ANTIDIABETIC ACTIVITY FROM SELECTED PLANTS

(A study of antidiabetic principles from *Aegle marmelos*)

Thesis submitted to the University of Calicut

for the partial fulfilment for the Degree of

DOCTOR OF PHILOSOPHY IN BIOCHEMISTRY

(Faculty of Medicine)

By

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July, 1999.

CERTIFICATE

This is to certify that this report " ANTIDIABETIC ACTIVITY FROM SELECTED PLANTS" (A study of antidiabetic principles from *Aegle marmelos*) is an authentic account of the work carried out by **P.T.C.PONNACHAN** under my supervision and guidance and no part thereof has been presented for any other degree.

Dr. K.R.Panikkar.

Supervising Guide

Date: 16.7.99

DECLARATION

I, Ponnachan,P.T.C. declare that my thesis entitiled **"ANTIDIABETIC ACTIVITY FROM SELECTED PLANTS"** (A study of antidiabetic principles from *Aegle marmelos*) is an authentic account of the work carried out by me under the supervision and guidance of Dr.K.R.Panikkar and no part thereof has been presented for any other degree.

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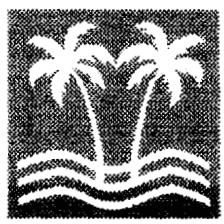

DEDICATED TO MY PARENTS

The essential part in Science is not a complicated mathematical formalism or a ritualised experimentation. Rather the heart of science is a kind of shrewd honesty that springs from wanting to know what the hell is going on !

Saul-Paul Sirag

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

CHAPTER I

INTRODUCTION

Diabetes mellitus is a disease associated with carbohydrate metabolism and to a certain extent metabolism of fats and proteins. Its prevalence among the human races ~~are~~ is very high and influenced by both genetic and environmental factors. The biochemical aspects regarding this are well known and it is now possible to control it if detected in time. Sushruta Samhitha (Kaviraj, 1981) and Charaka Samhitha (Vaidya Jadavaji, 1981) served as an ancient medical compendia for the early detection and treatment of this disease.

The term "diabetes" is a Greek word and means "to run through a siphon" and it was coined by two Roman Physicians Aretacus and Celsus(Volk and Wellman 1977) in 1st AD. They noted that a large amount of urine "runs through" the kidneys of those patients suffering from diabetes and they were the first to give a detailed clinical description of diabetes. Eventhough this disease was known centuries earlier the actual reason for the same remained a mystery. It was Thomas Cawley (Papaspyras, 1964) who for the first time associated diabetes with pancreas in the later part of the nineteenth century. Von-Mering and Minkowsky(Merring and Minkowsky 1889) produced diabetes in dogs by removing the pancreas. The disease was cured by administering insulin.(Banting and Best 1922) Initially it was believed that external pancreatic secretion has a role in maintaining normal blood sugar levels. But Hedon in 1893 observed that diabetes was absent in pancreatectomized dogs as long as pancreatic transplant remained intact. So there is the possibility that internal rather than an external secretory function ~~which~~ play a key role in blood sugar homeostasis. In 1893 itself Laguesse suggested that islets of Langerhans present in the pancreas may be

providing the internal secretion that regulate the blood sugar level. These cells were first described by Paul Langerhan in 1869. He observed at low magnification tiny, intensely yellow spots, measuring 0.1 to 0.2 mm, in rabbit pancreas left 2 or 3 days in Muller's fluid. Langerhan was not able to discover the actual function of these cells. But with the suggestion by Lagusse (1893) that these structures are the anatomical counterpart of the internal secretory function of the organ, many a work was done to establish the existence of a relationship between the islets and carbohydrate metabolism. This was first achieved by Schafer (1895) and Diamare (1899) . Both Dieckhoff (1895) and Ssobolew (1900) recorded the complete absence of islets in some cases of diabetes. In animal experiments, Ssobolew (1902), Dewitt (1906) and Mac Callum (1909) showed that pancreatic duct ligation is followed by atrophy of exocrine, parenchyma, whereas if the islets survive, diabetes does not develop. Mac Callum (1909) also demonstrated that the removal of such a duct ligated organ will induce diabetes.

In the meantime the histology of the pancreatic islets became a field for extensive study. Diamare (1899) and Schulze (1900) were the first to suggest that islets of Langerhans contain more than one type of cell. Lane (1907) named the two types of cells in the pancreatic islets as "A Cells" and "B Cells" depending on the morphological difference of the cells. Later Bloom (1931) identified a third type of cells in the islets and named it as "D cells".

1.1 Experimental Diabetes

When the basic facts concerning pancreatic islets were revealed and their role in human diabetes understood, attempt was made to produce the disease in experimental animals. Various workers have succeeded in developing diabetes

in animals by pancreatectomy (Merring and Minkowsky 1889), injecting with alloxan (Jacobs 1937), anti-insulin serum (Moloney and Coval 1955, Anderson et al., 1963) or mannoheptulose (Samaan 1962, Coore et al, 1963) all of which lead to a decrease in circulating insulin. It has also been noted that diabetes can be induced with growth hormone (Houssay 1936, Young 1953) and glucocorticoids (Ingle 1941). ~~Recently~~ Later on was it ~~is~~ proved that administration of streptozotocin (Elfellab et al, 1984, Levine 1961) produce diabetes. Another substance found to produce hyperglycaemia in experimental animals is diasoxide (Juan et al, 1970).

Alloxan is usually used to develop diabetes in animal models since most animals are susceptible to it and the dose required to produce diabetes varies with the animal. If a small dose is given diabetes will not develop and a higher dose will lead to severe kidney damage with uraemia (Goldner and Gomori 1943). The route of administration of alloxan varies with the animal used. The intravenous method is preferred for larger animals while the subcutaneous method for smaller animals. In rats the intravenous diabetogenic dose is 50-60 mg/kg whereas the subcutaneous dose is 150-200 mg/kg of the body weight. The intraperitoneal administration of alloxan is also found to produce diabetes in rats, but the degree of liver damage is greater and so it is not preferred. The guinea pigs appear to be resistant to diabetogenic action of alloxan (Mirsky 1945), so also duck, owls and chicken (Scott et al, 1945).

The speed with which alloxan is injected is important. For a diabetogenic dose given intravenously too slowly may not produce diabetes. If the entire dose of alloxan is given within 2-3 minutes, diabetes will develop. Presumably when the injection is given too slowly the alloxan is inactivated or neutralised before an effective concentration is attained in the pancreas. Although

alloxan is extensively used for the production of experimental diabetes, the mechanism of its action is not known. It is well known that alloxan is unstable in physiological solutions. Its half life at pH 7.4 and 37C is less than one minute (Paterson et al, 1949). Moreover, there are indications that alloxan injected in laboratory animals is decomposed rapidly as well (Archibald 1945). This was illustrated by Leech (Leech and Bailey 1945) who showed that alloxan injected intravenously can be detected in blood for less than 5 minutes. Based on these observations, it is widely held that the diabetogenic action of alloxan takes place within a few minutes after injection. The observation of Gomori and Goldner (1945) that clamping of a portion of a dog's pancreas protects the animal against development of alloxan diabetes is in support of this view. But Bilic and Felber (1970) showed that temporary interruption of arterial blood flow to the pancreas just prior to alloxan administration and the release of the blood flow ten minutes after alloxan treatment do not protect rats against the development of alloxan diabetes. They also showed after intravenous injection of alloxan, a substance giving a fluorescent product with alloxan reagent was detected in blood after four hours. The substance remained at higher levels, when animals prior to alloxan treatment were injected with reduced glutathione. There was also a prolonged detection of alloxan during incubation in whole blood and in buffer containing reduced glutathione.

Another diabetogenic substance is streptozotocin, an antibiotic extracted from *Streptomyces achromogenus*. In addition to its diabetogenic action, it has antimicrobial and antitumoral activity (Garret 1960, Herr et al., 1967). The development of diabetes in dogs and rats treated with streptozotocin was first reported by Rakieten et al., (1963) who attributed its action to disruption of pancreatic islets. Subsequent investigators (Evans et al., 1965, Arison et al., 1966)

confirmed this diabetogenic action in the rat, but suggested that diabetes resulted from degranulation of pancreatic B cells rather than from actual destruction of B cells. Junod et al., (1967) concluded that streptozotocin has a highly effective B cell cytotoxicity similar to but more specific and with wider margin of safety than alloxan. Several studies in rats and mice have documented a triphasic pattern in glucose and insulin levels after streptozotocin administration (Junod et al., 1969). There is a mild hyperglycaemia during the first two or four hours after injection, presumably due to mobilisation of liver glycogen. This is followed by the release of insulin from damaged B cells with resultant elevation of serum insulin and consequently profound hypoglycaemia which is most marked at seven to ten hours after injection. Within 24 hours there is permanent hyperglycaemia associated with low serum insulin levels and negligible pancreatic insulin content. It has been reported that the acute diabetogenic action of streptozotocin can be prevented by simultaneous administration of nicotinamide (Schein et al., 1967) and the toxicity could be correlated with decrease of NAD^+ concentration in the liver.

The effect of mannoheptulose on insulin secretion in man was studied. Following glucose infusion, mannose heptulose was given. The serum insulin dropped nearly zero and blood glucose level rose. But when tolbutamide was given at this stage, there was an immediate insulin response and fall of blood glucose. Thus mannoheptulose is a useful tool for blockage of insulin release which can be overcome by tolbutamide.

Hoffman and Whistler (1968) reported that 5 thio-d-gluco pyranose, when given interaperitoneally to rats in a dose of 50 mg/kg elevated blood sugar four or five times the normal level in two hours. Administration of insulin simultaneously

prevented this hyperglycaemic effect. This compound was recovered in the urine unaltered within 24 hours. It inhibited the glucose uptake by rat diaphragm while glucose uptake by rat liver slices was enhanced.

In addition, certain benzothiadizine compounds also produce hyperglycaemia in experimental animals (Rubin et al., 1962). Chlorothiazide and trichloromethazide have mild hyperglycaemic activity (Dollery et al., 1962). Diaoxide and its analogue AO - 25 also produce hyperglycaemia in man (~~Fitz~~ Fitzgerald and Keen 1964). It has been found that the latter two compounds inhibit insulin secretion both *in vivo* and *in vitro*.

1.2 Classification of Diabetes

The most generally applicable classification is that proposed by the British Diabetic Association (~~Gerald~~ Fitzgerald and Keen, 1964).

1. Potential diabetes: which exhibits glucose tolerance level normally but are considered as significantly greater risk of developing diabetes than general population.
2. Latent diabetes: have normal glucose tolerance but do not have a diabetic glucose tolerance test in the past usually during a pregnancy, an infection, other kind of stress or when obese.
3. Asymptomatic or subclinical or chemical diabetes: are persons with inappropriate hyperglycaemia. They have no significant glycosuria and are without any other symptoms or complications of the disease.
4. Prediabetes: is a retrospective classification defining the period of a diabetic before a diagnosis could be made.

Another type of classification is based on the amount of sugar present in the blood (Khosla and Goel, 1983).

1. Mild diabetes
2. Moderate diabetes
3. Severe diabetes

1. Mild diabetes: In this group the fasting blood sugar level will be below 100 mg/100 ml and the post prandial blood sugar will be less than 188 mg.
2. Moderate diabetes: In this the fasting blood sugar will be between 100-150 mg/100 ml and the post prandial blood sugar will be more than 188 mg and less than 250 mg.
3. Severe diabetes: In this the fasting blood sugar level will be above 150 mg/100 ml and the post prandial blood sugar level will be more than 250 mg.

1.3 Different forms of Diabetes Mellitus

1.3.1 General

Insulin-dependent diabetes mellitus (IDDM; also called type I)
Non-insulin dependent diabetes mellitus (NIDDM; also called type II)
Gestational diabetes mellitus.

1.3.2 specific

Maturity -onset diabetes of youth (MODY; glucokinase gene mutations)
Mutations of the insulin receptor (including leprechaunism)
Insulin gene mutations

Tropical diabetes (chronic pancreatitis associated with nutrtional or toxic factors)

Diabetes secondary to pancreatic disease or surgery

Diabetes associated with genetic syndrome, e.g., Prader-Willi syndrome

Diabetes secondary to endocrinopathies

1.4 Diagnosis of diabetes

Individuals who are genetically destined to become diabetic may not develop overt evidence of their disease until late in life although their liability to the disease has been determined at the time of conception. The period which precedes the development of the overt disease is designated as the prediabetic state and exists until a definitive diagnosis may be made by the accepted method of testing. In order to detect the homozygous carrier of the diabetic trait, various tests have been devised which depend upon the determination of the adequacy of glucose metabolism under conditions of stress. This can be done by the administration of cortisone or by noting the reaction of blood sugar to the injection of tolbutamide.

The cortisone glucose tolerance test reveals a number of individuals whose response is abnormal and who are presumed to be in the prediabetic state. The incidence of such reactions is much higher in those with diabetic history as compared to those without such diabetic relatives.

1.5 Method of treatment of Diabetes Mellitus

Virtually all forms of diabetes mellitus are due to a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin (insulin resistance). These abnormalities lead to alterations in the metabolism of carbohydrates, lipids, ketones, and amino acids; the central feature of the syndrome is being hyperglycaemia.

Insulin is given to patients only when the quantity produced by the subject is not sufficient and the usual procedure is to administer a drug that can induce the pancreatic islets to produce insulin. A large number of drugs can cause hypoglycaemia or hyperglycaemia or may alter the response of diabetic patients to their existing therapeutic regimes. A summary of drugs with hypoglycaemic or hyperglycaemic effects and their presumed sites of action is given in Table I

1.6 Oral Hypoglycaemic Agents

In contrast to the systematic studies that led to the isolation of insulin, the sulfonylureas were discovered accidentally . In 1942, Janbon and colleagues noted that some sulfonamides caused hypoglycaemia in experimental animals. These observations were soon extended, and I-butyl-3-sulfonylurea (carbutamide) became the first clinically useful sulfonylureas for the treatment of diabetes. This compound was later withdrawn because of adverse effects on the bone marrow, but it led to the development of the entire class of sulfonylureas.

Table 1

Drugs that Cause Hypoglycemia or Hyperglycemia (Adapted from Koffler et al., 1989).

DRUG	Possible site of action: Pancreas	Liver	Periphery	Other
Drugs with hypoglycemic effects				
β-Adrenergic receptor antagonist		+	+	+
Salicyclates	+			
Indomethacin*				
Naproxen*				
Ethanol		+		+
Clofibrate			+	
Angiotensin converting enzyme inhibitors			+	
Li^+		+	+	
Theophylline	+			
Ca^{2+}	+			
Bromocryptin			+	
Mebendazole	+			
Sulfonamides				+
Sulbactam/Ampicillin*				+
Tetracyclin*				
Pyridoxin		+		
Pentamidine†	+			
Drugs with Hyperglycemic Effects				
Epinephrine	+	+	+	
Glucocorticosteroids		+	+	
Diuretics	+		+	
Diazoxide	+			
Oral contraceptives	+		+	
β_2-Adrenergic receptor agonists	+	+	+	
Ca^{2+} -Channel blockers	+			
Phenytoin	+			
Cloinidine	+			+
H_2 - Receptor blockers	+			
Pentamidine†				+
Morphine	+			
Heparin				+
Nalidixic acid				?
Sulfin pyrazone*				
Marijuana				+
Nicotine*				

* Although these drugs are reported to have an effect on control of diabetes, there are no conclusive data about their effects on carbohydrate metabolism

† Short term effect is insulin release and hypoglycemia

1.7 Chemistry of Sulfonylureas

The sulfonylureas are divided into two groups of generations of agents. All members of this class of drugs are substituted arylsulfonylureas. They differ by substitutions at the para position on the benzene ring and at one nitrogen residue of the urea moiety. The first group of sulfonylureas include tolbutamide, acetohexamide, tolazamide and chlorpropmide. A second generation of hypoglycaemic sulfonylureas has emerged. These drugs glyburide (glibenclamide), glibizide and gliclazide are considerably more potent than the earlier agents. The structural formulae of the different drugs which are commonly used in the management of diabetes are presented in Fig.I

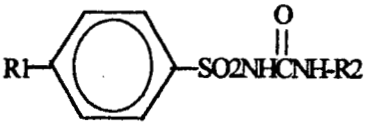
1.8 Mechanism of action of the drugs

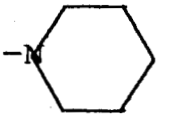
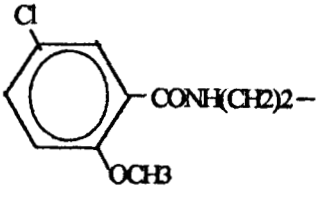
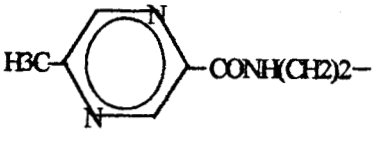
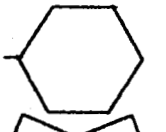
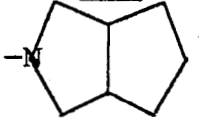
Sulfonylureas cause hypoglycaemia by stimulating insulin release from pancreatic cells. Their effects in the treatment of diabetes, however, is more complex. The acute administration of sulfonylureas to NIDDM patients increases insulin release from the pancreas. Sulfonylureas may also further increase insulin levels by reducing hepatic clearance of the hormone. In the initial months of sufonylurea treatment, fasting plasma insulin levels and insulin responses to oral glucose challenges are increased. With chronic administration, circulating insulin levels decline to those that existed before treatment, but, despite this reduction in insulin levels, reduced plasma levels are maintained. The reason for this is not clear, but it may relate to reduced plasma glucose allowing circulating insulin to have more pronounced effects on its target tissues, and to the fact that chronic hyperglycaemia *per se* impairs insulin secretion.

Figure 1

Structural formulae of the Sulfonylureas

General formula



First Generation Analogs	R1	R2
Tolbutamide (ORAMIDE, ORINASE)	H3C–	–C4H9
Chlorpropamide (DIABINESE)	Cl–	–C3H7
Tolazamide (TOLAMIDE, TOLINASE)	H3C–	
Acetohexamide (DYMELOR)	H3CCO–	
Second Generation Analogs		
Glyburide (Glibenclamide, MICRONASE, DIABETA, GLYNASE		
Glibizide (GLUCOTROL)		
Gliclazide (DIAMICRON, others)	H3C–	

It should be noted that there is no measurable acute stimulatory effect of sulfonylureas on insulin secretion during chronic treatment. This is thought to be due to down regulation of cell surface receptors for sulfonylureas on the pancreatic B cell. If chronic sulfonylurea therapy is discontinued, pancreatic B cell responsiveness to acute administration of the drug is restored. This raises the question of whether or not NIDDM patients who are responding poorly to maximal doses of a sulfonylurea would benefit from a short period of withdrawal from the drug. Sulfonylureas also stimulate the release of somatostatin, and they may suppress the secretion of glucagon slightly (Krall, 1985)

The effects of the sulfonylureas are initiated by binding and blocking an ATP-sensitive K^+ channel, which recently has been cloned (Philipson and Steiner, 1995). The drugs thus resemble physiological secretagogues which also lower the conductance of this channel (Ribalet and Ciani, 1987; Boyd, 1988) Reduced K^+ conductance causes membrane depolarization and influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels.

1.9 Biguanides

Metformin and phenformin were introduced in 1957, and butformin was introduced in 1958. The latter was of limited use, but metformin and phenformin were widely used. Metformin is absorbed mainly from the small intestine. The drug is stable, does not bind to plasma proteins, and is excreted unchanged in the urine.

Metformin is antihyperglycaemic, not hypoglycaemic. It does not cause insulin release from the pancreas and does not cause hypoglycaemia, even

in large doses. Metformin has no significant effects on the secretion of glucagon, cortisol, growth hormone or somatostatin.The main cause of reduced glucose levels during metformin therapy appears to be an increase in insulin action in peripheral tissues and reduced hepatic glucagon output due to inhibition of gluconeogenesis. Metformin also may decrease plasma glucose by reducing the absorption of glucose from the intestine.

1.10 Other Oral Hypoglycaemic Agents

1.10.1 Ciglitazone, Pioglitazone

These agents are thiazolidinediones. They are antihyperglycaemic in a variety of insulin-resistant and diabetic animal models. Like biguanides they do not cause hypoglycaemia in diabetic or normal persons. Ciglizone reduces plasma glucose, insulin, and lipid concentrations after oral administration in several insulin-resistant animal models. The reduction in plasma insulin levels follows a fall in plasma glucose concentrations, which is thought to be due to an effect of the drug to decrease insulin resistance in liver, skeletal muscle , and adipose tissue. The administration of these agents to normal animals does not potentiate insulin effects. Thiazolidinediones appear to augment insulin action in insulin-resistant animals by increasing the number of glucose transporters.

1.10.2 Glucosidase Inhibitors

Glucosidase inhibitors such as acarbose reduce intestinal absorption of starch, dextrin and disaccharides by inhibiting the action of intestinal brush

border glucosidase. Inhibition of this enzyme slows the absorption of carbohydrates; the postprandial rise in plasma glucose is blunted in both normal and diabetic subjects.

1.11 REVIEW OF LITERATURE

Role of plant products in treatment of Diabetes Mellitus

Long before the use of insulin indigenous remedies have been used for the treatment of diabetes mellitus. Nagarajan et al.. (1978), Atta-ur-Rahman and Khurshid Zaman (1989) and Ivorra et al.. (1989) prepared a comprehensive review of the materials used in the treatment of diabetes. Some of the earlier workers mentioned the use of herbal and mineral preparations for the treatment of this disease. Nadkarni's (1954) Indian Materia Medica gives the names of 42 plants which are considered to be useful in diabetes. Mukherji (1957) has given a detailed account of about a dozen of the important indigenous plants with regard to their pharmacognosy, chemistry, pharmacology and therapeutic uses. Ajgaonkar (1960-61) also describes potential plants for similar use. Aiman (1970) listed 35 plants which have been tested clinically for their curative properties in the last two decades. Chaudhary and Vohra (1970) have reviewed the work done on 21 plants for their antidiabetic activity. Karnick (1972) enumerates some aspects of 16 crude Indian drug plants, frequently used as a cure for diabetes in Ayurvedic system of medicine. Israili (1977) gives a detailed account of diabetes with references to Unani system of medicine and lists drugs with hypoglycaemic antidiabetic activity. A comprehensive review of various plants and the parts used for the treatment of diabetes is presented in Table II

There is an increasing demand from patients to use natural antidiabetic agents. This is more because insulin cannot be used orally and oral

Table 2

No	Name of Plant	Family	Common Name	Part Used	Nature Extract
1	Achyranthes aspera Linn.	Amaranthaceae	Apang	Whole plant	Alcoholic extract
2	Aconitum carmichael	Ranuculaceae		Roots	Aqueous methanol water
3	Adhatoda varica Nees	Acanthaceae	Arusa	Leaves and roots	Non nitrogenous principle
4	Aegle marmelos (L) Correa	Rutaceae	Bel	Leaves and roots	Alcoholic extract
5	Crataeva marmelos L.	Rutaceae	Burg	Leaves	Aqueous extract
6	Alima plantago var. orientale	Allismataceae		Roots	
7	Allium cepa Linn.	Lilliaceae	Onion	Bulbs	Onion extract
8	Allium sativa Linn.	Liliaceae	Garlic	Bulbs	Fresh juice
9	Alnus nepalensis D. Don.	Betulaceae	Udis	Whole plant	
10	Aloe barbadensis Mill	Liliaceae	Kuwar	Stem bark	
11	Aloe vera Auct. non. Mill	Liliaceae	Gundal	Leaves	Amorphous solid
12	Anacardium occidentale Linn	Anacardiaceae	Kaju, Cashew	Leaves, Bark	Bark extract
13	Anemarrhena asphodeloides	Lilliaceae		Rhizomes	Aqueous extract
14	Anethum graveolens L.	Umbelliferae	Sowa	Fruits	Glycans, anamerans A, B, C and D

No	Name of Plant	Family	Common Name	Part Used	Nature of Extract
15	Anthocleista nobilis	Potaliaceae		Bark from the trunk	Aqueous and alcoholic extract
16	Anthocleista rhizophoroides	Potaliaceae		Bark from the trunk	Aqueous and alcoholic extract
17	Dimorphantus elatus Miq	Araliaceae		Bark	Aqueous extract
18	Atractylodes japonica	Compositae		Rhizomes	Aqueous extract
19	Averrhoa bilimbi L.	Oxidaceae	Bilimbi	Leaves	Juice or decoction
20	Melia azadirach indica L.	Meliaceae	Margosa tree	Leaves	Aqueous extract
21	Barleria cristota Linn.	Acanthaceae	Bansa siyah	Plant	Alcoholic extract
22	Bauhinia candicans	Caesalpiniaceae		Leaves	Aqueous extract
23	Bauhinia variegata Linn	Caesalpiniaceae	Kachnar	Seeds and white flowers	Ethanolic extract
24	Biophytum sansitivum (L) DC	Oxalidaceae		Plant	plant insulin
25	Brassica napiformis L.H. Bailey	Cruciferae			Glucokinin
26	Bryonia alba L.	Cucurbitaceae	Bryoni	Plant	Methanolic extract
27	Vinca rosea Linn.	Apocyanaceae	Periwinkle	Leaves	Alkaloids

No	Name of Plant	Family	Common Name	Part Used	Nature of Extract
28	Centaurea aspera	Compositae		leaves	Mixed aqueous extract
29	Centaurea calcitrapa L.	Compositae		Leaves	Aqueous extract
30	Ceriopa tagal (Perr.)	Compositae	Fangal	Bark	Decoction
31	Cinnamomum cassia Blume	Lauraceae	Cinnnamomum	Leaves	Cinnamaldehyde
32	Eleutherococcus senticosus	Araliaceae	Gingsend	Roots	Aqueous extract
33	Ephedra gerardiana Wall.ex.Stapf	Ephedrales	Ephedra	Herb	Aqueous extract
34	Ficus bengalensis Linn.	Moraceae	Banyan tree	Bark	Bengalnoside
35	Ficcus glomerata	Moraceae	Rumbal	Bark	Alcoholic extract
36	Ficus religiosa	Moraceae	Fig tree	Root bark	Aqueous extract
37	Galega officinalis	Papilionaceae	French liliac	Whole plant	Alcoholic extract
38	Gymnema sylvestre R.Br.	Asclepiadaceae		Leaves	Gymnemic acid
39	Hammada salicornica	Chenopodiaceae		Leaves and stem	Ethanolic extract
40	Hedyotis biflora	Rubiaceae		Dried whole plant	Aqueous extract
41	Hordeum vulgare Linn.	Gramineae	Barley	Rootlets	Water soluble residue
42	Lithospermum erythrorhizon	Boraginaceae	Shikon	Roots	Aqueous extract
43	Lupinus albus Linn.	Papilionaceae		Seeds	Infusion of alkaloid
44	Momordica charantia Linn.	Cucurbitaceae	Bittergourd	Fruits	Aqueous extract

No	Name of Plant	Family	Common Name	Part Used	Nature of Extract
45	Morus alba Linn.	Moraceae	Whilte mulberry	Root bark	Infusion
46	Murraya koenigii Linn.	Rutaceae	Curry leaf	Leaves	Infusion
47	Ocimum sanctum Linn	Labiatae	Tulsi	Whole plant	Aqueous decoction
48	Olea europaea Linn	Oleaceae	Olive	Leaves	Hydro alcoholic extract
49	Pterocarpus marsupium Roxb.	Leguminosae	Vajasar	Hear wood	Crude drug
50	Rauwolfia serpentina Benth.ex.Kurz.	Apocynaceae	Sarpagandha	Fruits	Total alkaloids
51	Saccharum officinarum Linn.	Graminae	Sugar cane	Stem	Polysaccharide fraction
52	Securigera securidica Linn.	Papilionaceae		Seeds	Chloroform extract
53	Tecoma stans (Linn) Juss.	Bignoniaceae	Padal	Stem	Aqueous decoction
54	Eugenia cumini (L) Druce	Myrtaceae	Black berry	Seeds	Aqueous extract
55	Trigonella foenum-graecum Linn.	Papilionaceae	Fenugreek	Seeds	Alkaloids
56	Veratrum album Linn.	Liliaceae	False helle	Leaves	Alkaloids
57	Veratrum calcifonicum	Liliaceae		Leaves	Alkaloids
58	Vinca erecta	Apocynaceae		Whole plant	Vinsumine (alkaloid)
59	Zeya mays Linn.	Graminaceae	Maize	Styles	Mcerated decoction
60	Zingiber officinale Roscoe	Zingiberaceae	Ginger	Rhizomes	Freshly extracted roots

hypoglycaemic agents have many side reactions and toxicity. Besides after a certain period synthetic oral hypoglycaemic agents do not remain effective in lowering the blood sugar in chronic stages of diabetes.

Even though a number of plants have been claimed as hypoglycaemic, no drug has been developed which has stood the test of full scale clinical trials. Assessment in some clinical trials is only based on symptomatic improvement or on simple urine test and for some other trials only fasting blood sugar level has been adopted. Very rarely glucose tolerance test is done. But the antidiabetic action of a drug cannot be claimed until the response of therapy is observed on glucose tolerance test fully and this is the better and cheaper investigation. On the basis of this parameter, we can declare the response of therapy and hypoglycaemic action of a drug. Glucose metabolism can be corrected in so many ways even without therapy i.e., by diet control, exercise, yoga etc.

There is a great deal of controversy regarding the ethics of clinical trial with crude drugs and especially the newer ones. A section of the scientists feel it highly unethical to administer an unknown drug to human beings and simultaneously to deprive him from the known treatment during that time. Their objections are valid and are based on high ethical values, but it has limitations and their judgement is from a single angle.

So with all the limitations prevalent, many workers in the field of bio-chemistry have successfully carried out the work to lower the blood glucose level in experimental animals and human volunteers with the hypoglycaemic plants. There are different formulations such as the whole plant, root, leaf powder, seeds,

flowers etc. which has profound hypoglycaemic activity. Some of such plants that show hypoglycaemic activity are the following:

The aqueous extract of *Bambus dendrocalamus* leaves caused significant lowering of blood sugar in both normal and alloxan-treated rabbits. The effect persisted for about 96 h (Bapat et al., 1969). The alcoholic extract of *Barleria cristata* was found to have hypoglycaemic activity in albino rats (Dhar *et al.*, 1968). A glycoside isolated from the bark of *Ficus bengalensis* produced a hypoglycaemic effect in normal rabbits but not in diabetic animals (Deshmukh *et al.*, 1960). The aqueous extract exerted no significant effect on the fasting blood sugar level of normal rabbits but a significant lowering of fasting blood sugar was observed in alloxan diabetic rabbits (Vohra et al., 1970 and Vohra et al., 1970). The aqueous extract showed no hypoglycaemic activity on normal human beings although it showed slight hypoglycaemic activity in diabetic patients (Joglekar et al., 1963). On oral administration to normal fasting rabbits flavonoids A, B, C were active as hypoglycaemic agents, of which compound B showed maximum activity (Brahmachari and Augusti, 1964). The milky sap of the same plant caused a lowering of blood sugar (Gupta, 1966). -sistosteryl-D-glycoside isolated from the bark of *Ficus religiosa* showed hypoglycaemic activity in alloxan diabetic rats (Ambika and Rao, 1967).

The alcoholic extract of the leaves of *Gymnema sylvestre* and *Tribang shila* caused significant reduction in blood sugar in normal rats but produced marked and significant reduction in anterior pituitary-treated hyperglycaemic animals (Gupta and Seth, 1962). Aqueous extract of the oriental crude drug "byakujutsu", *Atractylodes japonica* rhizomes, showed hypoglycaemic activity in mice (Konno et al., 1985). Quinquefolan A, B and C, glycan of *Panax*

quinquefolium roots exerted significant hypoglycaemic activity in mice (Oshima et al., 1987). Quinquefolan A exhibited prominent hypoglycaemic effects. Intraperitoneal injection of Quinquefolan A to alloxan induced hyperglycaemic mice also lowered blood glucose level. Combined aqueous methanol extracts of the rhizomes of *Dioscera japonica* and *Dioscera batatus* exerted variable hypoglycaemic effects on i.p. dosing in normal mice. Glycans A, B, C, D, E and F all exhibited remarkable hypoglycaemic effects on normal and alloxan induced diabetic mice (Hikino et al., 1986). Oryzarans A, B, C and D of *Oryza sativa* roots significantly lowered the sugar levels in normal and alloxan induced diabetic mice (Hikino et al., 1986). An aqueous extract of rice bran of *Oryza sativa* exhibited significant hypoglycaemic effects on i.p. administration to normal and alloxan induced hyperglycaemic mice (Hikino et al., 1988).

Glycans (aconitans A, B, C and D) of *Aconitum carmicbaelin* roots exhibited hypoglycaemic effects in normal and alloxan induced hyperglycaemic mice (Konno et al., 1985). Mucilages and deacetylated product of Plantago mucilage showed marked hypoglycaemic activity (Tomoda et al., 1987). The decoction of *Artemesia akyssinica* significantly decreased the blood glucose level in alloxanized mice over a period of 6 hrs. However, the decoction produced a significant increase in blood glucose level after 2 hrs and 4 hrs of drug administration in normal mice. The same increase has been observed in mice treated with normal saline (Mossa, 1985).The effect of powdered *Cuminum nigrum* seeds on blood glucose of normal and alloxan treated hyperglycaemic rabbits has been observed (Akhtar and Ali, 1985). Anameram A, B, C and D from *Anemarrhena asphodeloides* rhizomes afford significant hypoglycaemic effects in normal and alloxan induced hyperglycaemic mice (Takahasi et al., 1986). The leaves of *Eriobotrya japonica* has significant hypoglycaemic activity in normal

and alloxan diabetic rabbits (Noreen et al., 1988). Extracts of leaves and flowers of *Centaurea corubionesis* reduced blood sugar levels in rats with glucose induced hyperglycaemia, but had no effect on alloxan diabetic animals (Chukla et al., 1988). The dried sap of *Aloe vera* induced hypoglycaemia in normal mice after 5 days of treatment and in alloxan-induced diabetic mice after 3 days of treatment (Ghannam et al., 1986). Moreover, a plant extract in use by diabetic individuals that contain "aloe" exhibited a hypoglycaemic effect on the glucose tolerance test in normal and streptozotocin induced diabetic rats treated for 1 week. Treatment with the extract did not significantly alter insulin levels or intestinal glucose absorption (Al-Awadi et al., 1985). Mossa (1985) indicated that an aqueous extract of *Aloe vera* was found to possess no significant hypoglycaemic activity in alloxanised mice. The aqueous extract of stem and leaflets of *Artemisia herba-alba* caused a decrease in ~~elevated~~ blood sugar and remission of diabetic symptoms in 15 diabetic patients with no side effects (Al-Waili, 1986). The ethereal extract of leaves of *Bauhinia candicans* had a hypoglycaemic effect on alloxan diabetic rats but had no effect in normal rats (Lemus et al., 1986). The aqueous extract of seeds of *Bixa orellana* had a hypoglycaemic effect on dogs (Morrison, 1982). The dried product of chloroform extract soluble in oil produced a non insulin mediated hypoglycaemic effect in the oral glucose tolerance test in dogs; an alcohol soluble agent of the chloroform extract had a hyperglycaemic effect in dogs (Morrison and West, 1985). The aqueous extract of leaves of *Bridelia ferruginea* lowered the fasting blood sugar levels of maturity-onset diabetic patients (Iwu, 1983). The methanolic extract of leaves of *Bryonia albasic* decreased the blood sugar in rats with alloxan diabetes after 20 days (Panosyan et al., 1981). The ethanol extract of root bark of *Bumelia sartorum* elicited a hypoglycaemic effect in normal rats and rabbits. In alloxan induced mild diabetic rats, the daily administration of ethanol extract for 12 days

lowered the blood glucose from the 8th day of treatment. Moreover, the extract improved glucose tolerance in alloxan induced diabetic rats. In addition, the extract enhanced glucose uptake in skeletal muscle and inhibited glycogenolysis in the liver (Nobrega et al., 1985). The aqueous extract of fruits of *Capsicum annum* caused a dose-dependent decrease in fasting blood glucose levels in rats. The hypoglycaemic effect of the extract may involve both the inhibition of intestinal glucose transport and its action upon systemic glucose metabolism (Monsereenusorn, 1980). Hypoglycaemic effect of aqueous extract of the leaves of *Cecropia obtusifolia* was proved in alloxan diabetic mice when the extract was given either orally or i.p. route (Perez *et al.*, 1984). An aqueous extract (15 mg/ml i.v.) exhibited hypoglycaemic effect in normal and pancreatectonized dogs. In both groups of animals, an increase in the concentration of triglyceride was observed. In intact normal animals there was no corresponding increase in insulin ~~plasma~~ Plasma levels. This result suggests that the effect of the plant extract may not be related to stimuli of the B - pancreatic cells (Mellado and Lozoya, 1984). The aqueous extracts of *Centaurea aspera*, *Centaurea calcitrapa*, *Centaurea melitensis* and *Centaurea solstialis* elicited hypoglycaemic effects in normal mice (Masso et al., 1979). The aqueous extract *Centaurea aspera* produced hypoglycaemia in rats and mice while showing no central and autonomic nervous system or local anaesthetic activity in rats and the extract caused transitory hypotension in the anaesthetized rat and cat and induced contractions in isolated guinea pig ileum (Masso, 1980). The hexanic extract of whole plant of *Cluytia richardiana* administered i.p. to normal rabbits caused reduction in blood glucose (Tilmisany and Ajabnoor, 1986). The leaves and fruits of *Coccinia indica* has hypoglycaemic effect and an improvement on oral glucose tolerance in patients with maturity onset diabetes. The maximum effect was observed after three weeks of treatment (Azadkhan et al., 1980). In animals, the administration of a

suspension of dried powdered leaves with milk caused a reduction in blood glucose levels in alloxan diabetic dogs but did not induce hypoglycaemia in a normal group of animals. Moreover, the drug reduced blood glucose during glucose tolerance test conducted in both normal and diabetic dogs (Singh et al., 1985). Oral administration of *Cuminum nigrum* seeds as well as the water and methanol extracts decreased blood glucose levels in normal and alloxan diabetic rabbits. Total blood lipids were not influenced by this substance in either normal or diabetic rabbits (Akthar and Ali, 1985). The whole fruit extract of *Cyanopsis tetragonolobus* showed significant hypoglycaemic activity in normal rabbits and the seeds had a hypoglycaemic effect in glucose fed fasting rabbits. In alloxan diabetic animals, the decoction was effective (Pillai et al., 1980). A hydroalcoholic extract of tubers of *Dioscorea dumetorum* administered to fasting normal rabbits induced a hypoglycaemic effect accompanied by some serious toxic reactions (Akubue and Mittal, 1982). Later it was demonstrated that the toxic reactions could be separated from hypoglycaemic activity through fractionation of the extract by solvent partition. Whereas the alkaloid containing fraction was hyperglycaemic in fasting normal mice, the whole extract and the fraction containing steroidal derivatives evidenced significant hypoglycaemic activities in fasting normal mice or rabbits and in severe alloxan diabetic rabbits (Undie and Akubue, 1986). The alcoholic extract of leaves of *Eriobotrya japonica* exerted a significant hypolgycaemic effect in normal rabbits but had no effect on alloxan treated rabbits. This suggests that the extract acts by initiating the release of insulin from pancreatic B cells of normal rabbits (Noreen et al., 1988). It was demonstrated that the oral administration of seeds of *Eugenia jambolana* to rats for 15 days caused a lowering of blood glucose accompanied by a significant increase in pancreatic cathepsin B activity. The effect was comparable to those of chloropropamide (Bansal et al., 1987). Oral administration of powdered aerial

parts of *Euphorbia prostrata* or methanol extract decreased the blood glucose levels of normal rabbits but had no effect on alloxan treated rabbits. This suggests that this plant acts probably by initiating the release of insulin from the pancreatic B cells of normal rabbits (Akhtar et al., 1984). The aqueous extract of *Ganoderma lucidum* reduced blood glucose and insulin levels 10 min after glucose influsion and increased blood insulin without affecting blood glucose 30 and 60 min after oral glucose infusion. Glucose absorption from the small intestine was not inhibited by water extract. In addition, the water extract inhibited the elevation of blood glucose induced by intravenous infusion of adrenaline without elevating the blood insulin level (Kimura et al., 1988). An active fraction from the seeds of *Lupinus albus* (4 mg/kg p.o.) exhibited a hypoglycaemic effect on normal rabbits (Cabo et al., 1983) and glucose or diazoxide induced hyperglycaemic rabbits but had no effect in alloxan or streptozotocin hyperglycaemic rats, which suggest that the presence of B pancreatic cells is required for activity (Cabo et al., 1983. Several extracts of flowers, stem and leaves of *Lythrum salicaria* possessed hypoglycaemic activity in normal and in glucose induced hyperglycaemic rabbits. There was an increase in circulating insulin in normoglycaemic rabbits. The root was inactive (Cadavid and Calleja, 1980). The other extract was the most active extract and it caused a significant reduction in blood glucose in normal rats and a corresponding increase of circulating insulin. Moreover, the other extract (50 mg/ml) increased insulin secretion in isolated rat islets of Langerhans (Lamela et al., 1985). A demethoxy derivative of leucocyanidin 3-0-beta-D-galactosyl cellobioside isolated from the bark of *Ficus bengalensis* demonstrated antidiabetic action. On oral administration, it decreased blood sugar significantly both in normal and moderately diabetic rats (Vinodkumar and Augusti, 1989). The aqueous extract of stems and leaves of *Tecoma stans* administered intravenously to an anaesthetised dog produced an immediate fall of arterial blood

pressure and a transient hyperglycaemia. Later, a blood glucose lowering effect is observed. The early transient hypotensive and hyperglycaemic response is attributed to the presence of histamine releasing substance in Tecoma extracts. The blood glucose lowering effect persists and supports the existence of specific hypoglycaemic principle in the extract (Meckes Lozoya and Lozoya, 1989). The aqueous extract of roots of *Anthodeista vogelli* possess favourable hypoglycaemic activity in a dose dependent manner on normal and hyperglycaemic animals compared to chloropropamide as a standard (Abuh et al., 1990). The hexane fraction of *Swertia chirayita* possibly acts through its insulin releasing effect (Chandrasekhar et al., 1990). Discoretine isolated from the aqueous fraction of the methanol extract of *Dioscorea dumetorum* tubers when administered intraperitoneally to normal and alloxan diabetic rabbits produced significant hypoglycaemic effect at a dose of 25 mg/kg. The fasting blood sugar in normoglycaemic rabbits was reduced from 112 mg/100 ml to 55 mg/100 ml after 4 hrs. In alloxan diabetic rabbits, the blood sugar was lowered from 520 mg/100 ml to 286 mg/100 ml at the same time interval (Iwu et al., 1990). The ethanolic extract of *Borassus flabellifer* produced hypoglycaemic and hypochloestrolemic effect in mice (Raishankar et al., 1989). The aqueous extract of *Salacia reticulata* significantly decreased the blood glucose in streptozotocin induced diabetic rats as shown by Serasinghe et al. (1990). The *Curcuma longa* rhizome extract showed blood glucose lowering activity in alloxan induced diabetic rats (Tank et al., 1990). The ethanolic extract of *Salacia macrosperma* showed significant hypoglycaemic activity in alloxan induced diabetic rabbits and normal rabbits (Venkateswarlu et al., 1990). Tomoda *et al.* (1990) have shown that the glycan isolated from the seeds of *Malva verticillata* shows significant hypoglycaemic activity. Administration of rind extract of *Punica granatum* showed significant hypoglycaemic activity in mild diabetic albino rats (Zafar and Singh, 1990). Singh

et al., 1991 have shown that the alcoholic extract of *Ficus glomerata* bark reduced the blood sugar levels in experimental albino rats within a fortnight. The effect was found to be permanent. The intraperitoneal administration of the methanolic extract of *Prunus davidiana* produced a significant hypoglycaemic effect. Total blood lipids were also decreased by these substances (Choi et al., 1991). Nagaraju et al. (1991) have shown that the ethanolic extract of wood of *Pterocarpus santatinus* showed hypoglycaemic activity in fasted, fed, glucose loaded and streptozotocin diabetic albino rats. Ahmad et al., (1991) showed that the feeding of ethyl acetate soluble fraction of an absolute ethanol extract of Pterocarpus marsupium wood for 5 days to alloxan diabettic rats significantly lowered blood sugar levels with corresponding increase in blood level. Kiho et al., (1992) reported that the ethanol precipitate fraction (RG-WP) obtained from the hot water extract from rhizome of Rehmannia glutinosa mainly composed of pectin like polysaccharides exhibited hypoglycaemic activity in normal and strptozotocin induced mice by intraperitoneal administration of the fraction. The results obtained after chemical modification and proteinase treatments of RG-WP to normal mice significantly increased the activities of hepatic glucokinase, and glucose 6 phosphatase dehydrogenase, but decreaed those of glucose 6 phosphatase and phosphofructokinase. RG-WP stimulated the secretion of insulin and reduced the glycogen content in the liver of normal mouse. An aqueous extract prepared from the leaves of *Bignonia tuira* was tested for hypoglycemic activity in both normal and diabetic rats by Medeiros et al., (1992). Pre treatment with plant extract (10ml/kg of a 10 percent solution orally for 4 consecutive days) lowered the blood sugar levels by 58.7 percent in alloxan induced diabetic rats. Al Hader et al., (1993) reported the effects of the volatile oil extracted from *Nigella sativa* seeds on the levels of glucose and insulin in rabbits. The i.p. administration of the volatile oil of *Nigella sativa* seeds (50mg/kg) to fasting normal and alloxan

diabeitc rabbits produced significantly hypoglycemic effects. These effects were consistent and time dependent. The administration of the volatile oil was not found to alter basal insulin levels in different animal groups. It was reported by Presanna Kumar et al., (1993) that the oral administration of the pectin isolated from the fruits of *Coccinia indica* at a dose of 200mg/100g BW/day showed a significant hypoglycaemic action in normal rats. The pectin administration resulted in a significant reduction in blood glucose and an increase in liver glycogen. Glycogen synthetase activity was highly significant. Incorporation of labelled glucose into hepatic glycogen was also found to be higher. A significant reduction in phosphorylase activity was noted in the pectin administered groups. Neem oil extracted from *Azadirachta indica* produced a significant blood glucose lowering activity in normal as well as alloxan induced diabetic rats at +3h and +6h. The decrease in blood glucose levels was more pronounced in hyperglycaemic rats. (Dixit et al., 1992). Dimethoxy ether of leucopelargonidin 3-O-alpha-L-rhamnoside isolated from the bark of the Indian Banyan tree *Ficus bengalensis* at a dose of 100mg/kg on oral administration to alloxan diabetic dogs showed a significant hypoglycemic action. It was also noted that there was an increase in the serum insulin (Augusti et al., 1994). The hypoglycemic activity of a decoction from juniper "berries" *Juniperus communis* both in normoglycaemic and in streptozotocin diabetic animals has been reported by de Medina et al., (1994). Juniper decoction decreases glycaemic levels in normoglycaemic rats at a dose of 250mg/kg. This effect can be achieved through an increase of peripheral glucose consumption and a potentiation of glucose induced insulin secretion. The effect of the butanol extract of *Zizyphus spina* christii leaves as well as christinin A, its main saponin glycoside has been investigated in normal and streptozotocin diabetic rats by Glombitza et al.(1994). In normal rats, treatment in both cases for one and four weeks produced insignificant changes in all studied parameters.

However, in diabetic rats, both treatments significantly reduced serum glucose level, liver phosphorylase and glucose-6-phosphatase activities, and significantly increased serum pyruvate level and liver glycogen content after 4 weeks treatment. There was also marked improvement in glucose utilization in diabetic rats in both cases. Serum insulin and pancreatic cAMP levels showed significant increase in diabetic rats treated for a period of 4 weeks with butanol extract. Kato and Miura (1994) reported that the methanol extract of rhizomes of *Polygonatum officinale* administered orally to streptozotocin induced diabetic mice, significantly reduced the blood glucose. Park and Cho (1994) investigated the effect of *Commelina communis* on the blood glucose level in alloxan induced diabetic rats and the biochemical properties of glucose-6-phosphate dehydrogenase from the rat livers. *Commelina communis* extract decreased blood glucose level in the alloxan induced diabetic rats ~~was~~ and the loss of body weight was recovered. Administration of the plant protein fractions elicited the significant increase of glucose-6-phosphate dehydrogenase activity and liver weight which were decreased in the diabetic rat liver. They suggest that the hypoglycemic activity of the extract is associated with the action of the enzyme on the rat liver. Hypoglycemic effect of *Artemisia herba* alba was reported by Al Shamaony et al. (1994). Their study revealed that the feeding of diabetic rats and rabbits with 0.39g/kg body weight of the aqueous extract of the aerial part of the plant for 2-4 weeks showed a significant reduction in blood glucose level, elevation of glycosylated haemoglobin level and hypoliposis effect, in addition to the protection against body weight loss of diabetic animals. Kumud et al. (1995) reported that S-methyl cysteine sulphoxide (SMCS), a sulphur containing amino acid isolated from *Allium cepa* showed antidiabetic and antihyperlipidemic effects. Oral administration of SMCS daily at a dose of 200mg/kg body weight for a period of 45 days to alloxan diabetic rats controlled significantly their blood

glucose and lipids in serum and tissues and altered the activities of liver hoxokinase, glucose-6-phosphatase and HMG CoA reductase towards normal. The effects of SMCS were comparable to those of glibenclamide and insulin. Khan et al. (1995) studied the effect of *Murraya koeniji* and Brassica juncea on carbohydrate metabolism using rats as experimental animals. Both showed significant hypoglycemic action. There was an increase in the concentration of hepatic glycogen and glycogenesis, as evident from the increased activity of glycogen synthetase, and decrease in glycogenolysis as evident from the decreased activity of glycogen phosphorylase and gluconeogenic enzymes. Khosla et al. (1995) reported a dose dependent hypoglycaemic activity of *Trigonella foenum graceum.* Oral administration of 2g and 8g/kg of seed powder to normal and alloxan induced diabetic rats significantly reduced the blood glucose in both the groups. Similarly Ali et al..(1995) treated alloxan diabetic and normal rats with whole seed powder of *Trigonella foenumgraecum.* The powder, its methanolic extract and the residue remaining after methanol extraction has significant hypoglycaemic effects when fed simultaneously with glucose. The water extract of the methanol extractive free residue of the seed powder showed significant hypoglycaemic activity at different prandial states. The soluble dietary fibre (SDF) fraction showed no effect on the fasting blood glucose levels of non diabetic or NIIDM model rats. However, when fed simultaneously with glucose, it showed a significant hypoglycaemic effect in NIIDM rat models. Chemical analysis showed that SDF is the key factor which is responsible for the hypoglycaemic effect of Trigonella foenu graecum seeds. However, compounds other than SDF are also involved in the hypoglycaemic activity. Investigations were carried out to evaluate the effect of the hot water extract of black tea *Camella sinensis* on streptozotocin induced diabetes in rats by Gomes et al. (1995). The extract significantly reduced the blood glucose level and was found to

possess both preventive and curative effects on experimentally produced diabetic rats. Chung and Lee (1995) reported the antidiabetic activity and effect on hepatic glutathione metabolism of polysaccharide from *Trichosanthes kirilowii* in alloxan diabetic rats. The polysaccharide inhibited the increase of blood glucose, triglyceride levels and LDH activity, but cholesterol did not change. It increased protein bound -SH, non protein bound -SH glutathione level and inhibited the decrease of glutathione S-transferse. Basnet et al. (1995) showed that Bellidifolin, isolated from *Swertia japonica* as a potent hypoglycaemic agent in streptozotocin induced diabetic rats by both oral and intraperitoneal administration. Bellidifolin significantly lowered the loaded glucose level in normal as well as diabetic rats; so also it lowered blood triglyceride level significantly. It stimulated glucose uptake activity in fibroblast cells expressing human insulin receptors. Jimenez et al. (1995) reported the antidiabetic activity of *Salvia lavandulifolia* extract on rats pancreotomized by streptozotocin. At a dose of 10mg dry residue/kg the extract produced an increase in Langerhan islet number and size and an increase in pancreatic insulin content. When both *Salvia lavandulifolia* extract (10mg/kg) and glibenclamide (1mg/kg) were administered concurrently to streptozotocin diabetic rats, a significant decrease in glycemic level and mortality index was achieved. The aqueous extract of *Artemisia herba alba* produced an initial hyperglycaemia in normoglycaemic and alloxan treated rabbits and mice. The extract significantly increased gastrointestinal transit time and the reaction time to thermal stimuli, but had no effect on the activity of alkaline phosphatase or concentration of creatinine and urea in plasma. Histopathological examination indicated mild hydropic degeneration in hepatocytes and proximal convoluted tubules. The duodenum showed mild oedema of the substantia of the mucosal propria. The extract also exhibited mild anti microbial activity. (Marrif et al., 1995). Romal et al. (1995) reported the antihyperglycaemic effect of 12 edible plants on 27 healthy rabbits,

submitted weekly to subcutaneous glucose tolerance test (GTT) after gastric administration of water, tolbutamide or a traditional preparation of plant. Tolbutamide, *Cucurbita ficifolia, Phaseolus vulgaris, Opuntia sativus* and *Cuminum cyminum* decreases significantly the area under the glucose tolerance curve and the hyperglycaemic peak. The glycemic decrease caused by *Psidium guajava, Brassica oleracea* and *Lactuca sativa* were not significant. The integration of a menu that includes the edible plants with hypoglycaemic activity for the control and prevention of diabetes mellitus may be possible. Oral administration of the chloroform extracts of *Parkia speciosa* empty pods to alloxan induced diabetic rats produced a significant reduction in blood glucose levels. A hypoglycaemic assay guided extraction, isolation and structure elucidation gave stimast-4-en-3-one. It produced 84% at 100mg/kg body weight compared to 111% activity of glibenclamide at 5mg/kg body weight dosages. The minimum effective dose which produced statistically significant hypoglycaemic effect was 50mg pericarp/kg body weight. Hypoglycaemic effect was not observed in healthy rats. The said compound is therefore identified as a new oral hypoglycaemic agent occurring naturally in nature as reported by Jamaluddin et al. (1995).Sharma et al. (1996) showed that the oral administration of 50% ethanolic extract of *Cinnamomum tamala* leaves significantly lowered plasma glucose levels in normoglycaemic and streptozotocin hyperglycaemic rats. The extract also exhibited hypercholesterolemic and anti hyperglyceridemic effects in streptozotocin hyperglycaemic rats.

Recently the effect of oral administration of ethanolic extracts of *Luffa aegyptiaca* seeds and Carrissa edulis leaves on blood glucose leaves both in normal and streptozotocin diabetic rats was reported by El-Fiky et al., (1996). Treatment with both extracts significantly reduced the blood glucose levels both

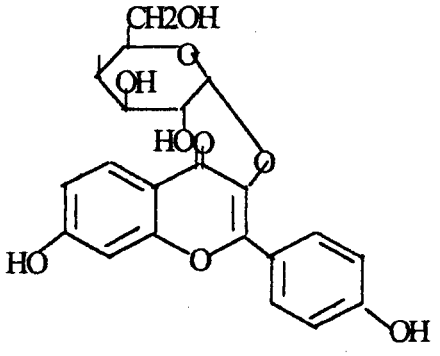
in normal and streptozotocin diabetic rats during the first three hours of treatment. *Luffa aegyptica* extracts decreased blood glucose levels with a potency similar to that of biguride, metformin. Hypoglycaemic efficacy of an aqueous extract of *Ficus carica* leaves on streptozotocin diabetic rats has been reported by Perez et al. (1996). The extract induced a significant hypoglycaemic effect after either oral or intra peritoneal administration. Body weight loss was prevented in treated diabetic rats and the survival index was significantly affected by plasma insulin levels. *Ficus carica* aqueous extract showed a clear hypoglycaemic activity in treated versus non treated diabetic rats. Subramoniam et al, (1996) reported that the oral administration of the methanol extract of the aerial parts of *Artemisia pallens* led to a significant blood glucose lowering effect in glucose fed hyperglycaemic and alloxan induced diabetic rats. The effect of the drug was dose dependent and significant at 100mg/kg levels in glucose fed rats. In fasted normal rats, the extract caused a moderate hypoglycaemic effect at a higher dose (1000mg/kg).Enigbokan et al., (1996) showed the hypoglycaemic activities of *Opuntia ficus-indica, Opuntia lindheimeri* and *Opuntia robusta* in streptozotocin induced diabetic rats using oral and parenteral routes of administration. When the aqueous extracts of the three *Opuntia* species were administered intravenously, they produced hypoglycaemic effect. However, when the extracts were adminstered orally, only *Opuntia lindheimeri* produced hypoglycaemic effect. A dilute aqueous solution of *Opuntia lindheimeri* dialysate also produced hypoglycaemic effect when adminstered intravenously. Intraperitoneal administration of all *Opuntia* species extracts failed to produce hypoglycaemic effects. The concentration and potency of active hypoglycaemic principle in *Opuntia cacti* have been reported to be species specific. Eno et al., (1996) reported that the crude extract from the leaves of *Elaephorbia drupifera* reduced the blood glucose levels in rats that received the extract 2h before glucose

administration. The extract was found to be faster and more effective than glibenclamide in lowering the blood glucose levels in fasting rats.

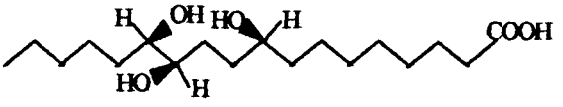
There may be several possible mechanisms of action of the active principle from plant sources. They may act on B cells of the pancreas and stimulate the secretion of insulin, inhibit A cells or hyperglycaemic factor, enhance the effect of insulin and adrenaline or assist in inhibiting the synthesis of glucose-6-phosphate phosphatase, fructose diphosphatase, pyruvate carboxylase or phosphoenol pyruvate carboxykinase and stimulate the synthesis of glucokinase. Since the plant products are found to be effective in the management of diabetes there is an earnest effort among the scientists to isolate the active principle and characterise it. Some of the compounds that show hypoglycaemic activity in experimental animal models which were purified and characterised are given in Figure ~~11~~ 2.

It is all too common a misconception that "all that is natural is good", and a number of highly toxic compounds have been isolated from plants, for instance many of the tannin containing plant extracts are toxic to the liver while many extracts containing alkaloids have side effects on a number of different parts of the body. The fact that most of the plant materials have been used for generations for treatment is, however, convincing evidence that many of the herbal prescriptions are reasonably safe but scientific toxicological trials are still necessary (Atta-ur-Rahman 1985). This will involve detailed toxicological tests both on crude plant extracts as well as on the purified substances which show hypoglycaemic activity. At the same time, the synergistic actions of different compounds in a crude extract cannot be ignored. Extensive studies in animals cannot entirely eliminate the possibility of unanticipated injurious effects of plant

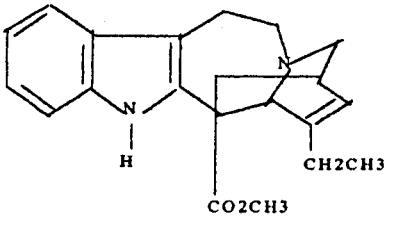
Figure 2



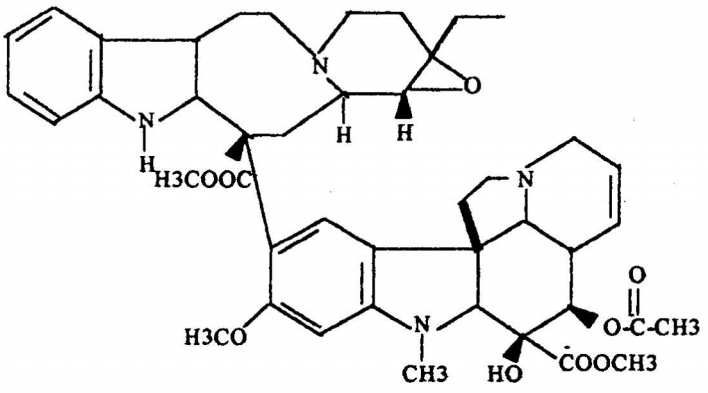
Kaempferol-3-galactoside



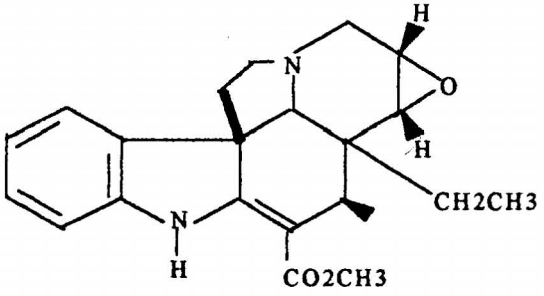
Trihydroxyoctadecadienoic acid



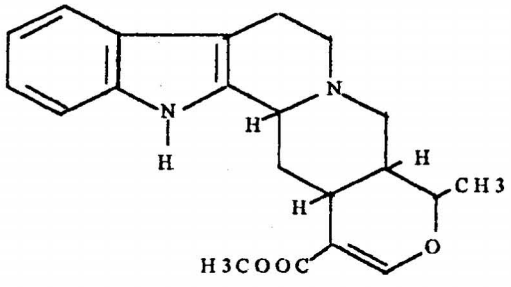
Catharanthine



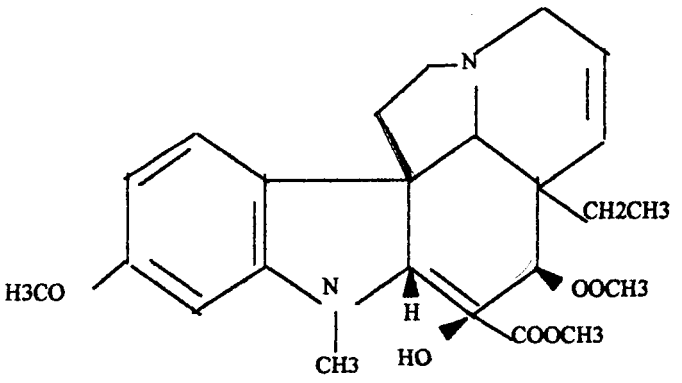
Leurosine



Lochnerine



~~Tetrahydroalstonine~~



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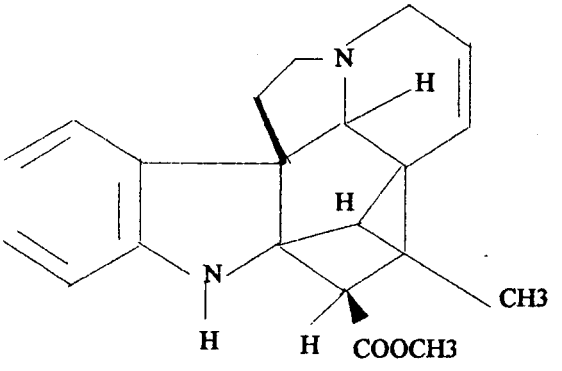

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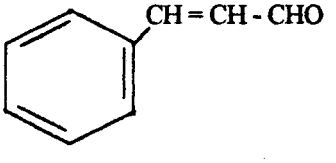
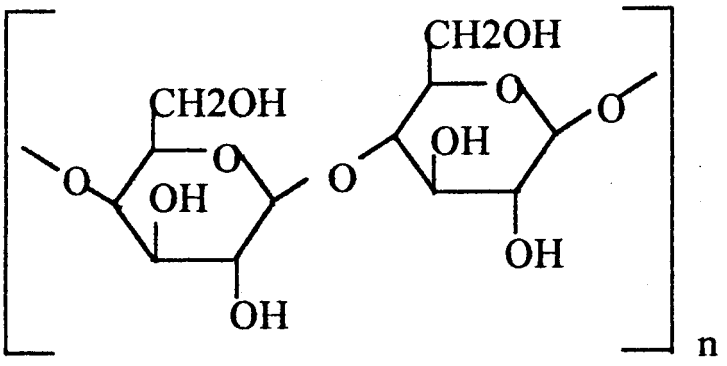
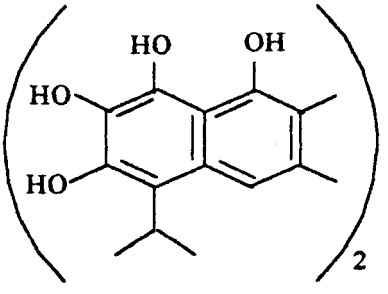


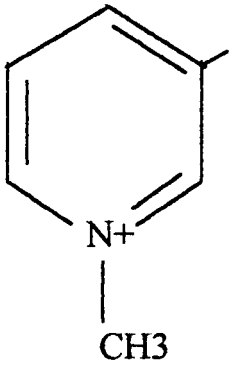
Cinnamaldehyde



γ- Cellulose



Gossypol



Trigonelline

derived drugs. Care must therfore be exercised before herbal extracts can be used either in indigenous or modern systems of medicine. Moreover, many of the plants show only very weak activity which may not be of therapeutic utility.

Aims and Objectives

Diabetes is a disease known for a long time and inspite of research carried out at international levels there is no suitable medicine to completely cure the disease except to control and prevent other complications. India with different varities of plants is a good source of plant products for the treatment of various diseases including diabetes. The Indian system of medicine has utilised the plant products for controlling diabetes and the plant *Aegle marmelos* is used by selected physicians in the state of Kerala. Its antidiabetic principle has been studied by many others. However, detailed investigations have not been carried out to assess the antidiabetic property of this plant. In order to unravel the efficacy of this plant product, experiments ~~are~~ were carried out using Swiss albino rats as the model to achieve the following aims:

1. **To establish the hypoglycaemic effect of the leaves of *Aegle marmelos*.**

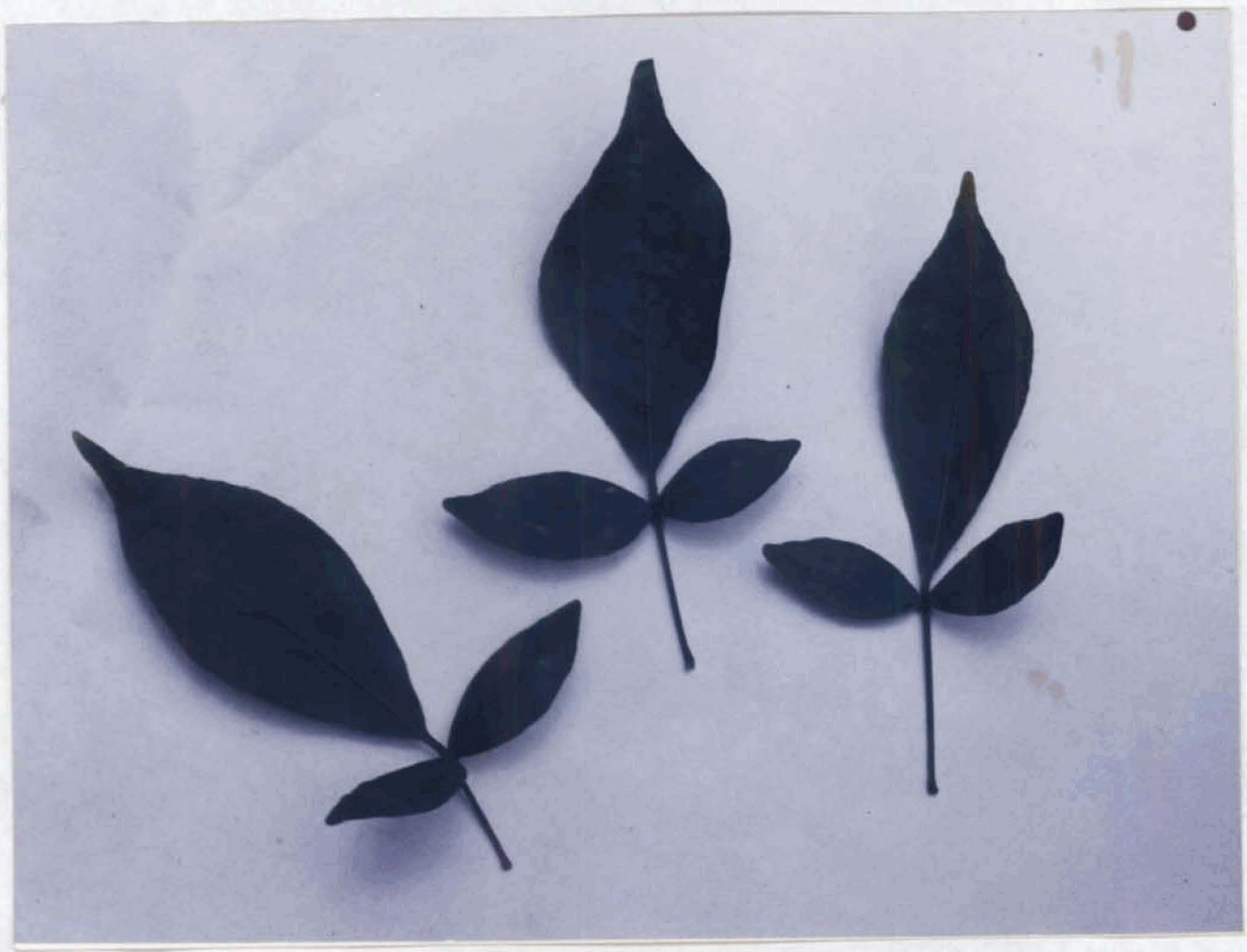
2. **To identify the most suitable solvent that would elicit the hypoglycaemic response.**

3. **Isolation and identification of the hypoglycaemic principle from the most desirable extract.**

Aegle marmelos

Family - Rutaceae

Trifoliate leaves of ***Aegle marmelos***



The following parameters were studied in the experimental animals for establishing the anitdiabetic effect of the leaves of *Aegle marmelos.*

Body weight

Blood glucose

Blood urea

Serum cholesterol

Liver glycogen

Serum insulin

Glucose Tolerance Test

Glucose uptake by diaphragm muscles

Glucose uptake by spleen cells

Glucose uptake by intestinal segments

Lipid peroxidation

Aegle marmelos (Linn) Correa

This plant is commonly known as "bael fruit tree" and is usually confined to the tropical regions of the world. It is comparatively a small tree which is spinous and has numerous branches which bear trifoliate leaves. The leaflets lanceolate, membranous, subcrenulate and pubescent. The tender leaves are confined to the apical regions of branches and new leaves are added throughout the year.

Chemical constituents of leaves of *Aegle marmelos*

The pulp contains mucilage, pectin, sugar, tannin, volatile oil, bitter principle, 2 per cent ash and a balsamic principle. Fresh leaves yields on distillation a yellow green oil with a peculiar aromatic odour- Marmelosin. The roots, leaves and bark when extracted with different solvents and analysed found to contain sugars, tannins and alkaloids mainly.

CHAPTER II

MATERIALS AND METHODS

CHAPTER II

MATERIALS AND METHODS

2.1. Introduction

Plant products have played a major role in finding a proper drug for diabetes and also in synthesising it by either chemically or biosynthetic methods and made available for human beings. The methods adopted for testing and trial of active principle as a source of medicine in animals are to be carried out by well known established fool proof methods. In this chapter we are describing the different methods to find the effectiveness of the drug in experimental diabetes. The animals used are Swiss albino rats as they are found to be more ~~near to~~ Similar in response to the drugs as in humans. ~~humans and effective against diabetic patients~~.

2.1.1. Material used

To detect the antidiabetic principle, fresh tender leaves of *Aegle marmelos* Correa ~~was~~ were selected for the work. The leaves were collected from the apical region of the healthy branches of the trees, which are usually seen in the temple premises of Kerala. The leaves contain an active antidiabetic principle as reported by Chatterjee and Bose (1952), Dhar *et al.* (1968), Chakraborthy and Poddar (1984).

2.1.2. Chemicals used

Alloxan	Sigma Chemical Company, St.Louis, USA.
M199	Gibco, BRL, Paisley.

Trypsin-EDTA	Gibco, BRL, Paisley.
PBS	Sigma Chemical Company, St.Louis, USA.
Glucose assay kit	Ortho Diagonostic Systems.
Radioimmunoassay kit	BRIT, Bombay.
Polyethylene glycol	
Bovine Serum Albumin	
Copper Sulphate	
Sodium potassium tartrate	
Folin-Ciocalteau reagent	
Sodium hydroxide	
Cholesterol	

2.1.3. Equipments Used

1. UV Visible Spectrophotometer - Bausch and Lomb Spectronic 1001
2. High Speed Cold Centrifuge - Remi
3. Colorimeter - Erba AE-11M
4. Lyophilizer - Labconco
5. I^{125} Gamma Counter IC4702 - ECI Ltd.
6. Oven - MC Dalal & Co.
7. Electric Monopan - Dhona, Special Instruments Consortium
8. Titertek Multiskan Plus - Cecil Instruments, UK.
9. Microtiter Plates - Libro, Virginia, USA.
10. Water Jacketed Incubator - Forma Scientific, UK.
11. Digital Ultraviolet Spectrophotometer - Cecil Instruments, UK.

2.1.4. Preparation of whole leaf powder

Fresh tender leaves of authentic species were collected and washed well to fray dirt and other impurities which are likely to be present in plants. The leaves were dried in the shade of the sun and pulverised to a powder about 1mm in diameter. The leaf poder ~~in~~ was kept in dry condition under low temperature till use.

2.1.5. Preparation of whole leaf powder suspension

Ten grams of leaf poder was mixed with 1g of gum acacia . The 100 ml of distilled water was added and stirred for 10 min. This was adminstered orally to diabetic animals.

2.1.6. Preparation of aqueous extract

Ten grams of leaf powder was mixed with 100 ml of distilled water and stirred well for 2h. It was kept at 4^0C overnight and the supernatant was collected. This was centrifuged for 5min and the active extract separated. This extract was administered orally to diabetic animals.

2.1.7. Extraction with other solvents

The extraction of the leaf powder was carried out in a similar way as described in 2.1.6 with petroleum benzene, choloroform and methanol. The organic solvent from the extract was allowed to evaporate off in a water bath and

the residue was resuspended in distilled water. This was administered orally to diabetic animals.

2.1.8. Preparation of alkaloids from leaf powder

Twenty grams of the dried leaf powder was mixed with 100 ml of petroleum ether and stirred for 30 min to remove the fatty material. This was followed by extraction with methanol. After the removal of the solvent, water was added to the residue and the mixture was acidified to pH 2 with 10 per cent HCl. It was kept for 10 days at 4^0C to obtain a clear solution which was then decanted. Water soluble non-basic organic substances were then separated by extraction with chloroform. The solution was then basified to pH 10 using 5 per cent sodium carbonate. It was then extracted with chloroform to extract free bases. The solvent was removed and the aqueous mixture of alkaloids was lyophilized and 1 g/100 ml sample was prepared to study the antidiabetic activity.

2.2.1. Animals and maintenance

Inbred Wistar strains of Swis abino male rats (Rattus norvegicus) were purchased from The Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy, Trichur, Kerala. They were housed in ventilated cages having paddy husk, kept in air conditioned animal house and fed with mouse chow (Lipton India) and water ad libitum.

2.2.2. Method to induce diabetes in animals

The animals were anaesthetised using diethyl ether and through the femoral vein alloxan monohydrate (Sigma) in cold buffer was injected. The dose was 60 mg/kg. After 5 days the blood sugar level was monitored and those animals which had blood sugar above 150 mg/100 ml were used as the diabetic animals for the experiment.

2.2.3. Experiment set up

The animals were divided into the following groups. Each group had six rats, two in each cage.

Group 1 - Kept as the normal group

Group 2 - Physiological saline injected through the femoral vein and was taken as the control group

Group 3 - Alloxan injected through the femoral vein and kept without any treatment to study the diabetic nature

Group 4 - Alloxan injected and one unit of insulin injected on all days after 5 days and the injection continued throughout the experimental period.

Group 5 - Alloxan injected and the drug administered orally after 24 hrs and the treatment continued throughout the experimental period.

Group 6 - Alloxan injected and the drug administered after 5 days and the treatment continued throughout the experimental period.

Group 7 - Alloxan injected and the aqueous extract given orally after 5 days and the treatment continued throughout the experimental period.

Group 8 - Alloxan injected and the alkaloids given orally after 5 days and the treatment continued throughout the experimental period.

Group 9 - Alloxan injected and the euglucon (a biguanide) given orally after 5 days and the treatment continued throughout the experimental period.

Before starting the experiment the body weight and the blood glucose were estimated. The blood was taken from the tail vein. After every 5 days the body weight and the blood glucose were monitored in all groups of animals. After 30 days the animals were sacrificed. The blood was collected from the heart and the serum separated was stored at -20^{0}C for insulin estimation. The visceral organs such as heart, brain, liver and kidney were removed, and kept at -20^{0}C for the estimation of liver glycogen, total protein and cholesterol.

2.2.4. Testing for diabetes

The method adopted here was to test the blood glucose and detect the presence of glucose in urine. We found that the collection of blood was easier from the tail vein by a standard accepted procedure. In several experiments we used the heparinised blood rather than the serum or whole blood. Plasma was separated and subjected to blood glucose estimation by utilising glucose oxidation reaction, the details of which are given elsewhere. The metabolic effect of the drug in reducing the blood sugar level were assessed by a standard protocol system by slight modification.Diabetes was induced in rats by injecting alloxan at a dose of 60mg/kg in buffer through the femoral vein. Diabetes was developed within 5 days and the blood glucose level was maintained by administration of insulin. The lowering of the blood sugar level was noted by different doses of the drug and the optimum concentration was thus obtained.

Several experiments to be carried out are done using the animals as set up in 2.2.3

In the case of extraction we have found detectable amount of the active principle in the aqueous extract. This was further concentrated by freeze drying. By this process a powdery material was obtained.

2.3. Methods

2.3.1. Estimation of glucose

The blood glucose was estimated by using glucose assay kit purchased from Ortho Diagnostic Systems.

Glucose is oxidised by glucose oxidase (GOD) to give gluconic acid and hydrogen peroxide is broken by peroxidase (POD) to water and oxygen. The latter oxidises phenol which combines with 4-Aminophenazone to give a red coloured complex. The intensity of the red coloured complex is proportional to the concentration of the glucose in the specimen under test. The intensity of the coloured complex is measured colorimetrically at 530 nm. The standard curve was prepared and the amount of glucose present in the samples was calculated.

2.3.2. Isolation and estimation of glycogen

Exactly 1.5 g of liver was digested in 2 ml of 30 per cent KOH by keeping it in a boiling water bath for 20 min. The tubes were cooled in ice. Then 0.2 ml of saturated sodium sulphate was added and mixed thoroughly. The

glycogen was precipitated by adding 5 ml of ethanol (95 per cent v/v). It was allowed to stand in ice for 5 min and the precipitate was removed by centrifugation. The supernatant was discarded and the precipitated glycogen was dissolved in 5 ml of water with gentle warming. It was diluted with distilled water to 10 ml and mixed thoroughly.

Then 1 ml of glycogen solution was taken in a calibrated test tube and 1 ml of 1.2 M HCl was added. It was kept in a boiling water bath for 2 hrs. After incubation one drop of phenol red indicator was added and it was neutralised with 0.5 M NaOH. The glucose content in the above sample was determined.

2.3.3. Estimation of Urea

The urea was estimated by using the Berthelot Reaction.

The ammonia formed from urease action reacts with phenol in the presence of hypochlorite to form an indophenol which reacts with the alkali to give a blue coloured compound. Nitroprusside acts as a catalyst increasing the rate of the reaction. The intensity of the colour is proportional to the concentration of urea and it is measured colorimetrically at 630 nm. The standard curve is prepared and the amount of urea present in the samples was calculated from this.

2.3.4.Estimation of cholesterol

The cholesterol was estimated by using Lieberman-Burchard reagent.

The solvent system includes acetic acid, acetic anhydride and concentrated sulphuric acid. The reaction involves the 3-hydroxy 5-ene part of the

cholesterol molecule which is first hydrated to form cholesta - 3, 5 - diene and then oxidised by the sulphuric acid to link two molecules together as bis-cholesta - 3, 5 diene. This material can be sulphonated by concentrated sulphuric acid which are highly coloured. The intensity of the colour depends on the concentration of cholesterol and this can be measured colorimetrically at 650 nm. The standard curve is prepared and the amount of cholesterol present in the samples were calculated from this.

2.3.5. Estimation of protein

The protein was estimated by the Lowry Method.

In a two step reaction, Folins reagent reacts with the aromatic amino acids in protein after treatment with alkaline copper to give a blue colour. This is the principle of this method. The following reagents were prepared.

Solution A : contains 1 ml $CuSO_4$ $5H_2O$ (1%) plus 1 ml sodium potassium tartrate (2%) and 98 ml 2 per cent Na_2CO_3 in 0.1 N NaOH. Solution B : Folin - Ciocalteau reagent 1 : 1 in distilled (prepared immediately before use).

BSA standard 1 mg/ml in distilled water. The test was as follows. Protein samples (100µl) was diluted to 1.2 ml with distilled water and 6 ml of solution (A) was added, mixed and incubated at room temperature for 10 min. Then 300µl of solution B was added with constant mixing and kept for 30 min at room temperature. The colour developed was read in a spectrophotometer at 750 nm against the reagent blank and with a BSA standard. The optical densities were converted to milligram protein from the standard curve.

2.3.6. Estimation of insulin

The serum insulin was estimated using the radioimmunoassay kit (BRIT, Bombay). This method is based on the competition of unlabelled insulin in the standard or samples and radioiodinated (I-125) insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin are separated by the second antibody polyethylene glycol (PEG) separation method. Insulin concentration of samples was quantiated by measuring the radioactivity associated with the bound fraction of samples and standards.

2.3.7. Glucose tolerance test (GTT)

The rats were divided into four groups of six each and were fasted for 18 hrs. The first group was kept as the control. The second group was given 500 mg/kg body weight the whole leaf powder, the third group 1 g/kg body weight the aqueous extract and the fourth group 100 mg/kg body weight the alkaloids given an hour prior to the administration of 1.5 g/kg body weight of glucose. The blood sugar levels of all the animals were estimated after intervals 30, 60 and 90 min respectively.

In an identical experiment diabetic animals were used to study the glucose tolerance.

2.3.8. Determination of lipid peroxidation

The ascorbic acid induced non-enzymatic lipid peroxidation using liver homogenate was determined by the thiobarbituric acid (TBA) method (Bishayee and Balasubramaniyan, 1971). Rat liver homogenate (25%, 100µl) in cold tris HCl buffer (0.2 M, pH 7.0) was incubated for 1 hr at 37°C with 150 nM KCl (100µl), 0.3 nM ascorbic acid (100µl) and tris HCl buffer (0.2 M, pH 7.0) in a total volume of 500µl. Test materials were also added during the incubation period. Controls without test materials were also kept. After incubation, 20 per cent trichloroacetic acid (1 ml) was added and the mixture heated in a boiling water bath for 15 min. Tubes were then cooled to room temperature and centrifuged at 2000 g. Optical density of the supernatant was measured. The amount of lipid peroxide was expressed as nanomoles of malonaldehyde formed in each tube.

2.3.1.1. Glucose uptake studies

These studies were to be carried out to ascertain whether glucose can be absorbed by the intact cells and the criteria which requires this process. It can also give an idea regarding the role, if any, in this felicitated transport for our extract. The glucose uptake in intact cells can be carried out by studying the effect on isolated diaphragm and the effect on individual cells can be understood by utilising spleen cells.

2.3.1.2. Isolation of diaphragm and the study of glucose uptake with the extract

Normal healthy rats of 90 - 120 days and weighing 180 - 200g were used. The animals were sacrificed by cervical dislocation and the diaphragm muscle was carefully removed asceptically into a petri dish containing cold PBS. The diaphragm was cut into bits weighing 100 - 150mg and these pieces were transferred to 2ml PBS and pre incubated for 10min at 37°C. The tissue was transferred to 2ml PBS having 400mg/100ml glucose. Then 500µl of the aqueous leaf extract was added and in the controls 500ul of distilled water. The whole experiment set up was incubated at 37°C for 60min. After incubation the glucose in the medium was estimated.

In an identical experiment the alkaloids extracted from the leaf in a volume 100µl was added and the amount of glucose in the medium estimated after 60 min.~~was estimated~~.

2.3.1.3. Preparation of single cell suspension from spleen

The rats were sacrificed as described in 2.3.1.2 and the spleen was removed asceptically into a petri dish containing cold M199 medium supplemented with 10% foetal calf serum. The spleen was teased with a toothed forceps and the pieces transferred to a homogeniser which has cold M199 with 10% foetal calf serum. The spleen pieces were gently homogenised. The homogenate was passed through a sieve to remove solid particles. The cells were treated with trypsin - EDTA for 5min and then washed thrice with HTPBS. The cells were washed twice in the medium containing 10% foetal calf serum and the viability of the cells were checked by trypan blue exclusion method. The cell suspension which showed 90% viability were selected and $1x10^6$ cells were transferred to 96 well

microtiter plates. Volume in each well was made up to 250ul with complete medium. Then 50ul of aqueous extract was added to the wells and the controls 50ul of distilled water. The plate was incubated in a humudified incubator at 37C and the glucose content in the medium of the experimental and control wells were estimated after 30min, 60min and 90min.

In a similar experiment as described, alkaloids extracted from the leaves in a volume 10µl was added into the wells and the glucose content of the medium was estimated.

2.4.1. Invitro study of glucose uptake by intestinal segments of rats.

Normal healthy rats of 90 - 120 days old and weighing 180 - 200 g were selected . The animals were sacrificed by cervical dislocation and the small intestine was cut off and transferred to a petri dish containing PBS. The intestine was cut into bits of 1cm to 2cm and the contents inside the intestine were carefully removed by forcing PBS through it with a syringe. The intestinal segments were made inside out and it was tied with a thread and suspended in 2ml PBS with 400mg/100ml glucose in a test tube. The 500µl of the aqueous extract was added to the experimental group and 500µl of PBS to the control tubes. The experimental set up was put in an incubator at 37^{0}C and glucose content of the medium estimated after 30min, 60min and 90min, respectively.

In an identical experiment 100µl of alkaloid extract of the leaf powder was added and the glucose content of the medium estimated.

2.6. Statistical evaluation

Statistical significance of the datas obtained were determined using student's "t" test (Lutz, 1978). The value of "t" was determined using the formula

$$t = \frac{\bar{X} - \bar{Y}}{\sqrt{S\left(\frac{1}{nx} + \frac{1}{ny}\right)}}$$

Where X and Y are the arithmetic means of the two sample groups, nx and ny being the two sample sizes.

The value of S was found out using the equation

$$S = \sqrt{\frac{(nx-1)Sx2+(ny-1)Sy2}{nx+ny-2}}$$

Where Sx is the standard deviation of X and Sy that of Y nx + ny -2 represents the degrees of freedom from which statistical significance (P value) was deduced with the help of "t" distribution table.

CHAPTER III

ANTIDIABETIC ACTIVITY OF THE WHOLE LEAF POWDER OF *AEGLE MARMELOS*

CHAPTER III

Antidiabetic activity of the whole leaf powder of *Aegle marmelos*

3.1.Introduction

In Ayurvedic system of medicine plants and plant products have played a crucial role in the treatment of diabetes mellitus . The patients prefer natural antidiabetic agents since most of these have no side effects and toxicity and also because the prolonged treatment of diabetes with oral hypoglycaemic agents is not effective in lowering the blood glucose level.

3.2.Materials and Methods

The whole leaf powder of *Aegle marmelos* was prepared as described in 2.1.4 and the leaf powder suspension was constituted as described in 2.1.5. Inbred Wistar strain male albino rats were used for the experiment and the rats were maintained as described in 2.2.1. The animals were made diabetic by intravenous injection of alloxan monohydrate as per the procedure described in 2.2.2.

3.2.1. Experiment set up

The animals were divided into the following groups. Each group has six rats, two in each cage.

Group1: Kept as normal group.

Group2: Physiological saline injected though the femoral vein and was taken as the control.

Group3: Alloxan injected and kept without any treatment to study the diabetic nature.

Group4: Alloxan injected and 1 unit of insulin injected on all days after 5 days of alloxan injection.

Group5: Alloxan injected and the whole leaf powder (500mg/kg) administered orally after 24h and the treatment continued on all days for 30 days.

Group6: Alloxan injected and the whole leaf powder (500mg/kg) administered orally after 5 days and the treatment continued on all days for 30 days.

Before starting the experiment the body weight and blood glucose was estimated. Then the blood glucose and body weight of the animals were monitored every 5 days till 30 days. At the end, the animals were sacrificed by cervical dislocation and blood was collected from the heart and the serum separated to estimate the insulin, cholesterol and urea. The liver was used to estimate the glycogen content.

3.3. Results

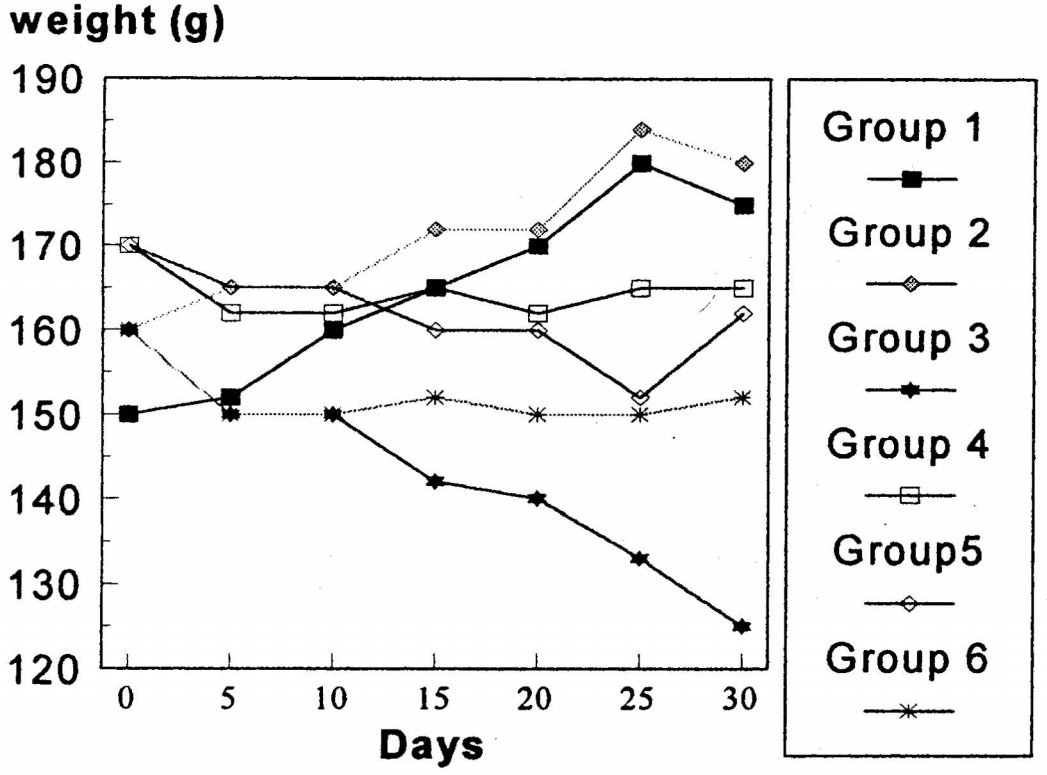
In the normal animals and saline injected control group the body weight was increasing till the end of the experiment. But in the alloxan injected diabetic group left without any treatment, it was noted that the body weight decreased substantially during the first five days and then went on decreasing. In the insulin treated group, the decrease in body weight was not significant when compared with the diabetic group. If the whole leaf powder is given after 24h of alloxan injection, a sudden decrease in body weight is prevented in the initial days

eventhough the gaining of weight is not at par with the normal controls. If the whole leaf powder is administered after five days of alloxan injection, the drop in body weight of that group is prevented when compared with the diabetic group (Fig3.1).

The blood glucose content of the different groups in the experiment are represented inTable 3.1. In the normal and saline injected control animals the blood glucose level was maintained at a range 74mg/dl to 84mg/dl throughout the experimental period. In the alloxan injected group left without any treatment, the blood glucose level increased substantially and this trend continued till the end of the experiment period. But in insulin treated group, the increase in blood glucose was prevented. In the whole leaf powder treated group, after 24hrs of alloxan injection the increase in blood glucose was not significant though there was a slight increase when compared with the normal control. In the whole leaf powder treated group, after 5days of alloxan injection the abnormal increase in blood glucose was prevented.

The blood urea level of the different groups of animals in the experiment after 30days is presented in Table3.2. In the normal and saline injected control group, the blood urea value was on an average 24mg/dl. In the diabetic group there was a significant increase in blood urea (58mg/dl $P<.05$) when compared with the normal control group. In the insulin treated group the blood urea level was 34mg/dl. In the groups where the whole leaf powder was administered after 24hrs and 5 days of alloxan injection, the blood urea level was 27mg and 44mg/dl respectively.

Fig 3.1 . The weight of the animals (g) treated with the whole leaf powder of *Aegle marmelos.*



Group 1 - Normal animals
Group 2 - Saline injected control
Group 3 - Diabetic group
Group 4 - Diabetic and insulin treated (1 unit of insulin daily)
Group 5 - Diabetic and leaf powder treated after 24 h of alloxan injection (500 mg/kg daily)
Group 6 - Diabetic and leaf powder treated after 5 days of alloxan injection (500 mg/kg daily).

Table 3.1. The blood glucose (mg/dl) levels of animals treated with the whole leaf powder of *Aegle marmelos*.

Animal Status	Days						
	0	5	10	15	20	25	30
1. Normal group	78 ± 0.89	76 ± 1.0	76 ± 0.92	82 ± 1.6	76 ± 1.6	80 ± 1.1	82 ± 0.8
2. Saline injected control group	82 ± 0.9	80 ± 1.2	84 ± 0.7	80 ± 0.2	84 ± 1.7	80 ± 0.9	84 ± 1.2
3. Diabetic group	80 ± 0.6	152 ± 7.8	187 ± 11	242 ± 7.0	257 ± 12	292 ± 16	347 ± 15
4. Diabetic and insulin treated group	79 ± 0.2	152 ± 9.0	130 ± 6.0	121 ± 5.0	109 ± 8.0	97 ± 5.0	92 ± 7.0
5. Diabetic and leaf powder treated after 24 h	83 ± 1.0	105 ± 9.8	105 ± 4.8	100 ± 7.1	94 ± 8.1	92 ± 6.0	90 ± 4.8*
6. Diabetic and leaf powder treated after 5 days	80 ± 1.7	162 ± 8.9	145 ± 6.7	129 ± 7.9	129 ± 4.2	110 ± 5.1	102 ± 6.1*

Values are mean ± S.D of 6 animals in a group

* $P < 0.05$ compared to the diabetic group

Table 3.2. The blood urea (mg/dl) level of animals treated with the whole leaf powder of of *Aegle marmelos*

Animal status	Blood urea (mg/dl)
1. Normal group	24.27± 3.87
2. Saline injected control group	24.17 ± 3.2.5
3. Diabetic group	58.81 ± 4.77
4. Diabetic and insulin treated group	34.94 ± 3.97
5. Diabetic and leaf powder treated after 24h of alloxan injection	27.40 ± 7.98*
6. Diabetic and leaf powder treated after 5 days of alloxan injection	44.67 ± 8.75*

Values are mean ± SD of six animals in a group

* $P<0.05$ compared to diabetic group

The serum cholesterol levels of animals in the experiment after 30 days are presented in Table3.3. In the normal and saline injected control group it ranged from 83mg/dl to 86mg/dl. In the diabetic group left without any treatment the serum cholesterol level shot up to 190mg/dl. In the insulin treated group the serum cholesterol value was 107mg/dl. But in the groups treated with the whole leaf powder after 24h and 5days of alloxan injection the cholesterol level was 99mg and 102mg/dl respectively.

The liver glycogen of animals in the experiment after 30 days is presented in Table3.4. In the normal and saline injected control group the glycogen level was 2g/100g of wet tissue. In the diabetic group left without any treatment the glycogen level was 1.34g/100g of wet tissue. But in insulin treated group the glycogen level was 1.97g/100g of wet tissue. In the groups treated with the whole leaf powder after 24h and 5days of alloxan injection the glycogen content of the liver was 1.92g and 1.85g/100g of wet tissue respectively.

The serum insulin level in the experimental animals after 30days are presented in Table3.5.

In the saline injected control group the serum insulin level was 11.7μU/ml. In the diabetic group it was 5.7μU/ml. But in the whole leaf powder treated group the serum insulin level was 12.2μU/ml.

3.4. Discussion

The increase in growth rate in the normal and the control group from the initial stage of the experiment till the end of the experiment is due to normal

Table 3.3. The serum cholesterol level of animals treated with the whole leaf powder of *Aegle marmelos*

Animal status	Serum cholesterol (mg/dl)
1. Normal group	86.62 ± 3.65
2. Saline injected control group	83.71 ± 6.76
3. Diabetic group	190.71 ± 12.75
4. Diabetic and insulin treated group	107.32 ± 7.86
5. Diabetic and leaf powder treated after 24h of alloxan injection	99.51 ± 6.72*
6. Diabetic and leaf powder treated after 5 days of alloxan injection	102.32 ± 7.86*

Values are mean ± SD of six animals in a group

* $P<0.05$ compared to diabetic group

44 B

Table 3.4. The liver glycogen (g/100g of wet tissue) level of animals treated with the whole leaf powder of *Aegle marmelos*

Animal status	Glycogen (g/100g)
1. Normal group	2.71 ± 0.12
2. Saline injected control group	2.57 ± 0.42
3. Diabetic group	1.34 ± 0.07
4. Diabetic and insulin treated group	1.97 ± 0.14
5. Diabetic and leaf powder treated after 24h of alloxan injection	1.91 ± 0.07*
6. Diabetic and leaf powder treated after 5 days of alloxan injection	1.85 ± 0.15*

Values are mean ± SD of six animals in a group
* $P<0.05$ compared to diabetic group

Table 3.5. The serum insulin (μU/ml) level of animals treated with the whole leaf powder of of *Aegle marmelos*

Animal status	Serum insulin (μU/ml)
1. Saline injected control group	11.7 ± 0.3
2. Diabetic group	5.7 ± 0.5
3. Diabetic and leaf powder treated after 24h of alloxan injection	12.8 ± 0.6*
4. Diabetic and leaf powder treated after 5 days of alloxan injection	12.1 ± 0.3*

Values are mean ± SD of six animals in a group
* $P<0.05$ compared to diabetic group

growth since at the beginning of the experiment they were only two months of age and were in growing stage till the end of the experiment. In the diabetic group though they were at par with normal and control group in the beginning of the experiment, subsequent to the induction of diabetes their growth rate began to decline substantially from day five. This declining trend continued throughout the entire phase of the experiment, eventhough these animals were also in the growing stage. From this it can be inferred that the induction of diabetes has a negative effect on the growth of animals. The blood glucose level in the normal and control groups remained almost steady. But in the diabetic group (alloxan induced) the blood glucose level increased continually reaching a value 340mg/dl after 30 days. Alloxan injection has been proved to produce severe diabetes by several earlier workers {Giri et al. (1986), Hukeri et al. (1988), Sharma and Mitali (1990), Neera Singh et al. (1990), Iwu et al. (1990) }. The decline in growth rate and increase in blood glucose in diabetic group appears to be highly correlated. It is also observed that in contrast to normal and control group, the diabetic group has an increased blood urea level. Similar observations have been made by Yokozawa et al (1988) by treating alloxan diabetic rats with roots of *Panax gingseng* and there was a significant decrease in blood urea level. Similarly the diabetic group was found to have a high serum cholesterol level in contrast to low serum cholesterol values in normal and control group. The increase of serum cholesterol in diabetic animals is well documented{Yokozawa et al. (1984), Srivastava and Joshi (1990), O'meara et al (1990)}. It is observed that there is a depletion of liver glycogen in alloxan induced diabetic group compared to the normal and control groups. Similar observations have been made by Kimura et al. (1981).

Insulin treatment improved the body weight in the diabetes induced animals when compared with the diabetic group. The improvement in the growth rate of groups 4 and 5 when compared with diabetic group. was significant, But there was no significant difference between the normal and insulin treated group. From this it appears that insulin treatment is capable of enhancing the metabolic activity of diabetic animals like that of normal animals. In group 5 the mean growth rate was better on the fifth and tenth days of post diabetes induction. The growth rate was comparable to that of group 4 during the rest of the periods. There was no significant difference between groups 4 and 5. In group 6, mean body weight was substantially low during the entire period of the experiment though it was significantly higher than group 3. In group 4 the blood sugar values decreased substantially by the 25th day and there was no significant difference with the normal group. The increase in blood glucose in group 4 was substantial compared to the control group. In group 5 the mean glucose level was less on the 5th day compared to groups 3,4 and 6. In group 6 the declining trend in blood glucose was slow even though at the end of 30 days the blood glucose level was significantly less when compared with the diabetic group. Blood urea values in groups 4, 5 and 6 were significantly less when compared to group3. The mean blood urea value was less in group5 when compared to groups4 and 6. There was no significant difference between groups1, 2, 4, 5 and 6. Serum cholesterol value in group4, 5 and 6 was significantly less compared to group3 whereas the difference between groups1, 2, 4, 5 and 6 was not significant. In groups4, 5 and 6 the liver glycogen was significantly higher than that of group3 though less than that of groups1 and 2. The serum insulin level in the diabetic group was significantly less when compared with the normal and whole leaf powder treated groups. It is of importance that the whole leaf powder treated group has shown serum insulin value even higher than the Saline injected control group.

From the above it can be construed that insulin treatment reduces the serum glucose level, improves metabolic activity, growth rate and overall performance of the animal. It also reduces the serum cholesterol and blood urea values. This can be attributed to the physiological and molecular mechanism involved in the carbohydrate metabolism in animals and the key role played by insulin (Pilkis and Park, 1974). The whole leaf powder treatment after 24h of alloxan injection, was found to be superior to insulin treatment as far as body weight, blood glucose level, serum cholesterol level and such parameters were concerned. The treatment of the animals after 5 days showed less efficiency when the above parameters are taken into consideration. It was reported by Shanmugasundaram et al. (1981) that the administration of leaves of *Gymnema sylvestre* in alloxan diabetes rats significantly reduced the blood glucose. Similar result was reported by Cheng and Yang (1983) after treating the diabetic mice with the fruits of *Psidium guajava.* The treatment of diabetic rabbits with leaf suspension of *Salvia lavandulifolia* at a dose of 250mg/kg decreased blood glucose as reported by Jimenez et al. (1986). Giri et al. (1986) reported that the feeding of *Cajanus cajan* seeds to alloxan diabetic rats decreased blood glucose, blood urea, serum cholesterol and increased liver glycogen. It has been documented by Schmidt et al. (1990), Yoon (1990) and Xu et al. (1992) that alloxan injection selectively destroys the pancreatic B cells. The whole leaf powder treatment in alloxan injected animals had a mean serum insulin value which was higher than the normal control group. This observation testifies the fact that whole leaf powder offers protection in the case of alloxan induced diabetes. It was reported by Chucla et al. (1988) that oral feeding of the leaves and flowers of *Centaurea corcubionensis* increased the circulating insulin in normoglycaemic rats. Leucocyanidin isolated from the bark of *Ficus bengalensis*

and administered orally increased serum insulin in diabetic animals as reported by Vinod Kumar and Augusti (1989). Administration of leaf powder 24h post injection of alloxan appears to reduce the severity of diabetes induction. Prolongation of leaf powder treatment after alloxan injection appears to influence the severity of diabetes induction only to a milder level compared to earlier treatment i.e., 24h of alloxan injection. This possibly indicates the presence of in the leaf powder some factors which might have ed and stimulated protect the pancreatic B cells from alloxan induced damage and necrosis.

Conclusion

The whole leaf powder of *Aegle marmelos* administered orally to alloxan induced diabetic rat models showed a significant control of blood glucose when compared with non treated animals. The initiation of treatment after 24h of alloxan injection seemed to give a sort of protection to the B cells of pancreas since it was noticed that the blood glucose value was less in this group when compared with that group where the leaf powder treatment was started after 5 days of alloxan injection. ~~Similar was the pattern in all the other parameters studied~~.

CHAPTER IV

DETECTION OF SOLVENT THAT ELUTES THE ANTIDIABETIC PRINCIPLE FROM *AEGLE MARMELOS* LEAVES

CHAPTER IV

Detection of the solvent that elutes the antidiabetic principle from *Aegle marmelos* leaves

4.1. Introduction

The whole leaf powder of *Aegle marmelos* showed pronounced hypoglycaemic activity in alloxan diabetic rats. In order to isolate the active principle different solvent systems were used such as petroleum benzene, chloroform, methanol and water. When these extracts are tested in alloxan diabetic rat models, they will give an insight as to which solvent the antidiabetic principle from the leaves elutes out and also will help to isolate and purify the antidiabetic principle.

4.2. Materials and Methods

The whole leaf powder of *Aegle marmelos* was prepared as described in 2.1.4. Ten grams of leaf powder was mixed with 100 ml of petroleum benzene and stirred for 2h at 4^{o}C. It was allowed to stand for 1h and the supernatant collected. The residue was eluted with chloroform, methanol and water in the same pattern. The organic solvent from the extract was removed in hot water bath and the residue was resuspended in 50ml distilled water. This was administered orally at a dose of 5ml/kg body weight daily in diabetic rat models.

4.2.1. Experiment set up

Inbred Wistar strain male albino rats were used for the experiment and the rats were maintained as described in 2.2.1. The animals were made diabetic by intra venal injection of alloxan monohydrate, as per the procedure described in 2.2.2. The animals were divided into the following groups. Each group had six rats, two in each cage.

Group1: Kept as normal group.

Group2: Alloxan injected and kept without any treatment.

Group3: Alloxan injected and petroleum benzene extract treated.

Group4: Alloxan injected and chloroform extract treated.

Group5: Alloxan injected and methanolic extract treated.

Group6: Alloxan injected and aqueous extract treated.

Before administering alloxan, body weight and blood glucose was estimated. These parameters were monitored every 5days after starting the experiment till the 30th day when the animals were sacrificed.

4.3. Result

In the normal group there was an increase in the body weight and in the diabetic group without any treatment, the body weight showed a decreasing trend. In the animal groups treated with extracts eluted with petroleum benzene, chloroform and methanol, there was a significant decrease in body weight ($P<0.05$) and in the aqueous extract treated group there was no significant decrease in the body weight when compared with the normal group. The blood glucose level of the different animal groups are given in Table 4.1. It was noticed that the blood glucose level was maintained to the normal level in the aqueous

Table 4.1. The blood glucose (mg/dl) levels of animals treated with different solvent extracts of leaves of Aegle marmelos

Animal Status	Days						
	0	5	10	15	20	25	30
1. Normal group	78 ± 0.89	76 ± 1.0	76 ± 0.92	82 ± 1.6	76 ± 1.6	80 ± 1.1	82 ± 0.8
2. Diabetic group	80 ± 0.74	152 ± 7.8	187 ± 11	242 ± 7.0	257 ± 12	292 ± 16	347 ± 15
3. Petroleum benzene extract treated group	83 ± 0.17	201 ± 12	210 ± 15	210 ± 21	227 ± 21	247 ± 18	321 ± 14
4. Chloroform extract treated group	87 ± 1.62	190 ± 9.6	207 ± 12	205 ± 9.8	206 ± 12	225 ± 19	247 ± 18
5.Methanolic extract treated group	80 ± 1.70	192 ± 8.9	215 ± 6.7	229 ± 7.9	239 ± 4.22	220 ± 5.1	232 ± 6.1
6. Aqueous extract treated group	82 ± 2.57	167 ± 12	157 ± 11	127 ± 17	111 ± 10	101 ± 9	92 ± 12*

Values are mean ± S.D of 6 animals in a group

* $P < 0.05$ compared to the diabetic group

extract treated group only . In the petroleum benzene extract treated group, the increase in blood glucose was very high when compared with the chloroform and methanolic extract treated group. But there was no significant decrease in blood glucose in groups 3,4 and 5 when compared with the normal group.

4.4.Discussion

Many workers have reported the antidiabetic activity of the aqueous extract of a variety of plants in different alloxan induced diabetic animal models. Abuh et al. (1990) reported that the aqueous extract of *Anthocleista vogelli* showed hypoglycaemic activity in a dose related fashion in rats, mice and rabbits. The aqueous extract of *Salacia reticualata* has profound hypoglyacemic activity as reported by Sersinghe et al. (1990). In diabetic rabbits, the aqueous extract of *Momordica charantia* fruits significantly decreased the blood glucose.(Akhtar et al. 1981). The whole plant aqueous extract of *Tecoma stans* showed blood sugar controlling activity (Perez et al. 1984); the aqueous extract of *Tephrosia purpurea* seeds fed orally to diabetic rabbits showed hypoglycaemic activity (Rahman et al. 1085).In the aqueous extract,it is likely that the active principle is separated and that may be the reason for the prevention of the abnormal increase of blood glucose in the group of animals treated with this.

Conclusion

The blood glucose was taken as a key parameter for the manifestation of diabetes mellitus and it was noted that the aqueous extract showed the maximum activity in alloxan diabetic rat models by maintaining the blood glucose

at par with normal controls. The treatment with other extracts did not give a significant decrease in blood glucose in alloxan diabetic rat models. Since the aqueous extract showed the maximum antidiabetic activity, further investigations to isolate the active principle ~~was~~ were carried out with the aqeuous extract.

CHAPTER V

ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *AEGLE MARMELOS*

CHAPTER V

Antidiabetic effect of the aqueous extract of *Aegle marmelos* leaves

5.1. Introduction

Plant extracts are used in the treatment of diabetes mellitus in the Ayurvedic system of medicine. Usually a mixture of different parts of plants is boiled with water in controlled conditions and the supernatant is collected and used as a hypoglycaemic agent. In most cases this will be effective in controlling the disease. The active principle in most of these cases remain a mystery eventhough the aim is achieved. If the active principle can be isolated it will act as a key to get an idea about its structure and to synthesise it artificially. Many such products are purified and extensive studies have been done in diabetic animal models.

5.2. Materials and Methods

The aqueous extract of *Aegle marmelos* leaf powder was prepared as described in 2.1.6.

5.2.1.Experiment set up

The animals were divided into the following groups. Each group has six rats, two in each cage.

Group1: Kept as normal group.

Group2: Physiological saline injected though the femoral vein and was taken as the control.

Group3: Alloxan injected and kept without any treatment to study the diabetic nature.

Group4: Alloxan injected and 1 unit of insulin injected on all days after 5 days of alloxan injection.

Group5: Alloxan injected and the aqueous extract (1g/kg) administered orally after 24h and the treatment continued on all days for 30 days.

Group6: Alloxan injected and the aqueous extract (1g/kg) administered orally after 5 days and the treatment continued on all days for 30 days.

Before starting the experiment, the body weight and blood glucose ~~was~~ were estimated. Then the blood glucose and body weight of the animals were monitored every 5 days till 30 days. At the end, the animals were sacrificed by cervical dislocation and the blood was collected from the heart and the serum separated to estimate the insulin, cholesterol and urea. The liver was used to estimate the glycogen content.

5.3. Results

In normal animals and saline injected control group, the body weight was increasing gradually till the end of the experiment. But in alloxan injected diabetic group left without any treatment the body weight decreased substantially during the first five days and this tendency continued till the end of the experiment where the animals became very weak. In insulin treated group the decrease in body weight was not significant when compared with the diabetic group. If the aqueous extract is administered after 24h of alloxan injection, a sudden decrease in body weight is prevented in the initial days even though the ~~gaining of~~ weight gain is at par with the normal controls. If the aqueous extract is

given after five days of alloxan injection, the drop in body weight of that group is prevented when compared with the diabetic group (Fig 5.1)

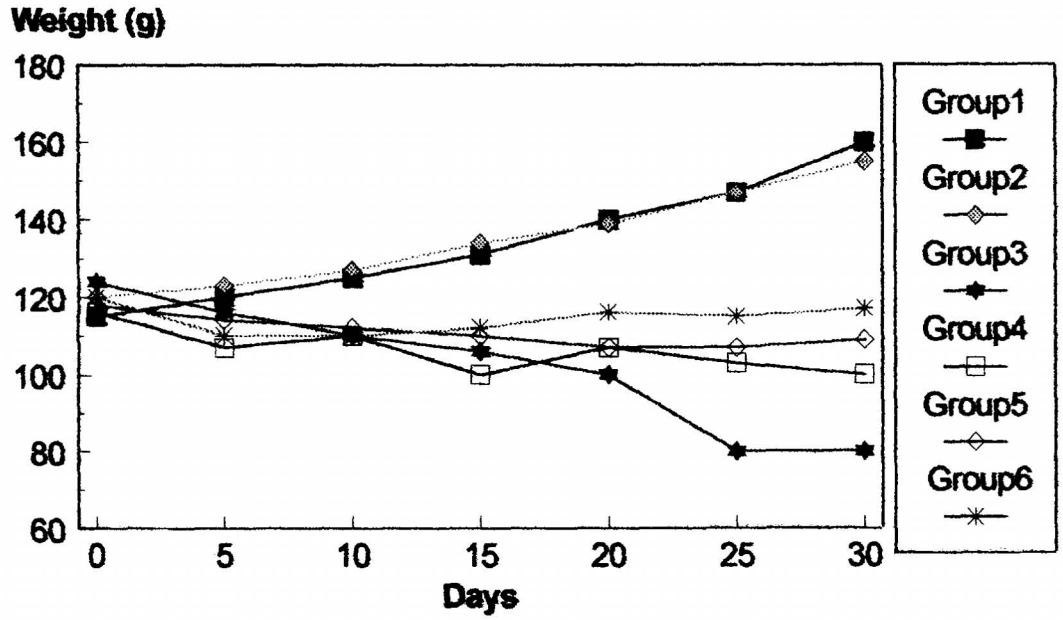
The blood glucose level of the different experimental animal groups are presented in Table 5.1. In the normal and saline injected control group, the blood glucose level was maintained at a range 76mg/dl to 84mg/dl throughout the experimental period. In the alloxan injected group left without any treatment the blood glucose increased substantially and from an initial value of 75mg/dl to 387mg/dl at the end of 30days. But the insulin treated group, the abnormal increase in blood glucose was prevented and at the end of 30days it was only 94mg/dl. In the aqueous extract treated group after 24h of alloxan injection the increase in blood glucose was not significant and there was only a slight increase when compared with the normal control. In the aqueous extract treated group after 5days of alloxan injection the abnormal increase in the blood glucose was prevented.

The blood urea level of different groups of animals in the experiment after 30days is presented in Table 5.2. In normal and saline injected control group, the blood urea value was 22mg/dl and 23mg/dl respectively. In the diabetic group the blood urea value shot up to 62mg/dl. In the insulin treated group the blood urea value was 35mg/dl where as in the aqueous extract treated group after 24h of alloxan injection it was 29mg/dl and in aqueous extract treated group after 5days of alloxan injection it was 41mg/dl.

The serum cholesterol levels of the animals in the experiment after 30days is presented in Table 5.3. In the normal and saline injected control group it ranged between 82mg/dl and 84mg/dl respectively. In the diabetic group left

60A*

Figure. 5.1. The weight of animals (g) treated with the aqueous extract of *Aegle marmelos*



Group1- Normal animals
Group2- Saline injected control
Group3- Diabetic group
Group4- Diabetic and insulin treated (1 unit of insulin daily)
Group5- Diabetic and aqueous extract treated after 24h of alloxan injection (1g/kg daily)
Group6- Diabetic and aqueous extract treated after 5days of alloxan injection (1g/kg daily)

60.B

Table 5.1. The blood glucose (mg/dl) levels of animals treated with the aqueous extract of *Aegle marmelos*.

Animal Status	Days						
	0	5	10	15	20	25	30
1. Normal group	76 ± 1.89	74 ± 1.0	78± 2.92	80 ± 2.6	77 ± 1.9	82 ± 1.7	80 ± 01.8
2. Saline injected control group	81 ± 1.3	83 ± 2.4	82 ± 2.5	78 ± 1.2	81 ± 2.8	84 ± 2.9	81 ± 3.2
3. Diabetic group	75 ± 3.6	162 ± 12	182 ± 15	237 ± 17	269 ± 17	312 ± 26	387 ± 11
4. Diabetic and insulin treated group	79 ± 2.7	157 ± 10	137 ± 8.0	125 ± 7.0	112 ± 8.0	94 ± 5.0	94 ± 9.0
5. Diabetic and aqueous extract treatedafter . 24 h	81 ± 2.0	115 ± 11	109 ± 3.9	111 ± 14	97 ± 16	91 ± 7.0	89 ± 4.8*
6. Diabetic and aqueous extract treated after 5 days	76 ± 2.4	172 ± 12	152 ± 5.9	129 ± 18	119 ± 8.2	109 ± 8.9	104 ± 9.5*

Values are mean ± S.D of 6 animals in a group

* $P < 0.05$ compared to the diabetic group

60.C

Table 5.2. The blood urea (mg/dl) level of animals treated with the aqueous extract of *Aegle marmelos*

Animal status	Blood urea (mg/dl)
1. Normal group	22.57± 2.57
2. Saline injected control group	23.38 ± 2.52
3. Diabetic group	62.92 ± 6.37
4. Diabetic and insulin treated group	35.983 ± 4.45
5. Diabetic and aqueous extract treated after 24h of alloxan injection	29.43 ± 6.58*
6. Diabetic and aqueous extract treated after 5 days of alloxan injection	41.91 ± 7.95*

Values are mean ± SD of 6 animals in a group

*$P<0.05$ compared to diabetic group

60 D

Table 5.3. The serum cholesterol (mg/dl) level of animals treated with the aqueous extract of *Aegle marmelos*

Animal status	Serum cholesterol
1. Normal group	84.17± 2.48
2. Saline injected control group	82.46 ± 3.42
3. Diabetic group	192.54 ± 6.65
4. Diabetic and insulin treated group	108.53 ± 5.41
5. Diabetic and aqueous extract treated after 24h of alloxan injection	91.49 ± 8.48*
6. Diabetic and aqueous extract treated after 5 days of alloxan injection	98.72 ± 9.72*

Values are mean ± SD of 6 animals in a group

*$P<0.05$ compared to diabetic group

without any treatment, the serum cholesterol value shot up to 192mg/dl. In the insulin treated group the serum cholesterol value was 108mg/dl. But in the animal group treated with the aqueous extract after 24h of alloxan injection and 5days of alloxan injection it was 91mg/dl and 98mg/dl respectively.

The liver glycogen content of animals in the experiment after 30days is presented in Table 5.4. In the normal and saline injected control groups it was 2g/100g of wet tissue. In the diabetic group left without any treatment, there was a significant decrease in the glycogen content of the liver and it was only 1.25g/100g wet tissue. In the animal group treated with insulin the liver glycogen content was 1.76g/100g wet tissue. But in the aqueous extract treated group after 24h and 5days of alloxan injection, the abnormal decrease in liver glycogen was prevented and it was 1.97g and 1.84g/100g wet tissue respectively.

The serum insulin level in the different groups of experimental animals are presented in Table 5.5.

In the saline injected control group, the serum insulin level was 11.7μU/ml. But in the diabetic group left without any treatment there was a significant decrease in the quantity of insulin and it was 5.7μU/ml. In the aqueous extract treated group after 24h of alloxan injection the serum insulin level was 8.7μU/ml and in the group treated with aqueous extract after 5days of alloxan injection it was 8.7μU/ml.

5.4. Discussion

Many workers have reported the antidiabetic activity of the aqueous extract of a variety of plants in different alloxan induced diabetic animal models.

Table 5.4. The liver glycogen (g/100g of wet tissue) level of animals treated with the aqueous extractof of *Aegle marmelos*

Animal status	Liver glycogen
1. Normal group	2.11± 0.87
2. Saline injected control group	2.82 ± 0.91
3. Diabetic group	1.25 ± 0.21
4. Diabetic and insulin treated group	1.76 ± 0.55
5. Diabetic and aqueous extract treated after 24h of alloxan injection	1.97 ± 0.85*
6. Diabetic and aqueous extract treated after 5 days of alloxan injection	1.84 ± 0.71*

Values are mean ± SD of 6 animals in a group

*P<0.5 compared to diabetic group

Table 5.5. The serum insulin (μU/ml) level of animals treated with the aqueous extract of of *Aegle marmelos*

Animal status	Serum insulin (μU/ml)
1. Saline injected control group	11.7 ± 0.3
2. Diabetic group	5.7 ± 0.5
3. Diabetic and aqueous extract treated after 24h of alloxan injection	8.7 ± 0.8*
4. Diabetic and aqueous extract treated after 5 days of alloxan injection	8.5 ± 0.4*

Values are mean ± SD of 6 animals in a group

*P<0.5 compared to diabetic group

Abuh et al. (1990) reported that the aqueous extract of *Anthocleista vogelli* showed hypoglycaemic activity in a dose related fashion in rats, mice and rabbits. The aqueous extract of *Salacia reticulata* has profound hypoglycaemic activity as reported by Shersinghe et al. (1990). In diabetic rabbits, the aqueous extract of *Momordica charantia* fruits significantly decreased the blood glucose (Akhtar et al., 1981). The whole plant aqueous extract of *Tecoma stans* showed blood sugar controlling activity (Perez et al., 1984); the aqeuous extract of *Tephrosia purpurea* seeds fed orally to diabetic rabbits showed hypoglycaemic activity (Rahman et al., 1985). Karunanayake et al., (1984) reported that the aqueous extract of *Aegle marmelos* had significant hypoglycaemic activity in alloxan diabetic rats. The water extract of *Schefflera leucantha* exhibited hypoglycaemic activity as reported by Satayavivad et al., in 1996. Sharma et al., (1996) reported that an aqueous extract of *Aegle marmelos* leaves exhibited significant hypoglycaemic and antihyperglycaemic effects in normoglycaemic and streptozotocin diabetic rats respectively. It also significantly increased plasma insulin levels of diabetic rats. The extract did not show any sign of toxicity and the LD50 was greater that 10g/kg when given orally. The aqueous extract treated group in our experiment responded in a pattern similar to the whole leaf powder treated groups. This means that the active principle may be eluted with water extraction which is turning out to be effective in the treatment of alloxan diabetic animals and the parameters we have studied is maintained almost in a normal level.

Conclusion

The aqueous extract of *Aegle marmelos* is effective in the treatment of alloxan diabetic animal models. The blood glucose when monitored every 5 days after the induction of diabetes prevents the abnormal increase in the

blood glucose and it is almost brought to the normal level within 30 days. It is noted that if the aqueous extract is administered after 24h of alloxan injection gives a sort of protection to the animals and diabetes is not manifested. The other parameters such as blood urea, cholesterol, glycogen and serum insulin are also maintained to the normal level in the treated groups whereas in diabetic group left without any treatment these parameters were significantly different from the control groups.

CHAPTER VI

ISOLATION OF ALKALOIDS FROM *AEGLE MARMELOS* LEAVES AND ITS ANTIDIABETIC ACTIVITY

CHAPTER VI

Isolation of alkaloids from *Aegle marmelos* leaves and its antidiabetic activity

6.1. Introduction

Isolation of the active principle from the plant parts that shows hypoglycaemic activity in diabetic animal models, is a crucial step in getting an insight into the actual mechanism by which the said substance is acting. Since the active principle is eluted with water extraction, an effort was made to analyse the contents in the aqueous extract. Since many have reported the activity of the alkaloids from different plants that have hypoglycaemic activity, the aqueous extract of *Aegle marmelos* was lyophilised and qualitative test for the presence of alkaloids was done. ~~There were the alkaloids~~ Since alkaloids were present in the extract, ~~and so~~ it was isolated and tested in diabetic animals.

6.2. Materials and Methods

The alkaloids from *Aegle marmelos* leaf powder were prepared as described in 2.1.8.

6.2.1. Experiment set up

The animals were divided into the following groups. Each group had six rats, two in each cage.

Group1- Saline injected control

Group2- Diabetic group

Group3- Diabetic and alkaloid fraction treated after 5 days of alloxan injection (100mg/kg body weight)

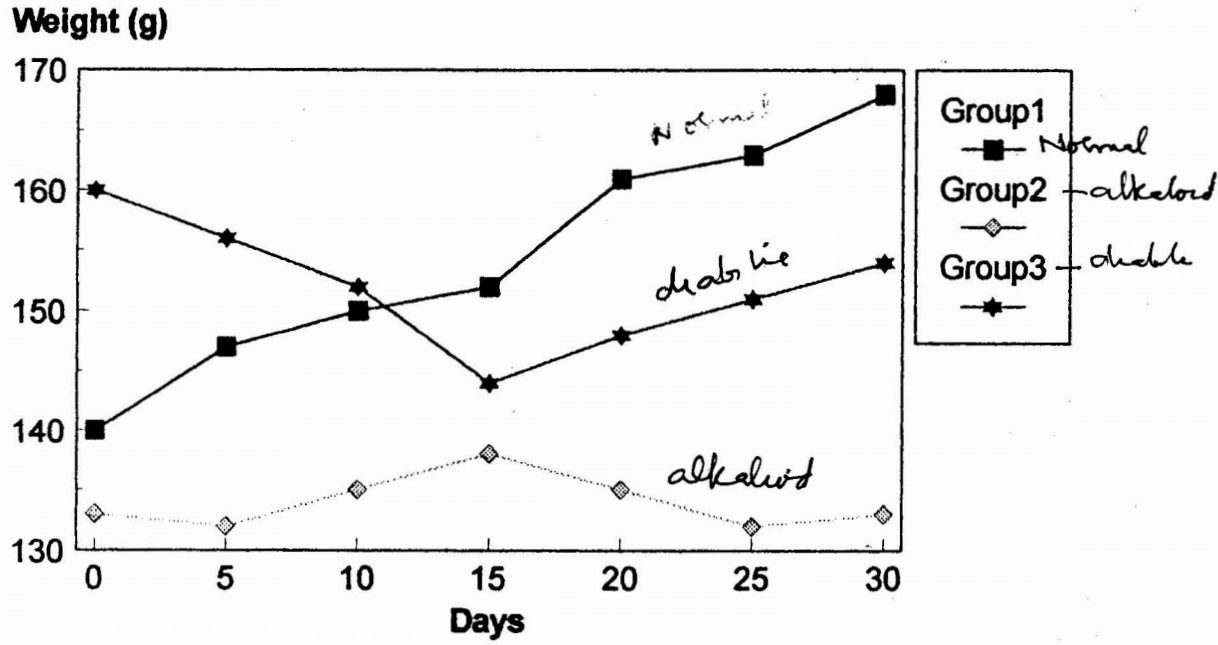
Before starting the experiment the body weight and blood glucose of all the animals were estimated. Then the blood glucose and body weight of the animals were monitored every 5 days till the 30th day. After 30 days the animals were sacrificed by cervical dislocation and the blood was collected from the heart and the serum separated to estimate the insulin, cholesterol and urea. The liver was used to estimate the glycogen content.

6.3. Results

In the saline injected control group the body weight was increasing gradually till the end of the experimental period. In the alloxan injected diabetic group the body weight was decreasing if no treatment was given and the fall in weight was very significant in the initial days. But in the alkaloid fraction treated group, the decrease in body weight was not significant when compared with the normal saline injected control and it was noticed that there was a gain in body weight after 10 days of treatment. In the diabetic group the body weight dropped for 15 days and after that there was an increase in the body weight at a slow pace but never reaching the weight at the start of the experiment (Fig. 6.1)

The blood glucose level of the normal control, diabetic group without any treatment and the diabetic with treatment are given in Table 6.1. In the control group the blood glucose was maintained at 76 mg/dl to 84 mg/dl. In the diabetic group without any treatment the blood glucose showed a tendency to increase significantly throughout the experimental period and reached a value of 340 mg/dl at the end of thirty days. But when the treatment of alkaloid mixture given daily to the diabetic group, the blood glucose decreased considerably and at the end of thirty days it was at par with the normal control group.

Figure 6.1. The weight of the animals treated with the alkaloid fraction of *Aegle marmelos* leaves



Group1- Saline injected control
Group~~3~~ Diabetic group
Group~~2~~ Diabetic and alkaloids fraction treated after 5days (100mg/kg)

corrections made
Supervising teacher

Table 6.1. The blood glucose (mg/dl) levels of animals treated with alkaloids fraction of *Aegle marmelos* and an antidiabetic drug- Euglucon

Animal Status	Days						
	0	5	10	15	20	25	30
1. Saline injected control group	78 ± 2.5	76 ± 1.9	82 ± 3.5	84 ± 2.7	79 ± 2.2	79 ± 2.7	84 ± 3.8
2. Diabetic group	83 ± 1.9	169 ± 7.8	187 ± 14	212 ± 11	266 ± 14	322 ± 22	340 ± 17
3. Diabetic and alkaloids treated after 5 days	80 ± 1.6	161 ± 11	137 ± 7.0	112 ± 10	97 ± 2.7	91± 5.9	89 ± 4.2*

Values are mean ± S.D of 6 animals in a group

* $P < 0.05$ compared to the diabetic group

The blood urea level of different groups of animals in the experiment after 30 days is presented in Table 6.2. In the saline injected control group the blood urea value was 23mg/dl. In the diabetic group without any treatment the blood urea level was 56mg/dl but in the diabetic group treated with the alkaloids the blood urea level was only 34mg/dl.

The serum cholesterol level of animals in the experiment after thirty days is presented in Table 6.3. In the normal control group the serum cholesterol level was 89mg/dl. In the diabetic group without any treatment the serum cholesterol value was 182mg/dl and in the diabetic group given the treatment there was a significant decrease in the serum cholesterol level and it was only 156mg/dl.

The liver glycogen content of the animals in the experiment after thirty days is presented in Table 6.4. In the normal control group the liver glycogen content was 2.67g/100g of wet tissue. In the diabetic group without any treatment it was 1.27/100g wet tissue. In diabetic group treated with alkaloids there was no significant decrease in the liver glycogen and it was 2.51g/100g wet tissue.

The serum insulin level in the different groups of experimental animals are presented in Table 6.5. In the normal group the serum insulin level was 11.7μU/ml. In the diabetic group left without any treatment the serum insulin level was 5.7μU/ml and in the alkaloid treated group it was 8.9μU/ml.

Table 6.2. The blood urea (mg/dl) level of animals treated with the alkaloid fraction of *Aegle marmelos*

Animal status	Blood urea (mg/dl)
1. Saline injected control group	23.84 ± 4.32
2. Diabetic group	56.82 ± 8.37
3. Diabetic and alkaloid fraction treated after 5days of alloxan injection	34.76 ± 5.89*

Values are mean ± SD of 6 animals in a group

*P<0.5 compared to diabetic group

Table 6.3. The serum cholesterol (mg/dl) level of animals treated with the alkaloid fraction of *Aegle marmelos*

Animal status	Serum cholesterol (mg/dl)
1. Saline injected control group	89.00± 6.27
2. Diabetic group	182.96 ± 11.27
3. Diabetic and alkaloid fraction treated after 5days of alloxan injection	156.00 ± 14.39*

Values are mean ± SD of 6 animals in a group

*P<0.5 compared to diabetic group

Table 6.4. The liver glycogen(g/100g of wet tissue) level of animals treated with the alkaloid fraction of *Aegle marmelos*

Animal status	Liver glycogen (g/100g)
1. Saline injected control group	2.67 ± 0.74
2. Diabetic group	1.27 ± 0.92
3. Diabetic and alkaloid fraction treated after 5days of alloxan injection	2.51 ± 0.75*

Values are mean ± SD of 6 animals in a group

*P<0.5 compared to diabetic group

Table 6.5 The serum insulin (μU/ml) level of animals treated with the alkaloids of *Aegle marmelos*

Animal status	Serum insulin (μU/ml)
1. Saline injected control group	11.7 ± 0.3
2. Diabetic group	5.7 ± 0.5
3. Diabetic and alkaloids treated after 5 days of alloxan injection	8.9 ± 0.7*

Values are mean ± SD of 6 animals in a group

*P<0.5 compared to diabetic group

6.4. Discussion

The alkaloids extracted from the leaves of *Aegle marmelos* were separated and administered orally at a dose 100mg/kg body weight to diabetic rats and it was noted that the decrease in the body weight was prevented where as in the diabetic animals without any treatment the body weight was decreasing significantly when compared with the normal group. It can be presumed that the alkaloids treatment prevents the weight loss. Monitoring of the blood glucose of the treated group clearly indicates that there is no significant increase in the blood glucose and it is almost at par with the normal saline injected control. There are many workers who have documented the hypoglycaemic activity of several alkaloids separated from different plants that showed hypoglycaemic activity in experimental diabetic animal models. Monro et al., (1989) reported that the alkaloids separated from the leaves of *Talauma ovata* showed hypoglycaemic activity in alloxan diabetic rats. The alkaloids containing fraction of tubers of *Dioscorea dumetorum* showed significant hypoglycaemic activity in severe diabetic rats (Undie and Akube, 1986). *Trigonelline*, the alkaloid extracted from the seeds of *Trigonella foenumgraceum* showed antidiabetic activity in diabetic animals as reported by Shani et al., (1975). The alkaloids extracted from the leaves of *Veratrum album* showed hypoglycaemic activity as reported by Machova et al., (1958). Sharma et al., (1980) who isolated an alkaloid *marmeline* from unripe fruits of *Aegle marmelos* and has reported that it showed antidiabetic activity in alloxan induced diabetic animal models. The blood urea, serum cholesterol and liver glycogen in the alkaloids treated group was almost at par with the control group and significantly different from the diabetic group. It was documented that the alkaloids extracted from the leaves of *Ziryphus mauritiana* significantly reduced the blood urea and increased liver glycogen in diabetic

animals and also showed hypoglycaemic activity as reported by Mohapatra et al., (1976). The serum insulin level in the alkaloid treated group was significantly different from the diabetic group and this may be due to the release of proinsulin or the conversion of proinsulin to insulin and it can be assumed that this activity is brought about with the action of alkaloids fed to the diabetic group.

Conclusion

The alkaloids extracted from *Aegle marmelos* showed hypoglycaemic activity in alloxan induced diabetic rats at a dose of 100mg/kg. It is also noted that the increase in the dose does not make a significant change in the different parameters we have studied.

CHAPTER VII

COMPARISON OF ANTIDIABETIC ACTIVITY OF ALKALOIDS OF AEGLE MARMELOS LEAVES WITH AN ANTI DIABETIC DRUG

CHAPTER VII

Comparison of the antidiabetic activity of alkaloids of *Aegle marmelos* leaves with an antidiabetic drug- Euglucon

7.1. Introduction

A number of oral hypoglycaemic agents are available in the market now a days to treat diabetes mellitus and it has been proved beyond doubt that all these act by stimulating the pancreatic islets to release more proinsulin into the blood which is converted to active insulin. Clinical studies have demonstrated that these drugs are ineffective in completely pancreatectomized patients and in insulin dependent diabetic subjects. On the other hand, they are effective in non insulin dependent diabetic patients in whom the pancreas retains the capacity to secrete insulin. In administering a plant product as an antidiabetic agent, one of the key factors to be looked into is the comparison of the said product with a standard anti diabetic drug commonly used. If the different parameters studied shows a significant similarity with the administration a drug commonly used, it can be recommended for the management of diabetes mellitus.

7.2. Materials and Methods

The alkaloids from *Aegle marmelos* leaf powder were prepared as described in 2.1.8. The antidiabetic drug Euglucon tablets were powdered and suspended in water at a concentration of 100mg/10ml

7.2.1 Experiment set up

The animals were divided into the following groups. Each group had six rats, two in each cage.

Group1- Saline injected control

Group2- Diabetic group

Group3- Diabetic and alkaloid fraction treated after 5 days of alloxan injection

Group4- Diabetic and Euglucon treated after 5 days of alloxan injection

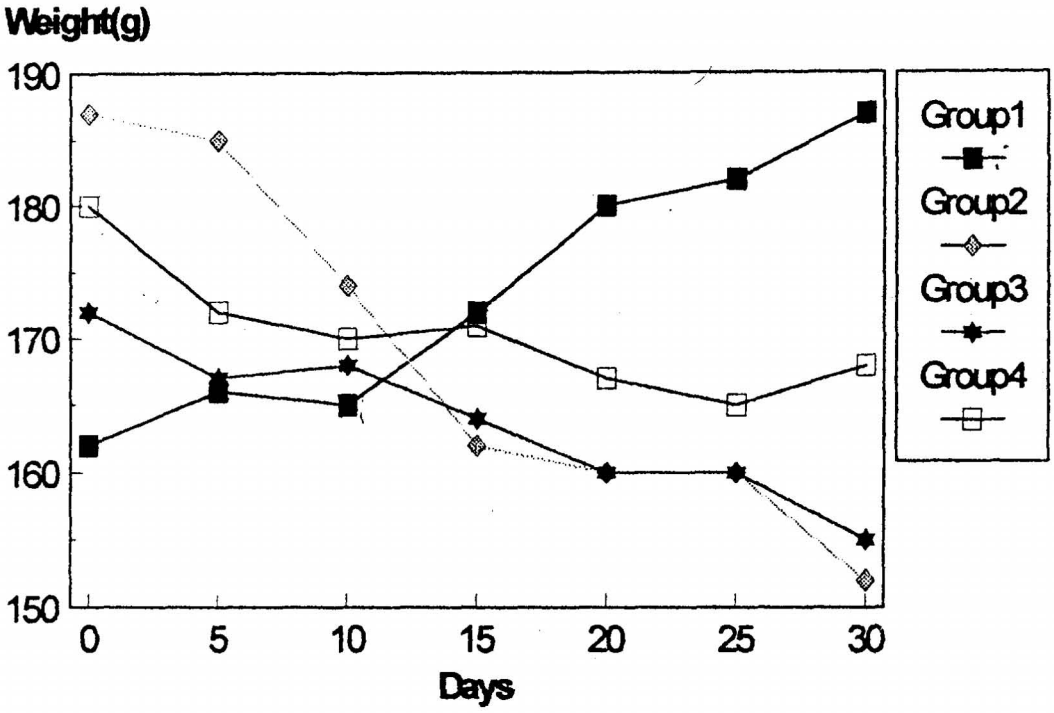
Before starting the experiment the body weight and blood glucose of all the animals were estimated. Then the blood glucose and body weight of the animals were monitored every 5 days till the 30th day. At the end of 30 days the animals were sacrificed by cervical dislocation and the blood was collected from the heart and the serum separated to estimate insulin, cholesterol and urea. The liver was used to estimate the glycogen content.

7.3. Results

The body weight of the saline injected control group increased till the end of the experiment. But in the alloxan injected diabetic group left with out any treatment, there was a significant decrease in the body weight when compared with the normal group. In the groups treated with the alkaloids and the antidiabetic drug euglucon, there ~~were no significant~~ was decrease in the body weight ~~and remained more of less at the same level as in the beginning~~. (Fig. 7.1)

The blood glucose level of the different experimental animal groups is given in Table 7.1. In the saline injected control group the blood glucose was

Figure7.1. The weight of the animals treated with alklaloids of *Aegle marmelos* leaves and a standard antidiabetic drug-Euglucon



Group1- Saline injected control group
Group2- Diabetic group
Group3- Diabetic and the alkaloids fraction treated (100mg/kg) after 5 days
Group4- Diabetic and Euglucon treated (100mg/kg) after 5days

Table 7.1. The blood glucose (mg/dl) levels of animals treated with alkaloids fraction of *Aegle marmelos* and an antidiabetic drug- Euglucon

Animal Status	Days						
	0	5	10	15	20	25	30
1. Saline injected control group	78 ± 2.5	76 ± 1.9	82 ± 3.5	84 ± 2.7	79 ± 2.2	79 ± 2.7	84 ± 3.8
2. Diabetic group	83 ± 1.9	169 ± 7.8	187 ± 14	212 ± 11	266 ± 14	322 ± 22	340 ± 17
3. Diabetic and alkaloids treated after 5 days	80 ± 1.6	161 ± 11	137 ± 7.0	112 ± 10	97 ± 2.7	91± 5.9	89 ± 4.2*
4. Diabetic and Euglucon treated after 5 days	82 ± 2.7	158 ± 12	147 ± 6.8	141 ± 11	132 ± 4.9	121 ± 11	99 ± 7.2*

Values are mean ± S.D of 6 animals in a group

* $P < 0.05$ compared to the diabetic group

maintained at 78mg/dl to 84mg/dl throughout the experimental period. In the diabetic group left without any treatment the blood glucose increased and reached a value of 340mg/dl. In the diabetic and alkaloid treated group, the blood glucose decreased from 161mg/dl to 89mg/dl at the end of 30 days. In the euglucon treated group also there was a decreasing trend in the blood sugar level and reached the value 99mg/dl from 158mg/dl at the end of 30 days.

The blood urea level of different groups of animals is given in Table 7.2. In the saline injected control group it was 24mg/dl. In the diabetic group left without any treatment, it was 53mg/dl. In the alkaloid treated group, the abnormal increase in blood urea was prevented and it was 29mg/dl. In the euglucon treated group it was 33mg/dl.

The serum cholesterol level of animals in the experiment after thirty days is presented in Table 7.3. In the saline injected control group the serum cholesterol level was 81mg/dl. In the diabetic group left without any treatment, it was 173mg/dl. But in the alkaloid treated group the cholesterol level was 89mg/dl and in euglucon treated group it was 112mg/dl.

The liver glycogen of the animals after thirty days is presented in Table 7.4. In the saline injected control group it was 2.87g/100g of wet tissue. In the diabetic group left without any treatment it went down to 1.29g/100g of wet tissue. In the diabetic group treated with the alkaloids, there was no significant decrease in the liver glycogen and it was 2.67g and so also in the euglucon treated group there was no significant decrease in liver glycogen where the value was 2.65g/100g of wet tissue.

Table 7.2. The blood urea (mg/dl) level of animals treated with the alkaloid fraction of *Aegle marmelos* and an antidiabetic drug- Euglucon

Animal status	Blood urea (mg/dl)
1. Saline injected control group	24.37 ± 5.82
2. Diabetic group	53.39 ± 7.57
3. Diabetic and alkaloid fraction treated after 5days of alloxan injection (100mg/kg body weight)	29.66 ± 7.32*
4. Diabetic and Euglucon treated after 5days of alloxan injection (100mg/kg body weight)	33.38 ± 8.49*

Values are mean ± SD of 6 animals in a group

*P<0.05 compared to diabetic group

Table 7.3. The serum cholesterol (mg/dl) level of animals treated with the alkaloid fraction of *Aegle marmelos* and an antidiabetic drug- Euglucon

Animal status	Serum cholesterol (mg/dl)
1. Saline injected control group	81.37 ± 7.22
2. Diabetic group	173.59 ± 11
3. Diabetic and alkaloid fraction treated after 5days of alloxan injection (100mg/kg body weight)	89.56 ± 9.12*
4. Diabetic and Euglucon treated after 5days of alloxan injection (100mg/kg body weight)	112.34 ± 15*

Values are mean ± SD of 6 animals in a group

*P<0.05 compared to diabetic group

Table 7.4. The liver glycogen(g/100g of wet tissue) level of animals treated with the alkaloid fraction of *Aegle marmelos* and an antidiabetic drug- Euglucon

Animal status	Liver glycogen(g/100g)
1. Saline injected control group	2.87 ± 0.22
2. Diabetic group	1.29 ± 0.61
3. Diabetic and alkaloid fraction treated after 5days of alloxan injection (100mg/kg body weight)	2.67 ± 0.76*
4. Diabetic and Euglucon treated after 5days of alloxan injection (100mg/kg body weight)	2.65 ± 0.15*

Values are mean ± SD of 6 animals in a group

*P<0.05 compared to diabetic group

The serum insulin level of different group of animals in the experiment is presented in Table 7.5. In the saline injected control group, the serum insulin level was 11.7μU/ml. In the diabetic group left without any treatment, there was a significant decrease in the serum insulin level and the value was 5.7 μU/ml. In the diabetic group treated with alkaloids the serum insulin level was 8.9μU/ml and in the euglucon treated group it was 9.5μU/ml.

7.4. Discussion

There was a significant decrease in the body weight of the diabetic animals left without any treatment when compared with the saline injected control group. The manifestation of diabetes might have adversely affected the normal metabolic activities of animals and there may be the utilisation of the fat and proteins for energy requirement and the glucose excreted through the urine. But in the alkaloid and euglucon treated groups the decrease in the body weight is prevented because there may be the triggering of the islet cells to have a normal quantity of serum insulin in their body. This argument becomes more realistic while observing the blood glucose values. In the diabetic group, the blood glucose value has increased significantly when compared with the control group, whereas in the treated groups, it is almost at par with the normal control group.Taking into consideration the other parameters such as blood urea, serum cholesterol and liver glycogen in the treated groups they are almost maintained at the normal level where as in the diabetic group left without any treatment these values were significantly different. It was reported by Padmini and Chakrabarthi (1983) that the fruits of *Eugenia jambolana* when administered orally to streptozotocin diabetic rabbits significantly reduced the blood glucose and maintained the normal weight, blood urea, serum cholesterol and liver glycogen. When the

Table 7.5. The serum insulin (μU/ml) level of animals treated with the alkaloids of *Aegle marmelos* and an antidiabetic drug- Euglucon

Animal status	Serum insulin (μU/ml)
1. Saline injected control group	11.7 ± 0.3
2. Diabetic group	5.7 ± 0.5
3. Diabetic and alkaloids treated after 5 days of alloxan injection (100mg/kg body weight)	8.9 ± 0.7*
4. Diabetic and Euglucon treated after 5 days of alloxan injection (100mg/kg body weight)	9.5 ± 0.5*

Values are mean ± SD of 6 animals in a group

*P<0.05 compared to diabetic group

diabetic rabbits were treated with the antidiabetic drug Phenformin, almost the same pattern is shown when the different biochemical parameters are analysed. Santakumari et al., (1984) reported that when tolbutamide, an antidiabetic drug at a dose of .25g/kg is given to alloxan induced diabetic rabbits, it reduced the blood glucose and they compared the activity to *'Dia-Dev'* an Ayurvedic formulation. So it can be construed that the alkaloids present in *Aegle marmelos* leaves behaved in a way similar to that of an anti diabetic drug.

Conclusion

The alkaloids are potential candidates for the treatment of diabetes mellitus since the study shows that the blood glucose is maintained at the normal level which is always considered as a prime factor in the treatment of the disease. Certainly the whole leaf powder is considered to be more effective and the aim is to isolate the active principle and to purify it. In this context, we got a mixture of alkaloids and by further purification the different components in it can be separated.

CHAPTER VIII

GLUCOSE TOLERANCE TEST WITH THE WHOLE LEAF POWDER, AQUEOUS EXTRACT AND ALKALOIDS

CHAPTER VIII

Glucose Tolerance Test in normal and diabetic rats with the whole leaf powder of *Aegle marmelos*, aqueous extract and alkaloids

8.1. Introduction

Glucose tolerance is an artificial load, because in day-to-day life such large amount of glucose does not enter into the blood. However, the GTT is a well standardised test and is highly useful to diagnose diabetes mellitus. Usually in normal individuals, if glucose load is given orally there is a spike in the blood glucose level which reaches the normal level within 90 minutes. The administration of an antidiabetic drug before 30 minutes and then giving a glucose load will prove the potential of the drug in lowering the blood glucose.

8.2. Materials and Methods

Normal healthy male rats were selected and were divided into four groups, six rats in a group.

Group1- Kept as control and 1.5g/kg glucose solution was fed orally

Group2- 500mg/kg leaf powder administered and after 30 minutes 1.5g/kg glucose solution given

Group3- 1g/kg aqueous extract administered and after 30 minutes 1.5g/kg glucose solution given

Group4- 100mg/kg alkaloid administered and after 30 minutes 1.5g/kg glucose solution given

The blood of all experimental and control animals were collected at 0h, 30m, 60m, 90m, 120m and 180m. The blood glucose of all the samples were estimated.

Glucose tolerance test was done in the diabetic animals also in the same pattern as described above.

8.3. Results

The blood glucose values in normal animals are presented in Table 8.1. Both in normal and experimental animals the initial blood glucose value ranged between 82mg/dl and 84mg/dl. In the normal animals after 30m of glucose load the blood glucose was 137mg/dl. But in groups 2, 3 and 4 it was 125mg/dl, 132mg/dl and 127mg/dl respectively. After 90m in the control group the blood glucose level was 99mg/dl and in group 2, 3 and 4 it was 89mg/dl, 102mg/dl and 92mg/dl respectively. By the end of 180m all the animal groups showed the normal blood glucose level (Fig. 8.1)

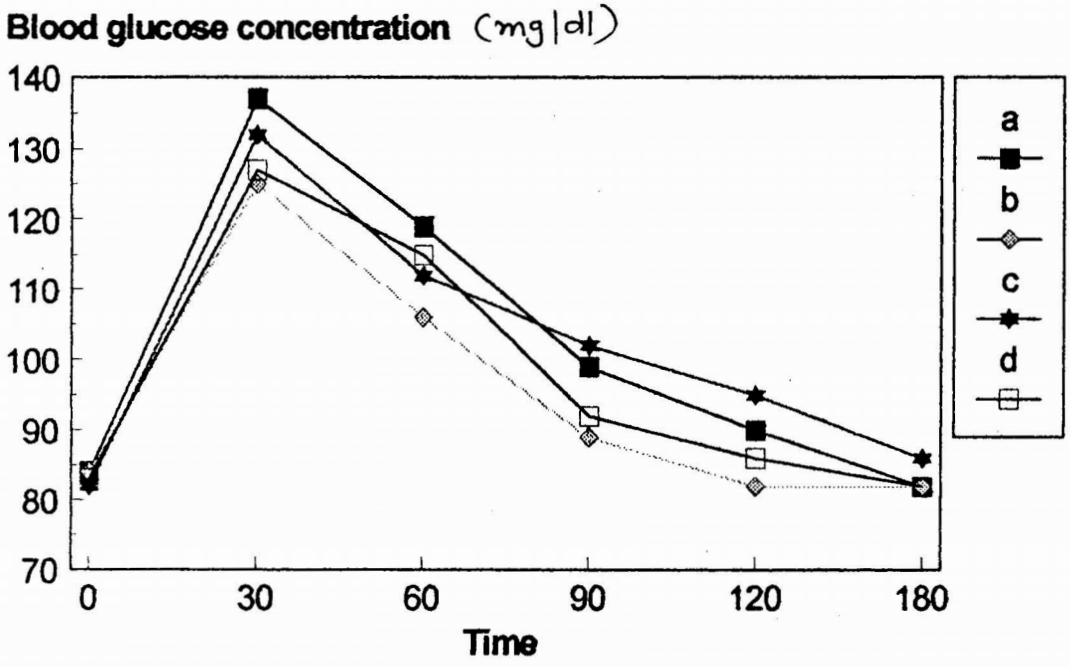
In the diabetic animals the control group had an initial blood glucose value of 376mg/dl and upon administration of glucose solution it shot up to 399mg/dl in 30m, then to 419mg/dl in 60m and then came down to 406mg/dl in 90m. In group 2, the initial blood glucose level was 402mg/dl which went up to 424mg/dl in 30m, then to 400mg/dl in 60m and 381mg/dl in 90m. In group3 and 4 the initial blood glucose level was 392mg/dl and 397mg/dl respectively. In the first 30m of glucose administration it was 412mg/dl and 426mg/dl, after 60m it was 403mg/dl and 412mg/dl and after 90m it was 390mg/dl and 401mg/dl respectively.(Table 8.2) After this the blood glucose level showed decreasing trend and at the end of 180m it was even less than the initial blood glucose value.(Fig.8.2.)

Table 8.1. Glucose tolerance test in normal animals.

	0 hour	30 minute	60 minutes	90 minutes
1. Control 1.5g/kg glucose solution given orally	84±1.62	137±3.35	119±4.62	99±2.43
2. Oral administration of whole leaf powder (500mg/kg) 1/2 h prior to oral administration of glucose solution (1.5g/kg)	84±1.27	125±4.75	106±3.84	89±1.98
3. Oral administration of aqueous extract (1g/kg) 1/2 h prior to oral administration of glucose solution (1.5g/kg)	82±1.75	132±3.62	112±3.97	102±2.83
4. Oral administration of alkaloid fraction (100mg/kg) 1/2 h prior to oral administration of glucose solution (1.5g/kg)	83±1.27	127±7.92	115±4.74	92±3.92

Values are mean ± SD of 6 animals in a group

Fig. 8.1.Glucose tolerance test (GTT) in normal animals.



a - Control - 1.5g/kg glucose solution given orally

b - 500mg/kg whole leaf powder given orally half an hour before the oral administration of 1.5g/kg glucose solution.

c- 1g/kg leaf powder aqueous extract given orally half an hour before the oral administration of 1.5g/kg glucose solution.

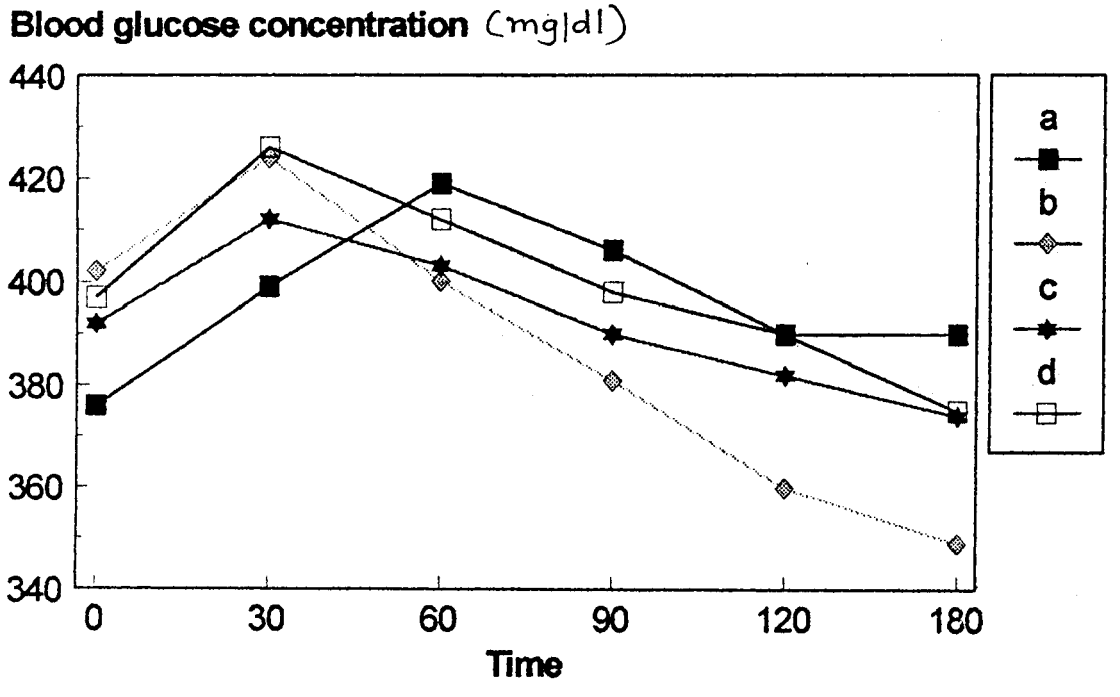
d - 100 mg/kg of alkaloid fraction given orally half an hour before the oral administration of 1.5g/kg glucose solution.

Table 8.2. Glucose tolerance test in diabetic animals.

	0 hour	30 minute	60 minutes	90 minutes
1. Control 1.5g/kg glucose solution given orally	376±19	399±14	419±14	406±9.0
2. Oral administration of whole leaf powder (500mg/kg) 1/2 h prior to oral administration of glucose solution (1.5g/kg)	402±12	424±9.0	400±10	381±9.0
3. Oral administration of aqueous extract (1g/kg) 1/2 h prior to oral administration of glucose solution (1.5g/kg)	392±11	412±9.0	403±7.0	390±9.0
4. Oral administration of alkaloid fraction (100mg/kg) 1/2 h prior to oral administration of glucose solution (1.5g/kg)	397±8.0	426±10	412±9.0	401±11

Values are mean ± SD of 6 animals in a group

Fig. 8.2. Glucose tolerance test (GTT) in diabetic animals



a - Control - 1.5g/kg glucose solution given orally

b - 500mg/kg whole leaf powder given orally half an hour before the oral administration of 1.5g/kg glucose solution

c - 1g/kg leaf powder aqueous extract given orally half an hour before the oral administration of 1.5g/kg glucose solution

d - 100mg/kg of alkaloid fraction given orally half an hour before the oral administration of 1.5g/kg glucose solution

8.4. Discussion

Glucose tolerance test is usually practised in the medical field to give an insight into the fate of an individual, whether he is prone to diabetes. In our experiment, the idea was to test the said drug in the presence of a glucose load in rats and to compare it with the control. In the control group of normal animals, the serum glucose value rose to a peak in 30m and began to drop from 60m onwards reaching a value of 99mg/dl in 90m. In the whole leaf powder treated group, the serum glucose value was less than the control group at all stages of study. The aqueous extract treated group had a lesser serum glucose value compared to the control group at 30 and 60m while the value was higher than control group at 90m. It is very interesting to note that the alkaloid fraction treated group had the serum glucose value comparable to whole leaf powder treated group, with better hypoglycaemic effect than the aqueous extract treated group at 30m. The hypoglycaemic effect of the whole leaf powder treated group was higher at 60m when compared to all other groups.Similarly at 90m also, the whole leaf powder treated group and alkaloid fraction treated group showed pronounced hypoglycaemic activity compared to control and aqueous extract treated groups. Chen and Xie (1987) reported that from the aerial parts of *Coptis chinensis* an alkaloid berberine was extracted and when given to normal and alloxan diabetic mice improved glucose tolerance. Similarly, dried sap of *Aloe vera* exhibited glucose tolerance in normal mice (Al-Awadi et al., 1985). Fernando et al., (1989) documented that the aqueous extract of the whole plant of *Astercanta longifolia* markedly improved the glucose tolerance in rats. *Pinitol*, a compound isolated from the leaves of *Bougainvillea spectabilis* increased glucose tolerance in normal rats when given orally as reported by Narayanan et al., (1987).Perez et al., (1996) reported that oral administration of the chloroform extract of bark of *Agarista mexicana* and leaves of *Verbesina persicifolia* altered glucose

tolerance in alloxan induced diabetic rats and enhanced glucose uptake in the skeletal muscles and significantly inhibited glycogenolysis in the liver. It was documented by Rodriguez et al (1992) that the oral administration of aqueous extract of *Hexachlamys edulis* to normoglycaemic and alloxan diabetic rats improved glucose tolerance . In this context it can be inferred that the whole leaf powder treatment has the potential to induce hypoglycaemia better than the aqueous extract or the alkaloid fraction treated group. The alkaloid treatment appears to be superior to aqueous extract treatment in inducing hypoglycaemia in normal animals.

In the diabetic animals also the hypoglycaemic effect of the whole leaf powder treatment was more pronounced at all stages of the experiment. The mean serum glucose values of the aqueous extract treated group and alkaloid fraction treated group were comparable. All the three types of treatments were significantly effective in inducing hypoglycaemia on comparison with the diabetic control group. The results in normal and diabetic animals establish that the whole leaf powder of *Aegle marmelos* has significant hypoglycaemic potential in normal as well as alloxan induced diabetic animals. The aqueous extract of whole leaf powder as well as the alkaloid components of the whole leaf were also effective in reducing the serum glucose levels. These observations appear to arise on the fact that the active principles of this plant might increase the serum insulin level and also ~~felicitate~~ facilitate better glucose absorption and utilisation by tissues. These results also indicate that there might be some other active principle other than the alkaloid extract and the components present in the aqueous extract which might have hypoglycaemic effect or a potentiating effect on the alkaloid or the aqueous extract principle.

CHAPTER IX

IN VITRO STUDY WITH AQUEOUS EXTRACT AND ALKALOID IN DIAPHRAGM MUSCLES,SPLEEN CELLS AND INTESTINAL SEGMENTS

CHAPTER IX

IN VITRO STUDY

9.1 Introduction

The blood glucose concentration depends on a wide variety of factors and its concentration at any time is the net result of an equilibrium between the rates of entry and removal of glucose in circulation. As such, all the factors that exert influence upon the entry or removal become of importance in the regulation of blood glucose concentration. Furthermore, when the renal reabsorptive capacity for glucose is exceeded, urinary loss of glucose becomes an additional factor influencing the maintenance of blood glucose concentration.

In vitro studies are to be carried out to ascertain whether glucose can be absorbed by the intact cell and the criteria required for this process. It can also give an idea regarding the role of the extract in the facilitated transport of glucose across the cell. This can be studied either in intact cell or in isolated cell suspension. The glucose uptake in intact cells can be carried out on isolated diaphragm and small intestine. Spleen cell suspension can be used as individual cells for studying the effect of the extract on glucose uptake. Further, lipid peroxidation with liver homogenate in the presence of the extract will give an idea about the percentage of inhibition by scavenging the free radicals.

9.2 Methods used

9.2.1. Isolation of diaphragm and the study of glucose uptake with the extract

Normal healthy rats of 90 - 120 days and weighing 180 - 200g were used. The animals were sacrificed by cervical dislocation and the diaphragm muscle was carefully removed asceptically into a petri dish containing cold PBS. The diaphragm was cut into bits weighing 100 - 150mg and these pieces were transferred to 2ml PBS and pre incubated for 10min at 37°C. The tissue was transferred to 2ml PBS having 400mg/100ml glucose. Then 500ul of the aqueous leaf extract was added and in the controls 500ul of distilled water. The whole experiment set up was incubated at 37°C for 60min. After incubation the glucose in the medium was estimated.

In an identical experiment, the alkaloids extracted from the leaf in a volume 100µl was added and the amount of glucose in the medium estimated after 60m.

9.2.2. Preparation of single cell suspension from spleen

The rats were sacrificed as described in 2.3.1.2 and the spleen was removed asceptically into a petri dish containing cold M199 supplemented with 10% foetal calf serum. The spleen was teased with a toothed forceps and the pieces transferred to a homogeniser which had cold M199 with 10% foetal calf serum. The spleen pieces were gently homogenised. The homogenate was passed through a sieve to remove solid particles. The cells were treated with trypsin - EDTA for 5min and then washed thrice with HTPBS. The cells were washed twice in the medium containing 10% foetal calf serum and the viability of the cells were checked by trypan blue exclusion method. The cell suspension which showed 90% viability were

selected and $1x10^6$ cells were transferred to 96 well microtiter plates. Volume in each well was made up to 250µl with complete medium. Then 50µl of aqueous extract was added to the wells and the controls 50µl of distilled water. The plate was incubated in a humudified incubator at 37°C and the glucose content in the medium of the experimental and control wells were estimated after 30min, 60min and 90min.

In a similar experiment as described, alkaloids extracted from the leaves in a volume 10µl was added into the wells and the glucose content of the medium was estimated.

9.2.3. Glucose uptake by intestinal segments of rats

Normal healthy rats of 90 - 120 days old and weighing 180 - 200 g were selected . The animals were sacrificed by cervical dislocation and the small intestine was cut off and transferred to a petri dish containing PBS. The intestine was cut into bits of 1cm to 2cm and the contents inside the intestine were carefully removed by forcing PBS through it with a syringe. The intestinal segments were made inside out and it was tied in a thread and suspended in 2ml PBS with 400mg/100ml glucose in a test tube. The 500µl of the aqueous extract was added to the experimental group and 500µl of PBS to the control tubes. The experimental set up was put in an incubator at 37°C and glucose content of the medium estimated after 30min, 60min and 90min.

In an identical experiment 500µl of alkaloid extract of the leaf powder was added and the glucose content of the medium estimated.

9.2.4. Determination of lipid peroxidation

The ascorbic acid induced non-enzymatic lipid peroxidation using liver homogenate was determined by the thiobarbituric acid (TBA) method (Bishayee and Balasubramaniyan, 1971). Rat liver homogenate (25%, 100µl) in cold tris HCl buffer (0.2 M, pH 7.0) was incubated for 1 hr at 37°C with 150 nM KCl (100µl), 0.3 nM ascorbic acid (100µl) and tris HCl buffer (0.2 M, pH 7.0) in a total volume of 500µl. Test materials were also added during the incubation period. Controls without test materials were also kept. After incubation, 20 per cent trichloroacetic acid (1 ml) was added and the mixture heated in a boiling water bath for 15 min. Tubes were then cooled to room temperature and centrifuged at 2000 g. Optical density of the supernatant was measured. The amount of lipid peroxide was expressed as nanomoles of malonaldehyde formed in each tube.

9.3. Results

The glucose uptake by diaphragm muscles in response to the aqueous extract and alkaloids of *Aegle marmelos* is presented in Table 9.1 and 9.2 respectively. In the aqueous extract test group there ~~had been~~ was a significant decrease in the glucose content of the medium when compared to the control. But in alkaloids added test group there was no significant decrease in the glucose content of the medium incubated with diaphragm muscles, when compared with control.

The glucose uptake by spleen cells in response to the aqueous extract and alkaloids of *Aegle marmelos* leaves is presented in Table 9.3 and 9.4 respectively. In both there had been a significant decrease in the glucose content of the medium when compared with the control.

Table 9.1. Glucose uptake by diaphragm muscles in response to the aqueous extract of *Aegle marmelos* leaves.

Concentration of glucose without tissue	After incubation with 100mg of tissue (60min)	
	Control	Test (Aqueous extract 500µl)
300mg/dl	283 ±7	236±4*

Values are mean ± SD of six experiments

* $P < 0.05$ compared to control group

Table 9.2. Glucose uptake by diaphragm muscles in response to the alkaloid fraction of *Aegle marmelos* leaves.

Concentration of glucose without tissue	After incubation with 100mg of tissue (60min)	
	Control	Test (Alkaloid fraction 500µl)
300mg/dl	280±7	248±2*

Values are mean ± SD of six experiments
* $P < 0.05$ compared to control group

Table 9.3. Glucose uptake by spleen cells (1 x 10^6 cells) in response to the aqueous extract of Aegle marmelos leaves.

	Glucose content (mg/dl) in the medium after incubation at 37°C.			
	0 min	30 min	60 min	90 min
Test	800	780±26	622±32	572±42*
Control	800	786±17	684±21	653±29

Values are mean ± SD of six experiments

* P l< 0.05 compared to control group

Table 9.4. Glucose uptake by spleen cells (1 x 10^6 cells) in response to the alkaloid fraction of Aegle marmelos leaves.

	Glucose content (mg/dl) in the medium after incubation at 37°C.			
	0 min	30 min	60 min	90 min
Test	800	741±29	657±12	609±37*
Control	800	786±17	684±21	653±29

Values are mean ± SD of six experiments

* P l< 0.05 compared to control group

The glucose uptake by intestinal segments in response to the aqueous extract and alkaloids of *Aegle marmelos* leaves is presented in Table 9.5 and 9.6 respectively. There was a significant decrease (P<0.05) in glucose content of the medium in both the experiments, compared to the controls.

The lipid peroxidation in rat liver homogenate with aqueous extract and alkaloids of *Aegle marmelos* leaves is presented in Figs 9.1 and 9.2 respectively. The alkaloids ~~are~~ were more effective above 50µg concentration and 50% inhibition was seen at a dose of 150µg.

9.4. Discussion

The glucose uptake by diaphragm muscles can be construed as an increase in glucose uptake by tissues in the presence of the extract. The aqueous extract was capable of considerably enhancing the glucose uptake compared to alkaloidal fraction. It was reported by Welhinda and Karunanayake (1986) that the fruit juice of *Momordica charantia* could significantly increase glucose uptake by rat diaphragm muscles *in vitro*. The aqueous extract may have the factors that may be triggering the glucose utilisation by intact tissues and this may be lacking in the alkaloid fraction. But when cell suspension was used, both the aqueous extract and alkaloid fraction showed a significant activity by increasing the glucose uptake by the cells when compared with the control. Similarly, when intestinal fragments are used there was a significant decrease in the glucose content of the medium when the aqueous extract and alkaloids are added. It can be inferred that since the aqueous extract and alkaloids are administered orally, it may be reaching the intestine and can activate the intestine to absorb the glucose and utilise it in an efficient way.So an abnormal increase in the glucose level is checked.This really tally with our result

Table 9.5. Glucose uptake by intestinal segments in response to the aqueous extract of *Aegle marmelos* leaves.

	Glucose content (mg/dl) in the medium after incubation at 37°C.			
	0 min	30 min	60 min	90 min
Test	400	329±25	241±29	201±42*
Control	400	381±17	321±19	293±11

Values are mean ± SD of six experiments

* P l< 0.05 compared to control group

Table 9.6. Glucose uptake by intestinal segments in response to the alkaloid fraction of Aegle marmelos leaves.

	Glucose content (mg/dl) in the medium after incubation at 37°C.			
	0 min	30 min	60 min	90 min
Test	400	341±14	295±37	227±25*
Control	400	381±17	321±19	293±11

Values are mean ± SD of six experiments

* P l< 0.05 compared to control group

Fig. 9.1. Lipid peroxidation in rat liver homogenate with the aqueous extract of *Aegle marmelos* at different concentrations.

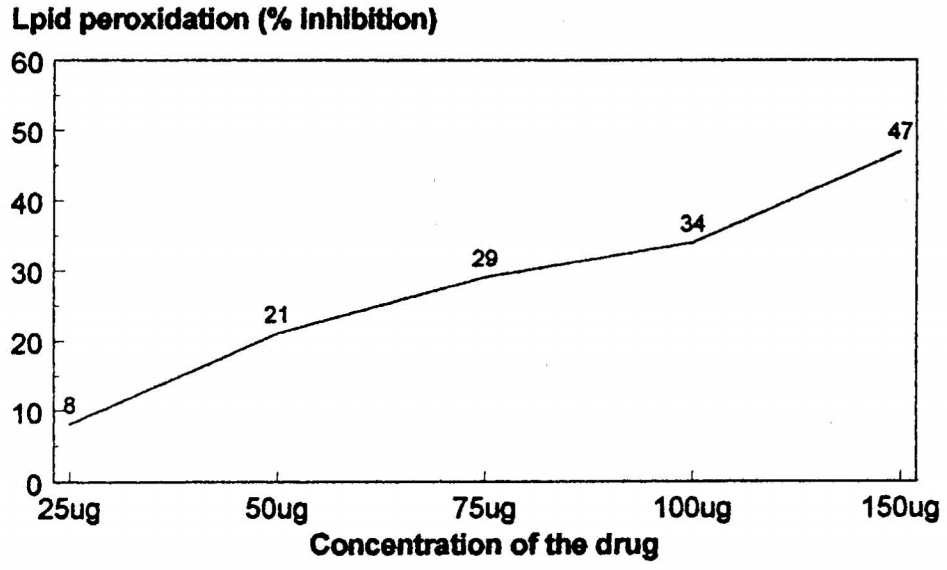
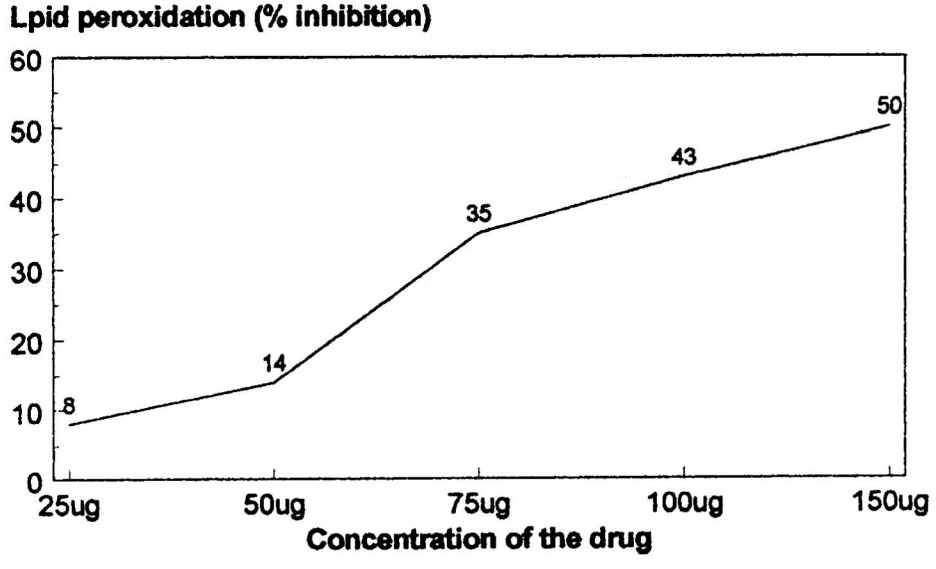


Fig. 9.2 Lipid peroxidation in rat liver homogenate with alkaloids of *Aegle marmelos* leaves at different concentrations.



since the adminstration of the aqueous extract and alkaloids of Aegle marmelos in alloxan induced diabetic rats controlled the blood glucose level.

It has been suggested by Yoon (1990) that the possible mechanism of action of alloxan in beta cells of islets include the generation of oxygen free radicals and alteration of endogenous scavengers of these reactive species, mainly in rodents. Inhibition of lipid peroxidation by the active principles present in aqueous extract as well as alkaloids indicates the potential of these ingredients in the leaves of *Aegle marmelos* to scavenge the free radicals formed in alloxan treatment, preventing the tissue damage and cytotoxicity to the B cells of pancreas and thereby preventing the incidence or reducing the intensity of damage by reducing the severity of diabetes. It was observed that an aqueous extract at a dose of 75µg was capable of 29 per cent inhibition and at a dose of 150µg the percentage of inhibition was 47. The alkaloidal fraction was more potent compared to aqueous extract. At 75µg dosage the percentage of inhibition was 35 and at a dose of 150µg, the inhibition was 50 per cent. It is reported by Song et al., (1992) that *Coptis chinesis* has very strong inhibitory effect on lipid peroxidation in normal and diabetic animals suggesting that Coptis could protect against diabetes induction by alloxan that probably was due to the fact that the drug was able to inhibit alloxan inducing free radicals. Yoshisaki et al., (1996) reported that the ethanol extracts of *Ephedra sinica* and *Nebumbo nucifera*, were significantly and dose dependently effective in eliminating superoxides in the plasma. In vitro study by Ip et al., (1997) for antioxidant activities with the crude water extract of the root of *Polygonum multiflorum* reported its ability to protect against CCl_4 induced hepatotoxicity in rats and to scavenge ferri-heme oxidants. Panda and Kar (1997) reported that the root powder of *Ashwagandha* possess free radical scavenging activity which may be responsible for it pharmacological activity.

CHAPTER X

GENERAL DISCUSSION

CHAPTER X

General Discussion

The internal secretions of anterior pituitary, adrenal cortex, medulla and pancreas are closely associated with carbohydrate metabolism. The hormone secreted by B cells of pancreas play the key role in carbohydrate metabolism. After the successful extraction of insulin by Banting and Best (1922) and elucidation of insulin structure by Sanger (1960), soon its precursor proinsulin and its structure were also identified ~~was quickly known~~. It has been subject to many reviews (Kitabchi, 1977; Schade and Eaton, 1985; Raptis and Dimitriadis, 1985; Taylor, 1995).

Insulin is transported in circulation bound to a B globin. At a tissue, insulin binds to receptors on the cell membrane. The insulin receptor is a very large glycoprotein on the surface of almost all the cells. The principal sites of insulin are in the initial phases of glucose metabolism. Insulin first binds to insulin receptors of target cell plasma membranes and then facilitates glucose entry into cells such as muscles and fat by activation of glucose transporters. With increased accumulation of glucose, metabolic scheme is enhanced and glucose utilisation is increased.

Insulin influences the metabolism of glucose by liver cells, the central organ of glucose homeostasis. The insulin receptors are not significantly regulated by insulin so the liver cell is freely permeable to glucose. Therefore, the major action of insulin in liver cell is after the initial transport step. The principal step is the first phosphorylation of glucose to form glucose-6-phosphate in the reaction catalysed by glucokinase. This glucokinase reaction is rate limiting and glucokinase activity is

influenced by insulin. Additionally, the effect of insulin on other key unidirectional phosphorylative steps directs glucose metabolism toward utilisation and fatty acid synthesis. An important effect of insulin is to increase the activity of pyruvate dehydrogenase system, which increases acetyl co enzyme, thereby promoting increased fatty acid synthesis and oxidation of carbon dioxide via Kerb's cycle.

Long before the use of insulin, indigenous remedies have been used for the treatment of diabetes mellitus. There is an increasing demand among patients to use the natural antidiabetic agents because insulin cannot be used orally and oral hypoglycaemic agents have many side reaction and toxicity. Traditional Indian system of medicine (Ayurveda) has relied on the curative property of leaves of *Aegle marmelos* in the treatment of diabetes even in Shustruta Samhita and Charaka Samhita.The leaves of *Aegle marmelos* were commonly used in the treatment of diabetes mellitus in the Ayurveda system of medicine and its antidiabetic principle was reported by Chatterjee and Bose (1952), Dhar et al., (1968), Chakraborthy and Poddar (1984), Sharma et al., (1996),Sharma et al., (1996), Reddy et al., (1996).

This background information necessitated a detailed analysis on the antidiabetic effect of *Aegle marmelos* and also on the active ingredient which might have tremendous potential as therapeutic agents in the treatment of diabetes which has an epidemic incidence of 5-10% among Indian population. Diabetic epidemic has been projected to reach an alarming proportion of about 20% by early decades of next century.

The diabetogenic potential of alloxan among rats could be confirmed in the present study. The growth rate among the normal and control groups showed an

upward trend in the beginning reflecting the growing stage. The growth rate of the diabetes induced rats began to decline substantially during the entire phase of the experiment. The blood glucose level of diabetic rats shot upto 340mg/dl after 30 days of alloxan injection. The diabetic rats also has elevated serum cholesterol, blood urea and is in agreement with earlier workers. {Giri et al. (1986), Hukeri et al. (1988), Sharma and ~~Mitali~~ Majumder (1990) } Administration of insulin improved body weight and reduced blood glucose level, improved metabolic activity and over all performance of the animals. The administration of whole leaf powder after 24h of alloxan injection was superior to insulin treatment taking into account body weight , blood glucose level, serum cholesterol, blood urea, liver glycogen and serum insulin. Treatment after 5 days of alloxan injection with the whole leaf powder was inferior to insulin administration. The higher serum insulin level of the whole leaf powder treated animals is indicative of its protective role in alloxan induced diabetes. The reduction of severity of diabetes induction in animals which are administered whole leaf powder 24h of alloxan injection is confirmative of its protective role from diabetes induction. Delay in administration of whole leaf powder for 5 days reduced its protective effect indicating that the active principles of the whole leaf powder has a protective effect on pancreatic β cells on alloxan induced damage and necrosis.

In an attempt to isolate and identify the active principle the whole leaf powder of *Aegle marmelos* were extracted using petroleum benzene, chloroform, methanol and water. These extracts were tested in alloxan diabetic rats and it was confirmed that the aqueous extract alone had a pronounced hypoglycaemic effect in alloxan induced diabetes.

As early as 1984, Karunanayeke et. al. reported that the aqueous extract of *Aegle marmelos* leaves has significant hypoglycaemic activity in alloxan induced diabetic rats. In the present study also, aqueous extract treated animals had a reduced serum glucose, serum cholesterol and blood urea. This again is confirmed by Sharma et. al., in 1986, who could demonstrate hypoglycaemic and antihyperglycemic effect of *Aegle marmelos* leaves in normoglycaemic and streptozotocin induced diabetic rats.

The aqueous extract of *Aegle marmelos* contain mucilage, pectin, sugar, alkaloids, tannin, volatile oil, bitter principle etc. (Indian Materia Medica). There were many reports that the alkaloids from different plant parts have antidiabetic activity. So the alkaloids from the leaves of *Aegle marmelos* were isolated and these were tested in alloxan diabetic rats. It could be confirmed that the alkaloids had hypoglycaemic activity in diabetic rats and so also the blood urea and serum cholesterol level were kept at par with the normal animals. There was also an increase in the body weight when compared with the diabetic group. This finding is in accordance with fully ~~endorsed by~~ Sharma et. al.(1981), who could isolate an alkaloid from the fruits of *Aegle marmelos* and tested in diabetic animal models.

The antidiabetic effect of alkaloids derived from *Aegle marmelos* leaves were compared for the first time with a synthetic antidiabetic drug-Euglucon. The decrease in body weight in Euglucon and alkaloid treated groups were comparable. Thirty days after treatment the glucose level was significantly lower when compared with Euglucon treated groups. Similarly, blood urea and serum cholesterol levels were lower in alkaloid treated groups. Though this is the first report of comparison of antidiabetic principle of *Aegle marmelos* with a synthetic drug, similar studies

have been reported by Padmini and Chakravarthi (1983) on the fruits of *Eugenia jambolana*. They compared the effect of the fruit extract with an antidiabetic drug Phenformin in diabetic rabbits. It probably appears that the success of the present study has a potential application in the management of diabetes mellitus especially in the Indian context where, *Aegle marmelos* can be propagated with limited cost.

Glucose tolerance test (GTT) conducted with the whole leaf powder, aqueous extract and alkaloids of *Aegle marmelos* leaves in the normal animals showed the superiority of the whole leaf powder over aqueous extract and alkaloids in glucose clearance. In the diabetic animals also whole leaf powder was superior to aqueous extract and alkaloid fraction of the leaves. The alkaloid treatment was superior to aqueous extract in normal animals while aqueous extract was superior to alkaloid fraction in diabetic animals as far as glucose clearance is concerned.

The results of the present study on glucose uptake by diaphragm muscles, spleen cells and intestinal segments modulated by aqueous extract and alkaloids of *Aegle marmelos* leaves substantiates the hypothesis that hypoglycaemic effect might be by ~~encouraging~~ enhancing the cellular uptake of glucose. Welhinde and Karunanayake (1986) ~~could~~ also ~~find~~ observed enhanced glucose uptake with the fruit juice of *Momordica charantia* in rat diaphragm muscles. This result endorse the confirmation of the hypoglycaemic effect of *Aegle marmelos*, which could also be possibly due to enhanced cellular transport of glucose molecules.

One of the possible mechanism suggested by Yoon (1990) on alloxan induced damage of β cells of pancreatic islets is the generation of free oxygen radicals and alteration of endogenous scavengers. Lipid peroxidation inhibition assay

using the aqueous extract and alkaloids of *Aegle marmelos* demonstrated the ability of these principles in inhibiting the lipid peroxidation to the tune of 21% at 50μg and in the alkaloid fraction a 75μg concentration inhibited the lipid peroxidation to 35%. This might be one of the basic mechanisms affording protection to the pancreatic cells from chemically induced diabetes mellitus. Similar studies on free radical scavenging by various plant extracts have been reported by Song et. al. (1992), Yoshisaki et. al. (1996), Ip et. al. (1997) and Panda and Kar (1997).

The results of the present study point out the possibility of utilising *Aegle marmelos* and its alkaloids as a potential antidiabetic agent. The results could confirm the ability of this plant in increasing the insulin level, enhancing the glucose uptake by cells, reduction of blood urea and cholesterol and keeping the blood glucose level almost at par with the normals. ~~Though~~ To a limited extent, the results are confirmative of inhibition of lipid peroxidation and free radical scavenging which have a definite role in affording protection to the β cells of pancreatic islets by diabetogenic chemicals and preventing the incidence of diabetes mellitus.

CHAPTER XI

SUMMARY AND CONCLUSION

CHAPTER XI

SUMMARY AND CONCLUSION

The present study substantiates that the leaves of *Aegle marmelos* has the potential antidiabeitc activity preventing the manifestation of diabetes in alloxan injected rats fed the leaf powder orally. Alloxan selectively destroys the β cells of pancreas. If the treatment would have been started with in 24h of alloxan injection the abnormal decrease in body weight, liver glycogen, serum insulin and increase in blood glucose, blood urea and serum cholesterol could be prevented. It was noted that the treatment of the whole leaf powder even after 5 days of alloxan injection was effective in preventing diabetic symptoms. To isolate the active principle, different extracts were given to diabetic rats. The aqueous extract showed the maximum activity in preventing the abnormal increase in blood glucose and decrease in body weight. That means the active antidiabetic principle may be soluble in water. The administration of alkaloids isolated from the leaf powder was found to be effective in preventing the abnormal increase in blood glucose, blood urea, serum cholesterol and decrease in serum insulin, liver glycogen and body weight in diabetic rats. The anidiabetic activity of the *Aegle marmelos* leaves was found to be on par with an antidiabetic drug, Euglucon.

Glucose tolerance test (GTT) conducted with the whole leaf powder, aqueous extract and alkaloids of *Aegle marmelos* leaves in the normal animals showed the superiority of the whole leaf powder over aqueous extract and alkaloids in glucose clearance. In the diabetic animals also whole leaf powder was superior to aqueous extract and alkaloid fraction of the leaves. The alkaloid treatment was

superior to aqueous extract in normal animals while aqueous extract was superior to alkaloid fraction in diabetic animals as far as glucose clearance is concerned.

The glucose uptake by diaphragm muscles, spleen cells and intestinal segments modulated by aqueous extract and alkaloids of *Aegle marmelos* leaves substantiates the hypothesis that hypoglycaemic effect might be by ~~encouraging~~ enhancing the cellular uptake of glucose. .

Lipid peroxidation inhibition assay using the aqueous extract and alkaloids of *Aegle marmelos* demonstrated the ability of these principles in inhibiting the lipid peroxidation to the tune of 21% at 50μg and in the alkaloid fraction a 75μg concentration inhibited the lipid peroxidation to 35%. This might be one of the basic mechanisms affording protection to the pancreatic cells from chemically induced diabetes mellitus.

It can be assumed that the whole leaf powder was showing the maximum activity because all the constituents were supplied to the diabetic animals by this way. It may be preventing the damage to the β cells of islets and maintain normal insulin secretion. So also the whole leaf powder may be converting the proinsulin to active insulin. But when the alkaloids ~~are~~ were tested even though hypoglycaemic activity was exhibited the efficiency of it, when compared with the whole leaf powder, was less. Recently Das et al (1996) reported that the administration of the aqueous extract of *Aegle marmelos* leaves altered the function of pancreatic β cells and the acinar cells. The changes in the acinar cells were coarsening of endoplasmic reticulum and alterations in their secretory function. The changes observed in the liver were dilation of veins, loss of usual concentric arrangement of hepatocytes,

liver fibrosis and decrease in glycogen content. The kidney tubules were thickened and the glomerulus were expanded. The treatment reversed the altered parameters to near normal suggesting the regeneration of the damaged pancreas.

The results of the present study point out the possibility of utilising *Aegle marmelos* and its alkaloids as a potential antidiabetic agent. The results could confirm the ability of this plant in increasing the insulin level, enhancing the glucose uptake by cells, reduction of blood urea and cholesterol and keeping the blood glucose level almost at par with the normal controls. Though to a limited extent the results are confirmative of inhibition of lipid peroxidation and free radical scavenging which have a definite role in affording protection to the β cells of pancreatic islets by diabetogenic chemicals and preventing the incidence of diabetes mellitus.

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Effect of leaf extract of *Aegle marmelose* in diabetic rats

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Alloxan induced animal model was used to evaluate the potential antidiabetic effect of *A. marmelose* leaf extract. The diabetic animals were given insulin injection and another group *A. marmelose* leaf extract orally. It maintained the weight of the animals near to the control rats but a significant decrease in weight was noted in diabetic animals without any treatment. The blood glucose level in treated animals were near to that of control ones. Also a significantly increased glucose tolerance was observed in animals orally given the leaf extract prior to the experiment. A significant decrease in liver glycogen (1.24 ± .07 g/100 g of wet tissue) was observed in diabetic rats which was brought to almost the normal level (1.84 ± .14 g/100 g) with leaf extract treatment. Blood urea and serum cholesterol increased (62.66 ± 3.50 and 192.67 ± 13.64 mg/dl) significantly in alloxan diabetic rats. The leaf extract treatment decreased the blood urea and serum cholesterol (37.83 ± 3.97 and 99.20 ± 8.43 mg/dl) to that of control ones. A similar effect was seen with insulin treatment. The results indicate that the active principle in *A. marmelose* leaf extract has similar hypoglycaemic activity to insulin treatment.

Long before the use of insulin indigenous remedies have been used for the treatment of diabetes mellitus. There is an increasing demand by patients to use the natural products with antidiabetic activity. This is because insulin cannot be used orally and continuous insulin injection have many side effects and toxicity[1,2]. Besides certain oral hypoglycaemic agents are not effective in lowering the blood sugar in chronic diabetic patients[3].

Plant extracts have been used by various investigators as hypoglycaemic agents[4-12]. Recently Chandrasekhar *et al.*[13,14] showed hypoglycaemic activity in selected Curcurbitaceae plants of Indian origin and in *Swertia chirayita*. In the present study we have investigated the antidiabetic effect of *Aegle marmelose* leaf extract in the alloxan diabetic rats.

Materials and Methods

Chemicals used—All chemicals and reagents used in the study were of analytical grade. Glucose estimation was done by using glucose assay kit. Blood urea was estimated by the Urease method using Berthelot Reaction[15]. Serum cholesterol was estimated by using Liebermann-Burchard Reagent[16]. Isolation and estimation of liver glycogen was done according to Plummer[17].

Methods of preparing crude extract from A. marmelose leaves—Fresh tender leaves were collected, dried in shade and powdered. 10 g of leaf powder was mixed with 100 ml of distilled water and stirred for 2 hr. It was kept overnight at 4°C and the supernatant was collected. This was used as the crude leaf extract to study the antidiabetic effect in alloxan induced diabetes. The effective dose (the quantity of extract that can bring down the glucose level in the blood to the normal level) was 1 g/kg weight of the animal. The extract showed antidiabetic activity if kept at 4°C for 2 weeks.

Animals used for experiment—Albino rats (Wistar strain) of 2-3 months old were selected for all the experiments. Rats were divided into 6 groups of 6 each:

Group I, kept as normal group.

Group II, given physiological saline through the femoral vein and was taken as the control group.

Group III, alloxan injection (60 mg/kg body wt) was given through the femoral vein and kept without any treatment to study the diabetic nature.

Group IV, alloxan injected and one unit of insulin given on alternate days after 5 days.

Group V (a), alloxan injected and leaf extract given orally after 24 hr and the traetment continued for 30 days.

Group V (b), alloxan injected and leaf extract given orally after 5 days and the treatment continued for 30 days.

On every 5th day from the start of the experiment, body weight was taken and the blood glucose estimated. All animals were sacrificed after 30 days of the experiment. The blood urea, serum cholesterol

and glycogen was estimated from the samples of these animals.

Results and Discussion

A decreasing trend in the body weight was noted in alloxan induced diabetic rats. On treatment of such rats with insulin and leaf powder extract the body weight was brought back to the initial level (Table 1). This indicates that the leaf powder extract is having an action similar to that of insulin.

A significant increase ($P<0.01$) was observed in the blood glucose after 5 days of alloxan injection and a steady increase in the group of animals given no treatment (Fig. 1). But when group IV was given insulin a gradual decrease in blood glucose was observed which was kept almost to the control ones. A similar effect was observed when leaf extract was administerd to group Vb. But a daily administration of the leaf extract in group Va maintained the glucose level near to that of control ones. A hypoglycaemic effect of *A. marmelose* was first reported by Dhar *et al.*[18]. In the present study the antidiabetic effect of *Aegle marmelose* is substantiated by using alloxan induced diabetic animal model. A similar study was carired out by Akhtar *et al.*[19] in normal and alloxan diabetic rabbits using *Momordica charantia* fruits, which significantly decreased the blood glucose level.

Table 2 presents he effect of glucose tolerance test carried out in control and experimental rats treated with leaf powder extract 30 min prior to the start of the

Table 3—Liver glycogen in normal, control and experimental rats

[Values are mean ± SE of 6-8 separate determination in each group]

Group no. & animal status	g/100 g wet wt of tissue
I Normal group	2.11 ± .04
II Saline injected (control)	2.11 ± .07
III Diabetic (alloxan injected)	1.24* ± .05
IV Diabetic and insulin treated	1.75 ± .08
V Diabetic and leaf extract treated	1.84 ± .14

*$P<0.05$ compared to groups I, II, IV & V.

Table 4—Blood urea and serum cholesterol in normal, control and experimental rats

[Values are mean ± SEM of 6-8 separate determination in each group]

Group no. & animal status	Blood urea mg/dl	Serum cholesterol, mg/dl
I Normal	22.66 ± 2.80	84.16 ± 2.78
II Saline (control) injected	24.00 ± 2.09	84.83 ± 4.53
III Diabetic (alloxan injected)	62.66* ± 3.50	192.67* ± 13.64
IV Diabetic and insulin treated	34.33 ± 2.06	108.17 ± 9.47
V Diabetic and leaf extract treated	37.83 ± 3.97	99.20 ± 8.43

*$P<0.01$ compared to groups I, II, IV & V.

Table 1—Weight (g) of normal, control and experimental rats
[Values are mean ± SE of 6-8 rats in each group]

Group & animal status	Initial	5 days	10 days	15 days	20 days	25 days	30 days
I Normal Group	115 ± 5	120 ± 4	125 ± 6	131 ± 6	140 ± 8	147 ± 6	160 ± 7
II Saline injected (control)	120 ± 7	123 ± 4	127 ± 7	134 ± 7	139 ± 5	147 ± 6	155 ± 9
III Diabetic (alloxan injected)	124 ± 5	116 ± 8	110 ± 7	106 ± 5	100 ± 5	80 ± 8	80* ± 5
IV Diabetic and insulin treated	116 ± 7	107 ± 5	110 ± 4	100 ± 7	107 ± 5	103 ± 4	100 ± 6
V Diabetic and leaf powder treated	118 ± 6	114 ± 7	112 ± 5	110 ± 4	107 ± 6	107 ± 5	109 ± 8

*$P<0.05$ compared to the zero day of the experiment.

Table 2—Glucose tolerance test in control and treated rats
[Values are mean ± SE of 6-8 separate determination in each group]

	Wt (g) of animals	Blood glucose in mg/100 ml			
		0 hr	30 min	60 min	90 min
I Control 1.5 g/kg glucose solution given, po	268 ± 7	78.3 ± 1.2	130 ± 1.2	114 ± 0.9	98 ± 1.4
II Experimental: 1 g/kg leaf powder extract given (po) 1/2 hr before oral administration of 1.5 g/kg glucose solution	276 ± 5	78.3 ± 1.5	115.5 ± 1.2	99.3 ± 1.1	83.7* ± 1.5

*$P<0.05$ compared to control.

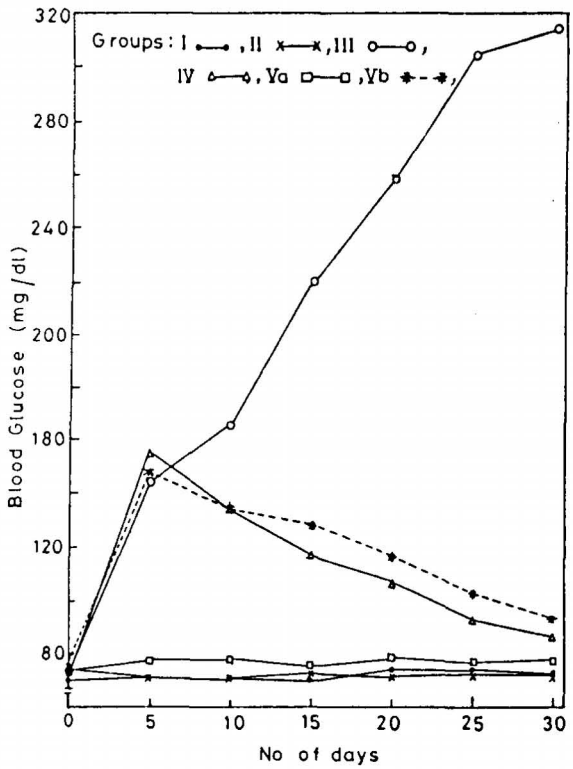

Fig. 1—Blood glucose levels is normal, control and experimental rats after varying intervals (group I—normal; group II—control (saline injected); group III—diabetic (alloxan injected); group IV—diabetic and insulin treated; group Va—diabetic and leaf extract treated 24 hr after alloxan injected and group Vb—diabetic and leaf extract treated 5 days after alloxan injection.

text. A significant increase ($P<0.05$) in glucose tolerance was observed. The liver glycogen level was significantly decreased ($P<0.05$) in diabetic rats, whereas, those treated with leaf powder extract maintained the glycogen in par with control rats (Table 3).

The blood urea and serum cholesterol levels increased significantly ($P<0.01$) in diabetic rats. In treated animals the blood urea and serum cholesterol were brought back to that of control rats. Giri *et al.*[9] reported a similar effect with the aqueous extract of *Cajanus cajan* is alloxan diabetic rats. It is a known fact that the kidney functioning is disturbed in diabetic condition. The treatment with leaf extract may have normalised the kidney function as indicated by the reversal of blood urea and cholesterol levels. Thus our results suggest that the active principle from *A. marmelose* leaf extract is effective for the treatment of diabetes.

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