7.1 INTRODUCTION

Diabetes mellitus is a metabolic illness in which mortality rate is increased worldwide\textsuperscript{1,2}. Recently diabetes is a serious health problem of World people. Diabetes mellitus, a metabolic disorder of several etiologies and it is characterized by chronic hyperglycemia with disturbances of fat, carbohydrate and protein metabolism due to imperfections in insulin action, insulin secretion or both\textsuperscript{3}. In diabetes, chronic hyperglycemia is associated with long term damage, dysfunction which finally leads to organ failure such as kidney, eyes, nerves and cardiovascular systems\textsuperscript{4}.

7.1.1 Etiological classification of diabetes mellitus

Classification of diabetes mellitus etiologically was reported by Kuzurya and Matsuda\textsuperscript{5}. Working on similar basis, the new WHO\textsuperscript{6} and indeed ADA\textsuperscript{7} classification includes both etiological types and clinical stages of diabetes and various other types of glucose intolerance.

Type I diabetes mellitus:

Type I diabetes is due to insulin deficiency due to destruction of β-cell absolutely. It may be

- Immune mediated diabetes and

- Idiopathic diabetes

Type II diabetes mellitus:

It usually varies from insulin resistance with insulin deficiency to predominant insulin resistance with secretory defect.
Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually. The prevalence may range from 1 to 14% of all pregnancies, depending on the population studied and the diagnostic tests employed.

7.1.2 Impaired glucose tolerance (IGT)

Impaired glucose tolerance is considered to be a class in early classification of WHO. Impaired glucose tolerance is a fasting glucose concentrations lower than that is needed for diagnose of diabetes but greater than normal reference value.

7.1.3 Risk factors for diabetes mellitus

- Obesity
- Diabetes occurs by family history
- Ethnicity / race (African American, Asian American)
- Age ≥ 42 years
- Hypertension ( BP≥ 140/90)
- Identification of impaired fasting glucose or impaired glucose tolerance previously
- Baby born over 4.5 kg or H/O Gestational diabetes mellitus
- HDL ≤ 35 mg% and/ or triglyceride ≥ 250 mg%
- Polycystic ovary syndrome
- Stress induces hyperglycemia
Infections, Myocardial infarction, Trauma, Pregnancy, Stroke, Emotional stress, Drugs like glucocorticoids, estrogens, sympathomimetics, nicotinic acid.

7.1.4 TYPE I DIABETES

It results from insulin deficiency due to destructive injury of β-cells of pancreas. A major role in destruction of β-cells of pancreas is autoimmune process. In many patients, auto antibodies to islet of β-cell antigens are noticed about 70% - 90%, especially at early period after onset of diabetes\(^0\) (Fig 7.1)

![Type 1 Diabetes: Insufficient Insulin](image)

Fig 7.1: Type I diabetes

7.1.4.2 Epidemiology and etiology of type I diabetes

Type I diabetes is most serious chronic diseases of childhood that affects approximately 1.4 millions in united states and 10 - 20 millions in World\(^1\). A prime target to prevent type I diabetes is made due to high incidence of severe mortality, morbidity and expenditure for health care\(^2\).
The important common cause in type I diabetes (over 90 cases) is autoimmune destruction of islet of pancreatic β-cells is mediated by T-cell. However it is probable that environmental factors trigger the onset of diabetes in individuals with an inherited predisposition (Fig 7.2).

![Diagram of schematic evolution of type I diabetes]

**Fig 7.2: Schematic evolution of type I diabetes**

The preclinical period is noticed by presence of auto antibodies to pancreatic β-cell antigens such as GAD 65 (Glutamic acid decarboxylase) or ICA512 (called also as IA-2) or insulin and proceeds the onset of hyperglycemia. Several prospective studies have reported that auto antibodies may appear early in childhood and presence of two or more antibodies is highly determine for development of diabetes\(^{13}\).
Environmental factors acts as triggers or regulators of the T-cell mediated autoimmune destruction that results in β-cell injury, insulitis and loss of β-cell mass. As β-cell function decrease, there is a loss in first phase insulin response to intravenous glucose; subsequently, this leads to glucose intolerance (pre-diabetes) and eventually the clinical onset of overt diabetes.

7.1.5 TYPE II DIABETES

Type II diabetes is also called as onset diabetes in adult or non-insulin dependent diabetes mellitus (NIDDM). It is described as increased concentration of blood glucose with relation to relative insulin deficiency and insulin resistance\(^\text{14}\).

![Type II Diabetes](image)

**Fig 7.3:** Mechanism of Type II diabetes

Type II diabetes is increasingly identified in children in similar to increase in obesity rates\(^\text{15}\) that is during childhood there is change in life style and also in dietary patterns\(^\text{16}\) (Fig 7.3). In type II diabetes, though it is not unknown there is a
little chance of developing ketoacidosis as compared to type I diabetes\textsuperscript{17}. Nonketonic hyperglycemia is one of the dangerous effect of type I diabetes.

7.1.5.1 Epidemiology and etiology of type II diabetes

It is most commonly occurring diabetes which constitutes about 85\% of cases in Caucasian populations. Several clinical risk factors is associated with type II diabetes such as

- Obesity
- Age
- Race geographical location
- Family history of diabetes
- Physical inactivity
- Previous gestational diabetes

In United States, prevalence of children with overweight is increased sharply over the last 3 decades to 15\% in 1999-2000 survey\textsuperscript{18}.

7.1.5.2 Risk factor for type II diabetes

About 80\% of type II diabetic subjects are obese and risk of developing diabetes increases gradually as weight (kg)/ height (m)\textsuperscript{2}, body mass (BMI) increases\textsuperscript{19}.

During past 50 years, there is sudden increase of developing type II diabetes and some related disorders such as hypertension, metabolic syndrome and obesity due to rapid life style change. Although this epidemiological change includes better hygiene, improved nutrition, many communicable diseases control and improvement
of quality healthcare opportunity which brings increased long life but also leads to rapid rise of new age diseases like heart diseases, obesity and diabetes.\textsuperscript{20}

Fig 7.4: Risk factors for type II diabetes

The increased risk of developing diabetes is associated with truncal or central obesity a condition in which fat is deposited subcutaneously and at intraabdominal (visceral) sites. In obesity, fat also deposited in other sites (mainly within islet cells, liver and muscle) and may lead to metabolic defects like insulin resistance\textsuperscript{21} (Fig 7.4).

7.1.5.3 Pathogenesis of type II diabetes

Both $\beta$-cell dysfunction and insulin resistance are initial stages of glucose intolerance. Usually, there is a decline in both insulin action and insulin secretion in those who progress from normal glucose to IGT and diabetes. Both environmental and genetic factors contribute to both IR and $\beta$-cell failure (Fig 7.5).
7.1.6 Insulin resistance and obesity

Main feature of insulin resistance syndrome is the elevation of free fattyacids levels\textsuperscript{22}. Expression of UCP2 gene in β-cell is shown to up-regulated by fattyacids which leads to impairment of insulin secretion stimulated by glucose in islet β-cells of pancreas of rats fed with diets rich of high fat\textsuperscript{23}. Fattyacids also observed to increase the uncoupling protein 2 in several tissues. It will give protection against ROS produced when there is increase in rate of coupling of mitochondrial respiration and rate of fattyacid oxidation. Indeed, deficiency of uncoupling protein 2 in mice macrophages generates more ROS than wild-type mice macrophage\textsuperscript{24}. This mechanism leads to protection of β-cell against oxidative damage which leads to death, but it cause insulin secretion impairment (Fig 7.6).
7.1.7 Mechanism of insulin resistance

Obesity is one of main cause of diabetes by causing insulin resistance; the best relationship is being with amount of visceral fat. By lipolysis visceral fat generates large amounts of non-esterified fatty acids (NEFAs). NEFAs inhibit insulin secretion by increasing accumulation of tiglyceride with β-cells and also lead to impairment of glucose uptake, increases gluconeogenesis in liver and muscle utilization. In addition, adipose tissue produces cytokines, such as resistin, IL-6 and tumor necrosis factor α (TNF-α) that interfere with insulin action. There is often increased sympathetic nervous system activity in obesity, which might also
increase lipolysis, reduced muscle blood flow (and thus glucose delivery) and directly affect insulin action\textsuperscript{25} (Fig 7.7).

![Diagram of insulin resistance mechanism](image)

**Fig 7.7: Mechanism of insulin resistance**

6.1.8 Oxidative stress and insulin resistance

Oxidative stress not only occurs in diabetic complication\textsuperscript{26-28}, but also associated to insulin resistance in vivo and in vitro\textsuperscript{29-31}. The increase in glucose and free fatty acid leads to oxidative stress together with stress sensitive signaling pathway activation\textsuperscript{30,32} which then weakens the insulin secretion and insulin action
which leads to overt type II diabetes. The multiple serine kinase cascades activation\textsuperscript{33-35} or protein tyrosine phosphatases inhibition\textsuperscript{36,37} is caused by increase in reactive molecules (Fig 7.8).

Fig 7.8: Role of serine kinase activation in insulin resistance induced by oxidative stress

The increased synthesis of reactive molecules or reduced capacity to eliminate reactive molecules leads to oxidative stress and improper regulation of intracellular signaling usually results to pathological condition including insulin resistance\textsuperscript{30,38}.

The elevation of cytokines, free fattyacid and others by a variety of factors including hyperglycemia increase reactive oxygen species (and/or nitrogen) production that leads to oxidative stress which in turn activates multiple stress
sensitive serine/threonine kinase signalling cascades such as IKKβ and others. The activation of kinase leads to phosphorylation of various targets like IRS proteins (IRS-1 and IRS-2) and insulin receptors. Discretion of threonine and/or serine sites (pS/T) by increased phosphorylation decrease the degree of phosphorylation of tyrosine stimulated insulin\textsuperscript{39,40}. Consequently there is decrease in downstream signaling molecule activities (eg., phosphatidylinositol 3- kinase; PI3K) which results in decreased insulin action (insulin resistance) and glucose transport\textsuperscript{41,42}.

7.1.9 DIABETIC COMPLICATIONS

Diabetic complications is divided into 2 important types such as

Acute complications

Long term complications

7.1.9.1 Acute complications

There are two major types of acute complications and they are diabetic ketoacidosis in type I diabetes and Non- ketogenic hyperosmolar coma in type II diabetes.

7.1.9.1 (a) Diabetic ketoacidosis

Diabetic ketoacidosis results from complete lack of insulin in the body so it starts to burn fatty acids which lead to the production of acetone ketone bodies\textsuperscript{43}. Diabetic ketoacidosis is a serious threatening problem to life in diabetes patients. Because of absence of insulin and related increased glucagon level leads to increased glucose release by liver and by gluconeogenesis.
7.1.9.1 (b) Hyperosmolar non- ketogenic coma (HONKC)

Non-ketotic hyperosmolar coma (non-ketotic hyperglycemia) is a diabetic coma associated with high mortality in type II diabetes which results due to intense dehydration. This occurs as a result of not taking enough fluids or due to loss of fluids from events like burns, pneumonia, a recent operation and stroke or by some drugs like diazoxide, phenytoin, diuretics and glucocorticoid. Non-ketotic coma is precipitate by stroke, myocardial infarction, infection or other acute illness. The serum glucose levels is usually greater 33 mmol/l (600 mg/dl) due to insulin deficiency and results a serum osmolarity of greater than 350 mOsm leads to polyuria (an osmotic diuresis).

7.1.9.2 Chronic complications

The diabetic patient is affected by a series of complications that cause premature mortality and morbidity.

7.1.9.2 (a) Diabetic neuropathy

Diabetic neuropathy is due to the damage of nervous system due to diabetes, especially related to peripheral nerves (motor neurons, pain fibers, autonomic nerves) which result from micro vascular injury which involves vasa nervorum (small blood vessels which supply nerves). It therefore affects all systems and organs since all are related. This is the most frequently encountered chronic complication of diabetes (Fig 7.9).
7.1.9.2 (b) Diabetic nephropathy

It is also called as Kimmelsteil-Wilson syndrome. In kidney glomeruli, angiopathy of capillaries leads to a progressive kidney disease called intercapillary glomerulonephritis which is characterized by diffuse glomerulosclerosis and nephritic syndrome\(^4\) (Fig 7.10).
Fig 7.10: Pathological patterns of diabetic nephropathy

Diabetic nephropathy involves two distinct pathological patterns.47

A) Diffuse glomerulosclerosis: widening of glomerular basement membrane and mesangial thickening.

B) Nodular glomerulosclerosis: large accumulation of periodic acid-Schiff positive material in the periphery of glomerular tufts, the Kimmelstiel-Wilson lesion

7.1.9.2 (c) Diabetic retinopathy

Diabetic retinopathy is a microvascular disease in which micro vessel supplies blood in retina of eye is affected, which finally becomes blindness. It is an ocular appearance of systemic diseases and occurs about 80% in diabetic patients for 10 years or more.48 Retinopathy blocks the flow of oxygen to the retinal cells.
Hyperglycemia induces death of intramural pericyte and basement membrane thickening leads to incompetence of the vascular walls. These damages change the formation of the blood-retinal barrier and also make the retinal blood vessels become more permeable. Tiny blood vessels are weak to poor blood sugar control. The small blood vessels in retina are damaged due to increase amount of glucose and/or fructose accumulation. During early stage, there is no change in the vision called non-proliferative diabetic retinopathy (NPDR).

When disease develops, severe NPDR becomes a proliferative or advanced stage. A new fragile blood vessel grow along with retina due to absence of oxygen in retina and a transparent, gel like vitreous humor which fills the eye inside leading to blood vessels bleeding, cloud vision and destroys retina.

7.1.10 INDUCTION OF DIABETES

7.1.10.1 Alloxan

During research work on experimental diabetes, initially used chemical diabetogen is alloxan. Alloxan is a cyclic urea analogue of chemical composition 2,4,5,6- tetra-oxo-hexa hydropyrimidine and it is freely water soluble and slightly acidic with PKG 6.63. Because of it short half life i.e., less than one minute, best way of administration is by pancreatic artery infusion.

\[
\begin{align*}
\text{NH} & \quad \text{CO} \\
| & \quad | \\
\text{CO} & \quad \text{CO} \\
| & \quad | \\
\text{NH} & \quad \text{CO}
\end{align*}
\]

Fig 7.11: Alloxan (2,4,5,6 tetra-oxo-hexa hydropyrimidine)
7.1.10.2 Mode of action of alloxan

Diabetic model of alloxan induces type I diabetes (insulin dependent diabetes mellitus) without causing insulin resistance\textsuperscript{51} and also cause functional and structural changes in kidney and liver and metabolic alterations due to membrane bound enzyme inhibition\textsuperscript{52}.

Alloxan inhibits insulin secretion induced by glucose from $\beta$-cells of pancreatic islets of langerhans. It is also reported that rapid and selective accumulation of alloxan in $\beta$-cells in comparison to non $\beta$-cells. Various studies showed that alloxan may affects ion channels and membrane potential in $\beta$-cells\textsuperscript{53}. Alloxan also oxidized glutathione by auto-oxidation and it is one of the free radical endogenous sources. Reactive oxygen species mediates cytotoxic action of alloxan.

7.1.10.3 Mechanism of alloxan

Alloxan is toxic glucose analogues which usually accumulate in pancreatic islets $\beta$-cells via GLUT, a transporter of glucose. In presence of intracellular thiols, mainly glutathione, alloxan generates reactive oxygen species (ROS) mainly with glutathione in cyclic redox reaction forms dialuric acid, a reduction product. Auto-oxidation of dialuric acid leads to super oxide radicals, hydrogen peroxide and finally catalyzed to hydroxyl radicals\textsuperscript{54}.

Hydroxy radicals are mainly responsible $\beta$-cell death, which possess low antioxidative defense capacity and leads to a state of insulin dependent alloxan diabetes.
Augmented influx of Ca^{2+} from extracellular fluid
Exaggerated mobilization of Ca^{2+} from intracellular stores
Limited elimination of Ca^{2+} from cytoplasm

Fig 7.12: Mechanism of alloxan induced ROS generation in beta cells of not pancreas. Gka-Gki- glucokinase active respectively.

HA - Alloxan radicals
(Ca^{2+}) I - Intracellular calcium concentration.

In pancreatic β-cell, reduction occurs in presence of several reducing agents such as cysteine, ascorbate, reduced glutathione and protein bound sulfhydryl
groups (-SH). Alloxan reacts with two - SH groups of sugar binding site of glucokinase which results to the formation of disulfide bond and enzyme is inactivated. The reduction of alloxan leads to formation of dialuric acid which then re-oxidized with alloxan forms reactive oxygen species (ROS) and superoxide radicals. These radicals release ferric ions from ferritin which then reduced to ferrous and ferric ions. Superoxide radicals also undergo dismutation by superoxide dismutase to form hydrogen peroxide. Finally, this hydrogen peroxide in presence of ferrous ion leads to the formation of highly reactive hydroxyl radical based on Fenton reaction. Alloxan also increases concentration of cytosolic free Ca^{2+} in pancreatic β-cell. These Ca^{2+} ion causes insulin release along with ROS which will damage pancreatic islets β-cell (Fig 7.12).

7.2 MATERIALS AND METHODS

7.2.1 Plant material

The Decalepis hamiltonii root used for the investigation was purchased from a plant supplier in Chennai, Tamil Nadu, India. Plant roots were authenticated taxonomically at Plant Anatomy and Research Center, Chennai, Tamil Nadu, India.

7.2.2 Preparation of extract

Decalepis hamiltonii root was dried in shade, crushed to coarse powder. Using soxhlet apparatus the root powder was extracted with petroleum ether (60 - 80°C) to remove fat and then with 90% methanol. Under reduced pressure the solvent was evaporated and obtained filtrate was used for further studies.
7.2.3 Animals

In the present study, healthy Wistar male albino rats weighing about 150 to 200 gms was used. The rats were kept in a polypropylene cage at controlled conditions of temperature (25 ± 2°C) with 12 hour light-dark cycles. Before the study, all animals were maintained for 7 days. The animals were fed with water ad libitum and standard pellet obtained from Sai-durga feeds and foods, Bangalore, India. The approval of the present study was obtained from Institutional Animal Ethical Committee of JSS College of Pharmacy, Proposal number IAEC/P.Cog/06/2010-2011.

7.2.4 Oral glucose tolerance test (OGTT)

Oral GTT was conducted in normal rat fasted overnight (18 h). Rats were divided into 4 groups and each group consists of six rats.

Group I : Normal control received orally 0.3% Carboxy methyl cellulose.

Group II : Received orally 7 mg/kg bwt glibenclamide, a reference drug.

Group III : Received orally 200 mg/kg bwt of methanolic extract of Decalepis hamiltonii dissolved in 0.3% carboxy methyl cellulose

Group IV : Received orally 400 mg/kg bwt of methanolic extract of Decalepis hamiltonii dissolved in 0.3% carboxy methyl cellulose.

After treatment for 30 minutes, all groups were orally loaded with 2 g/kg bwt of glucose. Blood samples were collected just prior to glucose administration and after glucose loading at 30, 60, 120 and 150 minutes. By using commercial kit, blood glucose levels were measured.
7.2.5 Hypoglycemic activity of normal rats

Healthy Wistar male albino rats weighting about 150 to 200 gms undergoes overnight fasting and rats were divided into 4 groups and each group consist of six rats.

Group I : Normal control received orally 0.3% carboxy methyl cellulose.

Group II : Normal rats received orally reference drug glibenclamide (7 mg/kg bwt).

Group III : Normal rats received orally methanolic extract of Decalepis hamiltonii (200 mg/kg bwt) dissolved in 0.3% carboxy methyl cellulose.

Group IV : Normal rats received orally methanolic extract of Decalepis hamiltonii (400 mg/kg bwt) dissolved in 0.3% carboxy methyl cellulose.

Blood samples were collected before treatment and after treatment at 1, 2 and 4 hour and by using commercial kit, glucose levels were determined.

7.2.6 Hypoglycemic activity of diabetic rats

Induction of diabetes

To induce diabetes in male Wistar rats, 150 mg/kg of alloxan monohydrate dissolved in normal saline were administered intraperitoneally to an overnight fasted rats. After 1 hour, all the animals were fed with water ad libitium and standard pellet. After 72 h, blood glucose levels were estimated and rats with greater than 180 mg/dl blood glucose level were utilized for study.

Healthy male Wistar albino rat’s weighing about 150 to 200 g was fasted overnight and rats were divided into five groups and each group consists of six rats.
Group I : Normal control received 0.3% carboxy methyl cellulose orally.

Group II : Diabetic rats received alloxan monohydrate orally (150 mg/kg bwt).

Group III : Diabetic rats received orally glibenclamide (7 mg/kg bwt).

Group IV : Diabetic rats received orally methanolic extract of Decalepis hamiltonii (200 mg/kg bwt) dissolved in 0.3% carboxy methyl cellulose.

Group V : Diabetic rats received orally methanolic extract of Decalepis hamiltonii (400 mg/kg bwt) dissolved in 0.3% carboxy methyl cellulose.

Blood samples were collected before treatment and after treatment at 1, 2 and 4 days and by using commercial kit, the glucose levels were determined.

7.2.7 Biochemical analysis

At the end of experiment, animals undergo overnight fasting and then by cervical decapitation, rats were sacrificed and the blood samples was collected and then allowed to clot. Serum was separated by centrifugation for 10 minutes at 2500 rpm. Serum glucose, alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol, triglycerides were determined.

Estimation of serum glucose

The estimation of serum glucose was done by Oxidase method⁶¹ [Appendix 16].
Assay of serum marker enzymes

Aspartate transaminase was assayed by Reitman and Frankel method [Appendix 6].

Alanine transaminase was assayed by Reitman and Frankel method [Appendix 7].

Estimation of lipids

Total cholesterol was estimated by Zak’s method [Appendix 17].

Triglycerides were determined by Foster and Dunn [Appendix 18].

Estimation of Glycogen

Liver was removed out and washed in a normal saline and stored at -80°C to assay glycogen content by using Anthrone reagent [Appendix 19].

7.2.8 Histopathological studies

After sacrificing animals, pancreas from each rat was removed and placed in 10% formalin and immediately followed processing by paraffin technique. Paraffin sections of 5μm thickness were cut and stained with hematoxylin and eosin (H&E) for histopathological examination. The β-cell granulation of pancreatic islets of langerhans was viewed under microscope.

7.2.9 Statistical analysis

Statistical analysis was conducted by using one way analysis of variance (ANOVA) followed by Duncan’s multiple range test. The values are mean ± SD of six rats in each group. Statistical significance was considered at p< 0.05.
7.3 RESULTS

Serum glucose, total cholesterol, triglycerides, aspartate transaminase, alanine transaminase were significantly elevated while liver glycogen significantly decreased in diabetic rats as compared to normal group.

7.3.1 Effect of MEDH on oral glucose tolerance of normal rats

Blood glucose levels of control, glibenclamide (7 mg/kg) and methanolic extract of Decalepis hamiltonii (200 mg and 400 mg/kg) after glucose administration orally (2 g/kg) at different time points (0, 30, 60,120, 150 minutes) was showed in Table 7.1 and Fig 7.13. The blood glucose level was increased to peak at 30 minutes in all groups. In glibenclamide and 400 mg of MEDH treated group, there was a reduced level of blood glucose at 150mins as compared to control group.

Table 7.1: Effect of MEDH on oral glucose tolerance of normal fasted rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dl)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fasting</td>
<td>Post treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>60 min</td>
<td>90 min</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td>72.58±0.65</td>
<td>76.66±0.92</td>
<td>74.38±1.15</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>76.46±1.10</td>
<td>198.93±1.29</td>
<td>152.71±0.68</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>77.23±0.84</td>
<td>199.67±1.01</td>
<td>159.82±1.48</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td>70.69±0.98</td>
<td>193.13±0.99</td>
<td>151.97±1.07</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group I - 0.3% CMC; Group II - Glibenclamide + glucose; Group III - MEDH (200 mg/kg) + glucose ; Group IV - MEDH (400 mg/kg) + glucose.
7.3.2 Effect of MEDH on blood glucose level of normal fasted rats

Blood glucose levels of euglycemic rats at 0, 1, 2 and 4 hour of administration were showed in Table 7.2 and Fig 7.14.

Table 7.2: Effect of MEDH on blood glucose level of normal rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dl)</th>
<th>Fasting</th>
<th>Time (h) after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Group I</td>
<td>72.1±0.58</td>
<td>70.16±0.89</td>
<td>68.96±0.68</td>
</tr>
<tr>
<td>Group II</td>
<td>76.33±0.77</td>
<td>66.4±0.90</td>
<td>58.34±0.70</td>
</tr>
<tr>
<td>Group III</td>
<td>70.17±1.24</td>
<td>68.34±0.72</td>
<td>62.23±0.92</td>
</tr>
<tr>
<td>Group IV</td>
<td>75.09±1.27</td>
<td>65.5±0.80</td>
<td>57.66±0.92</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group I - 0.3% CMC; Group II - Glibenclamide + glucose; Group III - MEDH (200 mg/kg); Group IV - MEDH (400 mg/kg).
Administration of glibenclamide (7 mg/kg) and methanolic extract of Decalepis hamiltonii (200 mg and 400 mg/kg) on euglycemic rats was not significant at 1 hour, but it was significant at 4th hour than normal control rat.

7.3.3 Effect of MEDH on blood glucose level of alloxan induced diabetic rats

Blood glucose levels of normal and diabetic rats at 0, 1, 2, 4 hour of administration were showed in Table 7.3 and Fig 7.15. Blood glucose level was increased significantly in diabetic group than normal control rats. Administration of glibenclamide (7 mg/kg) and methanolic extract of Decalepis hamiltonii (200 mg and 400 mg/kg) reduced blood glucose level in diabetic rats than normal control rats. Treatment with glibenclamide and 400 mg of MEDH at 4th day resulted in significant hypoglycemic effect of diabetic group.
Table 7.3: Effect of MEDH on blood glucose levels of alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dl)</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>1</td>
</tr>
<tr>
<td>Group I</td>
<td>72.48 ± 1.18</td>
<td>73.93 ± 2.52</td>
</tr>
<tr>
<td>Group II</td>
<td>204.8 ± 2.49</td>
<td>204.05 ± 1.74</td>
</tr>
<tr>
<td>Group III</td>
<td>201.9 ± 0.97</td>
<td>186.47 ± 0.89</td>
</tr>
<tr>
<td>Group IV</td>
<td>203.4 ± 1.19</td>
<td>187.39 ± 1.31</td>
</tr>
<tr>
<td>Group V</td>
<td>202.78± 0.98</td>
<td>181.19 ± 1.87</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group I - 0.3% CMC; Group II – Diabetic control; Group III - Glibenclamide ; Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).
7.3.4 Effect of MEDH on serum AST, ALT and liver glycogen of alloxan induced diabetic rats

Serum activities of AST, ALT and liver glycogen level of normal and diabetic rats induced by alloxan were showed in Table 7.4 and Fig 7.16 & 7.17.

Table 7.4: Effect of MEDH on serum AST, ALT and liver glycogen of alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>Glycogen (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>49.05 ± 2.4a</td>
<td>55.56 ± 3.29a</td>
<td>3.5 ± 0.16a</td>
</tr>
<tr>
<td>Group II</td>
<td>190.74 ± 5.48b</td>
<td>189.16 ± 2.44b</td>
<td>0.85 ± 0.034b</td>
</tr>
<tr>
<td>Group III</td>
<td>79.06 ± 2.59c</td>
<td>87.8 ± 2.87c</td>
<td>3.06 ± 0.18a</td>
</tr>
<tr>
<td>Group IV</td>
<td>119.78 ± 2.83d</td>
<td>120.6 ± 2.86d</td>
<td>1.8 ± 0.13c</td>
</tr>
<tr>
<td>Group V</td>
<td>81.9 ± 2.63c</td>
<td>90.07 ± 1.90c</td>
<td>2.7 ± 0.24a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P ≤ 0.05 by DMRT. (Group I - 0.3% CMC; Group II – Diabetic control; Group III - Glibenclamide; Group IV - MEDH (200mg/kg); Group V - MEDH (400mg/kg).
The serum AST and ALT activity was significantly increased and liver glycogen content was decreased in diabetic control rats than normal control rats. Administration of glibenclamide (7 mg/kg) and methanolic extract of Decalepis hamiltonii (200 mg and 400 mg/kg) decreased the activities of AST, ALT significantly and increased glycogen content in diabetic rats than normal control rats.

![Fig 7.17: Effect of MEDH on serum liver glycogen of alloxan induced diabetic rats](image)

7.3.5 Effect of MEDH on serum cholesterol and triglycerides of alloxan induced diabetic rats

In diabetic rats, cholesterol and triglycerides levels were significantly increased than normal control rats. Cholesterol and triglycerides levels were decreased to nearly normal after administration of glibenclamide (7 mg/kg) and methanolic extract of Decalepis hamiltonii at dose of 200 mg and 400 mg/kg/bwt (Table 7.5 and Fig 7.18).
Table 7.5: Effect of MEDH on serum cholesterol and triglycerides of alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>129.16±6.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>149.84±3.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>260.83±6.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>235.5±5.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>132.16±5.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>163.8±4.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>160.79±7.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>187.03±6.14&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>144.5±4.54&lt;sup&gt;d&lt;/sup&gt;</td>
<td>168.7±2532&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group I - 0.3% CMC; Group II – Diabetic control; Group III - Glibenclamide; Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).

7.3.6 HISTOPATHOLOGICAL RESULTS

Histopathology of pancreas of normal rats showed normal acini and normal pancreatic beta cells [Plate 7.1 (a)]. But in alloxan induced diabetic rat, there was severe damage of beta cell of islets of pancreas [Plate 7.1 (b)].
Plate 7.1: Histopathology of pancreas

(a) Group I  (b) Group II

(c) Group III  (d) Group IV

(e) Group V

Group I: Normal control; Group II: Diabetic rats; Group III: Glibenclamide; Group IV: 200mg MEDH and Group V: 400mg MEDH
Treatment with control drug glibenclamide and methanolic extract of Decalepis hamiltonii root at dose of 400 mg/kg bwt showed restoration of gathering of pancreatic beta cells to nearly normal [Plate 7.1 (c) and (e)]. Whereas treatment with 200 mg methanolic extract of Decalepis hamiltonii root showed partial restoration of beta cells [Plate 7.1 (d)].

7.4 DISCUSSION

The present study was done to assess the antidiabetic activity of methanolic extract of Decalepis hamiltonii root against alloxan induced diabetic rats.

In India diabetic patients were increasing day by day which may be due to life style change, food pattern change i.e. change from traditionally diet rich of fiber to diet rich of sugary fast food and also due to genetic basis. Since the nature of diabetes was chronic, it requires treatment for long term in order to prevent the complications arising because of persistent high blood glucose level. In modern healthcare system, pharmacotherapy was available treat diabetes which included insulin and 16 oral hypoglycemic drugs.\(^6\)

In developing countries like India, it was impossible for most of diabetic patients to use these synthetic drugs on regular basis because of economic control. These synthetic drugs were also associated with several adverse effects. For treatment of diabetes mellitus there was increase in use of traditional indigenous plants that were widely available in India and there are more than 150 plant extract and some of their secondary metabolites in plant including tannins, flavonoids, alkaloids etc were used to treat diabetes.\(^6\)
Alloxan was used as a diabetogen in the present study. Alloxan induces diabetes by destroying pancreatic partially by producing relative oxygen species. Present study was conducted to evaluate antidiabetic effect of methanolic extract of Decalepis hamiltonii root of normal and diabetic rats induced by alloxan. Alloxan, beta cytotoxin, induced diabetes by destroying insulin secreting pancreatic β-cells which resulted to decrease in endogenous release of insulin that created the situation for decreased utilization of glucose by tissues\textsuperscript{69}.

In current study, the level of serum glucose of alloxan induced diabetic rats was decreased significantly by administration of methanolic extract of Decalepis hamiltonii root as compared to diabetic control rats. Antidiabetic activity of methanolic extract of Decalepis hamiltonii root was due to promotion of secretion of insulin by closing K\textsuperscript{+} - ATP channels, depolarization of membrane and stimulating calcium influx, an initial important step for secretion of insulin. Various other plants were also reported to possess insulin stimulatory and antidiabetic effects\textsuperscript{70}. The bioactive antidiabetic principles in plants are alkaloids, flavonoids, triterpenoids, phenolics and sterols\textsuperscript{71}. Flavonoids were believed to recover the damaged β-cell in diabetic rats induced by alloxan\textsuperscript{72}. The effective antihyperglycemic agents in plants were phenolics.

Some plants possessed properties similar to some well known sulfonylurea drugs like glibenclamide, in normoglycemic animals they reduced blood glucose. Glibenclamide enhanced the pancreatic β-cell activity which in turn resulted in secretion of insulin in excessive amounts, which caused reduction in level of blood glucose. Like plant extract, glibenclamide also significantly decreased the blood glucose of diabetic rats. The present findings of destruction of pancreatic islet β-cell by alloxan coincides

178
with earlier suggestion of Jackson and Bressler\textsuperscript{73} in which sulfonylurea possessed extra pancreatic antihyperglycemic mechanism of action next to capacity of insulin secretion and the uptake of glucose into, and by tissues utilization.

The metabolic complication of both experimental and clinical diabetes is hyperlipidemia. Previous studies revealed that hyperlipidemia and hyperglycemia were the common characteristics of diabetes mellitus induced by alloxan\textsuperscript{74}. In the present study, total cholesterol and triglycerides were significantly decreased in rats by methanolic extract of Decalepis hamiltonii as compared to diabetic controls. The reduction in cholesterol level may be due to inhibitory effect of methanolic extract of Decalepis hamiltonii on rate- regulatory enzyme (HMG CoA reductase) of cholesterol biosynthesis\textsuperscript{75} or by stimulating effect of glucose utilization by peripheral tissues\textsuperscript{76}. A relative molecular ordering of residual phospholipids was due to increased cholesterol concentration which resulted in decrease of membrane fluidity\textsuperscript{77}.

Accumulation of triglycerides was important risk factor of coronary heart disease (CHD). The triglyceride level was increased significantly in diabetic rats that may be due to absence of insulin. In normal condition, lipoprotein lipase was activated by insulin which leads to triglyceride hydrolysis\textsuperscript{78}. But in diabetic condition due to lack of insulin lipoprotein lipase was not activated resulting in hypertriglyceridemia. In diabetic rats, methanolic extract of Decalepis hamiltonii root decreased the triglyceride level in tissues which leads to progression of coronary heart disease.
The abnormally increased level of serum lipids concentrate the ion in diabetes mellitus results to increased mobilization of free fatty acids from fat deposits (adipose tissue) due to less utilization of glucose. The action of methanolic extract of Decalepis hamiltonii may increase the activity of enzymes of bile acid synthesis and its excretion and this leads to decreased in serum cholesterol and triglycerides. The lipid lowering effect of extract may be due to action of flavonoids and other phenolic compounds, di and triterpenoids, steroids and glycosides. Normalized rate of lipogenesis was due to the insulin-like activity of triterpenoids or activating normoglycemia by the insulinotropie effect of flavanoids or the lipid lowering property of phenolic compounds.

The enzymes involved in the conversion of aminoacids to ketoacids are aspartate transaminase and alanine transaminase. The elevated activities of transaminases lead to inflammatory hepatocellular disorders. Hepatic dysfunction may be induced by increased activities plasma AST and ALT in diabetes.

Larcan et al found that in diabetic patients, liver was necrotized which support our findings. In type 2 diabetes, chronic mild elevation of transaminases was frequently found. Therefore, plasma alanine transaminase and aspartate transaminase activity was increased which resulted from enzymes leakage into blood stream from cytosol of liver. On other side, treatment of methanolic extract of Decalepis hamiltonii to diabetic rats leads to decreased in activity of plasma ALT and AST than diabetic control rats.

In diabetes, glucose synthesis was impaired in skeletal muscles and liver of rat because of markedly decreased level of glycogen content in skeletal muscle.
and liver in diabetes. Insulin was stimulator of glycogen synthase system. On other side, insulin inhibited the glycogenolysis and due to absence of insulin, glycogenolysis was not inhibited by insulin so decreased the glycogen content of liver. Since alloxan induced the pancreatic β-cell destruction leads to marked reduction of insulin levels, so it was rational that glycogen level decreased in tissues because for glucose influx they depend on insulin. Treatment with methanolic extract of Decalepis hamiltonii prevented the depletion of glycogen content in liver of diabetic rats which was mainly due to insulin release from β-cell.

The above findings confirmed the hypoglycemic activity of root of methanolic extract of Decalepis hamiltonii.
7.5 REFERENCE


5 Kuzuya T, Matsuda A. A classification of diabetes on the basis of etiologies vs. degree of insulin deficiency. Diab Care 1997; 20: 1185-97.


48 Kertes PJ, Johnson TM. Evidence Based Eye Care Philadelphia, PA: Lippincott Williams and Wilkins.


189


