INTRODUCTION

Medicinal plants have played an important role in Indian culture since Rig Veda (5600 BC) where about 67 medicinal plants were recorded. It is estimated that 80 % of about 4 billion population have to rely on traditional medicines due to high cost of modern medicines, lack of availability of required medicines and personal preferences. Out of 250,000 higher plants, more than 80,000 have medicinal value and India occupies unique position among world’s 12 biodiversity centers. It is identified that about 20,000 plants have good medicinal value and 7500 species are used by traditional communities (Miura et al., 2004). Diabetes mellitus is one of the most common diseases affecting millions of people. At least, 30 million people throughout the world suffer from diabetes mellitus. Diabetes is becoming a major menace in the last 10 years. In Indian, the situation is expected to become much worse in the years to come because of food habit and sedentary life style. Life expectancy may be halved by this disease, especially in developing countries where its prevalence is increasing and adequate treatment is often unavailable. Diabetes not only kills, but it is a major cause of adult blindness, kidney failure, neuropathy, heart attack and strokes (Dhasarathan and Theriappan, 2011).

Diabetes mellitus is a chronic disease characterized by deranged secretions and effects of insulin and glucagons, extensive disturbances of carbohydrates, proteins and lipid metabolism, thickening of capillary basement membrane throughout the body leading to microangiopathy and macroangiopathy and long term complications will affect eye, kidney, nervous system and circulatory system (Dhasarathan and Theriappan, 2011). Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia (Ugochukwu et al., 2003), lipoprotein abnormalities (Scoppola2001), raised basal metabolic rate (Owu et al., 2006), defect in reactive oxygen species (ROS) scavenging enzymes (Kesavulu et al., 2000) and high oxidative stress induced damage to pancreatic β-cells (Nayeemunnisa, 2009). Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when the fatal complications are taken into account (Trivedi et al., 2004). Diabetes mellitus is the most important non infective epidemic disease to hit the globe in the present millennium (Chaurasia et al., 2011).
the year 2025, India shall have the maximum number of diabetes in the world making it, the” Diabetes capital of the world” (Hillary et al., 1998). Diabetes mellitus is among the most common disorders in developed and developing countries. The disease is increasing rapidly in most parts of the world (Tiwari and Madhusudhana, 2002). In the recent past many hypoglycaemic agents are introduced, still diabetes and related complications continue to be a major medical problem not only in developed countries but also in developing countries. In 1995, the World Health Organization (WHO) reported that approximately 150 million persons worldwide had diabetes mellitus, and this number may well be double by 2025 (Bnouhamet et al., 2006). Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterised by glucose intolerance and chronic hyperglycemia (Kantarova et al., 2006). Diabetes Mellitus is the name given to a group of disorders characterised by chronic hyperglycemia, polyuria, polydipsia, polyphagia, emaciation, and weakness due to disturbance in carbohydrate, fat, and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action (Deb and Dutta, 2006).

Diabetes mellitus (DM) is classified into two major categories: type 1 and type 2 diabetes (Skyler, 2004). As a devastating disease, diabetes is affecting approximately 3% of the population worldwide, 90% of which suffer from type 2 diabetes (Skyler, 2004). Diabetes is mainly grouped into insulin-dependent diabetes mellitus (IDDM) (Type 1-diabetes) and insulin-independent diabetes mellitus (IIDM) (Type 2- diabetes). Type 1-diabetes is caused by a deficiency in insulin secretion from pancreatic $\beta$ cells. Type 2-diabetes mellitus is related to damage in the insulin signaling pathway. Chemical compounds that selectively damage pancreatic $\beta$-cells constitute a class of diabetogenic drugs. Alloxan, a cyclic urea derivative, is a potent diabetogenic agent that has been widely used for the induction of experimental type 1 diabetes (Rho et al., 2000). Although the two types of diabetes have distinct pathogeneses, hyperglycemia and various life-threatening complications resulting from long-term hyperglycemia are common to both (Abraira et al., 1995). Type 1A diabetes describes the immune-mediated form of type 1 DM; while type 1B is the non-autoimmune idiopathic form (American Diabetes Association, 1997). Type 1 DM accounts for about 10% of all cases of DM and it affects
approximately 20 million people worldwide (Libman et al., 1993). The pyrimidine derivative 2,4,5,6 [1H,3H]-pyrimidinetetrone known as alloxan has been widely used to induce diabetes mellitus in animals for many decades (Rerup, 1970; Oberley, 1988). Among chemical compounds that rapidly and selectively damage pancreatic cells, alloxan exhibits the most potent toxicity; hence the mechanism of its action has been the subject of many investigations. Streptozotocin or alloxan is often used to induce hyperglycemia in rats and mimic diabetic patients to evaluate hypoglycemic and related effects of antidiabetic compounds and extracts (Kordowiak et al., 2000). It has been reported that alloxan rapidly and selectively accumulates in $\beta$-cells in comparison with non-$\beta$ cells (Gorus et al., 1982).

Oxidative stress has been implicated in the development of many pathophysiological conditions including diabetes (Sies, 1991; Shishehbor and Hazen, 2004). Diabetes mellitus, an endocrine and metabolic disorder characterized by chronic hyperglycemia produces multiple biochemical impairments and oxidative stress especially an increased susceptibility to lipid peroxidation that plays role in the progression of the symptoms of diabetes. Several hypotheses have been postulated to explain the development of free radicals in diabetes which include auto oxidation of glucose, enzymatic and non-enzymatic glycation of proteins with increased formation of glucose derived advanced glycosylation end products (AGEs), enhanced glucose flux through polyol pathway (Oberlay, 1988) and reduction of antioxidant defence (Lipinski, 2001).

Despite the great strides, made in understanding and management of diabetes, the disease and disease related complications are increasing unabated due to multiple defects, in its pathophysiology (Lvora et al., 1989). In the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in the long run. So, the search for improved and safe natural antidiabetic agents is ongoing and World Health Organization has also recommended the development of herbal medicine in this concern (Schmincke, 2003). The holistic approach of herbs has accelerated the global efforts to harness and harvest medicinal plants having multiple beneficial effects (Rao et al., 1997). Some of them have been evaluated and their active principles isolated, however, the search for novel antidiabetic drugs continues (Nagarajan et al., 1987). Spices are dried herbs they are
known to exert several beneficial physiological effects including the antidiabetic influence (Dragland et al., 2003). Among the spices, fenugreek seeds (Trigonella foenumgraecum), garlic (Allium sativum), onion (Allium cepa), and turmeric (Curcuma longa) have been experimentally documented to possess antidiabetic potential. In a limited number of studies, cumin seeds (Cuminum cyminum), ginger (Zingiber officinale), mustard (Brassica nigra), curry leaves (Murraya koenigii) and coriander (Coriandrum sativum) have been reported to be hypoglycaemic (Srinivasan, 2005). Most bioactive spices constituents are collectively called phytochemicals. The large majorities of these phytochemicals are redox active molecules and therefore defined as antioxidants (Carlson et al., 2010).

Many Indian medicinal plants are reported to be useful in diabetes (Kirthikar and Basu, 1995; Nadkarni and Nadkarni, 1996). Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems (Noor et al., 2008). Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines in the present day drugs for many rural populations in developing countries. India is well known for its herbal wealth. Medicinal plants like Trigonella foenum graecum, Allium sativum, Gymnema slyvestre and Syzigium cumini have been studied (Grover et al., 2002) for treatment of diabetes mellitus. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability (Valiathan, 1998). Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical antidiabetic activity (Grover et al., 2002; Dineshkumar et al., 2009). Medicinal plants and their products have been widely used for treatment of diabetic populace all around the world with less known scientific basis of their functioning (Patwardhan et al., 2004; Said et al., 2007). Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-diabetic activity. In the type 2 diabetic model rats, the ethanol extract of stem bark of Bridelia ndellensis showed an antihyperglycemic effect comparable to that of glibenclamide when fed simultaneously with glucose. The ethanol
extract of *B. ndellensis* had no hypoglycemic effect in type 1 diabetic rats in fasting and postprandial glucose load conditions and, in type 2 diabetic rats in fasting condition. Thus, the extract may act on β-cells like sulfonylurea drugs to stimulate insulin secretion (Sokeng *et al*., 2005). Similar results have been reported with *Anacardium occidentale* aqueous leaf extract (Sokeng *et al*., 2001). The *Bridelia ferruginea* bark extract achieved a reduction in plasma glucose levels especially in glucose induced hyperglycemic rats. This implies that the methanol extract has anti-diabetic properties and may thus be useful in the management and treatment of diabetes mellitus (Kolawole *et al*., 2006). The methanolic stem bark extract of *B. grandis*, had hypoglycaemic effects in type 2 diabetic mice (Njamen *et al*., 2011). *Bridelia retusa* bark was screened for their medicinal properties as reported by Tatiya *et al*. (2011). The methanolic extract of the bark of *B. retusa* collected from Maharashtra and Andhra Pradesh regions and the plant collected from the region of Maharashtra was found to be superior with respect to extraction yield and radical scavenging activity. These differences may possibly be related to the natural climatic differences which occur over a particular geographical area to be influenced by several climatic factors (Banerjee and Bonde, 2011). A number of factors influence the concentration of the active constituent’s, some of which are time and period of collection, geographical origin and climatic conditions. Sometimes, the influence of these factors may lead to even absence of active constituents in the same plant collected from different regions, as evidenced by several research reports (Houghton., 1998; Bilia, 2002; Marcus *et al*., 2002; Banerjee and Bonde, 2011), hence the present study was undertaken. In the first chapters, the phytochemical analysis has revealed the presence of bitter principles, flavonoids, saponins, coumarins, reducing sugars, proteins, tannins, quinones and glycosides which may be responsible for the various biological activity. The antimicrobial activity of the ethanolic extract was highest against broad range of microbes in comparison to other extract along with hepatoprotective, nephroprotective and *in vivo* antioxidant activity (Cordeiro and Kaliwal, 2011) was highest for the ethanolic extract was noted in earlier chapters. Hence, ethanolic extract of the stem bark material of *Bridelia retusa* was used in the present investigation to study *in vivo* antidiabetic activity in alloxan induced diabetes in female Sprague Dawley rats.
MATERIALS AND METHODS

Animals

Adult virgin Sprague Dawley rats aged 90 days weighing between 100-200 gm were used in the experiments. The rats were maintained in P.G. Department of Studies in Zoology, Karnataka University, Dharwad and permitted by local ethical committee. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. Standard rats pellet diet “Gold Mohar’ (Hindustan Lever company, Mumbai) was provided along with water ad libitum. The rats were maintained under normal day/night schedule (12L:12D) at room temperature 25±2°C.

Induction of diabetes

Diabetes was induced in the female rats by intraperitoneal administration of alloxan monohydrate in concentration of 150 mg/kg body weight dissolved in normal saline. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 5 percent glucose solution was kept in bottles in their cages for next 24 h to prevent hypoglycemia. After 5 days, the rats with blood glucose above 250 mg/dl were used for the experiment. Initial and final body weight was recorded in the experiment. The blood glucose level (BGL) was monitored after alloxanisation in blood samples collected by amputation of the tail tip under mild anesthesia. A drop of blood was placed on a blood glucose test strip (Glucocard ™ 01 sensor) and was inserted into a glucometer ARKRAY Glucocard 01-mini Blood glucose testing system.

Experimental Schedule

Rats were divided into six groups of ten rats each as follows. Saline was gavaged orally to group I with normal food and water (negative control) and group II, alloxan of 150 mg/kg was administered to rats (diabetic control) by intraperitoneal injection. The diabetic rats of groups III were given 500 mg/ kg glibenclamide (glib) in saline for 15 days. Groups IV, V and IV were given two doses 100 and 150 mg/kg of ethanolic to
diabetic rats for 15 days. The rats were monitored for change in body weight and food consumption during the experiment. All the fasted rats were necropsied by mild ether anesthesia after 16 days. The organs were dissected out and washed with ice-cold saline immediately. A portion of the tissue was homogenized using a potter-Elvejham homogenizer, and the supernatant was used for estimation of biochemical parameters, dehydrogenases and phosphatases, glucokinase, glucose 6-phosphatase, and fructose 6-phosphatase, hexokinase and oxidative parameters. Blood glucose level of all the animals was noted after 5, 10 and 15 days after administration of alloxan.

**Serum biochemical parameters**

Blood was withdrawn from the jugular vein of anesthetized rats into clean tubes and allowed to stand for 30 min. Blood samples (without anticoagulant) collected were allowed to coagulate followed by centrifugation to obtain serum for estimation. The serum was collected after centrifugation. The estimation of serum parameters was performed for cholesterol, ASAT (Serum Gluatic Oxaloacetic Transaminase, SGOT) and ALAT (Serum Glutamic Pyruvic Transaminase, SGPT) as described in chapter III.

**Preparation of tissue homogenate**

The tissue homogenate was prepared by taking 1 g of fresh organ, sliced and homogenized in 15 ml of cold sucrose 250 mM with Potter Elvehjem homogenizer for 2 min and the pellet was discarded. The supernatant was used as enzyme source for estimation of biochemical contents such as DNA, RNA, protein, glycogen and activity of enzymes such as LDH, ACP, AKP and oxidative stress parameters were carried out as per the methods mentioned in chapter III and also hepatic glucokinase (Ananthi et. al., 2003), glucose-6-phosphate (Sowmia et. al., 2009) and fructose-6-phosphate (King et. al., 1965).

**Biochemical estimations**

**Estimation of glucokinase activity**

Glucokinase activity was estimated by the method described by Ananthi et. al. (1962).
**Principle:** Glucose is phosphorylated with ATP (Adenosine triphosphate) in the reaction catalysed by Hexokinase (HK). The product, glucose-6-phosphophate (G6P) is then oxidized with concominant reduction of NAD (Nicotinamide dinucleotide) to NADH in the reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). The formation of NADH causes an increase in the absorption at 340 nm. The increase is directly proportional to the amount of glucose in the sample.

Take 2 ml of tissue homogenate, add 1 ml of glucose, 0.5 ml ATP, 0.1 ml MgCl₂, 0.4 ml KH₂PO₄, 0.4 ml KCl, 0.4ml NaF and 2.5 ml Tris HCl buffer. Then incubate for 5 min at room temperature and immediately transfer 1 ml of reaction mixture to the tubes containing 1 ml of 10% TCA. Consider this as zero sample. Incubate the reaction mixture at room temperature for 30 min. After incubation take 1 ml reaction mixture and add 1ml 10 % TCA. Consider this as 30 min sample. Centrifuge the tubes for 10 min at 3000 rpm. Take the supernatant and add 4 ml o-toludine reagent. Simultaneously blank and standard were prepared. The green color was read at 620 nm against blank. The enzyme activity was expressed as µmoles glucose phosphorylated/hr/mg protein of tissue.

**Estimation of glucose-6-phosphate (G6P) activity**

Glucokinase activity was estimated by the method described by Sowmia et. al. (2009).

To 0.3 ml of homogenate, add 3 ml substrate and 0.7 ml citrate buffer. Incubate the reaction mixture for 1 hr at 37°C. Then add 1 ml of 10% TCA and centrifuge at 3000 rpm for 10 min. Separate the supernatant and make the volume to 1ml with distilled water. To this add 1 ml ammonium molybdate and 0.4 ml ANSA reagent. Incubate for 20 min, the blue color developed was read at 640 nm against the blank. Simultaneously blank and standard were prepared. The activity was expressed as µmoles inorganic phosphate liberated/min/mg protein of tissue.
Estimation of fructose-6-phosphate (F6P) activity

Fructose-6-phosphate activity was estimated by the method described by King et. al. (1965).

Take 0.1 ml homogenate and add 1.2 ml of Tris HCL buffer, 0.1 ml substrate, 0.25 ml MgCl₂, 0.1 ml KCl and 0.25 ml EDTA serially. Incubate the mixture for 15 min at 37°C and addition of 10 % TCA. Centrifuge at 3000 rpm and collected the supernatant which was made upto 1 ml with distilled water. Add 1 ml ammonium molybdate and 0.4 ml of ANSA. After 20 min the blue color obtained was read at 660 nm against blank. Simultaneously blank and standard were prepared. The activity was expressed as µmoles inorganic phosphate liberated/min/mg protein of tissue.

Statistical analysis

Statistical calculations were carried out as per the method mentioned in Chapter II.
OBSERVATIONS

a. Effect of ethanolic extract of stem bark of *Bridelia retusa* (EESBB) on body and organs weight in alloxan induced diabetic rats (Table 4.1; Graph 4.1).

**Body weight**

In control rats, body weight gain was 37.83 g which on exposure to alloxan was 15.17 g. The body weight after treatment with glibenclamide, 100 and 150 mg of ethanol extract to alloxan treated rats was 18.0, 23.17 and 29.17 g, respectively.

The present study revealed that the decrease in body weight was significant in alloxan treated group when compared with that of normal control. The significant increase in weight was observed in the rats of alloxan treated group along with ethanolic extract and it was also significantly higher than that of standard drug in alloxan treated group.

**Organs weight**

**Liver**

In rats of control group, the mean weight of the liver was 4.34 g. On exposure to alloxan the mean weight of the liver was 2.88 g. The treatment with glibenclamide, 100 and 150 mg of ethanol extract to alloxan treated rats, the mean weights of the liver was 3.69, 3.67 and 4.86 g, respectively.

The findings of the present study indicated that the weight of the liver showed significant decrease in rats of alloxan exposed group when compared with that of normal control. On treatment with both the doses of ethanol extract to alloxan exposed rats showed significant increase in the weight of the liver when compared with that of alloxan treated rats and it was also significantly higher than that of standard drug in alloxan treated group.
Kidney

The mean weight of the kidney in control and alloxan treated control rats was 0.83 and 0.65 g, respectively. The mean weights of the kidney on treatment with glibenclamide, 100 and 150 mg of ethanol extract to alloxan treated control rats was 0.70, 0.81 and 0.89 g, respectively.

The present study suggested that the decrease in the weight of the kidney was significant in alloxan treated group when compared with that of normal control. The administration of ethanolic extracts to rats treated with alloxan showed significant increase when compared with that of alloxan treated control group and it was also significantly higher than that of standard drug in alloxan treated group.

Pancreas

In normal rats, the mean weight of the pancreas was 0.64 mg while in alloxan treated control group was 0.41 mg. The treatment with glibenclamide, 100 and 150 mg of ethanol extract to alloxan control rats, the mean weights of the pancreas was 0.43, 0.48 and 0.49 mg, respectively.

The present results suggested that there was significant decrease in the weights of the pancreas on alloxan exposure when compared with that of normal control rats. Both the doses of 100 and 150 mg of ethanol extract when administered to alloxan treated rats showed increase in weights which was significant when compared with that of alloxan treated control group and it was also significantly higher than that of standard drug in alloxan treated group.

b. Effect of ethanolic extract of stem bark of Bridelia retusa S. (EESBB) on blood glucose and serum parameters (SGOT, SGPT and cholesterol) of alloxan induced diabetic rats (Table 4.2; Graph 4.2).

Blood glucose

The blood glucose levels after 5, 10 and 15 days of normal rats was 106.33, 106.60 and 105.83 mg, respectively and alloxan treated rats showed 347.0, 207.0 and 169.67 mg,
respectively. The blood glucose after 5, 10 and 15 days of alloxan treated rats along with glibenclamide was 348.67, 115.50 and 78.50 mg respectively, 100 of ethanol extract was 349.17, 137.17 and 81.17 mg, respectively and 150 mg extract was 348.50, 115.67 and 75.84 mg.

The findings of the present study indicated that the blood glucose levels in alloxan treated rats were significantly increased when compared with that of normal control. However, a significant decrease was observed in the blood glucose levels of alloxan exposed rats on treatment with ethanol extracts when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the blood glucose levels of plant extract exposed to alloxan treated rats when compared with that of alloxan treated group.

**Cholesterol**

The cholesterol content in the serum of normal and alloxan treated rats was 137.04 and 281.59 mg, respectively. The level of cholesterol in alloxan induced diabetic rats on treatment with glibenclamide, 100 and 150 mg of ethanol extracts was 193.58, 177.56 and 164.60 mg, respectively.

The present study indicated that the level of cholesterol in the serum of alloxan treated group showed a significant increase when compared with that of normal control. However, there was a significant decrease in the cholesterol content in the serum of the alloxan treated rats along with plant extracts which was significantly lesser than that of standard drug in alloxan treated group. Thus, the results revealed recovery in the level of cholesterol content in the serum of rats exposed to alloxan after treatment with both plant extracts when compared with that of alloxan treated control group.

**Aspartate aminotransferase (ASAT) / Serum Glutamic Oxaloacetic transaminase (SGOT)**

The activity of SGOT of normal was 103.30 and alloxan treated rats showed activity of 163.80 IU respectively. The SGOT activity of alloxan treated rats along with
glibenclamide, 100 and 150 mg of ethanol extract was 153.0, 157.70 and 152.0 IU, respectively.

The findings of the present study indicated that the SGOT activity of alloxan treated rats was significantly increased when compared with that of normal control. However, a significant decrease was observed in the SGOT activity of alloxan exposed rats on treatment with ethanol extracts when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of SGOT of plant extract exposed to alloxan treated rats when compared with that of alloxan treated group.

**Alanine aminotrasferase (ALAT) / Serum Glutamic Pyruvic transaminase (SGPT)**

The activity of SGPT of normal and alloxan treated rats was 49.90 and 64.03 IU respectively. The SGPT activity of alloxan treated rats along with glibenclamide, 100 and 150 mg of ethanol extract was 53.77, 59.86 and 53.39 IU, respectively.

The results indicated that the SGPT activity of alloxan treated rats was significantly increased when compared with that of normal control. But there was a significant decrease in the SGPT activity of alloxan exposed rats on treatment with plant extracts when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of SGPT of plant extract administered to alloxan treated rats when compared with that of alloxan treated group.

c. **Effect of ethanolic extract of stem bark of Bridelia retusa (EESBB) on biochemical contents (DNA, RNA, proteins and glycogen) in the liver, kidney and pancreas of alloxan induced diabetic rats (Tables 4.3, 4.4 and 4.5; Graphs 4.3, 4.4 and 4.5).**

**Deoxyribosenucleic acid (DNA)**

**Liver**

The DNA content in the liver of normal and alloxan treated rats was 3.96 and 1.54 mg, respectively. The level of DNA in the rats treated with glibenclamide, 100 and 150 mg of ethanol extract along with alloxan was 2.69, 2.84 and 3.18 mg, respectively.
The results of the present study indicated that the level of DNA in the liver of alloxan treated rats showed a significant decrease when compared with that of normal control. However, there was a significant increase in the level of DNA in the liver of alloxan treated rats along with plant extracts. Thus, the results revealed recovery in the level of DNA content in the liver of rats exposed to alloxan along with plant extracts when compared with that of alloxan treated control group.

**Kidney**

The level of DNA in the kidney of normal and alloxan treated rats was 2.96 and 1.24 mg, respectively. On treatment with glibenclamide, 100 and 150 mg of ethanol extract along with alloxan was 1.68, 1.48 and 2.48 mg, respectively.

The findings of the present study indicated that in alloxan treated rats the level of DNA in the kidney showed a significant decrease when compared with that of normal control. Thus, the results indicated recovery in the level of DNA in rats exposed to alloxan along with plant extracts when compared with that of alloxan treated control group.

**Pancreas**

The level of DNA in the pancreas of normal and alloxan treated rats was 3.34 and 1.35 mg, respectively. On treatment with glibenclamide, 100 and 150 mg of ethanol extract along with alloxan was 2.37, 3.31 and 3.58 mg, respectively.

The findings of the present study indicated that in alloxan treated rats the level of DNA in the pancreas showed a significant decrease when compared with that of normal control. Thus, the results indicated recovery in the level of DNA in rats exposed to alloxan along with plant extracts when compared with that of alloxan treated control group.
Ribonucleic acid (RNA)

Liver

The RNA content in the liver of normal control was 2.96 and in alloxan treated rats was 2.58 mg. The level of RNA on treatment with glibenclamide, 100 and 150 mg of ethanol extract was 2.85, 2.67 and 2.95 mg, respectively in alloxan treated rats.

In the present study, the level of RNA in the liver of alloxan treated group showed a significant decrease when compared with that of normal control. However, a significant increase in the RNA content was observed in the liver of alloxan treated rats along with plant extracts when compared with that of alloxan treated group. Thus, the results indicated recovery in the level of RNA in rats exposed to alloxan along with plant extracts when compared with that of alloxan treated control group.

Kidney

The level of RNA in the kidney of normal and alloxan treated rats was 2.08 and 1.16 mg, respectively. The level of RNA in the kidney of alloxan exposed rats treated with glibenclamide, 100 and 150 mg of ethanol extract was 1.39, 1.46 and 1.96 mg, respectively.

The present study thus suggested that the level of RNA in the kidney of alloxan treated rats showed a significant decrease when compared with that of normal control. However, there was significant increase in the RNA content in the kidney in alloxan treated rats along with plant extracts when compared with that of alloxan treated control group. Hence, the results indicated recovery in the level of RNA on treatment with plant extracts in rats exposed to alloxan when compared with that of alloxan treated control group.

Pancreas

The level of RNA in the pancreas of normal and alloxan treated rats was 2.79 and 2.04 mg, respectively. The level of RNA in the kidney of alloxan exposed rats treated with
glibenclamide, 100 and 150 mg of ethanol extract was 2.47, 2.13 and 2.89 mg, respectively.

The present study thus suggested that the level of RNA in the pancreas of alloxan treated rats showed a significant decrease when compared with that of normal control. However, there was significant increase in the RNA content in the pancreas in alloxan treated rats along with plant extracts when compared with that of alloxan treated control group. Hence, the results indicated recovery in the level of RNA on treatment with plant extracts in rats exposed to alloxan when compared with that of alloxan treated control group.

**Protein**

**Liver**

The protein content in the liver of normal and treated alloxan rats was 180.70 and 170.50 mg, respectively. The level of protein in the rats treated with glibenclamide, 100 and 150 mg of ethanol extract was 180.50, 175.60 and 178.50 mg, respectively.

The present study indicated that the level of protein in the liver of alloxan group rats showed a significant decrease when compared with that of normal control. However, there was a significant increase in the protein content in the liver of the alloxan treated rats on administration of plant extracts. Thus, the results revealed recovery in the level of protein content in the liver of rats exposed to alloxan after treatment with plant extracts when compared with that of alloxan treated control group.

**Kidney**

The level of proteins in the kidney of normal and treated alloxan rats was 170.42 and 162.07 mg, respectively. The level of proteins in the kidney treated with glibenclamide, 100 and 150 mg of ethanol extract in alloxan treated rats was 170.31, 166.55 and 169.73 mg, respectively.
The findings of the present study suggested that in alloxan treated rats the level of proteins in the kidney showed a significant decrease when compared with that of normal control. However, the proteins content in the kidney showed a significant increase in the alloxan treated rats on administration of plant extracts when compared to alloxan treated control group. Thus, the recovery in the level of proteins in rats exposed to alloxan after treatment with plant extracts when compared with that of alloxan treated control group was evident in the present result.

**Pancreas**

The level of proteins in the pancreas of normal and treated alloxan rats was 195.43 and 176.46 mg, respectively. The level of proteins in the pancreas treated with glibenclamide, 100 and 150 mg of ethanol extract in alloxan treated rats was 187.44, 186.50 and 185.71 mg, respectively.

The findings of the present study suggested that in alloxan treated rats the level of proteins in the pancreas showed a significant decrease when compared with that of normal control. However, the proteins content in the pancreas showed a significant increase in the alloxan treated rats on administration of plant extracts when compared to alloxan treated control group. Thus, the recovery in the level of proteins in rats exposed to alloxan after treatment with plant extracts when compared with that of alloxan treated control group was evident in the present result.

**Glycogen**

**Liver**

The level of glycogen in the liver of normal and alloxan treated rats was 36.92 and 25.53 mg, respectively. In the liver of alloxan exposed rats, the level of glycogen on treatment with glibenclamide, 100 and 150 mg of ethanol extract was 36.45, 35.54 and 36.15 mg, respectively.

In the present study, the level of glycogen in the liver of rats of alloxan treated group showed a significant decrease when compared with that of normal control.
However, a significant increase in the level of glycogen was observed in the liver of the alloxan treated rats along with the plant extracts. Thus, the results indicated recovery in the level of glycogen in rats exposed to alloxan along with plant extracts when compared with that of alloxan treated control group.

**Kidney**

The level of glycogen in the kidney of normal and alloxan treated rats was 37.49 and 36.23 mg, respectively. The level of glycogen on treatment with glibenclamide, 100 and 150 mg of ethanol extract was 37.25, 37.17 and 37.27 mg, respectively in alloxan induced diabetic rats.

The present study suggested that the level of glycogen in the kidney of alloxan treated rats showed a significant decrease when compared with that of normal control. However, there was significant increase in the glycogen content in the kidney in alloxan treated rats along with plant extracts. Hence, the results indicated recovery in the level of glycogen on treatment with plant extracts in rats exposed to alloxan when compared with that of alloxan treated control group.

**Pancreas**

The level of glycogen in the pancreas of normal and alloxan treated rats was 34.44 and 23.07 mg, respectively. The level of glycogen on treatment with glibenclamide, 100 and 150 mg of ethanol extract was 33.74, 29.57 and 30.88 mg, respectively in alloxan induced diabetic rats.

The present study suggested that the level of glycogen in the pancreas of alloxan treated rats showed a significant decrease when compared with that of normal control. However, there was significant increase in the glycogen content in the pancreas in alloxan treated rats along with plant extracts. Hence, the results indicated recovery in the level of glycogen on treatment with plant extracts in rats exposed to alloxan when compared with that of alloxan treated control group.
d. Effect of ethanolic extract of stem bark of *Bridelia retusa* (EESBB) on hepatic glycolytic enzymes activities in alloxan induced diabetic rats (Tables 4.6; Graphs 4.6).

**Hexokinase**

The hexokinase activity of normal rats was 0.79 and in alloxan treated rats was 0.46 µmoles respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 0.73, 0.69 and 0.78 µmoles of hexokinase activity, respectively.

The findings of present study indicated that the hexokinase activity of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the hexokinase activity on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

**Glucokinase**

The glucokinase activity of normal rats was 4.15 and in alloxan treated rats was 2.71 µmoles. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 3.95, 3.62 and 4.05 µmoles of glucokinase activity, respectively.

The findings of present study indicated that the glucokinase activity of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the glucokinase activity on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of glucokinase of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.
Glucose-6-Phosphotase (G6P)

The G6P activity of normal rats was 110.73 and in alloxan treated rats was 150.57 µmoles respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 120.27, 140.27 and 120.70 µmoles of G6P activity, respectively.

The findings of present study indicated that the G6P activity of alloxan treated rats was significantly increased when compared with that of normal control. However, there was a significant decrease in the G6P activity on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of G6P of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

Fructose-6-Phosphotase (F6P)

The F6P activity of normal rats was 48.87 and in alloxan treated rats was 56.41 µmoles respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 46.27, 50.85 and 47.15 µmoles of F6P activity, respectively.

The findings of present study indicated that the F6P activity of alloxan treated rats was significantly increased when compared with that of normal control. However, there was a significant decrease in the F6P activity on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of F6P of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.
e. Effect of ethanolic extract of stem bark of *Bridelia retusa* (EESBB) on enzymes activity in the liver, kidney and pancreas of alloxan induced diabetic rats (Tables 4.7, 4.8 and 4.9; Graphs 4.7, 4.8 and 4.9).

### Lactate dehydrogenase (LDH)

#### Liver

The activity of LDH in the liver of normal and alloxan treated rats was 12.63 and 13.83 µmoles respectively. The LDH activity in the liver of alloxan treated rats along with glibenclamide, 100 and 150 mg of ethanol extract was 13.83, 13.43 and 12.94 µmoles, respectively.

The results indicated that the LDH activity in the liver of rats treated with alloxan was significantly increased when compared with that of normal control but there was a significant decrease in the LDH activity in the liver of alloxan exposed rats along with plant extracts when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of LDH in the liver of alloxan treated rats along with plant extract when compared with that of alloxan treated group.

#### Kidney

The activity of LDH in the kidney of normal was 10.0 and in alloxan treated rats was 12.81 µmoles, respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 11.72, 10.72 and 10.70 µmoles, respectively.

The findings of the present study indicated that the LDH activity in the kidney of alloxan treated rats was significantly increased when compared with that of normal control. However, there was a significant decrease in the LDH activity in the kidney on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of LDH in the liver of plant extract treated to alloxan exposed rats when compared with that alloxan treated group.
Pancreas

The activity of LDH in the pancreas of normal was 10.10 and in alloxan treated rats was 12.11 µmoles, respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 11.22, 10.12 and 10.20 µmoles respectively.

The findings of the present study indicated that the LDH activity in the pancreas of alloxan treated rats was significantly increased when compared with that of normal control. However, there was a significant decrease in the LDH activity in the pancreas on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of LDH in the pancreas of plant extract treated to alloxan exposed rats when compared with that alloxan treated group.

Acid phosphotase (ACP)
Liver

The activity of ACP in the liver of normal and alloxan treated rats was 14.33 and 13.47 µmoles, respectively. The ACP activity in the liver of alloxan treated rats when administered with glibenclamide, 100 and 150 mg of ethanol extract was 14.66, 14.70 and 14.39 µmoles, respectively.

The results indicated that the ACP activity in the liver of alloxan treated rats was significantly decreased when compared with that of normal control. But there was a significant increase in the ACP activity in the liver of alloxan exposed rats on treatment with plant extracts when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of ACP in the liver of plant extract administered to alloxan treated rats when compared with that of alloxan treated group.
Kidney

The activity of ACP in the kidney of normal was 13.38 and in alloxan treated rats was 12.47 µmoles. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed ACP activity in the kidney as 12.77, 13.24 and 12.67 µmoles, respectively.

The findings of present study indicated that the ACP activity in the kidney of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the ACP activity in the kidney on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of ACP in the kidney of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

Pancreas

The activity of ACP in the pancreas of normal was 13.58 and in alloxan treated rats was 12.27 µmoles. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed ACP activity in the pancreas as 12.17, 11.24 and 12.16 µmoles, respectively.

The findings of present study indicated that the ACP activity in the pancreas of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the ACP activity in the pancreas on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of ACP in the pancreas of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.
Alkaline phosphotase (AKP)

Liver

The activity of AKP in the liver of normal and alloxan treated rats was 15.18 and 16.75 µmoles respectively. The AKP activity in the liver of alloxan treated rats on treatment with glibenclamide, 100 and 150 mg of ethanol extract was 16.63, 16.18 and 16.12 µmoles respectively.

The results indicated that the AKP activity in the liver of alloxan treated rats was significantly increased when compared with that of normal control. Although, there was a significant decrease in the AKP activity in the liver of alloxan exposed rats on treatment with both ethanol extract when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of ALAT in the liver of plant extract administered to alloxan treated rats when compared with that of alloxan treated group.

Kidney

The AKP activity in the kidney of normal was 14.48 and in alloxan treated rats was 16.75 µmoles respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 15.30, 14.54 and 14.30 µmoles of AKP activity in the kidney respectively.

The findings of present study indicated that the AKP activity in the kidney of alloxan treated rats was significantly increased when compared with that of normal control. However, there was a significant decrease in the AKP activity in the kidney on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of AKP in the kidney of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.
Pancreas

The AKP activity in the pancreas of normal was 14.48 and in alloxan treated rats was 16.75 µmoles respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed 15.10, 14.14 and 15.10 µmoles of AKP activity in the pancreas respectively.

The findings of present study indicated that the AKP activity in the pancreas of alloxan treated rats was significantly increased when compared with that of normal control. However, there was a significant decrease in the AKP activity in the pancreas on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of AKP in the pancreas of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

f. Effect of ethanolic extract of stem bark of Bridelia retusa S. (EESBB) on oxidative stress parameters (Lipid peroxidation, SOD, CAT and GST) in the liver, kidney and pancreas of alloxan induced diabetic rats (Table 4.10, 4.11 and 4.12; Graph 4.10, 4.11 and 4.12).

Lipid peroxidation (LPO)

Liver

The level of lipid peroxidation in the liver of normal and alloxan treated rats was 0.45 and 0.63 nmole, respectively. The lipid peroxidation level in the rats treated with glibenclamide, 100 and 150 mg of ethanol extracts was 0.47, 0.53 and 0.48 nmole, respectively.

The present study indicated that the level of lipid peroxidation in the liver of alloxan treated rats group showed a significant increase when compared with that of normal control. However, there was a significant decrease in the lipid peroxidation in the liver of the alloxan treated rats on administration of plant extracts. Thus, the results revealed decrease in the lipid peroxidation in the liver of rats exposed to alloxan along with plant extracts when compared with that of alloxan treated control group.
Kidney

The lipid peroxidation level in the kidney of normal and treated alloxan rats was 0.53 and 0.56 nmole, respectively. The level of lipid peroxidation in the kidney treated with glibenclamide, 100 and 150 mg of ethanol extract in alloxan treated rats was 0.54, 0.58 and 0.53 nmole, respectively.

The findings of the present study suggested that in alloxan treated rats the level of lipid peroxidation in the kidney showed a significant increase when compared with that of normal control. However, the lipid peroxidation in the kidney showed significant decrease in the alloxan treated rats when administered with plant extracts. Thus, the level of lipid peroxidation in rats exposed to alloxan with plant extracts decreased when compared with that of alloxan treated control group was evident in the present result.

Pancreas

The lipid peroxidation level in the pancreas of normal and alloxan treated rats was 0.37 and 0.79 nmole, respectively. The level of lipid peroxidation in the pancreas treated with glibenclamide, 100 and 150 mg of ethanol extract in alloxan treated rats was 0.74, 0.76 and 0.75 nmole, respectively.

The findings of the present study suggested that in alloxan treated rats the level of lipid peroxidation in the pancreas showed a significant increase when compared with that of normal control. However, the lipid peroxidation in the pancreas showed significant decrease in the alloxan treated rats when administered with plant extracts. Thus, the level of lipid peroxidation in rats exposed to alloxan with plant extracts decreased when compared with that of alloxan treated control group was evident in the present result.

Superoxide dismutase (SOD)

Liver

The activity of SOD in the liver of normal and alloxan treated rats was 5.93 and 4.47 units, respectively. The SOD activity in the liver of alloxan treated rats on
administration of glibenclamide, 100 and 150 mg of ethanol extract was 5.69, 5.11 and 5.58 µmoles, respectively.

The results indicated that the SOD activity in the liver of alloxan treated rats was significantly decreased when compared with that of normal control. Although, there was a significant increase in the SOD activity in the liver of alloxan exposed rats on treatment with both of ethanol extracts when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of SOD in the liver of plant extract administered to alloxan treated rats when compared with that of alloxan treated group.

**Kidney**

The activity of SOD in the kidney of normal and alloxan treated rats was 5.53 and 4.28 units, respectively. The SOD activity in the kidney of alloxan treated rats when administered with glibenclamide, 100 and 150 mg of ethanol extract was 5.64, 5.15 and 5.54 µmoles, respectively.

The findings of the present study indicated that the SOD activity in the kidney of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the SOD activity in the kidney on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of SOD in the kidney of plant extract administered to alloxan exposed rats when compared with that of alloxan treated group.

**Pancreas**

The activity of SOD in the pancreas of normal and alloxan treated rats was 5.16 and 4.71 units, respectively. The SOD activity in the pancreas of alloxan treated rats when administered with glibenclamide, 100 and 150 mg of ethanol extract was 5.30, 5.20 and 5.10 µmoles, respectively.
The findings of the present study indicated that the SOD activity in the pancreas of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the SOD activity in the pancreas on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of SOD in the pancreas of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

Catalase (CAT) 
Liver
The activity of CAT in the liver of normal and alloxan treated rats was 60.61 and 32.43 µmoles, respectively. The CAT activity in the liver of alloxan treated rats on treatment with glibenclamide, 100 and 150 mg of ethanol extract was 45.06, 35.46 and 43.11 µmoles, respectively.

The results indicated that the CAT activity in the liver of alloxan treated rats was significantly decreased when compared with that of normal control. But there was a significant increase in the CAT activity in the liver of alloxan exposed rats on treatment with plant extracts when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of CAT in the liver of plant extract administered to alloxan treated rats when compared with that of alloxan treated group.

Kidney
The activity of CAT in the kidney of 50.60 µmoles in normal and in alloxan treated rats was 38.81 µmoles respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed CAT activity in the kidney as 48.43, 35.80 and 44.65 µmoles respectively.

The findings of present study indicated that the CAT activity in the kidney of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the CAT activity in the kidney on
treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of CAT in the kidney of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

**Pancreas**

The activity of CAT in the pancreas of 35.61 µmoles in normal and in alloxan treated rats was 20.81 µmoles, respectively. The alloxan treated rats administered with glibenclamide, 100 and 150 mg of ethanol extract showed CAT activity in the pancreas as 25.60, 25.80 and 24.11 µmoles, respectively.

The findings of present study indicated that the CAT activity in the pancreas of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the CAT activity in the pancreas on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of CAT in the pancreas of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

**Glutathione s-transferase (GST)**

**Liver**

The activity of GST in the liver of normal and alloxan treated rats was 5.17 and 4.97 µmoles respectively. The GST activity in the liver of alloxan treated rats on treatment with glibenclamide, 100 and 150 mg of ethanol extract was 4.87, 4.67 and 4.89 µmoles respectively.

The results indicated that the GST activity in the liver of alloxan treated rats was significantly decreased when compared with that of normal control. Although, there was a significant increase in the GST activity in the liver of alloxan exposed rats on treatment with ethanol extract when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of GST in the liver of plant extract
administered to alloxan treated rats when compared with that of alloxan treated control group.

**Kidney**

The activity of GST in the kidney of normal and alloxan treated rats was 3.17 and 2.87 µmoles, respectively. The GST activity in the kidney of alloxan treated rats on administration with glibenclamide, 100 and 150 mg of ethanol extract was 3.48, 3.35 and 3.26 µmoles, respectively.

The findings of the present study indicated that the GST activity in the kidney of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the GST activity in the kidney on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of GST in the kidney of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.

**Pancreas**

The activity of GST in the pancreas of normal and alloxan treated rats was 5.17 and 3.97 µmoles, respectively. The GST activity in the pancreas of alloxan treated rats on administration with glibenclamide, 100 and 150 mg of ethanol extract was 4.87, 4.17 and 4.67 µmoles, respectively.

The findings of the present study indicated that the GST activity in the pancreas of alloxan treated rats was significantly decreased when compared with that of normal control. However, there was a significant increase in the GST activity in the pancreas on treatment with plant extracts in alloxan exposed rats when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of GST in the pancreas of plant extract treated to alloxan exposed rats when compared with that of alloxan treated group.
DISCUSSION

The world health organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for the treatment of diseases due to easy availability, economic and less side effects when compared to allopathic system of medicines. Nearly 2000 of natural drugs are mentioned in Indian Material Medica that have reported various pharmacological activities, out of these 1600 are from plant origin (Mukherjee, 2008). Herbal remedies have formed the basis of traditional medicine for millennia, and have formed the roots of modern pharmacology. While science from roughly the 1880's onwards has striven to isolate the active compounds found in medicinal herbs, the list is ever growing. Wound infections are most common in developing countries, such as Sub-Saharan African and South Asian countries, than in developed countries. Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide (Kumar et al., 2007). Diabetes mellitus (DM) is a common disorder associated with increased morbidity and mortality and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia due to defective insulin secretion, insulin action, or both, resulting in impaired carbohydrate, lipid, and protein metabolism (Andreoli et al., 1990; Lebovitz, 1994). Diabetes mellitus (DM) is a chronic disease which is caused either by inherited disability or acquired deficiency in production of the hormone insulin and its subsequent inability to regulate the blood glucose level and also where there is insufficient production of insulin, the insulin secreted is unable to regulate the blood glucose levels. The outcome of the above two conditions is that there is an increased level of blood glucose which in turn damages many of the vital organs (kidney, eye, etc.) of the body (Nagappa et al., 2003).

In most patients, pancreatic islets are infiltrated by immunocytes (insulitis). At diagnosis, hyperglycemia is evident, and destruction of β-cells of pancreatic islets is more than 90% complete (Ryu et al., 2001). Thus, in long-standing type 1 DM, β-cell mass is usually decreased to less than 1% of normal mass (Meier et al., 2005). On the other hand, insulin resistance, β-cell dysfunction and failure, are typical features of type 2 DM (Hayden, 2007). In such patients, the initial relative insulin deficiency may progress to
absolute deficiency, and the individual may depend on exogenous insulin for survival (Raskin et al., 2005). As the disease advances, the pancreatic islets are characterised by fibrosis (Ko et al., 2004), amyloid formation by β-cells (Marzban and Verchere, 2004), and β-cell deficit from apoptosis (Butler et al., 2003). Thus, structural deterioration of the pancreatic islets is a key factor in both type 1 and type 2 forms of DM. The link between chronic hyperglycemia and oxidative stress in β-cells has been documented (Robertson et al., 2004). Chronic hyperglycemia results in oxidative stress via autoxidation of glucose in the presence of transition metals (Wolff et al., 1989); decreased activities of antioxidant enzymes such as SOD and glutathione peroxidase (Blakytny and Harding, 1992); increased oxidative phosphorylation (Nishikawa et al., 2000), glycosylation of proteins (Wolff et al., 1989); and activation of the hexosamine pathway (Kaneto et al., 2001). Such increased, hyperglycemia-induced, intra-islet oxidative stress has been hypothesized and demonstrated to result in β-cell dysfunction and death (Donath et al., 1999; Robertson et al., 2003; Hong et al., 2007), as well as in fibrosis of pancreatic islets (Hayden et al., 2007). Thus, drugs that improve glycemia and/or oxidative stress have been reported to ameliorate or prevent islet lesions and fibrosis. In this regard, Ko et al. (2004) reported the beneficial effects of ramipril, an angiotensin converting enzyme (ACE) inhibitor, on the improvement of glycemia and prevention of islet fibrosis in Otsuka Long-Evans Tokushima fatty rats. And using a similar animal model, Yamabe and Yokozawa (2007) reported the protective effect of the polyherbal drug, Hachimi-jio-gan, on oxidative damage and fibrosis of the islets of Langerhans. Moreover, using islet cells isolated from Wistar rats, Beppu et al. (2003) reported that the free radical scavenging activity of Aloe arborescens is associated with the preservation of β-cells of pancreatic islets.

The pharmacological management of Diabetes mellitus has changed dramatically in the past few years with the introduction of many new medications, including α-glucosidase inhibitors, a biguanide, the thizolidinediones, insulin analogs, maglitinides and D-phenylalanine derivatives. These new agents have dramatically increased the number of options available to providers and patients (Unwin et al., 1999). Combination therapy has become common place for the management of hyperglycemia in patients with type II diabetes (Yki-Jarvinan, 2001). There are 1,200 species of plants representing 725
genera in 183 families extending from the marine algae and fungi with anti-diabetic activity. The mechanisms of action of most antidiabetics herbs are not clear, although a few have been documented. Diasulin is a polyherbal drug, which control glucose level by increasing glycolysis and decreasing gluconeogenesis with a lower demand of pancreatic insulin. It also regulates the activities of hepatic glucose metabolic enzymes (Pari et al., 2001). In India, herbal preparations have been used in the treatment of diabetes. A review carried out in April 2004 showed that a few herbs have been scientifically tested to have antidiabetic activity. These includes; *Momordrica charantia*, *Pterocarpus marsupium* and *Trigonella foenumgreacum*. Mechanisms such as the stimulating or regenerating effect on β-cells or extrapancreatic effects are proposed for the hypoglycemic action of these herbs (Saxera and Vikram, 2004). Natural compounds with antidiabetic activity in descending frequency of occurrence includes, complex carbon hydrates, alkaloids, glycopeptides, terpenoids, peptides, amines, steroids, flavonoids, lipids, coumarins, sulphur compounds and inorganic ions. The anti-diabetic mechanisms involved in hypoglycemic activity are numerous, including direct competitive antagonism with insulin, stimulation of insulin secretion, stimulation of glycogenosis and hepatic glycolysis, Pancreatic β-cell potassium channel blockers, cAMP (Cyclic adenosine monophosphate) stimulation, modulation of glucose absorption from the gut among others (Marles, 1996).

Alloxan is a hydrophilic compound, which readily decomposes at neutral pH (Lenzen and Munday 1991, Munday et al. 1993). Alloxan is selectively toxic to β-cells (Hammarstrom et al., 1966; Boquist et al., 1983) by preferentially accumulating in β-cells as glucose analogues through uptake via GLUT2 glucose transporter (Weaver et al., 1978; Gorus et al., 1982). Alloxan causes diabetes in animals through its ability to destroy the insulin-producing β-cells of the pancreas (Webb, 1966; Cooperstein and Watkins, 1981; Lenzen and Panten, 1988; Oberley 1988). Alloxan in the presence of intracellular thiols, generates ROS (reactive oxygen species) in a cyclic reaction with its reduction product, dialuric acid. It inhibits thiol-dependent enzymes such as glucokinase and hexokinase (Lenzen and Panten, 1988) and undergoes redox cycling in the presence of physiological reducing agents, generating ‘active oxygen’ species (Winterbourn and Munday 1989). It is generally believed that the latter species are involved in the initiation of the toxic changes
which lead to pancreatic beta cell death (Oberley 1988). The beta cell toxic action of alloxan is initiated by free radical formed in the redox reaction. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron catalysed reaction step, hydroxical radicals, these hydroxical radicals are ultimately responsible for the death of β-cells (Malaisse, 1982) with their particularly low oxidative defense capacity and ensuing state of insulin dependent ‘alloxan diabetes’. As a thiol reagent, alloxan selectively inhibits glucose induced insulin secretion through its ability to specifically inhibit glucokinase (Lenzen and Panten, 1988) through oxidation of functionally essential thiol groups, there by impairing oxidative metabolism and glucose sensor function of the enzyme in the β-cells. In 1938, Wohler and Liebig synthesized a pyrimidine derivative called alloxan, in 1943, alloxan became of interest in diabetes research when Shaw Dunn and McLetchie reported the induction of diabetes by alloxan due to specific necrosis of β-cells in experimental animals (Jorna et al., 1997; Peschke et al., 2000). Evidence indicates that pancreatic β-cell damage induced by alloxan is mediated through the generation of cytotoxic reactive oxygen species (ROS) (Yamamoto et al., 1981; Malaisse and Lea, 1982; Takasu et al., 1991). Okamoto (1985) proposed that the primary target of ROS produced by alloxan is the DNA of the pancreatic β-cells that causes DNA strand breaks. Increase of cytosolic Ca\textsuperscript{2+} also plays an important role in the diabetogenesis of alloxan, in relation to radical generation and DNA fragmentation (Park et al., 1995).

The severe diabetes produced by alloxan results in blood sugar levels equivalent to a total pancreatectomy, hence sulphonylureas such as tolbutamide, which act mainly by stimulating insulin release from β-cells, show only a small hypoglycaemic effect in this instance. Therefore a test plant extract producing a significant hypoglycaemia (in a severely alloxan- diabetic animal) must be operating through a different mechanism. Moderate diabetic animals are recommended for use in testing drugs for use in Non insulin dependent diabetes mellitus (Williamson et al., 1996). For all animals a single dose of alloxan, 140 – 180 mg/kg (usually 150 mg/kg) is administered as a 5% w/v in distilled water injected intravenously into the marginal ear vein of rabbit or intraperitoneally in case of mice and rats. A rest period of seven days for rabbits, 12 days for rats and mice is allowed during which the animals have free access to food and water. Alloxan and its
reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. There after, highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells (Szkudelski, 2001). Alloxan causes massive destruction of the β-cells of islets of langerhans, not only destroys pancreatic β-cells but also damages the kidney (Gupta et al., 2005). The disease is progressive and is associated with high risk of atherosclerosis, kidney and nerve damage as well as blindness. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated to result in the development of the disease as well as its long-term complications (Bartosikova et al., 2003). The mechanism of alloxan has been fully described much earlier (Lazarow, 1954; Colca et al., 1983). Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that alloxan causes diabetes by rapid depletion of a cells, by DNA alklylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states (Janapati et al., 2008).

Pharmacological treatment of DM is based on oral hypoglycemic agents and insulin which have so many side effects (Andreoli et al., 1990). There is an increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents (Holman and Turner, 1991; Kameswararao et al., 1997; Kameaswara et al., 2001). The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest (Holman and Turner, 1991; Williams and Pickup, 1991; Kameswararao et al., 1997). The World Health Organization also recommended and encouraged this practice especially in countries where access to conventional treatment of diabetes is inadequate (WHO, 1980). WHO has however emphasized the fact that safety should be the over-riding criteria in the selection of herbal medicine for use in healthcare. Diabetes mellitus is probably the fastest growing
metabolic disease in the world and as knowledge of the multifactorial /heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes (Akhtar and Ali, 1984), but only a few have been scientifically evaluated. Alloxan is known for its selective pancreatic islet cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals (Zarrow et al., 1964; Nafisa et al., 2007). Alloxan is the most commonly used chemical for induction of diabetes mellitus. It is a well-known diabetogenic agent widely used to induce Type 11 diabetes in animals (Viana et al., 2004). Hence, the present study was initiated to identify antidiabetic properties in B. retusa stem bark extracts.

a. Effect of ethanolic extract of stem bark of Bridelia retusa (EESBB) on body and organs weight in alloxan induced diabetic rats.

Body weight

Studies have shown an association between hyperglycemia and decreased body weight of diabetic animals (Zafar et al., 2010). The present study revealed that the decrease in body weight in the rats of alloxan treated group, though increase in weight was observed in the rats of alloxan treated group along with ethanolic extract. In this respect, Badr El-Din (1997) attributed this to the inability to use carbohydrates including lipolysis, acidosis and glycogenolysis, disturbances in one or more of the metabolic pathways. Present observations are in agreement with the findings of Piyachaturawat et al. (1988), Habibuddin et al. (2008) and Lee et al. (2008). The loss of body weights in rats was observed on treatment with streptozotocin (STZ) was considered because of injurious effects of STZ which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions (Zafar et al., 2010). Perez et al. (1996) mentioned Ficus carica leaf extract prevented the weight loss in diabetic rats which may be due to insulin secretion (Farouque and Meredith, 2003). It has also been stated that streptozotocin by producing diabetes (hyperglycaemia) and hypoinsulinemia causes reduction in the body weight of diabetic animals (Zafar et al., 2010). Alloxan caused body weight reduction, which was reversed by the Phyllanthus amarus hydroalcoholic extract at 500 mg kg⁻¹ (Lawson-Evi et al., 2010) as also seen in the present study at 150 mg of ethanolic extract. The severe
weight loss as seen in the present study is regarded due to loss or degradation of structural proteins as structural proteins are known to contribute to body weight (Chen and Lanuzzo, 1982). *Amaranthus* species used in the study as reported by Girija et al., (2011) showed weight loss due to STZ was recovered by methanolic extracts which was attributed to the possible presence of essential amino acid and proteins in the extract which may also be so in the present study. Medium polar extract of *Stevia rebaudiana* at 200 and 400 mg for 10 days reversed weight loss due to alloxan which was lower than standard drug glibenclamide (Misra et al., 2011) contrary to the present study with 150 mg of ethanolic extract was better than standard drug. Normal healthy control was found to be stable in their body weight but diabetic mice showed reduction in the body weight, in an earlier study, the decrease of body weights was diminished by the *Euphorbia hirta* flower extract treatments after 14 days of treatment (Kumar et al., 2010) as also in present study. Alcoholic extracts of *Vinca rosea* exhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight (Ahmed et al., 2010).

Diabetes is associated with weight loss. The reversal of weight loss in extract-treated diabetic group indicates that the restorative effect of the extract may be due to the reversal of gluconeogenesis and glycogenolysis (Huang et al., 2000). Alloxan showed a significant decrease in body weight, this dehydration and loss of body weight is associated with diabetes mellitus (Huang et al., 1990). The loss in weight was expected in diabetic mice as diabetes affects the protein synthesis in the body (Ananthi et al., 2003). The total cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet of β-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug (Gorray et al., 1986). In the present study, the ethanolic extract was able to reverse the effects of alloxan on body weight which was best at 150 mg/ kg evidently better than standard drug glibenclamide. Various reports suggest that there is reduction in the body weight in diabetic rats (Rasch and Mogensen, 1980). Loss of body weight could be due to, dehydration and catabolism of fats and protein during diabetes mellitus (Hofteizer and Carpenter, 1973) as seen in present study. This prevention of loss in body weight by
extract in the present study may be due to increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control.

**Organs weight**

The findings of the present study indicated that the weight of the liver, kidney and pancreas showed decreased in rats of alloxan exposed group which on treatment with both the doses of ethanol extract showed increase in the weights of the liver, kidney and pancreas than in alloxan treated rats. The decrease in weights of liver, kidney and pancreas due to alloxan was elevated on treatment with *Stevia rebaudiana* extracts as reported by Misra et al., (2011). The decrease in the weight of pancreas could be attributed to the disruption and disappearance of pancreatic islets and selective destruction of insulin-producing cells (Kim et al., 2006; Heidari et al., 2008). Liener (1996) suggested that an increase in pancreatic growth might be caused by a diversion of amino acids from the synthesis of body tissue protein. Grant et al., (1997) found rapid pancreatic enlargement in rats fed with raw soya beans and attributed the increase in pancreatic growth to the presence of soya-bean lectin and trypsin inhibitors. On the basis of World Health Organization Criteria, more than 70% of patients with cirrhosis have oral glucose intolerance, and diabetes is more prevalent in these patients than in the general population (Kruszynska and McIntyre 1991). Diabetic nephropathy (DNP) is a major cause of illness and premature death in diabetic patients, largely through accompanying cardiovascular diseases and end-stage renal failure (Rasch and Mogensen, 1980). Diabetes induced by alloxan in rats results in development of nephropathy similar to early stage clinical diabetic nephropathy (Robbins and Cotran, 2004). Excessive production and accumulation of lipids can have devastating effect on renal structure and function (Yotsumoto et al., 1997). Most patients with liver cirrhosis are intolerant to oral glucose, even when their fasting blood glucose concentration is normal. Impaired regulation of carbohydrate metabolism includes glucose intolerance, hyperinsulinemia and hyperglucagonemia (Holdsworth et al., 1972; Silva et al., 1988). Insulin resistance in target organ such as muscle or adipose tissue and hyperinsulinemia seem to be the pathophysiologic basis of diabetes in liver disease. Thus the protective role of the extract best at 150 mg was observed due to prevention of weight loss in vital organs caused by alloxan.
Loss of organ weight has been used as criteria in the assessment of drug toxicity in animals (Boyd and Knight, 1963). Induction of diabetes by STZ leads to loss of body weight due to increased muscle wasting and loss of tissue proteins (Trinder, 1969; Yadav et al., 2008). Recently, Pandhare et al., (2011) reported after 30 days of aqueous extract treatment of *Sesbania sesban*, gain in body weight was observed in diabetic rats and the results were comparable with that of the standard drug glibenclamide as seen after 15 days in the present study. Doddamani and Kaliwal (2011) have reported the decrease in body and organs weight due to alloxan. Decrease in body, liver and kidney weights was observed in pesticides treated rats and mice (Adilaxmamma et al., 1994; Ratnasoorya et al., 1995; Kacker et al., 1999; Baliger and Kaliwal, 2000, 2002, 2003, 2004; Radhika and Kaliwal, 2002; Mahadeswami and Kaliwal, 2002; Shreelakshmi and Kaliwal, 2007; Kshreesagar and Kaliwal, 2008; Manwadi and Kaliwal, 2010). Rats treated with monocrotrophos and BHC showed decrease in liver and kidney weights (Sisodia, 1990; Shivanandappa and Krishnakumari, 1981). The decrease in relative weights of liver and kidney in the present study are in support of findings on treatment with xenobiotics reported earlier (Shivanandappa and Krishnakumari, 1981; Prazedzecki et al., 1969; Janardhan and Sisodia, 1990: Isabramanian et al., 1991; Ratnasoorya et al., 1995; Kacker, 1995; Semanogh et al., 2001). Pesticides may disrupt the normal functioning of the female reproductive organs (Bretveld et al., 2006). The protective effects of ethanolic extract was by reversed decrease in body and organs weight as was observed in the present study with best results at 150 mg/kg which was comparatively better than standard drug glibenclamide.
b. Effect of ethanolic extract of stem bark of *Bridelia retusa* (EESBB) on blood glucose and serum parameters (SGOT, SGPT and cholesterol) of alloxan induced diabetic rats.

**Blood glucose**

Glucose homeostasis involves the co-ordinate regulation of several metabolic pathways, including gluconeogenesis and glycolysis, which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response (Reaven, 1998). Generalised increase in the level of blood glucose during diabetes have been consistently reported both in animal models (Mathew and Augusti, 1975; Hamme *et al.*, 1991; Sharpe *et al.*, 1998; Tukuncu *et al.*, 1998) and humans especially those suffering from insulin dependent diabetes mellitus (Bell *et al.*, 1984). Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism causes an over exertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia. The findings of the present study indicated that the blood glucose levels of alloxan treated rats was increased, however, a decrease was observed in the blood glucose levels of alloxan exposed rats on treatment with ethanol extracts. Alloxan has been reported to cause a massive reduction in β–cells of the islets of Langehans and induce hyperglycemia (Aruna *et al.*, 1999). The present results indicated that there was gradual recovery in the blood glucose levels of plant extract exposed to alloxan treated rats from 5th to 15th day at 150 mg as also reported by Girija *et al.*, (2011) at higher concentration of 400 mg on 14th day of treatment with *Amaranthus viridus*, *A. caudatus* and *A. spinusus*. Crude ethanolic extract of *Aegle marmelos* has also been shown to possess blood glucose lowering effect in diabetic rats after 2 weeks of administration (Karet *et al.*, 2003) as also seen in the present study. Alcoholic leaf extract of *Aegle marmelos* in the dose of 250 mg/kg orally for a week has shown to have hypoglycemic effect (Sachdewa *et al.*, 2001) as observed at 150 mg/kg in the present study. Similar reports of hypoglycemic activity have been reported in alloxan induced diabetes mellitus by plant extracts of *Artemesia herba* (Shammuony *et al.*, 1994), *Terminalia catappa* (Nagappa *et al.*, 2003), *Croton zambericus* (Okokon *et al.*, 2006), *Tapianthus butungii* (Osinubi *et al.*, 2006), *Tinospora*
cordifolia (Prince and Menon, 2000) and Nymphaea stellata (Rajagopal and Sasikala, 2008) and Nymphaea odorata (Dodamani and Kaliwal, 2011).

Diabetes mellitus is a syndrome characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins, hence in the present study, the hyperglycemic activity indicates induction of diabetes may be due to glycogenolysis or gluconeogenesis (Guyton and Hall, 2000). Hyperglycemia and diabetes are imputed to the selective destruction of pancreatic β-cells that secret insulin and abnormalities in the regulation of peroxide and transition metal metabolism have been postulated in the development of the disease as well as its long term complication (Zheng et al., 2007). Chronic insulin deficiency and insulin insensitivity are the major causes of the decreased hepatic glucose utilization and increased glucose production in several animal models of type 2 diabetes, because insulin decreases the hepatic glucose output by activating glycogen synthesis and glycolysis, and by inhibiting gluconeogenesis (McGarry, 1992). In this study, increase in blood glucose level was observed on induction of diabetes mellitus in rat models, which was reduced in a dose dependent manner with the highest percentage reduction at 150 mg comparable to that reported by Ozougwu (2011) at 300 mg/kg of Allium cepa aqueous extract. Extract of Stevia rebaudiana at 200 and 400 mg for 10 days decreased the elevated blood glucose level in diabetic animals which was lower than standard drug glibenclamide (Misra et al., 2011) as also seen with both 100 and 150 mg of ethanolic extract in the present study.

The fundamental mechanism in diabetes mellitus involves the overproduction (excessive hepatic gluconeolysis and gluconeogenesis) and decreased utilization of glucose (Ashok et al., 2010). The extract like standard drug, glibenclamide may induce hypoglycaemia by stimulating insulin release and action, thereby enhancing cellular uptake and utilization of glucose in rats. It remains unclear whether the cellular glucose uptake may be due to increased insulin secretion or decreased insulin degradation rate. It is possible that the extract may act by undetermined ways apart from stimulating insulin production from the pancreatic islets since these would have been severely damaged by alloxan. The mechanism of the hypoglycaemic effects of the extract remains speculative,
therefore, further studies are required to unravel the pathway of its hypoglycaemic action and to shed more light on the hypoglycaemic constituents of the plants. It is however evident from this study that the extracts must contain hypoglycaemic agents capable of lowering blood glucose level in alloxan diabetic rats. Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β-cells. It is used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs. With this agent, it is possible to produce different grades of severity of the disease by varying the dose of alloxan used, these may be classified by measuring fasting blood sugar (FBS) levels: e. g. in rabbits moderate diabetes has been defined as an FBS level of 180–250 mg/dl, and severe diabetes as an FBS level of above 250mg/dl (Huralikuppi, 1991). Inhibition of the proximal tubular re-absorption mechanism for glucose in the kidney, if any, can also contribute towards blood glucose lowering effect (Sharma et al., 1983; Subramoniuin et al., 1998).

*Bridelia ferruginea* probably reduces plasma glucose levels, an effect which is more pronounced in hyperglycemic states than in normoglycemic states reported by Mathew et al., (2006). This is in agreement with present study and the reports of Ampofo (1977) and Iwu (1983) that aqueous extract of the leaves of *Bridelia ferruginea* had been shown to possess hypoglycemic activity. Mechanisms such as the stimulating or regenerating effect of β-cells on extra pancreatic effects are proposed for the hypoglycemic action of *Bridelia ferruginea*. This finding is also in consonance with the work done by Saxena and Vikram, (2004). In the NIDDM diabetic model rats, the ethanol extract of *B. ndellensis* showed an anythyperglycemic effect comparable to that of glibenclamide when fed simultaneously with glucose (Sokeng et al., 2005). Thus, the extract may act on β-cells like sulfonylurea drugs to stimulate insulin secretion. Similar results have been reported with *Anacardium occidentale* aqueous leaf extract (Sokeng et al., 2001). β-sitosterol, quercetin, quercetin-3-glycoside and epigallocatechin isolated from *B. ferruginea* (Addae-Mensah and Achenbach, 1985), have demonstrated hypoglycemic activity. *B. retusa*, which belongs to the same genus is likely to contain such compounds responsible for the observed antihyperglycemic effects. There was a noted blood sugar lowering effects of methanolic stem bark extract of *B. grandis* might be due to the
presence of either octadecan-1-ol and/or lupeol in extracts of *B. grandis* recently reported by Njamen *et al.* (2011). Like *Bridelia ferruginea* and *B. grandis* bark extract, *B. retusa* of *Bridelia* family achieved a reduction in plasma glucose levels especially in hyperglycemic rats. Thus it can be concluded that *B. retusa* also has hypoglycemic activity. Further chemical and pharmacological investigations are needed to elucidate in detail the active principles and the real mechanism of action of this plant extract.

**Cholesterol**

Hyperlipidemia certainly contributes to major risk factor for cardiovascular diseases (Nikkila and Kekki, 1973; Umesh, 2005). During diabetic state, insulin deficiency contributes to derangements of various metabolic and regulatory mechanisms in body. At normal state insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses triglycerides and prevents mobilization of free fatty acids (Briones *et al.*, 1984; Nikkila, 1984). However, insulin deficiency inactivates the lipoprotein lipase which promotes liver conversion of free fatty acids into phospholipids and cholesterol and finally discharged into blood which resulted into elevated serum phospholipid level (Shirwaikar, 2005; Pushparaj *et al.*, 2007). The prevalence of atherosclerosis and hyperlipidaemia among diabetics is on the increase worldwide. Alteration in serum lipids profile is known in diabetes, which are likely to increase the risk of coronary heart disease (Laakso, 1996; Steiner, 1999; Massing *et al.*, 2001). Hypercholesterolemia has been reported to occur in alloxan diabetic rats (Sharma *et al.*, 1996; Pushparaj *et al.*, 2000). Lipid profile which is altered in serum of diabetic patients (Orchard, 1990; Betteridge, 1994) appeared to be a significant factor in the development of premature atherosclerosis through increase in serum total cholesterol levels. The present study indicated that the level of cholesterol in the serum of alloxan treated group showed an increase which however decreased in the alloxan treated rats along with plant extract. Thus, the results revealed recovery in the level of cholesterol content in the serum of rats exposed to alloxan after treatment with plant extract best at 150 mg. Insulin deficiency is associated with hypercholesterolemia due to metabolic abnormalities (Murali *et al.*, 2002). This implies that plant extract may possess insulin-like activity which would be helpful to reduce the incidence of lipid born complications. The significant control on
serum lipids may prevent from simultaneous coexistence of hypercholesterolemia and reduce the cardiovascular risk factors (Murali, 2002). These findings are in agreement with previous studies carried out by Chakrabarti et al., (2005) using aqueous and methanolic seed extract dose (250 mg/kg) of Caesalpinia bonducella on alloxan induced diabetic rat models. The elevated serum cholesterol in diabetic rats was reduced by methanolic extract of Arthanema sesanoides treatment (Selvan et al., 2008). The reduction in serum cholesterol was observed in a dose dependent manner in the present study were in agreement with previous reports (Blumenthal, 1998; Ozougwu, 2011). The diabetic mice in an earlier study, showed increased serum cholesterol that decreased with the Euphorbia hirta flower extract treatments after 14 days of treatment (Kumar et al., 2010). The marked hyperlipidaemia that characterizes the diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Hardman and Limberd, 2001). A reduction in lipid profile could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics (Cho et al., 2002).

Considering in the present study the extract effects on lipid components, it can be assumed a potential hypolipidaemic agent which will be of great advantage both in diabetic conditions as well as the associated atherosclerosis or hyperlipidaemic conditions.

Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids (Huuponen et al., 1984) which accounts for the atherosclerosis, arteriosclerosis and severe coronary heart disease which leads to increase levels of transaminases, marker enzymes important in heart and liver damage (Vaishwanar and Kowale, 1976). Tjokroprawiro et al., (1983) found a significant decrease in blood sugar level in the onion treated diabetic patients. Orekhov and Grunwald (1997) found that garlic indirectly affects atherosclerosis by reduction of hyperlipidemia, hypertension, and probably diabetes mellitus and prevents thrombus formation. The extract showed similar effects to that of glibenclamide as also seen with Amaranthus species by Girija et al., (2011). Oral supplementation of Ecuta sativa oil before or after alloxan treatment resulted in lower serum glucose levels and improved lipid profile as compared with rats treated with alloxan alone (Missiry and Gindy, 2000). The hypoglycaemic and hypolipidaemic effects are thus
protective mechanisms against the development of atherosclerosis, hyperlipidaemia and hyperglycaemia common in diabetes mellitus.

**Serum Glutamic Oxaloacetic transaminase (SGOT) and Serum Glutamic Pyruvic transaminase (SGPT)**

The increased activity of serum glutamic oxaloacetate transminase (SGOT) also called as aspartate aminotransferases (ASAT) and of serum glutamic pyruvate transminase (SGPT) or alanine aminotransferases (ALAT) in diabetes is of clinical importance (Rao *et al.*, 1981) because elevated activity of SGOT is suggestive of cardiac damage and that of SGPT liver damage (Scott *et al.*, 1984). The findings of the present study indicated that the SGOT and SGPT activity of alloxan treated rats was increased, however, a decrease was observed in the alloxan exposed rats on treatment with ethanolic extract. Thus, the results indicated that there was recovery in the activity of SGOT and SGPT of plant extract exposed to alloxan treated rats when compared with that of alloxan treated group. Increased serum concentration of qualitative diagnostic enzymes such as SGPT and SGOT were observed in diabetic rats indicating an altered liver function and or liver mitochondrial injury. On treatment with methanolic extract of *Elaeodendron glaucum* significantly reversed the elevated marker enzymes i.e. SGOT and SGPT and restored to normal values indicating a revival of insulin secretion into circulations and also its protective effect (Garabadu *et al.*, 2011). Alcoholic extracts of *Mormodica charantia, Aegle marmelos* and *Embelica jambolana* showed a progressive dose dependent decrease in SGOT and SGPT activities with 500 mg/kg dose after 30 days of treatment which showed similar depressant effect to glibenclamide (300 μg/kg) on SGOT and SGPT activities of STZ diabetic rats (Sundaram *et al.*, 2009) as also seen in the present study at 150 mg of ethanolic extract. Elevation of biomarker enzymes such as SGOT and SGPT was observed in diabetic rats and indicates the hepatic damage. The hepatic damage was restored hepatocytes as the elevated transaminase activities was reduced by methanolic extract of *Arthanema sesanoides* treatment (Selvan *et al.*, 2008). The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activity (Ghosh and Suryawansi, 2001).
The serum, liver and kidney levels of ASAT and ALAT have also found to become elevated in diabetic induced animals, whenever disease process affects liver cell integrity (Vinitha et al., 1994). Transaminases (ASAT and ALAT) which are active in absence of insulin because of availability of amino acids in to blood of diabetes are responsible for the increased gluconeogenesis and ketogenesis observed in diabetics (Felig et al., 1970) Oral administration of *Terminalia arjuna* bark extract (250 and 500 mg/kg body weight) to lower the elevated serum enzyme levels was reported by Ragavan and Krishnakumari (2005) as also seen in the present study at 100 and 150 mg of extract. In view of this the extract mediated reduction in the levels of ALAT and ASAT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic, tissue damage caused by alloxan. This effect was in agreement with earlier studies, wherein the isoenzymes were recognized as markers for liver and muscle legions and the accepted view that serum level of transaminases return to normal and restores the normal function of liver and kidney (Palanivel et al., 2001). The activities of GOT and GPT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore, the activities of GOT and GPT in the circulation were indicators of hepatic damage. Plasma levels of ASAT were increased around twice that of normal in diabetic animals and diabetic animals treated with extract showed improvement. Recovery of plasma ASAT levels of diabetic rats towards normal shows that the dichloromethane methanol extract of *Catharanthus roseus* leaves and twigs extracts (Singh et al., 2001) had no adverse effect on liver functions. *Strychonous potatorum* seed extract made a significant increase in serum ASAT and ALAT in alloxan treated animals suggested that the reduction in diabetes is by inhibiting intestinal peristalsis (Dhasarathan and Theriappan, 2011). The administration of alcoholic extract caused a highly significant effect on enzymes of ASAT and ALAT on 15th day as also seen in the present study. Thus from the above mentioned earlier studies it is evident that the extract showed decrease in SGOT and SGPT activities which was almost that of standard drug. Necrosis occurrence in the liver causes release of these enzymes into the circulation. Enhancement of ASAT level in serum shows hepatic injuries like in viral hepatitis, infarction and muscular damages. ALAT, which mediated converting of alanine to pyruvate and glutamate, is special for liver and is good indicator of hepatic injuries.
Increased levels of the above enzymes are indicators of cell infiltration and functional disturbance of liver cell membranes (Drotman and Lawhan, 1978). Return of above enzymes serum levels to normal range due to turnip root ethanolic extract (Daryoush et al., 2011) received rats may be as a result from prevention of intracellular enzymes infiltration because of cell membrane stability or regeneration of new cells (Thabrew and Joice, 1987). This effect on serum ASAT and ALAT may be due to the modulatory effects of constituents of water-soluble fraction of Cassia auriculata ethanol extract preventing the leakage of the enzyme into circulation (Hakkim et al., 2007). Thus in the present study the protective role of the ethanolic extract of B. retusa was confirmed by reduced SGOT and SGPT activities in the serum which may be due to one or more constituent(s) of the extract.

Diabetes mellitus is characterized by metabolic dysfunctions that results in elevated blood and tissue concentrations of many metabolites, namely: glucose, cholesterol, triglycerides, fatty acids and ketone bodies (Krupp et al, 1985). Urenia lobata (leaves and roots) extracts significantly depressed typical diabetes indices such as elevated blood glucose and cholesterol in the diabetic rats (Onoagbe et al, 2010). Ragavan and Krishnakumari (2005) reported a decrease in blood glucose levels and serum activities ASAT and ALAT on administration of ethanolic stem bark extract of Terminalia arjuna. Ethanolic extract of flowers of Cassia auriculata was reported to decrease elevated levels of total serum cholesterol and blood glucose as well as serum activities ASAT and ALAT in diabetic rats (Hakkim et al, 2007). Thus from the present study, it can be concluded that the levels of total serum cholesterol and blood glucose increased along with SGOT and SGPT activities which that were raised in alloxan diabetic rats can be lowered by ethanolic extract of B. retusa.
c. Effect of ethanolic extract of stem bark of *Bridelia retusa* (EESBB) on biochemical contents (DNA, RNA, proteins and glycogen) in the liver, kidney and pancreas of alloxan induced diabetic rats.

**Deoxyribosenucleic acid (DNA) and Ribosenucleic acid (RNA)**

The nucleic acids i.e. Deoxyribosenucleic acid (DNA) and Ribosenucleic acid (RNA) are the molecular repositories of genetic information. The structure of proteins, and every biomolecules like glycogen, cholesterol, etc., and cellular components, is a product of the information programmed in nucleus of the cell (Lehninger, 2007). The results of the present study indicated that the level of DNA and RNA in the liver, kidney and pancreas of alloxan treated rats was decreased, however, there was a increase in the level of nucleic acids in alloxan treated rats along with plant extract. Thus, the results revealed recovery in the level of DNA and RNA content in the liver, kidney and pancreas of rats exposed to alloxan along with plant extract. Alloxan has been known to be diabetogenic and induces DNA strand breaks in isolated rat pancreas islets *in vitro* and *in vivo* to cause diabetes mellitus (Yamamoto *et al.*, 1981; Okamoto, 1985). It is known that alloxan can cause accumulation of enough reactive oxygen species (ROS) to induce an increase of Ca$^{2+}$ influx, which results in secondary reactions ultimately leading to the fragmentation of DNA of β-cells (Yamamoto et al., 1981). According to Okamoto's model (1985), alloxan induces islet DNA strand breaks, and poly (ADP-ribose) synthetase acts to repair the DNA breaks, consuming islet NAD; this rapid and marked depletion of islet NAD is assumed to induce diabetes mellitus. This seems to be of special importance in understanding the pathogenesis of insulin dependent diabetes mellitus. The exact mechanism of reversal of DNA and RNA damage due to extract in the present study still needs to be investigated which was best at 150 mg and comparable to glibenclamide. Similar reports have been indicated in diabetes induced by alloxan in mice which were reversed by *Nymphaea odorata* (Dodamani and Kaliwal, 2011). There is increasing evidence that alloxan causes diabetes by rapid depletion of a cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus (Janapati *et al.*, 2008).
Oxidative damage to cells in the organism in most of the diseases is characterized by tissue damage and the oxidative stress may be a consequence of this damage (Halliwell et al., 1999). Okamoto (1985) has proposed that the primary target of ROS produced from alloxan is the DNA of pancreatic β-cells, and that causes alloxan induced DNA strand breaks. The DNA fragmentation by alloxan is a critical step in the induction of alloxan-diabetes. Hyperglycaemia and diabetes also generates reactive oxygen species (ROS) which in turn, causes lipid peroxidation and membrane damage (Hunt et al., 1988). ROS generated by the autooxidation of carbohydrates and lipids may further auto-catalytically enhance the rate of autooxidation and propagate oxidative damage (Baynes et al., 1996). Similar reports on oxidative stress suggested that the manifestations of oxidative stress, their characterization in terms of oxidative damage to lipids and proteins, and their regulation at the level of RNA when studies on rat renal cortex for 6 weeks of streptozotocin induced diabetes was conducted (Kakkar et al., 1995). The damage to the DNA in the peripheral nerve have also been reported (Obrosove et al., 1999). The induction of diabetes by using alloxan monohydrate caused massive destruction of pancreatic β-cells that caused decreased production of proteins (Aruna et al., 1999). In the present study the decrease in DNA and RNA level may be due to decreased mitotic index and disturbed cell division (Saxena et al., 1993). The DNA fragmentation induced by alloxan may be mediated by Ca\textsuperscript{2+} dependent endonuclease, the activity of which is affected by ATP. This initiates the repair process involving the activation of poly (ADPribose) synthetase and the associated NAD utilization. Alloxan causes the depletion of cellular ATP and this is believed to be a result of a lack of NAD\textsuperscript{+}. It is believed that NAD depletion is so precipitous that it becomes irreversible and results in a virtual cessation of NAD-dependent metabolism leading to cell death. This is supported by the fact that nicotinamide supplementation and free radical quenchers can prevent alloxan-induced diabetes (Suresh et al., 2001). Thus the ROS released during the oxidative stress may have acted on DNA and RNA causing their degradation and thus resulting in nucleic acid damage which was indicated by the decreased levels in diabetic rats of the present study. The protection of the extracts against nucleic acid damage may be the possible reason of the increase especially effective at 150 mg in the present study.
Protein

Diabetic animals also showed an increased break down of muscles and other tissues proteins into amino acids due to enhanced proteases activity which in turn resulted into increased urea levels in the blood (Asayama et al., 1994). The present study indicated that the level of protein in the liver, kidney and pancreas of alloxan group rats showed a significant decrease which increased in the alloxan treated rats on administration with the plant extract. Thus, the results revealed recovery in the level of protein content in the liver, kidney and pancreas of rats exposed to alloxan after treatment with plant extract. *Aegle marmelos* alcoholic extract of leaves administered to diabetic animals also demonstrated decreased rates of protein catabolism when compared to untreated diabetic animals. However, the rate of protein catabolism was still higher than controls (Upadhya et al., 2004). Recently reports on administration of ethanol extract of wood and bark of *Pterocarpus marsupium* to the diabetic rats, the levels of protein was found to be restored in the normal rats (Mohan et al., 2011). These results were in accordance with the effect of *Wattakaka volubils* in diabetic rats (Maruthupandian et al., 2010). The relative fuxes of oxygen free radicals (OFR) formation (in H$_2$O$_2$ equivalents) through mitochondrial respiration (Chance et al., 1979) and degradation of glycated proteins (Baynes, 1991) may have some effect on the oxidative stress in diabetes.

Alcoholic extracts of *M. charantia, A. marmelos* and *E. jambolana* showed a progressive dose dependent increase in serum proteins with 500 mg/kg dose which showed similar depressant effect in glibenclamide (300 μg/kg) treated STZ diabetic rats (Sundaram et al., 2009). Similar depressant effect on serum total protein and albumin concentrations in STZ (Porte and Halter, 1981; Pepato et al., 1996; Sivajothi et al., 2007) and alloxan (Dodamani and Kaliwal, 2011) diabetic rats has also been reported by some workers. Increase in protein level in liver by extract of *Vernonia amygdalina* leaves and its fractions were reported in diabetic animals (Akah et al., 2009). Return of above total protein levels to normal range due to turnip root ethanolic extract (Daryoush et al., 2011) received rats may be result from effective control of total protein as an early improvement of functional and secretory mechanism of hepatic cells. During diabetes, there is increased protein catabolism with inflow of amino acids to liver, which feed gluconeogenesis and
accelerated ureagenesis, resulting in hypoproteinemia and hypoalbuminemia (Bhavpriya and Govindasamy, 2000) which may have been the case in the present study. Diabetic hyperglycemia induces elevation of the levels of urine total protein and considered as significant markers of renal dysfunction (Bretzel, 1997). There was a decrease in the protein in alloxan induced diabetic control which was attributed to the non enzymatic glycation of proteins seen in diabetes and responsible for long term complication (Vlassara et al., 1981) hence the oral administration of ethanolic seed extracts of Spondias mombin and Parinari polyandra (Iweala and Olundare, 2011) reversed the effect of alloxan on total protein concentration as also seen in the present study. In the present study, the extract was able to rectify the fall in the levels of protein caused due to alloxan in the vital organs in the present study best at 150 mg comparable to glibenclamide.

Glycogen

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Due to selective destruction of 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) decreases as they depend on insulin for influx of glucose (Whitton and Herns, 1975). In the present study, the level of glycogen in the liver, kidney and pancreas of rats of alloxan treated group showed a significant decrease which however, increased in the alloxan treated rats along with the plant extract, the results thus indicated recovery in the level of glycogen in rats exposed to alloxan along with plant extract. Oral supplementation of Ecuta sativa oil before or after alloxan treatment resulted in improved hepatic glycogen content (Missiry and Gindy, 2000). Aberration of liver glycogen synthesis or glycogenolysis in diabetes may be due to lack of or resistance to insulin, which is essential to activate glycogen synthase system. The significant increase of liver glycogen level in Stevia plant extract-treated groups may be due to reactivation of the glycogen synthase system by improving insulin secretion (Swanston-Flatt et al., 1990). As also in an earlier study, the hepatic glycogen concentration was significantly higher in the naringin treated group compared with the
diabetic group (Leelavinothan et al., 2010). In an earlier study, hepatic glycogen content decreased the diabetic control animals but with the extract of Sarcocephalus latifolius S. M. (Rubiaceae) and Daniella oliveri Rolfe (Caesalpiniaceae) roots (250 mg/kg) and glibenclamide increased the hepatic glycogen content (Iwueke and Nwodo, 2008) as seen at 150 mg of the extract in the present study. Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer, 1998). Increase in liver glycogen can be brought about by an increase in glycogenesis and/or decrease in glycogenolysis (Babu et al., 2003). So the extract might have stimulated glycogenesis and/or inhibited glycogenolysis in the diabetic rat liver as reported by Rajagopal and Sasikala (2008) in hydroethanolic extract of Nymphaea stellata leaves.

Treatment of the diabetic rats with Sarcocephalus latifolius and Daniella oliveri inhibited the depletion in glycogen content almost to that of normal, this was possibly due to either the stimulation of insulin release from β-cell (Lolitkar and Rao, 1996) or insulinomimetic activities of the extract giving rise to direct peripheral glucose uptake or a combination of the two. Reduction in hexokinase (HK), glucokinase (GK) and Phosphofructokinase (PFK) enzyme activities in diabetic animals has been reported to give rise to a depletion of liver glycogen (Seoane et al., 1996; Chattopadhyay, 1998). Some studies have demonstrated that hepatic glycogen content in untreated diabetic rats was higher than in treated and untreated nondiabetic rats (Chattopadhyay, 1998; Ugochukwu and Babady, 2003). In this study, the hepatic glycogen content was reduced significantly in diabetic controls as compared to the normal control animals. This is in agreement with earlier findings (Welihinda and Karunanayake, 1986; Bollen et al., 1998; Grover et al., 2000) who demonstrated that glycogen deposition from glucose is impaired in diabetic animals proportional to the severity of insulin deficiency (Gannon and Nuttal, 1997; Stalmans et al., 1997). Glycogen levels in liver which were low in diabetic animals, increased several folds in dichloromethane methanol extract of Catharanthus roseus leaves and twigs treated diabetic animals (Singh et al., 2001). After 14 days treatment with methanolic extract of Arthenema sesanoides, the liver glycogen elevated level were
decreased in diabetic rats (Selvan et al., 2008) as also in present study. In general, increased hepatic glucose production, plus decreased hepatic glycogen synthesis and glycolysis, are the major symptoms in type 2 diabetes that result in hyperglycemia. In the view of glycogen level, there may be three possible way of antidiabetogenic action, one possible way may be increased insulin level. Other possible ways of antidiabetic action of extract may be by preventing the inactivation of the glycogen synthetase and by synthesis of the glycogen synthetase (Jung et al., 2004). This effect on glycogen may be due to the modulatory effects of constituents of water-soluble fraction of Cassia auriculata ethanol extract through induction of insulin secretion (Hakkim et al., 2007). Thus the extract in the present study may have involved one or combination of the following mechanism(s) to ensure normalization of glycogen levels in all the organs analysed in the present study.

Under oxidative conditions in the diabetic rats, damage can occur to cellular biomolecules such as lipid, protein, carbohydrate and DNA (Hannon-Fletcher et al., 2000). Pancreatic, hepatic and renal protein, glycogen, DNA and RNA contents were reduced on alloxan exposure which were normalized by treatment with Nymphaea oorodata (Dodamani and Kaliwal, 2011). Pancreatic protein, pancreatic DNA and RNA contents were increased by soya bean lectin in both diabetic and non-diabetic rats as observed by others (Grant et al., 1990; Pusztai, 1991; 1993). Jordinson et al. (1997) concluded that 30 mg of raw soya flour and 84 μg of soya bean lectin plays a major role in the stimulation of pancreatic protein secretion. This may be due to oxidative damage, an increase in the rate of auto-oxidation of substrates (carbohydrates and proteins) declines the antioxidant defense (Halliwell et al., 1999). Hence, in the present study the biochemical content of DNA, RNA, proteins and glycogen of liver, kidney and pancreas were reduced in diabetic rats which were recovered by extract best at 150 mg and glibenclamide.
d. Effect of ethanolic extract of stem bark of Bridelia retusa (EESBB) on hepatic glycolytic enzymes activity in liver of alloxan induced diabetic rats.

Liver is the organ involved in glucose homeostasis and the main site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from lactate, amino acids and glycerol. These are the two important complementary events that balance the glucose load in our body (Bhavapriya and Govindaswamy, 2000). Liver is an insulin dependent tissue, which plays vital role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer et al., 1998).

Hexokinase and Glucokinase

Hepatic glucokinase is the most sensitive indicator of the glycolytic pathway in diabetes and its increase can increase the utilization of blood glucose for glycogen storage in the liver (Iynedjian et al., 1988). The findings of present study indicated that the glucokinase and hexokinase activity of alloxan treated rats was decreased which on treatment with plant extract showed an increase in the glucokinase and hexokinase activity. Thus, the results indicated that there was recovery in the activity of glucokinase and hexokinase of plant extract treated to alloxan exposed rats. In an earlier study, the effect of naringin in experimental diabetic rats increased hepatic glucokinase (Leelavinothan et al., 2010). Also, hepatic glycogen reserves are important for whole body glucose homeostasis and are markedly low in the diabetic state (Hombrook, 1970; Migliorini, 1971; Whitton and Hems, 1975). Activity of glucokinase, the first regulatory enzyme of glycolytic pathway was also increased by dichloromethane methanol extract of Catharanthus roseus leaves and twigs in vitro (Singh et al., 2001) also seen in the present study. Decreased activity of glucokinase is reported in diabetes (Chang et al., 1977; Storey and Bailey, 1978) and also in the present study. Thus the extract enhanced the decreased the activity of the enzyme in dose dependent manner with best result at 150 mg comparable to standard drug. The deficiency of insulin in mice treated with alloxan may be due to damage of β-cells of pancreas or reported to inhibit the activity of glucokinase directly (Lenzen and Panten, 1988). Similar results have been reported in rats treated with
hydroethanolic extract of *Nymphaea stellata* flower increased activity of hexokinase (Rajagopal and Sasikala, 2008) and treatment with *Hemidesmus indicus* extract increased the activity of hexokinase in the liver (Sowmia *et al.*, 2009).

**Glucose-6-Phosphatase (G6P) and Fructose-6-Phosphatase (F6P)**

Glucose-6-phosphatase and fructose-6-phosphatase are important regulatory enzymes in gluconeogenesis. Liver being the main organ is responsible for maintaining the homeostasis of the blood glucose (Arati and Sachadanandam, 2003). The gluconeogenic enzyme glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis (Mithievre *et al.*, 1996). These seem to be the consequence of the high glucose-6-phosphatase activities in a diabetic state (DeFronzo, 1988; Reaven, 1988; Guignot *et al.*, 1999). Hyperglycemia seems to enhance non-oxidative metabolism (glucose conversion to lactate) through increasing glucose-6-phosphate (G6P) (Vaag *et al.*, 1992). Increased glucose metabolism to lactate is associated with an increase in NADH/NAD\(^+\) ratio (Williamson *et al.*, 1993). The findings of present study indicated that the G6P and F6P activity of alloxan treated rats decreased the elevated activity observed in the alloxan exposed rats on treatment with plant extract. Thus, the results indicated that there was recovery in the activity of the enzymes with plant extract treatment to alloxan exposed rats. Glucose-6-phosphate dehydrogenase activity was decreased in diabetic state can result in the diminished functioning of the pentose phosphate pathway and thereby the production of reducing equivalent such as NADH and NADPH (Weber and Convery, 1996; Pannerselvam and Govindasamy, 2002) as may have been the case in the present study. G6P plays a critical role in blood glucose homoeostasis and its activity can also be considered as an index of the stability of the microsomal membrane (Van-Schaftingen and Gerin, 2002). Decreased activity of liver G6P was shown in the N-nitrosodiethylamine (NDEA) treated rats, which might be attributed to the increased lipid peroxidation caused by NDEA. Consistent with previous studies (Singh *et al.*, 2004), diallyl sulfide administration enhanced G6Pase activity significantly, compared to the N-nitrosodiethylamine treated group value, suggesting the ability of diallyl sulfide to preserve membrane integrity (Ibrahin and Nassar, 2008). In an earlier study, the
administration of naringin considerably increased the activity of glucose-6-phosphate dehydrogenase and decreases the activity of glucose-6-phosphatase, while the decrease in plasma glucose concentration causes the activation of the pentose phosphate pathway, inactivation of the sorbitol pathway and consequently an increase in the NADPH level (Sinclair, 1993). Hepatic glucose production is raised in diabetic state is associated with the impaired suppression of the gluconeogenic enzyme fructose 1,6-bisphosphatase. Gluconeogenic enzyme activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes (Pari and Murugan, 2005). The recovery of the enzyme activity indicates that the extract in the present study plays an essential role in controlling the glucose level in the blood by enhancing the enzyme activity. This may be due to increased insulin secretion which is responsible for the repression of the gluconeogenic principle enzymes and peripheral utilization of glucose.

The reduction in hepatic hexokinase is mainly due to leakage of these enzymes into the blood as a result of alloxan toxicity (Grover et al., 2000). Higher activity of glucose 6-phosphatase provides H\(^+\) which binds with NADP\(^+\) to form NADPH which is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, pentose phosphate pathway that is still active in liver provides NADPH, which converts acetyl radicals into long chain fatty acids during diabetes mellitus. Similar results were reported by other researchers in experimental diabetes (Grover et al., 2000). However treatment of alloxan diabetic rats with ethanol extract in the present study for 15 consecutive days could restore the normal metabolism by shifting the balance from lipids metabolism to carbohydrate metabolism as also reported by Rajagopal and Sasikala (2008) in hydroethanolic extract of *Nymphaea stellata* leaves and Dodamani and Kaliwal (2011) of ethanolic extract of *Nymphaea orodata* leaves. The increase in the enzyme activity of this glycolytic enzyme by the extract indicates protective role in diabetes. The activities of hepatic hexokinase (HK), glucokinase (GK) and Phosphofructokinase (PFK) in diabetic rats were decreased treated but treatment repeatedly for 21 days with the extract of *Sarcocephalus latifolius* (Rubiaceae) and *Daniella oliveri* Rolfe (Caesalpinioideae) roots (250 mg/kg) and glibenclamide in the diabetic control led to a rise in the activities of these
enzymes when compared with the diabetic control group of rats. The mechanism(s) of action of extract in the present study from the effect on these glycolytic enzymes, it seems to increase flux of glucose into the glycolytic pathway in an attempt to reduce high blood glucose concentration (Iwueke and Nwodo, 2008). The extract in the present study showed reversal of the abnormal enzyme activities in the diabetic rats thus indicating its beneficial effect in glycolytic pathways.

e. Effect of ethanolic extract of stem bark of Bridelia retusa (EESBB) on enzyme activity in the liver, kidney and pancreas, kidney and pancreas of alloxan induced diabetic rats.

Lactate dehydrogenase (LDH)

LDH is a cytosolic enzyme involved in biochemical regulation (i.e. interconversion of lactate to pyruvate) reactions of the body tissues and fluids, LDH products the cofactor (NAD+ / NADH) for glycolytic enzymes (Gupta et al., 1996). The results indicated that the LDH activity in the liver, kidney and pancreas of alloxan treated rats was increased but reduced in the alloxan exposed rats on treatment with plant extract. Thus, the results indicated that there was recovery in the activity of LDH in the liver, kidney and pancreas of alloxan treated rats along with plant extract. Increase in lactate dehydrogenase activity is also reported in diabetic rats (Chang et al., 1977; Lemieux et al., 1984). Serum, liver and kidney LDH is almost doubled in alloxan induced diabetic animals. This is line with finding of others (Maneemegalai et al., 1993; Dodamani and Kaliwal, 2011). Administration of Terminalia arjuna bark extract the LDH level was found to decreased and this was similar to that of normal rats as seen in the present study. Thus decreases of LDH activity in serum, liver and kidney, thus decreasesing the endogenous glucose production as reported by Ragavan and Krishnakumari (2005). Treatment with diallyl sulfide resulted in a significant decrease in liver LDH activity of N-nitrosodiethylamine treated rats. The increased activity of LDH could favor pyruvate (aerobic carbohydrate metabolism) against lactate (anaerobic), thus enhancing energy metabolism in the cell and reflecting restoration of normal cellular/metabolic function (Ibrahin and Nassar, 2008) as seen in the present study. Return of above LDH serum levels to normal range due to turnip root ethanolic extract (Daryoush et al., 2011) received
rats may be as a result from prevention of intracellular enzymes infiltration because of cell membrane stability or regeneration of new cells (Thabrew and Joice, 1987). The reduction in hepatic LDH are mainly due to leakage of these enzymes into the blood as a result of alloxan toxicity (Grover et al., 2000).

In a recent report, silymarin treatment to alloxan blocked changes in the enzyme activity of LDH that was increased in alloxan treated balb/c mice wherein the activities were brought to that of normal (Al-Jassabi et al., 2011) as also seen in the present study. Younathan (1962) reports the inhibition of NAD-linked steps in the tricarboxylic acid cycle by relatively small doses of alloxan (kidney), and it has been reported previously that effects of alloxan can be reduced by many factors including NADH (Lazarow, 1954). The possibility that high concentrations of coenzyme might protect against the adverse effects of alloxan has been considered (Graymore et al., 1968). Certainly, the results presented here suggest that an immediate and direct effect of alloxan on the coenzyme systems should be considered as a real possibility, and that this in turn would lead to the failure of the dehydrogenase systems and cellular death. Thus it can be noted that ethanolic extract used in the present study was effective in normalizing LDH activity in all the tissue which was best at 150 mg and comparable to glibenclamide.

**Acid phosphotase (ACP) and Alkaline phosphotase (AKP)**

AKP and ACP are ubiquitous in nature, their primary role of extra cellular phosphatases is to provide inorganic phosphate for cell growth by hydrolysis of external phosphate esters which do not penetrate the cytoplasmic membrane (Periaswamy et al., 1994) and also AKP is the prototype of these enzymes that reflect the pathological alteration in biliary flow (Ploa et al., 1989). During diabetes, the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (AKP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants (Singh et al., 2001). The results indicated that the ACP activity in the liver, kidney and pancreas of alloxan treated rats was decreased but increased when treated with plant extract. The results indicated that the AKP activity in the liver, kidney and pancreas of alloxan treated rats decreased the
elevated activity observed in the alloxan exposed rats on treatment with plant extract. Thus, the results indicated that there was recovery in the activity of ACP and AKP in the liver, kidney and pancreas of plant extract administered to alloxan treated rats. Increase in serum and tissue ACP and AKP activity may also be due to hepatocellular necrosis or cellular leakage that serves as a biomarker for chemical induced injury. Increase in ACP and AKP activity in all tissues and blood may be due to damage of organs, especially liver, bones, small intestine and kidney (Zimmerman et al., 1976). Acid phosphatase activity of liver of diabetic rats was also found to be increased and at a low dose of 75 mg/kg for 24 days ethanolic extract was reported to inhibit acid phosphatase activity and at higher doses i.e. 300 mg/kg stimulation was reported (Chauhan et al., 1979) as seen at 150 mg of ethanolic extract in the present study. Liver alkaline phosphatase activity was found to be significantly increased in diabetic animals and the treatment with dichloromethane methanol extract of Catharanthus roseus leaves and twigs further normalized the activity (Singh et al., 2001) as seen in the present study. Increase in alkaline phosphatase activity in testes and prostate at 300 mg/kg for 24 days by ethanolic extract of C. roseus leaves was reported by Chauhan et al. (1979). In diabetic rats, the administration of ethanol Strychnous potatorum seed extract exhibited a remarkable increment in AKP level in treated animals when compared to normal rats (Dhasarathan and Theriappan, 2011). Increase in alkaline phosphatase activity in liver by extract of Vernonia amygdalina leaves was also reported in diabetic animals (Akah et al., 2009). Oral administration of Terminalia arjuna bark extract (Ragavan and Krishnakumari 2005 and Pterocarpus marsupium wood and bark extracts (Mohan et al., 2011) to the diabetic rats lowered the elevated serum enzyme level. In a study observed earlier, an increase in the level of liver and kidney marker enzymes in alloxan induced diabetes indicated that alloxan administration produced hepatic damage and an increase in membrane transport is evidenced by enhanced activities of membrane bound enzymes, AKP and ACP in the kidney (Shanmugasundaram et al., 1983; Ragavan and Krishnakumari 2005). In an earlier study, serum ACP and AKP increased considerably in alloxan induced diabetic rats. Elevated level of these enzymes in diabetes may be due to extensive damage to liver in the experimental animals by alloxan which were normalized by water soluble ethanolic extract extract of Cassia auriculata (Hakkim et al., 2007). Thus the activities of the both
the enzymes were normalized by both the extract and glibenclamide with comparable results in all the organs of the present study.

The present findings are in agreement with recent reports of Dodamani and Kaliwal (2011). Estimating the activities of serum and tissue marker enzymes like AKP, ACP, ALT and LDH can make assessment of liver function, when liver and kidney cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra et al., 1998). The extract was thus capable of returning the activities of LDH, ACP and AKP back to normal and thus managing the irregularities observed in diabetic rats. Thus the extract has therapeutic, preventive and protective effects in diabetes in terms of hepatic, renal and pancreatic damage caused by alloxan which may be due to plant secondary metabolites (Trease and Evan, 1996)

f. Effect of ethanolic extract of stem bark of Bridelia retusa (EESBB) on oxidative stress parameters (Lipid peroxidation, SOD, CAT and GST) in the liver, kidney and pancreas of alloxan induced diabetic rats.

Diabetic experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation (Baynes and Thorpe, 1996; Ihara et al., 1999). Oxidative stress has been shown to be responsible, at least in part, for tissue damage and cell dysfunction (Kakkar et al., 1995). Glucose can be auto-oxidized in a cell-free system under physiological conditions via ene-diol tautomer formation which generates hydrogen peroxide; reactive intermediate such as hydroxyl and superoxide radicals, and ketoaldehydes (Brownlee et al., 1988; Packer, 1993). Transition metals such as iron are believed to be of crucial importance in the cascade of these reactions, as they catalyze auto-oxidation of glucose (Packer, 1993). Several studies have reported that glucose auto-oxidation can actually occur and could be responsible for increased oxygen radicals in diabetes (Monnier, 1990; Baynes, 1991; Santini et al., 1997).
Lipid peroxidation (LPO)

The oxidative stress in the diabetic animals measured by markers since free radical (CH3*) measurement is difficult due to their very short half-life and their low concentration. Therefore, indirect markers are commonly used to evaluate secondary products of lipid peroxidation such as thiobarbituric acid reactive species (TBARS) (Bonnefont-Rousselot et al., 2000). Lipid peroxidation is a free radical mediated process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, low concentrations of lipid peroxide are found in plasma and tissues. Oxygen derived free radicals generated in excess in response to various stimuli could be cytotoxic to several tissues. Most of the tissue damage is considered to be mediated by these free radicals by attacking the membranes through peroxidation of polyunsaturated fatty acids. The increase in oxygen free radicals in diabetes could be primarily due to increase in blood glucose levels, which upon auto-oxidation generate free radicals (Ivorra et al., 1989). The present study indicated that the level of lipid peroxidation in the liver, kidney and pancreas of alloxan treated rats group showed an increase which decreased on administration of plant extract. Thus, the results revealed decrease in the lipid peroxidation in the liver, kidney and pancreas of rats exposed to alloxan along with plant extract. Like in the present study, the levels of TBARS were increased in liver, kidney and pancreas of STZ-induced diabetic rats which might be due to an increase in the generation of free radicals by STZ reported by Malini et al., (2011). Moreover oxidative stress markers are lipid peroxidation and total antioxidant status. Elevated lipid peroxidation was also studied in STZ diabetic animal. Arthenema sesanoides methanolic extract and glibenclamide treated groups were significantly reduced the oxidation of lipids in kidney and liver (Selvan et al., 2008) as also seen in the present study. Auto-oxidation of glucose and the increased susceptibility of the tissues of the diabetic animals may be due to the activation of the lipid peroxidation system and observed remarkable increase in the concentration of TBARS (Prince and Menon, 2001) as seen in the present study. A marked increase in the concentration of TBARS has been observed in STZ induced diabetic rats (Shirwaikar et al., 2005). The increased susceptibility of the tissues of the diabetic animals may be due to the activation of the lipid peroxidation system. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting
from altered carbohydrate and lipid metabolism, increased generation of reactive oxygen species and decreased level of antioxidant defenses

*Aegle marmelos* alcoholic extract of leaves administered to diabetic animals reported (Upadhya *et al*., 2004) increased oxidative stress in diabetic rats, evidenced by higher MDA but on administration of the extract, the MDA levels was decreased as also seen in the present study. This indicates that in the presence of the extract there is an improvement in the oxidative stress. Increased oxidative stress in the tissues and blood of streptozotocin (STZ) diabetic rats was similarly reported. This was said to be a contributory factor in the development of the complications of diabetes (Kakkar *et al*., 1995; Curcio *et al*., 1995). Increased free radical induces LPO which refers to the oxidative degradation of lipids that impairs cell membrane functioning resulting into cell damage and leading to several pathologies and cytotoxicity (Hunkar *et al*., 2002; Hanachi *et al*., 2004). MDA (CH$_2$(CHO)$_2$), a reactive electrophile species which is one of the LPO end product analogy to advanced glycation end product used as a redox marker and measurement of elevated oxidative stress. Our present study showed an elevation of MDA level in diabetic rats suggesting the tissue peroxidative damages by alloxan. Oral administration of (Garabadu *et al*., 2011) methanolic extract of *Elaeodendron glaucum* (200 and 400 mg/kg) lowered the elevated levels of LPO as seen in present study (100 and 150 mg/kg) which suggests that it might prevent oxidative stress and provide protection to vital tissues like liver, kidney, etc. indicating its antioxidant activities. Oral supplementation of *Ecuta sativa* oil before or after alloxan treatment resulted in decrease in lipid peroxidation in the liver (Missiry and Gindy, 2000). Similarly the extract was capable of protecting against LPO and thus oxidative stress in liver, kidney and pancreas comparable to standard drug.

**Superoxide dismutase (SOD) and Catalase (CAT)**

The major antioxidant enzymes including SOD and CAT are regarded as the first line of the antioxidant defense system against reactive oxygen species generated *in vivo* during oxidative stress. SOD dismutase superoxide radicals to form hydrogen peroxide, which in turn is decomposed to water and oxygen by CAT, thereby preventing the
formation of hydroxyl radicals (Kamalakkanan and Stanley, 2003; Yao et al., 2005). The results indicated that the SOD and CAT activity in the liver, kidney and pancreas of alloxan treated rats was decreased, although there was an increase in the SOD and CAT activity in the liver, kidney and pancreas of alloxan exposed rats on treatment with 150 and 100 mg of ethanol extract with 150 mg showing better effects. Thus, the results indicated that there was recovery in the activity of SOD and CAT in the liver, kidney and pancreas of plant extract administered to alloxan treated rats. The enzymes SOD and CAT are major antioxidant defense systems of the body which protect the cell membrane and other cellular constituents against oxidative damage by free radical species (ROS) (Umamaheshwari and Chatterjee, 2009). The methanolic extract of Elaeodendron glaucum reported by Garabadu et al., (2011) also showed decreased activities as seen in present study.

Decreased concentration of total antioxidant enzymes in alloxan treated diabetic rats were observed due to their utilization during inhibition or destruction of free radical species which also indicates an imbalanced ROS production and antioxidant scavenging systems. SOD is a metalloprotein enzyme primarily involved to catalyze the superoxide anion radical (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$) and finally decomposition into H$_2$O and O$_2$ during detoxification reactions by CAT (Tripathi and Chandra, 2009; Mandlik et al., 2008). Catalase has been shown to protect against alloxan-induced islet DNA strand breaks (Okamoto, 1985), indicating that H$_2$O$_2$ generation might mediate the action of alloxan. In the present study, the activities of SOD and CAT were significantly reduced in liver, kidney and pancreas of diabetes induced rats which were almost normalized with ethanolic extract. Reports have shown that the activities of SOD and CAT were lowered in tissues of diabetic rats (Malini et al., 2011; Ihm et al., 1999) which coincide with the present study. The observed decrease may be due to the utilization of non protein thiols by increased oxygen free radicals produced in hyperglycemia conditions. SOD catalyses the conversion of super oxide anion to hydrogen peroxide and oxygen. In diabetic control group liver and kidney SOD level was reduced and it was improved by the methanolic extract of Arthenema sesanoides treatment reported by Selvan et al. (2008) as also seen in the present study. Matkovies et al. (1977; 1982) found that rats with STZ induced diabetes
had decreased SOD activity in liver, kidney, spleen, heart, pancreas, skeletal muscle, testis, and erythrocytes. Oral glutathione treatment restored the SOD levels in liver and retinal cortex. Catalase is a haem containing ubiquities enzyme, detoxify the H$_2$O$_2$ into water and oxygen. The level of CAT in liver and kidney was improved by methanolic extract of *Arthenema sesanoides* and glibenclamide. Antioxidants may have a role in the prevention of diabetes (Baynes *et al.*, 1999) in the present study. From the above reports, *in vivo* antioxidant status supports antidiabetic effect of the extract. Rats treated with ellagic acid significantly increased the levels of superoxide dismutase (SOD) and catalase (CAT) in STZ-induced diabetic rats suggesting the antioxidant property of a polyphenol ellagic acid (Malini *et al.*, 2011). Thus the decreased activities of antioxidant enzymes in the present study may be contributed by one or more phytoconstituents of the extract that needs further investigation.

**Glutathione s-transferase (GST)**

Glutathione-S-transferase (GST) work together with glutathione in the decomposition of hydrogen peroxide to non-toxic products at the expense of the GSH. Reduced activity may result from radical-induced inactivation as well as diabetes-induced glycation (Bruce *et al.*, 1982; Hodgson and Fridovich, 1975). GST an important role in initiating detoxification (Sedlack and Lindsay, 1968) by catalyzing the conjugation of GSH to the electrophilic foreign compounds for their elimination from the system. Decreased activities of GST in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of toxic products (Prince, 2001). Glutathione S-transferases (GST) 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 results indicated that the GST activity in the liver, kidney and pancreas of alloxan treated rats was decreased, although, there was an increase in the GST activity on treatment with ethanol extract when compared with that of alloxan treated control rats. Thus, the results indicated that there was recovery in the activity of GST in the liver, kidney and pancreas of plant extract administered to alloxan treated rats. The increment in
the activity of GST (El-Demerdash et al., 2005) is consistent with the induction in the
generation of free radicals. Increased GST activity might be one of the defense mechanism
in these animals to detoxify or neutralize the toxic metabolites, e.g. ketone bodies,
generated in liver by the diabetes. Anwar and Meki (2003) have suggested that garlic oil
may effectively normalize the impaired antioxidants status in streptozotocin induced-
diabetes. The effects of this 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 as observed in the present study by elevating the reduced
GST activity in liver, kidney and pancreas.

Natural antioxidant strengthens the endogenous antioxidant defenses from reactive
oxygen species (ROS) and restores the optimal balance by neutralizing the reactive
species. Like many chronic diseases, diabetes is widely believed to increase oxidative
stress. In diabetes an increase in oxidative stress arises due to compromise in natural
antioxidant mechanisms and an increase in oxygen free radical production (Baynes and
Thorpe, 1999). Several studies showed that alloxan produces a decrease in the activity of
the antioxidant enzymes during the development of alloxan-induced type I DM in liver,
pancreas and testis (Soto et al., 1998; El-Missiry, 1999). Increased SOD activity
accompanied by a significant increase in aldehydic products of lipid peroxidation,
indicating an increased hepatic oxidative stress which may also occur in other tissues in
alloxan-treated rats. The aldehydic products of lipid peroxidation such as MDA which is
more cytotoxic and stable than reactive oxygen species and react quickly with cellular
constituents (Esterbauner et al., 1991). Besides these negative effects MDA is modulators
of signal transduction pathways that disturb cellular activities (Grune et al., 1997). This in
turn may contribute to the disruption of intracellular and membrane redox state of many
cells including liver and β-pancreatic cells, hence disturbing glucose regulation. Oil
induced a decrease in hepatic lipid peroxidation, hence aldehydic concentration, and,
therefore, improves serum glucose regulation. The hepatic SOD activity was increased in
rats supplemented with Ecuta sativa oil (Missiry and Gindy, 2000) as compared with
diabetic rats. SOD is responsible for removal of superoxide radicals (Nishikimi et al.,
1972); thus, it may contribute to the modulation of redox state of liver cells as well as
other important secretory cells such as β-pancreatic cells. Different studies have shown that Diabetes mellitus is associated with the increased formation of free radicals and decrease in antioxidant potential. In both insulin dependent and insulin independent diabetes, there is increased oxidative stress (Nazirogolu et al., 2005). So, free radical scavenging and antioxidant effect may be responsible for its antidiabetic effect. It is possible that extract in the present study exerts its effect by causing hypoglycemia. The exact mechanism of antidiabetic activity is still unclear but it may be due antioxidant and free radical scavenging effect of the plant and presence of tannins and other phenolic compounds in the extracts.

A considerable amount of evidence has suggested that oxidative stress plays an important role in the pathogenesis and complication of diabetes mellitus (DM). DM has often been associated with increased oxidative stress in vivo. The increased in oxidative stress in diabetic patients occurred particularly in subjects with poor glycaemic control. Studies on diabetic subjects have demonstrated that increase in the production of free radicals, as a result of hyperglycaemia, resulted in glycosylation of proteins and production of more reactive oxygen species (ROS) (Hunt et al., 1988). Such an oxidative stress is important in the development of many complications of diabetes mellitus such as retinopathy and nephropathy (Baynes, 1991; Lyons et al., 1997). In addition, the oxidative stress is further exacerbated by the decrease in antioxidant enzymes activity, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (Lacka et al., 1995). Under these conditions, damage can occur to cellular biomolecules such as lipid, protein, carbohydrate and DNA (Hannon-Fletcher et al., 2000). Lipid peroxidation products in plasma and cell membrane have been used as the main biomarkers of oxidative damage (Draper and Hadely, 1990). A number of lipid peroxidation products mainly, MDA and 4-hydroxy-2-nonenal are known to interact with DNA (Vaca et al., 1988). A broad range of oxidation products have been described (Draper and Hadely, 1990), including lipid peroxides, which are the precursor for other reactive intermediates such as alkoxyl radicals, and hydroxyalkenals formed in lipid peroxidation reaction, including malondialdehyde (MDA). Such interaction can lead to cytotoxicity, genotoxicity and carcinogenesis (Esterbauer et al., 1991).
Overall, it can be concluded that in the present study, higher dose of 150 mg of ethanolic extract of *B. retusa* showed better effect comparable to standard drug glibenclamide. Thus the results obtained from the present study can be considered very much promising and comparable with glibenclamide, a standard drug used to treat diabetes mellitus by recovery of glucose, biochemicals, enzymes as well as protecting against oxidative stress. The most significant findings at 150 mg ethanolic extract of the present study are similar to that the aqueous leaves extract of *Murraya koenigii, Psidium guajava, Catharanthus roseus* at the dose of 500 mg/kg body weight for 15 days have shown beneficial effect not only on blood glucose but also on body weight in streptozotocin induced diabetic rats (Prasad *et al.*, 2009). It was found that methanolic whole *Vinca rosea* plant extract at high dose (500 mg/kg) is more effective than whole plant extract at low dose (300 mg/kg) after 14 days of treatment as seen in the present study at 150 mg after 15 days. Hence, the methanolic whole plant extract at high dose (500 mg/kg) was more effective and showed similar curative effect as standard glibenclamide (5 mg/kg) as also the case in the present study with ethanolic extract showing results comparable to the standard drug. This could be due to the possibility that some cells are still surviving to act upon by *Vinca rosea* extract to exert its insulin releasing effect (Ahmed *et al.*, 2010) as may have been the case in the present study. From the above information it may be stated that the antidiabetic activity of the extract may be by sensitization the insulin receptor or stimulation of the secretion of insulin from beta cell of Islets of Langerhans in pancreas of diabetic rats and it was supported by improved *in vivo* antioxidant status. Under oxidative conditions in the diabetic rats, damage can occur to cellular biomolecules such as lipid, protein, carbohydrate and DNA (Hannon-Fletcher *et al.*, 2000). The protein, DNA and RNA contents were increased by soya bean lectin in both diabetic and non-diabetic rats as observed by others (Grant *et al.*, 1990; Pusztai, 1991; 1993). This may be due to oxidative damage, an increase in the rate of auto-oxidation of substrates (carbohydrates and proteins) declines the antioxidant defense (Halliwell *et al.*, 1999).
Since the measurement of enzymes activities of aminotransferases (ASAT and ALAT) and phosphatases (acid and alkaline) is of clinical and toxocological importance as changes in their activities are indicative of tissue damage by toxicants or in disease conditions (Singh et al., 2001) thus carried out in the present study. The increase in the activities of plasma ASAT, ALAT, LDH, AKP and ACP indicates that diabetes may have induced hepatic dysfunction. Supporting these finding it has been found by Larcan et al. (1979) that liver was necrotized in diabetic patients. Therefore, the increment of the activities of ASAT, ALAT, LDH, AKP and ACP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993), which gives an indication on the hepatotoxic effect of alloxan. On the other hand, treatment of the diabetic rats with either onion or garlic caused reduction in the activity of these enzymes in plasma (El-Demedash et al., 2005) compared to the mean values of diabetic group. The present results are in agreement with those obtained by Ohaeri (2001) in rats. The reduction in liver enzyme activities is mainly due to leakage of these enzymes into the blood stream as a result of alloxan toxicity which leads to the liver damage. Amagase et al., (2001) concluded that garlic and onion juices exerted antioxidant and antihyperglycemic effects and consequently may alleviate liver and renal damage caused by alloxan-induced diabetes as also seen in the present study. Treatment of the diabetic rats with the aqueous suspension of the Lupinus albus, L. of family leguminosae, Cymbopogon proximus of family Gramineae, and Zygophyllum coccineum of family Zygophyllaceae restored the activities of the above enzymes to their normal level in plasma, liver and testes, thus indicating antihyperglycemic effects and consequently may alleviate liver and renal damage caused by alloxan-induced diabetes (Mansour et al, 2002) as also seen in the present study.

A good number of plants that are used to treat diabetes have been shown to contain phytochemicals that possess hypoglycaemic and/or anti-diabetic activities (Krupp et al, 1985; Ali et al, 1995; Kako et al, 1996; Tahz and Raza, 1996; Vedavanam et al, 1999; Kobayashi et al, 2000; Sharma et al, 2007). Some of the active constituents of the plants exert their actions by stimulating the release of insulin from the β-cells of the pancreas of normal and mildly diabetic animals (pancreatic mechanism) or by insulinomimetic effects
e.g. stimulation of cellular processes that consume glucose (extra-hepatic mechanism). Noor et al., (2008) reported the antidiabetic activity of Aloe vera in streptozotocin induced diabetic rats. They have also mentioned that there are two possible explanations for this finding, first, A. vera may exert its effect by preventing the death of cells and/or second, it may permit recovery of partially destroyed cells which may be so in the present study. Burcelain et al., (1995) reported that the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles. The methanolic extract of Elaeodendron glaucum reported by Garabadu et al., (2011) to have potential antidiabetic extract in alloxan-induced diabetic model through reduction of oxidative damage and modulating antioxidant enzymes in dose dependant manner as also seen in present study. It has been suggested that the active principles from plant sources might act by several mechanisms such as stimulating insulin secretion, increasing repair/proliferation of β-cells, enhancing the effect of insulin and adrenaline and increasing the antioxidative capability (Shanmugasundaram et al., 1990; Fayed et al., 1998). Thus these reports of earlier studies suggested that various plants proved to possess a wide variety of natural antioxidant constituents such as tannins, saponins, alkaloids, flavonoids, phenolic acids and poly phenols, etc. which enhances free radical scavenging activities and responsible to ameliorate change in antioxidant enzymes which may be helpful for treatment of diabetic related complications. Most of the plants have been found to contain substances like glycosides, alkaloids, flavonoids and terpenoids which are frequently implicated as having antidiabetic effects (Loew and Kaszkin, 2002). Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles (Oliver-Bever, 1986; Rhemann and Zaman, 1989). Flavonoids are known to regenerate the damaged β-cells in the alloxan diabetic rats (Chakravarthy et al., 1980) and phenolics are found to be effective antihyperglycemic agents (Manickam et al., 1997). Thus the protective effect of the ethanolic bark extract in the present study may be due to presence of the bioactive constituent(s) that needs to be investigated. There are reports that some plants of Achyrantes aspera, Glossostemon bruguieri and Ougeinia Oojetensis contains mucilages and minerals like calcium, zinc,
magnesium, manganese and copper had remarkable hypoglycaemic activity decreasing the blood glucose levels in diabetic rats within 15 days as seen in the present study (Akhtar and Iqbal, 1991; Ibrahim et al., 1997, Velmurugan et al., 2011). Hence along with phytoconstituents even the mineral composition of the extract may have played a vital role in the present study which also needs to be found out.

Numerous mechanisms of actions have been proposed for plant extracts. Some hypotheses relate to their effects on the activity of pancreatic \( \beta \)-cells (synthesis, release, cell regeneration/revitalization) or the increase in the protective/inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extract. Other mechanisms may involve improved glucose homeostasis (increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes, inhibition of intestinal glucose absorption, reduction of glycaemic index of carbohydrates, reduction of the effect of glutathione. All of these actions may be responsible for the reduction and or abolition of diabetic complications. The saponins will reduce the level of serum glucose levels, liver phosphorylase and glucose-6-phosphatase activities, and significantly increased the serum pyruvate level and liver glycogen. There was also marked improvement in glucose utilization in diabetic rats. Serum insulin and pancreatic cAMP levels showed significant increases in diabetic rats (Glombitza et al., 1994; Velmurugan et al., 2011). This suggests that anti-hyperglycemic action of *Bridelia retusa* might be involved in both pancreatic and extra pancreatic mechanisms.

Primary therapeutic purpose for treating type 1 diabetes is to reduce blood glucose levels. Various hypoglycemic medications have been prescribed in the hospitals and clinics which promote insulin sensitivity and reduce hepatic glucose output. However, some of these hypoglycemic medications may have some side effects. In case of type 1 diabetes patients who have been treated with insulin therapy for extended period of time compound about pain, bruise and even insulin allergy rash and dyspnoea (Lee, 2008). Therefore natural medicinal plants and foods that have antidiabetic functions but do not have harmful side effect have been focused on. In the present study, positive effect of
Bridelia retusa stem bark extract on blood glucose, biochemicals, enzyme activities in sera and organs and antioxidant was clearly seen in alloxan diabetic rats. From the results obtained, there was a beneficial effect of the extract for 15 days after the induction of alloxan. Possible explanation in regards to the differences in reduction of blood glucose level by the extract might be due to protection of pancreatic cells from further damaging or even enhancement of remaining beta cell function. Like Bridelia ferruginea (Addae-Mensah and Achenbach, 1985) and B. grandis bark extract (Njamen et al., 2011) which was attributed to the phytoconstituents, B. retusa also achieved a reduction in plasma glucose levels especially in hyperglycemic rats. The ethanolic extract of B. retusa was found to have potential antidiabetic activity in alloxan-induced diabetic model through reducing oxidative damage and modulating antioxidant enzymes in dose dependant manner. Although the exact chemical compound(s) responsible for the antidiabetic activity of the extract still remain speculative. More detailed studies using different doses and covering longer period of observation are needed before reaching a clear-cut conclusion. Further, isolation and establishment of exact mechanism of action of specific compound from the extract needs to be carried out in the future.
SUMMARY AND CONCLUSION

Adult female rats were treated with single dose of alloxan and blood glucose was monitored for 15 days. The diabetic rats with high glucose levels were treated with plant extract for 15 days at doses of 100 and 150 mg to evaluate the protective role in preventing diabetes, hepatic and renal disorders. The changes in biochemicals, enzyme activity and oxidative stress parameters caused by alloxan in serum, liver, kidney and pancreas was brought to normal by ethanolic extract and glibenclamide, standard drug. Such plants may help to discover new chemical classes of drugs that could serve as selective chemotherapeutic agents for the maintenance of health.

1. The weight loss due to alloxan was significant but was enhanced by treatment with plant extract in the present study. The toxic effect of alloxan in the present study was observed by significant decrease in the weight of liver, kidney and pancreas. Both the extracts were capable of rectifying the toxic effects but the effect of ethanol extract at 150 mg showed better results which may be due to its crucial role in increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein, thus total cell mass reflects the balance between the renewal and loss of these cells.

2. The increase in blood glucose was observed in diabetic rats indicating hyperglycaemic action of alloxan due to pancreatic β-cells damage was normalized by treating with ethanolic extracts of B. retusa thus confirming the protective effect of both the extracts though ethanol extract at 150 mg dose showed better results. Due to mechanisms such as the stimulating or regenerating effect of β-cells or extra pancreatic effects.

3. The concentration changes in cholesterol and inceased activities SGOT and SGPT observed due to alloxan in serum was normalized by treating with ethanolic extracts of B. retusa thus confirming the protective effect of both the extracts though ethanol extract at 150 mg dose showed better results indicates a revival of insulin secretion into circulations and also its protective effect. Considering the extract effects on lipid components, it can be assumed a potential hypolipidaemic.
agent which will be a great advantage both in diabetic conditions as well as the associated atherosclerosis or hyperlipidaemic conditions.

4. The biochemical content DNA, RNA, protein and glycogen were decreased in alloxan treated group and the activity in extract treated group was almost equivalent to that of normal group in the present study with ethanol at 150 mg showed more beneficial effects. This may be due to protection against alloxan induced damage to DNA, RNA and proteins with a simultaneous attack on polyunsaturated fatty acids, intoxication by free radicals, glyconeogenesis and damage of macromolecules.

5. The enzyme activity of LDH and AKP in the present study, increased significantly while that of ACP was decreased in alloxan treated group of liver, kidney and pancreas. Both the extract rectified the enzyme activities but the effect of ethanol extract at 150 mg showed better results which may be by prevention of cellular leakage, loss of integrity of cell membrane and bringing about stabilization of plasma membrane.

6. The changes in enzyme activity of hexokinase, glucokinase, glucose-6-phosphotase and fructose-6-phosphotase in the present study in alloxan treated group of liver, kidney and pancreas were normalized by both the extract but the effect of ethanol extract at 150 mg showed better results indicating its beneficial effect in glycolytic pathways.

7. The oxidative stress caused due to alloxan in the present study was observed by significant increase in lipid peroxidation and decrease in SOD, CAT and GST activity in the liver, kidney and pancreas. The extract was observed to significantly decrease lipid peroxidation and increase in SOD, CAT and GST activity by inhibiting free radicals in the liver, kidney and pancreas wherein 150 mg of ethanol extract showed more antioxidant effect.

8. Overall ethanolic extract at 150 mg showed a better protective effect comparable to standard drug, glibenclamide against alloxan induced toxicity when biochemicals,
enzyme activity and oxidative stress parameters were evaluated in liver, kidney and pancreatic tissues. The protection against alloxan induced toxicity which may be attributed to the individual or combined effect of phytoconstituents.

In conclusion, it can be said that the extract exhibited a protective effect and possessed anti-lipid peroxidative and antioxidant activities. The promising results obtained indicate extensive study on this plant will enable to exploit its potentials which may be attributed to the phytochemicals.