CHAPTER 7

DIABETES MELLITUS

7.1 INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder has now become an epidemic, which results from insulin deficiency, characterized by hyperglycaemia, insufficient insulin and insulin resistance or both, leading to altered metabolism of carbohydrates, protein, lipids and an increased risk of vascular complications [98], defect in reactive oxygen species scavenging enzymes [99] and high oxidative stress induced damage to pancreatic beta cells [100].

India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 [101].Diabetes mellitus is ranked seventh among the leading causes of death in the world and is considered third when its fatal complications are taken into account.If not cured or controlled it may even lead to acute or chronic complications causing retinopathy, nephropathy, neuropathy, ketoacidosis, microangiopathy and other related infections affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [102].

Different types of reported diabetes mellitus can be classified under following two categories:

Type 1 is insulin-dependent diabetes mellitus (IDDM), in which the body does not produce any insulin. It most often occurs in children and young adults. Type 1 diabetes accounts for 5–10% of diabetes.

Type 2 is noninsulin-dependent diabetes mellitus (NIDDM), in which the body does not produce enough, or improper use of secreted insulin for 90–95% of diabetes.

Type 2 diabetes is nearing epidemic proportions, due to an increased number of elderly people, and a greater prevalence of obesity and sedentary lifestyles.

In conventional therapy, type I diabetes is managed with exogenous insulin and type 2 with oral hypoglycaemic agents (sulphonylureas, biguanides etc,). In traditional practice medicinal plants are also used in many countries to control diabetes mellitus.

As a very common chronic disease, diabetes is becoming the third “killer” of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases because of its high prevalence, morbidity and mortality. Therefore once diagnosed, it should be well regulated by means of various therapeutically effective drugs. Besides, the therapy based on chemotherapeutic agents, the present century has progressed towards naturopathy. Thus, medicinal plants have an ever emerging role to play in treatment or management of lifelong prolonging diseases like diabetes mellitus, especially in developing countries where resources are meagre.

Diabetes mellitus (DM) has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated. Diabetes mellitus alone is accompanied with several other diseases infecting healthy

individuals. The treatment of each of such disease can be done by exploiting the herbal integrity of India. The plants in parts or as full can be used for curing any disorder related with diabetes mellitus. Moreover in some cases, extracts of plants are self-capable of treating the related disorders such as polyuria, polydypsia, glycosuria etc. along with curing the chronic disorders such as diabetes mellitus [103]. A large number of plant preparations have been reported to possess antidiabetic activity over last several decades.

Our Vedic literatures like Charak Samhita have already reported on the use of plants, herbs and their derivatives for treatment of diabetes mellitus. More than 400 plants have been incorporated in approximately 700 recipes which are used to treat diabetes mellitus in almost two thirds of the world population.

Some of the crude drugs with proved activity against Diabetes are *Aegle marmelos, Agrimonia pilosa, Allii Cepa, Allii sativi, Andrographis paniculata, Anisodust anguticus, Catharanthus roseus, Ephedra sinica, Euonyrnus alatusFructus Coini, Gymnema sylvestre,Momordica charantia, Nelumbo nucifera, Prunella vulgaris, Tribulus terrestris, Trigonella foenum* [104].

7.1.1 Diabetes Screening Models

One of the most straightforward ways of studying the effects of hyperglycaemia in an animal is to remove the pancreas, either partially or totally. The species of animal used is determined by several factors. In general, the smaller the animal, the more manageable and cheaper the experiment. Hence, pancreatectomised rats and mice are the most commonly used.

Alternatively Chemicals with diabetogenic effect in experimental animals such as rodents can be enumerated as Alloxan, Streptozotocin, Vacor, Dithizone, and 8-hydroxyquinolone.

A variety of agents are known to induce diabetes in experimental animals. Non-surgical methods of inducing hyperglycaemia by damaging the pancreas also exist. These include the administration of chemicals such as Streptozotocin and Alloxan [105]. Surgical and toxin-mediated pancreatic damage are valuable tools in studying the consequences of hyperglycaemia, e.g. the development of diabetic complications. When performed on female animals, they may be used to study the effect of gestational diabetes on the offspring [106].

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wohler and Justus von Liebig [107]. It is one of the usual substances used for the induction of diabetes mellitus apart from Streptozotocin [108].Alloxan, a chemical diabetogenic when administered to rodents by a single i.p. injection of 120 mg/kg of alloxan monohydrate in sterile saline and many other animal species induces an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these species, with characteristics similar to type 1 diabetes in humans.

A large number of *in vivo* studies have been conducted on animals to test the claimed activity have demonstrated the hypoglycaemic property of many plants, in glycaemia induced by the aforesaid agents have already reported in various literatures [109].Many studies have confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus. The effects of these plants may delay the development of diabetic complications and even assist in correcting the metabolic abnormalities. Moreover, during the past decade and especially in last few

years some of the new bioactive drugs isolated from hypoglycaemic plants showed anti-diabetic activity with more efficacy than synthetic oral hypoglycaemic agents. Therefore, plants as folk remedies are widely used to treat diabetes mellitus.

7.1.2 Herbal Formulations with Anti-Diabetic Property

Diabecon : This product was marketed by Himalaya&Co. The ingredients present in the formulation are *Gymnema sylvestre,Pterocarpus marsupium, Glycyrrhiza glabra, Casearia esculenta, Syzygium cumini, Asparagus racemosus, Boerhavia diffusa, Sphaeranthus indicus, Tinospora cordifolia, Swertia chirata, Tribulus terrestris, Phyllanthus amarus, Gmelina arborea, Gossypium herbaceum, Berberis aristata, Aloe vera, Triphala, Commiphora wightii, shilajeet, Momordica charantia, Piper nigrum, Ocimum sanctum, Abutilon indicum, Curcuma longa and Rumex maritime.s*

Diabeta : This product was marketed by Ayurvedic cure Ayurvedic Herbal Health Products- The ingredients present in the formulation are *Gymnema sylvestre*, *Vinca rosea* (Periwinkle), *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), *Pterocarpus marsupium* (Kino Tree), *Momordica charantia* (Bitter Gourd), *Syzygium cumini*(Black Plum), *Acacia arabica* (Black Babhul), *Tinospora cordifolia* and *Zingiber officinale* (Ginger).

Syndrex : This product was marketed by Plethico Laboratories.The ingredients present in the formulation are Germinated Fenugreek seed extract.

Diasulin : This product was marketed by Himalaya and Co. The ingredients present in the formulation are *Cassia auriculata, Coccinia indica, Curcuma longa, Emblica officinalis, Gymnema sylvestre, Momordica charantia, Scoparia dulcis, Syzygium cumini, Tinospora cordifolia, Trigonella foenumgraecum* [110].

7.2 MATERIALS AND METHODS

Reagents

Alloxan, Glipizide, Anaesthetic ether, Malondialdehyde (MDA), Thiobarbituricacid, Normal saline, 10%formalin, 50%ethanol, Hematoxylin, Eosin. GOD-POD Kit, BUN, Serum Creatinine, Total Cholesterol kits were procured from Span Diagnostics, Germany.

7.2.1 Experimental Design

Diabetes was induced by a single injection of Alloxan 60 mg/kg b.w. to rats fasting for at least 16 h through the tail vein in freshly prepared 10 mmol/l sodium citrate, pH 4.5. Blood glucose levels were measured daily 3 days prior and 7 days after alloxan administration. Development of diabetes mellitus was proven by sustained hyperglycaemia and glycosuria (diabetic rats had glycaemia > 16 mmol/l).

Group Separation

The rats were randomly divided into 5 groups (n = 6) as follows

Group I : Normal control animals I that received saline solution, i.p for 21 days.

Groups II : Diabetic control animals treated with alloxan (saline solution, i.p) for 3days and were left untreated for 21 days.

Group III : (Standard) diabetic rats treated with Standard drug Glipizide (0.5 mg/kg. P.o. daily) for 21 days.

Group IV : Diabetic rats treated with MELA 200mg/kg p.o. for 21 days.

Group V : Diabetic rats treated with 400mg/kg of MELA p.o. for 21 days.

The rats developed diabetes within 2 days after alloxanisation as evidenced by sustained hyperglycaemia and glycosuria.Only those animals with blood glucose level above 250mg/dl were included in the study.

Every week (from 1stweek to 3rdweek) on 1st, 7th, 14th, 21st day, blood samples were collected by retro-orbital puncture under light ether anaesthesia, then the serum was separated by centrifugation at 2000rpm for 15min in a cooling centrifuge and blood glucose levels were measured. On 21st day, glucose level was finally measured and the extract at both dose levels and the standard drug were administered for 21 days.

Blood samples were collected on 1st, 7th, 14th and 21st day by retro-orbital puncture under light ether anesthesia. The serum was then separated by centrifugation at 2000rpm for 15min in a cooling centrifuge and blood

glucose levels, Total protein, Serum creatinine, Serum cholesterol, Blood urea nitrogen were measured using commercially available kits from Span Diagnostics, Germany using Semi auto analyzer (May sun – 500e). On 21st day, the animals were sacrificed and pancreas was isolated to prepare pancreatic homogenate. This homogenate was used to study the lipid per oxidation products and antioxidant enzymes such as Lipid per oxidation level (LPO), Super Oxide Dismutase (SOD) and Catalase (CAT).

7.2.2 Effect of MELA on Blood Glucose Levels in Alloxan Induced Diabetic Rats

Blood glucose was measured by using commercially available GOD POD Kit from Span Diagnostics, Germany using Semi auto analyser (Maysun-500e).

a) Estimation of Blood Glucose Level by GOD - POD method

The intensity of colored complex produced is directly proportional to the concentration of Glucose content and is measured at 505nm or with green filter.10µl of the serum sample was added to 1 ml of the reagent volume and incubated at 37^{0}c for 15min and the absorbance was measured at 505 nm within 1 hour the preparation.

The concentration of glucose was calculated using the formula 7.1

$$\text{Glucose Concentration (mg/dl)} = \frac{\text{Abs of Sample}}{\text{Abs of Std}} \text{ X Conc. of Std} \qquad (7.1)$$

7.2.3 Effect of MELA on Biochemical Parameters in Alloxan Induced Diabetic Rats

Biochemical parameters like Serum Creatinine, Blood Urea Nitrogen, Total Protein, and Total Cholesterol were measured by using commercially available Kits from Span Diagnostics, Germany using Semi auto analyser (Maysun-500e).

a) Determination of Serum Creatinine (Jaffe kinetic method)

Procedure

In different tubes, pipetted out 1000 µl of working reagent into marked test tubes as Blank and Test respectively to the tube marked test added 100 µl Serum. Mixed well, and read decrease in the absorbance at 492 nm against reagent blank for 120 seconds at an interval of 60 seconds using semi auto analyzer by kinetic method. The units are expressed as mg/dL. Creatinine level was calculated using the following formula (7.2)

$$\text{Creatinine (mg/dL)} = \frac{\text{Abs of Sample}}{\text{Abs of Std}} \text{ X Conc. of Std} \qquad (7.2)$$

b) Determination of Blood Urea Nitrogen (BUN)

In different tubes 1000 µl of working reagent was added and then marked as blank and test and to this added 10µl working agent and serum respectively. Mixed well, incubated for 60 seconds at 37 ºC and read the decrease in absorbance at 340 nm against reagent blank measured for 60 seconds at an interval of 20 seconds using semi auto analyzer by kinetic method. The Units are expressed as mg/dL.

The results were calculated using the following formula (7.3)

$$\text{Blood Urea Nitrogen} = (\text{mg/dL}) = \frac{\text{Abs of Sample}}{\text{Abs of Std}} \text{ X Conc. of Std} \qquad (7.3)$$

c) Determination of Serum Total Cholesterol

In different marked test tubes as Blank and Test, pipette 2ml of working reagent and 20µl serum respectively. Mix well, incubate for 5 minutes and read the absorbance at 510 nm against reagent blank using semi auto analyzer by kinetic method. The results were calculated using the following formula (7.4).The Units were expressed as mg/dL.

$$\text{TotalCholesterol} = (\text{mg/dL}) = \frac{\text{Abs of Sample}}{\text{Abs of Std}} \text{ X Conc. of Std} \qquad (7.4)$$

d) Determination of Serum Total Protein (Biuret's method)

In tubes marked as Blank, 1000µl of Alk. $CuSO_4$ reagent and 20 µl Serum respectively were added and incubated for 10 min at room temperature. Add Lowry reagent 0.2 ml to each tubes Mix well, incubated for 30 min. The absorbance was read at 660 nm using colorimeter method. The results were calculated using the following formula (7.5)

$$\text{Total Protein} = (\text{g/dL}) = \frac{\text{Abs of Sample}}{\text{Abs of Std}} \text{ X Conc. of Std} \qquad (7.5)$$

7.2.4 Effect of MELA on Per Oxidation Product and Antioxidant Enzymes

Super oxide dismutase (SOD) [86], Catalase activity and the level of per oxidation product viz. Tissue Malondialdehyde (MDA) was measured in pancreas homogenate in a procedure similar to that of the procedure followed in the hepatoprotective activity was measured [136] in pancreas homogenate.

7.3 HISTOPATHOLOGICAL STUDIES

The isolated pancreas was fixed in 10% formalin solution. Then it was dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6µm thickness were prepared and stained with Haematoxylin and Eosin then examined under microscope.

7.4 STATISTICAL ANALYSIS

The data are expressed as mean ± SEM (n=6).Statistical significance was determined by one way ANOVA followed by Dunnet's test with $p < 0.05$ considered significant.

7.5 RESULTS

Effect of MELA on Blood Glucose Levels

Methanolic extract of Fruit pulp of *Limonia acidissima* was subjected to anti-diabetic activity in rats where Alloxan monohydrate (60 mg/kg b.w., i.p.) was used as the diabetogenic agent. A marked rise in blood glucose level was observed in diabetic control compared to normal control rats. Methanolic extract of *Limonia acidissima* (200 and 400 mg/kg) exhibited a dose dependent significant anti-hyperglycaemic activity on 1, 7, 14, 21 day post treatment. The extract at a dose of 200 mg/kg also caused reduction in blood glucose level but the results were found statistically insignificant.

The antihyperglycemic effect of MELA at 400mg/kg was found less effective than the reference standard, Glipizide as it produced a significant reduction in blood glucose when compared to diabetic control. The results are shown in Table 7.1.

Effect of MELA on Biochemical Parameters

Serum urea, serum creatinine and serum cholesterol levels were decreased significantly in a dose related manner by methanolic extract of *Limonia acidissima* (200 and 400mg/kg) after 21 days of treatment, where as serum protein level was increased significantly when compare to diabetic control group. However, the extract at a dose of 200mg/kg failed to reverse the altered biochemical parameters significantly. The results are shown in Table 7.2. and Figure7.3.

Table 7.1 Effect of MELA on Blood Glucose Level (BGL) in Alloxan induced diabetic rats

Treatment	**0 week**	**1st week**	**2nd week**	**3rd week**
Normal Control	101.46±3.81	132.92±2.86	124.70±3.23	120.45±2.23
Diabetic Control	190.32±2.01	314.41±1.91	382.25±2.86	413±2.20**
Standard Glipizide (5mg/kg)	407.20±2.48	308.34±3.47	236.99±5.91	160.47±2.37 *
MELA 200 mg/kg	396±1.83	376.47±1.81	326.51±1.23	292.39±2.91**
MELA 400 mg/kg	388.80±2.73	346.12±1.92	276.51±2.98	190.39±1.36**

Each value is represented as mean± SEM, No. of animals (n) = 6

**p<0.01vs.Normal Control,££p<0.01Vs.Diabetic Control,

One-way ANOVA followed by Dunnet's `t' Test.

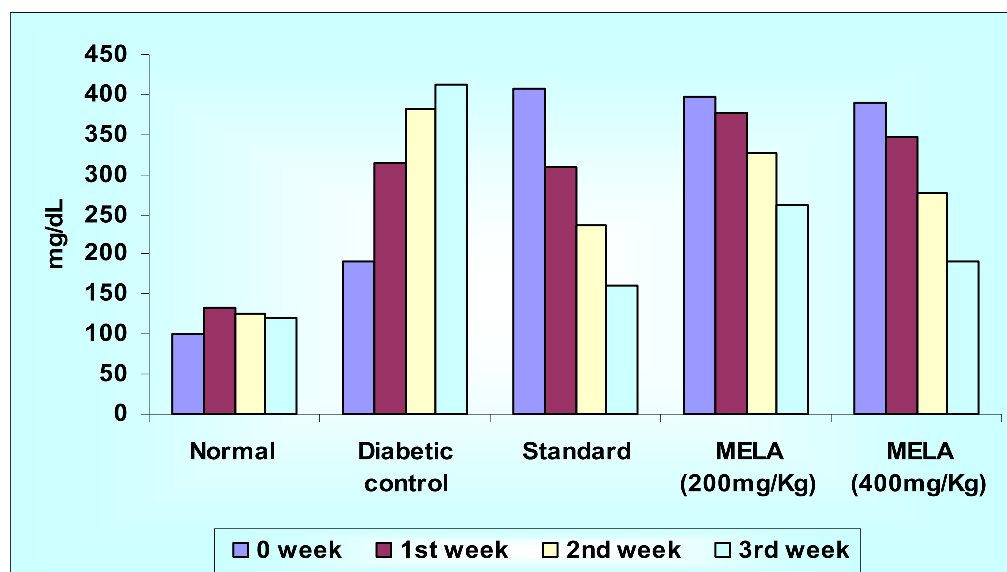


Figure 7.1 Effect of MELA on Blood Glucose Level (mg/dl) in Alloxan induced diabetic rats

Table 7.2 Effect of MELA on Biochemical Parameters in Alloxan induced diabetic rats

Treatment	Total Protein (g/dl)	Total Cholesterol (mg/dl)	Serum Creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)
Normal Control	6.96±0.02	1.28±1.79	0.50±0.01	42.66±1.22
Diabetic Control	3.77±0.05**	181.60±1.15*	0.79±0.008	70.79±0.88**
Standard Glipizide (5mg/kg)	6.68±0.08 **	91.948±0.56**	0.63±0.008**	50.83±0.87**
MELA 200 mg/kg	5.022±0.15	107.784±1.34*	0.71±0.007*	61.83±1.35*
MELA 400 mg/kg	6.05± 0.09**	97±1.64**	0.69 ±0.008**	5.52±1.47**

Each value is represented as mean± SEM, No of animals (n) = 6
*p<0.05 Vs Normal Control,**p<0.01 Vs Normal Control,£p<0.05 Vs Diabetic Control,££p<0.01 Vs Diabetic Control, One-way ANOVA Followed by Dunnet's `t' Test.

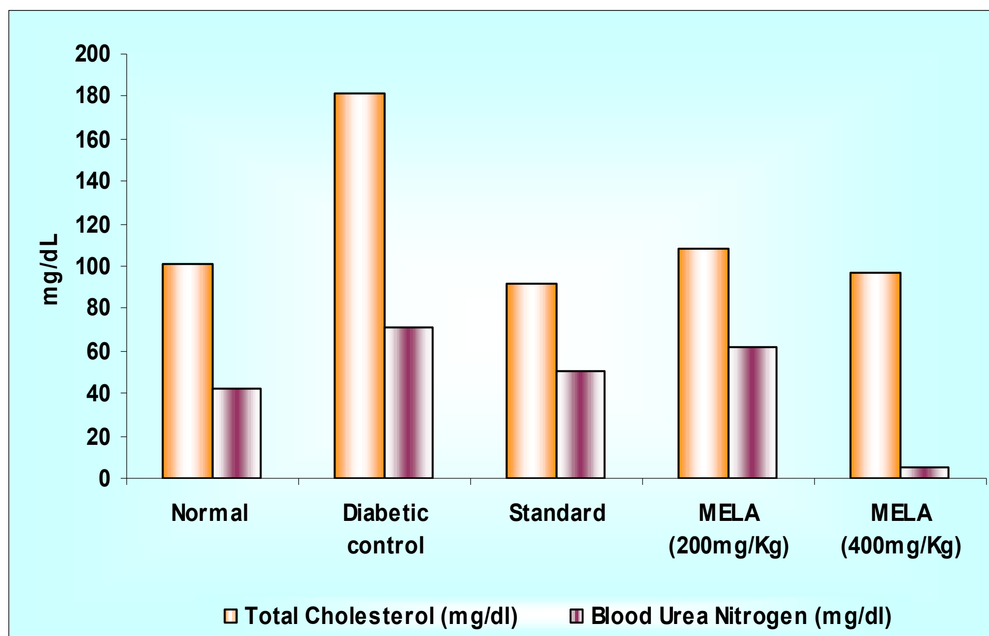


Figure7.2 Effect of MELA on Total cholesterol and BUN in Alloxan induced diabetic rats

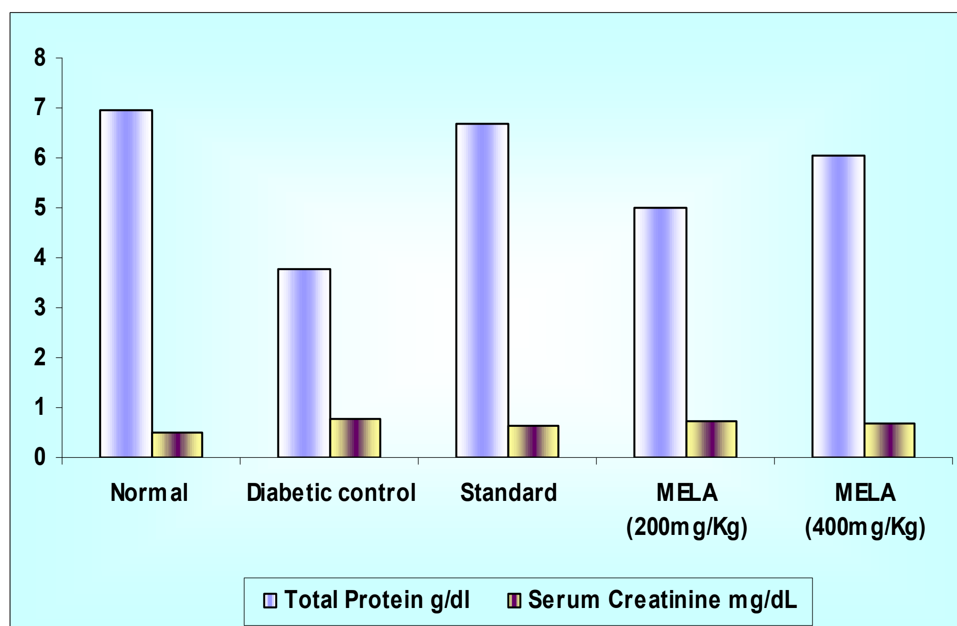


Figure 7.3 Effect of MELA on Total Protein and Serum creatinine in Alloxan induced diabetic rats

Table 7.3 Effect of MELA on Antioxidant enzyme levels in pancreatic homogenate of Alloxan induced diabetic rats

Drug Treatment	**Superoxide dismutase** (U/mg protein)	**Catalase** (k/mg protein)	**Lipid Per oxidation** (µ mole/g protein)
Normal Control	16.54±0.15	6.32±0.15	0.43±0.07
Diabetic Control	08.324±0.18	2.680±0.19	1.891±0.13
Standard Glipizide (5mg/kg)	15.148±0.33**	5.225±0.14**	0.34±0.01**
MELA 200 mg/kg	14.38±0.13**	3.946±0.16	0.295±0.04
MELA 400 mg/kg	13.859±0.21**	4.653±0.14**	0.234±0.01**

Each value is represented as mean± SEM, no.of animals (n) = 6

Diabetic control vs Std ** P<0.01, Diabetic control vs MELA ** P<0.01

One-way ANOVA followed by Dunett's `t'Test.

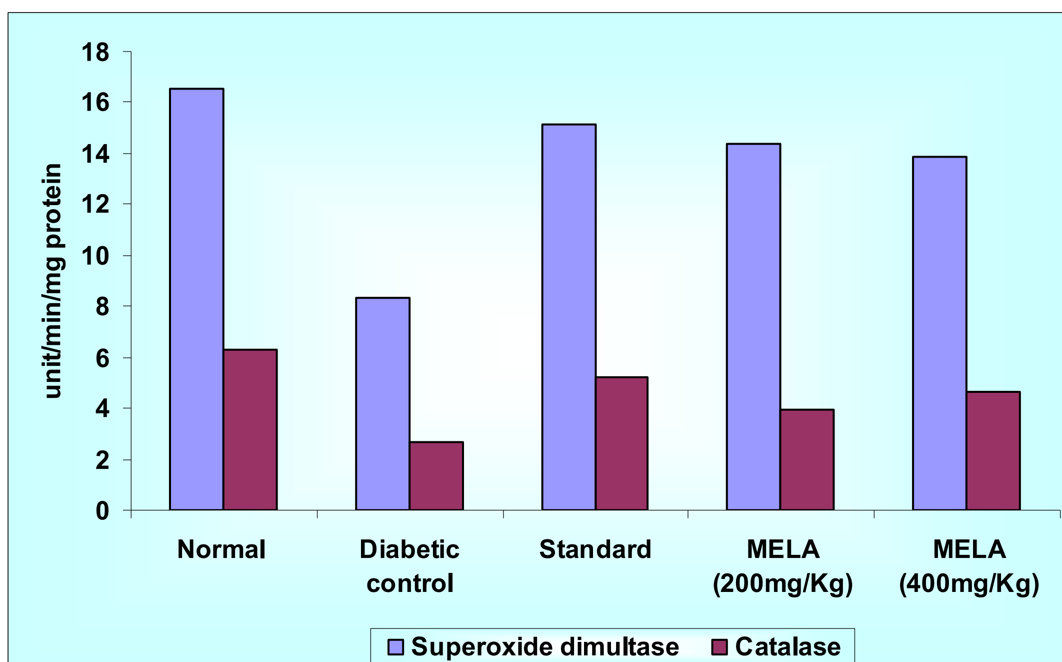


Figure 7.4 Effect of MELA on Antioxidant enzymes in Alloxan induced diabetic rats

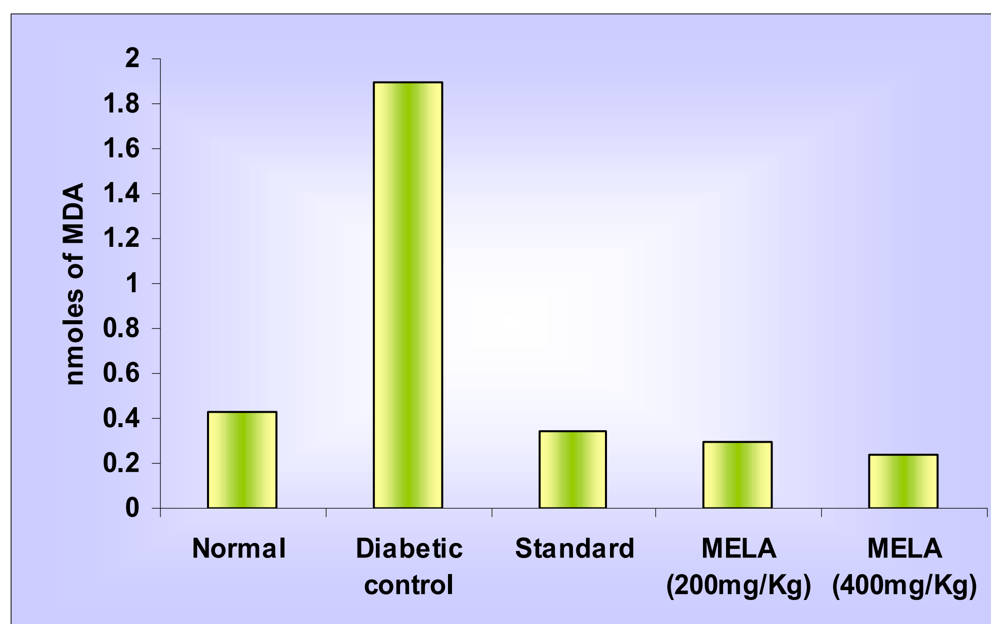
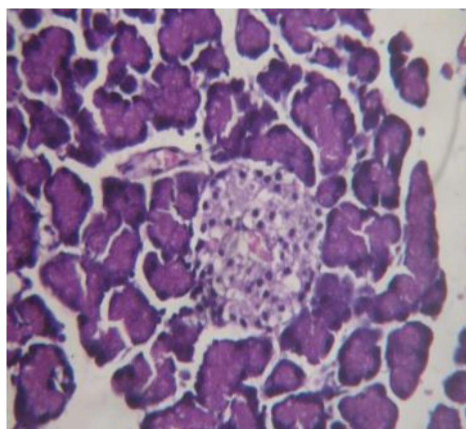
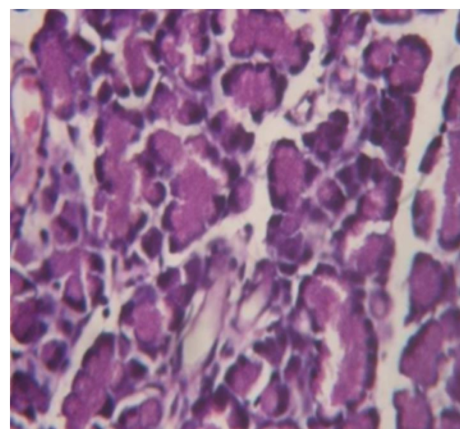


Figure 7.5 Effect of MELA on Lipid per oxidation in Alloxan induced diabetic rats

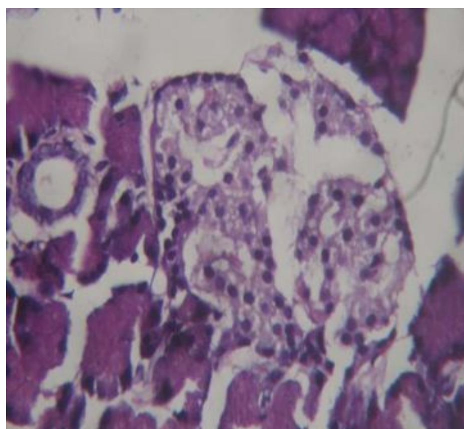
In this context, a marked increase in the concentration of TBARS was observed in liver of diabetic rats. Administration of the extract at both dose levels and Glipizide significantly decreased the levels of TBARS in diabetic rats (Table 7.3, Figure 7.5).



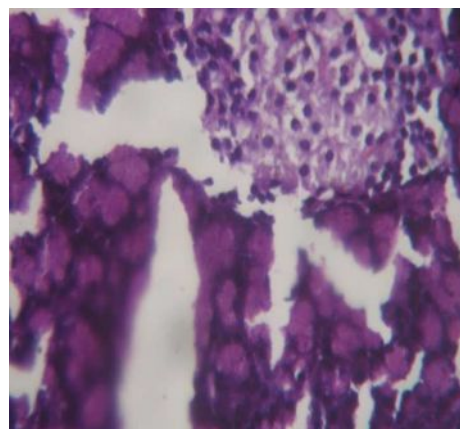
(a) Control rat



(b) Diabetic control



(c) Diabetic rat after Glipizide therapy



(d) Diabetic rats after MELA treatment

Figure 7.6 Photograph of Pancreatic islets with its acinar tissue (Magnification-400x)

Histopathological Studies

Section from the non-diabetic rats showed normal acini and islets while the section from diabetic control rats showed minute and reduced number of islets. Section from MELA extract treated diabetic rats showed good number of regenerating tiny islets, which could be comparable to that of the non-diabetic rats (Figure 7.6 (a) to (d)).

7.6 DISCUSSION

Experimentally induced diabetes in animals has the advantage of allowing the analysis of biochemical, hormonal and morphological parameters that takes place not only during the induction of a diabetic state, but also after it has taken place and during its evolution to a severe insulin deficiency or even death. This strategy has great advantages but it has to be considered that none of the animal models with induced diabetes corresponds exactly to the human type-2 diabetes mellitus. Nonetheless they provide adequate models to investigate the pathogenic mechanism that leads to hyperglycaemia and its consequences [111]. Alloxan became the first diabetogenic chemical agent. Alloxan (pyrimidine-2, 4, 5, 6-tetraone), a frequently used diabetogenic chemical, shows selective toxicity to β-cells of the islets of Langerhans. When Dunn and Letchie accidentally produced islet-cell necrosis in rabbits while researching the nephrotoxicity of uric acid derivatives. Alloxan is a specific toxin that destroys the pancreatic β cells, provoking a state of primary deficiency of insulin without affecting other islet types [112]. Hence, alloxan was selected to induce diabetes in the present study. Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Alloxan is one of the usual substances used for the induction of diabetes mellitus by damaging the pancreatic cells [113].

The diabetogenic action of alloxan is mediated by inhibiting the enzyme glucokinase through oxidation of the two thiol groups at the glucose-binding site of the enzyme. Its cytotoxic effect on β-cells seems to be associated with the cyclic redox process of the alloxan-dial uric acid interconversion, producing ROS, i.e. a superoxide radical anion, hydrogen peroxide, and a hydroxyl radical [114]. Due to this effect, alloxan can be used to generate a pathophysiological status, similar to type I diabetes. In addition, the low expression of antioxidant enzymes by these tissues relative to other tissues leaves β-cells particularly vulnerable to the effects of reactive oxygen species (ROS), a very important factor in the development of diabetes.

In diabetes, there is always a relationship between glucose homeostasis, lipid metabolism later renal function and enzyme activities [115]. We found that 21 day administration of MELA at a dose level of 400 mg/kg shows equal effectiveness in controlling diabetics when compared with diabetic rats treated with standard drug Glipizide than 200mg/kg of MELA thus it proved to have a hypoglycaemic effect on Alloxan –induced diabetic rats, indicated that, there was a regeneration of beta cells of islets of Langerhans. As a result, there was an increase in insulin level, which brought about homeostasis in the above mentioned biochemical parameters such as cholesterol, blood urea nitrogen, creatinine, total protein and in the antioxidant enzyme activities.

Our literature review also indicated the hepatoprotective property of MELA which results in the improvement of liver function .A subsequent increase in the uptake of blood glucose and its utilization may be an another mechanism of action of the extract. Other possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors.

It has been shown that MELA both 200 and 400mg/kg markedly improved the glucose tolerance in alloxan induced diabetes in rats as compared to control ($p<0.01$). Extract showed dose dependent effect, 200 and 400 mg/kg dose shows reduction in glucose level. More over MELA showed significant reduction in blood urea and creatinine in treated rats as compared to diabetic rats ($p<0.01$) but significantly increased total protein level.

In Diabetes mellitus, a variety of protein are subjected to non-enzymatic glycation and is thought to contribute to the long term complications of the disease [116]. The level of total plasma protein was found to be decreased in this study. This could be due to increased lipid per oxidation in the diabetic rats. The decreased plasma protein levels may also be ascribed to

- Decreased amino acid uptake.
- Greatly decreased concentration of variety of essential amino acids,
- Increased conversion of glycogenic amino acid to CO_2 and H_2O and
- Reduction in protein synthesis secondary to a decreased amount and availability of mRNA [117].

In alloxan-induced diabetic rats, increased food consumption and decreased body weight were observed. This indicates polyphagia condition and loss of weight due to excessive breakdown of tissue proteins [118]. Decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight by diabetic rats [119].An increase in the body weight and protein levels in the animals treated with MELA.

Increasing evidence from both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins [120]. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defence mechanisms may lead to the damage of cellular organelles and [121] increased lipid per oxidation and development of insulin resistance. These consequences of oxidative stress may promote the development of complications of diabetes mellitus.

Recent experimental and clinical studies have uncovered new insights into the role of oxidative stress in diabetic complications, suggesting a different and innovative approach to a possible "causal" antioxidant therapy. Possible sources of oxidative stress and damage to proteins in diabetes induced free radicals generated by autoxidation reactions of sugars and sugar adducts to protein and by autoxidation of unsaturated lipids in plasma and membrane proteins. The oxidative stress may be amplified by continuing cycle of metabolic stress, tissue damage and cell death, leading to increased free radical production and compromised free radical inhibitory and scavenger systems [122]. Under conditions of severe oxidative stress, free radical generation leads to protein modification. Proteins may be damaged directly by specific interaction of oxidants or free radicals with particularly susceptible amino acids. They are also modified indirectly, with reactive carbonyl compounds formed by the auto-oxidation of carbohydrates and lipids, with eventual formation of advanced glycation/lipoxidation end products [123].

As mentioned above, diabetes mellitus is a condition in which free radicals are involved both in human beings and in experimental models. It is

well known that alloxan administration causes severe necrosis of pancreatic β-cells. It has been suggested that alloxan induces the production of $H_2O_2$and of some free radicals, such as $O_2$and HO that first damage and later bring about the death of the cells [124].

Lipid per oxidation is a free-radical mediated propagation of oxidative insult to polyunsaturated fatty acids involving several types of free radicals and termination occurs through enzymatic means or by free radical scavenging by antioxidants[125].Lipid per oxidation end products measured as Thiobarbituric acid reactive substances and hydro peroxides were seen increased in plasma of alloxan-diabetic rats in this study. Medicinal plants with antioxidant properties may supply endogenous defence reactive oxygen species. The above fact can be taken as a possible reason for the glucose lowering effect of the extract.

The results from the present study also indicate that MELA can reduce the levels of blood urea nitrogen, serum creatinine, serum cholesterol and increase the serum protein and confirms the possibility that the major function of the extract are on the protection of pancreas, thereby reducing the causation of diabetes in the experimental animals.

Decreased protein content of blood serum in diabetic patients were reported [126] indicating elevated lipid per oxidation and reduced SOD and CAT activity and thereby decreased antioxidant defensive system. MELA significantly reduced the elevated LPO and significantly improved SOD and CAT activity in the treated animals at both the levels i.e., 200 and 400mg/kg.

In histopathological study, the light microscopic photograph islets from control rat appeared circular with the granulated beta cells appearing darker. Small and shrunken islets and destruction of beta cells were observed in the diabetic rats. Well formed islets and increased cell number were

observed in diabetic rats, after MELA therapy. The data presented in electron micrograph of the beta cell of normal and treated rats showed evidence for increased secretary granule synthesis and there by increased insulin secretion after the administration of MELA suggesting possible regeneration /repair of the islets of Langerhans in Alloxan treated rats.

7.7 CONCLUSION

In conclusion, the present study indicated a significant dose dependant anti-diabetic effect for the methanolic extract of *Limonia acidissima* (200 and 400 mg/kg) and supports its traditional usage in the control of diabetes. It is also concluded that the extract have strong antioxidant potential activity by *in vivo* studies. Further studies are under progress in our laboratory for the detailed studies in isolation of the compounds and pharmacological investigations of the constituents, which are responsible for the pharmacological activity reported traditionally.