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A Comparative Effects of Some Selected Medicinal Plants on Blood Sugar Level, Lipid Profile and Oral Glucose Tolerance Test in Normal and Alloxan-Induced Diabetic Rats

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A COMPARATIVE EFFECTS OF SOME SELECTED MEDICINAL PLANTS ON BLOOD SUGAR LEVEL, LIPID PROFILE AND ORAL GLUCOSE TOLERANCE TEST IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

A Thesis Submitted for the Degree of Doctor of Philosophy

By

Naznin Ara Khatune

June, 2016

Department of Pharmacy
Faculty of Science
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Declaration

I do hereby declare that the materials embodied in this thesis entitled "**A Comparative Effects of Some Selected Medicinal Plants on Blood Sugar Level, Lipid Profile and Oral Glucose Tolerance Test in Normal and Alloxan-Induced Diabetic Rats**" prepared for submission to the Department Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi, Bangladesh for the Degree of Doctor of Philosophy is original research work of mine and have not been previously submitted for any award of Degree or Diploma.

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Certificate

This is to certify that the materials included in this thesis entitled "**A Comparative Effects of Some Selected Medicinal Plants on Blood Sugar Level, Lipid Profile and Oral Glucose Tolerance Test in Normal and Alloxan-Induced Diabetic Rats**" are original research work conducted by **Naznin Ara Khatune**, Reg. No. 1231, session 2008-2009, Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi, Bangladesh. The thesis contains no material previously published or written by another person except when due reference is made in the text of the thesis.

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Dedicated To
My Parents And Family

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-:Abbreviations:-

%	:	Percentage
°C	:	Degree Celsius
ALP	:	Alkaline Phosphatase
BHT	:	Butylated Hydroxy Toluene
BW	:	Body Weight
CK-MB	:	Creatinin Kinase Myocardium Type B
DM	:	Diabetes Mellitus
DPPH	:	1, 1-Diphenyl-2-Picrylhydrazyl
FCR	:	Folin–Ciocalteu Reagent
FPG	:	Fasting Plasma Glucose
g	:	Gram
HDL	:	High Density Lipoproteins
hr	:	Hour
IAMEBBC	:	Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee
ICDDRB	:	International Centre for Diarrhoeal Disease Research, Bangladesh
IDDM	:	Insulin Dependent Diabetes Mellitus
IGT	:	Impaired Glucose Tolerance
kg	:	Kilogram
LDL	:	Low Density Lipoproteins
mg	:	Milligram
min	:	Minute
ml	:	Milliliter
MRDM	:	Malnutrition-Related Diabetes Mellitus
NIDDM	:	Non-Insulin Dependent Diabetes Mellitus
OGTT	:	Oral Glucose Tolerance Test
OECD	:	Organization for Economic Co-operation and Development
OW/BW	:	Organ Weight to Body Weight Ratio
SGOT	:	Serum Glutamate Oxalate Transaminase
SGPT	:	Serum Glutamate Pyruvate Transaminase
TC	:	Total Cholesterol
TCA	:	Tri chloro Acetic Acid
TG	:	Triglycerides
VLDL	:	Very Low Density Lipoproteins

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ABSTRACT

Diabetes mellitus (DM) is a common and serious metabolic disorder throughout the world. Despite the availabilities of hypoglycemic agents from natural and synthetic sources, diabetes and its complications continued to be a major health care problem. The management of diabetes without any side effects is still a challenge; therefore plants continue to play an important role in the discovery of new compounds for the treatment of this disease. The study was undertaken to evaluate the comparative effects of some selected medicinal plants on blood sugar level, lipid profile and oral glucose tolerance test in alloxan-induced diabetic rats.

The dried coarse powders from plants were exhaustively extracted with ethanol by cold extraction. An effort was made to investigate the acute toxicity (LD_{50}) of the extracts. Diabetes was induced in Norwegian Long Evans rats by a single intra-peritoneal administration of alloxan monohydrate (110mg/kg). Preliminary hypoglycemic properties were investigated at 30, 60 and 90 mins after the oral administration of extracts in fasting rats. The qualitative presence of phytoconstituents in the extracts was also determined by standard methods. On the basis of preliminary screening the plant *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and *Urginea indica* (UIE) extracts with demonstrable hypoglycemic activity were further evaluated for their safety and efficacy in alloxan-induced diabetic rats. After induction of diabetes the rats were divided into several groups for oral administration of GAE (200, 400mg/kg), ANE (50, 100, 200 mg/kg) and UIE (12, 25 mg/kg) once daily for a period of 15 days. Metformin (150mg/kg) was used as standard drug. The survival rate, time course of changes in blood sugar levels (FBS) and body weights were measured after the treatment. We have also estimated serum triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL), serum glutamic-oxaloacetic transaminase (SGOT) and creatine kinase-myocardium type (CK-MB). To compare the effects of GAE200, ANE100 and UIE25 in diabetic rats, we have examined OGTT, lipid profiles, liver glycogen and histopathology. A comparative i*n-vitro* antioxidant activity including flavonoid content, phenolic content, total antioxidant activity, reducing power capacity and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity were also carried out. Finally, the bioactive principles from UIE were isolated and identified through bio-assay guided fractionations using chromatographic techniques and spectral analysis respectively.

Our results demonstrated that ethanol extracts from plants were non-toxic in rats. All the plants possessed hypoglycemic activities of which GAE (200mg/kg), ANE (100mg/kg) and UIE (25mg/kg) showed remarkable efficacy. Preliminary phytochemical analysis of GAE, ANE and UIE revealed the presence of triterpenoid, flavonoid, steroid, glycoside, saponin and tannin. The 15 days survival rate among the treatment groups was 100% ($p<0.001$ *vs*

DC). Oral administration of GAE, ANE and UIE significantly lowered the fasting blood sugar levels and the effects were dose-dependent. Oral ingestion of GAE, ANE and UIE significantly improved body weight and organ weight and reduced the levels SGOT and CK-MB. The data revealed that the level HDL increased and the level of TC and TG were significantly decreased with GAE, ANE and UIE at all dose levels. Also the increment of LDL, VLDL and LDL/HDL ratio were significantly attenuated after the treatment. Diabetic rats treated with GAE, ANE and UIE, showed significant improvement in oral glucose tolerance and restored the liver glycogen content. All the extracts have favorable effects on the preservation pancreatic morphology as evidenced by increase number of viable β-cells of the pancreas. Among the treatment groups UIE25 showed most significant reduction in blood sugar level, lipid profile, and restoration of liver glycogen and normalization of pancreatic β-cells architecture.

The free radical scavenging activity of extracts GAE, ANE and UIE showed considerable total antioxidant activity with IC_{50} of 76.45±0.21µg/ml, 43.25±0.15µg/ml and 27.50±0.021 µg/ml, respectively in DPPH scavenging assay. Among the three plant extracts the UIE showed the highest iron reducing capacity (absorbance 1.31 at 200µg/ml vs ascorbic acid standard 1.55 at 200 µg/ml), total phenol content (87.74mg of GAE/g dried extract), total flavonoid (67.72mg of quercetin/g dried extract) and total antioxidant (307.25 mg of ascorbic acid/g dried extract).

Two compounds UC-1 and UC-3 were isolated from chloroform fractions of UIE and were identified as 5,7-dihydroxy-2-methyl-4H-chromen-4-one and 5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one, respectively. These compounds were the first report of isolation from the plant *Urginea indica*. Further, rats treated with UC-1 and UC-3 showed a significant improvement in glucose tolerance, attenuated blood sugar levels and lipid profiles; and restored liver glycogen content. Oral ingestion of UC-1 and UC-3 significantly reduced the levels of SGOT and CK-MB as well as improvement in pancreatic β-cell architectures.

We concluded that the plant bark of *Grewia asiatica (Phalsa),* rhizome of *Alpinia nigra (Jangli Ada)* and bulb of *Urginea indica (Bon Pianj)* as well as isolated compounds UC-1 and UC-3 exhibited significant effects on the fasting blood glucose level, improvement in liver glycogen content, serum SGOT, CK-MB levels as well as organ protection. The beneficial effects of extracts as well as isolated compounds can be partially explained by the preservation of pancreatic β-cell structure through the suppression of oxidative stress. However, the exact mechanism by which UC-1 and UC-3 exerted its beneficial effects in alloxan-induced diabetic rats remained to be elucidated.

Chapter One

Introduction

1.1. Introduction

1.1.1 Plant as a Source of Drugs

Plants have been primary source of medicines in the traditional healthcare systems worldwide and even currently in developed and most of the developing countries. The approach to characterization and isolation of active ingredients from plants started in the late 19th century. Consequently, chemical constituents isolated are used as important drugs as such or as their derivative(s) today. During 1983 to 1994, 39% of the New Approved Drugs (NAD) was of natural molecules, products derived semi-synthetically from natural source and/or synthetic products [1]. Parallel to synthetic drugs, the use of natural products is growing exponentially around the world, because of their compatibility with the human body, cheapness and minimum side effects. During the last decade there has been a major increase in the use of medicinal plants all over the world particularly in U.S.A. and European countries. The global demand for botanical and plant derived drugs is expected to increase from US $19.5 billion in 2008 to US $32.5 billion in 2013, with an annual growth rate of 11.0% [2].

The number of higher plant species on this planet is estimated as 250,000 [3]. Of these, about 6% have been screened for biological activity and 15% are reported to be phytochemically characterized [4]. In Asia especially the southern region shares about 20% of the all known vascular plants in the globe including 7000-8000 species of medicinal plants [5]. According to the World Health Organization (WHO), about 65-80% of the world's population relies on traditional medicine for their primary health care [6]. The investigations on the therapeutic application of the plant have lead to the discovery of several clinically applicable drugs e.g. digoxin, morphine, reserpine, taxol, vincristine etc. In addition elucidation of the structure of active principles paved the way for synthesis and derivation for compounds with higher efficacy and lower adverse effect such as metfomine, oxycodon, teniposide, amiodarone etc. [7]. Accumulating evidences clearly showed that plants are rich source of bioactive chemical entities and thus, plants continue to engage the attention of scientists associated with drug discovery.

The beneficial effects of plants typically results from the secondary metabolites like alkaloids, tannins and phenolic compounds [8]. These secondary metabolites exert

their effects resembling to the endogenous substances like ligands, hormones, signal transduction molecules or even neurotransmitters. Although herbs have been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpasses their importance, for a while. However, the blind dependence on synthetic drug is over and people are returning to the naturals in terms of safety and security of health. However, scientific evidences need to be created for their efficacy through extensive pharmacological and chemical studies.

India has a rich heritage of usage of medicinal plants in the Ayurvedic, Siddha and Unani system. Many Indian plants have been investigated for their beneficial effects in different animal models and patients. Today, traditional medical practice is still the mainstay of health care delivery system especially in the rural areas of Bangladesh where conventional medical facilities are not within the reach of most people. About 85% of the population relies on it for their medical care [9]. Indigenous peoples have a long history and expertise in the use of medicinal plants and their knowledge can be documented as the basis for the development of lead compounds.

Among the South Asian countries, Bangladesh endowed with huge sources of herbal medicines. Out of 500 species of medicinal plants about 250 species are used for the preparation of traditional medicines in Bangladesh. Bangladesh has a number of indigenous people or tribes, including the Chakmas, Garos, Santals, Marmas, Oraon, Mrus, Rakhains, Tripuras and others. They have their own traditional medicinal practitioners, known as kabiraj [9]. However, majority of these plants have not yet been scientifically evaluated [10].

Currently, there are several initiatives taking place worldwide to support herb use in the maintenance of health and the prevention or treatment of disease. In fact, hundreds of thousands of scientists around the globe are reported on new plant-derived compounds and their biological activity. Herbs hold a great promise for improving health, but growth will not be possible without continuing research and increasing awareness of sustainable trade [11]. Attempts have been made by scientists to rationalize the scientific basis and practice of the Indian Traditional medicinal plants.

1.1.2. Diabetes the Silent Killer

Diabetes is one of the commonest endocrine disorders affecting mankind worldwide [12]. It may be defined as metabolic disease, initially characterized by loss of glucose homeostasis occurring due to defects in insulin secretion or insulin action resulting from impaired metabolism of glucose, lipids and other energy yielding fuels such as lipids and proteins [13]. It has now become an epidemic with a worldwide incidence of 10% in the general population [14]. The International Diabetes Federation (IDF) has predicted that the number of individuals with diabetes in the world will rise from 382 million in 2013 to 592 million in the year 2035 [15]. The prevalence of DM is increasing at a much faster rate in developing countries than in the developed nation. South Asian region including Bangladesh is the most vulnerable focus. In 2011, the IDF also estimated that 4.8 million people living in Bangladesh had Diabetes and by 2030, that number is expected to grow to 16.8 million. This explosion in diabetes prevalence will place Bangladesh among the top most eight countries in terms of diabetic patients in 2030 as shown in Figure 1.1 [16]. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades.

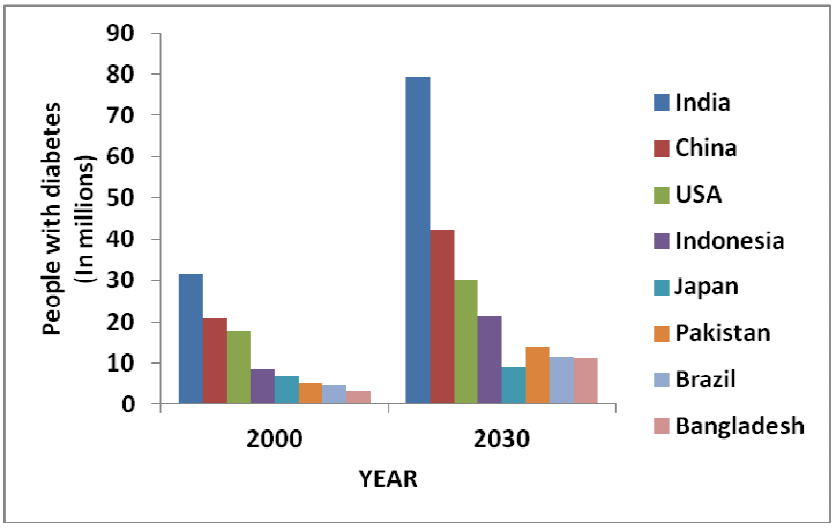


Figure 1.1: Current status of diabetes in top eight countries.

Diabetes is being projected as the World's main disabler and killer in the next 25 years [17]. Patients with diabetes experience significant morbidity and mortality from microvascular such as retinopathy, neuropathy, nephropathy and macrovascular complications such as heart attack, stroke and peripheral vascular disease. The

complications are far less common and less severe in people who have well-controlled blood sugar levels [18].

Diabetes is one of the costliest health problems in the world. Healthcare expenditures on diabetes were expected to account for 11.6% of the total healthcare expenditure in the world in 2010. Estimated global healthcare expenditures to treat and prevent diabetes and its complications were expected to be at least US $376 billion in 2010. By 2030, this expenditure is projected to exceed some US $490 billion [19]. Thus, Diabetes is one of the most prevalent and devastating chronic non-communicable diseases having serious health, economic and social consequences.

1.1.3. Pathogenesis of Diabetes Mellitus

Diabetes can be classified into three major categories: type 1 diabetes, type 2 diabetes and gestational diabetes mellitus [20].

1.1.3.1. Type 1 diabetes mellitus

Type 1 diabetes mellitus, known as insulin dependent diabetes mellitus or juvenile-onset diabetes is more prevalence among young people [21]. It results from an absolute deficiency of insulin, commonly caused by chronic autoimmune disease that results from a complex interaction of both genetic and environmental factors [22]. The pathogenesis of type 1 diabetes is summarized in Figure 1.2.

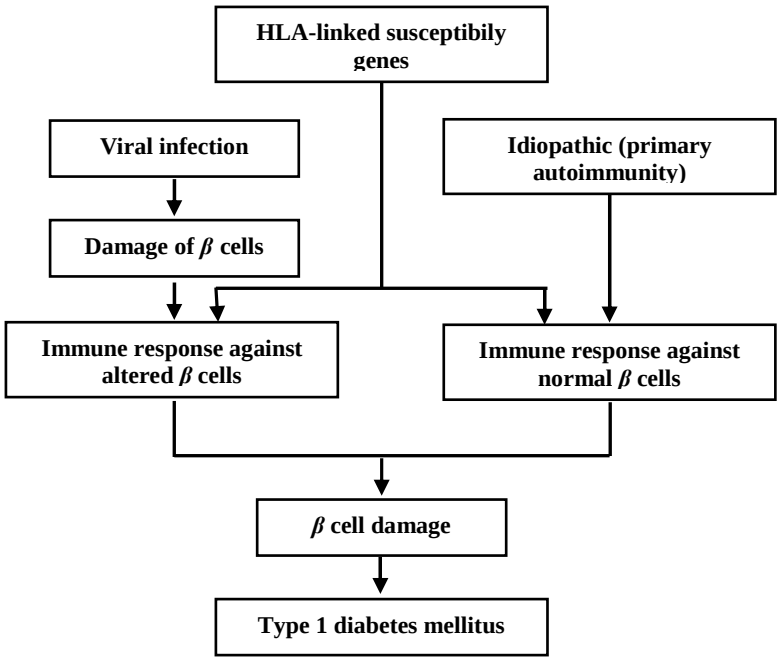


Figure 1.2: Pathogenesis of type 1 diabetes mellitus [23].

1.1.3.2. Type 2 diabetes mellitus

Type 2 diabetes mellitus, commonly known as non-insulin diabetes mellitus occurs in adult patients aged 40 years and above. It is a polygenic disorder with obesity related insulin resistance playing a major role in its onset and progression. It is characterized by excessive hepatic glucose production, decreased insulin secretion from pancreatic β-cells and insulin resistance in peripheral tissue such as muscle, adipose and liver [24]. There are convincing data to indicate a genetic component associated with insulin resistance [23]. Insulin resistance is a feature of the offspring of parents with type 2 diabetes. In type 2 diabetes, the insulin resistance has been suggested to have a co-dominant mode of inheritance [25]. Insulin resistance is also caused by acquired factors such as obesity, sedentary life style, pregnancy and hormone excess. The pathogenesis of insulin resistance and type 2 diabetes is summarized below (Figure 1.3). During its early stage, insulin resistance is compensated by hyperinsulinemia and thus, preserving normal glucose tolerance. Deterioration into impaired glucose tolerance occur where either insulin resistance increases or the insulin secretory responses decrease or both [26].

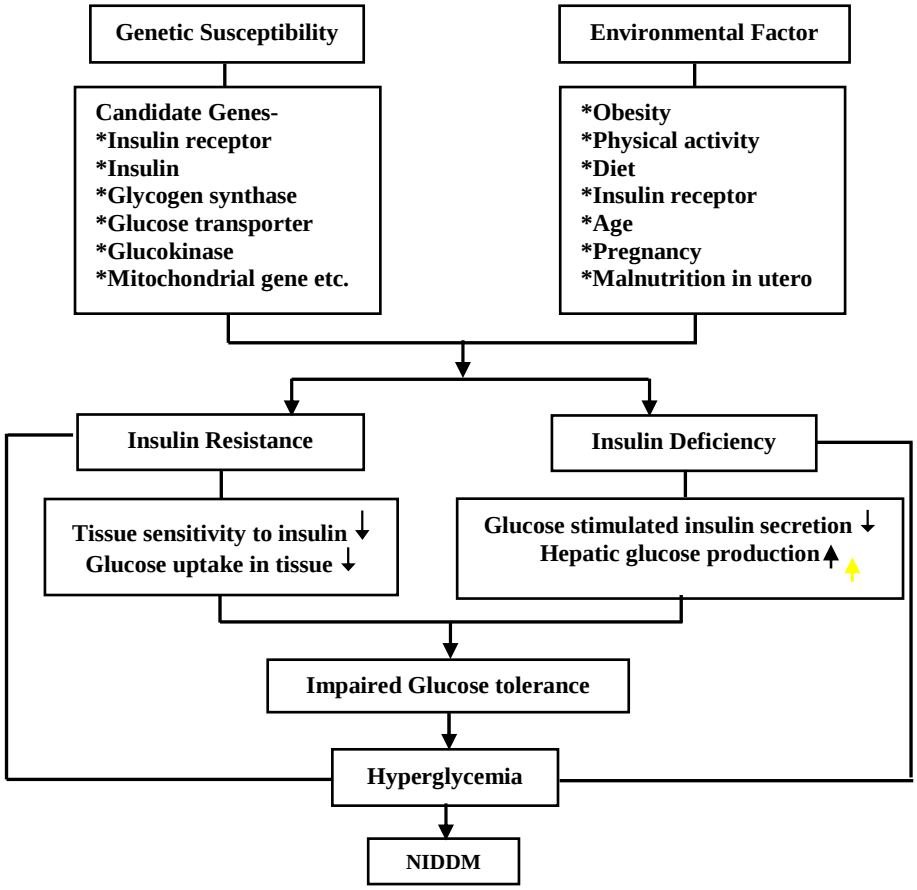


Figure 1.3: Progressive pathogenesis of type 2 diabetes mellitus [26]

1.1.3.3. Gestational diabetes

Gestational diabetes or Type III is a type of diabetes which develops during pregnancy and may improve or disappear after delivery. Even though it may be transient, gestational diabetes may damage the health of the fetus or mother, and about 40% of women with gestational diabetes develop type 2 diabetes later in life [27].In recent years, a sub-type of type 2 diabetes mellitus, known as the maturity onset diabetes of the young (MODY) has been identified in children and adolescents and affecting about 2% of people with diabetes. MODY is believed to be caused by mutations in the glucokinase that plays a prominent role in hepatic and pancreatic glucose metabolism [28].

1.1.3.4. Other forms of diabetes

Diabetes may develop as a consequence of other diseases or medication called **s**econdary diabetes. Davidson (1991) listed some causes of secondary diabetes, a term coined by Davidson (1991) as "other types" of diabetes, these include pancreatic diseases such as pancreatitis, surgery, cystic fibrosis and the use of other drugs not prescribed for the condition such as contraceptive pills, diuretics, steroids, genetic syndromes (extremely rare) and endocrine diseases such Cushing·s syndrome and Acromegaly [29].

1.1.4. Complications of Diabetes Mellitus

Uncontrolled hyperglycemia in both type 1 and type 2 diabetes lead to the development of both acute and long term complications [30]. Acute complications include diabetic ketoacidosis, nonketotic hyperosmolar coma and diabetic coma. Elevation of blood glucose level for longer period of time leads to damage to blood vessels by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins. It is now well established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications [31, 32]. There are also multiple abnormalities of lipoprotein metabolism in very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) in diabetes responsible for both microvascular and macrovascular diseases [33, 34]. Microvascular disease leads to retinopathy, neuropathy and nephropathy and macrovascular disease leads to

cardiovascular disease, mainly by accelerating atherosclerosis [35]. These disorders including coronary artery disease, leading to myocardial infarction (heart attack) or angina, stroke (mainly ischemic type), peripheral vascular disease contribute to intermittent claudication (exertion-related foot pain) as well as diabetic foot [34].

Chronically elevated blood glucose levels lead to increase production of mitochondrial reactive oxygen species (ROS), which activate a number of metabolic pathways such as the polyol pathway, formation of advanced glycation end product (AGE), hexosamine pathway and also the protein kinase C (PKC) pathway (Figure: 1.4) whose end products contribute to the development of long term complication of diabetes [30].

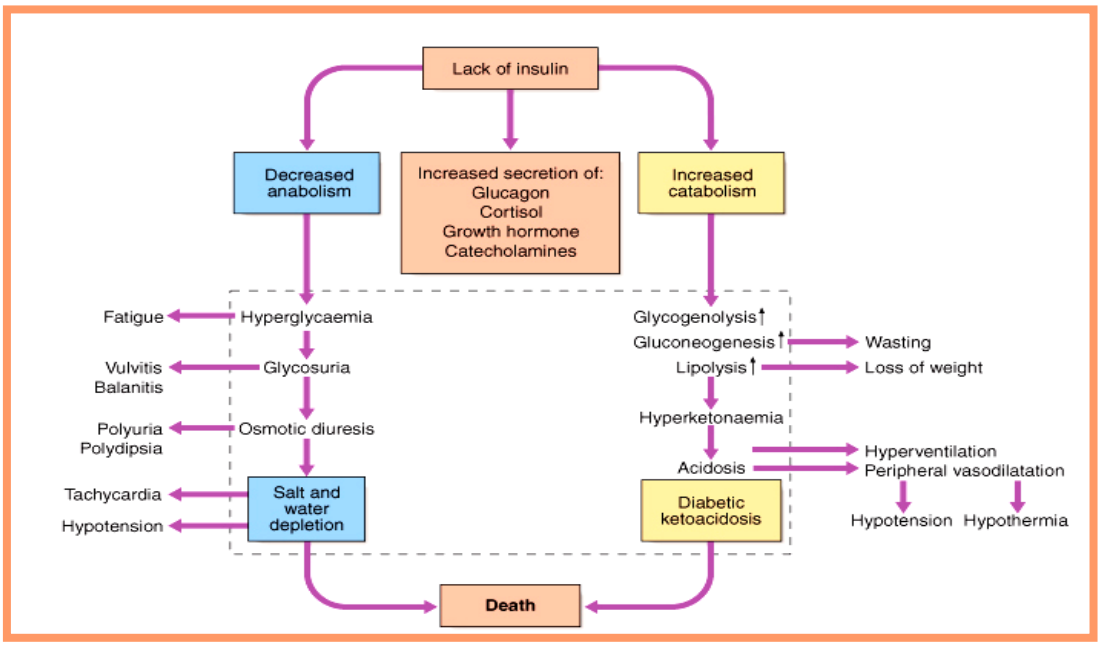


Figure 1.4: Metabolic pathways activated by chronically elevated blood glucose levels as well as the resultant long term complications of diabetes mellitus [29].

1.1.5. Treatment of Diabetes Mellitus and Its Limitations

The chronic hyperglycemia of diabetes can lead to multiple organ damage, which are devastating to the individual and very expensive to the health services [36]. Available evidence indicates that diabetes related complications can be prevented or delayed by achieving tight glycemic control [37]. Therefore, much effort has been devoted to the search and development of optimal therapeutic regimens for the management of diabetes [38]. Regardless of the type of diabetes, patients are required to control their blood glucose with medications and/or by adhering to an exercise program and a

dietary plan [25]. Insulin therapy by injection is given to those with type 1 DM and also to some patients with type 2 DM when oral hypoglycaemic drugs fail to lower blood glucose [39]. Patients with type 2 DM are usually placed on a restricted diet and are instructed to exercise, the purpose of which primarily is weight control. If diet and exercise fail to control blood glucose at the desired level, oral antidiabetic medications such as sulfonylureas, repaglinide, metformin, α-glucosidase inhibitors and thiazolidinediones (TZDs) are prescribed (either alone or in combinations of different classes) together with dietary restriction and exercise programs [40]. When hyperglycemia becomes severe, patients are usually switched to insulin injections with or without oral agents to improve insulin action [41].

Oral antidiabetic agents exert their actions by various mechanisms (Table 1.1) including reduction of hepatic glucose production, enhancement of insulin secretion by pancreatic β-cells, improvement of insulin sensitivity and inhibition of intestinal glucose digestion and absorption.

The use of these drugs is however, limited by the fact that they have their own drawbacks, ranging from the developing of resistance and adverse effects to lack of responsiveness in large segment of patients. These major adverse effects, such as potential hypoglycemia, weight gain, gastro-intestinal discomforts and also lactoacidosis prompt patients to stop taking anti-diabetic medications [42]. In addition to their potential side effects, many of the oral anti-diabetic agents have higher secondary failure rates [41]. Furthermore, despite the intensive use of current anti-diabetic agents more than 50% type 2 diabetic patients still exhibit poor glycemic control and some (18%) develop serious complications within six years of diagnosis [43]. So, clearly there is a need for new anti-diabetic agents.

1.1.6. Antidiabetic Plants in Traditional Medicines

1.1.6.1. Scope of alternative remedies

The limitations of currently-available oral antidiabetic agents either in terms of safety and efficacy coupled with the emergence of the disease into a global epidemic have encouraged a concerted effort to discover drugs that can manage type 2 diabetes more efficiently [44]. In addition, the world health organization has also recommended and

encouraged the use of alternative therapy especially in countries where access to the conventional treatment of diabetes is not adequate [45]. With increasing incidence of diabetes mellitus in rural population throughout the world, there is a clear need for the development of indigenous, inexpensive anti-diabetic drugs of natural origin [46].

Table 1.1: Pharmacologic therapy of type II diabetes and comparison of available agents.

Medication	Mechanism of action	Benefits	Risks and concerns
Sulfonylureas (glyburide, glipizide, glimepiride)	Binds to sulfonylurea receptor, stimulating insulin release.	Extensive experience; UKPDS data show improve microvascular outcomes; low cost; once daily dosing.	Hypoglycemia; weight gain; may impede ischemic preconditioning.
Meglitinides (Repaglinide, nateglinide)	Binds to sulfonylurea receptor, stimulating insulin release.	Targets postprandial glucose, mimics physiologic insulin secretion.	Hypoglycemia, weight gain, no long term experience, extensive frequent dosing (compliance)
Biguanides (Metformin)	Decreases hepatic glucose production.	Weight loss or weight neutrality; lipid and other nonglycemic vascular benefits; UKPDS data show improved macrovascular outcomes; once daily dosing available (sustained release product)	Diarrhea, lactic acidosis, many contraindications to consider before prescribing; lowers vitamin B_{12} levels (but no apparent hematologic indices)
α-glucosidase inhibitors (Acarbose)	Retards gut carbohydrate absorption.	Targets postprandial glucose; weight neutral; non-systemic.	Intestinal gas, expensive; frequent dosing (compliance)
Thiazolidiediones (Rosiglitazone, pioglitazone)	Activates PPARγ, increasing peripheral insulin sensitivity.	Addresses primary defect of T2DM; no hypoglycemia, lipid and other nonglycemic vascular benefits, potential antiatherosclerotic properties, potential for β-cell preservation, once daily dosing.	Liver monitoring still advised, edema, weight gain, no long term experience, slow onset of action, expensive.
Insulin	Increases insulin supply	Extensive experience, rapidly effective in all circumstances, no contraindications, UKPDS data show improved microvascular outcome, cardiovascular and mortality benefits demonstrated in acute settings, low cost (traditional forms, analogs are most expensive)	Hypoglycemia, weight gain, injections and more frequent glucose monitoring required, increases complexity, stigma.

PPARγ, peroxisome proliferator activator receptor γ; UKPDS, United Kingdom Prospective Diabetes Study. (Adapted with permission from Inzucchi SE: Yale Diabetes Facts and Guidelines. New Haven, Yale Diabetes Center, 2004.)

The management of type 2 DM (NIDDM) is possible with the drugs that can either lower the blood sugar level or restore the liver glycogen level. In modern system of medicine, there is no drug, which is reported to act by both mechanisms [47]. The hypoglycemic effect of several herbal extracts have been confirmed in human and animal models of type 2 diabetes and many of the conventional drugs have been derived from these medicinal plants. Metformin, a less toxic biguanides and potent oral glucose-lowering agent, was developed from *Galega officianalis* and used to treat diabetes [7].Out of dozens of oral medications for diabetes; only one medication i.e. metformin is approved for use in children with diabetes [48]. In developed countries the use of antidiabetic herbal remedies is reported to have been increasing since the introduction of insulin and synthetic oral hypoglycemic agents during the early part of the 20th century. This renewed interest in herbal antidiabetic remedies in developed countries is believed to be motivated by several factors, including side effects and high secondary failure rates [49]. However, in recent years, there has been a resurgence of interest in medicinal plants with hypoglycemic potential in developing countries.

1.1.6.2. Scientifically validated antidiabetic plants

The Natural Products Alert (NAPRALERT) database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi to plants with antidiabetic activity. Over half of the species have been used ethnopharmacologically in traditional medicine as antidiabetics, and some 50% of these traditional remedies have been studied experimentally [50].

The most commonly used medicinal plants whose blood glucose lowering effects have been tested and confirmed in different parts of the world (especially in India subcontinent) include: *Allium cepa (*Onion*), Allium sativum* (Garlic), *Aloe vera* (Ghritokumari), *Cinnamomum tamala, Coccinia indica* (Telakucha), *Gymnema sylvestre* (Gurmar), *Momordica charantia* (Bitter Melon), *Murrayi koningii* (Jhumka lata), *Ocimum sanctum* (Tulsi), *Panax ginseng* (Asian), *Trigonella foenum-graecum* (Methi), *Pterocarpus marsupium* (Pitshul) and *Syzigium cumini* (Kalojam) [51, 52]. In Bangladesh, the uses of traditional medicinal plants for the treatment of DM are in primary stage. Rahman *et al*., have reported 30 species from 18 families for the treatment of diabetes in Bangladesh [53]. Plant parts used, active principles as well as

the possible mechanism of action of commonly used medicinal plants are summarized in Table 1.2.

Table 1.2: List of scientifically validated antidiabetic plants.

Plant and Family	Plant part	Active ingredient	Mechanism of action	References
Allium cepa (Onion) **Alliaceae**	Onion bulbs	▪ S-methyl cysteine sulphoxide ▪ S-allylcysteine sulphoxide	▪ Stimulate insulin secretion ▪ Compete with insulin for insulin-inactivating sites in the liver	▪ [54]. ▪ [55, 56].
Allium sativum (Garlic), **Alliaceae**	Garlic gloves	▪ S-methyl cysteine sulphoxide-precursor of allicin and garlic oil	▪ Stimulate in vitro insulin secretion ▪ Inhibit glucose production by the liver	▪ [57] ▪ [58]
Aloe vera (Aloe barbadensis) **Aspholedeceae**	Leaf pulp and gel	▪ Phytosterols	▪ Stimulate synthesis and/or release of insulin ▪ Alter activity of carbohydrate metabolizing enzymes	▪ [59] ▪ [60].
Catharanthus roseus (Madagascar periwinkle) **Apocynaceae**	Fresh leaf juice	▪ Alkaloids: catharanthine, leurosine and vindolinine ▪ Tannins	• Increases hepatic utilization of glucose • Supress activities of gluconeogenic enzymes	▪ [61] ▪ [62].
Cinnamomum cassia (Chinese cinnamon) **Lauraceae**	Bark	▪ Cinnamaldehyde ▪ Cinnamic alcohol ▪ Methyl hydroxyl chaconne polymer	▪ Enhances insulin action ▪ Increase glucose uptake and glycogen synthesis	▪ [63] ▪ [64].
Coccinia indica **Cucurbitaceae**	Leaves	▪ Beta sitosterol	▪ Suppress glucose 6-phosphatase ▪ Stimulate glycogen synthase activity and reduction of phosphorylase activity	▪ [65] ▪ [66].
Fiscus bengalensis ***Moraceae***	Leaves and bark	▪ Lecoperlargonin derivative	▪ Increases insulin secretion ▪ Inhibit insulinase activity	▪ [67] ▪ [68].
Gymnema Sylvestre (Gurmar). **Asclepiadaceae**	Leaves	▪ Gymnemosides and gymnemic acid (from the saponin fraction) ▪ Triterpene glycosides	▪ Stimulate insulin secretion from rat islets. Decreases the activity of gluconeogenic enzymes ▪ Induce beta cell regeneration	▪ [69] ▪ [70].
Ginseng (Panax ginseng) **Araliaceae**	Root and leaves	▪ Polysacharides ▪ Ginsenosides ▪ (steroidal saponins)	▪ Slow digestion and absorption of CHO ▪ Affect NO mediated glucose transport	▪ [71] ▪ [72].
Mormordica charrantia (Bitter melon) **Cucurbitaceae**	Fruit pulp, seed, leaves and whole plant	▪ Charantin (a peptide) ▪ Insulin like polypeptide P ▪ ("vegetable insulin")	▪ Stimulatie insulin secretion ▪ Supress the activities of gluconeogenic enzymes ▪ Increases the number of beta cells in diabetic rats	▪ [73] ▪ [74] ▪ [75]
Murrayi koningii (Curry leaf) **Rutaceae**	Leaves	▪ Carbazole alkaloids ▪ Copolin-a-glucoside	▪ Stimulate insulin secretion ▪ Increases glycogenesis and decrease glycogenolysis and gluconeogenesis	▪ [76] ▪ [77, 78].
Ocimum sanctum (Holy basil) **Lamiaceae**	Leaves	▪ Pectins	▪ Stimulate insulin secretion	▪ [79, 80, 81]
Opuntia streptacantha (Cittrus colynthis) **Cactaceae**	Fruit	▪ Saponins and glycosidic components	▪ Stimulated insulin release from isolated pancreatic islets	▪ [82]
Polygala senega **Polygalaceae**	Rhizomes	▪ Triterpenoid glycosides ▪ (Senegin II)	▪ Increases insulin sensitiviry	▪ [83]
Polygonatum officinale ***Liaceae***	Rhizomes	▪ Unknown	▪ Decreased hepatic glucose output ▪ Increased insulin sensitivity	▪ [84] ▪ [52]
Pterocarpus marsupium **Fabaceae**	Bark	▪ Epicatechin and catechin (tannin) ▪ Pterostilbene (flavonoid)	▪ Prevent beta-cell damage in rats ▪ Regenerate functional pancreatic beta cells ▪ Enhances insulin release	▪ [85] ▪ [86] ▪ [51]
Syzigium cumini (Eugenia jambolana) **Myrtaceae**	Seeds, leaves and fruit pulp	▪ Mycaminose	▪ Stimulate kinases involved in peripheral utilization of glucose	▪ [67, 88].
Trigonella foenum graecum (Fenugreek) **Fabaceae**	Seeds	▪ Alkaloid-trigonelline, ▪ nicotinic acid, and coumarin ▪ 4-hydroxyisoleucine ▪ Galactomannan	▪ Slow down digestion and absorption of CHO ▪ Incease glucose induced insulin release	▪ [89] ▪ [90].

1.1.6.3. Mode of action of antidiabetic medicinal plants

Plant-based products have been popular all over the world for the centuries and herbal alternatives are proven to provide symptomatic relief and assist in the prevention of the secondary complications of diabetes. Some herbs have also been proven to help in the regeneration of *ß*-cells and thus overcoming insulin resistance. In addition to maintaining normal blood sugar level, some herbs are also reported to possess antioxidant activity and lipid lowering action. The majority of plants with blood glucose lowering activity appear to contain polysaccharides, glycosides and flavonoids. Several investigations indicated that a plant and/or its product may contain one or more active principles which may act synergistically to exert a greater blood glucose lowering efficacy through a combination of more than one mechanism [52, 87].

The mechanisms by which herbal antidiabetic remedies reduce blood glucose levels are more or less similar to those of the synthetic oral hypoglycemic drugs and are summarized below [91, 92):

- Stimulation of insulin synthesis and/or secretion from pancreatic *ß*-cells
- Regeneration of damaged pancreatic *ß*-cells
- Improvement of insulin sensitivity
- Mimicking the action of insulin (acting like insulin)
- Alteration of the activity of glucose metabolizing enzymes
- Slowing down the absorption of carbohydrates from the gut

Many of the herbal drugs used in diabetes also have antioxidant activity can reduce oxidative stress induced by high glucose level in the body [93]. However, most of the species of higher plants have not been screened for chemical or biologically active constituents [94]. So there is a big challenge to fully exploit medicinal biodiversity to look for phytochemicals with antidiabetic properties. On the basis of folkloric reputation and literature review we selected eight medicinal plants with antidiabetic activity available in Bangladesh [10]. Therefore, the research protocol was designed to compare the antidiabetic, lipid-lowering and antioxidant activities of plants extracts such as *Grewia asiatica* Linn., *Corcorus ollitoriou* Linn., *Alpinia nigra*, *Prunica granatum* Linn., *Jatropha curcas* Linn., *Cinamomum tamala* Nees., *Urginea indica* and *Hollarhena antidysenterica* (L) Wall., respectively and/or isolated compounds in alloxan-induced diabetic rats.

1.1.7. Alloxan-Induced Animal Model

Alloxan or mesoxalylurea is an organic compound based on a pyrimidine heterocyclic skeleton. This compound has a high affinity for water and therefore exists as the monohydrate.

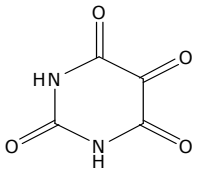


Figure 1.5: Alloxan.

Alloxan has two distinct pathological effects:

- It selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the *β*-cell.
- It causes a state of insulin-dependent diabetes through its ability to induce reactive oxygen species (ROS) formation, resulting in the selective necrosis of *β*-cells [95].

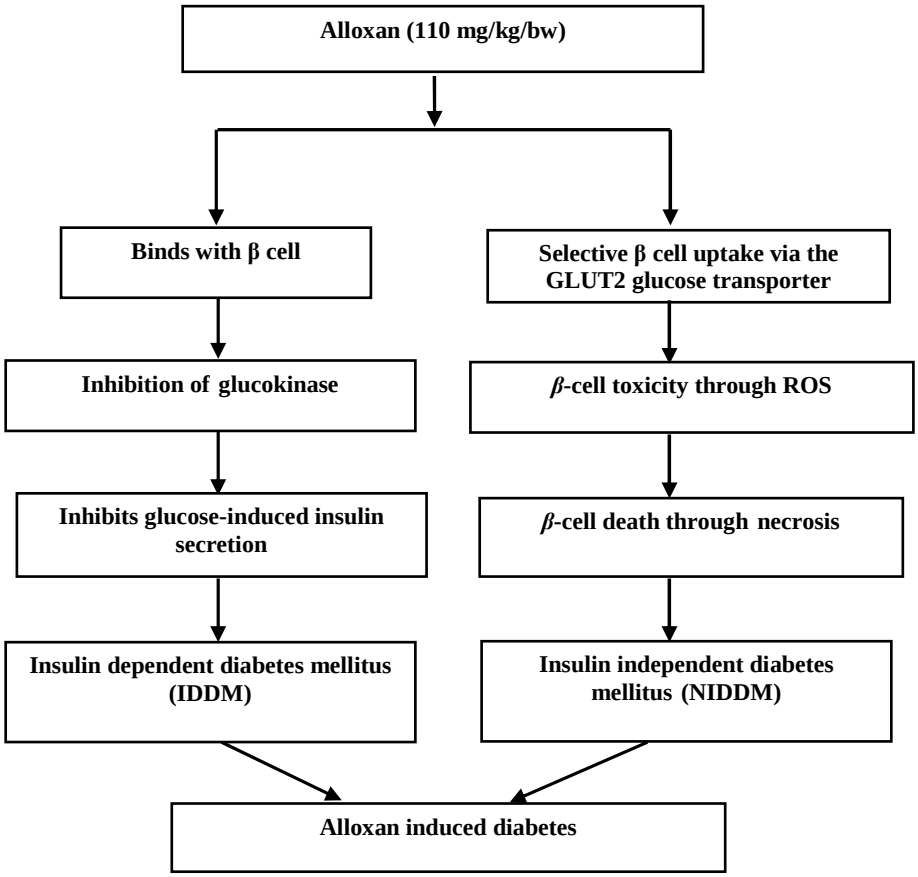


Figure 1.6: Schematic representation of the mechanism of action of alloxan.

Hyperglycemia can directly cause increased ROS generation. Glucose can undergo auto-oxidation and generate hydroxyl free radicals ($OH^{\bullet}$) [96]. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which results in increased intra cellular osmolarity, depleted glutathione reserves and enhanced production of free radicals (Figure 1.7) [97].

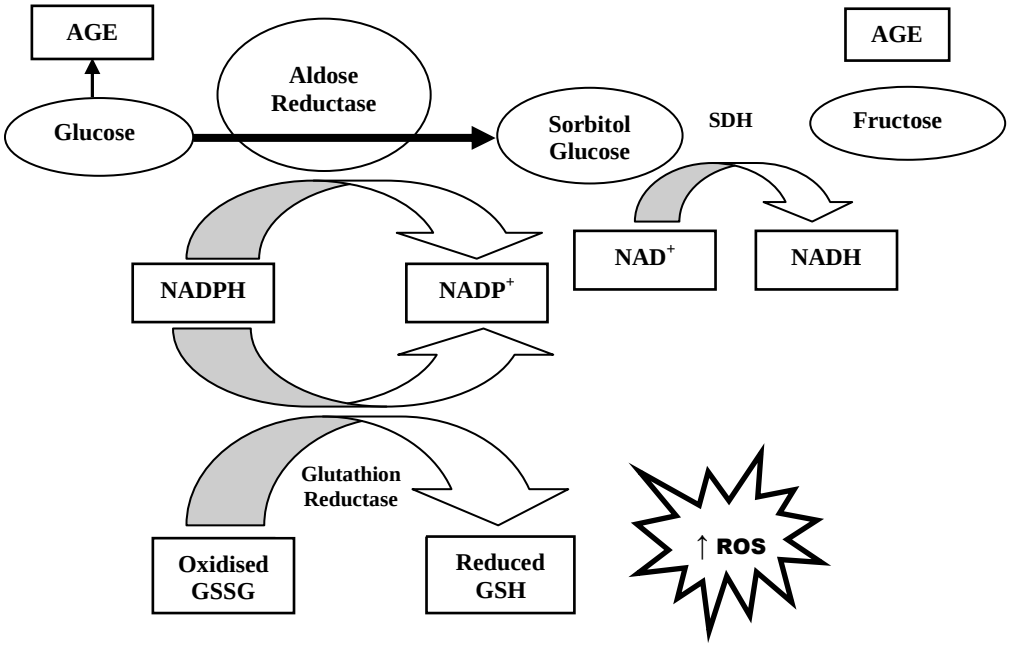


Figure 1.7: Hyperglycemia increases oxidative stress.

Free radicals are generated as byproducts of normal cellular metabolism can result in cell dysfunction and destruction resulting in tissue injury. The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by nonenzymatic and enzymatic catalase (CAT), glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) antioxidants [98]. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. This is particularly dangerous for the β-cells of islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses [99]. Furthermore, excessive generation of ROS has pathological consequences including damage to proteins, lipids and DNA [100].

1.2. Plants Under Study

The plants for the study were selected on the basis of their folkloric reputation and ethnobotanical reports for the treatment of diabetes and related complications are shown in the Table 1.3.

Table 1.3: List of selected medicinal plants and their used parts.

Local name	Botanical name	Family name	Plant parts	BNHVA*
Phalsa	*Grewia asiatica* Linn.	Tiliaceae	Stem bark	DACB- 33102
Jangli Ada	*Alpinia nigra (Gaertn.)* B. L. Burtt.	Zingiberaceae	Rhizome	DACB- 33200
Bon Piaj	*Urginea indica* Kunth	Liliaceae	Bulb	DACB- 33199
Tossa pat	*Corchorus olitorius* Linn	Malvaceae	Seed	DACB- 33232
Dalim	*Prunica granatum* Linn	Lythraceae	Stem bark	DACB- 33184
Tejpat	*Cinnamomum tamala* Nees et Eberm	Lauraceae	Stem bark	DACB- 33203
Kurchi	*Holarrhena antidysenterica* (L.) WALL	Apocynaceae	Stem bark	DACB- 33214
Bagbherenda	*Jatropha curcas* Linn.	Euphorbiaceae	Root	DACB- 33179

*Bangladesh National Herbarium voucher/ access no.

1.2.1. Taxonomy of the Selected Plants.

I. Grewia asiatica Linn.

Grewia asiatica Linn. (Tiliaceae) is an exotic bush plant considered horticulturally as a small fruit crop but also used as a folk medicine. The phalsa plant is native to the Indian subcontinent and Southeast Asia and now widely cultivated on a commercial scale in the northern and the western states of India, Pakistan, Philippines and other tropical countries [101].

Botanical Name : *Grewia asiatica* Linn.

Synonims : *Grewia subinequalis* DC.

Common Names:

Bengali/Vernacular Name: Phalsa, Phassa, Falsa. (Ben.)

English Name : Asiatic grewia, Phalsa (Eng.)

Distribution in Bangladesh

In Bangladesh it grows in the forests of Sylhet, Chittagong and different Sal forests and is also planted in many areas of the country [10].

Taxonomy of *Grewia asiatica* Linn [102]

Kingdom : Plantae

Subkingdom : Viridaeplantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Malvales

Family : Tiliaceae

Genus : *Grewia*

Species : *Grewia asiatica*

Figure 1.8: Fruits and leaves of *Grewia asiatica.*

II. Alpinia nigra (Gaertn.) B. L. Burtt.

Alpinia nigra (Gaertn.) Burtt (Zingiberaceae) is an aromatic herbaceous plant, grows well on riverside and can grow on land and is distributed thought-out tropic Asia, from India to Ceylon, Indo-china (Thailand, Philippines, Singapore, Myanmar, Borneo, Indonesia and Malaysia) [103]. It is not only an edible vegetable but also used in folk remedies. The members of family Zingiberaceae have been subjected to extensive chemical and pharmaceutical investigations because of their use in Indian and oriental medicines [104].

Botanical Name : *Alpinia nigra* (Gaertn.) B. L. Burtt

Synonims : *Alpinia allughas* (Retz.) Roscoe, *Zingiber nigrum* Gaertn, *Heritiera allughas.*

Common Names:

Bengali/Vernacular Name: Jangli Ada, Tara. (Ben.)

English name : Wild Ginger (Eng.)

Distribution in Bangladesh

In Bangladesh it grows by the side of streams and canals in all areas of the country [10].

Taxonomy of *Alpinia nigra* (Gaertn.) B. L. Burtt. [105]

Kingdom : Plantae

Subkingdom : Viridaeplantae

Division : Tracheophyta

Class : Liliopsida

Order : Zingiberales

Family : Zingiberaceae

Genus : *Alpinia*

Species : *Alpinia nigra*

Figure 1.9: Rhizomes and leaves of *Alpinia nigra*.

III. Urginea indica, Knuth.

Urginea indica, Knuth (Liliaceae), commonly called 'Indian squill' is a small, glabrous, bulbous, herb, grows best in dry, sandy soils, especially in the seacoast. It is medicinally very important and marketable bulbs are obtained after about 4-5 years [106]. It is a widely diffused plant occurring in plains of India and dry hills of lower Himalayas, Africa, Mediterranean region, Abyssinia, Nubia and Senegambia and Germany [107].

Botanical Name: *Urginea indica* Kunth.

Synonims **:** *S. indica*, Roxb.

Common Names:

Bengali/vernacular name : Bon Piaj, Jangli Piaj, Jangli Rashun (Beng.)
English name **:** Indian Squill, True Squill, Sea Onion (Eng.)

Distribution in Bangladesh
Grows wild in sandy-areas of Chittagong and Cox's Bazar [10].

Taxonomy of *Urginea indica* Knuth [108]

Kingdom **:** Plantae
Subkingdom **:** Tracheobionta
Division **:** Magnoliophyta
Superdivision **:** Spermatophyta
Class **:** Liliopsida
Order **:** Liliales
Family **:** Liliaceae
Genus **:** *Urginea*
Species **:** *Urginea indica*

Figure 1.10: Whole plant of *Urginea indica.*

IV. Corchorus olitorius Linn.

Corchorus olitorius Linn. (Tiliaceae) is an annual herb with slender stems. It (Jute) is an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh and in tropical Asian countries like the Philippines and Malaysia, as well as in tropical Africa, Japan, South America, the Caribbean and Cyprus [109].

Botanical Name: *Corchorus olitorius* Linn.

Common Names:

Bengali/Vernacular Name : Tossa pat (Beng.)
English Name **:** Jute, Jew's mallow. (Eng.)
Distribution in Bangladesh

Bangladesh is the second highest producer of jute. It is cultivated all over Bangladesh specially in Dhaka, Mymensing, Tangail, Comilla, Khulna, Jessore, Pabna, Rajshahi [10].

Taxonomy of *Corchorus olitorius* Linn [110]

Kingdom **:** Plantae
Subkingdom **:** Viridaeplantae
Division **:** Tracheophyta
Class **:** Magnoliopsida
Order **:** Malvales
Family **:** Malvaceae
Genus **:** *Corchorus*
Species **:** *Corchorus olitorius*

Figure 1.11: Leaves of *Corchorus olitorius*

V. Punica granatum Linn.

Punica granatum Linn. (Lythraceae) is a native of Iran and is extensively cultivated as fruit tree or ornamental or for medicinal purpose in Mediterranean region such as Spain, Morocco, Egypt, Afghanistan and Iran. It is commonly found in tropics and subtropics [111].

Botanical Name : *Punica granatum* Linn.

Common Names:

Bengali/Vernacular Name : Dalim, Anar (Beng.)

English Name : Pomegranate (Eng.)

Distribution in Bangladesh

It is cultivated and grown naturally all over Bangladesh [10].

Taxonomy of *Punica granatum* Linn. [112]

Kingdom	**:** Plantae
Subkingdom	**:** Viridaeplantae
Division	**:** Tracheophyta
Class	**:** Magnoliopsida
Order	**:** Myrtales
Family	**:** Lythraceae
Genus	**:** *Punica*
Species	**:** *Punica granatum*

Figure 1.12: Fruit of *Punica granatum*

VI. Cinnamomum tamala Nees et Eberm

Cinnamomum tamala Nees et Eberm (Lauraceae) is an evergreen tropical tree belonging to the family Lauraceae. It is found in India along the North -Western Himalayas, in Sikkim, Assam, Mizoram and Meghalaya. It is also found in tropical and sub-tropical Asia, Australia, Pacific region and South Asia such as Nepal, Bangladesh and Myanmar. Its leaves are used in Indian cookery and as bay leaves in Europe [113, 114].

Botanical Name : *Cinnamomum tamala* Nees et Eberm

Common Names:

Bengali/Vernacular Name: Tejpatta (Beng.)

English Name : Indian Bay Leaf, Bay Leaf (Eng.)

Distribution in Bangladesh

Cultivated commercially in Sylhet and different regions of Bangladesh [10].

Taxonomy of *Cinnamomum tamala* Nees et Eberm. [115]

Kingdom : Plantae
Subkingdom : Viridaeplantae
Division : Tracheophyta
Class : Magnoliopsida
Order : Laurales
Family : Lauraceae
Genus : *Cinnamomum*
Species : *Cinnamomum tamala*

Figure 1.13: Leaves of *Cinnamomum tamala*

VII. Holarrhena antidysenterica (L.) WALL.

Holarrhena antidysenterica (L.) WALL. (Apocynaceae) is a medicinal plant. It is indigenous throughout the Indian subcontinent and found all over the country in deciduous forests up to 900 meters [116].

Botanical Name : *Holarrhena antidysenterica* (L.) WALL

Common Names:

Bengali/Vernacular Name: Kurchi (Beng.)
English Name : Tellichery (Eng.)

Distribution in Bangladesh

Forests of Dhaka, Tangail, Chittagong, Chittagong Hill Tracts and Cox's Bazar, Sylhet and Dinajpur [10].

Taxonomy of *Holarrhena antidysenterica* (L.) WALL [117]

Kingdom : Plantae
Subkingdom : Tracheobionta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Asteridae
Order : Gentianales
Family : Apocynaceae
Genus : *Holarrhena*
Species: *Holarrhena antidysenterica*

Figure 1.14: Leaves of *Holarrhena antidysenterica*

VIII. Jatropha curcas

Jatropha curcas (Euphorbiaceae) is a large shrub, 3-4m high, native of topical America, occurring almost throughout India and in Andaman Island [113].

Botanical Name : *Jatropha curcas*

Common Names:

Bengali/Vernacular Name: Bagbherenda (Beng.)
English Name **:** Purging nut, Physic nut (Eng.)

Family **:** Euphorbiaceae

Distribution in Bangladesh

It is grown throughout the country and grown as a live fence in the villages [10].

Taxonomy of *Jatropha curcas* Linn [118]

Kingdom **:** Plantae
Subkingdom **:** Viridaeplantae
Division **:** Tracheophyta
Class **:** Magnoliopsida
Order **:** Malpighiales
Family **:** Euphorbiaceae
Genus **:** *Jatropha*
Species : *Jatropha curcas*

Figure 1.15: Leaves and fruits of *Jatropha curcas*

1.2.2 The Ethnopharmacological Importance of the Selected Plants

The medicinal importance of the selected plants are listed in Table 1.4.

Table 1.4: The medicinal importance of the selected plants.

Plant name	Plant parts	Pharmacological activity
Grewia asiatica Linn.	Stem Bark	Antidiabetic, demulcent, febrifuge and in biliousness, vata, kapha, urinary troubles and burning vagina etc. [119].
	Fruits	Astringent and stomachic, tonic and aphrodisiac, inflammations, burning sensation, troubles of throat respiratory, cardiac and blood disorders, fevers, vata, kapha, biliousness, diarrhoea and remove the dead foetus etc. [120].
	Seed	Anti-fertility. [121].
	Leaves	Pustular eruptions, antibiotic action etc. [121].
Alpinia nigra	Rhizome	Diabetes, stimulant, decoction; stomachic, carminative, laxative, antiflatulent, diuretic, expectorant, antifungal, headache, rheumatism, bronchitis, liver and kidney diseases, dyspepsia, gastric disease, vomiting, sea-sickness, chest pain, tubercular glands and impotence etc. [122].
Urginea indica	Bulbs	Diuretic, cardiac stimulant, expectorant, chronic bronchitis, asthma, hypoglycemic and anticancer etc. [123]. Also used in arthritis, rheumatism, coetaneous and subcutaneous parasitic infection; dropsy, male sterility, allergies and gout etc. [105].
Corchorus olitorius	Seeds	Gonorrhea, chronic cystitis, pain fever, and tumors. [112].
	Leaves	Control blood pressure, cholesterol build-up, diabetes and prevent heart disease, diarrhea, stomach ache, dysentery, cancer etc. [112].
Punica granatum	Flowers	Styptic to gum and useful in vomiting, ophthalmodynia, ulcers, pharyngodynia, epistasis etc. [114].
	Fruits	Anemia, hyperdypsia, hyperglycemia, pectoral diseases, splenopathy, bronchitis, dysentery and diarrhoea [114].
Cinnamomum tamala	Leaves	Hypoglycemic, stimulant and carminatives [124].
	Stem Bark	Anorexia, bladder disorders, dryness of mouth, coryza, diarrhoea, nausea, spermatorhea etc. [125].
Holarrhena antidysenterica	Stem Bark	Amoebic dysentery, diarrhea, asthma, bronchopneumonia and malaria [126].
	Root	Antibacterial [126].
	Fruits	Aanti-cancer and antihyperglycemia [127].
Jatropha curcas	Leaves	Glactagogue, rubefacient, suppurative and insecticidal, ulcer, tumor and scabies [113].
	Root	Anthelmintic [113].
	Seeds	Purgative, rheumatism and leprosy [113].

1.2.3. Biological Investigation and Scientific Reports on the Selected Plants

Some reported biological works of the selected plants are listed in the Table 1.5.

Table 1.5: Some reported biological works on the selected plant species.

Plant species	Plant parts	Pharmacological activity
Grewia asiatica	Fruits	Radioprotective [128], neuroprotective [129], antiemetic activities [130], antioxidant activity [131] etc.
	Leaves	Hypoglycemic activity [132, 133, 134].
Alpinia nigra	Rhizome	Antibacterial activity [135], antioxidant activity [136], anthelmintic efficacy [137].
Urginea indica	Bulb	Cure patients with ischemic heart diseases, cardiomiopathy and corpulmonale [138], antifungal activity [139], antitumor and anticancer activity [140].
Corchorus olitorius Linn	Seeds	Antibacterial or antifungal activity [141], antifertility activity [142].
Punica granatum Linn	Flower	Hypoglycemic activity [143], improved postprandial hyperglycemia [144]
	Fruit	Antibacterial activity [145], anticancer activity [146], antioxidant effect, anti-atheroscletic activity [147].
Cinnamomum tamala	Leaves	Antibacterial activity [148], Anti-inflammatory activity [149], antidiarrhoea [150].
Holarrhena antidysenterica	Stem bark	Antibacterial and antifungal activity [151], antioxidant activity [152].
	Seed	Antidiabetic and antihyperlipidemic [153].
Jatropha curcas	Fruit	Antimicrobial activity [154], anti- HIV activity [155], antiferlity activity [156].
	Bark	Wound healing activities [157].

1.2.4. Phytochemical Investigation and Scientific Reports on the Selected Plants.

Previous phytochemical studies on the selected plants have resulted in the isolation of alkaloids, glycosides, steroids**,** terpenoids, flavonoids, tannins, fats and many other phytochemicals. Some of the research work carried out is presented in Table 1.6.

Table 1.6: Some chemical compounds isolated from selected plant species.

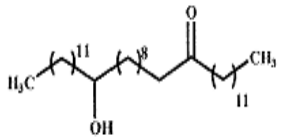
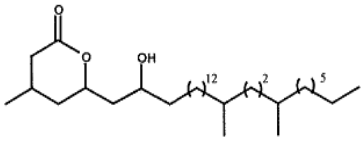
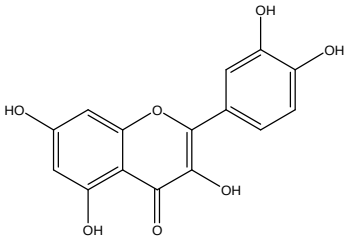
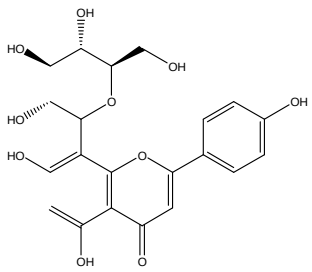
Plant species	Plant parts	Chemical constituents	Chemical structures
Grewia asiatica	Fruits	Anthocyanin-type cyanidin 3-glucoside [158], vitamins A and C, minerals, carotenes and dietary fibres etc [159].	
	Flowers	Grewinol, a long chain keto-alcohol, tetratricontane 22-ol 13-one [160].	 Grewinol
		A delta-lactone, 3, 21, 24 trimethyl- 5, 7-dihydroxyhetriacotanoic acid δ-lactone [161].	 Tetratricontane 22-ol 13-one
	Seeds	Oil that contains 8% palmitic acid, 11% stearic acid, 13.5% oleic acid, and 64.5% linoleic acid with 3% unsaponifiable [162].	
	Leaves	Flavonoids such as, quercetin, kaempferol, isorhamnetin, isorhamnetin 3-O-rhamnoside, vitexin, kampferol-7-O-glucoside and kampferol-3-O-(6″-O-E-p-coumaroyl)-glucoside [163].	 Quercetin  Vitexin

Table 1.6 contd…

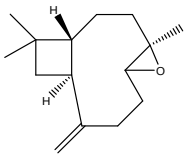
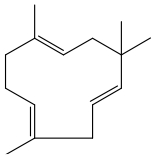
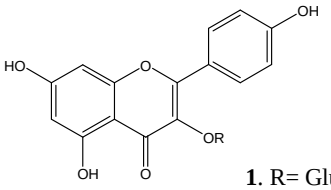
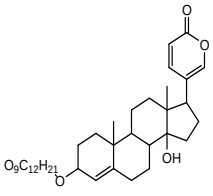
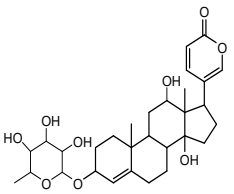
Plant species	Plant parts	Chemical constituents	Chemical structures
Alpinia nigra	Rhizome	β-caryophyllene oxide, α-pinene, linalool, α-phellandrene geranyl acetate, *β*-pinene and α-pinene and α-humulene etc [164].	 *β*-caryophyllen oxide  *α*- Humulene
	Seed	Two bioactive flavone glycosides, {astragalin (1) and kaempferol-3-*O*glucuronide (2)} [165].	 **1**. R= Glu **2.** R= GluUA 1. Astragalin, 2. Kaempferol-3-o glucuronide
Urginea indica	Bulb	Cardiac glycosides, scillaren-A and scillaren-B [166]. A novel cardiogenin, 6-desacetoxy scillirosidin [167]. Two bufadienolides (scilliphaeoside and anhydroscilliphaeosidin) [168]. Three flavonoid glycosides, 5,6-dimethyoxy-3',4''-dioxymethylene-7-O-(6''-beta-D-glucopyranosyl-beta-D-glucopyranosyl) flavanone (1), 5,4'-dihydroxy-3-O-alpha-L-rhamnopyranosyl-6-C-glucopyranosyl-7-O-(6''-para-coumaroyl-beta-D-glucopyranosyl) [139].	 Scillaren A  Scilliphaeoside

Table 1.6 contd…

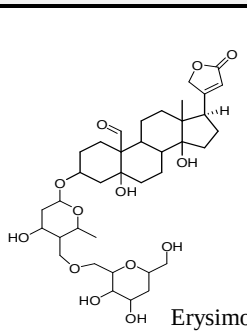
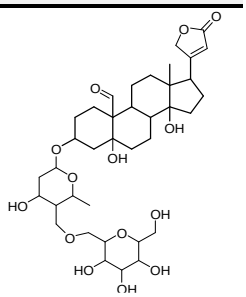
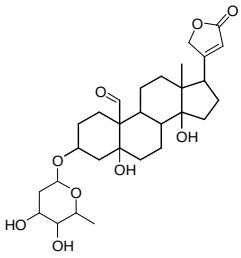
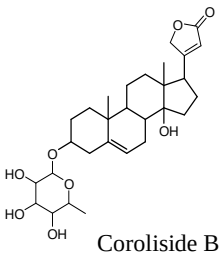
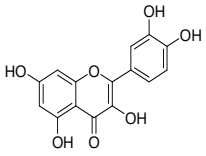
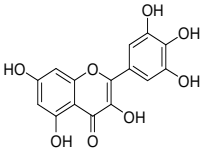
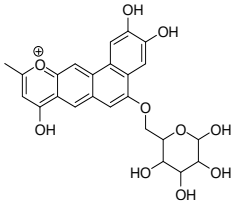
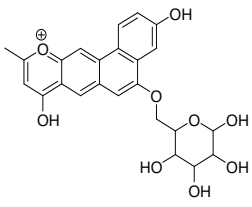
Plant species	Plant parts	Chemical constituents	Chemical structures
Corchorus olitorius Linn	Seed	Cardenolide glycosides, erysimoside, olitoriside, corchoroside A and coroliside. [169, 170].	 Erysimoside  Olitoriside  Corchoroside A  Coroliside B
Punica granatum Linn	Fruit	Phenolic compounds, quircetin, myricetin [171] The Anthrocyanine, cyanidin-3- glucose, cyanidin-3-rutinose, delphinidin-3- glucose and pelagonidin-3-glucose [172].	 Quircetin  Myricetin  Cyanidin-3- glucose  Pelagonidin-3- glucose

Table 1.6 contd…

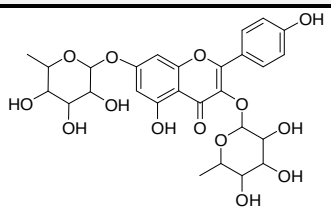
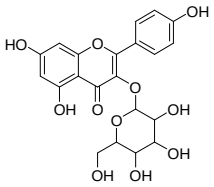
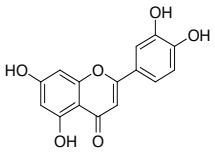
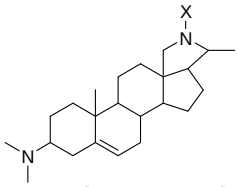
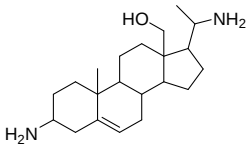
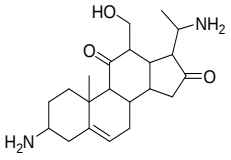
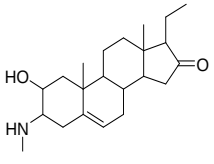
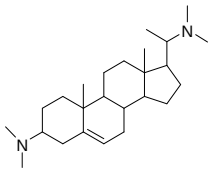
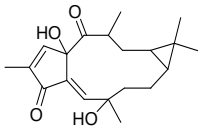
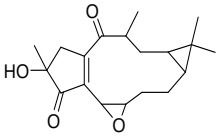
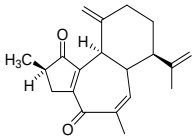
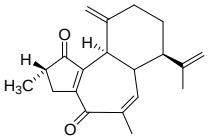
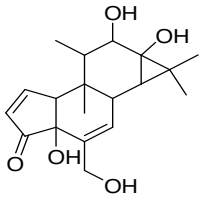
Plant species	Plant parts	Chemical constituents	Chemical structures
Cinnamomum tamala Nees	Leaves	Gglycosidic compound, kaempferol -3 -o-glycopyranoside, quercetin-3-o-sophoroside, Kaempferol 3,7-dirhamnoside and quercetin-3-o-rutinoside glycoside. Polyphenolic compounds, 3,4',5,7-tetrahyroxyflavone; 3,3',4',5,7-pentahydroxyflavone [173].	 Kaempferol 3, 7-dirhamnoside  Kaempferol -3-o-β-D-glucoside  3,4',5,7-Tetrahyroxyflavone
Holarrhena antidysenterica (L.) WALL	Bark	O-free Steroidal alkaloids (conessine, conissimine), conkurchine group alkaloids (including conessidine) and O-containing alkaloids (holarrhimine, holafrine, holarrhetine). Two new alkaloids – holacine and holacimine isolated from bark [174].	 Conessine X=CH_3 Conissimine  Holarrhimine
	Seed	Alkaloid Holarricine [175].	 Holarricine
	Leaves	O-containing alkaloids (kurchiphyllamine and kurchiphylline). Three new aminodeoxyglycosteroids – holarosine B and holantosines E and F. [176].	 Kurchiphyllamine  Kurchiphylline

Table 1.6 contd…

Plant species	Plant parts	Chemical constituents	Chemical structures
Jatropha curcas	Roots	Two lanthyrane diterpenes cuculanthyranes A and B, Curcusones A and B, and curcusones C and D [177].	 Cuculanthyrane A  Cuculanthyrane B  Curcusone A  Curcusone B
	Seed	Jatropherol-I [178]	 Jatropherol

1.3. Aims and Objectives

The main objectives of the present investigations were-

- ❖ Phytochemical and pharmacological screening of plants to evaluate the hypoglycemic properties of extracts from different plant.
- ❖ Safety and efficacy of plant extracts and comparative evaluation of their hypoglycemic, hypolipidemic and antioxidant activity in alloxan-induced diabetic rats.
- ❖ Isolation and identification of bioactive principles from the plant extract and further evaluation for their anti-diabetic potentials.

Chapter Two

Preliminary Screening of Plants

2.1. Introduction

Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes [50]. The hypoglycemic actions of some of these phytoconstituents have been evaluated and confirmed in animal models [179] suggesting that natural products could serve as a source for effective anti-diabetic agents. In this viewpoint, I have studied the preliminary pharmacological and phytochemical screening of selective medicinal plant species such as *Grewia asiatica* Linn., *Corcorus ollitoriou* Linn., *Alpinia nigra*, *Prunica granatum* Linn., *Jatropha curcas* Linn., *Cinamomum tamala* Nees., *Urginea indica* and *Hollarhena antidysenterica* (L) Wall., respectively which can be the basis for the development of new drugs [180] and can offer a new avenue to the development of new therapeutics in the management diabetes and its long term complications.

2.2. Materials and Methods

2.2.1. Collection of Plant Materials

Fresh plant parts from the eight selected medicinal plants were collected from different region and areas of Bangladesh. Fresh plant materials were washed under running tap water, air dried and then grinded to fine powder and then stored in airtight bottles.

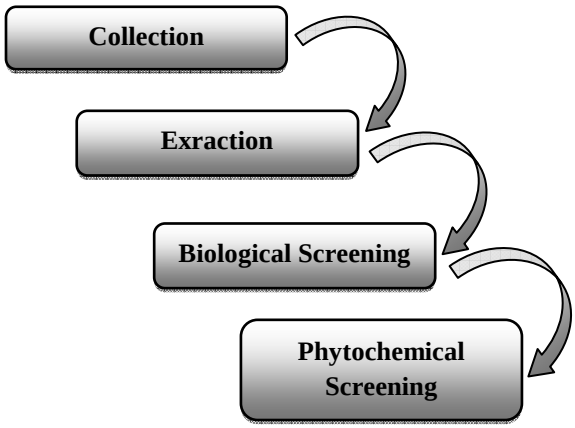


Figure 2.1: Preliminary screening protocol.

2.2.2. Preparation of Plant Extract

The powdered plant materials were extracted with rectified spirit (96% ethanol). The extracts were defatted with petroleum ether for several times. Then, the defatted

liquors were allowed to evaporate using rotary evaporator at temperature 40-45°C under reduced pressure. Finally, a highly concentrated crude ethanol extracts were obtained and kept in a desiccator to dry to give a solid mass.

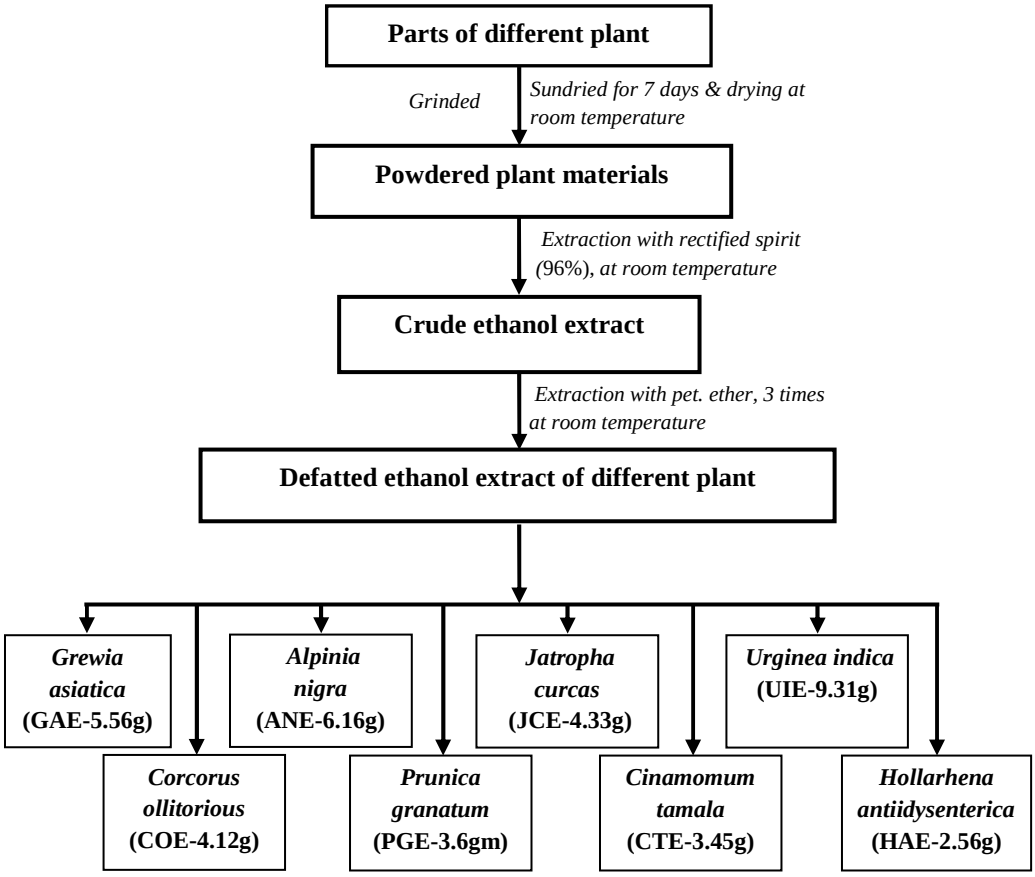


Figure 2.2: Schematric representation of extraction procedure. GAE, ethanol extract of *Grewia asiatica*; COE, ethanol extract of *Corcorus ollitorious*; ANE, ethanol extract of *Alpinia nigra*; PGE, ethanol extract of *Prunica granatum*; JCE, ethanol extract of *Jatropha curcas*; CTE, ethanol extract of *Cinamomum tamala*; UIE, ethanol extract of *Urginea indica*; HAE, ethanol extract of *Hollarhena antidysenterica*.

2.2.3. Biological Screening

2.2.3.1. Acute toxicity study

The acute oral toxicity study was carried out according to OECD guidelines [181]. No sign of toxicity and mortality were found even after administration of a limit dose of 2000mg/kg body weight of plant extracts for 3 days with exceptions for the plant extracts *Jatropha curcas* and *Urginea indica* whereby the administration of a limit dose was of 200mg/kg. Hence, one-fifth of LD_{50} doses of the ethanol extracts of all the eight plants *i.e.* 200 mg/kg/day p.o. of *Grewia asiatica*, 400 mg/kg/day p.o. of *Corcorus ollitorious*, 100 mg/kg/day p.o. of *Alpinia nigra*, 500 mg/kg/day p.o. of *Prunica granatum*, 20 mg/kg/day p.o. of *Jatropha curcas*, 400 mg/kg/ day p.o. of

Cinamomum tamala, 200 mg/kg/day p.o. of *Hollarhena antidysenterica* and 25 mg/kg/day p.o. of *Urginea indica*, respectively, were selected for the evaluation of antidiabetic effects.

2.2.3.2. Experimental animal

The most commonly used animal models for screening of antidiabetic activity are the alloxan induced diabetic animal models [182]. Alloxan exert their diabetogenic action when administered parenterally: intravenously, intraperitoneally or subcutaneously [182]. The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status.

Nine-weeks-old Norwegian Long Evans rats (140-170 g) purchased from ICDDRB, Dhaka, Bangladesh were kept in cages and maintained in well ventilated room under conditions of light and dark cycle. They were allowed with standard rat diet and water *ad libitum*. Throughout the study the animals were cared in accordance with the guidelines of our institution. The experimental protocol was approved by Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) at the Institute of Biological Sciences, University of Rajshahi, Bangladesh.

2.2.3.3. Induction of diabetes

After fasting 16hrs, diabetes was induced into rats by in intra-peritoneal injection (i.p.) of alloxan monohydrade (110 mg/kg), dissolved in saline. After 96 hrs, plasma glucose levels were measured by glucometer (Clever Check-TD 4226, Germany) using a blood sample from tail-vein of rat. Rats with blood sugar level higher than 11.5 mmols/l are considered as diabetic [95].

2.2.3.4. Assessment of hypoglycemic activity of extracts in diabetic rats

Diabetic rats were treated with plant extracts in one-fifth of LD_{50} doses of the respective ethanol extracts. The suspensions of extracts were prepared by using 0.5% methyl cellulose (MC) (SD fine chemicals, Mumbai, India) in water. Treatment with plant extracts was started 96 h after alloxan injection. After 1 hour of feeding of extracts blood samples were collected from the tail-vein of rats at 0, 30 and 60 mins. Plasma glucose level was estimated using glucose oxidase and peroxidase method [183].

2.2.4. Phytochemical Screening

Qualitative phytochemical analysis of the crude powder of the plant materials for the identification of phytoconstituents such as alkaloids, glycosides, tannins, steroids, phenols, terpenoids and flavonoid etc. were carried out using standard procedures described below [184, 185, 186].

Table 2.1: Phytochemical tests for the screening of plant extracts.

- **Alkaloids:** 200mg plant material in 10ml methanol, filtered. A 2ml filtrate + 1%HCl + Steam, 1ml filtrate + 6 drops of Mayor's reagents/Wagner's reagent /Dragendroff's reagent. Creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids.
- **Steroids (Liebermann-Burchard reaction):** 200mg plant material in 10ml chloroform, filtered. A 2ml filtrate + 2ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of steroids.
- **Terpenoids:** 2ml filtrate + 2ml acetic anhydride + conc. H_2SO_4, Blue-green ring indicated the presence of terpenoids.
- **Flavonoids:** Water extract of the plant was reduced to dryness on the boiling water bath. The residue was treated with dil. NaOH, followed by addition of dil. HCl solubility and color was noted. A yellow solution with NaOH which turns colorless with dil. HCl confirmed the presence of flavonoids.
- **Cardiac glycosides (Keller-kiliani test):** 2ml filtrate + 1ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4. Green blue color indicated the presence of cardiac glycosides.
- **Saponins (Frothing test):** 0.5ml filtrate + 5ml distilled water. Frothing persistence indicated the presence of saponins
- **Tannins and phenols:** 200mg plant materials in 10ml distilled water, filtered. 2 ml filtrate + 2 ml 15% $FeCl_3$. Blue-black precipitate indicated the presence of tannins and phenols.

2.3. Results and Discussion

On the basis of folkloric reputation eight selected medicinal plants (1 Kg each) were cold extracted with 96% ethanol and the % yields of extracts of the screened plants are shown in Table 2.2.

Table 2.2: The yield of different plant extracts.

Scientific name	Plant part	Yield (g)	Yield (%)
Grewia asiatica	Stem bark	5.56	1.1
Corcorus ollitorious	Seed	4.12	0.82
Alpinia nigra	Rhizome	6.16	1.23
Prunica granatum	Stem bark	3.65	0.73
Jatropha curcas	Root	4.326	0.86
Cinamomum tamala	Bark	3.45	0.69
Hollarhena antiidysenterica	Bark	2.56	0.512
Urginea indica	Bulb	9.31	1.86

The results of anti-hyperglycemic effect of all the eight plant extracts on the fasting blood sugar levels of diabetic rats are shown in table 2.3. Our study indicated that the doses GAE (200mg/kg), ANE (100mg/kg) and UIE (25mg/kg) showed remarkable and comparable glucose lowering efficacy (Table 2.3).

Table 2.3: Hypoglycemic effect of different plant extracts.

Scientific name	Plant part	Dose (mg/kg)	Blood glucose level (mmols/l)		
			0 min	30 min	60 min
Grewia asiatica	Stem bark	200	17.1 ± 2.00	13.6 ± 1.40	**12.3 ± 0.70**
Corcorus ollitorious	Seed	300	17.8 ± 0.14	15.8 ± 0.50	14.2 ± 0.15
Alpinia nigra	Rhizome	100	17.9 ± 1.70	13.9 ± 1.50	**12.2 ± 0.45**
Prunica granatum	Stem bark	400	18.8 ± 0.50	17.7 ± 0.40	15.2 ± 0.25
Jatropha curcas	Root	10	18.1 ± 0.25	17.4 ± 0.32	16.1 ± 0.54
Cinamomum tamala	Bark	400	17.2 ± 0.15	15.8 ± 0.14	14.8 ± 0.44
Hollarhena antiidysenterica	Bark	200	17.7 ± 0.35	15.2 ± 0.70	14.2 ± 0.22
Urginea indica	Bulb	25	18.4 ± 1.00	13.3 ± 2.00	**11.1 ± 0.70**

On the basis of hypoglycemic activity three plants *Grewia asiatica*, *Alpinia nigra* and *Urginea indica* were selected for phytochemical screening. The results of phytochemical analysis of the selected plant extracts are shown in Table 2.4.

Table 2.4: Preliminary phytochemical analysis of selected medicinal plants.

Secondary metabolites	**Inference**		
	Grewia asiatica	***Alpinia nigra***	***Urginea indica***
Alkaloids	-	-	-
	-	-	-
	-	-	-
Steroids and triterpenoids	+	+	+
	+	+	+
Flavonoids	+	+	+
	+	+	+
Glycosides	+	+	+
Saponins	+	-	-
Tannins	+	+	-

(+), indicates presence and (-), indicates absence

2.4. Conclusion

The present investigation indicated that among the selected eight (08) medicinal plant species, the stem barks of the plant *Grewia asiatica*, rhizomes of the plant *Alpinia nigra* and bulbs of the plant *Urginea indica* have demonstrable hypoglycemic activities. The phytochemical screening revealed the presence of steroids, terpenoids, flavonoids, glycosides, saponins and tannins which may be responsible for the glucose lowering activity of the extracts. However, further pharmacological investigations are required to know the effects of three (03) prominent plant extracts in diabetic rats.

Chapter Three

Rationale of the Study

3.1. Background

Diabetes mellitus is a debilitating and often life-threatening disease and the prevalence of diabetes is increasing among the world populations [187]. In addition to insulin currently available oral hypoglycemic agents are not effective because of their poor therapeutic outcomes. Lower efficacy, high cost and less availability are of great concern in the management of diabetes mellitus [188]. A scientific investigation of traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and therapeutic strategies. Thus, the development of new oral hypoglycemic agents and adjuncts to antidiabetic therapy is imperative [189].

Scientific evaluation of several Indian plant species has proved the efficacy of the botanicals in reducing blood sugar level [51]. There are numerous plants still await scientific investigation, which have mentioned in the indigenous systems of medicine. A large number of plants screened for their antidiabetic effect have yielded certain interesting leads, but till to date no plant-based drug has reached such an advanced stage of development [190]. Hence, the interest in herbal drug research continues with an expectation that some day, we would be able to bring a safer and more effective compound that will replace currently available oral synthetic drugs [17].

The three plants *Grewia asiatica* Linn, *Alpinia nigra* (Gaertn.) Burtt and *Urginea indica*, Knuth. were selected on the basis of their preliminary hypoglycemic and phytochemical screening. The plants are grown in Bangladesh and are distributed thought-out tropical Asia and have a folkloric reputation of hypoglycemic activity [10].

According to ethnomedical survey the plant *Grewia asiatica* (Family:Tiliaceae) have been used as antidiabetic, antifertility, antioxidant, antipyretic, analgesic, antibacterial agents [119, 120]. *Alpinia nigra* (Gaertn.) Burtt (Zingiberaceae) is widely used in the ayurvedic system of medicine in the treatment of diabetes mellitus, dyspepsia, vomiting, obesity, rheumatism, bronchitis, liver and kidney diseases [122].

Traditionally, the bulbs of *Urginea indica* Knuth (Family: Liliaceae) have been used as hypoglycemic, expectorant and cardiac stimulants; and also reported to have anticancer activity [105].

A comprehensive literature review showed that, a number of chemical constituents were isolated from the plants *Grewia asiatica* and *Alpinia nigra* but there were only few reports on the isolation of bioactive compounds from the plant *Urginea indica* (Table 1.6). However, the extracts and isolated compounds from the plant *Grewia asiatica* (Bark), *Alpinia nigra* (Rhizome) and *Urginea indica (*Bulbs*)* have not been so far evaluated for their anti-diabetic and hypo-lipidemic activity (Table 1.5). The present investigation was therefore undertaken to assess the hypoglycemic and hypolipidemic efficacy of ethanolic extract *of Grewia asiatica* (Bark), *Alpinia nigra* (Rhizome) and *Urginea indica* (Bulbs) in diabetic rats and isolation and identification of bioactive compounds from *Urginea indica* (Bulbs).

3.2. Study Protocol

The present study has given an insight into the phytochemical and biological investigations of selected Bangladeshi medicinal plants which includes:

1. **Preliminary phytochemical and pharmacological screening of plants.**
    - Collection of plants materials and extraction with rectified sprit (96% ethanol) by cold extraction method.
    - Preliminary phytochemical analysis of crude ethanol extracts from different plants.
    - Preliminary pharmacological (blood glucose lowering efficacy) screening of the crude ethanol extracts from different plants in alloxan-induced diabetic rats.

2. **Evaluation of the hypoglycemic, hypolipidemic and antioxidant activity of the plant extracts in alloxan-induced diabetic rats.**

    - Survival rates in rats after oral administration of plant extracts.
    - Time course of changes in (0, 5, 10 and 15 days) in blood sugar levels and body weights in diabetic rats.
    - Effects of plant extract on oral glucose tolerance tests (OGTT) in diabetic rats.
    - Effect of plants extracts on lipid profile (TC, TG, LDL, VLDL, HDL & LDL/ HDL ratio).
    - Measurement of liver glycogen, SGOT and CK-MB level after 15 days of oral treatment with plant extracts.

- Finally, analysis of in-vitro antioxidant activities of the extracts
  - Determination of total phenolic and flavonoid content of the plant extracts by Folin–Ciocalteu reagent and Aluminium chloride respectively.
  - Determination of the total antioxidant activity of the extractives by the method of Prieto *et al.,* (1999) and reducing power capacity by the method of Oyaizu *et al.,* (1986).
  - Observation of *in vitro* free radical scavenging activity of the different extract using DPPH assay.

3. Isolation and identification of bioactive principles from the biologically active plant extract and evaluation for their anti-diabetic potentials.

- Fractionation of the crude ethanol extract successively with petroleum ether, chloroform, ethyl acetate and finally with water.
- Evaluation of the blood glucose lowering efficacy of the crude fractions in diabetic rats.
- Isolation of pure compounds from most effective fraction by TLC and PTLC chromatographic analysis.
- Identification and characterization of pure compounds by 1D and 2D ^{1}H- and ^{13}C- NMR spectral analysis.
- Finally, evaluation of the anti-diabetic activity of isolated compounds in diabetic rats.

Chapter Four

Safety and Efficacy of Plant Extracts in Diabetic Rats

4.1. Introduction

In preliminary pharmacological screening, *Grewia asiatica*, *Alpinia nigra* and *Urginea indica* have been found to possess hypoglycemic activity as shown in Table 2.3. We investigated the safety and efficacy of the plant extracts in alloxan-induced diabetic rats after 15 days of oral administration. Primarily, we observed the survival rate, time course of changes in blood sugar and body weights after oral ingestion of plant extracts/drug in diabetic rats. Further, we have estimated various biochemical parameters, such as fasting blood sugar level (FBS), serum triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), enzymes serum glutamic-oxaloacetic transaminase (SGOT) and creatine kinase-MB (CK-MB) after 15 days of oral treatment.

4.2. Antidiabetic and Hypolipidemic Activity Assay

4.2.1. Materials and Methods

4.2.1.1. Drugs and chemicals

The standard drug, Metformin HCl was the generous gift sample obtained from Square Pharmaceuticals Ltd., Pabna, Bangladesh. Alloxan monohydrate was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Blood glucose concentration was measured by using a glucometer (One Touch Ultra, Japan). Plasma TG, TC, HDL, SGOT and CK-MB concentrations were analyzed using commercial diagnostic kits (Human, Germany). All other chemicals and solvents used were of analytical grade.

4.2.1.2. Plant materials

The plant parts were collected in fresh condition from matured plants. The rhizomes of the plant *Alpinia nigra* (Gaertn.) Burtt were collected from Meharchandi, the adjoining area of Rajshahi University campus, Rajshahi, during the month of January-February in 2010, the bulbs of *Urginea indica*, Knuth. were collected from sandy-areas of Chittagong during the month of April-May in 2010 and the stem barks of *Grewia asiatica* Linn. were collected from botanical garden of Rajshahi University, Rajshahi during the month of June-July in 2010, respectively.

4.2.1.3. Preparation of plant extracts

The collected plant parts were washed with running water, chopped into pieces and sun dried for several days. The dried plant materials were coarse powdered in an electrical grinder after overnight drying in oven below 50°C. The powdered plant materials were then extracted with rectified spirit (96% ethanol) in flat bottom glass containers (Aspirator). The bottles were kept at room temperature and allowed to stand for 7-10 days with occasional shaking and stirring. The extracts thus, obtained were filtered using cotton and then through filter paper (Whatman Fitter Paper No. 1). The filtrates were defatted with petroleum ether for several times. Then, the defatted liquors were allowed to evaporate using rotary evaporator at temperature 40-45°C under reduced pressure. Finally, a highly concentrated crude ethanol extracts were obtained and kept in a desiccator to dry to give solid masses.

4.2.1.4. Experimental animal

Nine-weeks-old Norwegian Long Evans rats (140-170g) purchased from ICDDRB, Dhaka, Bangladesh were kept in cages and maintained in well ventilated room under conditions of light and dark cycle (12hrs). They were allowed with standard rat diet and water *ad libitum*. Throughout, the study the animals were cared in accordance with the guidelines of our institution. The experimental protocol was approved by Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) at the Institute of Biological Sciences, University of Rajshahi, Bangladesh.

4.2.1.5. Induction of diabetes

After 16 hrs of fasting, diabetes was induced into rats by a single intra-peritoneal injection (i.p.) of alloxan monohydrade (110 mg/kg), dissolved in saline. After 96hrs, plasma glucose levels were measured by glucometer (One Touch Ultra, Japan) using a blood sample from tail-vein of rats. Rats with blood sugar level higher than 11.5mmols/l were considered as diabetic.

4.2.1.6. Experimental protocol design

After induction of diabetes, rats were divided into several groups for the oral administration of extracts/drugs or vehicle for 15 days. Age-matched healthy rats were used as normal control. The rats were divided into following groups-

1. Control Groups:

Group	Drug
Normal Control (NC)	Vehicle 0.5% MC
Diabetic Control (DC)	Vehicle 0.5% MC

2. Diabetic rats treated with standard drug Metformin

Group	Drug
Diabetic Standard (DS)	Metformin, 150mg/kg, 0.5% MC

3. Diabetic rats treated with different doses of *Grewia asiatica* extracts (GAE)

Group	Drug
GAE200	GAE 200mg/kg, 0.5% MC
GAE400	GAE 400mg/kg, 0.5% MC

4. Diabetic rats treated with different doses of *Alpinia nigra* extracts (ANE)

Group	Drug
ANE50	ANE 50mg/kg, 0.5% MC
ANE100	ANE 100mg/kg, 0.5% MC
ANE200	ANE 200mg/kg, 0.5% MC

5. Diabetic rats treated with different doses of *Urginea indica* extracts (UIE)

Group	Drug
UIE12	UIE 12mg/kg, 0.5% MC
UIE 25	UIE 25mg/kg, 0.5% MC

4.2.1.7. Time course of changes in blood glucose levels

The diabetic rats were subjected to oral administration of different doses of plant extracts daily for 15 days. Group NC and DC received vehicle, 0.5% MC and Group DS received metformin (150 mg/kg) in 0.5% MC. The blood glucose levels of rats

were measured on day 0, before the initiation and on days 5, 10 and 15 during the course of treatment. Blood samples were drawn from the tail-vein of rats. Blood glucose levels were measured by enzymatic analysis [183].

4.2.1.8. Measurement of body weight and organ weight

The body weight of rats from each group was measured at 0 day, before treatment and after 15 days of the oral administration of extracts/drugs. At the end of experiments, the rats were anesthetized, chest opened and blood samples were withdrawn directly from aorta and poured into blood collecting tube (K3, EDTA, Spain). The blood samples were centrifuged at 4000 rpm for 10 minutes (Ultra-centrifuge, Centurion, UK) and the plasma sample were freeze up at -40°C until further biochemical estimation. Rats were sacrificed and liver, pancrease and heart sample were removed and cleaned of the surrounding tissues. The organ weights (OW) were measured immediately and the ratio of organ weights to body weight (OW/BW) were calculated. Then, parts of pancreas were stored in 10% formalin for histopathology and liver samples were stored in a refrigerator at -40°C for glycogen estimations.

4.2.1.9. Analysis of lipid profile

The concentration of TC, TG and HDL cholesterol were measured colorimetrically by blood analyzer using commercially available wet reagent diagnostic kits (Human, Germany). The concentrations of low density lipoprotein (LDL) and very low density lipoprotein (VLDL)-cholesterol were calculated by using Friedewald formula, VLDL=TG/5, [LDL=TC-(HDL+VLDL)] [191]. The ratios of LDL to HDL cholesterol were calculated.

4.2.1.10. Estimation of serum glutamic oxaloacetic transaminase (SGOT) and creatinine kinase (CK-MB) levels

Estimations of CK-MB was done by immunoinhibition method using (Humazym UV-test) according to the manufacterer's protocol [192]. The liver enzyme such as serum glutamate oxaloacetate transaminase (SGOT) was determined by using kits obtained from commercial suppliers [193, 194, 195].

4.2.2. Statistical Analysis

Data were expressed as Mean±Standard error of mean (SEM). Statistical comparison were performed by one-way analysis of variance (ANOVA) followed by Tukey's test. Results considered as significant when $p<0.05$. Statistical calculations were done using MS Excel and graphs were prepared using Graph Pad Prism Software, version 5.00 for Windows.

4.3. Results and Discussion

4.3.1. *Grewia asiatica*

4.3.1.1. Survival rate

Table 4.1 shows the survival rate among the rats after 15 days of oral ingestion of GAE. Three of 5 (60%) rats died in Group DC between days 1 to15. None of the rats died in Groups DS, GAE200 and GAE400. The 15 days survival rate was significantly higher among the treatment groups than in Group DC ($p<0.001$).

Table 4.1: Survival rate in rats after oral administration of different doses of GAE.

Treatment and dose	0 Day	5 Days	10 Days	15 Days
Normal Control (NC)	100	100	100	100***
Diabetic Control (DC)	100	80	60	40†††
Diabetic Standard (DS)	100	100	100	100***
GAE 200 (200mg/kg)	100	100	100	100***
GAE 400 (400mg/kg)	100	100	100	100***

The results are expressed in percentages (%). Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received metformin 150mg/kg/day. † $p<0.05$, †† $p<0.01$ and ††† $p<0.001$ vs. NC *$p<0.05$, **$p<0.01$ and *** $p<0.001$ vs DC.

4.3.1.2. Hypoglycemic effect of GAE in alloxan-induced diabetic rats

Time course of changes in blood sugar levels were shown in Figure 4.1. On 0 day, before treatment the blood glucose levels were significantly higher in DC rats compared to NC ($p<0.01$). On day 5, 10 and 15, the blood sugar levels were significantly lowered in Groups DS, GAE200 and GAE400 when compared with Group DC rats. The effects of GAE on blood sugar levels were dose-dependent and no significant differences between Groups GAE200, GAE400 and Group DS were observed during the course of treatment.

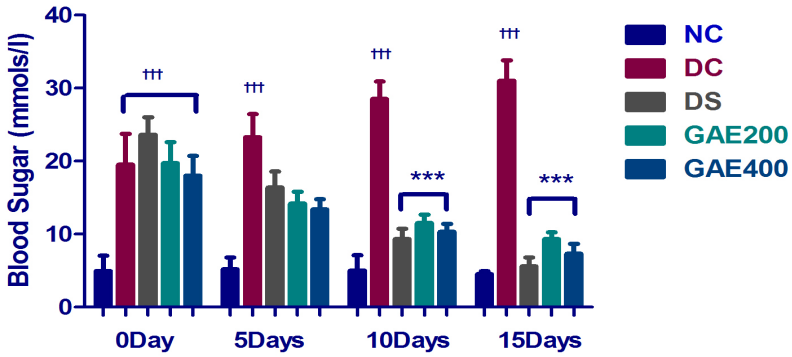


Figure 4.1: Time course of changes in blood sugar level after treatment of rats with *Grewia asiatica* extract. Data expressed in Mean±SEM. Control group received 0.5% methyl cellulose and standard group received 150mg/kg Metformin. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC *p<0.05, **p<0.01 and *** p<0.001 vs DC.

4.3.1.3. Changes in body weight, organ weight and their ratio

Body weight, organ weights and organ weight to body weight ratios are summarized in Table 4.2. Body weights and organ weights were decreased in Group DC compared to Group NC rats. Although treatment with GAE improved body weights, organ weights and organ weight to body weight ratios, the effects were not significant among the treatment groups.

Table 4.2: Effect of GAE on body weight, organ weight and organ weight to body weight ratio in alloxan-induced diabetic rats.

Group	BW (g)	HW (g)	LW (g)	PW (g)	H/B (g/kg)	L/B (g/kg)	P/B (g/kg)
NC	150±7	0.48±0.025	5.51±0.47	0.40±0.04	3.1±0.1	35.5±1.23	2.3±0.4
DC	120±6†	0.34±0.025†	3.5±0.22†	0.25±0.02†	2.6±0.2	26.5±2.7†	2.0±0.08
DS	143±7	0.49±0.03	5.1±0.81	0.38±0.04	3.2±0.22	34.2±2.5	2.2±0.26
GAE200	136±6	0.47±0.035	4.23±0.26	0.30±0.03	3.25±0.1	30±1	2.1±0.1
GAE400	130±5	0.45±0.023	4.11±0.25	0.26±0.035	3.3±0.23	30.5±1.1	2.0±0.02

The results are expressed as Mean±SEM. H/B, ratio of heart weight to body weight; L/B, ratio of liver weight to body weight; P/B ratio of pancreas weight to body weight. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

4.3.1.4. Alteration in lipid profile

The changes in lipid profile are shown in Table 4.3. The data revealed that both TC and TG levels were significantly elevated in Group DC compared to the Group NC rats. The level of TC and TG were significantly reduced after ingestion of GAE (200

and 400) and were comparable to NC rats. The plasma HDL level was significantly lower and the levels of VLDL, LDL and LDL/HDL ratio were significantly higher in Group DC. The increment of VLDL, LDL and LDL/HDL ratio were significantly attenuated and HDL level was restored among the treatment groups and was comparable to NC rats. The Group GAE 400 exhibited greater improvement in lipid profile among the treatment groups.

Table 4.3: Effect of GAE on lipid profile in alloxan-induced diabetic rats.

Group	[a]TC (mg/dl)	[b]TG (mg/dl)	VLDL	LDL (mg/dl)	HDL (mg/dl)	LDL/HDL
NC	70.0±7.4	71.7±6.6	14.3±4.5	37.6±5.6	29.0±5.7	1.3.0±0.37
DC	136.7±14.7†††	115.7±5.88†††	23.1±1.17†††	76.3±4.32†††	5.7±1.77†††	13.2±0.6†††
DS	79.0±6.27**	73.5±6.16***	9.8±3.6***	25.0±5.56***	41.5±2.12***	0.60±0.06***
GAE200	68.3±5.65***	83.7±5.01**	16.7±1**	18.0±3.74†***	29.3±3.62***	0.61±0.08***
GAE400	67.3±8.04***	77.0±3.74***	15.4±.74**	16.3±2.94†***	34.3±2.01***	0.48±0.05***

The results are expressed as Mean±SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.
[a]TC: Total cholesterol
[b]TG: Triglycerides.

4.3.1.5. Changes in plasma SGOT and CK-MB levels

Figure 4.2 shows the levels of SGOT and CK-MB after 15dyas of oral administration of GAE in diabetic rats. The level of SGOT and CK-MB were significantly higher in Group DC rats. Oral ingestion of GAE significantly reduced the SGOT and CK-MB levels and was comparable to NC rats. The effects of GAE on SGOT and CK-MB were dose-dependent.

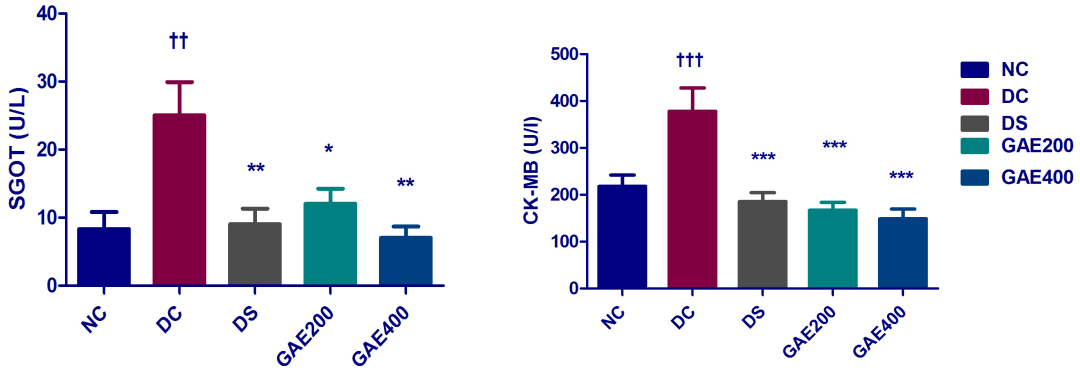


Figure 4.2: Effect of *Grewia asiatica* extract on SGOT and CK-MB in alloxan-induced diabetic rats. The results are expressed as Mean±SEM. Group NC and DC received 0.5% methyl cellulose and standard group DS received 150mg/kg metformin . † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

4.3.2. *Alpinia nigra*

4.3.2.1. Survival rate

The effects of different doses of *Alpinia nigra* extracts (ANE) on the survival rate of diabetic rats are shown in Table 4.4. Sixty (60%) rats died in Group DC during the course of treatment. The 15 days survival rate among the treatment groups was 100% ($p<0.001$).

Table 4.4: Survival rate in rats after oral administration of different doses of ANE.

Group	Total animals	Survivors	Deaths	Survival rate (%)
Normal control (NC)	5	5	0	100
Diabetic Control (DC)	5	2	3	40 †††
Diabetic standard (DS)	5	5	0	100 ***
ANE50 (50mg/kg)	5	5	0	100 ***
ANE 100 (100mg/kg)	5	5	0	100***
ANE 200 (200mg/kg)	5	5	0	100 ***

Data expressed in percentages (%). Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150 mg/kg metformin. *p<0.05, **p<0.01 and *** p<.001 vs. DC, †p<0.05, †† p<0.01 and ††† p<0.001 vs. NC.

4.3.2.2. Hypoglycemic effect of Alpinia nigra extracts (ANE) in diabetic rats

Time course of changes in the blood sugar levels in diabetic rats are shown in Figure 4.3. The blood glucose levels were significantly higher in Group DC compared to NC rats. Oral administration of ANE reduced blood sugar levels in diabetic rats during the course of treatment and the effects were dose-dependent. Group ANE100 and Group ANE200 rats showed significant glucose lowering efficacy between 10-15 days and were comparable to Group DS.

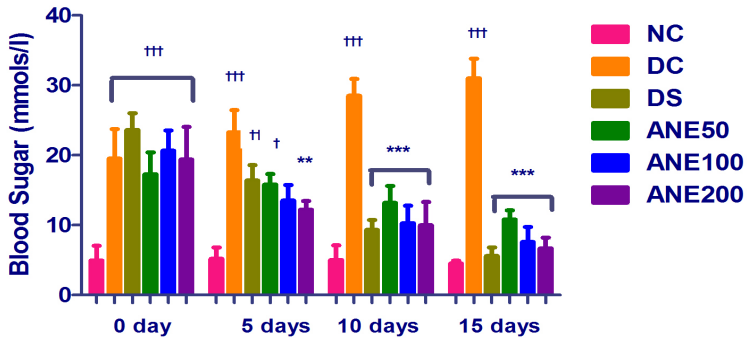


Figure 4.3: Time course of changes in blood sugar levels after treatment of rats with *Alpinia nigra.* Data expressed in Mean±SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

4.3.2.3. Changes in body weight, organ weight and their ratio

Body weight, organ weight and organ weight to body weight ratios are summarized in Table 4.5**.** No significant changes in the body weight were observed among the groups during the course of treatments. The liver weight and liver weight to body ratios were improved among the treatment groups.

Table 4.5: Effect of ANE on body weight, organ weight and organ weight to body weight ratio in alloxan-induced diabetic rats.

Group	BW (g)	HW (g)	LW (g)	PW (g)	H/B (g/kg)	L/B (g/kg)	P/B (g/kg)
NC	150±7	0.5±0.025	5.51±0.47	0.4±0.04	3.1±0.1	35.5±1.2	2.3±0.4
DC	120±6†	0.3±0.025††	3.5±0.22†††	0.3±0.02†	2.6±0.2	26.5±2.7†	2.0±0.08
DS	143±7	0.49±0.03	5.1±0.81	0.38±0.04	3.2±0.22	34.2±2.5	2.2±0.26
ANE 50	137±1.4	0..39±0.014	3.9±0.16	0.3±0.03	2.8±0.08	28.5±0.9	2.2±0.43
ANE 100	140±1.08	0.43±0.016	4.4±0.21*	0.4±0.06	3.0±0.11	34.2±1.23*	2.5±0.16
ANE 200	145±5.3*	0.44±0.029	5.1±0.35**	0.4±0.01	3.1±0.31	35.2±1.04*	2.58±0.22

The results are expressed as Mean±SEM. H/B, ratio of heart weight to body weight; L/B, ratio of liver weight to body weight; P/B ratio of pancreas weight to body weight. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

4.3.2.4. Alteration in lipid profile

The changes in lipid profile are shown in Table 4.6. Our results revealed that both TC and TG levels were significantly elevated in Group DC rats. However, the treatment with ANE significantly reduced both levels and the effect was comparable to NC rats. The plasma HDL level was significantly lower and the levels of LDL and LDL/HDL ratio were significantly higher in Group DC rats. The HDL was increased and increment of both LDL and LDL/HDL ratio were significantly restored among the treatment groups and the effects were dose-dependent.

Table 4.6: Effect of ANE on lipid profile in alloxan-induced diabetic rats.

Group	[a]TC (mg/dl)	[b]TG (mg/dl)	VLDL	LDL (mg/dl)	HDL (mg/dl)	LDL/HDL
NC	70.0±7.4	71.7±6.6	14.33±4.5	37.6±5.6	29.0±5.7	1.3±0.37
DC	136.7±14.7†††	115.7±5.88†††	23.13±1.17†††	76.3±4.32†††	5.7±1.77†††	13.2±0.6†††
DS	79.0±6.27**	73.5±6.16**	14.7±2.5***	25.0±5.56***	41.5±2.12***	0.60±0.06***
ANE50	85.5±10.1*	69.33±12.5**	13.86±2.1**	36.33±1.48***	23.66±3.34*	1.14±0.22***
ANE100	66±13.09***	62±2.12**	12.4±1.7**	26.66±1.92***	36.3±4.26***	0.63±0.22***
ANE200	65±7.07***	56±12.02**	11.2±1.5**	19.25±1.06†***	40±1.41***	0.48±0.01***

The results are expressed as Mean±SEM. HDL, high-density lipoprotein; LDL, low-density lipoprotein, LDL/HDL, ratio of low-density lipoprotein to high-density lipoprotein; Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.
[a]TC: Total cholesterol
[b]TG: Triglycerides.

4.3.2.5. Changes in SGOT and CK-MB levels

The levels of SGOT and CK-MB are shown in Figure 4.4. The relatively high levels of SGOT and CK-MB indicating inflammation and tissue damage in diabetic rats. However, treatment with ANE reduced the level of SGOT and CK-MB significantly.

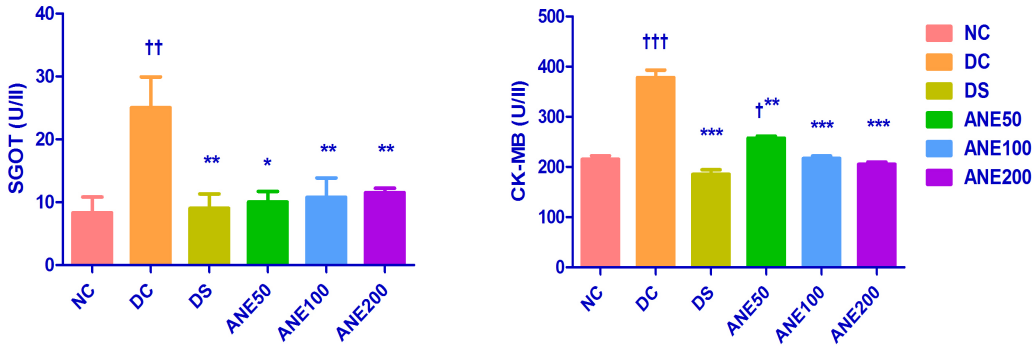


Figure 4.4: Effect of *Alpinia nigra* extract on SGOT and CK-MB in alloxan-induced diabetic rats. The results are expressed as Mean±SEM. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

4.3. 3. *Urginea indica*

4.3.3.1. Survival rate

Table 4.7 shows the survival rate among the groups of rats after 15 days of treatment. Three of 5 rats (60%) in Group DC died between days 1 to 15. None of the rats died in UIE 12, UIE 25 and Group DS. The 15 days survival rate were significantly higher among the treatment groups than in Group DC (p<0.001).

Table 4.7: Survival rate in rats after oral administration of different doses of UIE.

Treatment and dose	Total animals	Survivors	Deaths	Survival rate (%)
Normal control (NC)	5	5	0	100
Diabetic Control (DC)	5	2	3	40 †††
Diabetic standard (DS)	5	5	0	100 ***
UIE 12 (12mg/kg)	5	3	2	100 ***
UIE 25 (25mg/kg)	5	5	0	100 ***

The results are expressed as Mean±SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin.*p<0.05, **p<0.01 and *** p<.001 vs. DC, †p<0.05, †† p<0.01 and ††† p<0.001 vs. NC.

4.3.3.2. Hypoglycemic effect of Urginea indica bulb extracts (UIE) in alloxan-induced diabetic rats

Figure 4.5 shows the time course of changes in blood sugar levels. The blood glucose levels were significantly higher in Group DC rats. The extract, UIE showed significant glucose lowering efficacy and the effects were dose dependent.

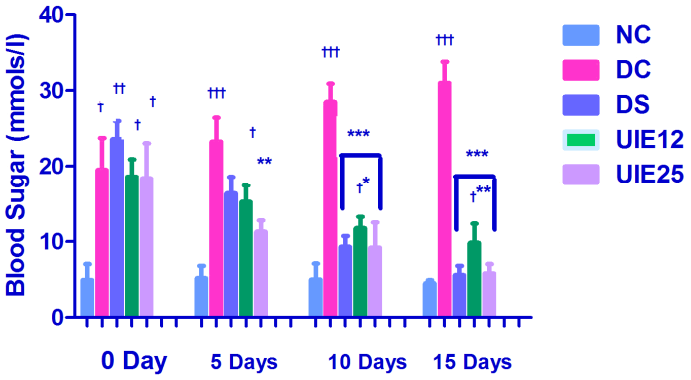


Figure 4.5: Time course of changes in blood sugar level after treatment of rats with *Urginea indica*. Data expressed in Mean±SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin. *p<0.05, **p<0.01 and *** p<.001 vs. DC and p<0.05, †† p<0.01 and ††† p<0.001 vs. NC.

4.3.3.3. Changes in body weight and organ weight in rats treated with UIE

Table 4.8 summarizes the body weight, organ weight and organ weight to body weight**.** No significant changes in organ weight to body weight ratio were observed among the experimental groups during the course of treatments. The results revealed that the liver and pancreas weight and their ratios were significantly improved in UIE25 rats.

Table 4.8: Effect of *Urginea indica* extract on body weight and organ weight in alloxan-induced diabetic rats.

Group	BW (g)	HW (g)	LW (g)	PW (g)	H/B (g/kg)	L/B (g/kg)	P/B (g/kg)
NC	150±7	0.48±0.025	5.51±0.47	0.40±0.04	3.1±0.1	35.5±1.23	2.3±0.02
DC	120±6†	0.34±0.025††	3.5±0.22†	0.25±0.02†††	2.6±0.2	26.5±2.7†	2.0±0.08
DS	143±7	0.49±0.03	5.1±0.81	0.38±0.04	3.2±0.22	34.2±2.5	2.2±0.26
UIE12	126±3	0.37±0.021†	4.06±0.36*	0.35±0.01**	2.9±0.18	32.1±1.9†	2.1±0.01
UIE25	141±1	0.47±0.042	5.37±0.15**	0.41±0.05***	3.37±0.24	38.06±2*	2.7±0.06*

The results are expressed as Mean±SEM. H/B, ratio of heart weight to body weight; L/B, ratio of liver weight to body weight; P/B ratio of pancreas weight to body weight. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

4.3.3.4. Alteration in lipid profile

Table 4.9 shows the changes in lipid profile after 15-days of oral treatment. Our results revealed that high level of TC and TG were significantly reduced among the treatment Groups and were comparable to Group NC rats. The increment of both LDL and LDL/HDL ratio were significantly attenuated when compared with DC rats. The

Group UIE25 exhibited greater improvement in lipid profile among the treatment groups and the effects were dose-dependent.

Table 4.9: Effect of *Urginea indica* bulb extract on lipid profile after 15 days in alloxan-induced diabetic rats.

Group	[a]TC (mg/dl)	[b]TG (mg/dl)	VLDL	LDL (mg/dl)	HDL (mg/dl)	LDL/HDL
NC	70.0±7.4	71.7±6.6	14.3±4.5	37.6±5.6	29.0±5.7	1.3±0.37
DC	136.7±14.7†††	115.7±5.88†††	23.13±1.17†	76.3±4.3†††	5.7±1.7†††	13.2±0.6†††
DS	79.0±6.27***	73.5±6.16***	14.7±2.5	25.0±5.5***	41.5±2.1***	0.60±0.06††***
UIE12	111.6±5.40†	104±2.31†††	14.8±5.6	50.3±5.3**	16.6±1.4†††	3.2±0.19***
UIE25	53.66±6.79***	51.66±2.41***	10.3±1.28**	15.8±1.4†***	54.3±2.1***	0.29±0.013†††***

Data expressed in Mean±SEM. VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL/HDL, ratio of low-density lipoprotein to high-density lipoprotein. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.
[a]TC: Total cholesterol; [b]TG: Triglycerides.

4.3.3.5. Changes in plasma SGOT and CK-MB levels

The levels of SGOT and CK-MB are summarized in Figure 4.6. In DC rats SGOT and CK-MB levels were elevated as compared to NC rats. The relatively higher levels of SGOT and CK-MB were significantly decreased and the effect was dose-dependent.

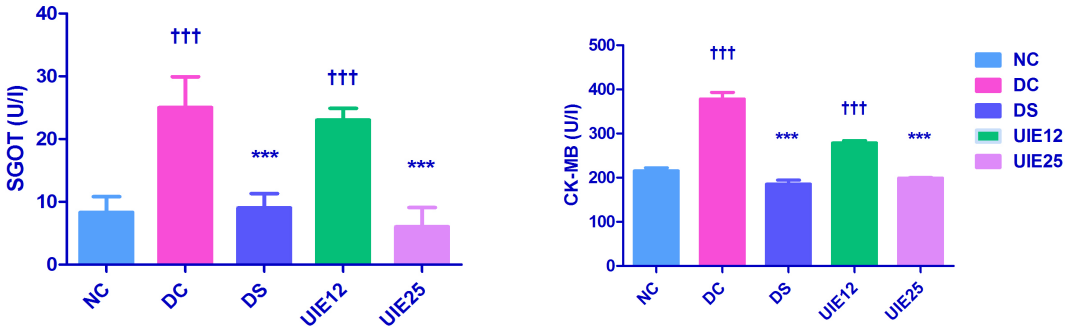


Figure 4.6: Effect of *Urginea indica* extract on SGOT and CK-MB in alloxan-induced diabetic rats. Each group comprised of 5 animals. The results are expressed as Mean±SEM. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

4.4. Conclusion

The present investigation indicated that the parts from the plants *Grewia asiatica, Alpinia nigra* and *Urginea indica* possessed antidiabetic and hypolipidemic properties. The extracts improved the organ weights, organ weight to body weight ratios, lipid profile and attenuated the SGOT and CK-MB levels in diabetic rats. However, we were interested to know the possible mechanism of their beneficial effects in alloxan-induced diabetic rats.

Chapter Five

Comparative Study of Plants in Diabetic Rats

5.1. Introduction

Ehanol extract of *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and *Urginea indica* (UIE) have been found to ameliorate high level blood sugar and lipid profile and thus improved survival rate in diabetic rats (Chapter 4). Therefore, the present study protocol was designed to compare the effects of the extracts from the three plants on OGTT, lipid profiles, liver glycogen and histopathology in alloxan-induced diabetic rats. This study also involved comparison of *in-vitro* antioxidant potential of the extracts from the three medicinal plants. On the basis of the comparable hypoglycemic activity the doses of the extracts were selected as GAE (200mg/kg), ANE (100mg/kg) and UIE (25 mg/kg), respectively (Table 2.3).

5.2. Materials and Methods

5.2.1. Comparison of Antidiabetic Activity

5.2.1.1. Experimental design

The animals, induction of diabetes and experimental design for the comparative antidiabetic activities were described earlier in Chapter 4, Section 4.2.1.4, 4.2.1.5, and 4.2.1.6.

5.2.1.2. Oral glucose tolerance test (OGTT)

Blood glucose levels of rats were measured after fasting over-night. After 1 hour of feeding of extracts and/drugs, all rats given a glucose load (2g/kg). Blood samples were withdrawn from the tail-vein of rats at 0, 30, 60 and 120 mins after glucose loading. Plasma glucose level was estimated using glucose oxidase and peroxidase method [183].

5.2.1.3. Analysis of lipid profile

The concentration of TC, TG and HDL cholesterol were measured colorimetrically by blood analyzer using commercially available wet reagent diagnostic kits (Human, Germany). The concentrations of low density lipoprotein (LDL) and very low density lipoprotein (VLDL)-cholesterol were calculated by using Friedewald formula, VLDL=TG/5, [LDL=TC-(HDL+VLDL)] [191]. The ratios of LDL to HDL cholesterol were calculated.

5.2.1.4. Estimation of liver glycogen content

The liver glycogen content was determined according to the method described by Tarnoky K. *et al*. [196]. Briefly, it utilizes the o-toluidine-glucose coupling reaction for the estimation of glycogen after trichloroacetic acid (TCA) extraction, precipitation by alcohol and hydrolysis.

5.2.1.5. Histopathological study

The histopathological studies of pancreas were carried out at the Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh. Briefly, for light microscopy pancrease were fixed in PBS containing 10% formalin. The tissues were washed in running tap water, dehydrated in the descending grades of isopropanol and finally cleared in xylene. The tissues were then embedded in molten paraffin wax. After embedding in paraffin, several transverse sections (5 μm) were cut from the mid organ level and stained with hematoxylin-eosin stain. The specimens were observed under light microscope for any pathological changes (CIA-102; Olympus, Tokyo, Japan).

5.2.2. *In-Vitro* Biological Assay for Antioxidant Activity

5.2.2.1. Determination of total phenolic content

The total phenolic contents of extracts from *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and of *Urginea indica* (UIE) were determined by the method described by Singleton *et al.*, [197] involving Folin-Ciocalteu reagent (FCR) as oxidizing agent and Gallic acid as standard.

The FCR actually measures a sample's reducing capacity. Chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotungstates - molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})_4$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI):

$$Mo\ (VI) + e^{-} \rightarrow Mo\ (V)$$

Materials:

Folin-ciocalteu reagent, Sodium carbonate, Methanol (Sigma, USA), Gallic acid (Wako pure chemicals Ltd., Japan), Micropipette (10-100 µl), Pipette (1-10 ml), UV-spectrophotometer (Shimadzu, USA).

Procedure:

1. 0.5 ml methanolic solution of extractives (or standard) at various concentration were introduced into test tubes.
2. 2.5 ml of Folin-ciocalteu reagent (diluted 10 times with water) was added into each of the test tubes.
3. 2.5 ml of sodium carbonate (7.5%) were added.
4. The test tubes were then incubated for 20 min at room temperature to complete the reaction.
5. Then absorbance at 760 nm was measured by UV-Spectrophotometer.
6. A typical blank solution contained all reagents except plant extract or standard.

The total phenolic content was expressed as Gallic acid equivalents in milligrams per gram extract as calculated from standard Gallic acid graph by the following formula:

$$C = (c \times V)/m$$

Where,

C = total content of phenolic compounds, mg/g plant extract, in Gallic acid equivalents;

c = the concentration of Gallic acid established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of different pure plant extracts, gm.

5.2.2.2. Determination of total flavonoid (TF)

Total flavonoid content of the different extracts from *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and of *Urginea indica* (UIE) were determined by aluminum chloride colorimetric method. Quercetin was used as standard [198].

Principle:

The content of total flavonoid in different extractives of plant extracts such as GAE, ANE and UIE were determined by the well known aluminum chloride colorimetric method. In this method aluminum chloride forms complex with hydroxyl groups of flavonoid present in the samples. This complex has the maximum absorbance at 510 nm.

Materials:

Aluminum Chloride, 1mM NaOH, Methanol (Sigma chemical company, USA), Quercetin (Wako pure chemicals Ltd., Japan), Micropipette (10-100 µl), Pipette (1-10 ml), UV-spectrophotometer (Shimadzu, USA).

Procedure:

Total flavonoid (TF) was determined by the following procedure by Ordonez *et al.* [199].

1. 0.5 ml methanolic solutions of extractives or standard at various concentrations were introduced into test tubes.
2. 2ml of distilled water was added into each of the test tubes.
3. Then 0.15ml of 10% $AlCl_3$ was added.
4. After 6 min 1 ml of 1M NaOH was added and volume is made up to 5ml with distilled water.
5. The solution was mixed and absorbance measured at 510 nm

The total content of flavonoid compounds in plant extracts was expressed as Quercetin equivalents in mg per gram of plant extracts as calculated from standard Quercetin graph was calculated by the following formula equation:

$$C = (c \times V)/m$$

Where,

C = total content of flavonoid compounds, mg/g plant extract, in Quercetin equivalent (GAE)

c = the concentration of quercetin established from the calibration curve, mg/ml

V = the volume of extract, ml

m = the weight of pure plant extracts, gm.

5.2.2.3. Determination of total antioxidant capacity

Total antioxidant capacity of different extracts from *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and of *Urginea indica* (UIE) were determined by the method described by Prieto *et al.* [200] with some modifications.

Principle:

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex in acidic pH. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI) and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

$$\text{Mo (VI)} + e \rightarrow \text{Mo (V)}$$

Materials:

Sulphuric acid (Merck, Germany), Sodium phosphate, ammonium Molybdate and Methanol (Sigma chemical company, USA), Ascorbic acid (Analytical or Reagent grade), Water bath, Micropipette (100-1000µl), Pipette (1-10ml), UV-spectrophotometer (Shimadzu, USA)

Procedure:

1. 0.5 ml solutions of different extractives or standard at different concentrations were taken into the test tubes.
2. 3ml of reaction mixtures (containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate) were added into each of the test tubes.
3. The test tubes were incubated at 95°C for 10 mins to complete the reaction.
4. Then absorbance at 700 nm was measured using a UV-Spectrophotometer against blank after cooling at room temperature.
5. A typical blank solution contained 3ml of reaction mixtures and the appropriate volume (0.5ml) of the same solvent used for the sample, and it was incubated under the same conditions.

5.2.2.4. Reducing power capacity assessment

The reducing power of different extracts from *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and of *Urginea indica* (UIE) were evaluated by the method described by Oyaizu *et al.* [201].

Principle:

In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

$$Fe^{3+}\text{-ferricyanide} + e \quad \rightarrow \quad Fe^{2+}\text{-ferricyanide}$$

Materials:

Potassium ferricyanide and Trichloro acetic acid (Merck, Germany), Ferric chloride, and Ascorbic acid (Sigma chemical company, USA), Phosphate buffer (Sigma-Aldrich, USA), Water bath, Centrifuge machine, Pipette (1-10 ml), UV spectrophotometer (Shimadzu, USA).

Procedure:

1. 0.25 ml solution of different extractives or standard at different concentration was taken into the test tubes.
2. 0.625 ml of potassium buffer (0.2 M) and 0.625ml of potassium ferricyanide (1%) solution were added into each of the test tubes.
3. The reaction mixture was incubated at 50°C for 20 mins to complete the reaction.
4. 0.625 ml of TCA (10%) solution was added into each of the test tubes.
5. The total mixture was centrifuged at 3000rpm for 10 mins.
6. 1.8 ml supernatant was withdrawn from the mixture and mixed with 1.8ml of distilled water.
7. 0.36 ml of ferric chloride (0.1%) solution was added to the diluted reaction mixture.

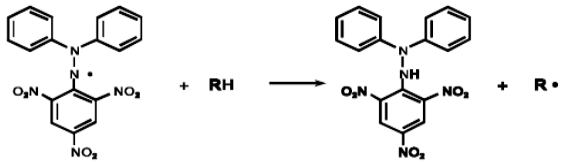
8. Then, absorbance at 700 nm was measured using a UV-Spectrophotometer against blank.
9. A typical blank solution contained all reagents except plant extract or standard and it was incubated under the same conditions as the rest of the samples solution.

5.2.2.5. DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

DPPH was used to evaluate the free radical scavenging activity of various compounds as well as extracts [202].

Principle:

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method antioxidant activity can be measured by observing color change from purple to yellow. The absorbance (517 nm) decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.



DPPH
517 nm

Materials:

DPPH and Methanol (Sigma, USA), BHT (Butylated hydroxy toluene), Pipette (1-10 ml), UV Spectrophotometer (Shimadzu, USA).

Procedure:

1. 2 ml methanol solution of the different extractives or standard at different concentration was taken into the test tubes.
2. 3 ml methanol solution of DPPH was added into each of the test tubes.
3. The test tubes were then incubated at room temperature for 30 mins in dark place to complete the reaction.

4. Then absorbance at 517 nm was measured using a UV-Spectrophotometer against blank.
5. A typical blank solution contained all reagents except plant extract or standard.
6. The percentage (%) of inhibition activity was calculated from the following equation:

$$I\% = [(A_0 - A_1)/A_0] \times 100,$$

Where,

I% is the percentage of scavenging activity

A_0 is the absorbance of the control, and

A_1 is the absorbance of the extract/standard.

Then % of inhibition was plotted against concentration and from the graph IC_{50} was calculated.

5.3. Results and Discussion

5.3.1. Comparison of Antidiabetic Activity

5.3.1.1. Effect on oral glucose tolerance test (OGTT) in diabetic rats

As shown in Figure 5.1, after oral glucose load, the blood glucose levels were significantly higher in DC rats. In Group DC, blood glucose concentration were peaked after 30 mins and remained high over 120 mins. Rats in Groups GAE200, ANE100 and UIE25 showed a significant attenuation in blood glucose concentration at 60 mins and 90 mins as compared with Group DC. The pronounced glucose tolerance was observed with Group UIE 25.

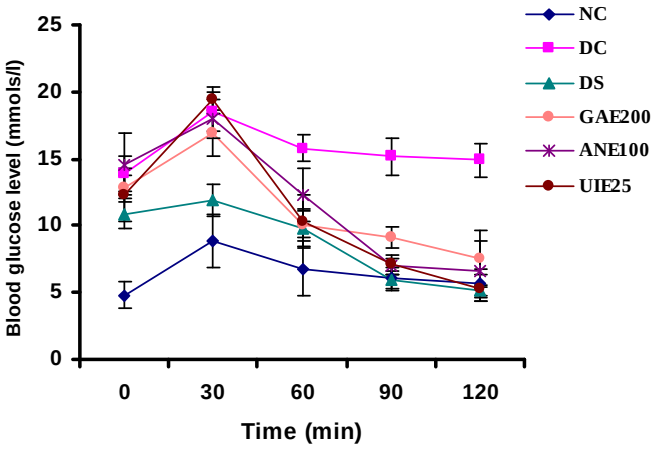


Figure 5.1: Comparative effect of GAE, ANE and UIE on oral glucose tolerance test (OGTT) in alloxan diabetic rats. The results are expressed as Mean± SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin.

5.3.1.2. Alteration in lipid profile

As shown in Figure 5.2 the levels of TC, TG, LDL, VLDL- cholesterol and LDL/HDL were significantly higher and HDL-cholesterol was lowered in Group DC than those in Group NC rats. Administration of GAE200, ANE100 and UIE25 extracts significantly decreased the levels of TC, TG, LDL and VLDL-cholesterol and LDL/HDL ratio while the higher level of HDL-cholesterol was normalized after the treatment. Among the treatment groups the most significant improvement in lipid profile was observed with Group UIE25.

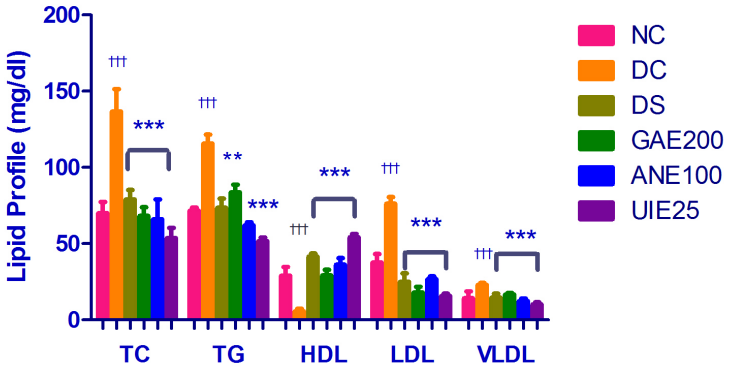


Figure 5.2: Comparative effect of GAE, ANE and UIE, on lipid profile in alloxan-induced diabetic rats. The results are expressed as Mean± SEM. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

5.3.1.3. Changes in liver glycogen

The level of liver glycogen is summarized in Figure 5.3. The liver glycogen content was lower in Group DC than that of Group NC rats. Oral ingestion of GAE200, ANE100 and UIE25 extracts significantly restored the liver glycogen content. However, the Group UIE25 exerted most prominent effect among the treatment groups.

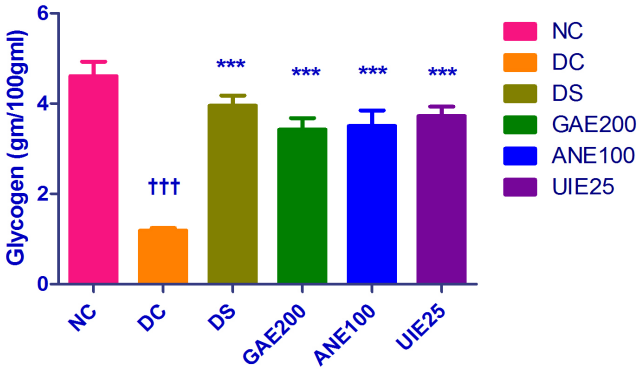


Figure 5.3: Effect of GAE, ANE and UIE on liver glycogen in alloxan-induced diabetic rats. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin Group. The results are expressed as Mean± SEM. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

5.3.1.4. Histopathology of pancreas

Figure 5.4 illustrates representative photographs of thin sections of pancreas stained with Hematoxylin-eosin where NC rats showed no architectural changes.

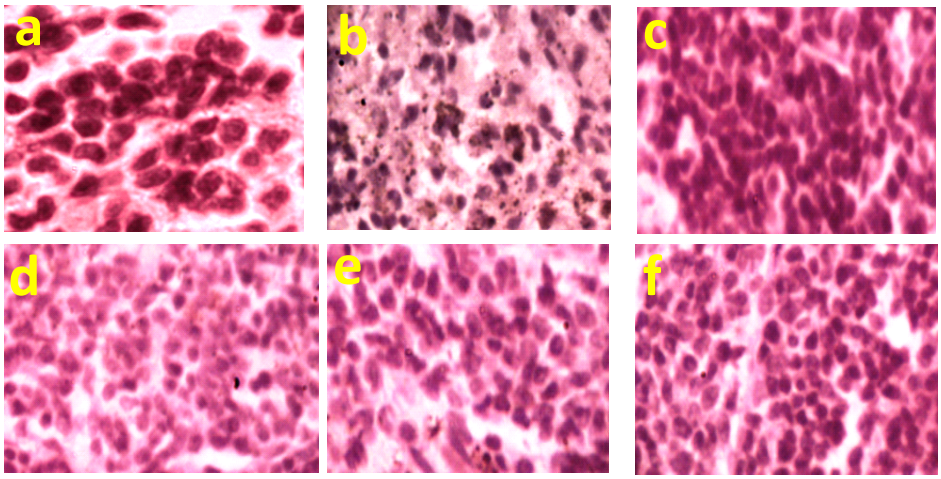


Figure 5.4: Comparative histopathological findings in the pancreas tissue of alloxan-induced diabetic rats. Treated groups (HE staining, magnification 400x). **a.** Group NC: normal, round islets with intact nucleus, **b.** Group DC: Islets damaged, shruken in size and loss of viable islets, **c.** Group DS: normal and round islets are visualized, **d.** Group GAE200 and **e.** Group ANE100 showed mild to moderate reduction in cellular damage and size of islets and **f.** Group UIE 25 showed almost normal cellular population and size of islets.

The islets from rats of Group DC showed cellular swelling due to extensive inflammations with complete loss of architecture of pancreatic β-cells. There was a reduction in the size and number of the islets in untreated DC rats. The diabetic rat treated with UIE25 restored normal cellular population size of islets, absence of islets damage and presence of hyperplasia. The Group GAE200 and ANE100 rats also showed mild to moderate reduction of necrosis of the islets of pancreas. There was a reduction in the size and increase in the number of the islets in these treatment groups. However, significant architectural improvement was observed with UIE25 than that of DC rats.

5.3.2. Comparative *In-vitro* Antioxidant Assay between the Plant Extracts

5.3.2.1. Determination of total phenolic content

The phenolic content of GAE, ANE and UIE were 44.65, 62.72, and 87.74 mg of gallic acid equivalent/gm of dried extract, respectively (Figure 5.6). The calibration curve for determination of phenolic content using gallic acid as standard is shown in Figure 5.5.

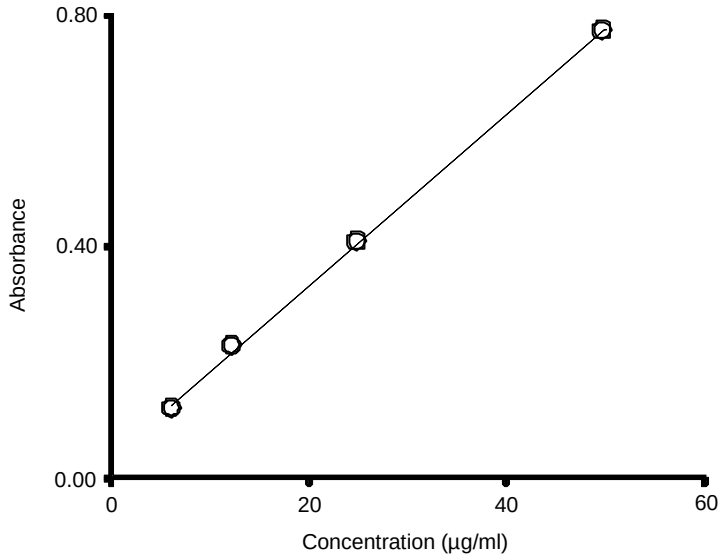


Figure 5.5: Calibration curve of gallic acid for the determination of phenolic content (R^2 = 0.999). Values are mean of triplicate experiments and represented as Mean± SEM

From the results it was evident that the phenolic content of UIE was higher than that of GAE and ANE. Hence, UIE fraction might serve as a good source for phenolic compounds as well as antioxidants.

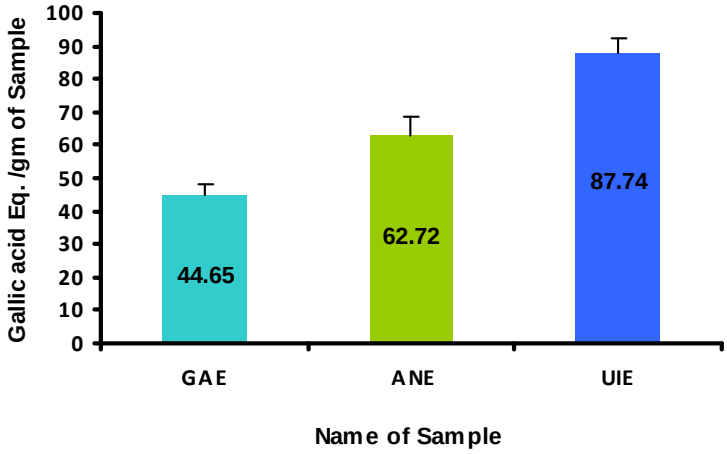


Figure 5.6: Total phenol content (mg/gm plant extract in gallic acid equivalent) ehtanol extracts of *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and *Urginea indica* (UIE).

5.3.2.2. Determination of total flavonoid

The flavonoid content of the extractives was calculated on the basis of the calibration curve for quercetin (Figure 5.7).

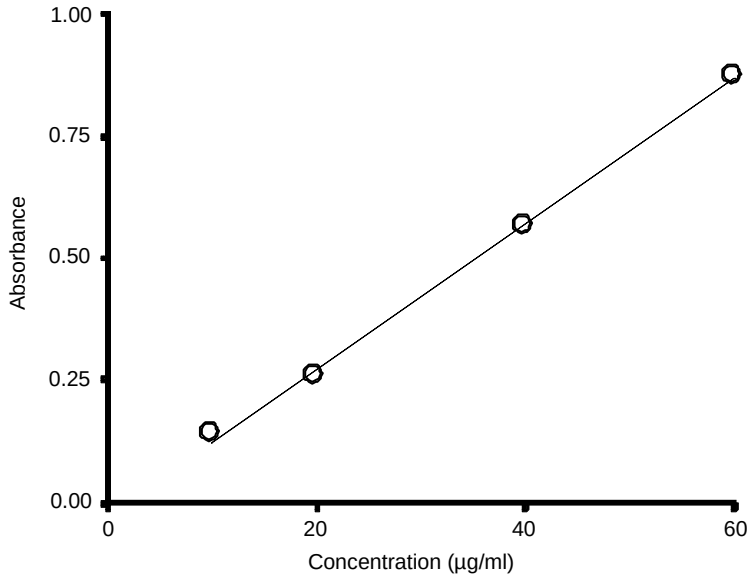


Figure 5.7: Calibration curve of quercetin for the determination of total flavonoid (R^2 = 0.998). Values are mean of triplicate experiments and represented as Mean ± SEM.

The flavonoid contents of GAE, ANE, and UIE were 39.11, 50.46, and 67.72 mg of QUE/gm of dried extractives, respectively (Figure 5.8). The results demonstrated that UIE can serve as a good source of flavonoid.

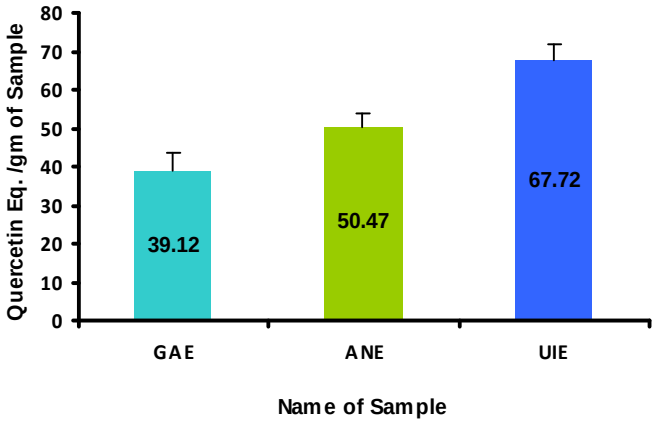


Figure 5.8: Total flavonoid contents (mg/gm plant extract in quercetin equivalent) of GAE, ANE and UIE. Values are mean of triplicate experiments and represented as Mean ± SEM.

5.3.2.3: Determination of total antioxidant content

The total antioxidant contents of GAE, ANE, and UIE were calculated on the basis of the calibration curve (Figure 5.9) for ascorbic acid and the results were expressed as mg of ascorbic acid/gm of extractives (Figure 5.10).

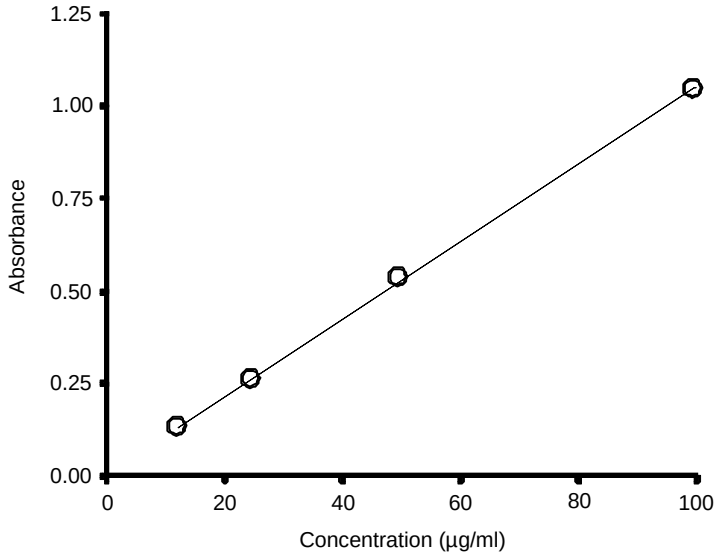


Figure 5.9: Calibration curve of ascorbic acid for the determination of total antioxidant contents (R^2= 0.999). Values are mean of triplicate experiments and represented as Mean ± SEM.

The total antioxidant contents of GAE, ANE, and UIE were 213.36, 247.63, and 307.25 mg of ascorbic acid Eq/gm of dried extractives, respectively. The results demonstrated that all extracts possessed antioxidant potentials of which UIE was the richest one.

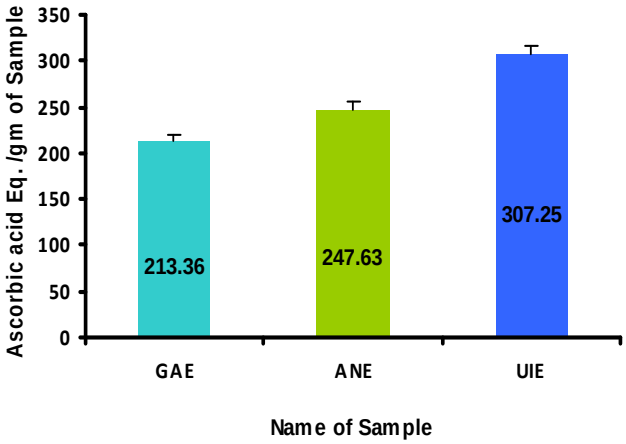


Figure 5.10: Total antioxidant activity of crude ethanol extract of *Grewia asiatica* (GAE), *Alpinia indica* (ANE) and *Urginea indica* (UIE). Values are mean of triplicate experiments and represented as Mean ± SEM.

5.3.2.4. Reducing power capacity

The Fe^{3+} reducing power of GAE, ANE, and UIE was determined using ascorbic acid as standard. The results are shown in Figure 5.11.

All the fractions, UIE, ANE, and GAE, showed significant reducing power capacity when compared with standard ascorbic acid at various concentrations. The results demonstrated that among the fractions UIE had most significant iron reducing capacity.

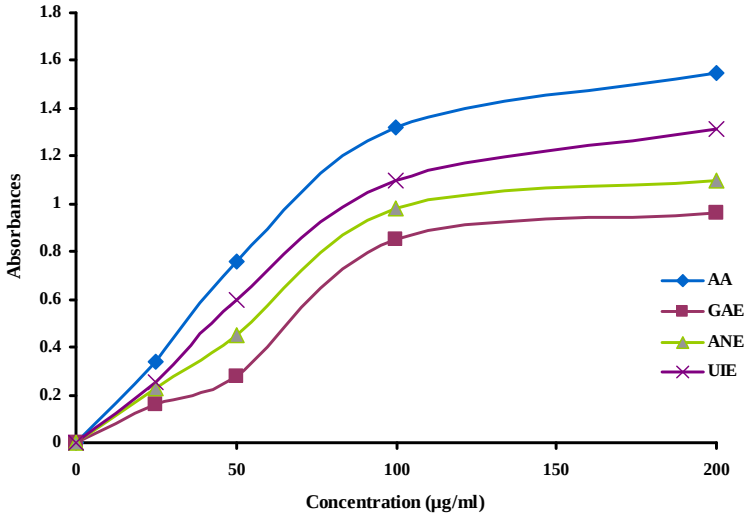


Figure 5.11: Reducing power capacity of ethanol extracts of *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and *Urginea indica* (UIE). Here, Ascorbic acid (AA)= Standard. Values are mean of triplicate experiments and represented as Mean ± SEM.

5.3.2.5. DPPH radical scavenging activity

The results of DPPH radical scavenging assays of different plant extracts and ascorbic acid standard are given in Figure 5.12**.** The scavenging activities of all the crude ethanol extracts were less than that of ascorbic acid standard. IC_{50} of ascorbic acid standard and ethanol extracts of *Grewia asiatica* (GAE), *Alpinia nigra* (ANE) and *Urginea indica* (UIE) were 12.50 µg/ml 76.45 µg/ml, 43.25 µg/ml and 27.50 µg/ml, respectively (Figure 5.13).

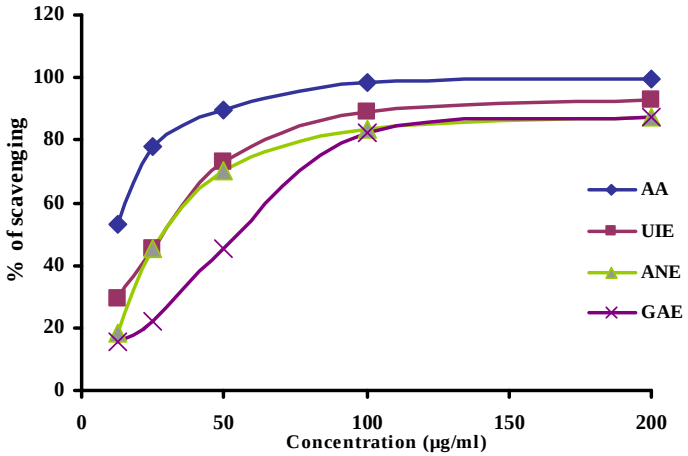


Figure. 5.12: DPPH radical scavenging activity of crude ethanol extracts of GAE, UIE, ANE and ascorbic acid (AA) standard. Values are mean of triplicate experiments and represented as Mean ± SEM.

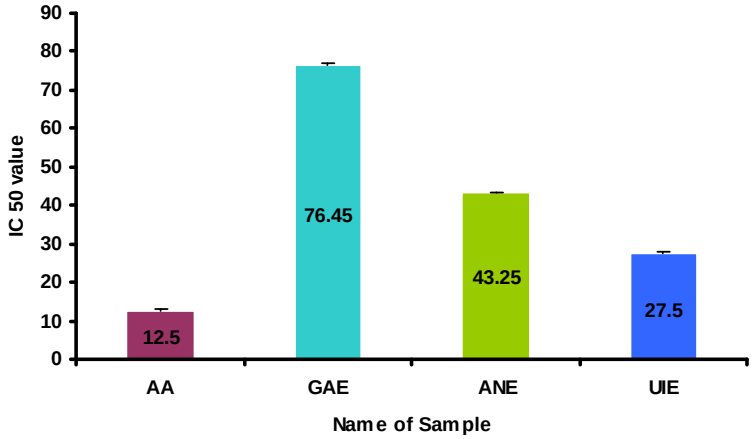


Figure 5.13: IC_{50} (µg/ml) values of different extracts for DPPH radical scavenging activity. Values are the mean of triplicate experiments and represented as Mean ± SEM.

5.4. Conclusion

In conclusion, the results of the present study clearly demonstrated that the ethanolic extract of the bark of *Grewia asiatica* (GAE200), rhizome of *Alpinia nigra* (ANE100), and bulb of *Urginea indica* (UIE25) exhibited significant antihyperglycemic and antihyperlipidemic activity. These extracts also showed significant improvement in OGTT, liver glycogen content as well as pancreatic *β*-cells architectures (Figure 5.4). The mechanism by which the plant extracts improved diabetic condition can be partially explained by the presence of considerable quantity of phenolic and flavonoid type of antioxidants within the plant extracts that exhibited free radical scavenging and reducing power capacity. Amongst the three aforesaid medicinal plants, *Urginea indica* (25mg/kg) showed the most significant antidiabetic activity in terms of its glucose ($p < 0.001$) and lipid lowering efficacy and restoration of liver glycogen content as well as antioxidant potencies. Considering all the results, my future work directed on the isolation and characterization of active principles from the *Urginea indica* bulbs extract.

Chapter Six

Isolation and Characterization of Bioactive Principles

6.1. Introduction

The ethanolic extract of *Urginea indica* bulbs (UIE), which is rich source of antioxidant has been found to possess remarkable hypoglycemic, hypolipidemic activities and also had beneficial effects on organ protection (Chapter-5). Present study was further designed to isolate the phytochemicals from the plant *Urginea indica* Knuth. (Family: Liliaceae), that will further strengthen the ethnomedicinal use of this plant in various herbal formulations for the treatment of diabetes.

6.2. Materials and Methods

6.2.1. Collection of Plant Material and Preparation of Plant Extracts

As described in experimental protocol Chapter 4, Section 4.2.1.2 and 4.2.1.3.

6.2.2. Solvent-Solvent Partitioning of Crude Extract

The whole extraction and partitioning process is shown in Figure 6.1. The powdered bulbs (1.5 kg) were washed, sun dried for several days and then grinded to coarse powder. The powdered plant materials were soaked with rectified spirit (96% ethanol) for 7-10 days with occasional shaking and stirring and the extracts were successively filtered through cotton and filter paper and concentrated under reduced pressure to get brownish mass (30 g).

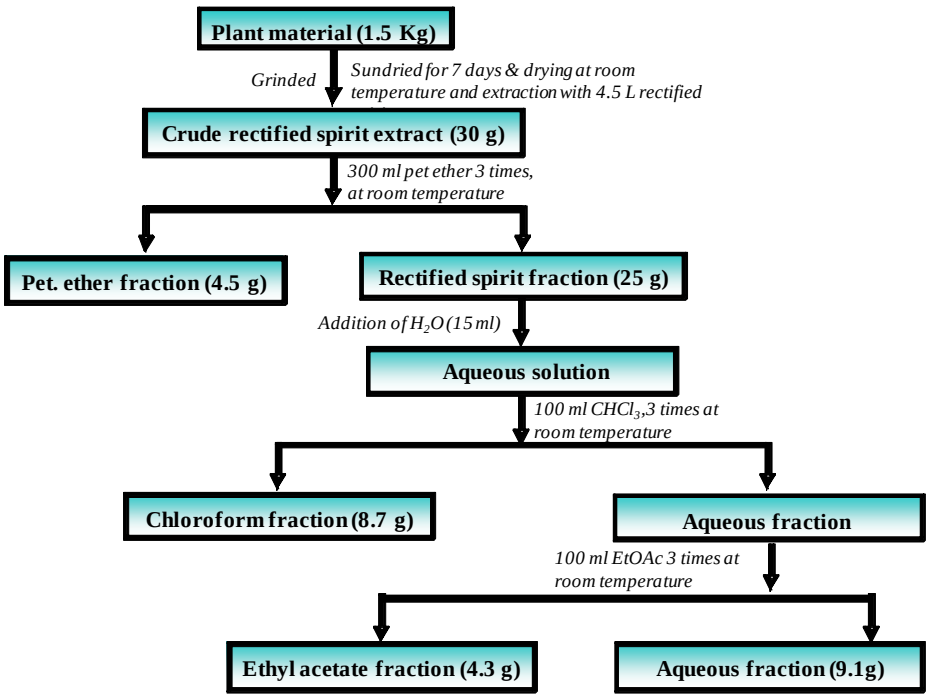


Figure 6.1: Schematic representation of solvent-solvent partitioning of the crude extract of *Urginea indica* Knuth. bulb.

The brownish mass (30 g) of the UIE was then defatted with petroleum ether (300 ml x 3) to get 25.0 g defatted extract. After adding H_2O (15 ml) the extract was further partitioned with $CHCl_3$ (100 x 3mL) and EtOAc (100 x 3mL) to get $CHCl_3$ (UIC, 8.7 g), EtOAc (UIE, 4.3 g) and aqueous (9.8 g) fractions, respectively.

6.2.3. Investigation of Chloroform Extract

A portion of the chloroform soluble extract (UIC, 4g) was prepared for further fractionation by column chromatographic method. UIC was mixed well with a small amount of silica gel in a mortar with pestle to get a free flowing powder and was subjected to a column chromatography (2.5id x 20cm). The column was eluted with the solvent/solvent systems as shown in the Table 6.1.

The elutes were collected in a number of conical flask marked from 1 to 12. Each fraction was spotted on TLC plates [*n*-hexane: ethyl acetate (5:1)] and was viewed visually, under UV and color changes were observed after spraying with vanillin-H_2SO_4 reagent.

Table 6.1: Solvent systems used in the column analysis of chloroform fraction.

Fraction no.	Solvent system	Proportion	Volume eluted (ml)
1	n-Hexane	100%	200
2	n-Hexane : Chloroform	99:1	200
3	n-Hexane : Chloroform	49:1	400
4	n-Hexane : Chloroform	20:1	200
5	n-Hexane : Chloroform	10:1	200
6	n-Hexane : Chloroform	5:1	400
7	n-Hexane : Chloroform	3:1	300
8	n-Hexane : Chloroform	2:1	300
9	n-Hexane : Chloroform	1:1	200
10	Chloroform	100%	300
11	Chloroform: Methanol	1:1	200
12	Methanol	100%	200

The fractions with similar spots were further combined into five sub-fractions (F1-F5) that were assayed for their hypoglycemic activity (Table 6.2). Fractions F-2, F-3 and F-4 showed hypoglycemic activity whereas F-1 and F-5 were shown to be inactive.

Table 6.2: Sub-fractionation of the chloroform fraction and their hypoglycemic activity.

Code of the similar fractions	Fraction number	Amount (mg)	Hypoglycemic activity in alloxan-induced diabetic rats
F-1	1 to 2	30	-
F-2	3 to 4	300	+
F-3	6 to 7	375	+
F-4	8 to 9	29	+
F-5	10 to 12	49	-

6.2.4. Analysis of Fractions 3, 4, 6 and 7

Fractions 3-4 showed one distinct spot with little tailing as well as impurities shown in TLC using solvent system *n*-hexane: ethyl acetate (5:1). The spot showed positive test under UV and vanillin sulfuric acid reagent and was further subjected to PTLC eluted by the same solvent system. The bands which glow under UV were scrapped off separately and dissolved in ethyl acetate and filtered through cotton plug and collected in a beaker. After evaporation under reduced pressure a colorless crystalline compound was obtained and designated as UC-1, yield 75 mg and mp.156 - 157°C.

The fractions 6 -7 showed similar single spot on TLC under UV and with vanillin-sulfuric acid reagent. These two fractions were combined and solvent was evaporated off to a certain volume and was allowed to stand overnight at refrigerator (4°C). A few needle shaped crystals were appeared. The crystals were washed with *n*-hexane and upon recrystallization from *n*-hexane-ethyl acetate afforded compound UC-3 (80 mg) as needles with mp. 165 - 166°C.

6.2.5. Analysis of Other Fractions

Although other fractions showed a number of spots but the amount of each fraction was relatively small, hence were not analyzed.

6.2.6. Test of Purity of the Isolated Compounds

From the chemical analysis two compounds UC-1 and UC-2 were isolated from chloroform fraction. The purity of the isolated compounds were checked by determining R_f value using

commercially available pre-coated silica gel plate (kieselgel 60 GF 254) as shown in Table 6.3.

Table 6.3: R_f values of the isolated compounds.

Compound code	Solvent system	R_f Value
UC-1	n-Hexane : Ethyl acetate (5: 1)	0.72
	Chloroform (100%)	0.68
UC-3	n-Hexane : Ethyl acetate (5:1)	0.53
	Chloroform (100%)	0.50

6.2.7. Properties of the Isolated Compounds

The isolated compounds were characterized by their physical, chemical as well as spectral data. In physical properties such as physical form, color, solubility, melting point and R_f values were recorded. Chemical properties included color reaction of the isolated compound with specific spray reagent. Finally, spectroscopic techniques e.g. ^{1}H-NMR, ^{13}C-NMR and 2D NMR analysis were carried out for structure elucidation.

6.2.7.1 Physical properties of compounds

a. Compound UC-1

Physical form : Needle
Color : Colorless
Melting point : 156 -157^{0}C
R_f values : Shown in the Table 6.3
Solubility : Shown in the Table 6.4

Table 6.4: Solubility data of the compound UC-1.

Solvent	Solubility
Cyclohexane	Insoluble
n-Hexane	Sparingly soluble
Petroleum ether	Insoluble
Diethyl ether	Sparingly soluble
Chloroform	Insoluble
Ethyl acetate	Insoluble
Acetone	Soluble
Methanol	Sparingly soluble
Acetic acid	Soluble
Water	Insoluble
DMSO	Soluble

b. Compound UC-3

Physical form : Needles

Color : White

Melting point : 165 - 166^{0}C

R_f values : Shown in the Table 6.3

Solubility : Shown in the Table 6.5

Table 6.5: Solubility data of the compound UC-3.

Solvent	Solubility
Cyclohexane	Insoluble
n-Hexane	Sparingly soluble
Petroleum ether (40-60^{0}C)	Insoluble
Diethyl ether	Sparingly soluble
Chloroform	Insoluble
Ethyl acetate	Insoluble
Acetone	Insoluble
Methanol	Sparingly soluble
Acetic acid	Soluble
Water	Insoluble
DMSO	Soluble

6.2.7.2. Chemical properties of compounds

Material and method

The melting point was measured by a Fisher melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-300 and DMX-500 instruments with standard pulse sequences operating at 400 and 100 MHz in ^{1}H and ^{13}C NMR, respectively. ^{1}H and ^{13}C spectra were referenced relative to DMSO-d_6 (δ = 2.50 and 39.51 for ^{1}H and ^{13}C NMR, respectively). 2D NMR spectra (COSY, TCOSY, HSQC, HMBC) were recorded using the manufacturer's software. Chemical shifts are given in δ values (ppm) and coupling constants (J) are given in hertz (Hz). Flash column chromatography was carried out with silica gel 60 (70-230 mesh, Merck, 50 id×220 mm) with a gradient elution of 100% n-hexane to 100% $CHCl_3$ followed by $CHCl_3$: MeOH (1:1).

TLC Analysis

Thin-layer chromatography (TLC) analysis was performed on precoated silica gel plates (Kieselgel 60, F254, 20×20 cm, 0.25 mm thick, Merck). Spots were detected

under UV light at 254 and 365 nm and by staining in solution of 1% vanillin-Sulfutic acid in ethanol followed by heating.

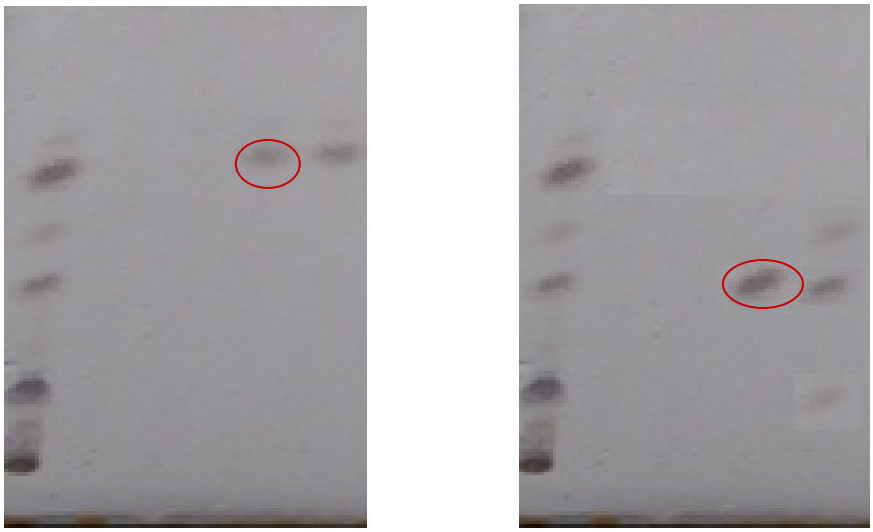


Figure 6.2: TLC of $CHCl_3$ fraction as well as isolated compounds. Solvent system, n-hexane : ethyl acetate (5:1). UC-1 (compound 1), UC-3 (compound 2), F-2, column fraction 2 and F-3, column fraction 3.

Preparative thin-layer chromatography (PTLC) was performed on silica gel plates (Kieselgel 60, F254, glass plate 20×20 cm, 0.5 mm thick, Merck). Spots were detected under UV light at 254 and 366 nm.

6.2.7.3. Spectral properties

UC-1 (crystal, 75 mg): 5,7-dihydroxy-2-methyl-4H-chromen-4-one; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.30 (3H, s, CH_3), δ 6.123 (olephenic 1H, s, H-3), δ 6.128 (1H, d, J = 2.4 Hz,H-6) and δ 6.28 (1H, d, J = 2.4 Hz, H-8) ppm.^{13}C-NMR (DMSO-*d*6, 100 MHz): δ 168.49 (C, C-2), 108.79 (CH, C-3), 182.59 (C, C - 4), 158.64 (C, C-5'), 99.61 (CH, C-6'), 165.05 (C, C-7'), 94.57 (CH, C-8),162.35 (C, C-9),104.22 (C, C-3), 20.75 (CH_3, at C-2) ppm. R_f 0.72 (5:1, n-Hexane:Ethyl acetate).

UC-3 (Crystal, 80 mg)**:** 5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one; ^{1}H NMR (DMSO-*d*6, 400 MHz): δ 2.33 (3H, s, CH_3), 6.54 (1H, d, J = 2.4 Hz, H-8), 6.31 (1H, d, H-6), 6.18 (olephenic 1H, s, H-3), 3.89 (3H, s, OCH_3 at C-7) ppm. ^{13}C-NMR (DMSO-*d*6, 100 MHz): δ 168.98 (C, C-2), 109.06 (CH, C-3), 182.80 (C, C-4),158.55 (C, C-5'), 98.69 (CH, C-6'), 165.94 (C, C-7'), 93.31 (CH, C-8), 162.12 (C, C-9),105.22 (C, C-3), 20.79 (CH_3, at C-2),56.88 (OCH_3 at C-6) ppm. R_f 0.53 (5:1, n-Hexane:Ethyl acetate).

6.3. Results and Discussion

6.3.1. Characterization

Compound UC-1 was obtained as colorless crystal with mp. 156-157^{0}C. The ^{1}H NMR spectrum (DMSO-d_6) showed a sharp signal at δ2.30 (3H), one set of doublets at δ 6.28 and 6.128 (J = 2.4 Hz) for meta coupled aromatic protons, one sharp singlets at δ 6.123 for olephenic proton (1H). These data indicate the presence of a flavonoid moiety. The flavonoidic nature of the compound was apparent from the UV spectrum, with absorption maxima at 275 and 324nm [203]. The ^{13}C NMR spectrum showed the presence of ten signals at δ 20.38, 94.18, 99.21, 103.85, 108.39, 158.26, 161.96, 164.62, 168.13, and 182.21.

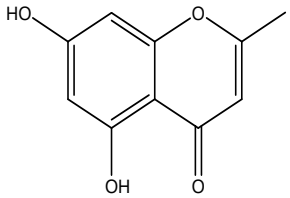


Figure 6.3: Structure of UC-1 (5,7-dihydroxy-2-methyl-4H-chromen-4-one)

Correlating ^{1}H NMR signals with ^{13}C NMR in gHSQC and gHMBC and comparing with known literature data, the structure of the compound UC-1 was determined as 5,7-dihydroxy-2-methyl-4H-chromen-4-one which was previously isolated from *Dysoxylum macrocarpum* [203] (Table-6.6).

Compound UC-3 (80 mg) was obtained as colorless crystal with mp. 165-166^{0}C. The ^{1}H NMR spectrum (DMSO-d_6) showed a sharp signal at δ 2.33 (3H), one set of doublets at δ 6.54 and 6.31 (J = 2.4 Hz) for meta coupled aromatic protons, two sharp singlets at δ 6.18 for olephenic proton (1H) and at δ 3.89 for methoxyl protons (3H). These data indicate the presence of a flavonoid moiety. The flavonoidic nature of the compound was apparent from the UV spectrum, with absorption maxima at 275 and 324nm [203]. The ^{13}C NMR spectrum showed the presence of eleven signals at δ 20.79, 56.88, 93.31, 98.69, 105.22, 109.06, 158.55, 162.12, 165.94, 168.98, 182.80.

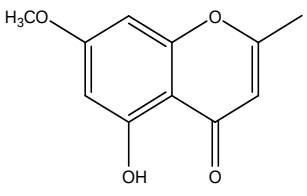


Figure 6.4: Structure of UC-3 (5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one)

Comparing ^{1}H and ^{13}C NMR of UC-1 with UC-3, it was found that both compound possess flavonoid nucleus. In addition, UC-3 showed a methoxyl peak at δ 56.88 which was absent in UC-1. Based on previously reported data (Table-6.6), the compound UC-3 was confirmed as 5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one which was also previously isolated from *Dysoxylum macrocarpum* [203].

Table 6.6: Comparison of ^{1}H NMR (recorded in DMSO-d_6) and ^{13}C NMR (recorded in DMSO-d_6) spectral data among pisonins B (recorded in $CDCl_3$), compound UC-1 and UC-3.

Position	Pisonins B		UC-1		UC-3	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	-	-	-	-	-	
2	-	167.0	-	168.137	-	168.98
3	6.02, q, 0.6	108.2	6.123, s	108..39	6.18, s	109.06
4		183.0	-	182.215		182.80
5		152.1	-	158.262		158.55
6		130.2	6.128, d, 2.4	99.211	6.31, d, 2.4	98.69
7		154.8	-	164.629		165.94
8	6.46, s	93.2	6.28, d, 2.4	94.187	6.54, d, 2.4	93.31
9		153.5	-	161.963		162.12
10		105.4	-	103.85		105.22
CH_3-2	2.35, d, 0.6	20.5	2.30, s	20.38	2.33, s	20.79
OCH_3-6	4.01, s		-	-		
OCH_3-7			-	-	3.89, s	56.88

* Data was determined carefully by details analysis of NMR pattern.

6.4. Conclusion

Using chromatographic technique, we have isolated two chromone derivatives, UC-1 and UC-3 from the $CHCl_3$ fraction of the bulb of *Urginea indica*. Based on the ^{1}H and ^{13}C NMR spectrum and a comparison with previously reported data, compound 1 (UC-1) was characterized as 5, 7 dihydroxy-2-methyl-4H-chromen-4-one and compound 2 (UC-3) as 5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one and these are the first report isolation from the plant, *Urgenia indica*. The plant extracts and its fractions showed remarkable hypoglycemic activities (Table-6.2). Hence, there is a possibility that the compounds isolated from the plant *Urginea indica* might have some beneficial effects in alloxan-induced diabetic rats. So, we have further investigated the effect of isolated compounds in diabetic rats.

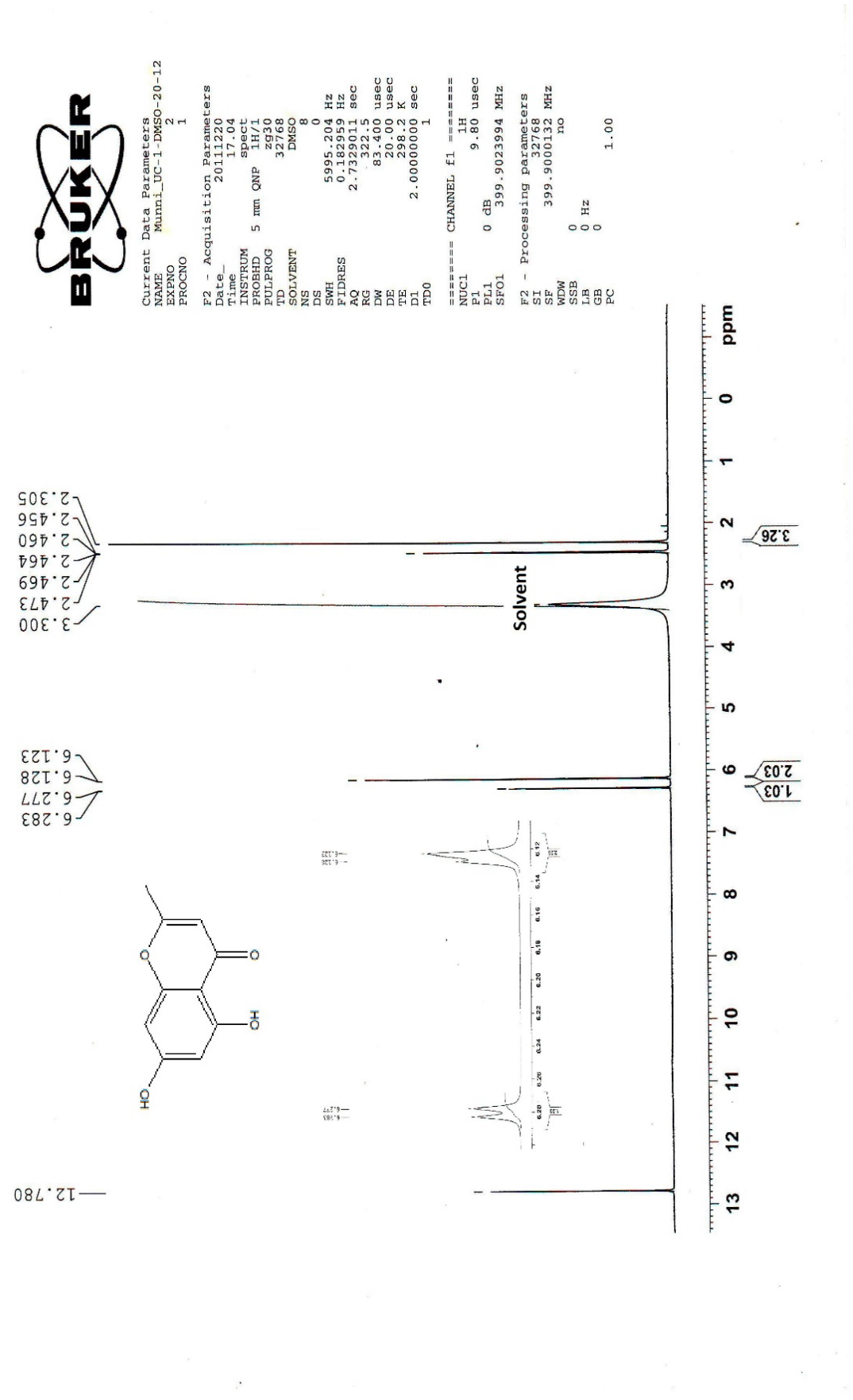


Figure 6.5: ^{1}H NMR spectrum compound UC-1 (DMSO-d_6, 400 MHz).

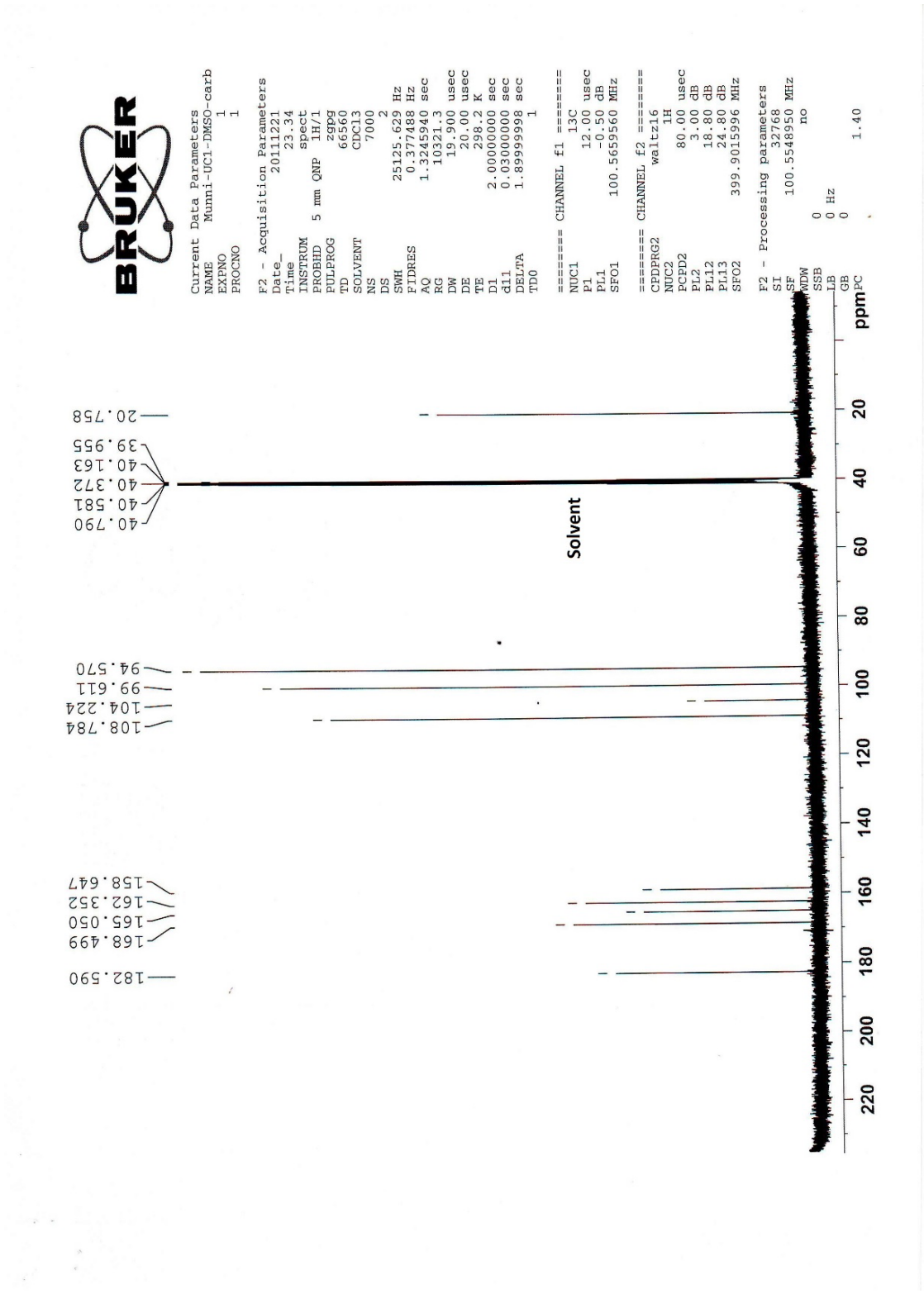


Figure 6.6: ^{13}C NMR spectrum of compound UC-1 (DMSO-d_6, 100 MHz).

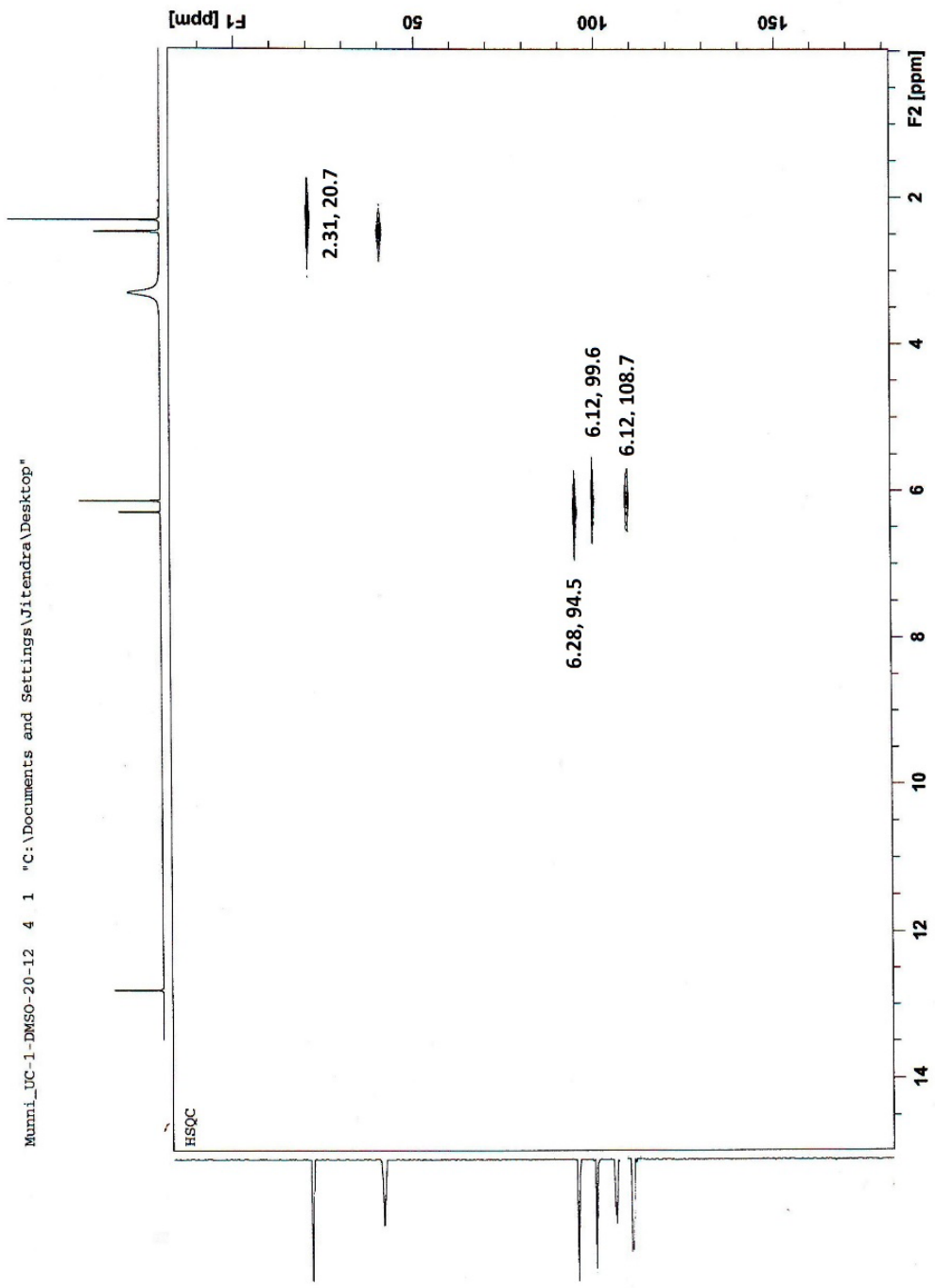


Figure 6.7: HSQC spectrum of compound UC-1 (DMSO-d_6, 100 MHz).

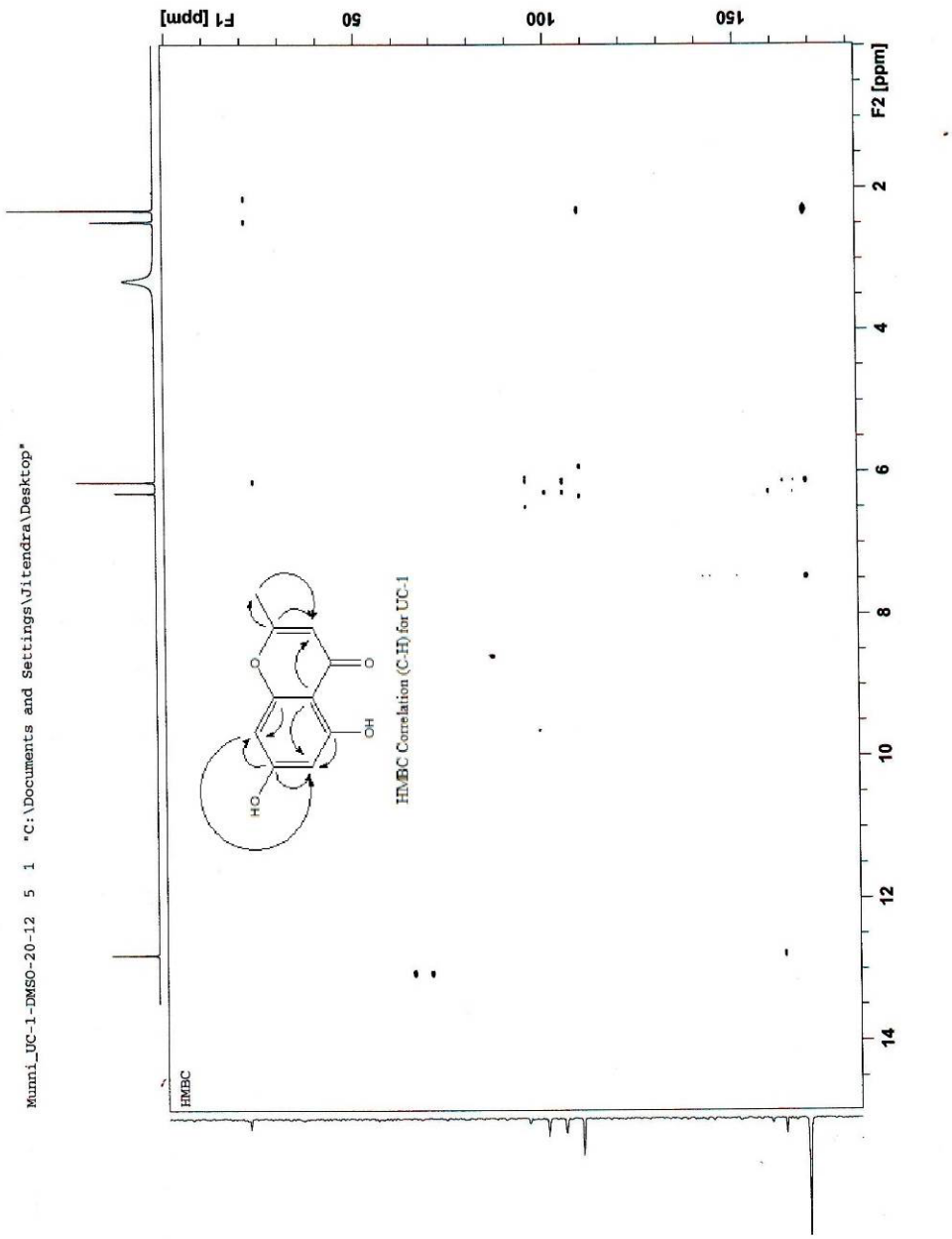


Figure 6.8: HMBC spectrum of compound UC-1 ((DMSO-d_6, 100 MHz).

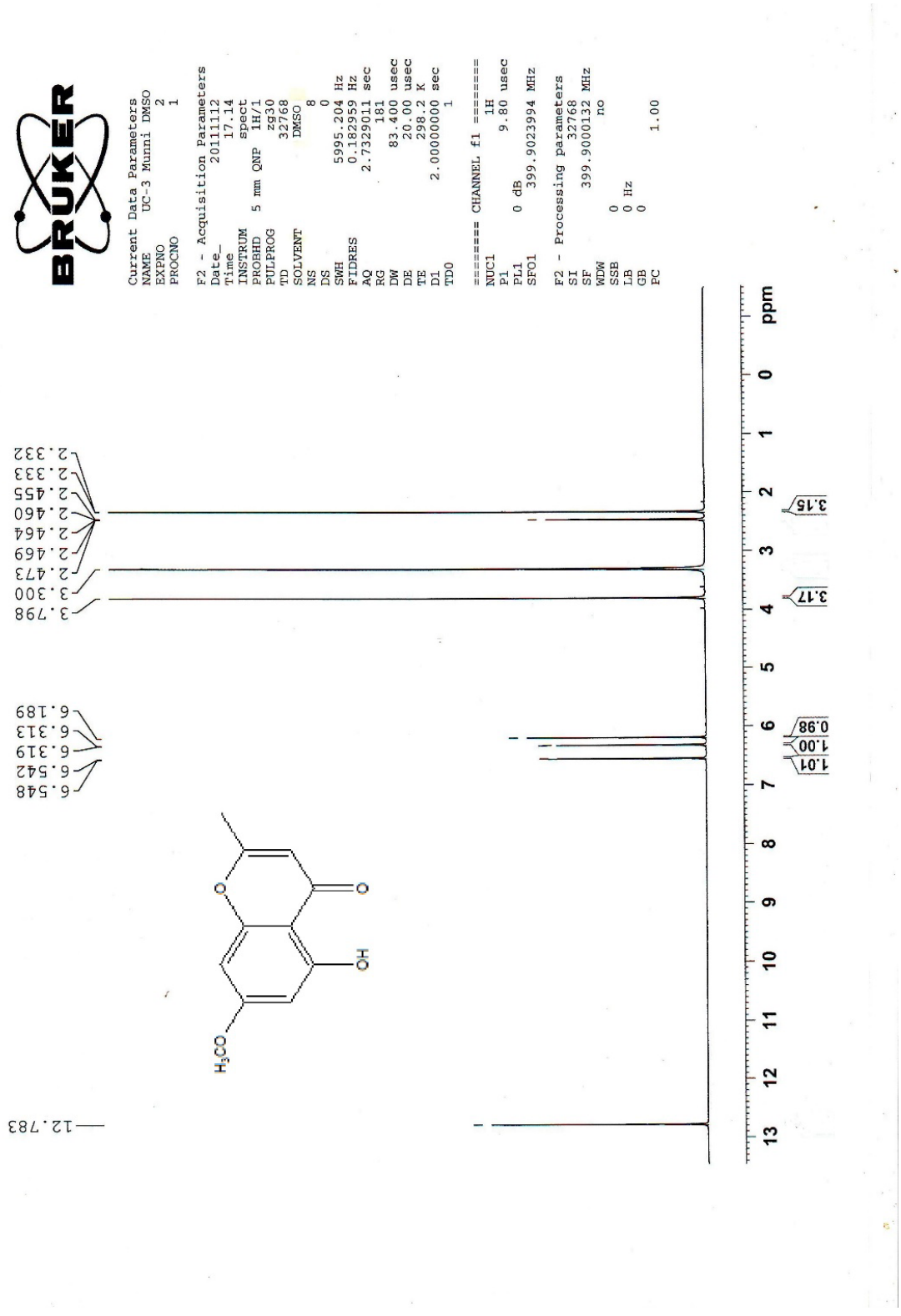


Figure 6.9: ^{1}H NMR spectrum of compound UC-3 ((DMSO-d_6, 400 MHz).

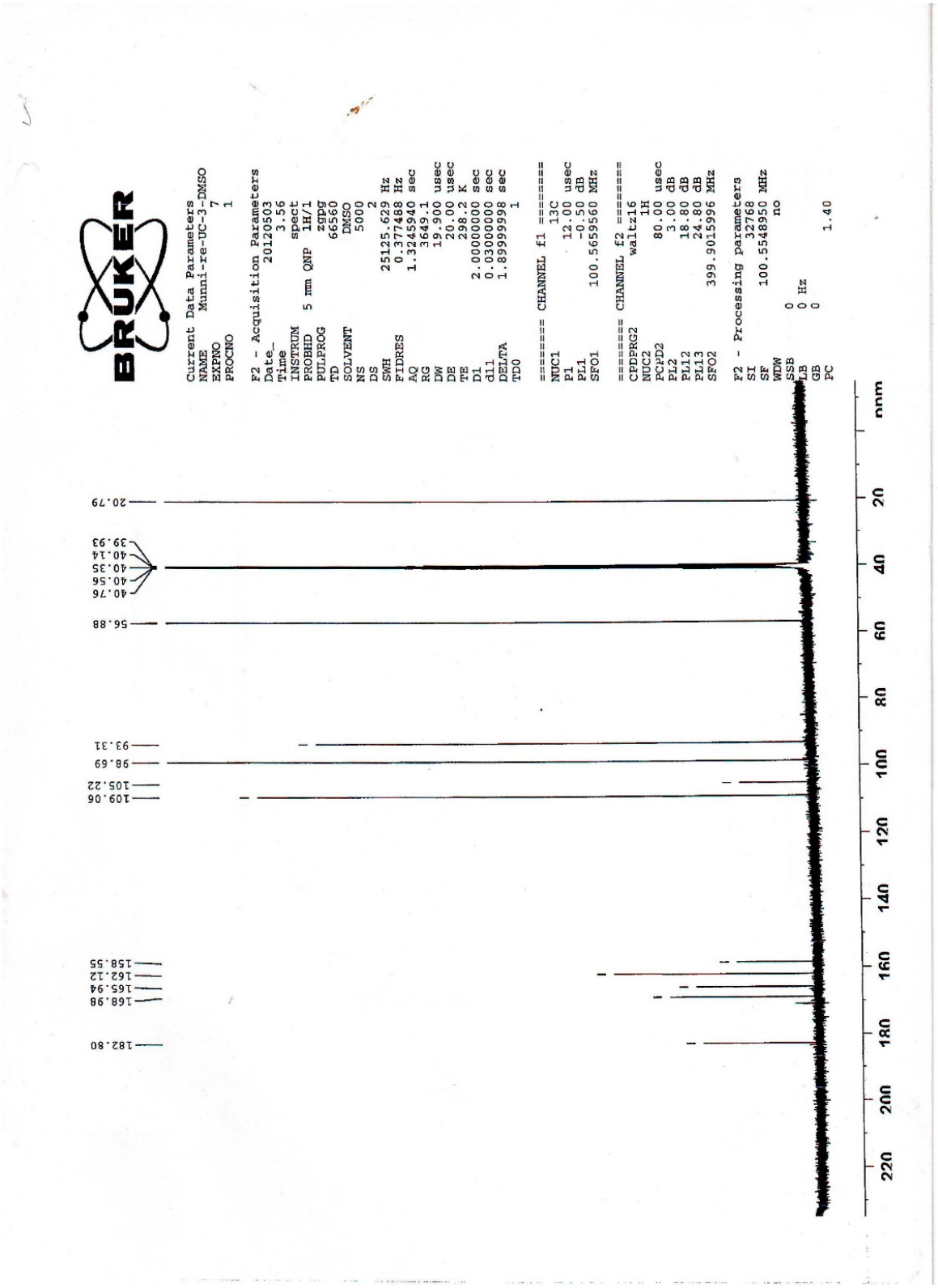


Figure 6.10: ^{13}C NMR spectrum of compound UC-3 ((DMSO-d_6, 100 MHz).

Chapter Seven

Pharmacological Investigation of Pure Compounds

7.1. Introduction

The compounds UC-1 and UC-3 are characterized as 2-methylchromone derivatives. These are the first report of isolation from the plant *Urginea indica*. Chromones and their derivatives are well known naturally occurring oxygen-containing heterocyclic compounds which reported to have important biological functions, such as antitumor [204], antihepatotoxic, antioxidant [205], anti-inflammatory [206], antispasmolytic, estrogenic [207] and antibacterial activities [208]. So, we consider the naturally occurring chromone derivatives might have some beneficial effects in alloxan-induced diabetic rats.

Several chromone derivatives have been reported from some plant species which are listed in the Table 7.1.

Table 7.1: Some reported chromone derivatives from different plants.

Plant Name	Isolated compounds	Refferences
Eugenia caryophyllata	5,7-dihydroxy-2-methylchromone *β*-D-glucopyranoside	[209]
Adina rubella	5-hydroxy-2- methylchromone	[210]
Pancratium maritimum	5,7- Dihydroxy-2-methylchromone and 5-hydroxy-7-methoxy-2-methyl-chromone	[211]
Polygonum cuspidatum	5-Carboxymethyl-7-hydroxy-2-methylchromone	[212]
Leucas inflata	2-methylchromone	[213]
Neochamaclea puluervlenta	2-methylchromone	[214]
Tussilago farfara	6-acetyl-7-hydroxy-2,3-dimethylchromone and 6-carboxy-7-hydroxy-2,3-dimethylchromone	[215]
Aloe vera	2-methyl-chromone	[216]

5,6,7-trihydroxy-2-methylchromone inhibits *α*-glucosidase, an enzyme that catalyses the final step in the digestive process of carbohydrate. Hence, *α*-glucosidase inhibitors can retard the decomposition and absorption of dietary carbohydrates to suppress postprandial hyperglycemia that is the 2-methylchromones reported to possess hypoglycemic activity [217].

Compounds UC-1 and UC-3 were also 2-methylchromones derivatives. So, there is a possibility that the compounds isolated from the plant *Urginea indica* might have some beneficial effects in diabetic rats. Therefore, I further investigated the effect of isolated compounds- UC-1, UC-3 and as well as the mother $CHCl_3$ fraction (UIC) after 7 days of oral administrations in alloxan-induced diabetic rats. The mother UIC fraction (from where the compounds were isolated) was also investigated for comparative study.

7.2. Antidiabetic Assay of UIC, UC-1 and UC-3

7.2.1. Materials and Methods

The animal, induction of diabetes and other materials and methods for the antidiabetic assay of UIC, UC-1 and UC-3 were same as described in Chapter 4, Section 4.2.1.

7.2.2. Experimental Protocol

Diabetic rats were divided into 5 groups for the oral administration of either extract, compounds and/ vehicle for 7 days -

I. Group NC (Vehicle 0.5% methyl cellulose, n = 5)
II. Group DC (Diabetic Control, Vehicle 0.5% MC, n=5)
III. Group DS (Diabetic Standard, Metformin HCl, 150mg/kg, op. n=5)
IV. Group UIC25 (Diabetic, UIC extract 25 mg/kg, n = 5)
V. Group UC-1 (Diabetic, UC-1 compound 5 mg/kg, n = 5)
VI. Group UC-3 (Diabetic, UC-3 compound 5 mg/kg, n = 5)

7.2.3. Biochemical Estimation

- FBS, OGTT, body weight changes
- Lipid profile, Liver glycogen, Enzymes SGOT, CK-MB
- Histopathology of pancreas

7.3. Results and Discussion

7.3.1. Effect of UIC25, UC1 and UC3 on OGTT

After oral ingestion of glucose the blood sugar levels were significantly higher among the diabetic rats. In group DC, blood glucose were peaked at 30 min and remained high over the next 90 min. Rats treated with UC1 and UC3 as well UIC25 showed significant improvement in glucose tolerance after 90 min of oral ingestion (Figure 7.1).

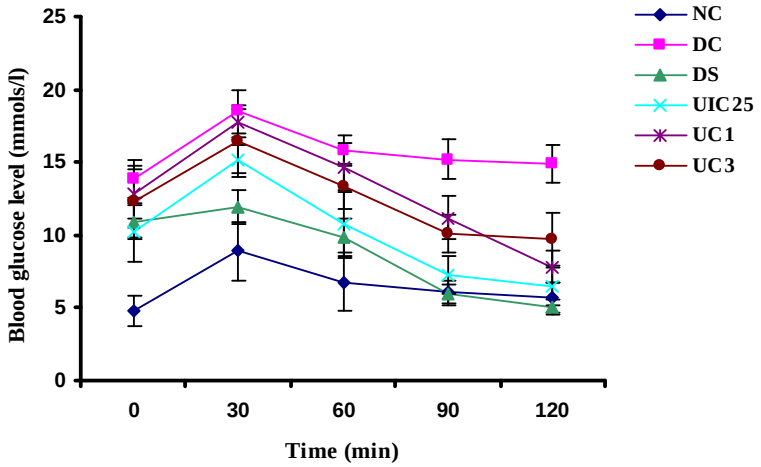


Figure 7.1: Effect of UIC25 as well as isolated compounds UC1 and UC3 on oral glucose tolerance test (OGTT) in alloxan diabetic rats. The results are expressed as Mean±SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150mg/kg metformin.† p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

7.3.2. Changes in Blood Sugar Levels with UIC25, UC1 and UC3

Figure 7.2 shows that the blood sugar level was significantly higher in the DC. After 7 days of oral ingestion of UC1, UC3 and UIC25 significantly reduces blood sugar levels and result was comparable to NC rats.

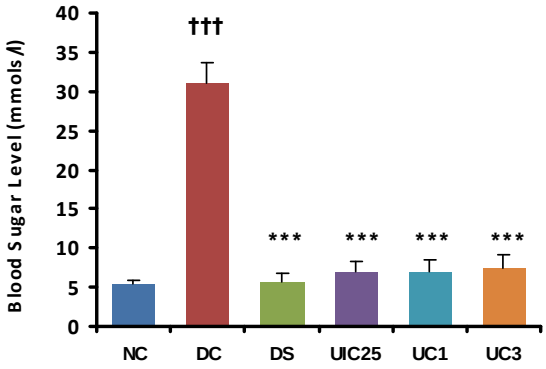


Figure 7.2: Effect of UIC25, UC1 and UC3 on blood sugar in alloxan induced diabetic rats. Each group comprised of 5 animals. The results are expressed as Mean±SEM. Control group received 0.5% methyl cellulose and standard group received 150mg/kg Metformin. † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

7.3.3. Effect of UIC25, UC1 and UC3 on Lipid Profile in Diabetic Rats

Figure 7.3 revealed that the level of TC, TG and LDL are significantly higher in diabetic rats. However, the compounds UC1, UC3 as well the fraction UIC25 decreased the values to normal levels. The level of HDL increased by the administration of UC1 and UC3 as well the UIC25.

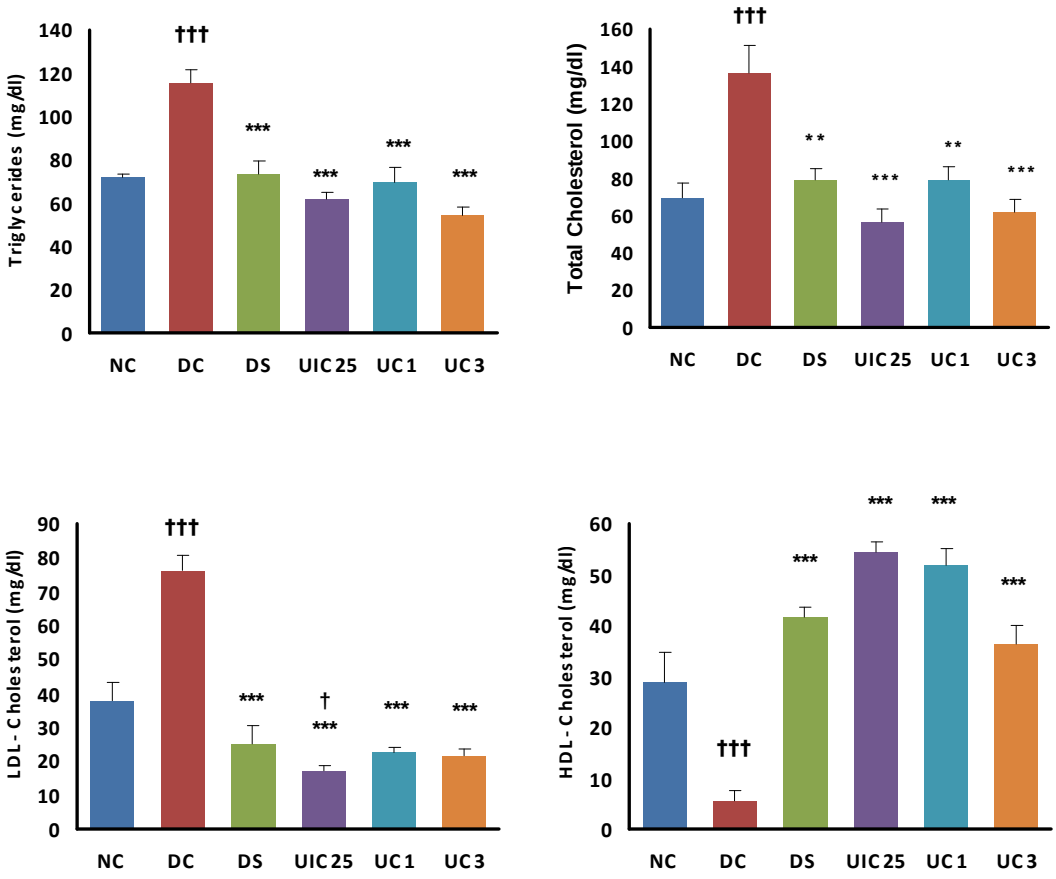


Figure 7.3: Effect of UIC25, UC1 and UC3 on lipid profile in alloxan induced diabetic rats. The results are expressed as means± SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

7.3.4. Effect of UIC25, UC1 and UC3 on Liver Glycogen Level, SGOT and CK-MB in Diabetic Rats

A significant decrease in the liver glycogen content was observed in DC rats. The values were significantly restored among the treatment groups. Oral ingestion of UIC25, UC1 and UC3 significantly reduced the elevated levels of SGOT and CK-MB (Figure 7.4).

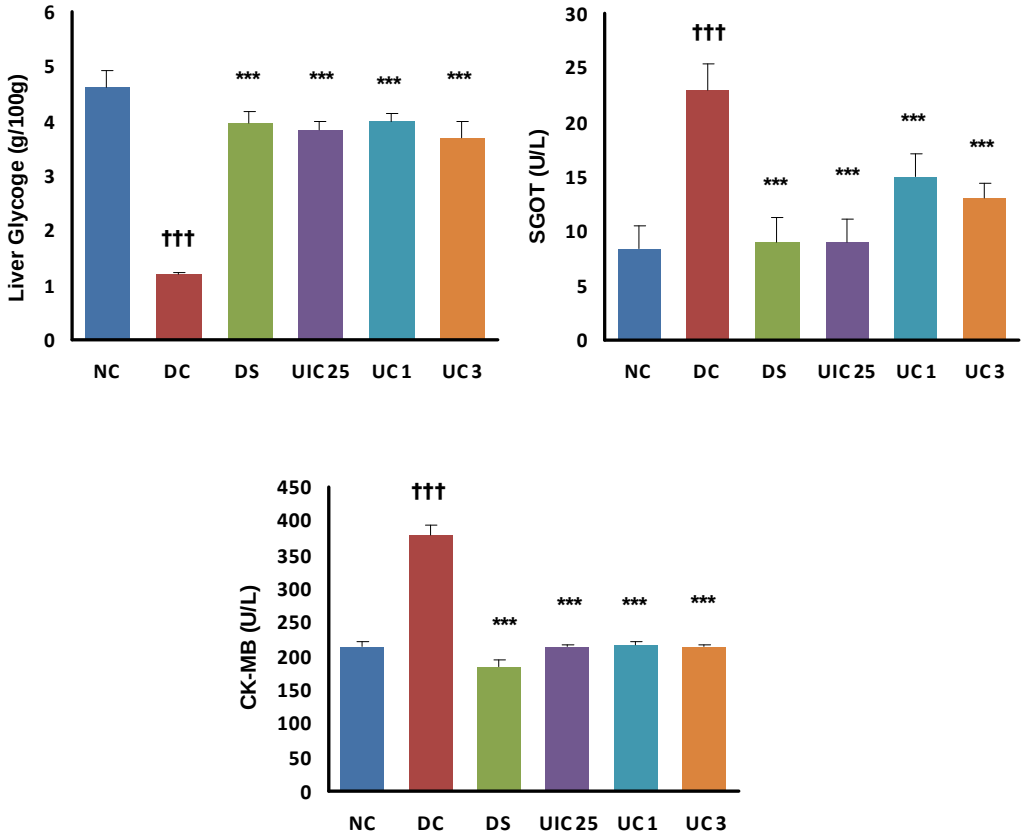


Figure 7.4: Effect of UIC25, UC1 and UC3 on liver glycogen content, SGOT and CK-MB in alloxan induced diabetic rats. Data expressed in Mean±SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and † p<0.05, †† p<0.01 and ††† p<0.001 vs. NC; *p<0.05, **p<0.01 and *** p<0.001 vs. DC.

7.3.5. Histopathology of Pancreas in Diabetic Rats with UIC 25, UC1 and UC3

Figure 7.5 represents the photographs of thin sections of pancreas stained with Hematoxylin-eosin. The diabetic rats treated with UC1 and UC3 as well as UIC showed reduction in cellular damage than that found in untreated Group DC. There was also a mild improvement in β-cell size and number of viable cells was observed.

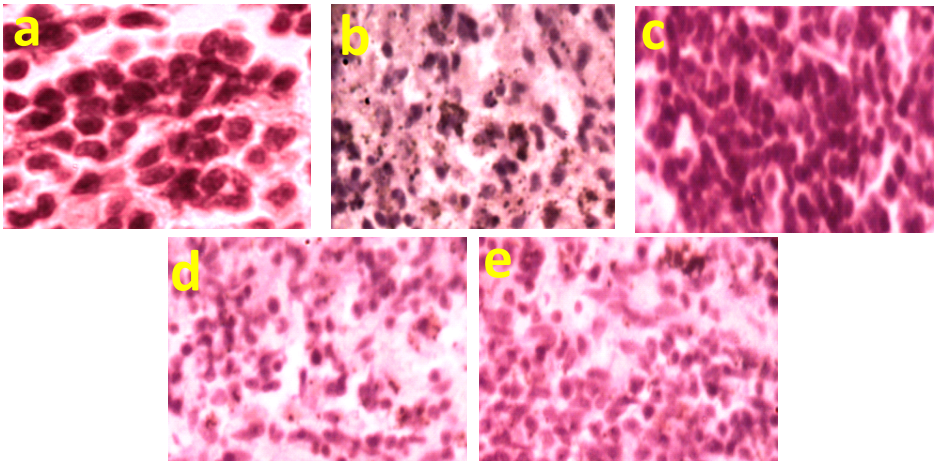


Figure 7.5: Histopathological findings in the pancreas tissue of alloxan-induced diabetic and treated groups (HE staining, magnification 400x). **a**. Group NC rats showed normal islets with intact nucleus. **b**. Group DC pancreas showed marked inflammation with damaged and shrunken cells of islets. **c**. DS almost normal islets visualized with round and intact nucleus. **d**. Group UC 1 and **e.** Group UC 3 showed reduction in cellular necrosis and increase in number cells of islets.

7.4. Conclusion

Compounds UC-1 and UC-3 have beneficial effects on blood sugar levels, lipid profile, OGTT and normalization of pancreatic β-cell architecture in diabetic rats.

Chapter Eight

Summary

Diabetes is multifactorial disease that has a significant adverse impact on health and mortality, particularly from cardiovascular diseases. Traditionally, the plant has been popularly used for the treatment of diabetes mellitus in India and subcontinents [218]. The plants provide a potential source of hypoglycemic drugs and many Indian plants have been investigated for their beneficial effects in diabetes. More than 800 traditional plants treatments for diabetes have been reported, however, only a few herbs have been scientifically evaluated [219, 220]. Bangladesh has endowed with huge sources of medicinal plant. However, majority of these plants have not yet been scientifically evaluated [10]. On the basis of folkloric reputation and few scientific reports we have selected eight (08) plants includes- 1. Grewia asiatica Linn. (stem bark), 2. Corcorus ollitorious Linn. (seeds), 3. Alpinia nigra (rhizome), 4. Prunica granatum Linn. (stem bark), 5. Jatropha curcas Linn. (root), 6. Cinamomum tamala Nees. (bark), 7. Urginea indica (bulb) and 8. Hollarhena antidysenterica (L) Wall. (stem bark) for the present study protocol. On the basis of preliminary screening the plant extracts with demonstrable hypoglycemic activity that is GAE, ANE and UIE were further evaluated for their safety and efficacy in alloxan-induced diabetic rats. Finally, we have evaluated the comparative effects of the plant extracts on blood sugar level, lipid profile and oral glucose tolerance test in alloxan-induced diabetic rats. We have also carried out investigation on the isolation and identification of bioactive principles from the plant extracts and further evaluation for their antidiabetic potentials.

Briefly, plant parts were collected from the different areas of Bangladesh, dried and the powdered plant materials were cold extracted with 96% ethanol. The extracts were subjected to acute toxicity testing and subsequently, preliminary pharmacological and phytochemical screenings were performed. The acute toxicity study showed that extracts from eight plants were safe up to a dose of 2000mg/kg body weight [181] with exception of *Urginea indica* and *Jatropha curcas* whereby the limit dose was 200mg/kg body weight. No sign of behavioral change and mortality was noted with extracts on 72hrs observation. Diabetes was induced into Long Evans rats by a single i.p. injection of alloxan monohydrade (110 mg/kg). In the present study, the diabetogenic effect of alloxan was in accord with previous studies [221]. In preliminary experiment the hypoglycemic activity of plant extracts were evaluated in alloxan-induced diabetic rats after oral ingestion of a single dose of the respective

extract (Table. 2.3). Among the eight plant extracts, *Grewia asiatica* Linn. (GAE, 200mg/kg), *Alpinia nigra* (ANE, 100mg/kg) and *Urginea indica* (UIE, 25mg/kg) showed remarkable glucose lowering efficacy and the effects were comparable. So, the plant extracts with demonstrable hypoglycemic activity were undertaken for their phytochemical analysis (Table. 2.4).

Preliminary phytochemical study of the three plants extracts revealed the presence of steroids, terpenes, glycosides, saponins, tannins and flavonoids. These constituents may in part be responsible for their hypoglycemic activity of these extracts either singly or in combinations. Several authors reported that some phytochemicals such as polysaccharides [222], terpenes and tannins [223], steroids [224] and alkaloids [225] have been implicated in the antidiabetic activities of plants. On the basis of preliminary screening the most prominent plant extracts that is GAE, ANE and UIE were further evaluated for their safety and efficacy in alloxan-induced diabetic rats. After induction of diabetes rats were divided into different groups for oral administration of extracts and/or vehicles for 15 days. Metformin was used as a standard drug and age-matched healthy rats were used as normal control.

Our results demonstrated that the 15 days survival rate among the treatment groups was 100%. Oral administration of GAE (200 and 400 mg/kg), ANE (50, 100 and 200 mg/kg) and UIE (12 and 25 mg/kg) significantly lowered the fasting blood sugar levels during the course of treatment. In OGTT the extracts at different doses increased the glucose tolerance suggesting the improvement of peripheral utilization of glucose in glucose loaded rats (Figure 5.1). The effects of GAE, ANE and UIE on blood sugar levels were dose-dependent. Among the treatment groups the effect of UIE25 was most significant in reducing elevated blood sugar level in alloxan-induced diabetic rats. The effect of GAE was in accordance with outcomes of Parveen *et al.* showed that different parts of *Grewia asiatica* possesses antihyperglycemic activity in alloxan-induced diabetic rabbits [133]. The mechanism by which the extract decreased fasting blood glucose levels in diabetic rats may be as result of stimulation of the residual pancreatic β-cells and probably by increasing peripheral utilization of glucose [226]. A number of medicinal plants have been reported to have an antihyperglycemic effect and a stimulatory effect on insulin release [227, 228]. The hypoglycemic action of the plant may also be due to increased glycogen synthesis, decreased degradation of glycogen and inhibition of gluconeogenesis in liver [76].

Induction of diabetes with alloxan is associated with characteristic loss of body and organ weight, which is due to increase in muscle wasting [229] and loss of tissue proteins [230]. Diabetic rats treated with GAE, ANE and UIE showed an improvement in body weight and organ weight, which may be due to its protective effects against muscle wasting [231]. Although treatment increased organ weight to body weight ratios, the most significant improvement in pancreas weight was observed with UIE25 (Table 4.8).

Hyperglycemia is accompanied with an increase in TC, TG, LDL and fall of HDL which is attributable to excess mobilization of fat from the adipose due to under peripheral utilization of glucose [232, 233]. Moreover, TC, TG, VLDL and LDL are atherogenic biomarkers responsible for atherosclerosis; however, a higher proportion of HDL is anti-atherogenic [234]. The data revealed that the level HDL increased and the level of TC and TG were significantly decreased with GAE, ANE and UIE. Also the increment of LDL, VLDL and LDL/HDL ratio were significantly attenuated after the treatment. The most significant improvement in lipid profile was observed with Group UIE25 (Figure 5.2). The regression of diabetic state by the administration of GAE, ANE and UIE improved the utilization of glucose and thereby suppressing the lipid mobilizations. Moreover, lipid lowering effects of extracts may also be due to low activity of cholesterol biosynthesis enzymes in liver and/or decreased level of lipolysis in adipose tissues which are under the control of insulin [235]. Recent studies indicated that triglycerides are independent risk factors for coronary heart diseases [235] and most of the anti-hyperlipidemic drugs do not decrease TG levels. Interestingly, in this study GAE, ANE and UIE lowered it significantly and this effect might be related to increase the endothelium bound lipoprotein lipase- a key enzyme which plays a major role in the transportation and metabolism of TGs of exogenous origin [236].

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues are a direct reflection of insulin sensitivity because insulin promotes glycogen deposition by stimulating glycogen synthetase [237]. Induction of diabetes with alloxan was associated with a marked reduction in liver glycogen stores which could be attributed to a decrease in the availability of the active form of enzyme glycogen synthetase, which in turn results from low level of insulin [237, 238]. Oral administration of GAE, ANE and UIE restored the liver glycogen content possibly

due to an increase level of insulin, which was evident by the preservation of pancreatic *β*-cells structure as revealed by microscopic examinations (Figure 5.4). The number of functionally intact *β*-cells in the islet organ is of decisive importance for the development course and outcome of diabetes. In alloxan-induced diabetes, (−)-Epicatechin [239] and *Vinca rosea* extracts [240] has been shown to act by *β*-cell regeneration. It was also suggested that regeneration of islet *β*-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs [241]. In our studies, the Group GAE200 and ANE100 showed partial reduction of necrosis of the islets of Langerhans. The diabetic rat treated with UIE25 restored normal cellular architecture of islets of Langerhan's and absence of islets damage. Among the treatment groups the significant architectural improvement was observed with UIE25.

SGOT and CK-MB are the reliable markers of liver and cardiac damage, respectively [239]. Oral ingestion of GAE, ANE and UIE significantly reduced the increased levels SGOT and CK-MB (Figure 4.2, 4.4 and 4.6). Among the treatment groups, ANE and UIE showed demonstrable attenuation of SGOT and CK-MB levels as noted by the improvement in liver and heart weights (Table 4.2 and 4.8).

Oxidative stress is one of the major pathogenic mechanisms considered to be implicated in diabetes [242]. Persistent hyperglycemia is associated with oxidative stress may result from an increased production of free radicals and compromised antioxidants defenses leading to the development of diabetic complications [243]. Lipid peroxidation, oxidative damage to DNA and protein may further complicate disease conditions in patients with type 2 diabetes [244]. Thus, treatment of diabetic patients with plant rich in antioxidants may prove to be advantageous in attenuating these complications. So, we further tested the extracts from three selected plants for their antioxidant property using different *in-vitro* methods. Our results indicated that the extract from the bark of *Grewia asiatica* Linn (GAE), rhizome of *Alpinia nigra* (ANE) and bulb of *Urginea indica* (UIE) showed the highest total antioxidant content. Among the three extracts the UIE had highest total antioxidant content whereas the *Alpinia nigra* (ANE) and *Grewia asiatica* Linn (GAE) had moderate one (Figure 5.10**)**. Also UIE showed the highest iron reducing capacity with absorbance of 1.31 at 200µg/ml whereas the GAE had the lowest reducing capacity with absorbance of 0.96

at 200µg/ml (Figure 5.11**)**. The results demonstrated that the plant *Urginea indica* (UIE) has the most antioxidant activity.

Polyphenols such as flavonoids, tannins and stilbenes are the most abundant antioxidants in the plant kingdom. Several studies reported positive correlation between phenolic content of plants with their antidiabetic activities [245, 246]. The antioxidant activity of the polyphenolic compound is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet, triplet oxygen and/or decomposing peroxides. Our results showed that among the three extracts, higher amount of phenolics was found in the UIE (87.74mg of gallic acid equivallent/g dried extract) than that of ANE (62.72mg of gallic acid equivallent/g dried extract) and GAE (44.65 mg of gallic acid equivallent/g dried extract) (Figure 5.6). These data indicated that *Urginea indica, Alpinia nigra* and *Grewia asiatica were* the significant source of phenolics. The observed antioxidant activities of the extract may be due to the presence of hydroxyl groups in phenolic compounds. Furthermore, flavonoids are the most ubiquitous groups of plant secondary metabolites and have good antioxidant potential. The flavonoid content of UIE, ANE and GAE were 67.72mg of quercetin equivalent/g dried extract, 50.46mg of quercetin equivalent/g dried extract and 39.11mg of quercetin equivalent/g dried extract, respectively (Figure 5.8). Among the extracts, the highest amount of flavanoid was found in UIE. In addition, other compounds, glycosides, terpenes, tannins and steroids have been found in UIE, ANE and GAE which might be responsible for their hypoglycemic and hypolipidemic activity (Table 2.4).

Radical scavenging activities are very important to prevent the deleterious role of free radical in diabetic disease. 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging is an accepted mechanism by which antioxidants act. Our results revealed that GAE, ANE and (UIE had DPPH radical scavenging activity with IC_{50} of 76.45±0.21 µg/ml, 43.25±0.15 µg/ml and 27.50±0.021 µg/ml, respectively (Table 5.8). Among the extracts, UIE had the highest free radical scavenging activity with IC_{50} of 27.50±0.021 µg/ml which is almost similar to that of ascorbic acid standard IC_{50} of 12.50±0.17 µg/ml and the lowest activity was found with GAE IC_{50} of 76.45±0.21 µg/ml (Figure 5.13). The results indicated that the extracts could serve as free radical scavengers, acting possibly as primary antioxidants and might protect

against pancreatic *β*-cell damage in alloxan-induced diabetic rats. It has been found that plants containing phenolics, flavonoids and tocopherols reduce the DPPH radicals by their hydrogen donating ability [247].

The results of the present study demonstrated that the ethanolic extract of the bark of *Grewia asiatica* (GAE200), rhizome of *Alpinia nigra* (ANE100), and bulb of *Urginea indica* (UIE 25) exhibited significant glucose and lipid lowering activity. Amongst the three aforesaid medicinal plants, *Urginea indica* (25mg/kg) showed the most significant antidiabetic activity in terms of its glucose and lipid lowering efficacy, organ protection as well as antioxidant potencies. Therefore, bioassay guided fractionation of *Urginea indica* bulb extract was carried out in order to isolate and characterize active principles responsible for the antidiabetic properties in alloxan-induced diabetic rats.

A number of chemical constituents were isolated from the plants *Grewia asiatica* and *Alpinia nigra* but there are only few reports on the isolation of pure compounds from the plant *Urginea indica* (Table 1.6). Therefore, the ethanol extract of *Urginea indica* bulbs was partitioned successively with pet-ether, chloroform and ethyl acetate (Figure 6.1). The successive fractions were further investigated for their antidiabetic activity. Among the fractionated extracts, chloroform extract (25mg/kg) was found to possess *in-vivo* hypoglycemic activity whereas pet ether and ethyl acetate extracts were devoid of activity [data not shown]. The $CHCl_3$ (4g) fraction was subjected to bioassay-guided fractionation using column chromatographic technique with n-hexane-$CHCl_3$, followed by $CHCl_3$-MeOH, which yielded five sub-fractions (F1-F5) (Table 6.1 and 6.2). Based on hypoglycemic activity F-2 and F-3 were further subjected to chromatography with n hexane-ethyl acetate as solvent to afford UC-1 (colorless crystal, 75mg, 5,7-dihydroxy-2-methyl-4H-chromen-4-one), and UC-3 (colorless crystal, 80mg, 5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one), respectively (Figure 6.2). Structure of UC-1 and UC-3 were determined by analysis of its 1D and 2D ^{1}H- and ^{13}C-NMR spectra and confirmed by comparing NMR data with previously reported derivative isolated from *Dysoxylum macrocarpum* [203] (Table 6.6). Both the compounds were flavonoid in nature and this is the first report of isolation of these two chromone derivatives from this plant *Urginea indica.*

Compounds such as flavanoids having phenolic group may be responsible for hypoglycemia and hypolipidemia by virtue of their antioxidant activity. It has been

reported that chromone derivatives may reduce post-prandial hyperglycemia by *α*-glucosidase inhibition [217]. However, no hypoglycemic activity of *Urginea indica* bulb as well as isolated compounds was previously reported. The wide variety of phytoconstituent with different molecular structure suggested that there is a possibility of different mechanisms involved in blood glucose lowering actions. Some have been shown to inhibit *α*-amylase while the others stimulating the release of insulin. For examples, alkaloids inhibit α-glucosidase and decrease glucose transport through the intestinal epithelium; polysaccharides increase the level of serum insulin, reduce the blood glucose level and enhance tolerance to glucose; flavonoids suppress the plasma glucose, cholesterol and triglycerides; and also increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets; and saponins stimulate the release of insulin and block the formation of glucose in the blood stream [248, 249].

Therefore, we have evaluated the hypoglycemic and lipid lowering effect of UC-1 and UC-3 in alloxan-induced diabetic rats after 7days of oral administration. Rats treated with UC-1 and UC-3 as well UIC25 showed a significant improvement in glucose tolerance, reduced blood sugar levels and attenuated high lipid profiles (Figure 7.1, 7.2, and 7.3). Oral ingestion of UC-1 and UC-3 significantly restored liver glycogen content and reduced the elevated levels of SGOT and CK-MB (Figure 7.4). In histopathological study the compounds UC-1 and UC-3 showed reduction in cellular damage and increased number of viable cells (Figure 7.5).

Our research work demonstrated that among the eight (08) selected plants' extracts, the stem barks of the plant *Grewia asiatica*, rhizomes of the plant *Alpinia nigra* and bulbs of the plant *Urginea indica* have significant hypoglycemic activity. Preliminary phytochemical analysis of three plant extracts indicated the presence of various bioactive constituents such as steroids, terpenes, flavonoids, glycosides and tannins (Table 2.4). The *Grewia asiatica* and *Alpinia nigra* extracts were found to be safe up to 2000mg/kg whereas *Urginea indica* was up to 200mg/kg. The extracts GAE (200 & 400mg/kg), ANE (50, 100 & 200mg/kg) and UIE (12 & 25mg/kg) improved the survival rates, OW, OW/BW, and attenuated SGOT and CK-MB levels in alloxan-induced diabetic rats. The comparative study clearly demonstrated that the among the three plants, *Urginea indica* (25mg/kg) showed the most significant antidiabetic activity in terms of its glucose and lipid lowering efficacy, restoration of liver

glycogen and architectural improvement in *β*-cells of pancreas as well as antioxidant potencies (Figure 5.10). This is the first report of antidiabetic activity of ethanolic extracts from the rhizome of *Alpinia nigra* and bulb of *Urginea indica* in alloxan-induced diabetic rats. Two chromone derivatives, UC-1(5, 7 dihydroxy-2-methyl-4H-chromen-4-one) and UC-3(5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one) were isolated from the bulb of *Urginea indica* and have found to be effective in reduction of blood sugar levels, lipid profiles, SGOT and restoration of liver glycogen.

We concluded that the plant *Grewia asiatica (Phalsa), Alpinia nigra (Jangli Ada)* and *Urginea indica (Bon Pianj)* as well as isolated compounds UC-1 and UC-3 exhibited significant effects on the fasting blood glucose level, improvement in liver glycogen content, serum SGOT, CK-MB levels as well as organ protection. The beneficial effects of extracts as well as isolated compounds can be partially explained by the preservation of pancreatic *β*-cell structure through the suppression of oxidative stress. However, the exact mechanism by which UC-1 and UC-3 exerted its beneficial effects in alloxan-induced diabetic rats remained to be elucidated.

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