BIOCHEMICAL BASIS FOR THE ANTIDIABETIC EFFECT OF CERTAIN PLANTS USED IN TRADITIONAL *AYURVEDIC* / *UNANI* MEDICINES

Thesis submitted
to the Pune University
for the award of

Degree of Doctor of Philosophy
in
Chemistry

Ms. Halim Eshrat Mohammed
(UGC)

NATIONAL CHEMICAL LABORATORY
(Council of Scientific & Industrial Research)
Pune - 411008 (India)

JANUARY 2002

Dedicated to the Memory
of my
beloved Parents

Certificate

This is to certify that the research work presented in the thesis entitled "*Biochemical basis for the Antidiabetic effect of certain plants used in traditional* ***Ayurvedic / Unani*** *medicines* " has been carried out by Miss Halim Eshrat Mohammed under my Supervision in the Division of Biochemical Sciences, National Chemical Laboratory Pune, for the award of the degree of Doctor of Philosophy in Chemistry. The work done and the thesis submitted are original and have not been submitted earlier to this or any other University.

Dr. (Mrs.) Mala Rao
(Co-Supervisor)
Scientist
Biochemical Sciences
National Chemical Laboratory
Pune

Dr. Paul Ratnasamy
Chief Supervisor
Director
National Chemical Laboratory
Pune

Date : January, 2002
Place : Pune

Declaration

I hereby declare that the present research work embodied in the thesis entitled ***"Biochemical Basis for the Antidiabetic effect of certain plants used in traditional Ayurvedic / Unani medicines"*** has been carried out by me under the ***supervision*** of ***Dr. Paul Ratnasamy*** and Dr. Mala Rao, National Chemical Laboratory Pune , for the award of Doctor Philosophy in ***Chemistry.***

This work is original and has not been submitted for any degree, diploma, or associateship of this or any other University.

Miss Halim Eshrat Mohammed

Biochemical Sciences Division
National Chemical Laboratory

Place: Pune
Date : January, 2002

Acknowledgment

I deem it a great pleasure to place on record my deep sense of gratitude and indebtedness to my research supervisor, ***Dr. Paul Ratnasamy*** Director National Chemical Laboratory Pune, for his support, invaluable guidance and constant encouragement throughout the period of the research work. I am specially thankful to him for having confidence in me which helped me to overcome many problems during my research work. I am indeed grateful to him for his constant support and full fledged cooperation .

I convey my special thanks to Dr. Mala Rao Scientist biochemical sciences division for her help and cooperation during my research.

I also take the opportunity of thanking to Dr. K.V. Raghavan, Director of Indian Institute of Chemical technology Hyderabad, for granting me permission to Utilize facilities in his institute and helpful comments at different stages of my work.

I am equally indebted to Dr. (Mrs.) Kaiser Jamil, Emeritus Scientist (Former Head Department of Biology and Biotechnology Division), for providing animal house facilities during the initial stages of my work at IICT.

I am deeply grateful to Ministry of Human Resource Development (Department of Secondary Education & higher Education) New Delhi. Government of India for accepting my research project and giving me research visa. I am also thankful to them for regularizing my overstay in India.

I would also like to thank the University Grants Commission (UGC), New Delhi, for the awards of ***Junio****r* and ***Senio****r* research fellowships.

I express my gratitude to my brother and sisters for their patience. Finally, I dedicate my thesis to the memory of my beloved father and mother.

Miss Halim Eshrat Mohammed

ABSTRACT

Abstract of the thesis entitled Biochemical basis for the "**Antidiabetic effect of certain plants use in traditional *Ayurvedic / Unani medicines*"** submitted to the University of Pune for the degree of *Doctor of Philosophy* in **Chemistry.**

Name of the research student	:	Halim Eshtat Mohammed
Name of the research Chief Guide	:	***Paul Ratnasamy***
Name of the Co - Guide	:	Dr. (Mrs.) Mala Rao.
Place of research	:	Division of Biochemical Sciences, National Chemical Laboratory , Pune-411008
Application dated	:	09.07.1999
Date of Registration No.	:	20.07. 1999
Reference No.PGS/Ph.D. / 2079	:	28.05. 2001

ABSTRACT

Diabetes mellitus **(DM)** is prevalent in all countries of the world. More than 30 million people are said to be affected throughout the world with this disease. Though insulin is widely accepted as an ideal choice for treatment of diabetes mellitus. The difficulty of repeated administration led to the search for the hypoglycemic agents. Diabetes mellitus (DM) is presently the most common non-communicable disease world wide and is the fourth or fifth leading cause of death in most developed countries. There is also substantial evidence that it is an epidemic in many developing and newly industrialized countries. Complications from DM such as cardiovascular disease (CAD), peripheral vascular disease (PVD), stroke, diabetic neuropathy, amputations, renal failure and blindness are on the increase. Therefore, it is certain that DM will be one of the most challenging health problems in the new millennium. Prevention and control programs are needed to stem the rising epidemic of DM and its complications. In 1997, an estimated 124 million people worldwide had diabetes mellitus, 97% of these had non-insulin dependent diabetes mellitus (NIDDM).

Deaths from cardiovascular disease predominate in-patients with diabetes of over 30-year's duration and in those diagnosed after 40 years of age. Patients with protein urea have a greatly increased risk of fatal cardiovascular disease. The frequency of coronary heart disease (CHD) in diabetes is related to that in the background population (e.g. it is low in diabetic patients in China and Japan). General risk factors for cardiovascular disease include smoking, obesity, hyperlipidaemia, hypertension, insulin resistance, haemostative and platelet

abnormalities, lack of exercise and a positive family history, Specific diabetes related risk factors may include hyperglycemia (especially for peripheral vascular disease) and hyperinsulinaemia. CHD in diabetic patients is associated with increased plasma cholesterol levels, with reduced HDL-cholesterol in NIDDM patients and possibly with increased triglyceride levels. The decreased insulin action and / or decrease insulin secretion Arterial disease may cause atherosclerosis.

Arterial disease may be manifested clinically as coronary heart disease (CHD),cerebrovascular disease, or peripheral vascular disease. Hypertriglyceridemia in diabetes therefore usually responds to intensified insulin treatment. LDL levels are also raised in association with poor glycaemia control, but a substantial improvement in blood glucose is required to lower LDL. Insulin stimulates LDL receptor activity. Preliminary evidence suggests that similar associations also apply in diabetes. Fibrinogen levels are raised in both IDDM and NIDDM, and are higher in those with cardiovascular complications. Many drugs with proven hypocholesterolemic activity are available clinically to ameliorate cases of individuals with premature arteriosclerosis and those with other risk factors, such as hypertension or diabetes mellitus. Treatment with insulin is essential for type I patients apart from insulin resistance; insulin therapy may lead to other complications like blurred vision and hypoglycemia. Insulin has yet been found in the treatment of type I diabetes. Plant materials which are cheap and are within the reach of the village folks are being used the world over as a remedy for DM. This prompted the scientific studies on the anti-diabetic

activity of the plant materials and many of these have been reported to show hypoglycemic activity in animals as well as human been. In many cultures of the world, herbal remedies are increasingly being employed in an attempt to achieve the same purpose. In India, for instance, the leaves of *Azadirachta Indica* (L.) (neem) is claimed to possess cholesterol-reducing effect and is used to treat patients with heart disease and obesity. For this reason it was decided to resolve this claim by investigating the effect of the crude extract of few Ayurvedic plants on the blood sugar, serum. Liver and kidney cholesterol of the wisteria rat. The effect on serum total protein, and albumin, were also examined in the same animal model.

The following thesis is divided in to 6 Chapters:

Chapter 1:

INTRODUCTION

It has become increasingly evident in recent years that a full spectrum of therapeutic agents for the treatment and prevention of human disease is far from being complete. In an attempt to fill in the gap, drug development research has now focused on traditional herbal remedies as a potential source for new and more effective medical therapies.

Despite considerable progress in the management of DM by synthetic drugs, the search for indigenous natural anti-diabetic agents is still going on. India being rich in its plant wealth. Several plants have been identified as the potential source of drugs in Indian system of Ayruveda medicine for the treatment of diabetes. Extracts of various plants have been shown to produce

hypoglycemia in normal and experimental diabetic animals. Some of the commonly studied plants are *Momordica Charantia, Allicin Cepa, Allium Sativum, Ficus begalansis, Eugenia jambolana, Abroma Augusta, Azadirachta Indica, Coccinia Indica, Curcuma Longa Indica and Ocimum sanctum. Since Abroma augusta , Azadirachta Indica , Coccinia Indica, Curcuma Longa and Ocimum sanctum.* are widely used in indigenous medicine both in *Ayurvedic* and *Unani.* Many of its products / chemical constituents are known to posses wide array of medicinal properties. Because of their diverse activity potential, there is a considerable hope of finding anti-diabetic properties in these plants.

Hence the present investigation reports the effect of certain *Ayurvedic* plant individually and or in combination with the other plants on alloxan / streptozotocin induced diabetic rats.

Chapter 2:

Preliminary studies on the hypoglycemic effect of *Abroma augusta* in alloxan Diabetes in rats

The hypoglycemic effect of the aqueous extract of *Abroma augusta* (Linn.) (Family: *Steculiceae*) was studied in normal as well as alloxan diabetic rats. Treatment of diabetic rats with 4 ml (4 gm dry weight) of aqueous extract of *A. Augusta* for 16 weeks resulted in gradual but significant fall in fasting blood glucose and improvement in glucose tolerance. Serum total and LDL cholesterol and triacylglycerol, which increased in diabetic rats, showed improvement. These results show that the water extract of *A. augusta* has both hypoglycemic and hypocholesterolemic effects.

Chapter 3:

Hypoglycemic, hypolipidemic and antioxidant properties of (*Ocimum sanctum* Linn.) (Tulsi) on streptozotocin induced diabetes in rats.

Since the time of Charaka and Susruta many herbal medicines in different oral formulations have been recommended for diabetes (Madhumeha). Crude drugs extracts from plant sources such as *Allium sativum (garlic). Azadirachta indica (neem) vinca rosea (nayantara), Gymnema sylvestra (meshashringe). Trigonella foenum graecum (fenugreek), Momordica Charantia (bitter gourd). Ficus bngalensis (banyan) Eugenia jambolana (bear)*.

Effect of oral administration of 200 mg / kg body weight (b.w) of the aqueous extract of the *Ocimum sanctum* (Tulsi) mixed with diet for eight weeks to streptozotocin induced diabetic rats, to analyzing blood glucose glucose tolerance, serum lipid profile and lipid peroxidation (LPO). Antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and one antioxidant reduced glutathione (GSH) in plasma and rat liver, lung, kidney and brain was studied. The treatment resulted in a significant reduction in blood glucose and serum lipids. The aqueous extract also decreased lipid peroxides formation

Thiobarbituric acid reactive substance (TBARS) and increased antioxidants in the tissues studied. The decrease in TBARS and increase in GSH, SOD, CAT and CAT clearly showed the antioxidant property of *Ocimum sanctum.*

Chapter 4:

Blood sugar lowering effect of water extract of *Azadirachta indica* (L.) (neem) and *Abroma Augusta* (Linn.) in diabetes rats.

Water extract of the dried powder of two plants, *Abroma augusta* (Fam: Sterculiaceae) root and *Azadirachta indica* (Fam: *Meliaceae*) (L), leaves combined together was administered. This treatment caused significant lowering of blood sugar in fasted rats and depressed the peak value in blood glucose during glucose tolerance test. The treatment resulted in a significant reduction in serum lipids. The aqueous extract also decreased lipid Peroxidase formation (TBARS), and increased antioxidant reduced (GSH) and superoxide dismutase (SOD) catalase (CAT) glutathione Peroxidase (GPX) and gluthione transferase (GT) It the plasma and the tissues liver, kidney, and muscle. It also prevented decrease in body weight shows that Abroma augusta root and Azadirachta Indica (Neem) leave when together as water extract have hypoglycaemic action as determined by assay in diabetic rats.

Chapter 5:

Hypoglycaemic and hypolipidemic effect of combination of *Coccinia indica* and *Abroma augusta* in alloxan diabetic in rats.

The hypoglycemic effect of *Abroma augusta* and *Coccinia Indica* is known individually . In *Ayurvedic* system of medicine in India, combination of plant extracts is used for the treatment of diabetes mellitus. So our aim has been to try the combined effect of *A. Augusta* and *C. indica* experimental animals on blood sugar glucose tolerance and, lipid profile.

Chapter 6:

Hypoglycemic and hypolipidemic effect of combination of *Curcuma Longa* constituents and *Abroma augusta* on blood glucose profile and antioxidant properties of STZ – induced diabetic in rats.

Since *Curcuma Longa* (Turmeric, Haldi) family Zingeberaceae is widely used in indigenous medicine both in *Ayurvedic* and *Siddha,* many of its products / chemical constituents are known to posses wide array of medicinal properties. The study will be aimed at assessing the role of *C.Longa* in the blood glucose profile, lipid peroxidation level and free radical formation in tissues. The effect of oral administration of (300 mg/kg body weight) of the aqueous extract of the turmeric active gradient *Curcumin* and *Abromine* powder mixed with diet was studied for 8 weeks on lipid peroxidation (LPO) and the antioxidant defense system in rat tissues, rat liver, lung, kidney and brain. This resulted in a significant reduction in blood glucose and in increased total hemoglobin. Decrease in free radical formation in the tissues was also observed. This study shows that *Abroma augusta* (whole plant) and fresh tuber

C. Longa showed decrease in thiobarbituric reactive substances (TBARS) and increase antioxidant and reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) indicating the antioxidant activity of *curcumin* and related compounds.

A part of this work has been published as.\

List of Research Paper Publications.

1. Halim Eshrat M. Ali Hussain, Kaiser jamil and Mala Rao (2001) Preliminary studies on the hypoglycemic effect of *Abroma augusta* in alloxan diabetic's rats. Indian *Journal of Clinical Biochemistry*, 16 **(1) :** 77 – 80.

2. Halim Eshrat M. Ali Hussain, Kaiser jamil and Mala Rao (2001) Hypoglycemic hypolipidemic and antioxidant properties of (Ocimum *sanctum* Linn.) (Tulsi) On streptozotocin induced diabetes in rats. *Indian Journal of Clinical Biochemistry*, 16 **(2):** 190 – 194.

3. Halim Eshrat M. Ali Hussain, Kaiser jamil Mala Rao (2002) Traditional Indian anti-diabetic plant *Azadirachta indica* (L.) in diabetic retinopathy in streptozoptocin induced diabetic rats. *Indian Journal Experimental Biology* NISCOM New Delhi. (**in Press).**

4. Halim Eshrat M. Ali Hussain, Kaiser jamil Mala Rao (2001) Hypoglycemic and hypolipidemic *Curcuma Longa* (Turmeric) and *Abroma augusta* on blood glucose profile and antioxidant properties of STZ – induced diabetic Wister rats. *Indian Journal of Clinical Biochemistry*. (**in Press**).

5. Halim Eshrat M. Ali Hussain, Kaiser jamil and Mala Rao (2001) Blood sugar lowering effect of water extract of *Azadirachta Indica* (L.) (neem) and *Abroma augusta* (Linn.) in diabetes rats. *Journal of Ethnopharmacology.* **(Communicated).**

6. Halim Eshrat M. Ali Hussain, Mala Rao Treatment with extracts of Vinca Rosea.prevents hyperglycemia and hyperinsulinemia in fructose rich diet in rats. *Diabetes Research and Clinical Practice*. (**Communicated**).

7. Halim Eshrat M. Ali Hussain, Kaiser jamil Mala Rao (2001) Hypoglycaemic and hypolipidemic effect of combination of Coccinia Indica and *Abroma augusta* in alloxan diabetic rats. *Diabetes Care*.

(**Communicated**).

8. Halim Eshrat M.Ali Hussain, Mala Rao, Kaiser Jamil, "Role of the Abroma augusta identity of Abromine ethanoic extract on streptozotocin – induced diabetic rats", Diabetes Research and Clinical Practice, **(Communicated).**

9. Halim Eshrat M. Ali Hussain, Mala Rao, Kaiser jamil . Effect of ′antidiabetis' herbal preparation on serum glucose and , cardiac and renal diseases in treatment of diabetes of India. *Diabetes Research and Clinical Practice.* (**Manuscript in Preparation**).

10. Halim Eshrat M. Ali Hussain, Mala Rao, Kaiser jamil , Role of the *Abroma Augusta* identity of Abromine ethanoic extract on streptozotocin – induced diabetic rats..*Diabetes Research and Clinical Practice .* (**Manuscript in preparation**).

11. Halim Eshrat M. Ali Hussain . Hypoglycemic compound of *Abroma augusta* STZ – induced diabetic Wister rats. *Journal of Ethnopharmacology* (**Manuscript in preparation**).

12. Halim Eshrat M. Ali Hussain, Mala Rao, Kaiser jamil Abstract entitled " Hypoglycemic hypolipidemic and antioxidant properties of traditional herbal medicine" has been accepted for oral presentation in **9th ASIAN PACIFIC CONGRESS CLINICAL**

BIOCHEMISTRY New Delhi, **13. March. . 2002 .** The **Abstract** is published in ***NATURE*** . www9thapccbnewdelhi.com.

13. Traditional Herbal Medicines, Book under Preparation.

Halim Eshrat M. Ali Hussain and Dr. Ram Dass Gautam Division of Entomology, Indian Agricultural Research Institute **(I.A.R.I).** New Delhi-110012.

14. **Medicinal use of Neem tree** (*Azadirachta indica*) A. Juss **. Book under Preparation Halim Eshrat M. Ali Hussain and Dr. R.P. Singh**, Indian Agricultural Research Institute, **(I.A.R.I) ,** New Delhi-110012.

15. Paper presented on "**Hypoglycaemic Effect of *Abroma-augusta* in Alloxan Diabetic Rats"** in International Conference on "Women : Biotechnology, Environment and Non-conventional Energy " October 17th to 21th , 2000, IICT, Hyderabad.

16. Accepted Poster Presentation on 13. March . 2002 in 9 the ASIAN PACIFIC CONGRESS OF CLINICAL BIOCHEMISTRY, NEW DELHI . The Abstract included in the **Abstract** Book. Web site www. **9thapccbnewdelhi.com**

17. Participated in International Conference at "INTERNATIONAL CONFERENCE ON IICT" , Hyderabad-500007 , INDIA.

I also attended in diabetic camps in various Hospitals including All India Institute of Medical Science, Safdarjung Hospital, , New Delhi, Agra Medical College, Agra, U.P,. Nizam Hospital, Hyderabad, PGI Medical College, Chandigarh, etc. **A personal communication made to me by one the patient, who has recovered from the diabetics by using this herbal medicine, has been included in this book as appendix**.

ABBREVATIONS USED IN THE THESIS

ADP	Adenosine diphosphate
ATP	Adenosine troposphere
ATPase	Adenosine triphosphatase
CVD	Cerebrovascular disease
CAT	Catalane
C°	Degree Centigrate
DCCT	Diabetes Control Complications Trial
DM	Diabetes mellitus
ESRD	End stage renal disease
FPG	Fasting Plasma glucose
Fig	Figure
GAD	Glutamic acid decarboxylase
GSH	Glutathione reduced
GAD	Glutamic acid decarboxylase
GPX	Glutathionperoxidase
GSHPX	Glutathione reduced
IDDM	Insulin dependent diabetes mellitus
LPO	Lipid per oxidation
MDA	Malonyldialdehyde
Mg	Milligram
MRDM	Malnutrition Related Diabetes Mellitus
NIDDM	Non- Insulin Dependent Diabetes
OD	Optical Density
GOD	Glucose Oxidase
PVD	Peripheral vascular disease
PUFA	Poly unsaturated fatty acids
NLE	Neem Leaf Exteact

NEFA	Non Esterified Fatty Acid
%	Percent
STZ	Streptozotocin
SOD	Superoxide dismutase
TBARST	Thiobarbituricreactive Substances
TAG	Triacylglycerol
TBA	Thiobarbituric acid
µ l	Microlitre
WHO	World Health Organization
SOD	Superoxide dismutase
PBS	Phosphate Buffered Saline
h	Hour
OD	Optical Density
GOD	Glucose Oxides

CHAPTER - 1

GENERAL INTRODUCTION

Diabetes mellitus (**DM**) is prevalent in all countries of the world. More than 30 million people are said to be affected throughout the world with this disease.

It is presently the most common non-communicable disease worldwide and is the fourth or fifth leading cause of death in most developed countries. There is also substantial evidence that it is an epidemic in many developing and newly industrialized countries. Complications from DM such as CAD, PVD, stroke, diabetic neuropathy, amputations, renal failure and blindness are on the increase. (Amos, 1997). Therefore, it is certain that DM will be one of the most challenging health problems in the new millennium. Prevention and control programs are needed to stem the rising epidemic of DM and its complications. In 1997, an estimated 124 million people worldwide had DM, 97% of these had (non-insulin dependent diabetes mellitus). By the year 2010, the total number of people with DM is projected to reach 239 millions (McCarty, 1994). Regions with greatest potential are Asia and Africa, where DM rates could rise to 2 to 3 folds than the present rates. The projected increase in DM incidence in Asia is likely to be 3.6 to 11.4, 28.8 to 57.5, 8.6 to 19.5 and 21.7 to 44 millions in Western Asia, South Central Asia, South East Asia and East Asia respectively. Increase in complications will undoubtedly follow the prevalence of DM.

Diet - induced hyperglycemia occurs due to malnutrition in early life. Carbohydrates are major source of energy for all living organisms including humans. The major pathways of carbohydrate metabolism either begins or ends with glucose and it is the major form in which carbohydrate from the intestinal tract is presented to the cells of rest of the body. Glucose is continuously delivered to all tissues by the blood, which normally contains 80-90 mg% before meal and 80-120 mg% after meal. The dependence of various tissues on glucose varies widely.

Glucose is the only fuel used by few specialized cells i.e. brain and erythrocytes. Despite large changes in the input and utilization of glucose, the blood glucose levels are maintained at constant level in response to hormonal signals. So the concentration of glucose in the blood is subjected to tight regulation and homeostasis of glucose in blood is mainly maintained by cooperative activity of liver, muscle, kidney and adipose tissue and by the endocrine glands at the cellular and enzymatic levels.

The liver plays glucostatic role in response to hormonal signals and by levels of glucose itself, while the kidney operates by way of filtration and reabsoption. Glucose metabolism is defective in two very common metabolic diseases, obesity and diabetes, which in turn contribute to factors in the development of number of major medical complications including atherosclerosis, hypertension, small vesseldiseases, kidney diseases, blindness etc.

Consumption of diets highly enriched with saturated fats or simple sugars (e.g. glucose) can increase insulin concentrations, enhance adipose tissue deposition, reduce insulin sensitivity and impair glucose tolerance. These effects are seen even if total energy intake is not increased, but are more pronounced if this is greater, High - fat

feeding can also enhance diabetic features in rodents treated neonatal with streptozotocin or with ventromedial hypothalamic lesions (Pasco WS, et al 1990 & Pascoe WS, et al 1992).

Two major types of clinical syndromes due to increased hyperglycemia can be mentioned here. The first one is characterised by insulin dependence and onset in early age, with weight loss and ketonuria (Passing of ketone in urine). This is generally termed as type I or Insulin dependent Diabetes Mellitus (IDDM). Fortunately the type I diabetes or (insulin dependent diabetes mellitus) prevalent in south East Asia including India as in the west. Instead, young people in India have malnutrition related diabetes mellitus (MRDM), which can be efficiently controlled or reversed by proper and balanced diet.

The second is characterised by late onset, insensitivity to insulin and partial insulin deficiency. It is generally called Non-Insulin Dependent Diabetes Mellitus (NIDDM), or type ll. The prevalence of diabetes, especially NIDDM, is spiraling upwards, both in developed and developing countries. Fueled by rapid economic growth, the prevalence of diabetes has now reached unto 8% of the world population. Poorly controlled diabetes aggravates the risk of diabetes complications and particularly cardiovascular diseases (Mayes, 1993).

1. 1. EPIDEMIOLOGY

Chronic hyperglycemia in diabetes is associated with damage of tissue, dysfunction and ultimate functional failure of various organs shown in (Fig 3), especially in the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association, 1998). Several pathogenic processes are involved in the development of DM.

Autoimmune destruction of β-cells of the pancreas leads to insulin deficiency /release. It may also occur due to diminished tissue response to insulin at one or more points in the complex pathway of hormone action. Inadequate insulin release and impairment of insulin action frequently CO -exist. Long term complications of DM include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputation and charcoal joints (Fig.8).

Autonomic neuropathy may also cause sexual dysfunction along with gastrointestinal, genitourinary and cardiovascular symptoms. Patients with DM have a higher incidence of atherosclerotic, cardio-vascular, peripheral vascular and cerebrovascular disease.

Hypertension, abnormalities of lipoprotein metabolism and periodontal disease are often found in DM patients. The emotional and social impact of DM and the demand therapy may also result in significant sychosocial dysfunction in- patients and their families. Complication of diabetes mellitus the poorly controlled diabetes aggravates the risk of diabetes complications, particularly cardiovascular diseases. The clinical coups and prognosis for diabetic patients is influenced predominantly by the duration of the disease and degree of metabolic control exercised (WHO, 1994).

Major microvascular complications of DM include retinopathy, nephropathy and neuropathy. DM is the most common cause of adult blindness in developed countries (Klein and Moss, 1992), either due to retinopathy and cataract or due to glaucoma. Diabetic patients are 17 times more prone to kidney disease and diabetes is now the leading cause of end stage renal disease (ESRD) in USA. Microvascular disease including cardiovascular disease including coronary heart disease (CHD),

cerebrovascular disease (CVD) or stroke and peripheral vascular disease (PVD) are the common causes of morbidity and mortality among people with diabetes (Tuomilehto and Rastenyte, 1997)., Late complications of diabetic mellitus.

1.2. DIABETIC NEPHROPATHY

Diabetic nephropathy is a clinical syndrome characterized by persistent proteinuria (<70 µg/min) increased morbidity and mortality due to cardiac and cerebrovascular diseases (Anderson et al, 1983; Vibrate et al, 1983; parving et al, 1991). It is also the most common cause of hypertension in IDDM patients. Hypertension in diabetic nephropathy is mainly due to sodium retention as well as renin-angiotensin system (O Hare et al, 1983).

Anti-hypertensive treatment with metoprolol, hydrazine and diuretics (i.e.furosemide or thiazide) for 39 months reduced the decline in kidney functions in young type 1 diabetic's patents with nephropathy (Parving et al, 1983). Results of another study suggests that strict metabolic controls for 8 months may delay but not reverse the progression of diabetic nephropathy (Vannini et al, 1983) .

On the other hand, poor glycemic control is associated with the development and rapid progression of nephropathy (Viberti et al, 1987) (fig 7). Improved glycemic control is of greater value in the initial period of the disease, In addition, treatment of hypertension (in long term trials) and low protein diets. (In short term trials), (Ciavarella , 1987; Zeller 1991) have emerged as effective means of slowing the progression rate of nephropathy.

Among the new pharmacological treatment of diabetic nephropathy, aminoguanidine, a potent and specific inhibitor of glucose –mediated cross linking and

tissue damage in vitro (Brownlee et al, 1986) has been shown to ameliorate glomerular basement membrane thickness (Ellis and Good, 1991). It has also been shown to decrease albuminuria (Edelstein and Brownlee, 1992); Itakura et al, 1991; Soulis et al, 1991). However, a very limited amount of information is available on the efficacy of amino-guanidine in humans, (Makita et al, and 1992).

Clinical studies are currently ongoing USA, Europe till date, no clinical data on human diabetic nephropathy is available and therefore its clinical value in the treatment of diabetic nephropathy is as yet unknown.

1. 3. DIABETIC NEUROPATHY

Diabetic neuropathy it remains the most insidious, least understood, and most difficult to treat among all diabetic complications. Although the precise Pathophysiology is not known, metabolic hypothesis is the most credible theory to be accepted.

It is substantiated by the fact that glycemic control influences the onset and progression of diabetic neuropathy (DCCT Research group, 1993; Ziegler et al, 1988; Richard et al, 1993; Amthor et al 1995). In brain, nerve, eye and kindly tissue, where glucose transport is independent of insulin, hyperglycemia induces a high intracellular glucose concentration

As a result, hexokinase pathway gets saturated resulting in metabolism of glucose by the polyol pathway. Glucose gets converted to sorbitol by aldose reductase, which in turn gets converted to fructose by sorbitol dehydrogenate (Gabbay et al, 1966).

Accordingly, sorbital and fructose get accumulated. Glycation of proteins also form irreversible advanced glycosylated end products especially involving myelin proteins.

Studies performed in rat's show that aldose reductase inhibition is efficacious. (DCCT research group, 1993).

Diabetes Control and Complications Trial (DCCT) demonstrated that the degree of metabolic control strongly influences the development of neuropathy but despite near normal blood glucose control over 5 years, 8% still developed abnormal clinical neurological signs and 18% showed abnormal nerve conduction tests. (Cameron et al, 1986; DCCT research group, 1993). On the other hand, poor glycemic control is associated with development and rapid progression of nephropathy.

One of the consequences of hyperglycemia in human diabetes mellitus is increased metabolism of glucose by sorbitol pathway. This reaction is catalyzed by aldose reductase. Sorbitol is converted to fructose by sorbitol dehydrogenase. Aldose reductase is essential in human brain, nerves, aorta, muscles, erythrocytes and ocular lens. Though the presently available oral hypoglycemic are effective in controlling acute metabolic abnormalities, they are associated with side effects.

Countering the metabolic abnormalities of diabetes prevents its long-term complications i.e. nephropathy, neuropathy. The symptomatology, complications and treatment of DM stand out remarkably well in the present area of scientific observations of the disease (Ajgaonkar, 1984).

Even WHO suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas where we lack safe modern drug (WHO, 1994). Though insulin is widely accepted as an ideal choice for treatment of diabetes mellitus, the difficulty of repeated administration led to the search for oral hypoglycemic agents.

Some of the drugs such as Sulfonylureas and biguanidines are being used for this purpose. A variety of plant preparations have been mentioned in *Ayruveda* and other indigenous systems of medicine, which are claimed to be useful in diabetes mellitus and their complications. The symptomatology, complications and treatment of DM stand out remarkably well in the present area of scientific observations of the disease.

Some of the drugs based on sulfonylurea and biguanidines are being used for this purpose. Few of the metabolic effects that are associated with some acquired form of insulin resistance are potentially fully reversible but in case of genetic or inherited components associated with disorders such as non insulin dependent diabetes which are inherited as a complex trait are only partially reversible (Molar et al, 1996).

1. 4. NON- DRUG

In case of obese individuals, the body tries to maintain euglycemia by increasing the levels of endogenous insulin secretion resulting in hyperinsulinaemia. Nevertheless, the increase in body mass contributes to insulin resistance with glucose intolerance or non-insulin dependent DM.

Therefore, obesity remains an important modifiable component of the metabolic disturbance. Also postulated on basis of scientific evidence, among all the constituents of the food, the dietary fat composition may have greater effect on insulin sensitivity (Storlien et al, 1996).

It has been shown that sufficient exercise both in terms of intensity and duration exerts beneficial effects on subjects with Non-insulin dependent diabetes (Perseghin et

al, 1996). However, at the same time these patients are likely to be old and obese, which reduces compliance and are unable to participate in exercise programs.

Therefore, tackling obesity by means of dietary manipulation and exercise remains a difficult option and not many obese patients are able to adopt and sustain the changes required in their lifestyle (UK Prospective Diabetes Study Group, 1995).Smoking has been associated with insulin resistance (Law et al, 1992) and the result of a non-randomized prospective study of healthy middle aged American men shows that cigarette smoking may be an independent risk factor for non-insulin dependent diabetes (Rimm et al, 1995). Unlike adverse effects of smoking on the development of NIDDM, moderate alcohol consumption seems to have beneficial effects, as it was found to be associated with a reduced risk of developing diabetes, possibly reflecting positive effects on insulin sensitivity . Many specific oral anti-diabetic agents have appeared in recent reviews on the treatment of NIDDM (Leboviz; Groop, 1992).

The American Diabetes Association (ADA) has recently published a consensus statement on the pharmacological treatment of hyperglycemia, which includes four classes of agents (Fig. 10-A and 10-B)

a) INSULIN
b) SULFONYLUREAS
c) METFORMIN
d) ACARBOSE

The ADA consensus statement stated that hyperinsulinaemia associated with syndrome X may be a marker of insulin resistance rather than etiologic factor. The ADA consensus statement cautions about possible weight gain with Sulfonylureas and insulin, which could contribute to worsening of cardiovascular risk factor.

In the ADA (1997), statement, considerable emphasis has been placed on combination therapy. This is important and, in the future, management of diabetes is likely

to become more like the management of hypertension in which many clods of agents are used in combination to achieve optimal control. Much of work on combination therapy have recently been published in following areas.

Insulin

Treatment with insulin (Fig 9) is essential for type I patient's .The administration is carried out apart from insulin resistance; insulin therapy may lead to other complications like blurred vision and hypoglycemia. Insulin has yet been found in the treatment of type I diabetes.

Role of insulin in diabetes mellitus:

When the pancreatic extract was tried in human beings an initial hyperglycemic action was observed and further investigations led to the discovery of the hormone glucagon secreted from A cells of the pancreas. Insulin, a protein hormone has a wide range of metabolic action especially on carbohydrate metabolism. Once the β cells of the islets of Langerhans are destroyed by viral action, drug etc, the synthesis of insulin decreases or stops depending on the extent of the damage.

The biochemical characteristics of the disease are an increased blood glucose concentration resulting in glycosuria and ketonuria . If the disease remains untreated, ketoacidc sets in with polyuria, coma and finally death. Plant materials in the treatment of diabetes mellitus:

Plant materials, which are cheap and within the reach are being used by the village folks the world over since long, as a

remedy for diabetes mellitus. This prompted the scientific studies on the antidiabetic activity of the plant materials and many of these have been reported to show hypoglycemic activity in animals as well as human beings.

1.5. *Combination of drugs like Sulfonylurea with insulin metformin and insulin , Acarbose and sulfonylurea, produce side effects* .

Since in hypertension, the choice of anti-hypertensive agents depends on the range of side effects, the same is true for diabetes. For instance, treatment with Metformin (Fig 10(a) & (Fig. 10(b), may lead to better effects on both dyslipidemia and insulinemia than treatment with a Sulfonylureas.

It is clear that the treatment of insulin resistance has great therapeutic potential for the amelioration of type II diabetes. Although currently available clinical / pharmacological modalities are not directed to the treatment of impaired insulin action, recent efforts have focused on the development of insulin sensitizing agents.

Major break through in this area occurred in this area in 1982 with discovery of ciglitazone, a thiazolidinedione derivative, which was followed by a number of other compounds, such as pioglitazone, troglitazone, englitazone, and others. All share a common thizolidine-2-4-dione structure, with chemical modification to enhance their bioactivities. Troglitazone progressed to late stage clinical development and is currently available in the market. It was found to be effective in reducing plasma glucose, insulin, non-esterified fatty acids, triglycerides concentrations in genetically insulin resistant animals, including the *kka, ob/ob.* and *db/db* mouse and the Zucker fa / fa rat (Fujita et al, 1983; Fujiwara et al, 1988; Stevenson et al, 1990) as well as in fructose fed and high fat -

adapted rats (Lee et al, 1990;). Similar effects were seen in human studies of diabetic patients (Nolan et al, 1994; Kumar et al, 1996).

The one distinguishing feature of these insulin- sensitizing agents is their apparent lack of hypoglycemic activity in euglycemia animals, despite the potential sensitization of insulin action. This property differentiates thiazolidinedione from some other current therapies for type I diabetes, including insulin itself or insulin secretagogues such as Sulfonylureas, which potentially induce hypoglycemia.

Preliminary data regarding triglitazone suggests that it have comparable hypoglycemic effects as of other oral anti-diabetic drugs. Apart from diabetes, insulin resistance is also an essential feature of several other disease states such as obesity, hypertension, impaired glucose tolerance, and polycystic ovarian syndrome. Initial studies with troglitazone have been conducted in some of these conditions and the results have been promising

(Nolan et al, 1994).

Preliminary data suggests that troglitazone has beneficial effects on blood pressure in obese subjects, the data requires confirmation from other sources. Troglitazone appears to be equal in efficacy to Sulfonylureas or metformin in reducing glycosylated hemoglobin and also reduces triglyceride levels. Biguanidines on the other hand are effective in increasing insulin sensitivity. However, the effect of biguanides on long term complications has not been studied. The major therapeutic goal in-patients with NIDDM is to reduced blood glucose level along with reduction in obesity and to normalize lipid disturbances and blood pressure, in order to improve the well being of the patients and reduce the risk of development of late diabetic complications.

The contribution of modification of lifestyle and role of insulin sensitizing drugs in prevention of non- insulin dependent diabetes is still obscure. Until date, treatment aimed at decreasing release or oxidation of non esterified fatty acids have produce inconsistent results and the toxicity of drugs has also been a problem. Fibril acid derivatives designed primarily to reduce dyslipidaemias have also been tried in- patients with diabetes but they bring about only a modest improvement in correcting hyperglycemia. Other strategies employed recently include experimental therapies with three agents

The problems with these experimental therapies are either that they are too toxic or have limited efficacy, which restrict their therapeutic use. The use of appetite suppressants in obese patients, which are likely to develop non-insulin dependent diabetes, is questionable. An association between pulmonary hypertension and fenfluramine derivatives has recently focused attention on the risks and benefits for appetite suppressants.

A variety of herbal preparations have been mentioned in Ayruveda and other indigenous system of medicine, which are useful in diabetes mellitus. The hypoglycemic activities of many of these agents have been experimentally well documented. However, no work has been done to study their effects on insulin resistance, which is an essential draw back of presently available management protocol of DM.

The present therapy is grossly incomplete in view of the unmodified incidence of certain complications such as insulin resistance with the resultant hyperinsulinaemia and is blamed in the development of complications. At present, it is postulated that any agent that modulates insulin resistance is likely to have significant effect in the prevention of long term complications of diabetes mellitus and also in the day to day measures In

humans, also fructose induced insulin resistance has been found in obese (and normal subjects 1980) but not in well controlled diabetic subjects. The exact mechanism of fructose induced hyperinsulinaemia and hypertriglyceridemia are not known but various mechanisms have been proposed). Suppress activation of hepatic glucose -6-phosphatase and fructose-1, 6-di phosphates activity.

Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for indigenous natural anti-diabetic agents is still going on. India is rich in its plant wealth, Herbal medicinal is being used by an increasing number of patients who typically do not advisee their clinicians of concomitant use.

Several plants have been identified as the potential source of drug in Indian system of *Ayurvedic* and *Unani* medicine for the treatment of diabetes. Extracts of various plants have been shown to produce hypoglycemia in normal and experimental diabetic animals. Some of the commonly studdied plants *are Abroma augusta, Alliume cepa, and Allium sativum, Azadirachta indica (neem). Coccinia indica, Curcuma longa, Cymopsis tetragonolobus, Ficus bengalensis, Eugenia Jambolana, Ficus bengalensis, Gymnema, Sylvester, Momordica charantia, Musa paradisiacal, Pterocarpus marsupial, etc.*

Abroma augusta (Ultkambal) (Family *Sterculiaceae*) along with *Azadirachta indica* (neem), widely used are indigenous medicine both in *Ayurvedic* and *Unani*. Many of their products / chemical constituents are known to posses wide array of medicinal properties.

Because of their diverse activity potential, there is a considerable hope of finding anti-diabetic compounds from *Abroma augusta* roots- bark and *Azadirachia indica* (stem and bark). The plant *Abroma augusta* is a tall spreading shrub with 10-15 cm long leaves, which is 10-12 cm broad with 1 to 2.5 cm long petioles. The lower leaves are cordite, 3-5

lobed, but the upper leaves are ovate give space lancelet. Flowers are dark reds, 5 cm in diameter. Sepals are 2.5-cm long, lancelet, free nearly to the base. Petals are deciduous. Fruit is a capsule, 4 cm long, obpyramidal, nearly three times as long as the persistent calyx.

The plant grows in the warmer parts of West Bengal, Bihar, Tripura, etc. in India. The fresh mucilaginous sap is used for treating the menstrual disorders. Decoction of the dry root is also used for the same prepares. Ulatkambal is often adulterated with the roots of the plants having fibrous bark, such as Crotalaria and Courtrooms, a small genus of 10 species, distributed in tropical Asia and Australia. The bark of *A. augusta* yields good fibber.

The plant is medicinally very important and its roots and root-barks are used as emmenagogue, uterine tonic and in dysmenorrhoea. Root extract showed significant abortifacient activity in rats, the leaves and stems are reported to be very efficacious in gonorrhoea. The leaves and stem - barks are extensively used in the treatment of unusually painful and difficult menstruation and have high medicinal utility.

The plant is effective in the treatment of diabetes amenorroea, uterine and useful in neuralgic dysmenorrhoea. It is frequently used in *Ayurvedic* and Unani systems. In spite of the extensive research all over the world on chemical and biological investigations on *Abroma Augusta* not much information is available on bio-chemical aspects of the role of *A. augusta* constituents (pure products) in treating diabetic patients.

The present work is aimed at assessing the role of *Abroma augusta* and evaluating the biochemical basis for the anti-diabetic effect. The study will help in understanding the chemical molecular mechanism and their critical importance in diabetics.

Since *Abroma augusta* Linn known as ′Devil′s cottons in English and belongs to the natural order Family Sterculiaceae, commonly known as ′ULATKAMBA in Hindi and Bengali (**Plate no 1** A). It grows throughout the hotter parts of India from the United Provinces to Sikkim, Khasia Mountains and Assam. It is a mass tree, which either grows wild or is cultivated.

The root or root-bark is used in medicine as an emmenagogue in menstrual disorders. The fresh viscid sap is said to be more efficacious and is used in dysmenorrhoea in doses of 30 grains. It to be useful in the congestive and neuralgic varieties of dysmenorrhoea and thought that it regulates the menstrual flow.

It has been used in traditional Indian system of medicine, *Ayurveda* and *Unani*. The root bark is widely used in indigenous medicines both in *Ayurvedic* Sidha and Unani systems. It has been reported that abromine has been identified as betaine (Das Gupta, & Basu, 1970). The leaves contain occasional, taraxerol, β-sitosterol acetate and mixture of long chain fatty diols (Mukherjee et al., 1977). The plant is effective in the treatment of diabetes and in amenorroea (Kapoor, 1984).

Abroma augusta have the center of interest to biochemists and pharmacologists as they have been found to exhibit a variety of pharmacological properties besides alkaloid, abromine, and the sterols is isolated (S. K. Bhattacharya, et al., 1969).

In spite of the extensive research all over the world on chemical and biological investigations on *Abroma augusta* not much information is available on bio-chemical aspects of the role of *A. augusta* constituents (pure products) in treating diabetic patients. The study will help in understanding the chemical molecular mechanism and their critical importance in diabetics.

1.6. *AIMS AND OBJECTIVES OF THE PRESENT STUDY*

It is evident from the information presented above that quite a few medicinal plants have been studied in some detail for their hypoglycemic activity while there are very limited studies on others although there are indications either from *Ayurvedic* & *Unani* systems of medicine or from preliminary studies published.

Our knowledge on the usefulness of medicinal plants will increase if detailed studies are carried out with some plants, which have not been subjected to detailed investigation. The objective of the present studies have been to screen two such plants about which there is less information for their hypoglycemic activity, *Abroma augusta* and *Coccinia indica* have been chosen for preliminary screening. Out of these *A. augusta* has shown better activity.and it has been chosen this plant for further studies.

1) In *Ayurvedic* system of medicine it is customary to use combinations of plant extracts. It is therefore intended to study effect of the combination of the water extract of *A. augusta* in combination with another plant which is will studied namely *Azadirachta indica* (neem).

2) Since *Abroma - Augusta* has shown promising results the third objective was to purity at least partially the active hypoglycemic constituent of *A. augusta* and studies the hypoglycemic activity and the mechanism of action of the partially purified compound.

BIBLIOGRAPHY

Ajagonkar SS. Ancient Indian Medicine and diabetes mellitus. Diabetes Mellitus in Developing **countries.** *Edited by Bajaj JS, Interprint New Delhi*. 1984; 1-10.

American Diabetes Association. Screening for type II diabetes. *Diabetes Care*. 1998; 21 **(1)**: S20-S31.

American Diabetes Association (ADA). Clinical practice recommendations. *Diabetes Care*, 1997; 20 **(1**): 22-24.

Amos AF, Mc Carty D J , Zimmet P. the Rising Global Burden of Diabetes and its Complications: Estimates and projections to the year 2010. *Diabetic Medicine.* 1997;14: (**Suppl 5)** 1-85.

Author KE, Hal- Jogerson K, Berg TJ, Heler MS, sandeick I, Aagents O, Hensen KE. The effect of 8 years of strict glycemic controls on peripheral nerve function in IDDM Patients The Oslostudy. *Diabetologia*, 1995; **(37):** 579-584.

Andersen AR, Christians JS, Andersen JK, Kreiner S, Deckert T.Diabetic nephropathy in Type I (insulin dependent) diabetes: an epidemiological study. *Diabetologia*. 1983: 25 **(6)**: 496 –501.

Brownlee M, VIassara H, Kooney A, Uirich P, Cerami A. Aminoguanidine prevents diabetes Induced arterial wall protein cross- linking. *Science*, 1986; 232 **(4758):** 1629- 1630.

Brown, S B., Goldstein, J.L. Drugs used in the treatment of Hyperlipo proteinemias. In:Gilman. A.G., Rall. T.W., Nies, A.S., Taylor, P. (Eds.) Goodman and Gilman's *Pharmacological Basis of Therapeutics*. **Vol. 1**,Eight ed., New, York, 1992; pp. 874.

Cameron NE, Leonard MB, Ross I, Whiting PH. The effect of sorbinil on peripheral nerve Conduction velocity polyol concentrations and morphology in streptozotocin diabetic rats. *Diabetologia,*1986; 29 **(3):** 168-174.

Ciavarella A, Di Mizio G, Stefoni, Borgnino LC, Vannini P. Reduced albuminuria after dietary Restriction in insulin dependent diabetic patients with clinical nephropathy. *Diabetes Care.* 1987; 10 **(4):** 407-413.

Das Gupta, B. and Basu, K. chemical investigation of bark of *Abroma augusta* Linn. Identity of *Abromine* with betaine. *Experientia*. 1970; **(26):** 477.

Edelstein D, Brownie M. Aminoguanidine ameliorates albumin in diabetic hypertensive Rats. *Diabetologia*, 1992; 35 **(1):** 96-97.

EIlis EN, Good BH. Prevention ofglomerular basement membrane thickening by Aminoguanidine in experimental diabetes mellitus. *Metabolism,* 1991; 40 **(10):** 1016-1019.

Fujita T, Sugiyama Y, Taketomi S, Shoda T, Kawamatsu Y, I watsuka H, Suzuoki Z.Reduction of insulin resistance in obese and / or diabetic animals by 21-4-(1 methylcyclohexylmethoxy) benzyl thiazolidine 24 dione

(ADD-3870, U-63287, ciglitazone), a new antidiabetic agent. *Diabetes,* 1983; **(32)**: 804- 810.

Fuji wara T, Yoshioka S, Yoshioka T, Ushiyama I, Horikoshi h. characterization of a new and antidiabetic agent, CS-045: studies in KK and ob/ob mice and Zucker fatty rats.

Diabetes, 1988; **(37)**: 1849-1888.

Gabbay KH, Merol LO, Field RA. Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. *Science, 1966*; 151 (**707):** 209-210.

Itakura M, Yoshikawa H, Bannai C, Kato M, Kunika K, Kawakami Y, Yamoaka T, Yamishta K. Aminoguanidine decreasea urinary albumin asnd high molecular weight proteins in diabetic rats. *Life Science, 1991*; 49 **(12)**: 889- 897.

Kapoor, R.K. Diabetes mellitus. Indian J. homeopath. Med. 1984; (19): 114.

Klein R, Moss S. Visual impairment and diabetes. In: Alberti K, DeFronzo R, Keen H, Zimmet P, eds. *International Textbook of Diabetes Mellitus.* Chichester: Wiley. 1992; 1373- 1384.

Law CM, Barker DJ, Osmond C, et al. Early growth and abdominal fatness in adult life. *Jepidemiol Community Heath* 1992; (26): 184-186.

Lebovitz HE. Oral antidiabetic agents: the emergence of α-glycosides inhibitors. *Drugs,* 1992; 44 (Supple 3): 21-28.

Lee MK, Miles PDG, Khoursheed M, Gao KM, Mossa AR, Olefsky JM. Metabolic effects of troglitazone on fructose induced insulin resistance in the rat. *Diabetes,* 1990; (390): 1218-1227.

Mayes, W. Jr. Fighting a global epidemic with simple weapons. IDF Bull. 1993; 38 (2): 4. –6. American Diabetes Association. Screening for type II diabetes. *Diabetes Care, 1998*: 21 (1): S20-S31.

Makita Z, Viassara H, Rayfield E, Cartwright K, Friedman E, Rodby R, Cerami A, Bucala R.Hemoglobin AGE circulating marker of advanced glycosylation. *Science,* 1992; **(258)**: 5082 –5085.

Mc Carty D, Zimmet P. Diabetes 1994 to 2010: Global estimates and projections. Melbourne: *International Diabetes Institute* **(1994)**.

Molar D.E., Bjorjbaek C, Vidal -Puig A.Candidate genes for insulin resistance. *DiabetesCare*, 1996; **(19)**: 396-400.

Mukherjee, Ks. and Badruddoja sah. Chemical investigation of bark of *Abroma augusta* Linn. *J. Indian chem Soc.*, 1977**; (54):** 647.

Nolan JJ, Ludvik B, Beerdsen P, joyce M, Olefsky JM. Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N Eng. J Med,* 1994; (331): 1188- 1193.

OHare JA. Ferriss JB, Twomey BM, Brady D, O Sullivan DJ. Hypertension in diabetic nephropathy : Combined role of sodium retention and the reninangiotensin system. *Diabetes*. 1983; 32 (28): (Supple 1): 651-653.

Pascoe WS, Storlien LH. Inducement by fat feeding of basal hyperglycemia in rats with abnormal beta – cell function. Model for study of etiology and pathogenesis of NIDDM.

Diabetes, 1990; (39): 226- 233.

Pascoe W, Jenkins AB, Kusunoki M, Storlien LH. Insulin action and determinants of glycaemia .In a rat model of type II (non- insulin – dependent) diabetes mellitus. *Diabetologia*, 1992; (35): 208-221

Parving H H. Impact of blood pressure and anti-hypertensive treatment on incipient and overt Nephropathy, retinopathy and endothelial permeability in diabetic mellitus. *Diabetes Care*. 1991; 14 (3): 260-169.

Parving HH, Andersen A R, Smite UM, Oxenboll B, Christians JS. Diabetic nephropathy and arterial hypertension. *Diabetologia,* 1983; 24 (1): 10-12.

Perseghin G, Price TB, Petersen K. F., Roden M, Cline G. W., Gerow K, et al. Increased glucose transport - phosphorylation and muscle glycogen synthesis after exercise training in insulin - resistant subjects. *N Engl. J Med*, 1996; (335): 1357- 1362.

Richard P, Nilson BY, Rosenquest FC. The effect of long term intensified insulin treatment on the development of microvascular complications of DM. *NEJM*, 1993; (290): 304-309.

Rimm E. B, Chan J, Stampfer K.N, Colditz G.A. Willett W.C. Prosoective study of cigarette Smoking, alcohol use, and the risk of diabetes in men. *BMJ,* 1995; (310): 555-559.

Soulis Liparota, Cooper M, Parpazoglou D, Clark B, Jerums G. Retardation by aminoguinadine of development of albuminuria, mesangial expansion and tissue fluorescence in STZ induced diabetic rats. *Diabetes*. 1991; 40 (10): 1328-1333

S.K. Bhattacharya, R. Lal and P.K. Das, Ind. *J. Pharmac*. 1969; (1): 717

Storlien L. H., Baur L. A., Kriketos A. D., Pan D.A., Cooney G. J, Jenkins A. B, et al. Dietary Fat and insulin action. *Diabetologia*, 1996; (39): 621-636.

Stevenson RW, Hutson NJ, Krupp MN, Volkmann RA, Holland GF, Eggler JF, Clark DA, Mc Pherson RK, Hell XL, Danbury BH. Actions of a novel anti-diabetic agent englitazone in hyperglycemic hyperinsulinemic ob/ob mice. *Diabetes*, 1990; (39): 1218-1227.

Tuomiehto J, Rastenyte D. Epidemiology of microvascular disease and hypertension in diabetes mellitus. In: Alberti K, Zimmet P, De Fronozo R, KeenH, and Eds M. *International Textbook of Diabetes Mellitus*, 2-nd edn. Chichester, Wiley. 1997: 1559- 1583

Vernon P, Ciavarella A, Flammini M, Borgnino LC, Bacci L. Diabetic nephropathy and continuos subcutaneous insulin infusion. *Diabetologia.,* 1983; (25): 200-201.

Viberti GC, Bilous RW, Mackintosh D, Keen H. Monitioring glomerular function in diabetic Nephropathy : a prospective study. *Am J Med,* 1983; 74 (2): 256-264.

Viberti GC, Keen H, Wiseman MJ.Raised arterial pressures in parents of proteinuria insulin dependent patients. *BMJ*, 1987; (195): 575-577.

World Health Organization, Prevention of diabetes mellitus. Technical Report Series. No. 844, Geneva, *WHO.* 1994.

Zeller K, Whittaker E, Sullivan L, Raskin P, Jacobson HR. Effect of restricting dietary protein On the progression of renal failure in - patients with insulin dependent diabetes mellitus. *NEJM*, 1991; 324 (2): 78 - 84.

Zeigler D, Wiefels K, Danniehl K, Grie FA. Effect of one year of near normoglycemia on peripheral nerve function in type I insulin dependent diabetic patients. *Kim Wochenschr* 1988; (66): 388- 396.

.

CHAPTER - 2

PRELIMINARY STUDIES ON THE HYPOGLYCEMIC EFFECT OF *ABROMA AUGUSTA* IN ALLOXAN DIABETIC RATS

2.1. INTRODUCTION

D*iabetes* derives its origin from a Greek word '**diabaino'** which means to go through, and 'mellitus means sweet or sugar. Hence, the passing of sugar with urine may be the crude meaning of the word diabetes mellitus.The hallmark of DM is the inability to control blood glucose, which eventually passes through urine in extreme cases of diabetes mellitus. DM is a group of metabolic disorders characterized by hyperglycemia resulting from defects either in insulin secretion or insulin action or both (David, 1996).

Due to deficient action of insulin on the target tissues, the metabolism of carbohydrates, fats and proteins is altered. Chronic hyperglycemia in diabetes is associated with damage of tissue, dysfunction and ultimate functional failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association, 1998).

Several pathogenic processes are involved in the development of DM. Autoimmune destruction of β-cells of the pancreas leads to insulin deficiency /release. It may also occur due to diminished tissue response to insulin at one or more points in the complex pathway of hormone action.

Inadequate insulin release and impairment of insulin action frequently co-exist. Long term complications of DM include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputation and charcoal joints (Fig 8).

Autonomic neuropathy may also cause sexual dysfunction along with gastrointestinal, genitourinary and cardiovascular symptoms. Patients with DM have a higher incidence of atherosclerotic, cardio-vascular, peripheral vascular and cerebrovascular disease. Hypertension, abnormalities of lipoprotein metabolism and periodontal disease is often found in DM patients. The emotional and social impact of DM and the demand therapy may also result in significant psychosocial dysfunction in-patients and their families.

Two major types of clinical syndromes due to increased hyperglycemia can be mentioned here .

The first one is characterised by insulin dependence and onset in early age, with weight loss and ketonuria (passing of ketone bodies in urine). This is generally termed as type 1 or Insulin Dependent Diabetes Mellitus (IDDM).

The second is characterized by late, onset, insensitivity to insulin deficiency. It is generally called Non-Insulin Dependent Diabetes Mellitus (NIDDM) or type II. The young people in India have Malnutrition Related Diabetes Mellitus (MRDM), which can be efficiently controlled or reversed by proper and balanced diet. (Bajaj & Madan1993.).

In diabetes, though there is two to three fold increase in blood glucose concentration from the normal levels, the tissues themselves are starved of glucose. Apart from high glucose in blood, there is also an increase of fat and protein in the blood, which may accumulate. The body may break these down to obtain energy.

The normal fasting blood glucose of humans is 60-100 mg/dl, which may increase up to 350 mg/dl in cases of diabetes. The functioning of the various organs are affected and the body function for maintenance of homeostasis under-goes a long lasting change due to the increase of the glucose concentrations in the blood. This has negative effects on the tissues which are dependent on insulin for glucose transport (namely adipose, liver and muscle) and tissues which are independent of insulin for glucose transport like brain, kidney and red blood cells in different ways (Brown Lee et al, 1986). The cause of IDDM has been reported recently to be T cell mediated auto-immunity towards glutamic acid decarboxylase (GAD).

Variety of herbal preparations have been mentioned in *Ayurveda* and other indigenous systems of medicine which are useful in DM. The hypoglycemic activity of many of these agents is experimentally well documented. DM is prevalent in all countries of the world. More than 30 million of people are said to be affected throughout the world with this disease. Insulin is widely accepted as an ideal choice for treatment of DM.

However no work has been done to study their effect on insulin resistance in DM. This study was designed to evaluate the effect of two or three different herbal preparations on dietary fructose induced insulin resistance. Extracts of various plants have been shown to produce hypoglycemia in normal and experimental diabetic animals.

Some of the commonly studied plants are *Momrdica charantia, Allium cepa, Allium sativum, and Ficus bengulensis. Eugenia jambolana, Coccinia Indica* and *Abroma Augusta*. *Abroma augusta* Linn. N.O. (Sterculiaceae) is a deciduous shrub or small tree with velvety branches, (Kirtikar K.R. & Basu, B.D 1933). Growing wild throughout the hotter parts of India from Utter Prudish to Skim, Khasia Hills (4,000 ft.) It has also been known to grow in Java, Philippines and China." Devil's" Cotton is the name given to it in English; in Sanskrit it is called 'Pishach Karpas' and in Hindi, Gujrati, Marathi and Bengali, it is termed as 'Ulatkambal'. It was only recently that this plant has come into use as a drug in the modern system of medicine, and it has been recommended for the treatment of menstrual disorders, (Sirkar, B.M., 1872 and Thornton, F.H., 1872.), sterility, gonorrhoea and external sores (Watt, G., 1889).

The earliest chemical work on this drug plant has been described by Dymock, Warden and Whopper, 1890, where, the

presence of some mucilage's and starch in the aqueous extract was reported Chopra, 1933 and Ghosh 1929 made more systematic attempt

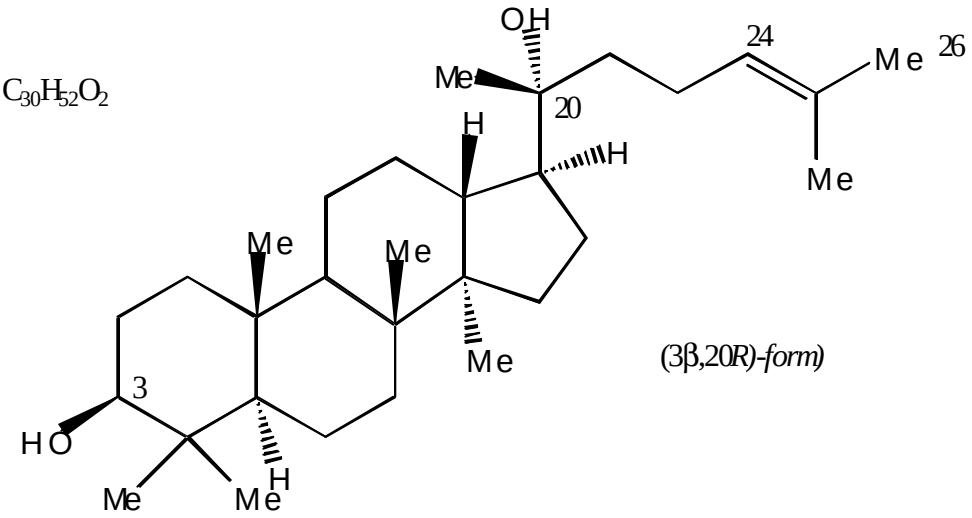
and presence of alkaloid (0.01%) and fixed oil. Bose K.C., 1932. Reported that the root –bark of this plant contains gum, wax, and Ashland resins. . The root was reported to contain an alkaloid, *Abromine,* (Srivastava et al 1956). ($C_6H_{13}NO_2$, 283-285

Hydrobromide, $C_6H_{13}NO_2$. HBr, crystalline powder from alcohol + ether, m.p 105º. Hydrochloride, $C_6H_{13}NO_2$. HCl, mp 128-129 º. The *Abromine* $C_6H_{13}NO_2$, mol wt 131.17 Composition is C 54.94%, H 9.99%, N 10.68 %, O 24.39 %. $H_2N(CH_2)_5COOH$.

(Fig 2.1.), Prep. From ∝ benzoyl aminocapronitrile:

(Figure 2.1)

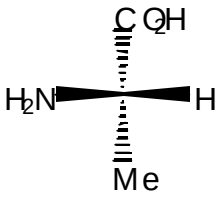
Dammar-24-ene-3,20-diol



$C_{30}H_{52}O_2$

(3β,20R)-form)

L-Alanine



$C_6H_{13}NO_2$

$C_6H_{13}NO_2$

A weighted amount of the powdered *Abroma augusta* roots was extracted in a soxhlet's apparatus with different solvents in succession and the following values were obtained: Petroleum ether extracted 0.58 %, ether about 0.8% chloroform 0.58 % ethylacetate o.68% and alcohol 0.50 %. For systematic examination, a larger amount of the powdered root was extracted in succession with petroleum ether, ether, alcohol, cold water and hot water. Water soluble base were found to be predominant.

The root-bark of *Abroma augusta* has the following constituents:

(Plate 1, B)

1. Mixed oil.
2. Resins.
3. Alkaloid, in minute quantities.
4. Water- Soluble bases. *Abroma augusta* is reported to be effective on treatment of diabetes and amennorrheas (Thakur, R.S., et al. 1989).

There are many studies which show that the root bark of *A. augusta* contains one alkaloid abromine (Srivastava, et al 1956) and the leaves contain some sterols (Adityachudhury, N. et al. 1969). Petroleum ether extract was found to contain antifertility agent Parkas, A., et al. 1975). In spite of the mention of its beneficial effect in diabetes mellitus, there are no detailed studies on its diabetes. We have therefore undertaken studies

on the hypoglycemic activity of *A. augusta*. Hypoglycemic effect of *Abroma augusta* in alloxan diabetic rats.

The effect of hypoglycemic extracts of *Abroma augusta* (fame - Sterculiaceae) studied in normal as well as on alloxan diabetic rats. Many herbal products including several metals and minerals have been described for the cure of diabetes in the indigenous systems of medicine .

This prompted us to take up the investigation on herbal extracts for the treatment of diabetes. Rats starved overnight received a single subcutaneous injection of alloxan (0.154 M, pH 4.5). After injecting alloxan, it was observed that the diabetic conditions started to set with varying time periods and varying doses. We could induce the type I type II type III condition in the rats, which parallel human subjects.

Diabetic rats when treated with aqueous extracts of the bark-root of the plant *Abroma-augusta* for 16 weeks shown significant changes in the blood glucose and improvemrnt in glucose tolerance and cholesterol leves.. Serum total and LDL cholesterol and triacylglyerol which increased in diabetic rats showed improvedment.

This study shows that the bark-root of *Abroma-augusta* has a hypoglycemic and hypocholesterolemic effects. The biochemical and physiological parameters of treated rats were determined and the mesangium were recorded photographically. These rats were treated with aqueous solution of the herb *Abroma augusta* and the biochemical changes were investigated over a period of 16^{th} weeks. The various

parameters analyzed showed a progressive change towards normal, and by 16 week all values like HDL cholesterol, fructose and plasma glucose level, when compared to corresponding values in control group, were found to be similar.

The results of doses 2.0 ml & 4.0 ml orally of the extracts and the biochemical changes were determined up to 16. The *Abroma augusta* principle not only prevented the elevation of blood glucose level brought down levels serum cholesterol, (LDL+VLDL) C, triacylglycerols and the ratios for total cholesterol/HDLC and (LDL+VLDL) C / HDLCto normal level The study shows that the same hypoglycemic principle has hypocholesterolemic effect also.

***The aim of the present study, which is a part of the overall studies on medicinal plants, is as follows*:**

- Preliminary study of *Abroma augusta* in alloxan diabetic rats.
- To induce diabetes in experimental animals which is necessary for studying the hypoglycemic activity of any drug.
- To study the hypoglycemic effect of the aqueous extract of *Abroma augusta* (Family: Sterculiceae) was studied in normal as well as alloxan diabetic rats.
- To diabetic rats with 4 ml (4 gm dry weight) of aqueous extract of *A. augusta* for 16 weeks and examine the effects on fasting serum glucose and improvement in glucose tolerance.
- To study total serum cholesterol, and LDL cholesterol and triacylglycerol which one increased in diabetic rats.

2.2. MATERIALS AND METHODS

Plant material: *Abroma augusta.* The air-dried root and bark were from Assam and Khasia Mountain, The plant was identified in a Botanical Reseat Institute, Lucknow. (Plate no 1 B.)

Preparation of the aqueous extract 100 gm of dried whole root-bark powder was extracted by soaking in 200ml of distilled water at room temperature for two days with frequent stirring. Then it was filtered. The residue was extracted three more times in a similar way. The extract was concentrated and stored in deep freeze. At the time of use, it was dissolved in water (1 gm dry residue in 1-ml water).

2.2.1. METHODS TO INDUCE EXPERIMENTAL DIABETES

(a) ALLOXAN INDUCED HYPERGLYCEMIA

Hyperglycemia and glycosuria occur after administration of alloxan (fig 11) in several species (Brunschwig et al, 1943;Baily and Baily, 1943; Tasaka et al, 1988). Investigators found that alloxan has a selective destructive effect on the β-cells of islet of Langerhans of the pancreas. However, this effect varied with species and the dose (Richard and Robert, 1983). Hyperglycemia and glycosuria occur after administration of alloxan in several species (Brunschwig et al, 1943;Baily and Baily, 1943; Tasaka et al, 1988).

(b) ALLOXAN

The cellular site of action of alloxan and exact mechanisms involved in its toxicity are not completely understood. Number of studies have shown that alloxan disrupts the integrity of the βcell plasma membrane (Watkins , et al, 1973) The site at which alloxan interacts with the cell membrane is uncertain. Some evidence indicates that alloxan acts at the site for sugar transport into the cell

(Howell and Taylor, 1967; Tomita et al, 1974). On the other hand, there is also evidence to suggest that alloxan acts at a glucoreceptor site responsible for insulin release, which is separate from the transport site (Grodsky et al, 1974; Miwa et al, 1975).

It has also been proposed those alloxan **(Fig 11**), leads to mitochondrial dysfunction and interferes with intracellular glucose oxidation (Brekke et al, 1980). There is a large variability in dosage of alloxan required to produce long-standing diabetes in different species, which is also compatible with life. In many experiments, a single dose (32 to 200 mg/kg) was adequate to produce desired degree of hyperglycemia, while in certain other studies it had to be repeated after 3 days. In the chornic state, hyperglycemia remains constant and blood glucose levels of 400 mg% or more can be expected after standard diabetogenic doses of alloxan and streptozotocin (STZ) **(Fig 12),** (Mansford and Opie, 1968; Hoftiezer and Carpenter, 1973).

High dosages of the β-cell toxin streptozotocin and alloxan induce severe insulin deficiency and IDDM with ketosis. Lower dosages calculated to cause a partial reduction of β-cell mass could be used to produce a mildly insulin-deficient state of NIDDM, without a tendency to ketosis (HO RS, Aranda CG, et al.1988). And (Bailey, Flattpr.1990) .The dosage is difficult to judge to create stable NIDDM without either gradual recovery or deterioration into IDDM. Streptozotocin is preferred because it has more specific cytotoxicity, to β-cell of he pancreas.

(c) EXPERIMENTAL INDUCTION OF DIABETES IN RATS

Healthy male albino Wistar rats (body weight (150-200gm) procured from Center for Cellular Molecular Biology (CCMB) Hyderabad were used in this study. The animals were fed on a pellet diet (Hindustan Lever, India).

Rats were devoid of any disease, at difference stages during the time of the experiment. Rats were starved over night and each rat received a single subcutaneous injection of alloxan monohydrate in freshly prepared sodium-acetate buffer (0.15 M, pH 4.5). The dose of alloxan was 20mg/100g-body weight in a volume of 0,10 to 0.15ml. The same volume of acetate buffer was given to each control rat. From next day, a single injection of 2 units of protamine-Zn insulin prepared in normal saline was given to each alloxan treated rat, for 6 days. This decreased the mortality

of the animals. Controls were given the same volume of normal saline instead of insulin.

(d) EXPERIMENTAL DESIGN

Animals were divided into 3 groups of 5 animals each. Group 1 served as healthy control and Group 2 were untreated diabetic rats. Animals of both the groups were given water for 16 weeks. Group 3 was diabetic rats given 4 ml (4 gm dry wt) of aqueous extract of *A. augusta* daily once using an intragastric tube for 16 weeks. Plasma glucose levels, fasting as well as in oral glucose tolerance test and serum lipid profile were determined at 0, 4, 8 and 16 weeks. The blood was collected retro-orbital from the inner cantos of the eye using Micro Hematocrit Capillaries, Mucous. The blood was collected in sodium fluoride and plasma was separated for plasma glucose determination. Serum was used for estimating lipids.

(e) PARAMETERS STUDIED

Plasma glucose: It was estimated by glucose oxide Peroxides method using kit from RANBAXY, New Delhi

SAMPLE PREPARATION

The blood was draw into eppendorf containing sodium fluoride and potassium oxalate in the ratio 1:3 as anti-coagulant. The sample was centrifuged at 3000rpm for 10 minutes and the aliquots of plasma separated were used for the estimation of glucose.

REAGENTS AND ENZYMES USED

Alloxan monohydrate Sigma.

Streptozotocin (STZ) Sigma.

Glucose oxides, $\geq$ 6.7 mg/dl

Horseradish Peroxides, $\geq$ 6.2 U / ml

4- amino antipyrine, 0.2×10^{-3} M

Phosphate buffer, 8×10^{-3} M, pH 7.0

Phenol, 86×10^{-3} M

Glucose standard, mg / dl

Pipes buffer, 40×10^{-3} M, pH 7.4

4-Chloropheno,l $5.4 \times 1o^{-3}$ M

Magnesium ions, 5×10^{-3} M

ATP $1. \times 10^{-3}$ M

Sodium azide, 0.05%

4- amino antipyrine, $0.4 \times 10^{-}3$ M

Standard solution, 2 mg / ml

Phosphotungstic acid, 0.55×10^{-3} M

Magnesium chloride, 25×10^{-3} M

Peroxidase, $\geq$0.5 U/ml

Glycerol kinase, $\geq$0.4U/ml

Gly -3-P-oxidase, $\geq$1.5 U/ ml

Lipase, $\geq$150U/ml

A. PROCEDURE FOR DETERMINATION OF CHOLESTEROL

200 µ l of sample was pipette out to 500 µ l solution (PPt). It was mixed, allowed to stand for 10 min at room temperature. The supernatant obtained after centrifugation at 12000 rpm for 2 minutes was separated off and the cholesterol was estimated as mentioned.

B. PROCEDURE FOR DETERMINATION OF TRIGLYCEROL

10 µl of plasma was added to 100µl of reagent and mixed well. This mixture was incubated for 5 minutes at 37 °C. Simultaneously a reagent blank and standard were also drawn. The absorbency of the blank, the sample and the standard were read the concentration of the triacylglycerol in sample was determined as mentioned.

1. GLUCOSE TOLERANCE TEST

The standard oral glucose tolerance test was performed in all rats at 0, 4, 8, 12 and 16 weeks from overnight fasted rats, and fasting blood was collected. Then glucose (2g/ kg) dissolved in water was given orally to the rats. The blood was collected at 0. 0,5,1,1.5 and 2 hrs, Plasma glucose was estimated in all the samples.

2. Estimation of glucose

Fasting blood was collected from rats in all the groups to determine plasma glucose the glucose was given orally then blood samples were collected at 30, 60, 90, and 120, min after administer for plasma glucose determination.

Plasma glucose was estimated by glucose oxide method using RANBAXY kits. In this method, the aldehyde group of the glucose is oxidized by glucose oxidase to give gluconic acid and hydrogen peroxide.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconate} + H_2O_2$$

The absorbency of the sample was read at 510- nm wavelength and glucose level was calculated by the formula.

$$\text{Concentration of glucose} = \frac{\Delta A \text{ sample}}{\Delta A \text{ Standard}} \times \text{Concentration of standard}$$

Where Δ A is the absorbency

A graph was drawn with blood glucose on Y-axis time on the X-axis. Area under the curve was calculated**.** (Fig 13).

SERUM LIPIDS

Total cholesterol, LDL- and HDL- cholesterol and triacylglycerol were estimated using kits from Dr. Reddy's Pathology Lab-Hyderabad following manufacturer's instructions.

HDL-CHOLESTEROL

Principle: Low-density lipoproteins (LDLand VLDL) and chylomicron fraction was precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation. The cholesterol concentration in HDL fraction, which remains in the supernatant, is estimated.

LDL- CHOLESTEROL

After estimating total cholesterol, HDL cholesterol, HDL cholesterol and triacylglycerol,

Triacylglycerols

The LDL cholesterol (mg/100ml)=Total Cholesterol

+ HDL (Cholesterol) /5

Friedwald. (1972). Estimation of the concentration of low -density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.

STATISTICAL ANALYSIS

All the data were statistically evaluated and the significance was calculated using students - test. All the results were expressed as mean ± SD.

$$\text{The LDL cholesterol (mg/100ml)} = \text{Total Cholesterol} \xrightarrow{\text{Triacylglycerol}} \text{HDL cholesterol/5}$$

Friedwald. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.

(a) TOTAL CHOLESTEROL

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino-antipyrine in the presence of phenol and peroxides.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterases}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestene-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-amino-antipyrine} \xrightarrow{\text{Peroxidas}} \text{quinoneimine} + H_2O$$

The absorbance at 500nm wavelength is determined and total cholesterol is calculated

$$\text{Cholesterol concentration in the sample} = \frac{\Delta \text{ A sample}}{\Delta \text{ Standard}} \text{ X Con of}$$

Total cholesterol, HDL cholesterol and triacylglycerols were estimated using standard kits of Randox, Mumbai, India. LDL cholesterol was calculated from the above measurement by using Friedwalds formula (Friedwald 1972).

$$\text{LDLC} = \text{TC} - \left(\text{HDLC} + \frac{\text{TAG}}{5} \right)$$

TC, LDLC, HDLC= total, low density and high density lipoprotein cholesterol respectively,

TAG = triacylglycerol.

2.2.2. ESTIMATION OF TRIACYLGLYCEROL

EXPERIMENTAL DESIGN

(a) LIPID PROFILE. In type 1 diabetic subjects with poor glycemic control, LDL particle are internalized and degraded by fibroblasts in vitro less efficiently than LDL from normal subjects.

These changes may be parallel to the pathogenesis of the accelerated atherosclerosis occurring in diabetics with hypertriglyceridemia. Thus, changes in apportions of HDL similar to those seen in human diabetes can be experimentally induced in rats. .

(b) HDL CHOLESTEROL

Principle: Low density lipoproteins (LDL and VLDL) and chylomicron fraction is precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in HDL fraction, which remains in the supernatant, is estimated as mentioned above.

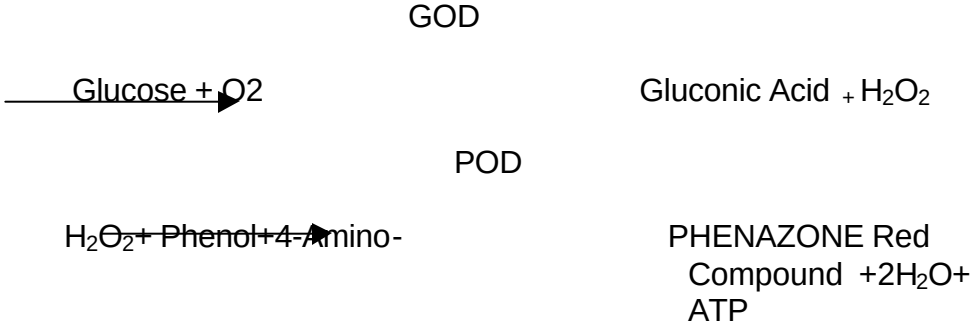
(c) LDL CHOLESTEROL

After estimating total cholesterol, HDL,LDL cholesterol and triacylglycerols. The LDL cholesterol was calculated by the following formula (Friedwald et al 1972)

LDL cholesterol (mg /100 ml) = Total cholesterol = triacylglycerols - HDL cholesterol

ESTIMATION OF GLUCOSE

Glucose is oxidized by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of Peroxidase (POD) oxidized the chromogen 4-aminophenazone +phenol to a red colored compound. The intensity of the red color produced is proportional to the glucose concentration and is measured at 505nm (Trinder et al, 1969).



2.2.3. ESTIMATION OF TRIGLYCERIDES

The levels of triglycerides were estimated using Enzopak kit, Reckon Diagnostics Pvt. Ltd., Baroda. The kit measures triglycerides using the glucose peroxidase (GPO) method. Conventional methods for the estimation of triglycerides have been chemical or enzymatic. In the enzymatic methods, triglycerides are hydrolyzed to release glycerol by use of lipase

(Fossati. et al.1982). There are various enzymatic methods to estimate liberated glycerol. In this kit, GPO and peroxides are used for quantitative estimation of serum triglycerides. This method is most specific due to the action of lipase to liberate glycerol, which is estimate.

The method is sensitive mainly because of higher molar extinction coefficient of the final color complex with the result that the sample Volume needed for these is less (Mc Gowan et al, 1983). Lipase hydrolyses serum triglycerides to glycerol and free fatty acids. The liberated glycerol is converted to glycerol-3-phosphate in the presence of ATP and glycerokinase, (GK). Glycerol-3-phoshateis oxidised by glycerol-3-phosphate oxidase (GPO) to yield hydrogen peroxide. Hydrogen peroxide thus generated reacts with ESPAS (N-ethyl-N-sulfopropylaminoanisidine) and 4- aminoantipyrine in the presence of peroxidase to form a colored complex. The intensity of the color so developed is proportional to triglycerides concentration and is measured spectrophotometrically at 546nm (530-570nm or with green filter)

Lipase.

Triglycerides + H_2O → Glycerol + Fatty Acids

GK

Glycerol +ATP → Glycerl-3-phosate +, ADP

GPO

Gly3-PhOSH. + O_2 → Dihydroxyacetone Phosphate + H_2O_2

Peroxidase

H_2O_2 +4-Aminoantipyrine + ESPAS → Quinone imine +3 H_2O

Triglycerides (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ X 100

LDL cholesterol (mg/100ml) = Triacylglycerol

Total Cholesterol HDL cholesterol

2.3. RESULTS AND DISCUSSION

These study reports not only the anti-hyperglycemic effect but also for the first time the hypolipidemic effect of *A. augusta.* Results in (Table 2.1) show the effect of water extract of *A. augusta* on the fasting plasma glucose (FPG) of normal and untreated diabetic rats. It can be seen that treatment with 4 ml of water extract of *A. augusta* brought down the FPG level to normal value. Lower dose (2ml) showed partial improvement .

Treatment of diabetic rats with 4 ml of extract showed considerable improvement in glucose tolerance also (Table2.2). These results point out that the diabetic rats showed abnormal glucose pattern. After treatment for 16 weeks with *A. augusta* the glucose tolerance pattern was normal. Since the untreated diabetic rats had very high FPF 300.7 $\pm$ 50.9 mg/dl (Table 2.1), and 2.1a it is quite possible that their pancreas is considerably damaged. Since treatment of the diabetic rats with *A. augusta* extract brought down the FPG to normal value, one can expect that *A. augusta* extract can either repair the damaged pancreas or the extract stimulated directly the utilization of glucose by various tissues.

In view of the observations of Pugazhenthi, S. and Murthy, P.S. (1981) that extracts of some medicinal plants contain not only hypoglycemic but also hyperglycemic compounds, it is quite possible that *A. augusta* extract also contains both hypoglycemic and hyperglycemic compounds. In such a case, the activity observed with *A. augusta* extract

in the present study would be the net effect of the activities of the two compounds with opposite effects. If so purification of the active hypoglycemic compound (s) would show hypoglycemic activity at a much lower dose considering this possibility.

We are trying to purify the hypoglycemic constituent of *A. augusta*. In the diabetic rats there was increase in total LDL- cholesterol, LDL/HDL ratio and triacylglycerols (Table 2.3). After treatment with 4ml. Of *A. augusta* extract, there was significant lowering of the serum lipids. There was slight increase in HDL- cholesterol. This indicates that the water extract of *A. augusta* has effect on the lipid metabolism of diabetic rats also.

There are reports that some plants with hypoglycemic constituents have hypolipidemic effect also (Puri, et al 1994 and Shukla, et al 1995). It is anticipated that isolation of the active hypoglycemic compound (S) and detailed work on its effect on carbohydrate and lipid metabolism and blood insulin levels would throw light on mechanism of hypoglycemic action of *Abroma . augusta.*

Table 2.1. Effect of water extract of *Abroma augusta* on the fasting plasma glucose Level in diabetic rats.

Group	Plasma glucose mg/dl, mean ± SD 0 weeks	16 weeks
Normal	95.5 ± 26.6	84.0 ± 29.3
Diabetic Untreated	166.7 ± 50.4[b]	300.7 ± 50.9[a]
Diabetic + *Abroma augusta* (4ml) treated	164.6 ± 26.9[b]	107 .5 ± 26.6[b]

a = p < 0.001

b= p < 0.05

Table No 2.2. Plasma glucose level after 16 weeks in diabetic rats (GTT).

Diabetic control	300.7 ± 10.88
Normal (Control)	84.0 ± 9.30[a]
Diabetic +*Abroma augusta* (2ml)	119.6 ±29.03[a]
Diabetic + *Abroma augusta* (4ml)	95.2±7.46[a]
Diabetic Control + *Abroma,auguata*	99.8±2 .48[a]

a= p < 0.001

Table 2. 3. Effect of 4 ml of *A.broma. augusta* extract on glucose tolerance in diabetic rats after 16 weeks treatment

Plasma glucose mg/dl, mean ± S. D					
Group	**0 hr.**	**0.5 hr.**	**1hr.**	**1.5 hr.**	**2hr.**
Normal	96. 5 ± 26.9	142.6±14.8	135.2±30.2	118.5±11.4	106.0±14.0
Diabetic Untreated	155.7 ±42.0[b]	240.6±78.1[b]	271.0±91.1[b]	284.7±81.8[b]	275.0±85.2[a]
Diabetic treated	89.8 ±25.2	115.0 ±21.1[c]	116.4±16.0	104.0±11.4	99.8±25.0

a =p <0.001
b= p <0.01
c= p < 0.05

Effect of *Abroma augusta* on serum lipid & glucose profile .The results obtained after treatment with *A. augusta* (4 ml) extracts are presented in (Table 2. 4)and (Table 2.5). As seen the (Table 2.4) there was a marginal progressive decrease in the values of total cholesterol, LDL cholesterol and total triacylglycerol at 4th and 8th week but it was not statistically significant. The value returned very close to the baseline (0-week) by 16th week. Similarly the HDL cholesterol values increased marginally (Table 2.5), but not significantly till the 8th week and returned very close to the baseline (0-week) by 16th week. But as seen in (Table 2 .5) the LDL cholesterol to HDL cholesterol ratio was significantly lower at 8th week than at '0'week. This decrease was maintained till 12th week. But by 16th week, the value returned to the baseline. The percentage change in the serum lipid profile at 8th week in *Abroma. augusta* with 2-ml extract was not

significant (Table 2.6). The change were somewhat more significant in *Abroma,* 4 ml extracts. The general parameters of the control , diabetic and *Abroma* . treated diabetic rats shown in (Table 2.1 a).

.

Table 2. 4. Plasma glucose levels 2ml aqueous 0f *Abroma – augusta.*

No. of week	Plasma glucose (mg/dl) Fasting	0.5 h	1h	1.5 h	2h	Diabetic control
Diabetic control	116.2± 11.6	164± 10.12	153±28.68	127±26.78	119±29.03	280.8±37.09
Normal (Control)	103.4 ±7.92	142±19.78	130.4±16.1	118.2±5.8	111.4±8.56	249±18.39
Diabetic +*Abroma* (2ml)	100.2± 5.58*	138.2 8.1*	125.9 9.2*	123.4 11.9*	110.4±9.76	245.9±15.13
Diabetic + *Abroma* (4ml)	99.4 ±15.61	144.2±18.68	127.8±10.32	123.6±16.31	111.4±16.6	250.5±20.50
Diabetic + *Abroma*	115±14.31	148.8±22.78	132.8±13.6	122.6±11.19	119.6±6.54	260.75±22.6

(All values are mean ± S D., *P < 0.05compared baseline value.)

The comparison of glucose tolerance curves at '0 "week and 8th Week have been shown in (Figure 13). The-percentage change in the indicators of glucose tolerance at 8thweek in case of 2ml *Abroma* extract is compared with the corresponding changes in the 4ml extract of *Abroma . augusta* (Table 2.3.). As seen in the figure 13, the change in the plasma glucose values.

Table 2.5. Effect of 4ml of aqueous extract of *Abroma augusta* .on serum Lipid profile Lipid fraction (mg/dl).

No. of weeks	Lipid fraction (mg/dl)				Triacylglycerol (mg/dl)
	Total Chlo	HDL-Chol.	LDL-Chol	LDL/HDL	
0	190±14.81	47.4±7.3	106.24±10	2.29±0.43	181.8±18.79
4	189±22	48.4±8.36	105.4±16.52	2.23±0.50	176±23
8	186±19.64	51.3±8.36	99.7±16.81	1.99±0.44*	175±15.79
12	191.6±13.93	50.6±5.54	104.96±11.6	2.10±0.33*	180.2±16.84
16	195.8±16.72	47.2±6.87	111±12.81	2.40±0.50	187.6±15.17

(All values are mean ± S D. *, P < 0.05 compared to baseline value).

Table. 2.6. Effect of treatment with 2ml water extract of *A. augusta* on the serum Lipid profile in diabetic rats.

Group	TC(mg/dl)	LDLC(mg/dl)	HDLC(mg/dl)	LDLC/HDLC	TAG(mg/dl)
Normal	168.6±16.9	78.0±12.2	44.9±13.3	1.7±0.3	115.6±42.6
Diabetic Untreated	246.0 ±14.4[a]	152.2±12.6[a]	45.0±12.0	3.3±0.4[a]	181.8±18.8[a]
Diabetic Treated	186.0 ±14.8	99.7±16.8[b]	51.3±8.4	1.9 ±0.4	175.0 ±15.8[a]

a = p < 0.001
b= p < 0.05

3.4. CONCLUSION

In conclusion water extract of tulsi possesses a wide range of beneficial effects to diabetic animals by reducing fasting blood glucose, improving glucose tolerance and serum lipid profile. The beneficial effect were seen in the various tissues of diabetic rats such as lungs, liver, kidney and brain as reflected in decrease in the lipid peroxidation products and increase in the overall antioxidant status of the animals. *In vitro* hyperglycemic studies:

It is evident that hyperglycemia is also one of the factors for adverse effects identified in erythrocytes of type 2 diabetic patients.

In order to confirm the oxidative damage under diabetic conditions and to understand the differential response of erythrocytes *to in vivo* hyperglycemia, the present study has been extended to examine the effect of in *vitro* hyperglycemia by incubating the erythrocytes of healthy persons at normal and elevated levels of glucose in the medium. Further from the in vitro studies it is evident that hyperglycemia is also one of the factors for adverse effects identified in erythrocytes of type 2 diabetic patients. It is therefore evident that *Ocimum sanctum* has atidiabetic activity. The water extract of the plant has been shown for the first time to have antioxidant properties.

BIBLIOGRAPHY

Agrawal, P. Rai, V. and Singh, R.B. Randomized placebo-controlled, single blind trial of Holy bass leaves in-patients with non-insulin dependent diabetes mellitus *Int .J . Cline. Pharmacol,* 1996; 34 **(9)**, 406- 409.

Bhargava, K. P. and Singh N. Anti-stress activity of *Ocimum sanctum Linn*. *Ind. J. Med. Res,* (1991) 73, 443-451.

Brunschwig A, Allen JG, Goldner MG, Gomori G. Alloxan. *J Med Assoc.*, 1943; 122: 966.

Bailey CC and Bailey OT. Production of diabetes mellitus in rabbits with alloxan, A Preliminary report. *J Am Med Ass*, 1943; **(122):** 165-1166.

Bailey OT, Bailey CC, Hagan WH. Alloxan diabetes in the rabbit: a consideration of the morphologic and physiologic changes. *Am J Med Sci,* 1944; 208: 450.

Bailey CJ, Flatt PR, Models for testing new hypoglycemic drugs. In: Bailey CJ, Flat PR, E

Baynes,J. WRole of oxidative stress in developments of complications of diabetes. *Diabetes*, 1991; **(40):** 405-412.

Chattopadhya, R.R. Hypoglycemic effect of Ocimum sanctum leaf extract, in normal and Streptozotocin diabetic rats. *Ind. Exp. Biol.* 1993; (31): 891-893.

Deneke S M and Fanburg B L: Regulation of Cellular glutathione. *Am J Ohysiol ?* 257 (Lung cells Mol Physical): 1989, L163- L 173.

Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N. and Ray, Screening of Indian plants For biological activity. Part1. Ind. J. Exp. Biol. 1968, **(6):** 232-238.

Gupta, S) Pharmacognosy of leaves of different species of Ocimum (Tulsi): *Ind. J. Med. Res.,*967; 2 **(1):** 35 –38.

Halloween B and Guttering J M C: *In free radicals in Biology and Medicine.* Halliwell B and Gutteridge J M c **(eds)** *calerendron press*, *Oxford*, 1989; 87.

Ho RS,Aranda CG, Tilery SJ et al. In vivo and in vitro glucose metabolism in a low dose streptozotocin rat model of non insulin dependent diabetes. In: ShafrirE, Renold AE, eds. Lesions from Animal Diabetes Frontiers *in Diabetes Research*. *London*: Libbey, 1988: 288-294.

Higuichi Y: Lipid peroxides and ∝-tocopherol in rat streptozotocin induced diabetes mellitus. *Acta Med Oklayama*. 1982. **(36):** 165-175.

Hussein, H.E.M.A., Jamil, K., and Rao, M. Preliminary studies on the hypoglycemic effect Of *Abroma augusta* in alloxan diabetic rats. *Ind. J. ciln. Biochem,* 2001.16 **(10):** 77- 80.

Hiroshi, O., Nobuko, O. and Kunio, V. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. *Biochem*, 1979**. (5):** 351-358

Karpen C W, Cataland K A, JR Arnold J H, Cornwell D D and panganamala R V: Restoration of the prostacycline thromboxane A_2 balance in diabetic rat: Influence of vitamin E. *Diabetes*, 1982; **(35):** 947-951.

Kazumi T, Yoshino G, FuJiii S, Baha S. Tumorigenic action of streptozotocin on the pancreas and kidney in male Wistar rats. Cancer Res. 1978; 38 **(7):** (2144-2147

Mohan, V. Ramachandran, A. and Viswanathan, M. Tropical diabetes. In. The diabetes Annual I, Eds. Aiberti. K.G.M.M., Krall, L.P.Elsevier *Science, Amsterdam*. 1985. P. 82-92.

Nadkarni. A.K, In: Nadkarni, K.M. (Ed.) *Indian Materia Medica*. **3rd** edition. Popular Book Depot. Bombay. 1954. P. 1209.

Pfatterott C, Meiselman H J and Hochstein: The effect of MDA on erythrocyte deformabiligy, *Blood,* 1982, 59 **(1):** 12-15.

Poliodoro, G. Dt., Arduini. R. G.L. A. and Federici, G.. Superoxide dismutase, reduced Glutathione and Tba- reactive products in erythrocytes of patients with multiple Sclerosis. *Int .J Biochem*, 1984, 16 **(5):** pp. 505-509.

Pugazhenthi, S. and Murthy, P.S. (Studies on isolation and effect of three orally active Hypoglycemic principles Kakara Ib, IIIa, and IIIb from bittergourd. *Dia.Bulletin* 1989**; (9):** 73-76.

Pushpagadan, P. and Sobti, S.N. (Medicinal properties of Ocimum (Tulsi) species and some Recent investigations of their efficacy, *Indian drugs*, 1977; **(14):** 207-208.

Rai. V. Iyer. U.VEffect of Tulsi (Ocimum sanctum) leaf powder supplementation on blood Sugar levels, serum lipids and tissue lipids in diabetic rats*. Plant Foods Hum. Nutr,* 1997. 50 **(1)**: 9-16.

Richard H, Bell JR, Robert J, Hye). Animal model of diabetes mellitus: Physiology pathology, *J Surgical Roes*, 1983 **; (38):** 433-460.

Satyavati . G.V. , Raina, M.K. and Sharma ,M. Medicinal plants of India*, Indian Concial of Medical Research*, New Delhi. P 65-68.

Shukla, R., Sharma, S.B., Puri, D., Prabhu, K.M. and Murthy, P.S. (2000) Medicinal plants for treatment of diabetes mellitus. *Ind.J.clin. Biochem,* 1976. 15 (August, supple.), 169- 177.

Stankora L, Riddle M, Larned J rt. al: plasma ascorbate concentrations and RBC dehydroascorbate transport in-patients with diabetes mellitus. *Metabolism,* 1984; **(33):** 347-353.

Sadikot S-M and Raheja B S): Antioxidant supplementation decreases free radical damage in diabetics. *Jour Dia Assoc. India,* 1991. 31 **(4):** 90- 94.

Sadikot S-M: Diabetes hyperyrnsion and insulin resistance hyperinsulinaemia syndrome, *Jour Diab Assoc. India*, 1993. 33 **(4)**: 93-100

Tappel. A L: Lipid peroxidation damage to cell components. *Federation proceedings.* 1973 .32 **(8),** 1870-1874.

Towill L. E, Drury J S, Hammons A S and Holleman J W: Reviews of the environmental effect of pollutants III Chromium. (1978) EPA / 600/ 1 –73 / 023. *Environmental Protection Agency*, Washington. Vernon, R. Psychoneuroendocrine influences on immunocom pretence and neoplasm *Science,* 1981; 5 **(212):** 1100-1110.

Wendel, AGlutathione peroxidase. In: *Methods in Enzymology*, Ed. Jakoby, W.B. Acadamic Press, New York, U.S.A. 1981. **Vol. 77**, p.327-333.

Yew M S): Effect of streptozotocin diabetes on tissue ascorbic acid and dehydroascorbic acid. *Horm Metab Res*, 1983; **(15):** 158-161.

Yagi, K. (Lipid peroxides and human disease. Chem. Phys. Lipids. 1987. 45, 357-402.

Yoshino G, Kazumi T, Morita S and Baba S. Insulin and glucagon in rats with islet cell tumors induced by small doses of streptozotocin. *Canada J physiol Pharmacol,* 1981**; (59):** 818 -819

CHAPTER - 3

HYPOGLYCEMIC, HYPOLIPIDEMIC AND ANTIOXIDANT PROPERTIES OF (*OCIMUM SANCTUM (*LINN) (TULSI)ON STREPTOZOTOCIN INDUCED DIABETIC RATS.

3.1. INTRODUCTION

(a) METABOLIC DISTURBANCES IN DIABETES MELLITUS

In DM the major changes are related to the metabolism of glucose, lipids and amino acids. The changes are mainly the result of a low insulin / glucagoen ratio.(Fig 14), describes the complication due to DM. Insulin deficiency decreases the uptake of glucose by cells. High glucagoen level decreases the hepatic fructose 2, 6-bisphosphate level, there by decreasing the utilization of glucose.

The insulin dependent enzymes are also less active. Net effect is inhibition of glycolysis and stimulation of gluconeogenesis leading to hyperglycemia. When the blood glucose level exceeds the renal threshold, glucose is excreted in urine. The loss and ineffective utilization of glucose leads to breakdown of fat and protein. This leads to loss of weight. To compensate the loss of glucose and protein, patient will take more food (polyphagia).

The need for fatty acid breakdown to meet the energy requirements would lead to production of more acetyl CoA. The enzyme carnatine acyltransferase is activated by a low insulin/ glucagoen ratio since; the malonyl CoA level is low. There is increased mobilization of TAG from adipose tissue as evidenced by high free fatty acid (FFA) level of plasma.

The availability of oxaloacetate is limited in the TCA cycle .The stimulation of gluconeogenesis is mainly responsible for the depletion of oxaloacetate. The excess of mitochondria acetylCoA therefore is diverted to ketone bodies leading to ketogenesis.

This is more in IDDM. The net effect is the increased mobilization and utilization of fat for meeting energy requirements. There is resultant hyperlipidemia especially an increase in

non-esterified fatty acids (NEFA), TAG and cholesterol level of plasma. Increased breakdown of proteins for providing substrate for glucogenesis and the absence of anabolic effect of insulin are responsible for muscle wasting. Early development of cataract of lens is due to the increased rate of orbital formation, caused by the hyperglycemia.

Glycosylation of retinal proteins and retinal microvascular abnormalities lead to retinopathy and blindness. Moreover, glycosylation of lysine residues of lens proteins also cause cataract formation. Peripheral neuropathy with paresthesia is very common. Decreased glucose utilization and its diversion to orbital on Schwa cells may be the cause for neuropathy.

Hyperglycemia and glycosuria occur after administration of alloxan in several species.

Brunschwig et al, 1943;Baily; 1943 and Baily, 1944; Investigators found that alloxan has a selective destructive effect on the β-cells of islet of Langerhans of the pancreas. However, this effect varied with species and the dose Richard

and Robert, 1983). High dosages of the β-cell toxin streptozotocin and alloxan induce severe insulin deficiency and IDDM with ketosis.

Lower dosages calculated to cause a partial reduction of β-cell mass could be used to produce a mildly insulin-deficient state of NIDDM, without a tendency to ketosis (HO RS, Aranda CG,and et al.1988). and (Bailey, Flattpr.1990) .

The dosage is difficult to judge to create stable NIDDM without either gradual recovery or deterioration into IDDM. Streptozotocin is preferred because it has more specific β-cell cytotoxicity, but sensitivity to this agent varies with species, strain

Lipid peroxidation (LOP)): One of the major determinants of cellular deferability is membrane mechanical behavior. The capacity of erythrocyte / reticulocyte to survive in the circulation is generally thought to be a consequence of factors that affect their mechanical properties (Pfafferott et al; 1982).

Among the biological processes likely to affect the mechanical behavior of the membrane are those which involve the changes in lipid composition and peroxidation of endogenous membrane Phospholipids Reticulocyte and erythrocyte membranes are labile to lipidperoxidation owing to their content of polyunsaturated lipids and to the fact that they are directly exposed to molecular oxygen and involve in the generation of free radical intermediates and semi-stable peroxides. (Tappel, 1973). Increased free radical production is said to mediate tissue injury in wide range of diseases and DM is no exception (Halliwell and Gutteridge, 1984; and Stankora, et al, 1984).

Diabetics have been shown to have increased levels of free radical activity and are more exposed to oxidative stress

(Sadikot and Raheja, 1991). Further evidence of oxidative stress can be gathered from earlier reports on antioxidant status mainly, ascorbic acid and Vitamin E (Vit E) levels . (Sadikot S M,1993).

Many studies indicated decreased ascorbic acid and increased dehydroascorbate, a primary oxidation product of ascorbic acid levels in plasma of both human and animal diabetics (Yew et al 1983; similarly decreased Vit E level are also reported in diabetic patients both IDDM and NIDDM and in experimentally induced diabetic rats (Higuichi , 1982; Karpen et al, 1982). Which indicate the presence of endogenous oxidative stress in diabetic condition.

(b) STREPTOZOTOCIN

Rakietan et al, 1963, reported diabetogenic effect of an antibiotic streptozotocin (STZ) (Fig 12). The reported incidence of islet cell adenomas in rodents with chronic STZ diabetes at 1 year varies from 5 to 99% but is clearly high enough to limit the effectiveness of the model to the studies of approximately 6 months duration.

The islets cell tumors which occur after STZ administration contain large amounts of insulin and can secrete enough insulin to reverse the diabetes (Kazumi et al, 1978; Yoshino et al, 1981). However, much less information is available in the literature about reversal of hyperglycemia and development of islet cell tumors in alloxan induced diabetes. At the same time, reversal of

diabetes has been reporred to be as soon as 3 months after injection, the risk of reversal being directly proportional to the duration of diabetic. However, in conclusion approximately 90% of animals become diabetic after one month of alloxan administration , remain so for an 1 year (Bailey et al, 1944). High dosages of the β-cell toxin streptozotocin and alloxan induce severe insulin deficiency and IDDM with ketosis.

Lower dosages calculated to cause a partial reduction of β-cell mass could be used to produce a mildly insulin-deficient state of NIDDM, without a tendency to ketosis (Ho , Aranda ,et al, 1988). And (Bailey and Flatt PR.1990). The dosage is difficult to judge to create stable NIDDM without either gradual recovery or deterioration into IDDM. Streptozotocin is preferred because it has more specific β-cell cytotoxicity, but sensitivity to this agent varies with species, strain.

3.2. MATERIALS AND METHODS

PREPARATION OF WATER EXTRACT

Fresh *Ocimum sanctum Linn* (Tulsi) leaves were collected, cleaned air-dried and powdered in a grinder.120g of air-dried powder were extracted over night with 170 ml of water with magnetic stirring. The water extract was separated and the residue was re-extracted with water. The combined water extract was concentrated in a lyophilize. Oral administration of extract was in the dose of 200mg/kg-body weight (b.w).

ANIMALS

Healthy male albino Wistar rats (200-250 gm) procured from Center for Cellular and Molecular Biology, Hyderabad were used in this study. The animals were fed on a pellet diet (Hindustan Lever, India) and water ad labium.

3.2.1. INDUCTION OF DIABETES IN RATS

Rats were made diabetic by intraperitoneal (i.p) injection of streptozotocin (STZ) 60 mg/kg in citrate buffer, pH 6.3. Five animals injected with same volume of citrate buffer acted as non-diabetic healthy controls. The rats were kept in separate metabolism cages. The diabetic rats were further divided into two groups of untreated and treated (five each). One group of untreated diabetic rats was orally administered saline daily (0.1ml/ 100 mg body weight) and the other (treated group) was orally administered daily water extract of tulsi 200 mg/Kg b.w per day. The extract or saline were administered daily orally in the morning for 8 weeks by bulged steel tube. The body weight was recorded weekly. At the end of the experiment animals were killed and tissues were collected and frozen in deep freezer (-4°C)

ESTIMATIONS

5 -ml blood was collected from the ear vein at the beginning and the end of the experiment(8 weeks). Erythrocytes and plasma were separated. Plasma glucose, total cholesterol, LDL, VLDL- and HDL-cholesterol and triglycerides were estimated as described earlier by us (Hussain et al 2001). Lipid peroxidation products were estimated as thiobarbutaric acid reactive substance (TBARS) in

plasma and tissues (Pugazhenthi, et al, 1989). Among the antioxidants reduced glutathione was determined by the method of (Hiroshi et. al 1979). Reduced glutathiones superoxide dismutase and catalase were estimated by method of wendel, A. 1981.

ESTIMATION OF SERUM PARAMETERS

a) **Total cholesterol:**

PRINCIPLE

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino-antipyrine in the presence of phenol and peroxides.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol fattyacid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxides}} \text{Cholestene-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-amino-antipyrine} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + H_2O$$

The absorbance at 500nm wavelength is determined and total cholesterol is calculated

$$\text{Cholesterol concentration in the sample} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{conc. of standard}$$

c) Triacylglycerol

PRINCIPLE

The triacylglycerols are determined after enzymatic hydrolysis with lipases. The indicator is quinoneimine formed from hydrogen peroxide, 4-amino-phenozone and 4-chlorophenol under catalytic influence of Peroxidase

$$\text{Triacylglycerol} + H_2O \xrightarrow{\text{Lipases}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol -3-Phosphates + ADP}$$

$$\text{Glycerol -3-Phosphates} + O_2 \xrightarrow{\text{Oxidase}} \text{dihydroxyacetone}$$

$$\text{Peroxidase} \xrightarrow{\text{Phosphates} + H_2O} H_2O_2 + \text{4-amino-antipyrine} + 4\ \text{-chlorophenol}$$

$$\text{quinoneimine} + HCl + H_2O$$

The absorbance at 500 nm is read and triacylglycerol is calculated by

$$\text{Triacylglycerol concentration in sample} = \frac{\Delta\ A\ \text{sample}}{\Delta\ A\ \text{standard}} \times \text{Conc. Of standard}$$

b) **HDL Cholesterol**

Principle: Low density lipoproteins (LDL and VLDL) and chylomicron fraction are precipitated quantitatively by addition of phoshotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in HDL fraction, which remains in the supernatant, is estimated as mentioned above.

c) **LDL Cholesterol**

After estimating total cholesterol, HDL cholesterol, and triacylglycerol, the LDL cholesterol was calculated by the following formula

LDL cholesterol (mg/100ml) = Triacylglycerol

TotalCholesterol ——————▶ H D L cholesterol

Table 3. 2. Effect of treatment for 8 weeks with water extract of tulsi on plasma sugar Level in glucose tolerance test (GTT) in rats.

Group	0hr.	0.5 1hr.	1hr	1.5 h	2hr.
Normal	84.0 ±6.5	150.6±4.3	148.2±5.6	110.5±6.7	88.4±5.6[b]
Diabetic	160.7±14.3	240.5±77.2	270.0±90.0	282.5±81.4	273.1±83.2
Diabetic + tulsi	82.0±2.1[a]	90.0±2.7[a]	88.3±3.2[b]	85.9±4.0	74.5±3.7

a = p < 0.001
b = p < 0.05

Table 3.3: Effect of treatment for 8 weeks with water extract of tulsi extract oF Lipid profile in rats.

Group	TC (mg/dl)	LDLC (mg/dl)	HDLC (mg/dl)	LDLC/ HDLC	TAG (mg/dl
1. Normal	160.0 ± 14.4	82.2±8.79	46.87±8.6	1.77 ± 0.13	110.6±5.2
2. Diabetic untreated	235.0 ± 16.0	165.84±6.7	48.34±2, 50	3.83 ± 0.19	188.1± 5.30
3. Diabetic + tulsi	173.±1.24	92.5±15.6	49.2±7.3	2.0±0.4	132.0±12.6

Table 3.4: Effect of treatment for 8 weeks with water extract of *Ocimum sanctum* (tulsi) on plasma and erythrocyte reduced glutathione glutathione peroxides and glutathione transferase in diabetic rats.

Control	Reduced glutathione		Glutathione peroxide		GlutathioneS-transferase	
	Plasma	Erythrocyte	Plasma	Erythrocyte	Plasma	Erythrocyte
Control	7.362±.. 531.2	241±17	27.082±1.91	27.668±.2.0	27.082±1.92	27.668±2.0
Control + tulsi	8.435.±.632	164±13.5[a]	52.951±1.71[a]	23.2.±65.1	30.2I ±6.5	20.041±1.82
Diabetic. Untreated	5.46±.542[a]	148±10[a]	28.467±3.245	21.051±1.90[a]	56.951±1.75[a]	15.04±10.9[a]
Diabetic + tulsi	7.150±691	150±12.2[a]	26.327±2,252	36.225±3.234[a]	30.2526±1.97	26.65±1.84

a = p < 0.001

Table 3.5: Effect of water extract of. *Ocimum sanctum* treatment for 8 weeks on lipid peroxidation. SOD and CAT activities in erythrocytes in rats.

Groups	LPO	SOD	CAT
Control	285.89±23.0	380.4±44.3	271.8±4.1
Control + tulsi	138.8±6.3[a]	243.7±2.2[a]	175.4±3.0[a]
Diabetic untreated	406.8±6.2[a]	323.6±1.5[a]	295.8±3.0[b]
Diabetic treated with tulsi	208.4±10.5[a]	298.2±6.3[a]	2 68.3±11.0

a = p < 0.001
b = p <0.05

Table 3.6: **Effect of treatment for 8 weeks with water extract of *Ocimum sanctum* (tulsi) in lipid peroxidation in rats.**

LPO (n moles /g tissue) mean ± SD					
Group	**Plasma**	**Liver**	**Lungs**	**Kidney**	**Brain**
1. Control	35±3.8	95.0 ± 14.3	108± 18.4	168.2± 28.0	210.4± 12.7
2. Diabetic	68±5.4[a]	270.o.± 56.2[a]	98.0±10.0	215± 60.2[b]	263.5±51.0
3.Diabetic 200mg *O.sanctum*	25.0±1.5.[a]	85.2 ±14.2[b]	70.0±11.5[a]	140.5±25.3	190.3 ±13.8[b]

a = p < 0.001
b = p < 0.05

Table 3.7: **Effect of treatment for 8 weeks with water extract of tulsi on Body weight and hemoglobin**

	Body weight (g)	**Hemoglobin**
Control	180 ±7[a]	14.2±3.9[a]
Diabetic untreated	130±3.3	10.3±1.9
Diabetic treated with tulsi	148±7[a]	12.9±1.3[a]

a = p < 0.005

3.4. CONCLUSION

In conclusion water extract of tulsi possesses a wide range of beneficial effects to diabetic animals by reducing fasting blood glucose, improving glucose tolerance and serum lipid profile. The beneficial effect were seen in the various tissues of diabetic rats such as lungs, liver, kidney and brain as reflected in decrease in the lipid peroxidation products and increase in the overall antioxidant status of the animals. *In vitro* hyperglycemic studies:

It is evident that hyperglycemia is also one of the factors for adverse effects identified in erythrocytes of type 2 diabetic patients.

In order to confirm the oxidative damage under diabetic conditions and to understand the differential response of erythrocytes *to in vivo* hyperglycemia, the present study has been extended to examine the effect of in *vitro* hyperglycemia by incubating the erythrocytes of healthy persons at normal and elevated levels of glucose in the medium. Further from the in vitro studies it is evident that hyperglycemia is also one of the factors for adverse effects identified in erythrocytes of type 2 diabetic patients. It is therefore evident that *Ocimum sanctum* has atidiabetic activity. The water extract of the plant has been shown for the first time to have antioxidant properties.

3.3. RESULTS AND DISCUSSION

Oral administration of tulsi extract for 8 weeks reduced fasting plasma glucose by (35%) when compared with initial value (172.5±6.43 mg/dl) in diabetic animals (Table 3.1). But when compared with diabetic untreated animals the percent reduction in plasma glucose was 62%. Improvement in glucose tolerance also was seen after *Ocimum sancimum Linn* (tulsi)treatment (Table 3.2). For example, 2 hrs after oral glucose load, the plasma glucose in untreated diabetic animals was 273.1 ± 83.2 mg/dl. But in the tulsi treated diabetic animals the blood glucose was nearly normal (74.5 ± 3.7 mg/dl.). The effect of *Osmium sanctum Linn* (tulsi) treatment (Table 3.2). Tulsi extract on the plasma lipid profile of diabetic rats is shown in (Table 3.3.) Excepting HDL cholesterol, which was unaffected, all the other plasma lipid parameters increased in diabetic untreated animals.

After treatment with *Ocimum sanctum Linn* tulsi extract for 8 weeks, the values came back to nearly normal. In plasma and erythrocytes the antioxidant reduced glutathione and the antioxidant enzymes glutathione reductase and glutathione peroxidase (Table 3.4) were decreased in diabetic animals when compared with those in controls.

However, after treatment with tulsi extract for 8 weeks, there was improvement in all the above parameters. In the erythrocytes, there was decrease in the levels of superoxide dismutase and catalase in diabetic rats and after treatment with water extract of tulsi there was improvement in the levels of these two antioxidant enzymes (Table 3.5).

In diabetic animals the lipid peroxidation products (TBARS) were elevated in the tissues, which were reduced in diabetic animals treated with *OCIMUM SANCTUM LINN* tulsi (Table 3.6).

There was improvement in body weight and total hemoglobin in blood (Table 3.7).

The results of a randomized placebo-controlled, single blind trial of holy basil (Tulsa) leaves in NIDDM patients indicated a significant decrease in fasting and post parricidal blood glucose levels after treatment when compared to treatment with placebo leaves (Agrawal, et al 1996). It is suggested that *Ocimum sanctum* potentates the action of exogenous insulin in normal rats. Recently the plant is reported to possess anti-stress or adaptogenic properties in animals and man (Baynes, .1991).

Stress is known to produce immune suppression, and studies revealed that tulsi has immunomodulatory activity (Vermon, 1981). In our studies treatment of diabetic rats with tulsi extract showed considerable improvement in fasting blood glucose and in glucose tolerance also (Table 3.2). These results confirm the work of earlier workers. *Ocimum sanctum* also corrected the abnormal lipid profile seen in diabetic rats as reflected by the decrease in serum

total and LDL and cholesterol level (Table 3.3). *O. sanctum* has been shown to possess antioxidant properties in general (Yagi, 1987).

But we have carried out detailed studies on the effect of water extract of tulsi in diabetic rats, We have demonstrated for the first time that in the various tissues of diabetic rats after treatment with water extract of tulsi, there was increase in the level of not only the antioxidant compound reduced glutathione but also the activity of antioxidant enzymes glutathione peroxides, glutathione reducats superoxide dismutase and catalase (Table 3.4, & 3.5).

It is now well established that oxidative stress as indicated by increase in superoxide anion and lipid peroxidation products in diabetes causes some complications (Baynes, 1991and Vermon, R. 1981). Any drug which reduces the levels of the lipid peroxidation products in diabetics would be helpful. The elevated levels of the lipid peroxidation product (TBARS) in diabetic animals were also reduced (Table 3.6).

BIBLIOGRAPHY

Agrawal, P. Rai, V. and Singh, R.B. Randomized placebo-controlled, single blind trial of Holy bass leaves in-patients with non-insulin dependent diabetes mellitus *Int .J . Cline. Pharmacol,* 1996; 34 **(9)**, 406- 409.

Bhargava, K. P. and Singh N. Anti-stress activity of *Ocimum sanctum Linn*. *Ind. J. Med. Res,* (1991) 73, 443-451.

Brunschwig A, Allen JG, Goldner MG, Gomori G. Alloxan. *J Med Assoc.*, 1943; 122: 966.

Bailey CC and Bailey OT. Production of diabetes mellitus in rabbits with alloxan, A Preliminary report. *J Am Med Ass*, 1943; **(122):** 165-1166.

Bailey OT, Bailey CC, Hagan WH. Alloxan diabetes in the rabbit: a consideration of the morphologic and physiologic changes. *Am J Med Sci,* 1944; 208: 450.

Bailey CJ, Flatt PR, Models for testing new hypoglycemic drugs. In: Bailey CJ, Flat PR, Eds. *NI*

Baynes,J. WRole of oxidative stress in developments of complications of diabetes. *Diabetes*, 1991; **(40):** 405-412.

Chattopadhya, R.R. Hypoglycemic effect of Ocimum sanctum leaf extract, in normal and Streptozotocin diabetic rats. *Ind. Exp. Biol.* 1993; (31): 891-893.

Deneke S M and Fanburg B L: Regulation of Cellular glutathione. *Am J Ohysiol* ? 257 (Lung cells Mol Physical): 1989, L163- L 173.

Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N. and Ray, Screening of Indian plants For biological activity. Part1. Ind. J. Exp. Biol. 1968, **(6):** 232-238.

Gupta, S) Pharmacognosy of leaves of different species of Ocimum (Tulsi): *Ind. J. Med. Res.,*967; 2 **(1):** 35 –38.

Halloween B and Guttering J M C: *In free radicals in Biology and Medicine.* Halliwell B and Gutteridge J M c **(eds)** *calerendron press*, *Oxford*, 1989; 87.

Ho RS,Aranda CG, Tilery SJ et al. In vivo and in vitro glucose metabolism in a low dose streptozotocin rat model of non insulin dependent diabetes. In: ShafrirE, Renold AE, eds. Lesions from Animal Diabetes Frontiers *in Diabetes Research*. *London*: Libbey, 1988: 288-294.

Higuichi Y: Lipid peroxides and ∝-tocopherol in rat streptozotocin induced diabetes mellitus. *Acta Med Oklayama*. 1982. **(36):** 165-175.

Hussein, H.E.M.A., Jamil, K., and Rao, M. Preliminary studies on the hypoglycemic effect Of *Abroma augusta* in alloxan diabetic rats. *Ind. J. ciln. Biochem,* 2001.16 **(10):** 77- 80.

Hiroshi, O., Nobuko, O. and Kunio, V. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. *Biochem*, 1979**. (5):** 351-358

Karpen C W, Cataland K A, JR Arnold J H, Cornwell D D and panganamala R V: Restoration of the prostacycline thromboxane A_2 balance in diabetic rat: Influence of vitamin E. *Diabetes*, 1982; **(35):** 947-951.

Kazumi T, Yoshino G, FuJiii S, Baha S. Tumorigenic action of streptozotocin on the pancreas and kidney in male Wistar rats. Cancer Res. 1978; 38 **(7):** (2144-2147

Mohan, V. Ramachandran, A. and Viswanathan, M. Tropical diabetes. In. The diabetes Annual I, Eds. Aiberti. K.G.M.M., Krall, L.P.Elsevier *Science, Amsterdam*. 1985. P. 82-92.

Nadkarni. A.K, In: Nadkarni, K.M. (Ed.) *Indian Materia Medica*. **3rd** edition. Popular Book Depot. Bombay. 1954. P. 1209.

Pfatterott C, Meiselman H J and Hochstein: The effect of MDA on erythrocyte deformabiligy, *Blood,* 1982, 59 **(1):** 12-15.

Poliodoro, G. Dt., Arduini. R. G.L. A. and Federici, G.. Superoxide dismutase, reduced Glutathione and Tba- reactive products in erythrocytes of patients with multiple Sclerosis. *Int .J Biochem*, 1984, 16 **(5):** pp. 505-509.

Pugazhenthi, S. and Murthy, P.S. (Studies on isolation and effect of three orally active Hypoglycemic principles Kakara Ib, IIIa, and IIIb from bittergourd. *Dia.Bulletin* 1989**; (9):** 73-76.

Pushpagadan, P. and Sobti, S.N. (Medicinal properties of Ocimum (Tulsi) species and some Recent investigations of their efficacy, *Indian drugs*, 1977; **(14):** 207-208.

Rai. V. Iyer. U.VEffect of Tulsi (Ocimum sanctum) leaf powder supplementation on blood Sugar levels, serum lipids and tissue lipids in diabetic rats. *Plant Foods Hum. Nutr,* 1997. 50 **(1)**: 9-16.

Richard H, Bell JR, Robert J, Hye). Animal model of diabetes mellitus: Physiology pathology, *J Surgical Roes*, 1983 **; (38):** 433-460.

Satyavati . G.V. , Raina, M.K. and Sharma ,M. Medicinal plants of India*, Indian Concial of Medical Research*, New Delhi. P 65-68.

Shukla, R., Sharma, S.B., Puri, D., Prabhu, K.M. and Murthy, P.S. (2000) Medicinal plants for treatment of diabetes mellitus. *Ind.J.clin. Biochem,* 1976. 15 (August, supple.), 169- 177.

Stankora L, Riddle M, Larned J rt. al: plasma ascorbate concentrations and RBC dehydroascorbate transport in-patients with diabetes mellitus. *Metabolism,* 1984; **(33):** 347-353.

Sadikot S-M and Raheja B S): Antioxidant supplementation decreases free radical damage in diabetics. *Jour Dia Assoc. India,* 1991. 31 **(4):** 90- 94.

Sadikot S-M: Diabetes hyperyrnsion and insulin resistance hyperinsulinaemia syndrome, *Jour Diab Assoc. India*, 1993. 33 **(4)**: 93-100

Tappel. A L: Lipid peroxidation damage to cell components. *Federation proceedings.* 1973 .32 **(8),** 1870-1874.

Towill L. E, Drury J S, Hammons A S and Holleman J W: Reviews of the environmental effect of pollutants III Chromium. (1978) EPA / 600/ 1 –73 / 023. *Environmental Protection Agency*, Washington. Vernon, R. Psychoneuroendocrine influences on immunocom pretence and neoplasm *Science,* 1981; 5 **(212):** 1100-1110.

Wendel, AGlutathione peroxidase. In: *Methods in Enzymology*, Ed. Jakoby, W.B. Acadamic Press, New York, U.S.A. 1981. **Vol. 77**, p.327-333.

Yew M S): Effect of streptozotocin diabetes on tissue ascorbic acid and dehydroascorbic acid. *Horm Metab Res*, 1983; **(15):** 158-161.

Yagi, K. (Lipid peroxides and human disease. Chem. Phys. Lipids. 1987. 45, 357-402.

Yoshino G, Kazumi T, Morita S and Baba S. Insulin and glucagon in rats with islet cell tumors induced by small doses of streptozotocin. *Canada J physiol Pharmacol,* 1981**; (59):** 818 -819

CHAPTER - 4

BLOOD SUGAR LOWERING EFFECT OF WATER EXTRACT OF *AZADIRACHTA INDICA* (L.) (NEEM) AND *ABROMA AUGUSTA* (LINN.) IN DIABETIC RATS.

4.1. INTRODUCTION

Many herbal products have been described for the care of diabetes mellitus in ancient literature of *Ayurveda* in India. Many herbs which have been shown to have hypoglycemic action in animals and humans have been recently reviewed (Gupta, 1994; Shukla et al , 2000.). *Artemisia herbal* alibi is also reported to have hypoglycemic effect (Twig and Bad 1988) *Azadirachta indica (L*) (Plate No 3), is one of the most common and well-known Indian bites; bark, leaves and fruits are commonly used in fevers and various skin diseases.

It is used in *Ayurvedic* preparations, ″Nimbadi Kwatha " for fevers, ″Nimbadi Anjana ″, for the eyes, and ″Neem Taila ″, as a local insecticide and antiseptic. In *Unani* system, the leaves are used in ″Hab Mussafi Hun″. For blood purification . Seeds in ″Zamad Bawasir ″and ″Herb Bawasir Badi″, for piles, ″Herb Seya Chasm″, for applying to eyes (Suma), Zima Mochas″, for pimples on the face, and bark in ″Are Gaza″, for fevers.the gum exuded by *A. indicia* tree contains 35% proteinaceous material. The presence of free amino acids in the exuviate is of considerable importance.

The most abundant amino acid is aspartic acid, besides *serine* and t*hreonin*e. The carbohydrate portion was complex which on hydrolysis yielded D-

galactose, L- arabinose (Major components), mansons, xylose, focus and rhamnose. The acid content (28%) has methoxy-β-D-glucopyranosyl uronic acid)-D- galactose and 4-O-β-d-glucopyranosyluronic acid) -D- galactose. Extracts "of ripe leaves, tender leaves, fruits and flowers of *Azadiruchta indicia* (*Neem*) have been reported to possess anti-diabetic, and antiviral activity. (Rao, et al. 1969 Bhattacharji et al, 1953).

Effect of the extract of the leaves was studied on the cardiovascular system of anaesthetized monkeys and rabbits (Rao et al, 1969). *Neem* has been shown to possess a number of pharmacological. Effects like cardiovascular, antimicrobial and immunomodulatory (Dhawan and Patnaik, 1993). One of the properties of neem has been its hypoglycemic effect. Different parts like seed and leaf extract have been shown to possess hypoglycemic effect (Murky et al, 1978; Dixie, et al, .1986; Santoshkumari and Dar 1990).

The plant *Abroma augusta* (Fam: Stetrculiaceae) is effective in the treatment of diabetes and in amenorroea (Kapoor, 1984). *Abromine* the active constituent has been identified as betaine (Das Gupta and Basu, 1970). The leaves contain octacosanol, tarasxerol, β-sitosterol acetate and mixture of long chain fatty idols. (Mukherjee, and Badruddoja, 1977; and Mukherjee, et al. 1978).

Recently we (Hussein et. al. 2001), have demonstrated the antidiabetic activity of *A.augusta* waters extract. In *Ayurveda* some time extracts of more than one plant are combined and used for treatment. So we studied the combined

effect of *A. augusta* and *A. indica* (water extract) in alloxan induced diabetic rats and the results are reported in this paper.

KIDNEY PATHOLOGY

Early stage of diabetic nephropathy in alloxan as well as STZ treated animals is characterized by an increase in renal size, in the glomerular capillary filtration surface (1.e. increase in the amount of glomerular basement membrane) and in glomerular filtration rate (Carney et al, 1979; Osterby and Gunderson, 1980; Gotzche, et al, 1981). After about 4 months of alloxan or STZ induced diabetes, light microscopic features of experimental diabetic nephropathy begin to appear, becoming progressively more copious in the ensuing months (Steen et al, 1966; Fox et al, 1977; Hagg, 1974; and Rasch, 1981).

A number of studies have addressed the question, whether glomerulopathy resulting from chemically induced diabetes can be prevented or reversed with insulin or pancreatic transplantation. The answer to this question has obvious implications for the human disease. At this point, the data is incomplete, but it does appear that pancreatic transplantation early in the course of experimental alloxan diabetes can prevent development of mesangial enlargement and glomerular basement membrane thickness for the normal life span of the rats (Bell et al, 1980).

As far as reversal of established lesions is concerned, it has been shown that to a great extent the abnormalities in the mesangium can be reversed. However, results of few studies indicate that thickening of glomerular basement

membrane may not be reversed by treatment of the diabetes (Mauer et al, 1972; Bretzel et al, 1979; Steffes et al, 1979; Rash, 1979; Gotzche et al, 1981; Mauer et al, 1981; Rasch, 1981; whener et al, 1980).

NERVER PATHOLOGY

Diabetes induced by either alloxan / STZ causes altered conduction velocities in peripheral nerves of rats in a manner similar to human diabetes (Richard et al, 1983).

HERBAL PRODUCTS IN DIABETES

The Charak Samhita, (300 B.C) Sushrut Samhita and Vaghbhatt have laid more emphasis on the prophylactic and curative measures of DM and not mere symptomatic treatment. Though the presently available oral hypoglycemic is effective in controlling acute metabolic abnormalities, they are societal with side effects.

Therefore, we are critically lacking a safe pharmacological agent which can effectively increase glucose utilization, counter the metabolic abnormalities of diabetes and prevents its long term complications i.e. *Nephropathy*, *Neuropathy*, *Cataract* and Hypertension etc. Before the introduction of insulin in 1922, dietary measures and traditional plant therapies were being used to treat DM. Understanding of DM in Ayurvedic medicine dates back to 600-800 BC. The symptomatology, complications and treatment of DM stand out remarkably well in the present area of scientific observations of the disease (Ajgaonkar, 1984).

4. 2. MATERIALS AND METHODS

i) Studies to establish hypoglycemic activity.

ii) Screening of hypoglycemic activity of various herbal extracts:

iii**)** Oral glucose tolerance (GTT) test.

(a) ANIMALS: RATS

Blood collection:

Parameter to be studied:

Serum glucose: By glucose oxides / peroxides (GOD/ POD).

Thiobarbituric acid reactive substance, (TBARS), and increased antioxidants reduced glutathione (GSH) superoxide dismutase (SOD) catalase (CAT) glutathione peroxidase

(GPX) and glutathione transferase (GT), in the plasma and the tissues liver, kidney, and muscle.

Screening of antihyperglycemic activity of various herbal extracts;

Preparation of diabetic animals: Alloxan (20-mg/100 gm b/w)

Sample collection: Blood collection retro-orbitally from the inner canthus of the eye using Micro Hematocrit Capillaries, Mucous.

(b) PLANT MATERIALS

Abroma augusta and *Azadirachta indicia*: The root–bark of *A. Augusta* was obtained from Khasia hills in Assume and identified by Botanical Reseat Institute, Lucknow, India. The root–bark was air dried and ground in an electrical mill. Freshly collected *neem* leaves were air dried and powdered as mentioned above. Equal parts of *Abroma augusta* root powder and

Azadirachta. indicia leaves powder were mixed well, 25g of the powder was well extracted by soaking in distilled water (100ml) for two days in the cold (at 4°C), with frequent stirring. The extract was concentrated in a lyophilize and kept separately in airtight containers in a deep freeze until use.

(c) ANIMALS

Healthy male albino Wisteria rats (150-200 gram) from Center for Cellular Molecular Biology Hyderabad India were fed on a pellet diet (Hindustan Lever, India) and water ad labium.

(d) INDUCTION OF DIABETES

Rats were starved over night and each rat received a single subcutaneous injection of alloxan monohydrate (20 mg/100 gm b/w) in freshly prepared sodium-acetate buffer (0.15M, pH 4.5). The volume to be injected was kept between 100 µl –150 µl. The same volume of acetate buffer was given to each control rat. Next day a single injection of 2 units of protamine-Zn insulin prepared in normal saline was given to each alloxan treated rat. This procedure was continued for 6 days. It decreased the mortality of the animals. Controls were given the same volume of normal saline instead of insulin (Sochor et al, 1988). Fasting blood sugar was checked on 7th day. Animals with fasting blood sugar (>150 mg /dl) were used as diabetic rats.

BLOOD COLLECTION

The blood was collected retro-orbital from the inner canthus of the eye using Micro Hematocrit Capillaries, Mucaps. The blood was collected in oxalate-sodium fluoride in Eppendorf tubes.

Parameters studied:

Plasma glucose:

Plasma glucose was estimated by glucose oxidase method using the Kit from Ranbaxy Labs, Delhi.

Glucose tolerance test

The standard oral glucose tolerance test was performed on all animals at before and after 8 weeks of experiment.

EXPERIMENTAL DESIGN

Animal groups: Animas were divided into 5 groups of 5 each. Group 1 served as healthy controls. Group 2 was untreated diabetic rats, group 3, diabetic rats were given water extract of *Abroma augusta*, group 4 diabetic rats received *Azadirachta. Indicia* (*Neem*) and group 5 diabetic rats were treated with *A. augusta* and *A. indicia* (combined). The dose extract was 200mg /day for each animal. Rats of groups 1 and 2 were given 4 ml of salin daily in place of plant extracts. TC, LDLC, HDLC= total, low density and high density lipoprotein cholesterol respectively,

TAG= triacylglycerol.

LIPID PROFILE

In type I diabetic subjects with poor glycemic control, LDL particle which are internalized and degraded by fibroblasts in *vitro* less efficiently than LDL from normal subjects. These changes may be parallel to the pathogenesis of the accelerated atherosclerosis occurring in diabetics with hypertriglyceridemia HDL changes have been shown to occur with diabetes. In insulin deficient streptozotocin- treated rats, a dramatic increase in the Apo-I/ Apo-II ratio in LDL was found, which was due to a 30% increase in Apo-I and a 50% decrease in Apo-II. Thus, changes in apportions of HDL similar to those seen in human diabetes can be experimentally induced in rats: these alterations are associated with significant difference in the binding characteristics of HDL to the Apo-I receptor.

Serum lipid profile

Total cholesterol, HDL cholesterol and triacylglycerols were estimated using standard kits of Random, Mamba, India. LDL cholesterol was calculated from the above measurement by using Friedwalds formula (Friedwald 1972).

LDLC = TC- (HDLC + (TAG) / 5).

TC, HDLC, HDLC= total, low density and high density lipoprotein cholesterol respectively,

TAG = triacylglycerol

4.3. RESULTS AND DISCUSSION

Fasting plasma glucose (FPG) values, before and after treatment for 8 weeks in normal, diabetic untreated and diabetic treated rats with water extract of one plant only (A*. Augusta or A. indica*) or both the plants (*A. augusta* and *A. indicia*) *Neem* is shown in (Table 4.1). The fasting plasma glucose (FPG) values remained more or less the same in normal i.e. 99.4 ± 19.5 mg/dl before and 89.8 ± 5.21 mg/dl after 8 weeks. In diabetic rats even the initial (0 week) FPG values were higher (172.2 ±15.4 mg/dl) which increased to 285.6 ± 42.6 mg/dl by 8 weeks.

However after treatment with 200mg of water extract *of A. augusta* and *Azadirachta indicia (Neem*) the higher initial FPG values (167.9±98.5mg/dl) returned to the normal level (92.4± 2.8), There was improvement in the FPG, values after treatment with one plant only, but the improvements was less as compared with the mixture of the plants. Results in Table 4.2 show the mean FPG values of normal, diabetic untreated and diabetic treated groups during glucose tolerance test after 8 weeks. The fasting blood glucose values for normal, diabetic untreated and diabetic treated were with both the extracts were 83.0 ±6.5, 160.7 ±14.3 and 80.0 ±2.0mg/dl. In the glucose tolerance test in the case of normal animals the peak values were obtained in ½ hr and were 160.5 ±4.2 mg/dl and retained to initial values in 2 hours. In the case of diabetic untreated animals the blood values remained at a high level (above 240 mg/dl). However, in *Abroma augusta* plus *neem* treated animals the zero hour values were normal (80.2 ±2.0 mg/dl). The peak values seen at one hour were close to

normal. The values in the animals treated with only one plant were in between the diabetic untreated and diabetic animals treated with both the plants. This shows that treatment with *Abroma. Augusta* + *neem* for 8 weeks improved glucose tolerance.

Effect of the water extract of *A. augusta* and neem on serum lipid profile is shown in (Table 4.3) and (Fig 15). In the diabetic animals the TC, LDLC/ HDLC and TAG values were higher. But HDLC values were not much affected (initial values not shown in table). But after treatment with extract of *neem* the TC and LDLC values were almost normal. There was slight increase in HDLC values. This shows that treatment with *A. augusta* and neem improved lipid profile in diabetic.

Table 4.4 shows the levels of lipid peroxidation product (LPO); the antioxidant reduced glutathione (GSH) and the individual antioxidant in enzyme. Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPX) and Glutathione transferase (GT). The improvement with the water extract of both the plants combined was much higher than with either of the two plants alone. The diabetic untreated animals also showed higher lipid peroxidation products in the tissues heart, lung liver and muscle (Table 4.5), which were brought back to near normal values by treatment with water extract of the two plants. Results with a single plant are not shown .The diabetic untreated animals showed loss in body weight, reduction in total hemoglobin and sugar in urine (Table 4.6). After treatment with water extract the two plants, the body weight

and total hemoglobin were nearly normal. There was no sugar in urine. The diabetic untreated rats showed retinopathy

(Fig 4.1), damage to the muscles and skin of the tail (Fig 4.2), and injury to legs and slight edema in the Paws of the legs. The animals looked lethargic and sickly. (Fig 4.3). All these symptoms disappeared after treatment for 8 weeks with the water extract of the two plants *A. augusta* and *A. indica* (neem). The treated, diabetic animals showed normal behavior and were almost like the normal rats, which disappeared after treatment with water extract of the two plants *Abroma. augusta* and *Azadirachta indica* combined. (Fig 4.4). As compared to (Fig 4.5) normal rats. (Fig. 4.5).

The results of our present studies showed that treatment of alloxan diabetic rats with water extract (200mg) of *A.augusta* and *neem* daily once for 8 weeks brought down the fasting (i) plasma (ii) glucose (Table 4.1) and improved glucose tolerance (Table 4.2). The mechanism of action has not been investigated in this initial study. From the improvement in glucose tolerance one can presume that blood insulin levels are elevated during glucose tolerance test. It is quite possible that the extract directly stimulates the activity of enzymes of pathways of glucose utilization in the tissues. It is common knowledge that one of the complications of untreated diabetes mellitus is increase in total and LDL cholesterol and triacylglycerols.

Since the treatment with water extract of the two plants has brought down TC and LDLC to normal range (Table 4.3) and considerably decreased TAG levels, the treatment has effect not only on carbohydrate metabolism but

also on lipid metabolism i.e. it has antihyperglycemic and hypolipidemic effect. This means that this acts like some of those few plants, which have been reported to have both antihyperglycemic as well as hypolipidemic effects (Shukla et al 2001; Sharma et al 1997). Treatment with the individual plants also showed improvement, which was however better with the combination of the plants.

It is now well established that in diabetic animals there is an increase in the free radicals and lipid preoxidation products as a result of which there is derangement antioxidant system which is reflected in the decrease in the concentration of the antioxidants such changes were seem in the untreated diabetic animals (Tables 4.4 and 4.5).

Treatment with the water extract of the two plants was effective in bringing back the above parameters. This shows that this extract had strong antioxidant activity. The elevated levels of free radicals and lipid peroxidation products in the uncontrolled diabetes group 2 in animals and humans will lead to complications, in the eyes, kidneys and central nervous system. The untreated diabetic animals showed signs of *retinopathy* (Fig 4.1), damage to skin of the tail (Fig 4.2(a) & 4.2.(b), injury to legs and edema of paws (Fig 4.3), which disappeared after treatment with water extract of the two plants *Abroma augusta* and *Azadirachta indica* combined (Fig.4.4). Treatment with water extract was so effective that the above mentioned complications seen in the untreated diabetic rats, disappeared. The overall behavior of the rats was normal. In conclusion it can be stated that the *two plants A. augusta and A. indicia*, have a synergistic effect when given together. They have a strong anti-hyperglycemic, anti-hyperlipidemic effect and

are capable of preventing the free radical and lipid peroxidation product mediated damage and complications.

Table 4. 1. **Effect of water extract of *Abroma augusta* and *Azadirachta indicia* on the fasting plasma glucose level in rats**

Plasma glucose (mg/ dl) mean ± SD		
Group	0 weeks	8 weeks
Normal	99.4 ± 19.5	89.8 ± 5.21
Diabetic	172.2 ± 5.4[b]	285.6 ± 42.6[a]
Diabetic + *A. augusta*	165.6 ± 26.0[c]	105.4 ± 26.6
Diabetic + *A. indica*	169.7 ± 28.2[c]	100.4 ± 26.4
Diabetic + *.augusta A* + *Azadirachta indicia* (treated)	167.9± 98.5[a]	92.4± 2.8

a = p < 0.001
b = p < 0.01
c = p <0.05

Table 4.2. Plasma sugar level in glucose tolerance test (GTT) in normal, diabetic untreated and diabetic treated with 200mg water extract of *A. augusta and A. indica* after 8 weeks .

Group	**0hr.**	**0.5hr**	**1hr**	**1.5hr**	**2hr**
Normal	83.0 ±6.5	160.5±4.2	145.2±5.3	110.5±6.7	86.3±5.2
Diabetic	160.7 ±14.3[a]	240.5±77.2[b]	270.0±90.0b	282.5±81.4[a]	273.1±83.2[a]
Diabetic + *A. augusta*	89.8 ±25.2	115.0±21.1[b]	116.4±16.0	104.0±11.4	90.5±25.0
Diabetic + *A. indicia*	87.8±24.5	111.2±19.6[b]	114.3±15.2[b]	102.0±10.8	82.3±2.5
Diabetic + *A.augusta* + *A indicia*	80.1±2.00	92.0±2.5[a]	86.3±3.0[a]	84.7±2.3[a]	72.5±3.6[c]

a = p < 0.001
b = p <0.01
c = p <0.02

Table 4. 3. Effect of 200mg treatment for 8 weeks with water extract (200mg / kg /body) *augusta . A. inadic*.and both *Abroma augusta* and *Azadirachta indicia* on Plasma lipid profile.

Group	TC (mg/dl)	LDLC (mg/dl)	HDLC (mg/dl)	LDLC/ HDLC	TAG (mg/dl
1. Normal	160. ± 14.4	82.2±8.79	46.87±8.6	1.77 ± 0.13	110.6±5.2
2. Diabetic untreated	235. ± 16.0[a]	165.84±6.7[a]	48.34±2,50	3.83 ± 0.19[a]	188.5 ± 55.30[c]
3. Diabetic + *A.*	185.0 ±14.8	99.8± 16.6	51.3 ± 8.4	1.9±0.4	175.0 ± 15.7 [b]
4. Diabetic + *A. indica*	175 ±1.23	95.6±16.2	48.2 ±7.3	2.0 ±0.4	135.0 ±13.2[c]
5. Diabetic + *A. augusta* and *neem* treated	175. ± 1.26	91.15± 13.2	44.6±6.4	1.56± o.o3	141.0 ± 12.2[b]

a = p <0.001
b = p < 0.01
c = p <0.02

Table 4. 4. Effect of water extract *A. augusta* and neem treatment for 8 weeks on lipid Peroxidation SOD and CAT and glutatheone peroxidase, glutathione Transferase activities in erythrocytes in rats.

Groups	LPO	SOD	CAT	GPX	GT
Control	1.288 ±0.08	7.12±0.65	1.270±.09	1.92±0.10	1.0±0.05
Diabetic Untreated	406.8 ±6.2[a]	3.38±0.67[a]	295.8±3.0[a]	70.93±6.87[a]	1197±118[a]
Diabetic + a. *augusta*	173.4±6.5[a]	7.06±0.70	188.6±12.5[a]	115.97±12.2[a]	1548±146[a]
Diabetic + *A. indica*	163.5±7.4[a]	6.28±0.59	148 ±11.3[a]	97.08±8.24[a]	1423±137[a]
Diabetic + *A. Augusta*+ *A Indicia*	108.72±5.11[a]	8.00±0.66	268.3±11.0[a]	114.20±10.56[a]	1551±150[a]

a = p < 0.001

Units: SOD: Units /mg protein; CATL n moles of H_2O_2 .GPx: n moles of GSH oxidized /min /mg protein; GST: LPO: n moles of malondialdehyde (MDA) / mg protein GSH: n moles / g of wet tissue.

Units: SOD: Units /mg protein; CATL n moles of H_2O_2 .GPx: n moles of GSH oxidized /min /mg protein; GST: LPO: n moles of malondialdehyde (MDA) / mg protein GSH: n moles / g of wet tissue.

Table 4.5. TBARS in normal and experimental rats.

Concentration of TBARA (mM/100g wet tissue)				
Group	**Heart**	**Liver**	**Kidney**	**Muscle**
Control	0.45 ±0.004	0.90 ±0.006	1.25±0.06	4.30 ±0.42
Diabetic Control	0.12±0.02	1.46±0.05	1.78±0.05	104.2 ±6
Diabetic + A. augusta	0.66±0.07[b]	0.95±0.03	1.34±0.05	117.3±8
Diabetic + *A. indicia*	0.64±0.06	0.96±0.05	1.33.0.03[a]	123.2±6
Diabetic + *A. augusta and neem*	0.68±0.07[a]	0.93±0.04	1.35±0.02[c]	115.3″±13.2.[b]

a = p , 0.001
b = p < 0.01
c = p < 0.02

Table 4.6 Effect of water extract *A. augusta* and neem treatment on total Hemoglobin, change in body weight and Urine sugar of normal and experimental rats.

Group	Hemoglobin (g/100 ml)	Changes in body (weight (g)	Urine Sugar
Normal	16.0 ±4.2	35.6±3.2	-
Diabetic control	10.5 ±1.2	-16.5 ±3.0	+++
Diabetic + *A. augusta*	13.6 ±0.2	4.8±1.0	_
Diabetic + *A. indicia*	12.9 ±1.3[a]	5.8±1.5[b]	_
Diabetic + A. *augusta* + *neem*	14.0 ±0.3[a]	5.4±1.1[a]	_

- **reduction over all weight**

+ **Increase in body weight overall**

a = p < 0.001

b = p < 0.01

4.4. CONCLUSION

Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for indigenous natural antidiabetic agents is still going on.

Azadirachata indica (*F: Meliaceae, Hindi: neem*) has been shown to possess number of pharmacological effects like cardiovascular, antimicrobial, immunomodulatory . One of the properties of neem has been its hypoglycemic effect.

Different parts of plants like seed and leaf extract have been shown to possess hypoglycemic effect. It has shown that aqueous neem leaf extract (NLE) has moderate hypoglycemic activity and significant antihyperglycemic activity in

normal rats. Hence the antidiabetic activity of NLE was evaluated in animal models of IDDM and NIDDM. The NLE was compared with *Abroma Augusta* for blood sugar lowering effect in diabetes rats.

Water extract of the dried powder of two plants, *Abroma augusta* (Fam*: Sterculiaceae*) root and *Azadirachta indica* (*Fam: Meliaceae*) leaves combined together was administered (200mg / Kg body weight) orally to diabetic rats once a day for 8 weeks, This treatment caused significant lowering of blood sugar in fasted rats and depressed the peak value in blood glucose during glucose tolerance test.

The treatment resulted in a significant reduction in serum lipids. The aqueous extract also decreased lipid Peroxidase formation thiobarbituric acid reactive substance, (TBARS), and increased antioxidants reduced glutathione (GSH) superoxide dismutase (SOD) catalase (CAT) glutathione peroxidase (GPX) and glutathione transferase (GT), in the plasma and the tissues liver, kidney, and muscle. It also prevented decrease in body weight suggesting that *abroma augusta* roots and neem leaves when given together as water extract have hypoglycemic action, as determined by assay in diabetic rats. Thus this study shows that *Abroma augusta* roots and neem leaves when given together as water extract also have hypoglycemic action.

BIBLIOGRAPHY

Ajagonkar SS. Ancient Indian Medicine and diabetes mellitus. Diabetes Mellitus in Developing countries. *Edited by Bajaj JS, Interprint New Delhi*. 1984; 1-10.

Bell RH, Fernandez-Cruz L, Brimm JE, Sayers Ha, Lee S, Orloff MJ. Prevention by Whole pancreas transplantation of glomerular basement membrane thickening in Alloxan diabetes. *Surgery*, 1980; 88 (10): 31-40.

Bhattacharji Satyendra, Chittarajan Mita Salimuzzaman, Siddiqui. Chemical Examination of the trunk Bark neem (*Azadirachta indica syn. Melia azadirachta*) *J. SCI. Industry. Res*. 1953; 12 B, 154-156

Pretzel RG, Breidenbach G, Hofmann J, Federlin K. Islet transplantation in experimental diabetes of the rat. VI. Rate of regression in diabetic kidney lesions after isogenic islet cell transplantation: quantitative measurements. *Horm Metab Res*, 1979; 11 **(3):** 200-207.

Carney SL, Wong NL, Dirks JH. Acute effects of streptozotocin diabetes on rat renal function. *J Lab Clin Med*, 1979; 93 **(6)**: 950-961.

Das Gupta, B. and Basu, K.Chemical investigation of Abroma augusta Linn. Identity of abromine with betaine. *Experientia,* 1970; **(26):** 477-478

Dhawan, BN, Patnaik, Gk., Pharmacological studies for therapeutic potential. In neem researchand development. Randhawa N, S. and Panni B, S. (Eds.) Published by Soc Pesticide. *Sci., India.* 1993; 242-249

Dixit VP, Singh R, Jank R. Effect of neem seed oil on the blood glucose concentration of Normal and alloxan induced diabetic rats. *J Ethnopharmacol*, 1986; **(17)**: 95-98

Friedwald. Estimation of concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin. Chem*, 1972; **(18)**: 499-500

Fox CJ, Darby SC, Ireland JT, Sonksen pH. Blood glucose control and glomerular capillary basement membrane thickens in experimental diabetes. *Br Med J,* 1977; **(2)**: (6087): 605-607.

Gupta, SS, Prospects and perspectives of natural plant products in medicine. *Indian Journal of* Pharmacology*,* 1994; **(26)**: 5-9.

Gotzche O, Gundersen HJ, OsterbyR. Irreversibility of glomerular basement membrane accumulation despite reversibility of renal hypertrophy with islet transplantation in early experimental diabetes. *Diabetes*, 1981; **(30)**: 481.

Halim Eshrat M. Hussein, Kaiser Jamil and Mala Rao.*Indian Journal of Clinical Biochemistry,* 2001; 16 **(1),** 77-80.

Hagg E. Occurrence of immunoglobulin and complement in the glomeruliof rats with Long term alloxan diabetes. *Acta Pathol Microbial Scand,* 1974**; (A)** 82: 220.

Kapoor, R.K., Diabetes mellitus. *Indian J. Homeopath. Med,* 1984; **(1):** 114- 116.

Mauler SM, Michael AF, Fish AJ, Brown DM. Spontaneous immunoglobulin and complement deposition in glomeruli of diabetic rats. *Lab Invest*, 1972; 27 **(50)**: 488- 494.

McGowan MW, Artiss JD, StrandbergDR, zakBA. 1983. Peroxidase couplemethod for the Colcrimetric determination of serum triglycerides. *Clin chem*, 1983 ; **(29)** : 538- 542.

Mukherjee, Ks. and Badruddoja sah. Chemical investigation of bark of *Abroma augusta* Linn. *J. Indian chem. Soc.,* 1977; 54, 647.

Mukherjee, K, S. and Badruddoj Sah. Chemical examination of heart wood of *Abroma augusta* Linn. *Curr. Sci.* 1978; **(47):** 301-303.

Murty KS, Rao DK, Murty LBG. A preliminary study on hypoglycemic and anti hyperglycemic effect of A. *Indicia. Indian J Pharmacol*, 1978; **(10)** : 247-250.

Osterby R, Gunderson HJG. Fast accumulation of basement membrane material and the rate of morphological changes in acute experimental diabetic glomerular Hypertrophy.*Diabetelogia,* 1980**; (18):** 493.

Rao A.R. Savithri Sukumar, T.v., Paramasivam, S.Kamalakshi, A.R. Parashuraman, and M. Shantha. . *Ind. Jour. Med. Res. March* 1969; **(57):** 3- 16.

Rash R. Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. Kidney size and glomerular volume. *Diabetologia,* 1979; 16 **(2) :** 125-128.

Rash R. Studies on the prevention of glomerulopathy in diabetic rats. *Acta Endocrinol* [**Supple**] Copenh. 1981; 242-243.

Shukla, R., Sharma, S.B., Puri, D., Prabhu, K.M. and Murthy, P.S. . Medicinal plants for treatment of diabetes mellitus. *Ind. J. Clin. Biochem,* 2000; 15 **(August, Supple.),**169-177.

Santoshkumari KS, Devil KS. Hypoglycemic effects of medicinal plants. *Ancient Sci*. *Life*. 1990; **(9)**: 221-3.

Sochor, M., Ball, M.R. Baquer, and N.Z: Effect of thyroid hormones on the level of metabolic intermediates in diabetic rat liver. *Biochem. Int,* Jan: 1990; 16 **(1)**: 15-23.

Steen OT, Orskov H, Lundbaek K. Kidney lesions in rats with severe long-term alloxan diabetes. *Acta Pathol Micriobiol Scand,* 1966; 66: 1- 4.

Twaij, H.A.A., Al-Badr. A, A. Hypoglycemic activity of Airtimes herb alba. *Journal of Ethnopharmacology*, 1988; (24): 123-126.

Staffs MW, Brown DM. Basgen Jm, Matas AJM and Mauer SM. Glomerular basement membrane thickness flowing islet transplantation in the diabetic rat. *Lab Invest*. 1979; 41 **(2)**: 116-118.

Werner H, Koshers W, Starch M, Staudenmeir M. Effect of islet transplantation on the glomerular changes in streptozotocin diabetes rats. *Virtuous Arch Pathol Anat*, 1980; 388 **(2):** 137-157.

CHAPTER - 5

HYPOGLYCAEMIC AND HYPOLIPIDEMIC EFFECT OF COMBINATION OF *COCCINIA INDICA* AND *ABROMA AUGUSTA* IN ALLOXAN DIABETIC RATS

5.1. INTRODUCTION

Diabetes is prevalent in all countries of the world and is stated to be one of the important causes of death worldwide (Amos,et al. 1997). *Diabetes mellitus* (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects either in insulin secretion or insulin action or both (David, 1996.) The poorly controlled diabetes aggravates the risk of diabetic complications, particularly cardiovascular diseases (CVD) (Bajaj, J.S.and Madan,R. 1993).

Complications such as coronary artery disease (CAD) stroke, *neuropathy,* renal failure, *retinopathy* are known be associated with DM (Malinow, et al. 1978) Insulin and various types of antidiabetic drugs such as biguanidines and Sulfonylureas including some of the recently developed ones are available for the treatment of diabetes. But none can be termed as an ideal one due to their toxic side effects and some times diminution in response after prolonged use (Dixit , and S. Joshi. 1985, Shukla et al. 2000.).

The disadvantages of the drugs available at present are that they have to be given throughout the life of a diabetic person and produce side effects. A variety of plant preparations have been mentioned in *Ayurveda* and other

indigenous systems of medicine, which are claimed to be useful for the treatment of DM and their complications.

Even WHO have suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas where we lack safe modem drugs (World Health Organization. (1994). Some of the plants known to be useful in diabetes have been recently reviewed by (Shukla, et al, 2000) . In the on going search for more effective and safer drugs attent in is paid now a to plant drugs because they are believed to be free from side effects.

Some of the plants useful for the treatment of diabetes mellitus including those from which some active constituents were isolated have been recently revived. There are many studies on *Abroma augusta* which show that it exhibits antifertility effect.

We recently reporred that *Abroma augusta* possess not only antihyperglycemic but also hypolipidemic effect (Halim Eshrat M. Ali Hussain,et al. 2001) Petroleum ether extract was found to contain antifertility agents (Malhotra , 1991. Adityachaudhury, N. and Gupta, P.K.1969.). *Coccinia indica* (Plate 5 and 6), is also known to be a drug useful for the treatment of DM (Ramacandran K.et al 1983, . Reinhold. , 1953.). In this paper the combined effect of water extract of a mixture *Abroma augusta* and Coccinia indica in rats made diabetic with alloxan and fructose rich diet is reported

5.2. ***MATERIAL AND METHODS***

Plants *Abroma augusta* were collected from Khasia mountains in Assam and the identification was done in for *Botanical Reseach Institute*

Lucknow,India and were confirmed . *Coccinia Indica* was purchased from Delhi market. The roots of *A. augusta and C. indica* leaves were air dried , Powdered in a grinder for the mixture , the two were mixed in equal proportions .Preparation of water extracts 150 grm of mixture of two plants was extracted over night with 180 ml of water with mgnetic stirring . The water extract was separated and the residue was re extracted with water. The combined water extracts in each case was concentrated in lyophilizer .

(a) ANIMALS

Wistar albino rats were obtained from Centre for Cellular and Molecular Biology Hyderabad Adult rats of either sex weighing betwween 150-200 grms were selected for the study. The animals were acclimatized to laboratory conditions and divided into various groups animals were housed in the on a 12-h light and dark cycler.

(b) INDUCTION OF DIABETES

Rats were made diabetic by giving alloxan (20 mg / 100 g body wt) subcutaneously as described by us (Halim M. et al; 2001). Groups of animals: Animals were divided into five groups of five each. Group 1 animals served as healthy controls. While Group 2 was untreated diabetic rats. Rats of groups 3, 4, 5 were diabetic but treated for 8 weeks with extract of *Abroma augusta* alone *Coccinia indicia* alone and *Abroma augusta plus Coccinia indica* respectively. Each rat in groups 3,4 and 5 was given 300mg of the water extract daily once orally for 8 weeks . Blood samples were collected from overnight fasted rats at 0,

and 8 weeks. Blood glucose and serum total cholesterol, HDLcholesterol, triacylcholesterol; glycosylated hemoglobin was determined using kit from Random Mumba. Total proteins and albumin in serum were determined by the method of Reinhold and as described earlier.

5.3. RESULTS AND DISCUSSION

The results obtained with untreated diabetic rats and diabetic rats treated with *A. augusta* alone, *C. indica* alone and *A. augusta plus C. indica* on fasting blood glucose and GTT are in compared with normal healthy control as shown in Table 5.1 .It is seen that treatment with either *A. augusta or C.indica* alone at a dose of 300mg / kg body wt brought down fasting blood glucose from a higher value of 160.5 ±32.1 to normal levels.

There was improvement in glucose tolerance also. In the diabetic untreated rats the blood sugar was 269.0 ±92.2 even after 2 hrs of glucose load in G.T.T. But in the *A. augusta and C. indica* treated rats the 2- hr blood glucose value was in the normal range. The GTT pattern before treatment was abnormal The blood glucose values which were higher in the diabetic animals (169.5 ± 92.1) was brought down to 75.2 ± 1.0 when 300 mg of the extract of the mixture of the two plants would was administered.

This treatment also brought down the FBG and (after 2-hr test), blood glucose levels to normal range (Fig 5.2), as shown in earlier Chapter 4. When the diabetic rats were given the individual plants extract of *A. augusta or C. indica* the blood sugar level were 105.4 ±26.6 and 98.2 ±25.2 respectively. This

means that there was additive effect of the two plants given at lower dose when compared with the higher dose of the individual plants The exact mechanism of action of the plants either alone or in combination can not be explained. .

However it is possible that these plants increase blood insulin levels and also stimulate utilization of glucose by liver and extra hepatic tissues. Table 5.2 shows the fasting plasma levelThe changes in the lipid profile are shown in Table 2. Before treatment the total cholesterol, (TC), LDL cholesterol (LDLC) and TAG were higher than in normal animals. After 8 weeks the TC, LDLC, LDLC/HDLC and TAG values of treatment, were still higher in diabetic untreated animals than in normal animals. There was slight increase in HDLC in untreated diabetic animals. However in the case of diabetic treated animals, with A. *augusta* alone, total Cholesterol, LDL and LDLC/ HDLC values returned to normal value. There was not much of change in TAG values . probably because these plants could not show much effect on triacylglycerols. But further improvement in increase in HDLC value was seen. With *C. indicia* and *A. augusta plus C. indica* treatments similar values were obtained.

This shows again that the water extract of the mixture of two plants which contains less than 300mg of each of the two plants was as effective as 300mg of water extract of each of the two plants. Improvement in lipid profile is suggestive of the action of the two plants on enzymes of lipid metabolism. The untreated diabetic animals showed signs of *retinopathy (Fig.5.and 5.1a*), damage to the muscles and skin of the tail. *(Fig 5.2*), injury to legs

and slight edema in the paws of the legs. The animals looked lethargic and sickly (*as shown earlier chapter 4)* All these symptoms disappeared after treatment for 8 weeks with water extract of the two plants *A. augusta* and *C. indica*.

The extract treatment with water extract was so effective that the above mentiond complications seen in the untreated diabetic rats, disappeared. The treated, diabetic animals showed behavior almost like the normal rats *(Fig 5.3*). (Fig. 5.4) and

(Fig. 5.5) showing in the earlier Chapter 4.

The overall behaviors of the rats were normal. In conclusion it can be stated that the two plants *A. augusta* and *C. indica*, have a synergistic effect when given together. They have a strong anti-hyperglycemic, anti-hyperlipidemic effect. The total serum proteins, serum albumin, hemoglobin and body weight values did not change much even after treatment indicating that the plants did not affect other parameter. The body weight and albumin decreased slightly in diabetic rats. (Table5.4). But in treated rats the body was normal There was no change in total protein in all the groups (Table 5.5).

In order to know whether the hypolipidemic effect seen with the plants only in diabetic animals or whether it was also in hyperlipidemic animals with normal blood glucose, another experiment of short duration was performed. Two groups of normal rats (5 in each) were taken and both were given high fat diet (carbohydrate, 73%; protein 16%; fat, 3%; crude fibers 5%; mineral salt, 2%; vitamins 1 %, for the standard diet and carbohydrate, 56%; protein, 15%;

fat. 20%; crude fiber, 5%; mineral salt, 2%; and vitamins, 1% for the high – fat diet.). One group was kept as control while the other group received 300 mg of water extract of a mixture of the two plants. Total cholesterol and TAG values at the end of the experiment are given in Table 5.6. In the animals given high fat diet however there was increase in total cholesterol and TAG values when compared with normal rats in the animals treated withextract of water *A. augusta* plus *C. indica* the values were nearly normal.

This shows that the plant extract has prevented increase in cholesterol and TAG, confirming that these plants have hypolipidemic effect in normal rats. In the untreated high fat diet fed rats there was an increase in the weight of the liver, (probably due to fatty infiltration). But after treatment there was some reduction in the weight of the liver indicating that the process to bring back the liver weight has begun and probably longer duration of treatment might lead to better results.

The effect of *A. augusta* and *C. indica* on total hemoglobin and urine sugar on experimental rats is given in Table 5.7. It is seen that the effect of the two plants in combination gave excellent improvement in hemoglobin, as compared to individual plant extract treatments. Also the sugars level which showed high levels in urine deceased to normal levels.

The plasma lipid profile in rats fed with high fat diet showed improvement after treatment with mixture of the 2 plants *(A. augusta + C. indica*) as shown in (Table 5.8).

Coccina indica this belongs to the family *Cucurbitaceae.* In Hindi and Punjabi this is termed *Kandari* and in Tamil and Malayalam *Kovakkai.* Juice from leaves and roots of this plant is advised in the treatment of diabetes. But subcutaneous injection of the enzyme and alkaloid extracted from the fresh juce of *Coccina indica* on rabbit's lowereither blood or urine sugar.

It is reported that pressed fresh juice of the plants also showed a similar negative result. Clinical trials with fresh juice on diabetic patients by the (Chopra and Bose, 1925). Two did not elicit any positive response but De and Mukherji (1953) observed antidiabetic properties of water-soluble portion of the roots of this plant on alloxan diabetic rabbits. When the rabbits were treated for 0-8 weeks with the extract, there was a persistent hypoglycemic effect for 3-4 weeks even after discontinuing the treatment De and Mukerji, (1953). Found that both alcohol extract and the water-soluble part of the alcohol extract of sun-dried and defeated root lowered the blood glucose level in normal and alloxan diabetic rabbits.

The alcohol extract showed 50% and 6-8% hypoglycemic potency in normal and alloxan diabetic rabbits as compared to tobutamide. (Mukerji, et al; 1972), observed 35% blood sugar reduction in normal guinea pigs within the first hour of the administration of the water soluble alkaloid principle. Ethanol extract too showed a similar reduction though the petroleum ether extract was comparatively less active. Both the petroleum ether and the ethanol extract were subjected to further solvent extraction and only chloroform extract from the ether extract showed a blood sugar level lowering similar to the water soluble principle.

(Khan, et al; 1980) observed a marked improvement in the glucose, glucose tolerance of 16 patients with maturity onset diabetes on treatment with homogenized and freeze – dried leaves of *Coccina indicia.* No renal injury was noticed.

Table 5.1. Effect of water extract of *A. augusta* and *C. indicia* and mixture of the two plants on fasting plasma glucose in normal and alloxan diabetic rats untreated andtreated at a dose of 300-mg/kg-body wt daily once. (GTT).

Blood glucose (mg/dl), mean ± S.D					
Group	**0 hr.**	**0.5 hr.**	**1 hr.**	**1.5 hr.**	**2 hr.**
Normal	94.3 ± 21.0	140.2 ± 11.2	132.6 ± 27.3	116.2 10.0	102.0 ± 12.0
Diabetic untreated	160.5 ± 32.1[a]	245.0 ± 68.6	273.4 ±89.3[a]	290.6 ±82.6a	169.0 ± 92.2[a]
Diabetic + *A. augusta*	87.9 ± 25.6	112.2 ± 20.6	114.3 ± 15.0	102.0 ± 10.3	95.6 ±24.0
Diabetic + *C. indicia.*	82.0 ± 4.2	86.0 ± 4.3[a]	81.0 ± 3.9[a]	80.0 ± 3.2[a]	84.0 ±3.2[b]
Diabetic + *A. augusta* + *C. indica*	81.0 ± 3.5	84.0 ± 4.2[a]	86.0 ± 3.3[a]	81.0 ±3.6[a]	75.2 ± 1.0[a]

a = p < 0.001
b = p < 0.01
c = p < 0.02

Table 5.2: Effect of treatment for 8 weeks with water extract of *A. augusta* and *C. indica* on fasting plasma glucose level in rats.

Plasma glucose (mg/dl) mean ± SD		
Group	**0 weeks**	**8 weeks**
Normal	99.4 ± 19.5	89.8 ± 5.21
Diabetic	172.2 ±5.4[a]	285.6 ±42.6[a]
Diabetic + *A. augusta*	164.6 ± 25.0[a]	105.4 ± 26.6
Diabetic + *Coccinia .Indica*	158.6 ±23.2[a]	98.2 ± 25.2

Diabetic + *A.augusta* + *C. indicia*	166.9 ± 95. 4[a]	85.4 ±2.3

a = p < 0.001

Table 5.3. Effect of (300 mg / kg /body) weight treatment for 8 weeks with water extract *A. augusta and C. indica* and both *A. augusta + C. indica* on plasma lipid rofile in diabetic rats

Group	TC (mg/dl)	LDLC(mg/dl)	LC(mg/dl)	HDLC	TAG (mg/dl)
1. Normal	170.3 ±10.3	78.0 ±12.2	44.9 ± 13.3	1.7 ± 0.3	115 6 ± 42.6
2. Diabetic untreated	250.0 ± 14.9a	152.2 ± 12.6[a]	45.0 ± 12.0	3.3 ± 0.4[a]	181 .8 ± 18.8
3. Diabetic +*A. augusta*	175.0 ± 12.3	99.7±16.8	51.3 ±8.4	1.9 ±0.4	174.0 ± 15.8
4 .Diabetic + *C. indica*	187.0 ±14.9	95.6 ±15.7	50.2 ±8.5	1.6 ±0.3	135.0 ±13.2
5.Diabetic + *A. augusta*+ *C. indicia*	174.0 ±10.3	51.3± 6.3[c]	94.3 ± 13.3[a]	1.8 ±0.5	131.0±11.2

TC, LDLC, HDLC = total. Low density and high density lipoprotein Cholesterol
Number of animal is 5 in each group

a = p < 0.001

Table 5.4. **Effect of water extract of *A. augusta* and *C. indica* on various treatments on the body weight of rats.**

Group	Initial (Body weight (g))	After 8 weeks of Treatment Bodyweight (g))
1. Normal	180±12	210±6
2. Diabeticntreated	170±8	180±2
3. Diabetic+*A. augusta*	168±5	214±7
4 .Diabetic +*C. indica*	163±7	220±8
5. Diabetic +. *augusta*+ *C. indicia*	145± 7.6[a]	200± 2.9

a = p < 0.05

Table 5.5. Effect of water extract of *A. augusta* and *C. indica* on , total protein and albumin.kept on high – fat diet.

Albumin (g / l)	55.0± 1.4	45.0± 3.1[a]	52.9 ± 3.0
Total Protein (g/ 1)	69.0± 3.0	73.2±2.9	78.02± 3.8[d]

a = p < 0.001
d = p < 0.05

Table. 5.6: **Effect of water extracts *A. augusta* and *C. indica* treatment on total *Hemoglobin*, and *Urine sugar* of normal and experimental rats.**

Group	hemoglobin	Urine sugar
Normal	16.0 ± 4.2	-
Diabetic Control	10.5 ± 1.2[a]	+++
Diabetic + *A.augusta*	13.6 ± 0.2[b]	_
Diabetic + *C.indica*	12.9 ± 1.3[b]	_
Diabetic + *A.augusta* + *C.indica treated*	14.0 ± 0.3[a]	_

a = p < 0.01
b = p < 0.01
c = p < 0.05

Table. 5.7: Effect of water extract0f the two plants (combined effect) on plasma lipid profile in rats fed high fat diet.

TISSUE	Normal rats	High-fat fed rats	High -fat diet Plus extracts
Liver	7.82± 0.33	10.4 ± 0.41	9.8 ± o.18[b]
Kidney	0.99 ± 0.05	1.08 ± 0.08	0.99 ± 0.06[c]

[b] ***p<0.0005***
[C] ***p<0.01***

5.4. CONCLUSION

The hypoglycemic effect of *Abroma augusta* (Family*: Sterculiaceae*) and *Coccinia Indica (Fame: Cucurbitaceae*) is known individually. In *Ayurvedic* system of medicine in India*, combination of plant extracts* are used for the treatment of diabetes mellitus, The present study reports the combined effect of *Abroma augusta* and *Coccinia indica* on the blood sugar, glucose tolerance and lipid profile of diabetic in experimental animals. Diabetes was induced in albino rats with alloxan. One group of diabetic rats was kept as untreated group and another group of diabetic rats was treated with 300mg per day of water extract of *Abroma augusta* or *Coccinia indica* or mixture of *Abroma augusta* and *Coccinia indica* in equal proportions for 8 weeks.

Fasting blood sugar, glucose tolerance and serum lipid profile were compared in healthy, diabetic untreated and diabetic treated rats at after 0, and 8 weeks of treatment. After 8 weeks of treatment of alloxan diabetic rats as indicated above, the fasting blood sugar came down, to almost normal value, was observed and improvement in glucose tolerance and serum lipid profile. The effect of the mixture of *Abrama augusta* and *Coccinia indica* was more useful than with either of the twodrugs individually .

BIBLIOGRAPHY

ADA (1997) Clinical practice recommendations. *Diabetes cares*, 20 **(1).** 22-24.

Adityachaudhury, N. and Gupta, P.K. Chemical examination of *Abroma Augusta. J. India Chem. Soc*. 1969; 63 **(3):** 378-381.

Amos AF, McCarty DJ, Zimmet P. the Rising Global Burden of Diabetes and its Complications: Estimates and Projections to the year 2010. *Diabetic Medicine.* 1997; **(14):** (Supple 5) 1-85.

Thereon, DJ, 1994. Towards the safer used of traditional remedies. Br. Med. J. 308,673-674.

Balaj, J. S, Madan, R. Diabetes in tropics and developing countries. *IDF Bull*. 1993; 38 **(2):** 5-6.

Bhattacharji satyendra, Chittarajan mita Salimuzzaman Siddiqi. Chemical examination of the Trunk Bark neem (*Azadirachta indicia* sin. *Melee Azadirachta*); *J. Sci. Industries* 1953. **(12)**B: 154-158.

Chopra, R, N. and Bose, J. P. *Cephalendra indicia* (telakucha) in diabetes; *Ind. J. Med. Res.,* 1925; **(13):** 11-15.

Mukerji, De. Effects of *Coccina Indica* on alloxan diabetes in rabbits; *Ind. J. Med. Sci.,* 1953; **(7):** 665. -668.

Das Gupta, B. and Basu, K., Chemical investigation of *Abroma augusta* Linn. Identity of *Abromine* with betaine. *Experientia*, 1970**, (26):** 477-478.

David MN. The Pathophysiology of Diabetic Complications: How Much Does the Glucose Hypothesis Explain? *Ann Intern Med*, 1996; **174 (1pt2):** 86-89.

Dhawan, BN, Patnaik, Gk., Pharmacological studies for therapeutic potential. In neemresearch and development. Randhawa N, S. and Panni B, S. **(Eds.)** *Published by Soc. Pesticide. Sci., India;* 1993. 242-249.

Dixit, VP and S. Josh. Antiatherosclerotic effects of alfalfa meal ingestion in chicks: A, Biochemical evaluation. *Ind. J. Physical. Pharmacy*, 1985; **(29):** 47-50.

Dixit VP, Sinha R, Jank R. Effect of neem seed oil on the blood glucose concentration of Normal and alloxan induced diabetic rats. *J. Ethnopharmacol.*1986**; (17)**: 95-98.

Friedwald, Estimation of concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Cline. Chem*, 1972; **(18):** 499-500.

Gupta, SS, Prospects and perspectives of natural plant products in medicine. *Indian Journal of pharmacology,* 1994. (26): 5-9

Halim Eshrat M. Hussain, Kaiser Jamil and Mala Rao. *Indian Journal clinical Biochemistry*, 2001. 16 **(1):** 77-80.

Khan, A. K. A. Akhta, S. and Mahtab, H; Treatment of diabetes mellitus with Coccinia *indica*; *Br. Med. I.* 1980; **(280):** 1044

Malhotra BN. Compendium of Indian Medicinal Plants, vol2, Central Drug Research Institute, Lucknow and Publication and information *Directorate,* (**CSIR**), New Delhi, 1991: Pp. 137- 138.

Malinow, M.R., Mclaughlin, H.K.Natio, L.A., Lewis and W.P. McNulty. Effect of alfalfa meals on shrinkage (regression of atherosclerotic plaques during cholesterol: feeding

In monkeys. *Atherosclerosis*, 1978; **(30):** 27-43.

McGowan MW, Artiss JD, StrandbergDR, zakBA. Peroxides couple method for the colcrimetric determination of serum triglycerides. *Cline chem.*, 1984; **(29):** 538- 542.

Mukherjee, Ks. and Badruddoja sah. Chemical investigation of bark of *Abroma augusta* Linn. *J.Indian chem. Soc.*, 1977; **(54):** 647.

Mukherjee, K, S. and Badruddoj Sah. Chemical examination of heart wood of *Abroma augusta* Linn. *Curr. Sci*, 1978. **(47)**: 301-303.

Mukherji, K, Ghosh, N. C. and Datta, T. *Coccinia Indica* Linn. As potential hypoglycemic agent; *Ind. I. Exp. Biol,* 1972; **(10):** 347-379.

Murty KS, Rao DK, Murty LBG. A preliminary study on hypoglycemic and anti hyperglycemic effect of *A. indica*. *Indian J Pharmacy,* 1978; (10) : 247-250.

Rao A.R.Savithri Skimmer, T.V., Paramasivam, S.Kamalakshi,AR Parashuraman, and M. Shanghai. *Ind. Jour. Med. Res. March* 1969, **(57):** 3-6

Ramacandran K.and Subramanian, B. Scarlet gourd, Coccinia grandis, little-known tropical *Drug plant. Econ. , Bot,* 1983, **(37):** 380.

Reinhold. J.G., In: Reiner, M. (Ed.) *Standard methods in clinical chemistry*, Vol. 1**. Academic Press, 1953; New York, pp. 88-9.**

Santoshkumari KS, Devi KS. Hypoglycemic effects of medicinal plants*. Ancient Sci. Life* **(9):** 221-3.

Shukla, R., Sharma, S.B., Puri, D., Prabhu, K.M.andMurthy, P.S.Medicinalplants for treatment of diabetes mellitus. *Ind. J. Cline. Biochem*. 15 (August**, Supple**.), (2000), 169- 177.

Sochor, M., Ball, M.R. Baquer, and N.Z: Effect of thyroid hormones on the level of metabolic intermediates in diabetic rat liver. *Biochem. Int. Jan*, 1988; 16

Twaij, H.A.A., Al-Badr. A, A., Hypoglycemic activity of *Artemisia herb alba Journal of Ethnopharmacolog,* 1988, **(4):** 123-126.

World Health Organization. Prevention of diabetes mellitus. Technical Report Series. 1994; ***no.* 844**. **WHO**. *Geneva.*

CHAPTER - 6

HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECT OF COMBINATION OF *CURCUMA LONGA* CONSTITUENTS AND *ABROMA AUGUSTA* ON BLOOD GLUCOSE PROFILE AND ANTI-OXIDANT PROPERTIES OF STZ - INDUCED DIABETIC RATS

6.1. INTRODUCTION

Given a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes, systematic and intensive search in plants for new drugs to treat Type 2 diabetes mellitus seem to be of a great utility. This approach seems likely to increase the chances for discovering new drugs for the management of Type 2 diabetes mellitus. Out of the two types of diabetes, the incidence of non-insulin dependent diabetes mellitus (NIDDM) is much higher than the insulin dependent diabetes mellitus (IDDM).

Sulphonylureas and few biaguanides are valuable treatment for hyperglycemia in NIDDM, but they are unable to lower glucose concentration to within normal range and reinstate a normal pattern of glucose homeostasis permanently. Use of these therapies is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects (Melinda A. et al. 1988). Even insulin therapy does not reinstate a permanent normal pattern of glucose homeostasis, and carries an increased risk of atherogenesis and

hypoglycemia. World Health Organization has recommended that medicinal plant research warrant attention (W H O, 1980).

Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Earliest description of curative properties of medicinal plants is found in Rigveda (2500-1800 BC). Charaka Samhita and Sushruta Samhita give extensive description on various medicinal herbs. Information on medicinal plants in India has been systematically organized (Satyavati, GV, et al. 1987). The World Health Organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment of diabetes should be further investigated.

Medicinal plants have the advantage of having no or only few side effects. Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world. Till today metformin is the only ethical drug approved for the treatment of NIDDM patients, which is derived from a medicinal plant *Galega officials* historically used for treatment of diabetes in medieval Europe.

There are many anti-diabetic plants, which might provide useful sources for the development of drugs, which can be used in the treatment of diabetes mellitus. The literature on medicinal plants with hypoglycemic activity is vast. Since it is difficult to include all such plants in this small review, a few commonly used plants have been covered here**.**

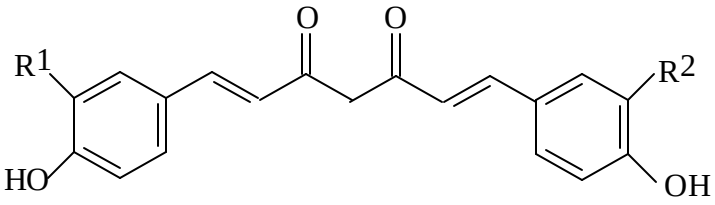
Toxicity studies have not been conducted for most of these plants. As many of these plants were used for many centuries and some time as regular

constituents of the diet, it is assumed that they do not have many side effects. However chronic consumption of large amounts of traditional remedies must always be taken with caution.

Since older times *Curcuma longa* (Turmeric) (Fam *Zingeberaceae),* commonly known as ***Haldi*** in Hindi has been used as spice, coloring agent and in traditional Indian system of medicine, Ayurveda. It is used in Indian medicines in several ways namely (i). It is an ingredient in the preparation of medicinal oils, ointment and poultice, (ii) it is used in diabetes and leprosy, (iii) it is used as stomachache, carminative, tonic, laxative, anthelmintic blood purifier, vermicide, as, antiseptic and cure for liver ailments. (iv). The raw juice is used to tear in gallstones, gall bladder complaints, and dental-troubles and for sore throat and common cold.parasitic skin diseases and pile cure. Curcumin, its major constituent has also been found to show anti-rheumatic action. The anti-rheumatic activity of 1200 mg of curcumin has been found to be comparable to that of 300 mg of phenylbutazone. The anti-inflammatory activity of curcumin analogues such as feruloyl, 4-hydroxy cinnamoyl methane and bis 9, 4-hydroxycinnamoyl0 methane was found to be comparable with sodium curcuminate and phenyl butane.

Demethylated derivatives of curcumin and ferric acid viz. are -3,4 dihydroxy cinnamoyl methane and caffeic acid is known potent inhibitors of lipid peroxidation (Fig No.16). Structures of curcumin and related compounds:

Figure No.16



R_1=R_2 = OCH : Curcumin

R_1= H_1R_2= : OCH: 4 –Hydroxycinnamoly1) (feruly1) methane

R_1= R_2=H : Bis) (4 – hydroxycinnamoy1) methane

This figure illustrates the structures of the Three Curcuminoids isolated from Curcuma Longa rhizomes. The side chains R_1 and R_2 of curcumin are two methoxy groups. 4- hydroxycinnamoly1 (feruloyl) methane has only one methoxy group at R_2. Bis (4- hydroxycinnamonyl) methane has a proton on both side chains R_1 and R_2.

1.Curcumin (trains, trains): 2. Bis 3, 4-Dihydroxycinnamoylmethane (trains, trains); 3. Ferulic acid (trains); 4. Caffeic acid (trains); 5. P-Hydroxycinnamic acid (trains); 6. O-Hydroxycinnamic acid (trains); 7. Cinnamic acid (trains); 8. 3.4.5-Trimethoxycinnamic acid 1: (tran). Show in (Fig 17). While conducting studies on the inhibition of ß- hydroxydeoxygeranosine formation by curcumin in mouse fibroblast cells, it was revealed that the compound was able to inhibit RA-induced tumor promotion by functioning as an hydroxyl radical scavenger to

prevent 8-OH & 6 formation within the DNA molecule (Shih, et al; 1993). The C. *longa* rhizomes has been reported to posses anti-diabetic properties as its alcohol extract possesses active constituents showing blood glucose lowering activity in alloxan induced diabetic rats. (Tank R. et al 1989).

Oxidative stress has been associated particularly with the development of complications in diabetes. (Jain. SK. et al 1989) Curcumin, (5-50 micro M) inhibited lipid peroxidation in a dose dependent manner. This inhibition was however reversed by adding high concentration of Fe 2 +. One of the major consequences of increased oxidative stress in lipid peroxidation, the oxidative degradation of lipids with more than two double bonds (C= C).

Endothelial injury in the vascular wall has been shown to be the initial event in the atherosclerosis and related problems of coronary heart diseases. Lipid droplet deposits in the aortic wall undergo peroxidative changes in presence of reactive species of oxygen, which eventually produce endothelial injury. Thus compounds that can scavenge the reactive species of oxygen and inhibit preoccupation of lipid could be useful as preventive agents against atherosclerosis.

Curcumins have also been reported to exhibit inhibitory effects on the lipid peroxidation induced by air on linoleic acid. They also inhibited the hemolysis of erythrocytes induced by hydrogen peroxide at low concentrations. It was inferred that the effects of the curcuminoids on hemolysis and lipid peroxidation of erythrocytes is presumably different from those of dl-1-tocopherols (Soudamini K. 1989.). Anti-oxidative components in the methanol extract of the rhizomes of

C. longa were identified as curcumins as their 50% inhibitory concentrations for the air oxidation of linoleic acid were significant, and were comparable to those of tochopherol. An extract of the crude Japanese drug ″Ukon ″containing rhizomes of *C. longa* exhibited intense preventive activity against carbon tetrachloride - induced liver injury. Turmeric based crude drugs were also found to exhibit anti-hepatotoxic activity (Hussein H.E. et al 2001).

. Although *C. longa* has been investigated for its various medicinal properties, but detailed studies on its anti-diabetic, antioxidant potential, lipid peroxidation level in diabetic rats are still lacking. It is therefore proposed to undertake study on the effect of *C. longa* and *A. Augusta* constituents on hyperglycemic, oxidative stress, lipid peroxidation level in diabetic rats.

6.1.1. TOXICITY STUDIES

Toxicity studies have not been conducted for most of these plants. As many of these plants were used for many centuries and some time as regular constituents of the diet. It is assumed that they do not have many side effects. Chronic consumption of large amounts of traditional remedies must always be taken with caution.

Since older times *Curcuma longa* (Turmeric) family *Zingeberaceae,* commonly known as *HLADI* has been used as spice, coloring agent and in traditional Indian system of medicine, Ayurveda. It is used in Indian medicines in several ways namely (i) it is an ingredient in the preparation of medicinal oils, ointment . (ii) It is used in diabetes and leprosy, iii).is used as stomachach, carminative, tonic, laxative, anthelmintic blood purifier, and vermicide, as

antiseptic and cure for liver ailments. (iv) The raw juice is used for stones, gall bladder complaints, dental-troubles and for sore throat and common cold. skin diseases and pile cure.

Curcumin, its major constituent has also been found to show anti-rheumatic action. The anti-rheumatic activity of 1200 mg of curcumin has been found to be comparable to that of 300 mg of phenylbutazone. The anti-inflammatory activity of curcumin analogues such as feruloyl, 4-hydroxy cinnamoyl methane and bis 9, 4-hydroxycinnamoy l0 methane was found to be comparable with sodium curcuminate and phenyl butazone (Bonin and Bker 1980). Have reported mutagenicity of turmeric oleoresin and pure curcumin in the presence d or in the absence of rat liver microsomal activation system in the Ames assay with S. typhinusium tester strains TA- 100 and TA-98. Continuous intake of turmeric does not create any toxicity in human body.

Demethylated derivatives of curcumin and ferric acid viz. is -3,4 dihydroxy cinnamoyl methane and caffeic acids are also the known potent inhibitors of lipid peroxidation . (Fig 16) Structures of curcumin and related compounds:*1.Curcumin* (trans, trans): 2. Bis 3, 4-Dihydroxycinnamoylmethane (trans, Trans); 3. Ferulic acid (Trans); 4. Caffeic acid (Trans); 5. P-Hydroxycinnamic acid (Trans); 6. O-Hydroxycinnamic acid (Trans); 7. Cinnamic acid (Trans); 8. 3.4.5-Trimethoxycinnamic acid 1: (Trans) are shown in (Fig, 17). While conducting studies on the inhibition of ß- hydroxydepxygeranosine formation by curcumin in mouse fibroblast cells, it was revealed that the compound was able to inhibit RA- induced tumor promotion by functioning as an

hydroxy radical scavenger to prevent formation within the DNA molecule (Shah, and Lin et al, 1993). The *C. longa* rhizomes have been reported to posses anti-diabetic properties as its alcohol extract possesses active constituents showing blood glucose lowering activity in alloxan induced diabetic rats. (Tank et al., 1989).

Oxidative stress has been associated particularly with the development of complications in diabetes as recently reviewed to (Brown et al 1993), Polyunsaturated fatty acids (PUFA) from peroxidation is essential to utilize their beneficial effects in health and in preventing diseases. Curcumin, (5- 50 micro M) inhibited lipid peroxidation in a dose dependent manner. This inbition was however reversed by adding high concentration of Fe 2 +. One of the major consequences of increased oxidative stress in lipid peroxidation, the oxidative degradation of lipids with more than two double bonds

(C= C).

Lipid peroxidation is broadly defined as the oxidative deterioration of polyunsaturated lipids and usually associated with the initial attack on such lipids of free radicals has been associated with the development of angiopathy also in diabetes . Lipid peroxidation is increased at a very early stage in experimental diabetes.

This increase was found both in kidney tissue, a primary site for development of complicated diabetic disease and liver. Oral administration of curcumin in mice peroxidation significantly lowered the increased peroxidiation of lipids in these tissues.

It also lowered the serum cholesterol level indicating that the use of curcumin helps in conditions associated with peroxide induced injury such as liver damage and arterial disease, (Jain et al, 1989). Dietary spice components have been screened for their protective effect against reactive oxygen species (ROS) induced lipid peroxide-mediated membrane. DNA damage and mutaganicity.

It has been reported that turmeric and its lipophilic principal curcumin are good antioxidants (Halli well et al, 1985). The injury in the vascular wall has been shown to be the initial event in the atheroclerosis and related problems of coronary heart diseases. Lipid droplet deposits in the aortic wall undergo peroxidative changes in presence of reactive species of oxygen, which eventually produce endothelial injury. Thus compounds that can scavenge the reactive species of oxygen and inhibit peroxidation of lipid could be useful as preventive agents against atherosclerosis.

Studies conducted on the effect of oral administration of curcumin on lipid peroxidation in various organs of mice like liver, lung, kidney and brain, revealed that curcumin significantly lowered the increased peroxidation of lipids in these tissues. It also lowered the serum and tissue cholesterol level in these animals, indicating that the use of curcumin helps in condtions associated with peroxide induced injury such as liver damage and arterial disease (Soudamini et al 1992).

Curcumins have also been reported to exhibit inhibitory effects on the lipid peroxidation induced by air on linoleic acid. They also inhibited the hemolysis of erythrocytes induced by hydrogen peroxide at low concentrations. It was inferred

that the effects of the curcuminoids on hemolysis and lipid peroxidation of erythrocytes are presumably different from those of dl-1-tocopherols (Sondamini et al 1989) .

Anti-oxidative components in the methnolextract of the rhizomes *of C. longa* were identified as cuecumins as their 50% inhibitory concentrations for the air oxidation of linoleic acid were significant. And were comparable to those of tochopherol. An extract of the ceude Japanese drug ″Ukon ″containing rhizomes of C. longa exhibited intense preventive activity against carbon tetrachloride - induced liver injury. Turmeric based crude drugs were also found to exhibit anti-hepatotoxic activity.

The presence of a new, water soluble peptide turmeric from turmeric as an efficient antioxidant, noncytotoxic antimutagen and Protestant of DNA thus providing evidence for the presence of chemopreventive agents in diet, indicate that dietary modulation of provident state-mediated diseases is a real possibility.

Although *C. longa* has been investigated for its various medicinal properties, but detailed studies on its anti-diabetic, antioxidant potential, lipid peroxidation level in diabetic rats are still lacking. It is therefore proposed to undertake study on the effect of *C. longa* constituents on anti-glycemic activity, antioxidant property, and lipid peroxidation level in diabetic rats.

6.2. MATERIALS AND METHODS

Fresh *Curcuma longa* rhizomes were procured from the market. The dried rhizomes were crushed into powder and soxhlet extracted with different solvent petroleum ether, benzene, ethylacetate etc. to yield different fractions: The rhizomes were also extracted with water.

(a) TESTING OF CURCUMA LONGA

The *C. longa* compounds were tested for their anti-oxidant potential and anti-diabetic activity by studying blood glucose, lipid peroxidation level in diabetic rats.

i) Induction of diabetes in rats

ii) Lipid peroxidation in red blood cells of rat was estimated by thiobarbituric acid reactivity (Jain,SK.et al.1989). Glucose, glycosylated haemolymph and serum lipid profile was estimated by using kits from standard companies.

iii) Lipid peroxidation in plasma (TBA method).

(b) METHODOLOGY FOR BIOASSAY.

The pure constituents of *C. longa* needed for the study were isolated, purified and Characterized as per the procedure described in Materials and Methods.

METHODS

Plant *Abroma Augusta* was purchased from Khasia Mountains in Assume and Turmeric (*Curcuma longa*) was purchased from Delhi market. The whole plant of *A. augusta* and *rhizome* of *C.longa* were air dried, Powdered in a grinder, mixture of the two were mixed in equal proportions before use.

(c) INDUCTION OF DIABETES IN RATS

The experiment animals were killed and tissues were collected and frozen in deep freezer (4°C). Healthy male albino wistar rats (150-200gm) procured from Indian Institute of Chemical Technology, Hyderabad were used in this study. The animals were fed pellet diet (Hindustan Lever, India) and water *ad libitum.* Rats were made diabetic by intraperitoneal injection of streptozotocin (STZ) 60 mg/kg in citrate buffer, pH 6.3. Five rats injected with same volume of citrate buffer acted as non-diabetic healthy controls. The rats were kept in separate metabolic cages. The diabetic rats were further divided into two groups of untreated and treated (five each). One group of untreated diabetic rats was orally administered saline daily (0.1ml/100mg b.w and the other (treated group) was orally administered daily water extract of combination of two plants of 300.mg / Kg body weight per day in the morning for 8 weeks by bulged steel tube. The body weight was recorded weekly.

(d) ESTIMATIONS.

Bood (5 ml) was collected from the vein at the beginning and the end of the experiment. Erythrocytes and plasma were separated. Plasma glucose, total cholesterol, LDL, VLDL- and HDL- cholesterol and triglycerides were estimated as described earlier by us (Hussein HE. et al 2001). Lipid peroxidation products were estimated as thiobarbituric acid active substance (TBARS) in plasma and tissues (Poliodoro,GD.et al 1984). Among the antioxidants, reduced glutathione was determined by the method of (Hiroshi,O.1979). Assay of antioxidant enzymes and protein were conducted as described earlier by us (Wendel, A. 1981).

Statistical analysis

All the data were statistically evaluated and the significance was calculated using student's-test. All the results were expressed as mean ±S.D.

6.3. RESULTS AND DISCUSSION

Many plants have been used for the treatment of diabetes mellitus in Indian system of medicine and in other ancient systems of the world, out of these only a few have been evaluated as per modern system of medicine. From many such plants only extracts have been prepared and their usefulness evaluated in experimental diabetes in animals. Most of them seem to act directly on pancreas (pancreatic effect) and stimulate insulin level in blood. Some have extra

pancreatic effect by acting directly on tissues like liver, muscle etc. and alter favorably the activities of the regulatory enzymes of glycolysis, gluconeogenesis and other pathways. Many of its products / chemical constituents are known to posses wide array of medicinal properties.

This study demonstrated was the Hypoglycemic and hypolipidemic effect of combination *of Curcuma longa* constituents and *Abroma augusta* (Linn.) on blood glucose profile and antioxidant properties of *STZ – induced diabetic rats.*

The present study has shown that Fasting plasma glucose (FPG) values, before and after treatment for 8 weeks is normal, diabetic untreated and diabetic treated with water extract of one plant only (A. *augusta or C. longa*) or both the plants *A. augusta and C. longa* are shown in (Table 6.1). The fasting plasma glucose (FPG) values remained more or less the same in normal i.e. 93.4 ± 24.3 mg/dl before and 89 .4 ± 6.2 2 mg/dl after 8 weeks. In diabetic rats even the initial (0 week) FPG values were higher 176 ± 50.2 mg/dl) which increased to 290.5± 10.7 mg/dl by 8 weeks. However after treatment with 300.mg of water extract of *A. augusta and C. longa the* higher initial FPG values (162.4 ± 22.6mg/dl came back to the normal level (89 .4 ± 6.2 2). There was improvement mixture of two plants. Same in the glucose tolerance test after 8 weeks.

Results (Table 6.2) shows the mean FPG values normal, diabetic untreated and diabetic treated group glucose tolerance test after 8 weeks. The fasting blood glucose values for normal, diabetic untreated and diabetic treated were both the extract 82.3 ± 20.0, 295.5 ± 11.1 and 72.2 ± 1.0 mg/dl. In the glucose tolerance test in the case of normal animals the peak values were

obtained in ½ hr and were 290.5 ±11.1 mg/dl and returned back to initial values in 2 hours.

We identified that this effect of combination of *Curcuma longa* and *Abroma augusta* not only showed anti-hyperglycemic effect but also for the hypolipidemic effect. . Results in (Table 6.2) showed the effect of water extract of *A. augusta* on the fasting plasma glucose (FPG) of normal and untreated diabetic rats. 92.3 ± 20.0, 295.5 ± 11.1 and 72.2 ± 1.0 mg/dl. However in *Abroma augusta* plus *Curcuma longa. The zero values were normal (* 72.2 ± 1.0 mg/dl) In the case of diabetic untreated animal the blood values remained a high level (above 270 mg/dl). This shows that treatment with *Abrma augusta* + *Curcuma longa* for 8 weeks improved glucose tolerance. .

Body weight. There was a decrease in weight in the diabetic rats, after treatment showed gain in weight as in control animals. Treatment of diabetic rats with extract of these two plants showed considerable improvement in glucose tolerance also (Table 6.1 and 6.2) and these results point out that the diabetic rats showed abnormal glucose pattern. After treatment for 8 weeks with *A.augusta* and *C. Longa* on fasting plasma glucose level in rats. Effect of 300mg/kg /body) treatment for 8 weeks with water extract A. augusta and *Curcumi on* Urea, Creatinine, Cholesterol and protein in (STZ diabetic rats (Table 6.3).Kidney mass Plasma Creatinine, glucose, Na^+K^+Concentration Urinary Na^+ K^+ Creatinine Excretion (Table 4). Turmeric *(Curcuma longa*), as well as it is active constituent Curcumin, inhibits lipid peroxidation. *Curcumin* and *Abroma*

augusta also decreases serum cholesterol levels in hyperlipidaemic rats. (Table 3). The results presented in (Tables 6.3) conform the lipid peroxide scavenging activity of *Curcumin* administration also reduced using erythrocyte ghost preparation .Scavenging activity of *Curcumin* administration also reduces lipid peroxidation (Salimath BP, et al 1985). Curcumin has been shown to inhibit cyclooxygenase activity (Huang, MT et al. 1988). From (Table 6.4): it is seen that the mixture of the extracts of the two plants *A.augusta* + *turmeric* has shown promising results of the treatment by lowering the food intake and reduction in body weight, and kidney mass. Analysis of blood plasmas, blood creatine and urinary creatin and urinary sodium levels appeared to have positive effect diabetic rats (Table 6.7). Positive effect of the extracts was also noticed in plasma cholesterol, phospholipids and erythrocyte cholesterol and phospholipid levels as seen in (Table 6.6). Effect of water extract of the combination of *A. augusta* and *C. Longa* for 8 weeks on Lipidperoxidation, SOD and CAT activities in erythrocytes in STZ diabetic rats (Table 6.5).The results presented in (Table 6.5). That glucose induces LOP in liver it comparable with diabetes in which hyperglycemia induces peroxidation of membrane lipids and causes cellular injury. The increase in SOD in liver at lower concentration of glucose could be the protective response by the liver cells to counteract the peroxidative stress in the tissue. Exposure of liver to elevated glucose levels result in the decreased activities of SOD, CAT, GST and GSH, which contributed to the increased lipid peroxides in the liver. It has been demonstrated that polyunsaturated fatty acids of mammalian tissues and body fluids undergo lipid peroxidation.

The positive effect of the two extracts was also seen in other biochemical parameter analyzed (TC, LDL- HDL and TAG) as presented in (Table 6.6). Normally diabetic rats tend to loose weight but after treatment with the two extracts, the body weight did not decline significantly (Table 6.7 and 6.8) it is therefore clear that *Curcumin* + *A. augusta* are a good combination for reducing not only the overall physiological and biochemical effects due to diabetes but also physical effects like body weight food and water intake. The combination of *A. augusta* and *C. Longa* on general parameter of STZ diabetic rats (Table 6.7).

Effect of water extract *A. augusta* and *C. lnga* on total hemoglobin, change in and Urine sugar of normal and experiment rats (Table 6.8, 6.9, & 6.10). These results point out after treatment with combination of mixture of two plants has improved. Treatment of rats with STZ / Alloxan is an established model for type I or Insulin - dependent diabetes. Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease, (Betteridge J. 1997). The liver and some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoproteins.

Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complication. (Brown GB et al. 1993). Many herbs and plant products have been shown to have antihyperglycemic and antihyperlipidemic property (Karunanayake, EH et al. 1993). In the preliminary chemical examination,

besides determining the % extractives and the ash content, the roots of *Abroma augusta* (Linn). have been shown to contain some alkaloid bases, reducing sugars and some phytosterols but glycosides have been found absent. An alkaloid, abromine m.p. 283°-285°with decomposition, its hydrochloride m.p. 230°and a phytosterol ($C_{30} H_{52} O_2$), m.p. 153-157°has been isolated. We plan to conduct further studies to better understand the mechanisms of action of this medicinal plant. There is every possibility of developing a few useful drugs from medicinal plants with a long history of human use.

***Structure of the Curcuminoids*.**

Curcmin, 4 – hydroxycinnamoyl (feruloyl methane and bis (4 – hydroxycinnamoyl) methane , as isolated from rhizomes of *Curcuma longa* (L), have been shown to have inhibitory effects on the lipid peroxidation. In the present study, the action of the natural curcuminoids was investigated on the lipid peroxidation of erythrocytes.

This fig shows the three curcuminoids isolated from *Curcuma longa* rhizomes.

The side chains R1 and R2 of curcumin are two methoxy groups. 4 – Hydroxycinnamyl (Fig 16).

(Feruloyl) methane has only one methoxy group at R2. Bis (4- hydroxycinnamoyl) methane has a proton on both side chains R1 and R2. (Fig 17).

Table. 6.1. Effect of water extract *of A.augusta* and *C. longa* on the Fasting plasma glucose level in rats.

Group	Plasma glucose mg/dl. (Mean±SD)	
	0	8 weeks
Normal	93.4 ± 24.3	83.0 ± 24.2
Diabetic untreated	176 ± 50.2[a]	290.5± 10.7[a]
Diabetic + *Abroma* treated	162.3±24.5[a]	104.5± 26.4
Diabetic + *Curcuma longa* treated	1705±6.40[a]	110.0±7.0[a]
Diabetic +*Abroma and Turmeric treated*	162.4 ± 22.6[a]	89 .4 ± 6.2 2

a = p < 0.001
c = p < 0.02

Table. 6.2. Effect of water extract of *A. augusta* and *Turmeric treated* and mixture of the two plants on fasting plasma glucose in normal and STZ-diabetic rats untreated and treated at a dose of 300-mg/kg-body wt daily once. (GTT).

Blood glucose (mg/dl), mean ± S.D					
Group	**0 hr.**	**0.5 hr.**	**1 hr.**	**1.5 hr.**	**2 hr.**
Normal	82.3 ± 20.0	140.2 ± 11.2	132.6 ± 27.3	116.2 10.0	102.0 ± 12.0
Diabetic untreated	295.5 ± 11.1[a]	245.0 ± 8.6	273.4 ±89.3[a]	290.6 ±82.6[a]	250.5.0 ± 9.2[a]
Diabetic + *A. augusta*	80.9 ± 25.6	112.2 ± 20.6	114.3 ± 15.0	100.0 ± 10.3	92.6 ±24.0
Diabetic + *Turmeric treated*	85.0 ± 4.2	86.0 ± 4.3	81.0 ± 3.9	82.0 ± 3.2[a]	83.0 ±3.2[a]
Diabetic + *A. augusta* + *Turmeric treated*	79.0 ± 3.5[a]	83.0 ± 4.3[a]	85.0 ± 3.2	81.0 ±3.6[a]	72.2 ± 1.0

Table. 6. 3. Effect of 300mg/kg /body) treatment for 8 weeks with water extract *A. augusta* and *Curcumi on* Urea, Creatinine , Cholesterol and protein in(STZ) diabetic rats.

Parameters	Normal	Normal + Mixture	Diabetic	Diabetic + Mixture
Urea (mg/dl)	26.8±3.2	28.5±5.4	68.8±3.1	43.6 2.0
Creatinine (mg/dl)	0.43±0.02	0.41±0.03	0.66±0.04	0.49±0.02
Cholesterol (mg/dl)	86±2.7	95±3.4	198±2.2	100±6.0
Protein (g/dl)	7.3±0.2	7.4±0,3	6.5 ± 0.1	7.00±0.08

Table 6.4. Kidney mass Plasma Creatinine, glucose, Na^+ K^+ Concentration Urinary Na^+ K^+ Creatinine excretion

Analysis	Non-diabetic untreated	Diabetic treated
Plasma [Na^+] mmol)	139 ± 2	134± 0.9*
Urinary Na^+excreted (mmol/1/2 4h)	750 ± 40	12.0± 45*
Plasma [k^+] (mmol/1)	5.0± 0.32	4.50 ± 0. 20
Urinary Creatinine Excreted /ml/24h	4.0 ± 0.2	4.5 ± 2.5
Plasma [creatinine] Mmol/1	30 ± 3.5	37 ± 3*

a= p < 0.05

Table. 6.5. Effect of water extract of the combination of *A. augusta* and *C. Longa* for 8 weeks on lipidperoxidation, SOD and CAT activities in erythrocytes in STZ diabetic rats

Group	LOP	SOD	CAT
Control	286.6±4.7	320 ±24.0	279.9 ±5.1
Diabetic untreated	410.9 ± 7.2	269.5 ±8.2	200.1±2.2
Diabetic + *A. augusta*	205± 11.2	293 ±10	258.0±10.0
Diabetic + *C. Longa*	211± 14	310.4 ± 1.8	269.2±4.6
Diabetic + *A. augusta* + *C. Longa*	209.5±10.8	330.5±2.00	395± 11.0

Table. 6. 6. Effect of 300mg aqueous extract of *Abroma augusta* and *Curcuma longa* on lipid Profile rats.

Group	TC (mg/dl)	LDLC(mg/dl)	HDLC (mg/dl)	LDLC/HDLC	TAG (mg/dl)
1. Normal	156.0 ± 13.4	80.2±8.79	45.87±8.4	1.5 ± 0.13	104.6±5.2
2. Diabetic untreated	245.0 ± 16.0[a]	170.84±7,4	49.35±2,50	3.93 ± 0.19[a]	190.1± 6.30[a]
3. Diabetic + *Abromaq augusta* and *Curcuma longa*	163.0 ± 1.54	92.15 ± 10.84	46.6±18.45	1.56± 0.03	130.0 ± 12.0[a]

a = p < 0.001 when compared with normal group.

Table. 6.7. The combination of *A. augusta* and *C. Longa* on general parameterof STZ diabetic rats.

General Parameter	Control	Diabetic	Diabetic + C. Long	Diabetic + A.augusta	Diabetic + C.Longa + A.augusta
Bodyweight (g)	215±10	140±8.0	182±1.4	210±3.0	213±3.5
Liverweight (g)	5.2±0.2	5.0+0.1	5.5+0.1	6.0+0.1	5.7 + 0.1
Kidney weight(g)	1.5±0.3	1.6±0.04	1.3±0.06	1.5±0.05	1.4±0.06
Protein (g/dl)	7.0±0.4	6.3±.02	6.2±.01	7.5±.3	6.8±0.07

Table. 6. 8. Effect of water extract *A. augusta* and *C. Inga* on total hemoglobin, and Urine sugar of normal and experiment rats

Group	Hemoglobin	Urine sugar
Normal	15.5 ± 4.0	-
Diabetic Untreated	11.5 ± 1.5	+++
Diabetic + *Abroma*+	13.9 ± 2.5	-
Diabetic + *C-longa*	14.5 ±1.9	-
Diabetic + Mixture	15.8 ±4.6	-

Table. 6. 9. Plasma, Erythrocytes of Controls and type 2 Diabetics.

	Cholesterol		Phospho lipids		Chol / P L	
Group	**Controls**	**Diabetics**	**Controls**	**Diabetics**	**Controls**	**Diabetics**
Plasma(μmole / g protein)	169. 35±6.21	230.26±3.26	221.15±3.95	318.3^{α}±5.0	0.75	0.72
Erythrocytes (μ mole / g Hb)	2.63 ±0.05 ^{b}	$3.04\pm0.08^{\alpha * b **}$	$2.45 \pm 0.06^{\alpha}$ **	2.7±0.06	1.07±0.02	1.28±0.05

Values are Mean ± S.D samples.

Significance between Controls and Diabetics type 2.

* **P < 0.001,** ** **P < 0.02** *** **P < 0.05**

Table. 6.10 Effect of water extract A. augusta and *C. Inga* on various treatments on The body weight of rats

Group	**Initial**	**Change in body weight (g)**
Normal	190±11.2	210± 7.0
Diabetic Untreated	160.5 ± 7	*200± 5.0
Diabetic + *Abroma*+ *C-longa*	175.9 ± 2.5	210± 1.0

.

****P< 0.05***

6. 4. CONCLUSIONS

Many plants have been used for the treatment of diabetes mellitus in Indian system of medicine and in other ancient systems of the world, out of these only a few have been evaluated as per modern system of medicine. From many such plants only extracts have been prepared and their usefulness evaluated in experimental diabetes in animals.

In some plants like *Alluvium cepa, Alluvium sativa, Ficus bengalensis, Gymnema Sylvester, Pterocarpus marsuplum etc.* active hypoglycemic principles have been isolated and their mechanism of action studied. Most of them seem to act directly on pancreas (pancreatic effect) and stimulate insulin level in blood. Some have extra pancreatic effect by acting directly on tissues like liver, muscle etc. and alter favorably the activities of the regulatory enzymes of glycolysis, gluconeogenesis and other pathways. Many of its products / chemical constituents are known to posses wide array of medicinal properties.

This study demonstrated that the Hypoglycemic and hypolipidemic effect of combination *of Curcuma longa* constituents and *Abroma augusta* (Linn.) on blood glucose profile and antioxidant properties of *STZ – induced diabetic rats.*

Dietary spice components have been screened for their protective effect against reactive oxygen species induced, lipid peroxidation. has been found to be an efficient antioxidant.

Effect of oral administration of 300.mg / Kg body weight of the aqueous extract of the turmeric's active gradient *Curcumin* and *Abromine* powdermixed with diet was studied for 8 weeks, on lipid peroxidation (LPO) and the antioxidant

defense system in rat tissues. In various organs of rats like liver and lung, kidney and brain was studied for 8 weeks resulted in a significant reduction in blood glucose and in increased total hemoglobin. The aqueous extract also resulted in decreased free radical formation in tissues studied.

This study shows that *Abroma augusta (*whole plant*)* and fresh tuber C. long in Control animals as well as those given *(Abromin that there was a* decrease in thiobarbituric reactive substances (TBARS) and increase reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) This clearly shows the antioxidant and property of the *Abromin* and *Curcumin*.

The effect of combination of *Curcuma longa* (Curcumin) constituents and *Abroma augusta(Abromine*) suggests that these changes initially counteract the oxidative stress in diabetes, however, a gradual decrease in the antioxidative process may be one of the factors which results in chronic diabetes. It is suggested that these changes initially counteract the oxidative stress in diabetes, however, a gradual decrease in the antioxidative process may be one of the factors, which results in chronic diabetes. The data indicate that these mixtures of two plants have use as an antidiabetic agent and in lowering oxidative stress in diabetes.

The effect of oral administration of (300 mg/kg body weight) of the aqueous extract of the turmeric active gradient *Curcumin* and *Abromine* powder mixed with diet studied for 8 weeks on lipid peroxidation (LPO) and the antioxidant defense system in rat tissues, rat liver, lung, kidney and brain. This

resulted in a significant reduction in blood glucose and in increased total hemoglobin. Decrease in free radical formation in the tissues was also observed.

This study shows that *Abroma augusta* (whole plant) and fresh tuber *C. Longa* showed decrease in thiobarbituric reactive substances (TBARS) and increase antioxidant and reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) indicating the antioxidant activity of curcumin and related compounds.

Since the plant products have fewer side effects, they have the potential as good hypoglycemic drugs. They may also provide clues for the development of new and better oral drugs for diabetes.

Curcuma longa (*Turmeric, Halidi*) from family *Zingeberaceas is* widely used in indigenous medicine both in *Ayurvedic* and *Siddha,* and many of its products / chemical constituents are known to posses wide array of medicinal properties. Because of their diverse activity potential, there is a considerable hope of finding anti-diabetic compounds from *Curcuma longa* rhizomes.

BIBLIOGRAPHY

Melinda A., Non insulin dependent diabetes mellitus treatment with sulphonylureas in Clinical Endocrinology and metabolism. Des natures, M. and Hale, P., Balliere- Tindall, *London.* 1988; P, 443-453.

World Health Organization.1980 Expert committee diabetes mellitus. Technical Report Second Series. No.**646**, *WHO.Geneva,* 1980 ; p. 61.

Satyavati, G.V. and Gupta, A. k. Medicinal plants of India eds Indian Council , *New Delhi;* 1987; Vol. II. **(4):** 18, 73.

Oubre, A.Y., Carlson, T.J., King, S.R. and Reaven, G.M. from plant to patient, an Ethanomedical approach to the identification of new drugs for the treatment of NIDDM. *Diabetologia;* . 1970 ; 4 0 **(5):** 614-617.

Shih, C.A., Lin J.K. Inhibition of 8-Hydroxydeoxyguanosive formation by curcumin in Mouse fibroblast cells. *Carcinogenrsis*; 1980 ; 14 **(4):** 709-712

Tank R, Sharma N, Sharma, I, dixit, VP, Anti-diabetic activity if *C.longa* in alloxan induced diabetic rats - *Indian drugs;* 1989 ; 27 **(11):** 587-589.

Brown G.B. Xue-Qiao, Sacco, D.E.and Alberts, J.J. Lipid lowering and plaque Regression. New insights into prevention of plaque disruption and clinical events in Coronary disease. *Circulation;* 1993; **(87):** 1781-1791.

Jain, S.K., robert, M., Duett, J., Herbst, JJ. (1989), Erythrocyte membrane lipid peroxidation and glycosylated haemoglobin in diabetes . Diabetes, 1989 ; (38): 1539- 1543

Hakino, H, (1985) Anti hepatotoxic activity of crude drugs. Yakugaku Zasshi . 1985 ; 105 **(2) :** 109-118.

Sondamini, K.K., Unnikrishnan, M.c., Soni, K.B. and Kuttan, R. Inhibition of lipid peroxidation and cholesterollevel in mice by curcumin. Indian J. Pharmacol 1992 ; (4): 234-243

Soudamini, K. K., Kuttan, R. Inhibition of chemical carcinogenesis by curcumins *J Ethnopharmacology*; 1989 ; **(27):** 227-233.

Bonin A..M , Baker RSV) . Mutagenicity testing of some approved food additives with Salmonella / microsome assay. Foodtechnology(1980; (Australia) 32 : 608 - 611.

Hussein, H.E.M.A., Jamil, K. and Rao, M. (2001) Preliminary studies on the hypoglycemic effect of Abroma *augusta* in alloxan diabetic rats. *Ind. J. clin. Biochem.* 16 **(1):** 77-80.

Poliodoro, G.D.T., Arduini., R.G.L A. and Federici, G. (1984) Superoxide dissimulates, reduced Glutathione and Tba- reactive products in erythrocytes of patients with multiple sclerosis. *Int. J. Biochem.* 1984 ; 16 **(50):** 505-509.

Hiroshi, O., Nobuko, O. and Kunio, V. Assay for lipid peroxides in animal tissues by Thiobarbituric acid reaction. *Anal. Biochem*. 1979; **(5):** 351-358.

Wendell, A. (1981) Glutathione peroxidase. In: Methods in Enzymology, Ed. Jakoby, W.B. Academic Press, New York, USA Vol. 1981 ; **(77):** P. 327-333.

Hussein, H.E.M.A., Jamil, K. and Rao, M. Hypoglycemic, hypolipidemic and Antioxidant properties of Tulsa (Ocimum sanctum Linn) on streptozotocin induced diabetes in Rats. *Ind. J. clin. Biochem*. 2001; 16 **(2):** 190-194.

Salimath BP, Sundaresh C.S. Srinivas L. Dietary compounds inhibit lipid peroxidation In erythrocyte membrane .*Nut Res*; 1985 ; **(6):** 1171-1178.

Huang MT, Smart RC, Wong CQ. Conney AH. inhibitory effect of Curcumin, Chlorogenicacide.Coffeic acid, and ferric acid on tumour promotion in mouse skin By 12-0-tertradecanoyl. phorobol , 1988; 13 **(48)** : 5941-5946.

Betteridge J. Lipid disorders in diabetes mellitus, in: J.C. Pickup, G. Willams (Eds.) TextBook of Diabetes, sec. Ond ed., *Blackwell Science, London*; PP, 1997 ; **(31):** 1-55.

Karunanayake, E.H. Tendon, K.H. Search of novel hypoglycemic agents from Medicinal plants, in: AK Sharma (Ed.) Diabetes Mellitus and Its Complications, An Update, *Macmillan* India LTD, *New Delhi, India*, pp.1993; 192-205.

Mata P, Alonso R, Lopez- Fare A, Orders JM, Lahore C, Caramel C, Codices R, Blanquez E, de Ova M . Effect of dietary fat saturation on LDL oxidation and monocyte adhesion too human Endothelial cells in vitro. *Arterioscler Thromb Vasc Biol*; 1996; **(16) :** 1347-1355.

SUMMARY

It has become increasingly evident in recent years that a full spectrum of therapeutic agents for the treatment and prevention of human disease is far from being complete. In an attempt to fill in the gap, drug development research has now focused on traditional herbal remedies as a potential source for new andmore effective medical therapies.

Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for indigenous natural anti-diabetic agents is still going on. India being rich in its plant wealth, Several plants have been identified as the potential source of drugs in Indian system of *Ayruveda* medicine for the treatment of diabetes.

Extracts of various plants have been shown to produce hypoglycemia in normal and experimental diabetic animals. Some of the commonly studied plants are *Momordica Charantia, Allicin Cepa, Allium Sativum, Ficus begalansis, Eugenia jambolana, Abroma augusta* , *Azadirachta indica, Coccinia indica , Curcuma Longa and Ocimum sanctum. Since Abroma augusta , Azadirachta indica , Coccinia indica , Curcuma longa and Ocimum sanctum.* are widely used in *indigenous medicine* both in *Ayurvedic* and *Sidda*, many of its products / Chemical constituents are known to posses wide array of medicinal properties.

Because of their diverse activity potential, there is a considerable hope of finding anti-diabetic properties in *alloxan / streptozotocin* induced diabetic rats. It is generally presumed by the word"*Ayurvedic* medicines for diabetes"that," means a permanent cure for diabetes, without a dose of hypoglycemic agents or a prick of Insulin. Presently available range of hypoglycemic or antidiabetic drugs in NIDDM or Insulin in IDDM has a limited role to play. Risk of drug tolerance is high with oral hypoglycemic agents. Thereby causing a raise in dosage or a change of drug. *Ayurvedic* medicines may help as "potentiators" for these drugs or play a supportive role in regulating the dosage of hypoglycemic.

Diabetes mellitus is a disease that affects more than 100 million people and may attain about five times more subjects in the next 10 years In the search for new compounds, and within the exploratioration of natural resources, the hypoglycemic effect of plants which are reputed as antidiabetic.

The aim of this investigation is to identify new potent antidiabetic compounds of plant origin. Deaths from cardiovascular disease predominate in-patient with diabetes of over 30-year's duration and in those diagnosed after 40 years of age. The complications and biochemical change due to diabetes mellitus are listed below (Table 1 and Table 2).

Table 1 : Biochemical Changes in Diabetes Mellitus

	AFFECTED PROCESS	RESPONSIVE TISSUES	CHANGE
A.	**CARBOHYDRATE METABOLISM**		
a)	Glucose utilization 1. Transport of glucose through cell membranes	Muscle & cell membranes	Decreases
	2. Glucokinase activity	Liver	Decreases
b	Glycogan synthesis	Muscle and liver	Decrease
c.	Gluconeogenesis	Liver	Increase
d.	Hepatic output of glucose	Liver	Increase
e.	Excretion of glucose	Kidney	Increase when renal threshold (180 mg/dl) exceeds
B.	**LIPID METABOLISM**		
a.	Lipolysis	Adipose	Increases
b.	Lipogenesis	Liver and adipose	Decreases
c.	Ketogensis	Liver	Increases
C.	**PROTEIN METABOLISM**		
a.	Transport of amino acids	Muscle, liver and adipose	Decreases
b.	Protein synthesis	Muscle, liver and adipose	Decreases
D.	**ACID BASE**		Metabolic acidosis

Table 2 : Complications of Diabetic Mellitus

Acute metabolic complications

- Ketoacidosis
- Hyper-osmolar nonketotic coma
- Lactic acidosis

Chronic complications

- Microvascular
- Retinopathy
- Nephropathy

Neuropathy

- Distal symmetric sensomotoric
- Focal and multifocal
- Autonomic

Macroangionpathy

- Ischemic heart disease
- Cerebrovascular disease
- Peripheral vascular disease

Associated conditions

- Dyslipidaemia
- Small dense LDL
- Low HDL and elevated triglycerides
- Elevated Lp (a)
- Lipoprotein oxidation and
- Hypertension

Patients with proteinuria have a greatly increased risk of fatal cardiovascular disease. The frequency of coronary heart disease (CHD) in diabetes is related to that in the background population (e.g. it is low in diabetic patients in China and Japan).

General risk factors for cardiovascular disease include smoking, obesity, hyperlipidaemia, hypertension, insulin resistance, hemostat and platelet abnormalities, lack of exercise and a positive family history, Specific diabetes related risk factors may include hyperglycemia (especially for peripheral vascular disease) and hyperinsulinaemia. CHD in diabetic patients is associated with increased plasma cholesterol levels, with reduced HDL-cholesterol in NIDDM patients and possibly with increased triglyceride levels. Figures (1.2. and3), showing HDL, LDL and peripheral cells with organs,VLDL containg endogenous, triglyceride and chylomicroses,containing deity triglyceride etc.

Atherosclerotic arterial disease may be manifested clinically as coronary heart disease (CHD), cerebrovascular disease or peripheral vascular disease**.** The effect of diabetes on atherosclerosis is different at each of these sites. The relative risk of arterial disease also varies widely with gender, go-graphical location, type and duration of diabetes. Mortality data from the Joslin Clinic (Marks HH, Krall LP. Joshin's Diabetes Mellitus 1971).

Diabetes confers a particularly high relative risk of peripheral vascular disease: about half of all lower limp amputations performed are on diabetic patients. The relative risk of amputation is highest below age 45, although the absolute risk increases with age. . These amputation figures increased in case of patients with microvascular disease and neuropathy, but in the 20-year Framingham study, the incidence of intermittent claudicating was increased 3.8-fold in men and six fold in women with diabetes as compared with non-diabetic subjects (Kannel WB, et al 1979).and (Pyorala K, et al.1987). Obesity adversely affect blood pressure, insulin sensitivity, blood glucose control and lipoprotein patterns, and weight reduction is an important aspect of diabetic treatment. However, epidemiological studies relating obesity to cardiovascular risk in diabetes have yielded conflicting results, with some finding a positive association . (Kannel WB, et al 1979). However, aerobic exercise reduces obesity and plasma insulin level and increases high-density lipoprotein (HDL) cholesterol, all of which are theoretically beneficial Cardiovascular status and the presence of other complications should be considered when giving advice about exercise. Homeostatic abnormalities, particularly raised fibrinogen and factor VII levels are strongly predictive of CHD in non-diabetic subjects. (Mead T, et al, 1986).

Preliminary evidence suggests that similar associations also apply in diabetes. Fibrinogen levels are raised in both IDDM and NIDDM, and are higher in those with cardiovascular complications. Fibrinolytic activity is

lower in NIDDM than IDDM and may be associated with ECG abnormalities. However factor VII levels are highest in diabetic patients with retinopathy and nephropathy, and have not been shown to be associated with large vessel disease. Clotting factors may nonetheless be an important link between large-and small vessel complications. In addition to their role in thrombus formation, platelets are involved in atherogenesis through their release of platelet-derived froth factor and chemotactic factors, which stimulate cellular proliferation in the atheromatous plaque. Measurements of platelet activity in diabetes have sometimes yielded conflicting results. (Betteridge DJ. 1987).

However, in vitro aggregation of platelets in response to ADP and collagen is increased in diabetes and is highest in those with complications. In vivo platelet activity, assessed from plasma levels of β-thromboglobulin and platelet factor 4, is elevated in non-diabetic subjects with vasculardisease and in uncomplicated diabetes, and is further raised in those with retinopathy. Thromboxane production is increased in non-diabetic subjects with CHD; amongst diabetic patients, it is also highest in those with Macrovascular complications.

Effects of diabetes on lipoproteins.

The commonest abnormality in diabetes hypertriglyceridemia due to an excess of very low-density lipoprotein (VLDL). (Albrink MJ. 1974). Lipoprotein lipase depends for its full activity on insulin and VLDL clearance

is reduced in poorly controlled patients with IDDM. In NIDDM patients, there is also overproduction of VLDL and apportion (apo) B. (Fig 2).

Insulin deficiency or resistance increases production of non-esterified fatty acids from adipose tissue by the action of hormone -sensitive lipase, and these provide a substrate for hepatic triglycerides synthesis. Hypertriglyceridemia in diabetes therefore usually responds to intensified insulin treatment. LDL levels are also raised in association with poor glycemic control, but a substantial improvement in blood glucose is required to lower LDL. (Poetry, et al. Diabetes 1980). (Fig 3). Insulin stimulates LDLreceptor activity. (Chat A, et al. 1979). Increasing IDL clearance, while non-enzymatic glycosylation of pa B reduces its affinity for the receptor, thereby slowing LDL removal. (Steinbrecher UP, et al 1984). HDL levels vary inversely with VLDL, since reduced lipoprotein activity impairs catabolism of VLDL and hence transfer of lipids and apportions to HDL.

ARTERIOSCLERIOSIS

May be due to decreased insulin action and /or decrease insulin secretion. Reaven, G.M.1980. many drugs with proven hypocholesterolemic activity are available clinically to ameliorate cases of individuals with premature arteriosclerosis and those with other risk factors, such as hypertension or diabetes mellitus (Brown and Goldstein, 1992).

In many cultures of the world, herbal remedies are increasingly being employed in an attempt to achieve the same purpose. In India, for

instance, the leaves of neem are claimed to possess cholesterol-reducing effect and are used to treat patients with heart disease and obesity. For this reason it was decided to resolve this claim cholesterol of the waster rat. Its effects on serum total protein, and albumin, were also examined in the same by investigating the effect of the crude extract of leaves of neem plant on the serum. Liver and kidney animal model.

The results of the Lipid Research Clinics Primary Prevention trial indicate that there is a positive correlation between plasma concentration of LDL-cholesterol and risk of coronary artery disease (Lipid Research Clinics Program, 1984a,b). This work, which was multicentre randomized, double – blind study showed that a 20% drug – induced reduction in LDL-cholesterol concentrations which resulted in the reduction of newly positive exercise tests (indicative of myocardial schema), angina pectoris, and coronary bypass surgery in the treated 25,20,and 21% respectively.

It has further been shown that the diminished and life prolonged when plasma lipids are lowered by hypocholesterolemic agents (Lipid Research Clinics Program, 1984 a and Lipid Research Clinics Program 1984b) Helsinki Heart Study, 1987).

The results of our investigations have yielded useful and intersting results. Based on our studies the extract of *Abroma augusta* has been shown for the first time to contain antidiabetic preterits

(Table 5.1). This extract in combination with other extracts of other medicinal plants like *Coccinia indica* and *Azadirachta indica* and *Ocimum sanctum* gave synergistic effects. This is therefore a novel study and the results are very significant, as there is a potential for their commercial expatiation. In this investigation it was essential to analyze all the parameters related to diabetes in all the chapters,, as the treatments were different combinations of plants.

BIBLIOGRAPHY

Albrink MJ. Dietary and drug treatment ofhyperlipidaemia in diabetics. *Diabetes,* 1974; **(23):** 913-918.

Marks HH, Krall LP. Onset Course, prognosis and mortality in diabetes mellitus.In Marble A,White P, Bradley RF, Krall LP, *Eds. Joshin's*, Diabetes *Mellitus* II Th edn. *Philadelphia*: Lea & Febiger, 1971; 209-254.

Jarrett RJ. The epidemiology of coronary heart disease and related factors in the contact of Diabetes mellitus impaired glucose tolerance. In: **Jarrett RJ, ed**. *Diabetes and Heart Disease*. Amsterdam: Elsevier *Publishers BV*, 1984; 1-23.

Kannel WB, Diabetes and glucose tolerance as risk factors for cardiovascular disease: the framingham Study. *Diabetes Care,* 1979; 120-126.

Betteridge DJ. Platelets and diabetes mellitus. In: Taylor KG, ed. Diabetes and the Heart. Tunbridgewells: *Castle House Publications,* 1987: 79-119.

Mead TW, Brozovic M, and Chakrabarti RR et al. homeostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet,* 1986; **(II)** 533-537.

Poetry, Dun FL, Raskin P. The effect of improved diabetic control on plasma lipid and lipoprotein levels. Comparison of conventional therapy and continuous subcutaneous insulin. *Diabetes*, 1980; **(29)**: 1001-1005

Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiological view. *Diabetes Metab Rev* 1987; **(3):** 464-524.

Reaven, G.M. Insulin – independent diabetes mellitus: Metabolic characteristics. *Metal. Clin. Exp*. 1980; **(29):** 445-454.

Chait A, Barman El, Albert JJ. Low-density lipoprotein receptor activity in cultured fibroblasts Mechanism of insulin-induced stimulation. *J Cline Invest*, 1979; **(64):** 1309-19.

Steinbrecher UP, Witztum JL. Glucosylation of low density lipoproteins to anextent comparable to that seen in diabetes slow their catabolism. *Diabetes,* 1982; **(31)**: 1029-1032.

Stevenson RW, Hudson NJ, Krupp MN, Volkmann RA, Holland GF, Eggler JF, Clark DA, M Pherson RK, Hell XL, Danbury BH. Actions of a novel anti-diabetic agent Englitazone in hyperglycemic hyperinsulinemic ob/ob mice. *Diabetes*, 1990**; (39):**1218-1227.

Brown and Goldstein J. L, Drugs used in the treatment of Hyperlipoproteinemias. In: Gilman. A.G., Rall. TW. Nies, A. S., Taylor, P. (Eds), Goodman and Gilman's

Pharmacological Basis of therapeutics. **Vol. I**, Eight ed. Mc Graw-Hill Inc, *New York*, 1992; pp. 874-894.

Lipid Research clinics program, 1984 b. the lipid research clinics coronary primary prevention Trial results. LI. The relation ship of reduction in incidence of coronary heart disease to cholesterol lowering. *Journal of American Medical Association*, 1984 b; **(251)**365- 374.

Lipid Research Clinics Program. The lipid research clinics coronary primary prevention trial Results. LI. The relation ship of reduction in incidence of coronary heart disease to Cholesterol lowering. *Journal of American Medical Association,* 1984 b**. (251):** 365- 374.