2.1 INTRODUCTION

DM is a metabolic disorder characterized by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both (American diabetes association, 2010). This is a most important and growing public health problem all over the world, with an estimated worldwide prevalence of 171 million people in 2000, expected to increase to 366 million people by 2030. In India, the number of people with diabetes is currently around 40.9 million, is expected to rise to 69.9 million by 2030 (Wild, et al., 2004). India leads the world with largest number of diabetic subjects thus earning the uncertain distinction of being termed the -Diabetes Capital of the World‖ (Mohan, et al., 2007). Hyperglycemia and hyperlipidemia are two important characters of DM, an endocrine based disease. Diabetic patients experience various vascular complications, such as atherosclerosis, diabetic nephropathy and neuropathy (Sheetz, 2002).

In diabetic research various chemicals have been used to induce diabetes in rodents, including STZ. The development of hyperglycemia, following STZ injection is primarily due to the direct pancreatic β-cell destruction, and resulting insulin deficiency (Luo, et al., 1998; Rerup, 1970).

Hyperglycemia and hyperlipidemia are important risk factors in the development of cardiovascular disease and metabolic disorders (Frishman, 1998). The basis of abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on its target tissues, resulting from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action (Devendra et al., 2004). Although the prevalence of diabetes is consistently increasing, an effective treatment is still lacking.
Current pharmacotherapeutics insufficiently reverse hyperglycemia, have limited tolerability and induce side effects (Eurich et al., 2007).

In experimental diabetes, enzymes of glucose metabolism are markedly altered and produce hyperglycemia, which leads to pathogenesis of diabetic complications. (Algandaby et al., 2010; Kondetiet al., 2009; Sharma et al., 2011). Defects in carbohydrate machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism pose an over exertion on the endocrine system leading to the deterioration of endocrine control (American diabetes association, 2004; Cheplick et al., 2004; Ramana and Srivastava, 2010). Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate metabolic enzymes and leads primarily to hyperglycemia (Fava, 2008; Tiwari and Madhusudana, 2002).

Diabetes is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism (Ogbonnia et al., 2008). The goals of managing DM are to optimize the control of blood glucose, reduce the effects of oxidative stress and normalize disturbances in lipid metabolism. Drug management of diabetes without associated untoward effect has also remained a challenge for conventional medical practice (Sabitha et al., 2011). Hyperglycaemia is associated with long term damage and failure of various organs such as eyes, kidneys, liver, nerves, heart, and blood vessels (Ramachandran et al., 2011). The abnormal changes in lipid and protein metabolism leads to progression of diabetic complications (Modak et al., 2007).

Lipid lowering therapy in diabetes is effective in reducing the risk of vascular complications. Metabolic syndrome had become a global epidemic, defined as a
cluster of three of five criteria: insulin resistance and glucose intolerance, abdominal obesity, hypertension, low high density cholesterol and hypertriglycerideremia (Deedwania et al., 2004). Hypercholesterolemia, or more specifically elevated plasma low density lipoprotein cholesterol (LDL-C), is an important risk factor for development and progression of atherosclerosis. Atherosclerosis is accompanied with the production of free radicals by endothelial and vascular smooth muscles. These free radicals initiate processes involved in atherogenesis through several important enzyme systems, including xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADP), oxidases and nitric oxide synthase. The hypercholesterolemic (HC) state leads to an increase in free radical production and thereby elevates lipid peroxides (Harrison et al., 2003). Sulphur compounds and its biochemical and physiological effects, including the effect on biliary lipid composition have been extensively investigated (Adamu et al., 1982; Saravanan et al., 2010).

Although several therapies are currently used in the treatment of diabetes, drawbacks such as cost, hypoglycemia, weight gain, gastrointestinal disturbances and liver toxicity are major concerns to search for alternative approach or medicine to treat or control diabetes (Prasad et al., 2009). The present study was undertaken to scientifically investigate effect of AMSB on carbohydrate and lipids metabolism in STZ induced experimental diabetic rats. The efficacy was compared with a standard hypoglycemic drug (glibenclamide).
2.2 MATERIALS AND METHODS

2.2.1 Chemicals

STZ, NADP and ATP were purchased from Sigma-Aldrich Chemical Corporation, USA. Commercial diagnostic kits were obtained from Qualigens Diagnostics (Mumbai, India) for determination of cholesterol, HDL-C and TGs. All other chemicals used were of analytical grade.

2.2.2 Experimental animals

Male albino wistar rats (150-180 g) were obtained from the IAM, Bangalore, Karnataka, India. They were maintained in the Animal house, Periyar University, Salem, India under standard environmental conditions (22–20°C, 45–64% relative humidity, 12 hrs dark light cycle). The animals were fed with standard rat feed pellet diet (IAM, Bangalore, India) and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. The studies were approved by the Institutional animal ethical committee (PU-TAEC/JULY 2011/06) of Periyar University, Tamil Nadu, India.

2.2.3 Acute toxicity study

Healthy adult male wistar rats were starved overnight, divided into three groups (n=6) and were orally fed with AMSB in increasing dose levels of 50, 100 and 150 mg/kg. The animals were observed continuously for 2h under behavioral, neurological and autonomic profiles. After a period of 24 and 72h, they were observed for any lethality or death (Barik et al., 2008).

2.2.4 Induction of experimental diabetes

Diabetes was induced in overnight fasted adult albino male wistar rats weighing 150-180 g by a single intraperitoneal injection of 55 mg/kg STZ (Kaleem, et al., 2006). STZ was dissolved in 0.1 M citrate buffer (pH 4.5). Hyperglycaemia
was confirmed by elevated glucose level (above 250 mg/dl) in blood, determined at 72 h.

2.2.5 Chronic experimental design

After the successful induction of experimental diabetes, the rats were divided into four groups comprising of six animals in each group as given below.

Group I : Normal control rats.

Group II : Diabetic control rats received (STZ in single dose 55 mg/kg/b.w.).

Group III : Diabetic rats administered with AMSB (150 mg/kg b.w) in aqueous solution orally for 45 days.

Group IV : Diabetic rats administered with glibenclamide (2.5 mg/kg b.w) in aqueous solution orally for 45 days (Rajiv Gandhi and Sasikumar, 2012). Body weight and blood glucose levels were measured periodically.

At the end of the experimental period, the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation, respectively.

2.2.6 Biochemical assays

2.2.6.1 Measurement of carbohydrate metabolic enzymes

Glucose levels were estimated by a commercially available glucose kit using the glucose oxidase method (Sigma Diagnostics, St. Louis, MO). Liver was immediately dissected, washed in ice cold saline to remove the blood and homogenised in 0.1 M Tris–HCl buffer, pH 7.4. The supernatant was used for enzyme activity assays. Hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase were assayed by the method of Lapeir and Rodnick (2001), Koida and Oda, (1959) and Gancedo and Gancedo, (1971), respectively and Glycogen synthase and
phosphorylase activities were assayed by the method of Leloir and Goldenber (1962) and Cornblath et al., (1963), respectively. Liver glycogen was assayed by the method of Ong and Khoo, (2000).

2.2.6.2 Measurement of lipid profile

Phospholipid was estimated by the method of Zilversmit and Davis (1950). The free fatty acid was measured by Falholt et al., (1973). Estimation of TC was carried out by the method of Zlatkis et al., (1953). TGs were estimated by the method of Foster and Dunn (1973) and HDL-C was estimated by the method of Burstein et al., (1970). The VLDL-C was calculated using the formula, TG/5 mg/dl. The serum LDL cholesterol was estimated by the method of Friedwald et al., (1972).

2.2.7 Statistical analysis

Results were expressed as the mean ± SEM. for statistical analysis of the data group means, were compared by one-way analysis of variance (ANOVA) followed by Duncan's post-test for multiple comparisons using SPSS/16.0. P<0.05 was considered to be statistically significant.

2.3 RESULTS AND DISCUSSION

STZ selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and resulting in a diabetic state (Lenzen, 2008; Suresh Kumar et al., 2012). The major mechanism underlying hyperglycaemia in DM involves the overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (Toft and Jenssen, 2005), and studies have shown that the level of blood glucose was elevated in STZ-induced diabetic rats. The administration of single high dose of STZ injection intraperitoneally can produce Type 1 diabetes by destroying the β-cells of the pancreas (Palsamya et al., 2008).
In acute study, administration of AMSB in treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weight and food consumption was observed between the groups of rats. Also, no gross pathological changes were seen. Thus, it was concluded that AMSB was safe at 150 mg/kg.

Table 2.1 shows assay of blood glucose in normal and experimental group of rats on day 0, 3, 20, 35 and 45. STZ induced diabetic rats (group II) showed significant \( P<0.05 \) elevation in blood glucose level compared to the normal control rats (group I). In group III and IV the supplementation of AMSB and glibenclamide respectively tended to bring blood glucose towards near normal levels compared to the diabetic control rats (group II).

Table 2.2 shows the changes in body weight of normal and experimental group of rats. There was a significant reduction in body weight in diabetic rats (group II) compared to control rats (group I). The administration of AMSB (group III) and glibenclamide (group IV) to diabetic rats restored the changes in level of body weight to near normal compared to diabetic rats (group II). In diabetic condition, elevated blood glucose, reduction in body weight, polyuria, polydipsia and polyphagia are usually perceived (Veeramani et al., 2007). STZ induction leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins (Chatterjee and Shinde, 2002; Salahuddin and Jalalpure, 2010).

Table 2.3 shows plasma levels of TC, TGs, phospholipids, free fatty acids and lipoproteins in the control and STZ-induced diabetic rats. When diabetic rats (group II) are compared with control rats (group I), the levels of plasma TC, TGs, phospholipids, free fatty acids, LDL-C and VLDL-C were significantly increased,
while the levels of HDL-C was significantly decreased. Administration of AMSB (group III) and glibenclamide (group IV) significantly reduced the TC, TGs, free fatty acids, LDL-C levels and significantly increased the HDL-C level when compared with diabetic rats (group II).

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Al-Shamaony et al., 1994; Kesari et al., 2007; Kumar et al., 2011a; Patil et al., 2011; Waqr, 2010). The abnormal high concentrations of serum lipids in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots (Al-Logmani et al., 2009). Hyperlipidaemia is a recognized as a common complication of DM. A reduction in insulin secretion causes a variety of derangements in metabolic and regulatory mechanisms leading to accumulation of lipids (Al-Attar, 2010).

Diabetes is associated with profound alterations in the plasma lipid, TGs and lipoprotein profile and with an increased risk of coronary heart disease (Maghrani et al., 2004). High level of total TC is one of the major factors for coronary heart diseases and it is well known that hyperlipidemia and the incidence of atherosclerosis is increased in diabetes (Tan et al., 2005). Coronary risk is associated with the elevated level of HDL-C was reported by Wilson (1988). Arcari et al., (2011) reported that, the increased level of LDL-C might be dangerous among the plasma lipid and the oxidation of LDL leads to its increased penetration of arterial walls. Excessive LDL-C in the blood tends to accumulate in the extracellular sub endothelial space of arteries, which are highly atherogenic and toxic to vascular cells thereby leading to atherosclerosis, hypertension, obesity and functional depression in some organs, etc. (Schneider et al., 2011). It has been demonstrated that insulin deficiency in DM leads to accumulation of lipids such as total cholesterol and TGs in diabetic
patients (Sharma et al., 2011). In the present study, we have perceived significantly increased levels of plasma TC, TG, VLDL and LDL as well as marked reduction in plasma HDL level in diabetic rats (group II). Administration of AMSB (group III) potentially decreased of plasma TC, TGs, phospholipids, free fatty acids, LDL-C and VLDL-C besides, the HDL-C level was increased in diabetic treated rats compared to STZ induced diabetic rats (group II). The above action could be beneficial in preventing diabetic complications like coronary heart diseases and atherosclerosis in diabetic condition.

Table 2.4 and 2.5 shows the efficacy of AMSB on TC, TGs, free fatty acids and phospholipids in liver and kidneys. TC, TGs, free fatty acids and phospholipids were increased significantly in the liver and kidneys of diabetic rats (group II). Supplementation of AMSB (group III) and glibenclamide (group IV), tended a significant reduction in TC, TGs, free fatty acids and phospholipids in both the tissues compared to the diabetic rats (group II).

The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Kumar et al., 2011b; Chi and Koh, 1982). In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses TGs and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia (Maruthupandian and Mohan, 2011; Pushparaj et al., 2007; Shirwaikar et al., 2004). The elevated phospholipid levels in serum are a consequence of elevated lipoproteins. This might have occurred in the diabetic rats as a result of lack of insulin which activates the lipase enzymes, hydrolyzing the stored TG and releasing large amounts of fatty acids and glycerol in the circulating blood (Shirwaikar et al., 2006). Consequently, the excess of fatty acids in the plasma may promote the hepatic conversion of fatty acids
into phospholipids and TC, the main product of lipid metabolism. The increased level of TG and TC in the blood of diabetic rats may lead to cardiovascular disease. The accelerated coronary heart disease has emerged as a leading cause of morbidity and mortality in diabetic patients worldwide (Ramachandran et al., 2012). High levels of TC and most importantly, LDL cholesterol are the predictors of atherosclerosis (Temme et al., 2002). In the present study, the AMSB significantly reduced the TC, TGs, free fatty acids and phospholipids in treated diabetic rats (group III) as compared to untreated diabetic rats (group II). These changes are beneficial in preventing diabetic complications as well as in improving lipid metabolism in diabetics (Kumar et al., 2010).

Table 2.6 showed the activities of hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase of control and diabetic induced experimental rats. The activity of hepatic hexokinase level was significantly decreased whereas elevation in glucose-6-phosphatase and fructose-1,6-bisphosphatase were also observed in STZ induced rats (group II) compared to control rats (group I). After treatment with AMSB (group III) and glibenclamide (group IV) hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase levels were normalized compared to diabetic untreated rats (group II).

Hexokinase plays an important role in the maintenance of glucose homeostasis (Saxena et al., 1992). The activity of hexokinase significantly decreased in the liver of diabetic rats (Sheela and Augusti, 1992). Hexokinase is the rate-limiting glycolytic enzyme that is severely impaired during diabetes. The activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase are increased in the liver during the diabetic condition (Hassan et al., 2009). Glucose-6-phosphatase and fructose-1,6-
bisphosphatase plays an important role in glucose homeostasis (Berg et al., 2001). Insulin deficiency results in the activation of gluconeogenic enzymes during diabetes. Glucose-6-phosphate is the key enzyme to maintain normal blood sugar level (Mayes, 2000). Treatment with AMSB has significantly normalized the activity of hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase enzymes.

Glucose-6-phosphate dehydrogenase is the key enzyme to maintain normal blood sugar level by diverting glucose-6-phosphate into the pentose phosphate pathway, thereby maintaining intracellular glucose-6-phosphate at optimum levels. Activity of glucose-6-phosphate dehydrogenase, the first regulatory enzyme of pentose phosphate pathway was found to be decreased in diabetic animals. As the glucose-6-phosphate dehydrogenase activity is decreased in diabetes, the available glucose-6-phosphate will be directed to either glycoprotein synthesis (Bihari et al., 1997) or gluconeogenesis instead of hexose monophosphate shunt, thereby decreasing the production of reducing equivalents such as NADH and NADPH. Insulin is reported to increase the activity of glucose-6-phosphate dehydrogenase in a dose dependent manner (Weber and Convery, 1966).

Table 2.7 exemplifies the effect of AMSB on glycogen, glycogen synthase and glycogen phosphorylase in experimental group of rats. Significant reduction was observed in hepatic glycogen and glycogen synthase with increase in the activity of glycogen phosphorylase in STZ induced rats (group II) when compared to control rats (group I). By administration of AMSB (group III) and glibenclamide (group IV), glycogen, glycogen synthase and glycogen phosphorylase were normalized.

Liver play an important role in maintenance of blood glucose level by regulating its metabolism. The conversion of glucose into glycogen in liver depends
on concentration of glucose and availability of insulin which stimulates glycogen
synthesis, which occur in presence of enzyme glycogen synthase and glycogen
phosphorylase (Stalmans et al., 1997). The decrease seen in hepatic glycogen content
in diabetes has been observed (Whitton and Hems, 1975). The significant increase in
the glycogen levels of the treated groups maybe because of reactivation of the
glycogen synthase system. Hence, improvement of glycogenesis process may be
another probable way of antidiabetic action (Maiti et al., 2004). Glycogen level,
primary intracellular storable form of glucose in various tissues is a direct reflection
of insulin activity as insulin promotes its deposition by stimulating glycogen synthase
and inhibiting glycogen phosphorylase (Sharma et al., 2010; El-Shenawy et al., 2006).

The liver glycogen content was markedly reduced in diabetic animals, which
is in proportion to insulin deficiency (Arunachalam and Parimelazhagan, 2013;
Chandramohan et al., 2008; Grover et al., 2002). Treatment of diabetic rats (group III)
with AMSB significantly restored the level of hepatic glycogen which may be due to
increased secretion of insulin from residual pancreatic β-cells attributed to stimulation
of glycogen synthase and inhibition of glycogen phosphorylase.

2.4 CONCLUSION

From this study, we can conclude that AMSB has potential antidiabetic action.
The present results show significant effect of AMSB on carbohydrate metabolism,
lipid profile in addition to its antidiabetic properties. These results support that the
AMSB may be used as an antidiabetic drug.
Table 2.1. Effect of AMSB on blood glucose level in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Group I: Control</td>
<td>92.38±8.12</td>
</tr>
<tr>
<td>Group II: Diabetic control (STZ:55mg/kg)</td>
<td>91.05±7.42&lt;sup&gt;a*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III: Diabetic + AMSB (150mg/kg)</td>
<td>92.73±8.74&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV: Diabetic + Glibenclamide (2.5mg/kg)</td>
<td>90.58±8.85&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D., n=6.

<sup>a</sup>P<0.05 Diabetic control (Group II) compared with control (Group I)

<sup>b</sup>P<0.05 AMSB (Group III) and Glibenclamide (Group IV) compared with diabetic control (Group II) group.
Table 2.2 Changes in body weight and urine sugar of normal and diabetic rats after
45 days of treatment with AMSB.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Group I: Control</td>
<td>160.87±3.16</td>
<td>182.46±4.79</td>
</tr>
<tr>
<td>Group II: Diabetic control</td>
<td>168.03±3.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139.12±1.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(STZ:55mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III: Diabetic + AMSB</td>
<td>162.59±2.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>158.73±2.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(150mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV: Diabetic + Glibenclamide (2.5mg/kg)</td>
<td>160.40±3.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>169.38±3.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D for six animals in each group

<sup>a</sup><i>P</i><0.05: <sup>a</sup>Significantly different from group I (Control)

<sup>b</sup><i>P</i><0.05: <sup>b</sup>Significantly different from group II (Diabetic control)
Table 2.3 Level of plasma lipoproteins, TC, TGs, phospholipids and free fatty acid concentrations in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>TGs (mg/dl)</th>
<th>Free fatty acid (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>54.17±2.021</td>
<td>21.03±1.06</td>
<td>29.89±1.036</td>
<td>94.72±1.08</td>
<td>90.2±3.81</td>
<td>65.6±2.81</td>
<td>91.3±3.74</td>
</tr>
<tr>
<td>Group II: Diabetic control (STZ:55mg/kg)</td>
<td>120.63±8.62^a</td>
<td>72.18±5.37^a</td>
<td>16.36±3.27^a</td>
<td>263.4±3.01^a</td>
<td>251.6±7.62^a</td>
<td>171.04±6.05^a</td>
<td>179.9±5.42^a</td>
</tr>
<tr>
<td>Group III: Diabetic + AMSB(150mg/kg)</td>
<td>60.45±2.71^b</td>
<td>31.48±2.70^b</td>
<td>20.17±0.16^b</td>
<td>118.6±2.73^b</td>
<td>93.1±0.67^b</td>
<td>72.18±2.36^b</td>
<td>106.01±3.18^b</td>
</tr>
<tr>
<td>Group IV: Diabetic + Glibenclamide</td>
<td>56.58±2.31^b</td>
<td>26.17±1.008^b</td>
<td>23.42±0.84^b</td>
<td>105.9±2.05^b</td>
<td>90.89±1.38^b</td>
<td>69.0±1.27^b</td>
<td>98.7±2.14^b</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D., n=6.

^a P<0.05 Diabetic control (Group II) compared with control (Group I)

^b P<0.05 AMSB (Group III) and Glibenclamide (Group IV) compared with diabetic control (Group II) group.