CHAPTER V
General discussion and conclusion

5.1. Ethno-botanical Survey

The survey in this investigation revealed that the population of the explored areas is still dependent on ethnomedicinal plants for their well being. Valuable datas on uses of 40 medicinal plants used by them in various ailments were enumerated. Most importantly, selection of the plants namely, *Trichosanthes bracteata* (Lam) voigt. (Cucurbitaceae), *Leucis plukenetii* (Roth.) (Lamiaceae) and *Cissampelos pareira* L. (Menispermaceae) were done on the basis of most frequently cited plants in the treatment of symptoms of diabetes for the present investigation. Screening of these plants revealed anti-hyperglycaemic activity of *Cissampelos pareira* when the aqueous leaf extract of these plants were administered to fructose-induced hyperglycaemic rats. In this context, it may be mentioned that number of other plants have also been reported to have anti-diabetic properties (Alyassin, *et al.* 1981; Sheela GC and Augusti KT, 1992; Chattopadhyay RR, 1999; Eidi A, *et al.*, 2005; Karthikeyan A, *et al.*, 2008; Wei Z, *et al.*, 2008; Patel VS, *et al.*, 2008; Mao XQ, *et al.*, 2009; Jia Q, *et al.*, 2009). In recent years, there has been a renewed interest to screen plant food materials and medicinal plants used in folk medicines, for a possible beneficial effect in diabetes (Gupta. 1983; Khare *et al.*, 1983; Prakash AO *et al.*, 1986; Baskaran *et al.*, 1990; Murakami *et al.*, 1996; Joffe DJ. and Freed SH. 2001; Persaud SJ. 1999; Liu B, Jones PM, Persaud SJ. *et al.*, 2009).

The rich cultural reservoir of traditional Indian medicine is supported by diverse cultural sources, which should be evaluated fully. It represents the people. The popular therapeutic habits and successes must to be retrieved and validated in order to use these informations to develop new cost-effective, safe and efficacious system of medicines. Efforts should also be made for their conservation and protection from over exploitation.

One of the informants lamented that due to merciless deforestation, many valuable medicinal plants with very potent curative properties are now missing from these areas. Due to the deforestation trends, traditional healers have to forage further and further from their local areas to procure the plants they use to heal their people. This confirms the observation during the survey that lush green forest areas could be seen right from the
Karbi Anglong-Hojai border areas just few years back (October 2003) but there are hardly any vegetation in these areas now (Fig. 4.21., Photograph taken in October 2007). Therefore, aggressive measures to control the deforestation from the Government side with proper planning by involving the local knowledgeable inhabitants of the surrounding areas is warranted in order to preserve such a valuable treasure of our state.

It has been observed that like most native peoples, the Bodos recognize an entire pharmacopoeia of medicinal plants as a part of a well-defined healing tradition. What is unique about this tradition is the use of a large number of forest species. They maintain intact traditional medical systems as part of their culture today, but, not unlike elsewhere, this knowledge is disappearing quickly for a multitude of reasons. These reasons are largely economic in nature and inextricably linked with the destruction of our life-sustaining forests and due to fundamental cultural divergence of the younger generations as they forsake their cultural traditions for the way of the “modern world” (Pesek T, et al., 2005). During the survey, it was observed that the Bodo inhabitant of village called Borthol in Karbi Anglong district comprising more than 500 Bodo families could not speak their language.

A cultural healing tradition that has used nature to treat both primary and complex ailments for over thousands of years has much to offer to the world and must not only be documented, recorded and saved as heritage, but can also be developed appropriately. The indigenous communities can join hands with researchers and Government for preserving the diverse flora for their own and others’ primary healthcare. It can also generate community-based micro-enterprises for ecologically sustainable community development and preservation of the forests and the deep cultural traditions there in.

5.2. Induction of diabetes in the rats

The significant increase in the serum glucose in the fructose fed rats may be due to elevated glucose production, associated with elevated rates of gluconeogenesis because of increased levels of circulating free fatty acids and lactates. The hepatic metabolism of fructose has important effects on both glucose and lipid metabolism (Mayes PA. 1993). Absorbed fructose is delivered to the liver via the portal vein. Fructose is phosphorylated in the liver by adenosine triphosphate to form fructose-1-phosphate. The reaction is catalyzed by the enzyme fructokinase. Fructose-1-phosphate is split by aldolase B into glyceraldehyde and dihydroxyacetone phosphate. Both can be converted to glyceraldehydes-3-phosphate. Thus, the fructose molecule is metabolized into 2 triose phosphate that bypass the main rate-controlling step in glycolysis, 6-phosphofructokinase. In contrast, hepatic glucose metabolism is limited by the capacity to store glucose as glycogen and, more importantly, by the inhibition of glycolysis and further glucose uptake resulting from the effects of citrate and ATP to inhibit phosphofructokinase. The products of fructose metabolism in the glycolytic pathway of the liver are glucose, glycogen, lactate, and pyruvate. Because fructose uptake by the liver is not inhibited at the level of phosphofructokinase, fructose consumption results in larger increases of circulating lactate than does consumption of a comparable amount of glucose. Consumption of high fructose continues to enter the glycolytic pathway distal to phosphofructokinase, and triacylglycerol production is facilitated (Elliott SS, et al., 2002). The significant increase in the serum triglyceride and glucose levels in this experiment may be due to above mentioned combined effects of prolonged high fructose and high carbohydrate source diet.

Significant weight gain was observed in the fructose-fed animals after 4 weeks of fructose feeding with significant increase in blood glucose, triglyceride and cholesterol levels. Highly significant increase in blood glucose levels were achieved only after 8 to 12 weeks of fructose feeding.

Considering the 3 ‘Rs’ (replacement, reduction, and refinement) (Russell WMS, and Burch RL, 1992), high fructose-fed rat model of type 2 diabetes was used in the present investigation because animal suffering could be minimized. Diabetes associated with obesity as a result of over nutrition, as in diabetic syndrome of human population develop in this model. More importantly, toxicity of chemicals on other vital organs of
the animals could be avoided. This was advantageous because evaluation of immune status was one of the objectives in this study. It must be mentioned here that diabetogenic agents like aloxan/streptozotocin has direct toxic effect on immune system (Rerup C, 1970; Uchigata Y, et al., 1983; Wilson GL, et al., 1988). However, this model has disadvantage of requirement of long period of dietary treatment increasing cost of maintenance of the animals. Moreover, it should be noted that frank hyperglycaemia developed only in those animals which were given diet with 80% or more of carbohydrate content (Rice and gram flour mixed with sugar syrup and soya oil). Therefore, the well known diabetogenic agent, aloxan (ALX) was also used in one of the experiments to assess the effect of CLE. ALX is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce type 1 and type 2 diabetes in experimental rat models. Due to the reported high incidence of ketosis and resulting mortality, low dose (40 mg/kg body weight) of this agent was used because it was observed that the percentage incidence of diabetic symptoms were quite variable and were not proportionately related to increasing doses of ALX as previously reported by other workers (Battel ML, et al., 1999) in these ALX treated animals. Therefore, high fructose-fed rats were treated with this low dose of ALX in this experiment. It was interesting to observe that all the animals exhibited significant hyperglycaemia, hyperlipidaemia and other symptoms within 7 days of administration of this drug and persisted till the end of the experimental period.

The ALX treated animals exhibit severe hyperglycaemia, glucosuria, hyperlipidaemia, polydypsia, polyphagia and other symptoms of uncontrolled diabetes and do also develop complications such as neuropathy, cardiomyopathy, well marked retinopathy and others (Srinivasan K, et al. 2005). In this study, the treated animals showed the following signs of the condition: moderate to severe hyperglycaemia, polydipsia (abnormal thirst), polyuria (increased urine volume) weight loss, asthenia (weakness due to the inability to use glucose as source of energy). Histological examination of the pancreatic tissues exhibited reduced % islets and β-cells volume with significantly reduced β-cell granulations.

There are evidences that reversal of hyperglycaemia due to pancreatic regeneration is early and common in case of ALX treated animals (Srinivasan K and
Ramarao K. 2007). However, it has been observed in this study that when high fructose-fed animals were treated with low dose of ALX, the animals exhibited long lasting hyperglycaemia and other symptoms. There was no mortality either in the ALX control group. This may be due to the low dose of aloxan. The long lasting hyperglycaemia and other symptoms exhibited by these animals are due to the administration of high fructose (and also high carbohydrate source diet) to the animals. The % β-cell granulation scores were also significantly lower in these animals when compared with that of the normal control animals. Moreover, similar kinds of results were obtained in these animals even after 12 to 16 weeks of administration of aloxan confirming long lasting hyperglycaemia and hyperlipidaemia in the control animals. Therefore, this interesting model of type 2 diabetes was also included in this study to assess the effect of CLE. The results in this study showed that there was significant reduction in the serum glucose, triglyceride, and cholesterol and percent glycosylated haemoglobin levels in this animal model also. Treatment with the CLE also revealed higher % β-cells granulation scores in the histological examination of aldehyde-fuchsin stained pancreatic tissues of these animals when compared with the % β-cells granulation scores in the fructose-aloxan diabetic control animals.

Careful selection of appropriate animal model, interpretation and extrapolation of the results obtained from these animal models to humans are critical for the investigators using animal models in the study of diabetes mellitus. Considering multiple factors in the production of the diabetes syndrome and long-lasting hyperglycaemia and other symptoms exhibited by the animals, this combination of high fructose and low dose aloxan-induced diabetic model may be good alternative and cost effective as compared to genetic and other models in the study of diabetes.

It is evident from the present studies that administration of high fructose in the rats significantly increased the serum triglyceride, cholesterol and glucose levels as previously reported by other investigators (Zavaroni I, et al., 1980; Thorburn AW, et al., 1989). There are also evidences that high fructose feeding induced impaired cellular insulin binding and insulin sensitivity in normal human subjects (Beck-Nielson H et al., 1980). Therefore the 'obesity epidemic' in India and many parts of the world appears to be emerged largely from changes in our diet and reduced physical activity. Most
important but not well-appreciated dietary changes has been the substantial increase in
the amount of dietary fructose consumption from high intake of sucrose and high fructose
corn syrup, a common sweetener used in the food industries (Elliot SS, et al., 2002).
There is an urgent need for increased public awareness of the risks associated with high
fructose consumption and greater efforts should be made to curb the supplementation of
packaged foods with high fructose additives.

5.3. Choice of anaesthetic drug in the study of diabetes

Careful choice of anaesthetic agent with sufficient efficacy and dosage to ameliorate pain
and distress in the animal experiments is vital to the investigators. Interaction of the
anaesthetic drug with vital organs and its effects on them must also be considered prior to
use. It must be mentioned here that use of ‘Ether’ in diabetes research must be avoided as
it was observed in this experiment that it elevated glucose levels in the normal rats. There
are references that ether stimulates autonomic nervous system and leads to the release of
adrenaline. As a result of this, glycogen is mobilized from both liver and muscle tissues
and a marked rise in blood sugar follows (Wylie and Churchill Davidson, 1986). Choice
of Ketamine hydrochloride as anaesthetic in this experiment was really a fruitful one.
The great advantage was its fewer side effects, its significant analgesic effect and
sufficient duration of action (30 minutes) (Reves et al., 2000) which could be prolonged
by giving sub-doses whenever required.

5.4. Effect of CLE on serum glucose, triglyceride and cholesterol levels
of hyperglycaemic rats

The results in the present study revealed that, Cissampelos leaf extract (CLE)
treatment significantly reduced the serum glucose, cholesterol and triglyceride levels in
the treated rats in a dose dependent manner as the treatment time progressed. The
reduction in the glucose levels may be due to improvement in the insulin action which
was impaired due to high fructose feeding and a high carbohydrate-source diet. The tight
control of glucose concentration in plasma is determined by a balance between glucose
absorption from the intestine, glucose production by the liver, and glucose uptake from
the plasma (Neil B, et al., 2005). In tissues such as muscle, fat, and liver, glucose uptake
and/or storage is regulated by insulin. In addition to promoting glucose utilization, insulin inhibits both basal and glucagon-stimulated hepatic glucose production, thus serving as the primary regulator of blood glucose concentration during fasting.

In mammals, up to 75% of insulin-dependent glucose disposal occurs in skeletal muscle (Neil B, et al., 2005). Adipose tissue also plays a special additional role in glucose homeostasis through its release of free fatty acids, tumor necrosis factor-α (TNF-α), leptin, Acrp 30/ adiponectin, and other adipokines that have been shown to contribute to insulin action and insulin resistance. Therefore, the significant reduction in the glucose levels after CLE treatment indicates improvement in the glucose utilization in the various insulin dependent and independent tissues and preservation of islet β-cells.

Increased FFAs and triglyceride inhibit insulin-stimulated glucose uptake at the level of glucose transport and/or phosphorylation, inhibit insulin-stimulated glycogen synthesis, and inhibit insulin-stimulated glucose oxidation (Groop LC, et al., 1989; Roden M, et al., 1996; Boden G and Shulman HI. 2002). FFAs have a special role in the insulin resistance associated with central obesity. Since central adipocytes are more resistant to insulin inhibition of lipolysis, there is an increased delivery of FFAs to the liver. This leads to increased accumulation of triglycerides that could also contribute to increased hepatic glucose output, reduced hepatic extraction of insulin, and hepatic insulin resistance. The significant reduction in the triglyceride and cholesterol levels of the CLE-treated rats indicates its significant beneficial effect on carbohydrate and fat metabolism.

The most characteristic feature of the β-cells failure is a specific defect in glucose sensing characterized by loss of first phase insulin secretion in response to a glucose stimulus. In this experiment, the fructose-fed rats exhibited impaired glucose tolerance in the oral glucose tolerance test indicating β-cells failure. The improvement in the glucose tolerance in the CLE-treated rats indicates its significant role in carbohydrate and fat metabolism and preservation of islet β-cells function.

5.5. Toxicity evaluation of CLE
Although it is the normal practice to determine the LD₅₀ value in the animals, now it is accepted to limit the study with an acute toxicity test using several doses including
reasonably high doses of the drugs (Babu V, et al., 2003). In this experiment, acute toxicity was tested up to a high concentration of 10g/kg body weight. The treated animals did not exhibit any toxic symptoms or abnormal behaviour even after administration of such a high dose. There was no mortality either.

Organs rich in GOT are heart, liver and skeletal muscles. When any of these organs are damaged, the serum GOT rises in proportion to the severity of damage (International Federation of Chemical Chemistry, 1976). In this experiment, the results did not show any indication of the above mentioned tissue damage as judged from the serum GOT levels of the treated rats as it did not vary significantly in comparison to normal control rats.

Elevation of serum GPT activity is found in liver and kidney diseases such as toxic hepatitis. Since serum GPT did not alter significantly after such a prolonged treatment, CLE may not have any toxic effect on these organs.

Elevation of Alkaline Phosphatase in serum of plasma is found in hepatitis and several other diseases. The results in this experiment reveals that CLE does not have any toxic effect on liver cells leading to elevation of this enzyme as there were no significant differences in the Alkaline phosphatase levels of normal control and the CLE-treated rats.

In the routine histopathological examination of the heart, liver and kidney tissues, no abnormal changes were observed in the morphology. To rule out any adverse effect on the pancreatic tissues, histological examination of the tissue was carried out. The pancreatic exocrine and endocrine cells appeared absolutely normal when compared with the tissues of normal control rats.

**5.6. Effect of CLE (100mg and 300mg) on serum glucose, triglyceride, cholesterol and insulin levels of hyperglycaemic rats**

The results in this experiment showed that CLE administration significantly reduced the serum glucose levels in the 50% fructose-induced hyperglycaemic rats in a dose dependent manner.

The extract also shows significant lowering effect on the triglyceride and cholesterol levels of these hyperglyaemic and hyperlipidaemic animals.
The mean triglyceride levels at the end of treatment period in case of CLE 100mg and 300mg-treated animals were 114.5 mg/dl and 125 mg/dl respectively. The mean triglyceride level in normal control was 99.6 mg/dl and in hyperlipidaemic control was 248 mg/dl. This clearly showed that there was significant reduction in the triglyceride level in the CLE treated group when compared with that of hyperlipidaemic control group (P<0.001).

Similarly, the continuous treatment with the CLE 100mg and 300mg also ameliorated the high cholesterol in the treated group to almost normal levels. Almost similar effect could be observed in the Pioz-treated group.

The serum insulin levels in the Pioz and CLE treated animals were higher in comparison to the levels of hyperglycaemic control animals after 4 weeks of treatment in the Pioz- treated group and after 3 weeks of treatment in case of CLE 100mg and 300mg-treated groups. These results implicate significant influence of the CLE on over all carbohydrate and fat metabolism and islet β-cell function in this hyperglycaemic and hyperlipidaemic rat model of diabetes. In addition to promoting glucose utilization, insulin inhibits both basal and glucagon-stimulated hepatic glucose production, thus serving as the primary regulator of blood glucose concentration during fasting state. Therefore, near normal levels of serum insulin in the fasted animals with significant reduction in serum glucose, triglyceride and cholesterol levels indicates that CLE may have a significant role in enhancing glucose utilization in the insulin dependent tissues like muscles and adipose tissues and also in liver.

5.7. Evaluation of immune status of the hyperglycaemic and normal rats treated with CLE

Herbal drugs are known to possess immunomodulatory properties and generally act by stimulating both specific and nonspecific immunity. (Ghaisas MM, et al, 2009). Many plants used in traditional medicine have immunomodulating activities. Some of these stimulate both humoral and cell-mediated immunity, while others activate only the cellular components of the immune system, i.e., the phagocytic function, without affecting the humoral or cell-mediated immunity. Some of these plants also suppress both humoral and cell-mediated immunity. Many plants with potential immunomodulatory
activity are reported, some of these have already been undertaken for evaluation of their activities in animals, and also to some extent in humans. (Subramoniam A, et al., 2000; Gayathri V, et al., 2005; Desai VR, et al., 2007; Madan J, et al., 2008; Krishna k, et al., 2009). Gayathri V, et al., (Gayathri V, et al., 2005) have reported that out of the three *Selaginella* species studied, the water extract of *S. involvens* has promising thymus growth stimulatory activity in adult mice and remarkable antilipid peroxidation property. Another plant, Guduchi (*Tinospora cordifolia*) has been referred as a plant of rasayana, which is being used as a rejuvenating herb in Ayurveda and other systems of medicine since many decades. Remarkable research work has been done on its immunomodulatory activity using its various extracts of different parts. Guduchi’s immunomodulatory property as an adjuvant therapy in diabetic patients with foot ulcers has been reported. In a prospective double blind randomized controlled study lasting for over 18 months in 50 patients, produced significantly better outcome with improvement in wound healing, indicating beneficial effects of immunomodulation for ulcer healing (Purandare H, et al., 2007). Madan J, et al., (Madan J., et al., 2008) have shown that administration of *Aloe vera* extract to swiss albino mice (300 mg/kg i.p.) daily for five days, significantly (*p* < 0.01) increases the total white blood cells count. Further, it increases humoral immune response, as demonstrated from the increase in plaque-forming cells in the spleen and circulating antibody titre.

Several other plants namely, *Acanthus ilicifolius* (Harcuch kanta, Acanthaceae); *adhatoda zeylanica* (arusa, acanthaceae); *Ajuga bracteosa* (Nilkanthi, Lamiaceae); *Alisma plantagoaquatica* (water plantain, Alismataceae); *Alstonia macrophylla* (apocynaceae); *Annona purpurea* (Annonaceae); *apium graveolens* (Ajmud, Apiaceae), *Avena sativa* (oat/Jeji, Poaceae) etc. are known for their immunomodulatory properties.

*Cissampelos pareira* (L.) Hirsuta (family: Menispermaceae) is a wound healer and antidote, paste of roots are used in fistula, pruritis, skin disorders and snake poison externally. Internally it is useful in anorexia, indigestion, abdominal pain, diarrhoea and dysentery (Anonymous, 1992). It is also used in cough and as it purifies breast milk, is used in various disorders of breast milk secretion. They are frequently prescribed for cough, dyspepsia, dropsy, urino-genital troubles such as prolapsus uteri, cystitis, haemorrhage and menorrhagia, and calcular nephritis (Kirtikar and Basu, 2001).
Cissampareine, a bis-benzyl-isoquinoline alkaloid, showed a significant and reproducible inhibitory activity against human carcinoma cells of the naso-pharynx in cell culture (Morita et al., 1993). Amareesh G, et al., have shown that the root of Cissampelos pareira possess a marked anticancerous agent (Amareesh G, et al., 2007).

The results in this experiment indicate that immune response in the hyperglycaemic animals is depressed as judged by the lower percent phagocytosis and haemagglutination antibody titre in the hyperglycaemic animals compared to the normal control animals. This may be due to oxidative stress associated with release of free radicals (Lyons TJ, Jenkins AJ, 1997; Baynes JW, 1991; Upadhya S, et al., 2004). It was interesting to observe that the percent phagocytosis was enhanced in the CLE treated normal and hyperglycaemic animals when compared with the hyperglycaemic control group. Significant enhancement in the antibody titre was also observed in the CLE-treated groups. During the survey the traditional healers reported that CLE leaf juice improves general body resistance if taken time to time. As reported by them, it is more frequently used by them during summer seasons to counteract influenza, jaundice and fever. Macrophages have a major role in modulating immune system. Phagocytosis of pathogens by macrophages initiates the innate immune response, which in turn orchestrates the adaptive response (Krishna k, et al., 2009). The primary target of most of the immuno-modulatory compounds is believed to be the macrophages which play a key role in the generation of an immune response. Activated macrophages produce not only effector molecules like free radicals and Nitric Oxide, but also produce Cytokines like TNF-α, IL1, IL6, IL12, etc. These cytokines mediate the effector response of other immune cell population. Since macrophages have major role in modulating immune response, the results indicate possible role of Cissampelos leaf extract in stimulation of immune response.

There are evidences that the relationship between inflammation and insulin resistance is not merely correlative but actually causative (pickup JC, et al., 1997; Festa A, et al., 2000; Grimble RF, 2002). Indipendent studies have recently indicated that obesity in rodents and humans is associated with increased infiltration of macrophages into adipose tissue. These findings suggest an intriguing mechanism for the increased production of proinflammatory peptides by adipocytes in obesity. With the onset of
obesity, secretion of low levels of TNF-α by adipocytes is believed to stimulate preadipocytes to produce MCP-1, a chemoattractant specific for monocytes and macrophages. It is believed that sufficient number of macrophages in the adipocytes cooperate with other cell types in initiating a vicious circle of macrophage recruitment and production of inflammatory cytokines, ultimately causing systemic insulin resistance (Hawkins M and Rossetti L, 2005). The increase in the macrophage indices and enhancement in the phagocytic activities of the peritoneal macrophages of the hyperglycamic *Cissampelos* extract-treated rats may be due to the above mentioned reasons. However, it should be noted that significant enhancement in the phagocytosis of peritoneal macrophages was observed in the normal control animals also.

Flavonoids are polyphenolic compounds that occur ubiquitously in plant origin. Over 4000 different flavonoids have been described (Hollman and Batan, 1997), and they are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids. Flavonoids have a variety of biological effects on numerous mammalian cell systems, *in vitro* as well as *in vivo*. They have been shown to exert antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, anti-inflammatory, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic and antiplatelet activities (Formica and Regelson, 1995).

Therefore, considering the results in this experiment, there is a scope to conduct isolation and characterization of active-marker compounds in the crude extracts of this particular species of *cissampelos* from Assam to evaluate its immunomodulatory properties in detail.

5.8. Glycaemic control in CLE-treated rats

These results clearly show that CLE has significant influence on the % HbA₁c (percent Glycosylated haemoglobin) and glucose levels of fructose and fructose-aloxan induced hyperglycaemic rats. In the fructose-fed hyperglycaemic animals, there were significant reduction after 8 weeks in the CLE 30mg-treated group and after 4 weeks onwards in the 100, 300mg and Pioz-treated groups of animals. In the fructose-aloxan induced hyperglycaemic rats also, significant reduction in %HbA₁c was observed. The CLE treatment reduced the %HbA₁c levels in a dose-dependent manner. Significant steady
reduction was observed in the CLE 300mg-treated animals right after 4 weeks of treatment till the end of the treatment period.

The observed increase in the levels of glycosylated haemoglobin in diabetic control group of rats was due to the presence of excessive amount of blood glucose. Throughout the circulatory life of red cell, glychaemoglobin is formed continuously by the addition of glucose to the N-terminal of the β-chain. This process, which is non-enzymatic, reflects the average exposure of haemoglobin to glucose over an extended period. During diabetes, the excessive glucose in the blood reacts with haemoglobin to form glycosylated haemoglobin (Alyassin D and Ibrahim K, 1981; Sheela GC and Augusti KT, 1992). Mechanisms by which increased oxidative stress is involved in the diabetic complications are partially known, including activation of transcription factors, advanced glycated end products (AGEs), and Protein kinase C. Glycosylated haemoglobin has been found to be increased over a long period of time in the diabetes mellitus (Bunn HG, et al., 1978). There is an evidence that glycation itself induce the generation of oxygen derived free radicals in diabetic condition (Gupta BL, et al., 1997). In this study, treatment with CLE showed significant decrease in the percent glycosylated haemoglobin levels in the treated animals. Since decrease in glycohaemoglobin level serves as an indicator of metabolic control in the diabetics (Trivelli LA, et al., 1971; Gonen B and Rubenstein AA, 1978; Gabbay KH, et al., 1977; Bates HM, 1978), the effect of CLE on the %HbA1c in this diabetic model of rat is noteworthy.

Insulin levels exhibited more complex behaviour. In the fructose induced hyperglycaemic fasted rats, no significant differences were observed in the insulin levels of the hyperglycaemic CLE treated, untreated and Pioz-treated animals when compared with the normal control animals although there was significant reduction in the glucose and percent glycosylated haemoglobin levels in these animals. However, significant differences in insulin levels were observed in these animals in fed state. The insulin levels were higher in all the groups in the fed state except in the hyperglycaemic control animals. In the Pioz-treated animal also, the levels were significantly higher when compared with the insulin levels in the fructose-fed hyperglycaemic animals (p<0.001). In the CLE treated groups, significant increase in the insulin levels were observed in
100mg and 300mg treated animals when compared with the levels of hyperglycaemic control animals \( p < 0.01 \) and 0.001).

In the fructose-alloxan induced hyperglycaemic animals also, there was no significant differences in the insulin levels of hyperglycaemic Pioz-treated, CLE treated and untreated animals when compared with the levels of hyperglycaemic control group although there was significant reduction in the glucose and percent glycosylated haemoglobin in these animals in fasted state. In the fed state, although the insulin levels in the pioz-treated and CLE 300mg-treated animals were higher than the hyperglycaemic untreated animals, it did not reach significant levels when compared with the levels in the normal control group. However, glucose and \( \% \text{HbA}_{1c} \) levels in these groups significantly reduced after treatment with the drugs.

5.9. Changes in the islet morphology of CLE-treated hyperglycaemic rats

The Pioz-treated and CLE-treated animals exhibited significantly higher \( \beta \)-cell granulation scores compared to the untreated groups in a dose and time dependent manner. It may be possible that CLE improves glucose utilization in the tissues and thus preserves islet \( \beta \)-cells. Further studies are required at molecular level to reveal the exact mechanism of action of *Cissampelos* leaf extract.

5.10. Effect of Pioglitazone

Pioglitazone (Pioz) have been administered to a variety of insulin-resistant obese and diabetic animal models (Fujiwara T. 1988; Fujiwara, T. *et al.*, 1991.; Lee MK, *et al.*, 1994; Miles, P.D.G. *et al.*, 1997; Miles, P.G. *et al.*, 1998;). These drugs have uniformly been shown to reduce plasma glucose levels in insulin-resistant diabetic mice and rats and concomitantly to lower insulinaemia. This combination of reduced glucose and insulin levels indicated that these agents improved insulin resistance, and this has been directly borne out by formal studies of insulin sensitivity in Pioz-treated animals. Thus, using the euglycemic glucose clamp technique, treatment with a variety of Pioz has been shown to improve insulin-stimulated glucose disposal, as well as insulin inhibition of hepatic glucose production in Zucker fatty rats (Fujiwara, T. *et al.*, 1991), Zucker diabetic fatty
rats (Fujiwara T. 1988; Fujiwara, T. et al., 1991), fructose-fed insulin resistant rats (Lee, MK. et al., 1994), TNF-α-treated insulin resistant rats (Miles PDG. et al., 1997), glucosamine-treated insulin-resistant rats (P.G. et al., 1998), as well as fat-fed rats (Kraegen EW, 1989). All of these represent standard models of genetic or acquired insulin resistance, some associated with obesity and some not, and, taken together, clearly demonstrate that Pioz can improve insulin action across a wide spectrum of insulin-resistant states, regardless of the underlying mechanisms. In this study, significant reduction in serum glucose, triglyceride, cholesterol and percent glycosylated haemoglobin was observed in the Pioglitazone-treated hyperglycaemic and hyperlipidaemic rats. The % β-cell granulations were also higher in the pancreatic islets of the animals treated with Pioz. When compared to the hyperglycaemic control groups.

5.11. Conclusion
Numerous plants have been used in traditional medicines for the treatment of diabetes. In recent years, there has been a renewed interest to screen plant food materials and medicinal plants used in folk medicines, for a possible beneficial effect in diabetes. (Dewanjee S, et al., 2008; Khan A, et al., 2009; Chen J, et al., 2009; Grace MH, et al., 2009; Mao XQ, et al., 2009; Jia Q, et al., 2009). Considerable amount of work has been carried out in this regard with Gymnema sylvestre (GS), a woody climber of the Asclepiadaceae family which has been known for many years for its anti-diabetic properties (Gupta and Variar, 1964; Chakavati, et al., 1966; Gupta, 1983; Khare, et al., 1983; Prakash AO, et al., 1986; Baskaran et al., 1990; Murakami et al., 1996; Joffe DJ. and Freed SH. 2001; Persaud SJ. 1999). Liu B, et al. have found that an extract of GS reversibly stimulates insulin secretion from MIN6 cells from isolated human islets. They are currently studying the intracellular signalling events activated by GS in β-cells (Liu B, Jones PM, Persaud SJ, et al., 2009). Many studies have also been carried out on bitter gourd (Momordica charantia) (Kavikumar S, et al., 1997) and ivy gourd (Coccinia indica) (Kamble SM, et al., 1996) both in experimental animals and human diabetic subjects. The hypoglycaemic influence is claimed to be mediated through an insulin secretagogue effect or through an influence on enzymes involved in glucose metabolism. There is scope for more extensive research in this field, especially to examine the long
term beneficial effect of vegetables/plants, to identify the active principle and to understand the mechanism of action, which is presently unclear. Since diet forms the mainstay in the management of diabetes mellitus, there is scope for exploiting the anti-diabetic potency of vegetables/plants to the maximum extent. Such plant food adjuncts possessing hypoglycaemic activity appear to hold promise as potential anti-diabetic agents.

The active principles of plants are often definite substances but in other cases they are complicated mixtures. The first class of these substances with medicinal properties is vegetable bases which include amines and alkaloids. A considerable number of medicinal drugs owe their curative properties to these bitter alkaloids. Another class of these active principles includes glycosides, essential or volatile oils, resins and antibiotic, each having their own functional significance. Lewis (Lewis WHO, 1992) has reported the use of *Stevia rebaudiana* (Asteraceae) leaves as a sweetener as well as for the treatment of diabetes mellitus since 1887 in India and other countries. Ivorra *et al.* (Ivorra MD *et al.*, 1989) has extensively reviewed active natural principles (polysaccharides, protein, flavonoids and related compounds, steroids, terpenoids and alkaloids) and crude extracts of 45 plant species which have been experimentally studied in last 10 years.

Medicinal plants used in indigenous medicines in crude forms for the management of diabetes mellitus, contain both the organic and inorganic constituents. It is known that certain inorganic mineral elements (potassium, zinc, calcium, traces of chromium, etc.) play an important role in the maintenance of normal glucose tolerance and in the release of insulin from β-cells of islets of Langerhans. *Cucuma longa, Acacia arabica* (babul), *Vinea rosea* (shada-phul), *Cordia myxa* (narvadi), *Musa paradisiaca* (plantain) and *phyllanthus emblica* used in Islamic systems of medicine have been reported to have large amounts (1.0 to 6.5 ppm) of chromium as compared to carbohydrates (Januja KM, 1991).

It is evident from the present investigation that the leaf extract of *C. pareira* reduced the level of serum glucose, triglyceride and cholesterol in fructose-induced and fructose-aloxan-induced hyperglycaemic and hyperlipidaemic rats. It also improved the tolerance for glucose in the treated animals. The ability of CLE in effectively controlling the increase in blood glucose levels in diabetic group of rats may be attributed to its anti-
hyperglycaemic effects. Further, the anti-hyperglycaemic activity of CLE was associated with an increase in serum insulin levels suggesting insulinogenic or other mechanism of activity of the CLE. The observed increase in the level of serum insulin indicates that CLE stimulates insulin secretion from the remnant of β-cells or regenerated β-cells. However, as previously mentioned, there was no significant increase in the serum insulin levels of the treated animals in fasted state although there were significant reduction in the glucose and %HbA1c of these animals. This indicates possible involvement of different mechanism of action other than stimulation of insulin secretion from the β-cells. It may be possible that CLE improves glucose utilization in the tissues and thus preserves islet β-cells. In this study, significantly higher β-cell granulations were observed in the pancreatic islets of the animals treated with CLE when compared with that of hyperglycaemic control animals. Further studies are required at molecular level to reveal the exact mechanism of action of *Cissampelos* leaf extract.

It may also be mentioned that the fructose-fed hypertensive rat is a model of acquired hypertension that also exhibits insulin resistance, hyperinsulinaemia, and hypertriglyceridaemia (Hwang IS, 1987). Several mechanisms have been proposed to mediate the link between hyperinsulinaemia/insulin resistance and hypertension in the high fructose rats (Verma S, 2000). The sympathetic nervous system is believed to be involved because both chemical sympathectomy (Verma S, *et al.*, 1999) and treatment with rilmenidine, an agent that decreases sympathetic outflow, (Penicaud L, *et al.*, 1999) have been shown to prevent fructose-induced hypertension. As previously mentioned, the alkaloid berberine and tetrandrine present in *Cissampelos* have been documented to have hypotensive, cholesterol lowering and cardiotonic actions (Floriani J, 1936; Feng PC, *et al.*, 1962; Mokkhasmit M, *et al.*, 1971; Yao WX, *et al.*, 2002; Issat T, *et al.*, 2006; Choi BH, *et al.*, 2006; Jantova S, *et al.*, 2007). Issat T, *et al.* have shown that berberine, a natural cholesterol reducing product, exerts antitumor cytostatic/cytotoxic effects independently from the mevalonate pathway. Since hypertension is associated with other symptoms of diabetes like hypertriglycerolaemia, hypercholesterolaemia and hyperglycaemia in this model, this particular species of *Cissampelos* from Assam may have beneficial effect in this model because CLE treatment significantly reduced serum triglyceride, cholesterol and glucose levels in the treated hyperglycaemic and
hyperlipidaemic animals. It may be possible that this particular species of *Cissampelos* may contain the alkaloids responsible for the significant lipid lowering properties that had been observed in these studies. Further studies are required to identify the active fractions present in this valuable plant.

The present study shows for the first time that the leaf extract of *Cissampelos pareira* has potential anti-hyperglycaemic activity in fructose-induced and fructose-alloxan-induced diabetic rats as judged from the significant reduction in serum glucose and percent glycosylated haemoglobin in the CLE 100mg and 300mg treated animals when compared with the levels in hyperglycaemic control animals. The high triglyceride and cholesterol in these animals were also ameliorated to almost normal levels. The significantly higher percent β-cell granulation scores in the CLE-treated animal gives further support to these findings. These effects are more or less similar to the effect of Pioglitazone which is a Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) agonist, used to treat type 2 diabetes in human (Diani AR, *et al*. 2003; Moritoh Y, *et al*., 2009).

Diabetes being multifactorial disease the treatment choice differs from patient to patient. Therefore, it is important to find out the biological activity of this herbal extract. Presently it is not possible to pin point the exact mechanism of action of the extract or to identify the active principle(s) responsible for such effect in this study. Therefore, further studies are required to explain more about the mechanism of the anti-diabetic activity of *Cissampelos pareira* and its compounds. Knowledge of the manner in which a chemical’s structure determine its activity or how it exerts its ultimate effects is not gained through in-vivo studies alone. In-vitro studies can predict the cellular or molecular effects of drug or toxins in human and can exhibit the complex physiological respects of whole organisms including signs and symptoms of injury. Therefore, there is a scope to conduct further studies in this direction to see the effect of CLE.

The leaves of *C. pareira* are ground into paste with water and used for diabetes by traditional healers in the remote villages of Karbi Anglong district of Assam without any known or recorded side effects. Although it is the normal practice to determine the LD₅₀ value, now it is accepted to limit the study with an acute toxicity test using several doses including reasonably high doses of the drugs (Babu V, *et al*. 2003). In this experiment, acute toxicity was tested up to a high concentration of 10g/kg body weight. The treated
animals did not exhibit any toxic symptoms or abnormal behaviour even after administration of such a high dose. There was no mortality either. Sub-acute toxicity evaluation also revealed normal levels of serum GOT, GPT and AP in the animals. *Cissampelos pareira* (2g/kg ethanolic extract) was found safe in acute and subacute toxicities in one such study (Amresh, et al., 2008). Chronic toxicity studies are further required for the support of the safe and sound use of this traditional herb. As it grows annually from perennial rootstock and is found in almost all districts of Assam (Kanjilal, Bor, 1976), it can be easily cultivated and used.