane positions of H-1 and H-2’ respectively; five doublet protons at \(\delta\) 1.91, 4.02, 4.88, 5.14 and 5.41 ppm which were assignable to the methane positions of H-13, H-3′, H-6′, H-6′ and H-2″ respectively; thirteen triplet protons at \(\delta\) 0.77, 1.36, 1.40, 1.43, 1.52, 1.63, 2.30, 2.34, 3.07, 3.38, 3.74, 3.91 and 4.85 ppm which were ascribable to the methane positions of H-8a, H-(7 & 12), H-8, H-12b, H-3, H-4, H-6, H-5, H-14b, H-10, H-4″, H-(4’ & 5’) and H-5’’ respectively; one quadruplet proton at \(\delta\) 1.77 ppm that was assigned to the methane position of H-11. The presence of seven hydroxyl groups were supported by the sharp singlet proton peak signals at \(\delta\) 5.01, 5.04, 5.08, 5.09 and 5.11 ppm, which undoubtedly attributed to the position of H-(4’ & 5’), H-(6’ & 3″), H-4″, H-5’’ and H-6” respectively. The singlet proton signal observed at \(\delta\) 5.23 ppm may be attributed due to the presence of the functional group of carboxylic acid (–COOH)
COOH) at the position of H-4a and the singlet peak at δ 5.37 ppm, which due to the presence of carbons of conjugated alkene (CH=C) that was assigned to the position of H-14, which is also supported by the IR stretching at 1564.36 cm\(^{-1}\) (Pateh et al, 2009; Jamal et al, 2009).

LC-MS spectroscopy showed the molecular ion [M]\(^+\) peak at 752.38 m/z value that correspond to a molecular formula, C\(_{40}\)H\(_{64}\)O\(_{13}\) which was consistent with the theoretical value of 752.43 and were supported by the molecular weight calculated by Rast’s procedure. Fragmentation ion peaks at m/z 734 correspond to the loss of hydroxyl unit from the 6’-carbon position of the parent molecule. The dehydration of fragment at m/z 734 (M - H\(_2\)O), on successive fragmentation would yield ion at m/z 692 with the loss of C\(_2\)H\(_4\)O. Moreover, the signal exhibited at m/z 604, could be attributed due to the cleavage of one Pyran moiety from the 2”-carbon atom position, with the loss of C\(_3\)H\(_6\)O\(_3\) from the parent molecule; which on successive fragmentation would yield ions at m/z 602 (corresponds to loss of C\(_3\)H\(_{10}\)O\(_3\)), m/z 586 (corresponds to loss of C\(_3\)H\(_{10}\)O\(_6\)), at m/z 456 (due to loss of C\(_{10}\)H\(_{16}\)O\(_{10}\)), so on. On the other hand, it could be revealed from the mass spectra that the peak exhibited at m/z 296 was equatorial with the agreement of cleavage of a Picene ring (Oleanolic acid glycoside) from the 10-carbon position with the loss of C\(_{30}\)H\(_{58}\)O\(_3\) from the parent molecule, which on successive fragmentation would yield ions at m/z 148 (due to loss of C\(_{35}\)H\(_{56}\)O\(_8\)), the intense peak at m/z 132 (due to loss of C\(_{35}\)H\(_{56}\)O\(_9\)) & a sharp peak at m/z 131 (due to loss of C\(_{35}\)H\(_{57}\)O\(_9\)); the further fragmentation of which would yield ions at m/z 115 (due to loss of C\(_{35}\)H\(_{57}\)O\(_{10}\)) & m/z 60 (due to loss of C\(_{38}\)H\(_{60}\)O\(_{11}\)).

Hence from the above discussion, it could be revealed that the isolated molecule is containing one heptamethyl-octadecahydro-picene moiety (Oleanolic acid glycoside nucleus) attached to a carboxylic acid at C-4a position, and one pyran moiety at the C-10 position, which could be attached to another pyran ring at C-2” position. The presence of the Oleanolic acid glycoside nucleus in the isolated molecule was further supported from the spectral data of the oleanolic acid glycoside that has been isolated and characterized by Jha et al (1984) and Hamburger et al (1989).

The reported experimental results and \(^{13}\)C-NMR, \(^1\)H-NMR and the Mass spectra reported in the present study led us to formulate the molecular formula of the compound as the C\(_{40}\)H\(_{64}\)O\(_{13}\), bearing the IUPAC nomenclature as: 2, 2, 6a, 6b, 9, 9, 12a- Heptamethyl- 10-[4’, 5’, 6’-trihydroxy-3’-(3”, 4”, 5”, 6”-tetrahydroxy-tetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy]-1, 3, 4, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-octadecahydro-2H-picene-4a-carboxylic acid (An Oleanolic acid glycoside derivative) (Fig. 4.32 of chapter-4).