

**STUDIES ON ORAL HYPOGLYCEMIC AGENTS FROM
SOME INDIAN MEDICINAL PLANTS**

**THESIS SUBMITTED
UNIVERSITY OF BOMBAY**

**FOR THE DEGREE OF
MASTER OF SCIENCE
IN
BIOCHEMISTRY**

**SUBMITTED BY
VILAS V. SHINDE**

**UNDER THE GUIDANCE OF
DR. S. T. NIKAM**

**HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
BOMBAY**

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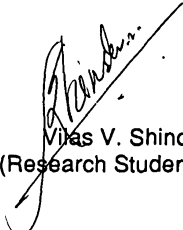
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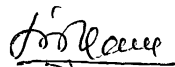
STATEMENT BY THE CANDIDATE

As required by the University Rule No. 108, I state that the work presented in the thesis entitled **"Studies on oral hypoglycemic agents from some Indian medicinal plants"** which I intend to submit is original in nature and, so far, has not been submitted for any degree of this or any other University. Wherever references have been made to work of others, it has been clearly indicated with sources of information in the 'Bibliography'.



Vitas V. Shinde
(Research Student)

Certified :



Dr. S. T. Nikam
(Research Guide)

A Word to say Thanks

Dr. S. T. Nikam, M.Sc., Ph.D.

Sir, I sincerely thank you for your great guidance and motivation in my research work.

Late Dr S. R. Sengupta, M.D., Director (1987-1993)

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REGULATION OF METABOLISM IN NORMAL AND DIABETIC CONDITION

The complex carbohydrates in food such as starch, lactose, sucrose, maltose etc. are rapidly hydrolysed by various digestive enzymes into monosaccharides which are then absorbed into portal circulation. After passing through the cell membrane glucose enters into several metabolic pathways. Glucose-6-phosphate derived from glucose or other sources like fructose or mannose, lactic acid, amino acid or glycerol can be converted to glycogen and stored in the tissues during glycogenesis. Glycogen storage is under the influence of insulin phosphorylase, adrenocortical steroids, glucagon, epinephrine, electrolytes and may be affected in diabetic condition. Supplies of blood glucose can be replenished from liver glycogen directly by glycogenolysis (Recant, 1955). Lactic acid is the end product of glycogenolysis and can be converted to blood glucose or to glycogen. During glycolysis, glucose-6-phosphate passes through number of reactions and finally converted into pyruvate. Then this pyruvate can be transformed into amino acid, alanine, by the addition of amino group. Acetyl-CoA may serve as a building block in the synthesis of amino acids, fatty acids, ketone bodies and other important products such as steroids. Acetyl-CoA enters Krebs Cycle, a major pathway for degradation of carbohydrate, protein and fat. The transamination of alanine and α -ketoglutarate results in the formation of pyruvate and glutamate. This reaction provides a link between carbohydrate and protein metabolism and represents a mechanism for the synthesis of amino acids from carbohydrate intermediates. In the liver some of the amino acids are converted to glucose following deamination. Amino acids have been shown to be glucogenic or gluconeogenic i.e. giving rise to liver glycogen or glucose. The released amino group is transformed into urea through urea cycle. It was found

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that in case of severe diabetes, large amount of sugar and ketone bodies are found in the urine on protein diet alone.

Dietary lipids consist of neutral fats, free fatty acids and steroids from animal and plant origin. With the help of enzymes the glycerides break down into glycerol and fatty acids which afterwards get absorbed by the lacteals and the lymphatic drainage system of the trunk. The absence of insulin or decreased destruction of insulin by insulinase, the metabolic functions of adipose tissue, strained muscle and liver gets altered in diabetic condition. The lack of insulin greatly reduces glycolysis and decreases the availability of oxaloacetate, proteogenesis and lipogenesis. Excess of acetyl-CoA which accumulate are then converted into ketone bodies.

When glucose uptake is diminished and thus both fatty acid synthesis and esterification of free fatty acids are impaired in diabetic state with the consequent net lipolysis and excess outflow of free fatty acid occurs in blood stream.

Protein metabolism is also severely affected. In deficiency state, insulin deficient muscle takes up and incorporates less amino acids into blood stream than does normal muscles (Manchester, 1972). Decreased protein synthesis coupled with enhanced catabolism results in a net loss of muscle protein and out pouring of amino acids into blood stream leading to muscular wasting despite the abundant supply of metabolic substrates for oxidation in the form of fatty acids and their metabolites. The predominant feature of liver metabolism in an insulin deficient organism is gluconeogenesis from precursors such as amino acids maintained and stimulated by simultaneous inflow and partial oxidation of free fatty acids. In addition, fatty acids flowing into the liver, are capable of phosphorylating free glycerol. These triglycerides

are then stored or secreted into blood stream in the form of particulate fat i.e. lipoproteins.

Persistent hyperglycemia is believed to be the major controllable factor which influences the development of diabetic complications. The excess amount of glucose in extracellular fluid increases its osmolar tension and draws fluid from intracellular space. This hyperosmolar fluid in kidney glomerulus urine causes the symptoms like polyurea, polydipsia and polyphagia. Loss of Na^+ & K^+ ions and intracellular dehydration cause lethargy, irritability, lack of concentration etc. The symptoms of dehydration are low volume pulse, low blood pressure, dry skin, low eye ball tension, dry turned tongue and cracked lips. The formation of acetone bodies leads to acidosis, coma and death.

The energy is mainly derived from protein and fat when glucose can not be burnt properly. In such conditions a patient develops loss of weight, weakness and fatigue.

The blurring of vision is usually associated with rapid changes in blood glucose control. Cataracts are also common in diabetic patients past middle age.

In diabetic atherosclerosis, deposition of lipids occur not only in arteries but also in arterioles. Lesions of the vascular system are responsible for coronary atherosclerosis, heart diseases, kidney diseases etc.

Glucose favours growth of fungi and bacteria. The presence of high concentration of glucose in the organs may lead to fungal and bacterial infections

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such as prostratitis, balantidiasis, vulvae tuberculosis, urinary tract infection etc. in diabetic patients.

A damage to peripheral nerves is common in diabetes. The common findings are numbness and tingling in the feet. Gastroparesis (partial or slight paralysis) may cause delayed gastrointestinal food absorption causing difficulty in the insulin treated patients or it may cause vomiting.

MANAGEMENT OF DIABETES MELLITUS

The aims of treatment are to achieve a normal metabolic state, avoid complications and allow the patient to lead a normal symptomfree life.

The traditional concepts for treating diabetes are diet, weight reduction, exercise, change in life style together with oral drugs and insulin. Weight reduction or caloric restrictions were found as basic foundations of diet therapy during the late fifties. Diets prescribed for diabetics have gone through remarkable changes in the insulin era, post insulin era and most importantly in the last two decades. Currently prescribed diets include high fibre and low fat diets (Chandalia et al, 1990). As early as 1935 Himsworth reported that carbohydrate tolerance in healthy adults was improved by increase in the proportion of carbohydrate in diet (Mann, 1987). The soluble fibre (e.g. Guar gum) forms a gel in the gut and retards the absorption of most nutrients, which lowers the glycemic indices of foods. The high fibre diet enhances postprandial serum insulin levels, either due to increased secretion or decreased degradation of insulin. The fibre vegetarian foods contain phytates and lectins which reduce the rate of digestion of starch and thereby diminish postmeal

hyperglycemia. The currently prescribed diabetic diets are low fat diets, only about 25-30% of the calories derived from fat. Because of the difficulties in maintaining diet therapy in diabetes, a number of dietary adjuncts have been considered to assist in the control of hyperglycemia (Horwitz, 1990). Alternative sweeteners such as aspartame and saccharine are useful in hyperglycemic excursions.

An exercise greatly increases the hypoglycemic action. With the exercise and the consequent burning of carbohydrates, the secretion of insulin helps to lower the blood sugar in the overweight patient having a mild diabetes.

INSULIN THERAPY

Insulin was discovered and purified for therapeutic use by Banting, Best and coworkers in 1922 and the details were reviewed by Bliss (1982).

Insulin is a protein with an empirical formula $C_{254}H_{377}O_{75}S_6$. Its structure was elucidated by Sanger (1959) for which he was awarded the Noble Prize. Insulin consists of two long polypeptide chains, α -chain has 21 amino acids and β -chain has 30 amino acids. These two chains are linked by 2-disulphide bridges within a chain. The 6 amino acids within the disulphide bridge of α - chain have been suggested as site of its binding to muscle and other tissues.

Insulin is required for all patients with NIDDM subjects. In the beginning, insulin preparations were quite shortacting and in order to abolish glycosuria, injections were given 4-6 times daily which was inconvenient. It was found that protamine, a fish derived protein delayed the absorption of insulin and this could be increased with

the addition of zinc. The pharmacological processes have led to the NPH (Neutral Protamine Hagedorn or Isophane) and lente preparations that are in use today.

The advanced biotechnology led to the production of human insulin from genetically programmed bacteria or yeasts or by substituting the different amino acids in already purified porcine insulin (Goeddel et al, 1979).

The major effect of insulin is to increase the utilization of glucose by most of body tissues and it does so by increasing the transportation of glucose across the cell membrane by phosphorylation.

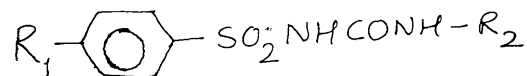
The side effects of insulin are common. They may be listed such as sweating, nervous instability, faintness, hunger, headache, rapid heart action, due to overaction with the development of hypoglycemia which can generally be prevented by readjustment of insulin type. Since insulin is a foreign protein, most of the patients experience slight reddening, discomfort, and itching at the site of injections after about first two or three weeks.

ORAL HYPOGLYCEMIC AGENTS

Over the past 30 years, or more, two groups of oral hypoglycemic agents, have been widely prescribed, the sulphonylureas (introduced 1954) and the biguanides (introduced 1957), both differ in chemical structure, pharmacological action and to some extent therapeutic indication.

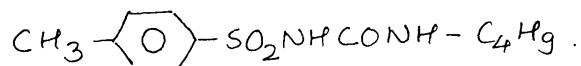
SULPHONYLUREASE

Sulphonylureas are related to the sulphonamides and were discovered by chance from the observation in 1942 when some patients were treated with a sulphonamide for typhoid fever developed symptoms of hypoglycemia. The sulphonylurea radical (SO_2NHCONH) is responsible for hypoglycemic activity. This chemical group is attached to a benzene ring and the substitution of different chemical groups R_1 and R_2 confer the difference in potency and duration of action. The general formula is

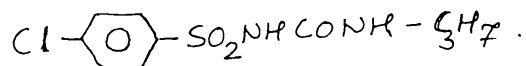


The available preparations are usually divided into two groups : so called first generation (introduced in 1954) and second generation (introduced in 1969) sulphonylurease.

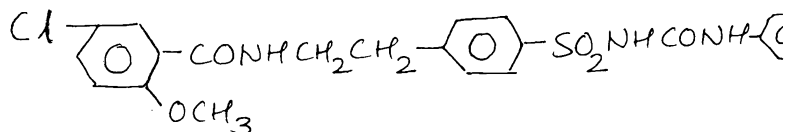
First generation sulphonylureas include Acetohexamide, Tolbutamide, Chlorpropamide, Tolazamide, Glymidine. While second generation sulphonylurease include Glibenclamide, Glibornuride, Glipizide, Gliquidone, Glidazide (Campbell 1990).

TOLBUTAMIDE

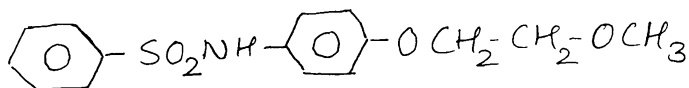
Tolbutamide is rapidly absorbed from the upper gastrointestinal tract and appears in the blood within 30 minutes of oral administration. The peak levels of the drug are found after 3 to 5 hours. The main chemical group i.e. carboxytolbutamide is not hypoglycemic. The average half life of this drug is 5-6 hours after oral or intravenous administration and there is no hypoglycemic effect after 12 hours (Smith et al, 1965).

CHLORPROPAMIDE

This drug was introduced into clinical practice in 1957, a year after tolbutamide. It is excreted unchanged in the urine without any metabolism in the body. Its half life is 36 hours and elimination from the body may take several weeks (Knauff et al, 1959). The maximum hypoglycemic effect occurs after 8 to 10 hours after oral administration and total duration of action of the drug was found more than 36 hours.

GLIBENCLAMIDE

Glibenclamide was synthesised in 1966. It is approximately 250 times more potent than glymidine and tolbutamide on molecular weight basis. Clinical studies indicate that the effective dose ranges from 2.5 to 30 mg per day. Side effects have been shown to be minimal (Mutalik et al, 1972). Its half life is 4 to 8 hours and lethal dose (LD₅₀) is above 50 mg/kg by mouth.

GLYMIDINE

Glymidine was introduced in 1964 as hypoglycemic agent functionally similar to be sulphonylureas. Glymidine appears in the blood within half an hour of oral administration. It has a half life of 3 to 8 hours.

Mechanism of the action of sulphonylureas

All sulphonylureas stimulate the pancreatic β -cells for insulin secretion. Therefore, some functional islet-cell tissue is necessary in the patients receiving these drugs. Perleg et al (1966) reported the effect of sulphonylureas on release of insulin within minutes of intravenous administration in man. An effect of

sulphonylureas to decrease hepatic glucose output, has been suggested by studies that have demonstrated decreased hepatic gluconeogenesis and glycogenolysis in the presence of these compounds.

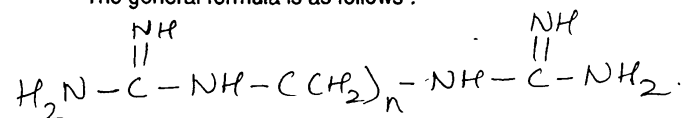
Side effects of sulphonylureas

These drugs could be more toxic and therefore are not recommended in diabetic subjects with severe hepatic or renal insufficiency or congestive heart failure and also during pregnancy. Sometimes, there may be gastrointestinal reactions or mild skin rashes. Other reactions include hepatic toxicity and prolonged hypoglycemic reaction.

Cholestatic jaundice has been observed in small number of patients given oral sulphonylurea therapy, particularly chlorpropamide. Following discontinuation of the drug there appears to be a reversible liver damage.

BIGUANIDES

The general formula is as follows :



These biguanides (formed by combination of two guanidine molecules) were noted to be 150 times more effective and less toxic than the parent guanidine. A number of drugs from biguanide series were found to have acceptable toxicity and

were clinically useful (Ezzele et al, 1981). These compounds, however, were found to be effective in diabetics, but not in the normal human subjects (Dhar et al, 1975).

The long term therapy with biguanides has been shown to lower plasma cholesterol and triglyceride levels (Schwartz et al, 1966) and this property has been promoted in diabetics with raised VLDL (very low density lipoproteins) values. The known examples from the class of biguanides are phenformin and metformin.

Mechanism of action of biguanides

The increase in the uptake of glucose in peripheral tissues while in oxidation by anaerobic glycolysis leads to decreased concentration of glucose. The studies in men have shown that phenphormin accelerates the turnover of glucose in nondiabetic subjects also (Belfiore, 1980). This turnover may be due to both increased hepatic output of glucose and increased peripheral utilization of glucose, the balancing of these two effects contributing for the inability to demonstrate hypoglycemic effects in most nondiabetic subjects.

Side effects of biguanides

The nonspecific side effects include nausea, vomiting, heart-burn, diarrhoea, constipation and a metallic taste. The dose related side effects include headache, tiredness and parasthesias. Waters et al (1978) noted the levels of blood lactate and pyruvate in diabetic subjects.

Review of Literature

REVIEW OF LITERATURE

The medicinal plants are a major reservoir of biologically active compounds. At present there are about 2,50,000 plant species among the world flora. The study of modern herbalism (phytotherapy) is rapidly emerging throughout the world. A Swiss-German physician and herbalist, Paracelsus introduced new methods and concepts for the beginning of chemical research which led to the development of synthetic products. Without going deeply into the mystic-scientific philosophy, Paracelsus had concentrated on what is now defined as the active principle of a plant. Then herbalist was transformed into a pharmacist to extract substances from herbs and formed new constituents.

According to Harborne (1984) the extraction of the active principle from plant depends on three factors viz. the texture of the plant material, its water content and type of substance being isolated. The precise procedure for obtaining constituents from dried plant tissues like leaves, seeds and root tubers by infusion, decoctions or maceration with water or suitable solvents have been employed.

Indian Materia Medica (Nadkarni, 1976) has described about 2,000 different drugs of plant origin as remedies for many diseases. Some of the antidiabetic plants amongst them can be listed as: *Abroma augusta* (P.H.T), *Aconitum ferox*, *Cassia auri*, *Cephalandra indica*, *Curcuma longa*, *Embllica myrobalan*, *Ipomoea digitata*, *Rubia cordifolia*, *Scilla indica* (P.H.T).

Tripathi et al (1979) have carried out the screening of the constituents of Madhawashava, a compound effective in diabetes mellitus. They have reported that

among 27 drugs 10 drugs have shown hypoglycemic action upto certain extent. Among these, roots of *Inula racemosa* Hook (Puskarmul) and leaves of *Cinnamomum tamala* Nees (Tejpatra) produced highly significant hypoglycemic activity. Thirty five antidiabetic plants were listed by Aiman (1970). The two investigators, Olive Bever and Zahad (1979) reviewed the relevant literature and comparatively listed 90 plants considering their botanical position, chemical constituents, and pharmacological actions, toxicity and other properties. The hypoglycemic action of endogenous growth regulators and trace elements from plants were also discussed (Mirsky et al, 1956). The hypoglycemic effect of 12 antidiabetic plants used in Mexico was studied in 27 healthy rabbits (Roman - Ramos et al, 1992). They found that eight of the studied plants, decreased significantly the hyperglycemia produced by the administration of 50% dextrose. The strongest effect was yielded by the decoction of *Guaiaacum coulteri*.

The immature fruits and roots of ochre have been shown to contain mucilages with glucose lowering activity in normal mice (Tomoda et al, 1987). The seeds of *Acacia arabica* (Babul), *A. benthami*, *A. modesta* (Kantosuriya) and *A. suma* (Shami), *A. catechu* (Khair) and *Albizzia moluccana* (Udala) were used in the diet, which showed hypoglycemic effect in normal rats (Singh et al, 1975 and 1976).

In alloxan-diabetic rabbits, oral administration of allicin (50-200 mg/kg b.w.) (Augusti & Mathew, 1975), the extracts of *Allium cepa* (onion) and *Allium sativum* (garlic) (Brahmachari and Augusti, 1961, 1962) reduced the blood sugar levels. Allylpropyl disulphide (APDS) from *A. cepa* and diallyl disulphide oxide (allicin) from *A. sativum* have been isolated and established their hypoglycemic activity. A reduction in circulating glucose was associated with increased serum insulin

concentrations in normal volunteers (Augusti and Benaim, 1975). Several fractions of onion with different organic solvents were prepared by Gupta et al (1977) and tested for their blood sugar lowering effect during oral glucose tolerance test in normal healthy rabbits. The maximum hypoglycemic effect was produced by the chloroformic extract which was 79.4% as compared to tolbutamide. Diphenylamine, isolated from the bulbs of *A. cepa* has also been claimed to exert hypoglycemic activity (Karawya et al, 1984). An aqueous extract of *A. cepa* improved glucose tolerance in normal mice and reduced glycemia in streptozotocin diabetic mice (Day & Bailey, 1986). Mukherjee (1957) also reported the hypoglycemic effects of *A. cepa*.

Althea mucilage-O was isolated from the roots of *Althea officinalis* (Tomada et al, 1987) and its hypoglycemic effect was noted.

Narayanan et al (1987) isolated a new antidiabetic compound, pinitol, from the leaves of *Bougainvillea spectabilis* and studied their hypoglycemic effect in normal fasting, alloxan - diabetic and orally glucose fed albino mice.

***Coccinia indica* syn. *C. grandis* syn. *C. cordifolia* (Bhimbee)**

This plant is known in Bengal for showing remarkable effects in reducing the amount of sugar in the urine of diabetic patients. It has a reputation of being Indian substitute for insulin. Water soluble fractions of alcoholic extract of roots revealed hypoglycemic properties in alloxan-diabetic rabbits (De et al, 1953). Gupta (1963) has shown that this plant also caused considerable inhibition of the hyperglycemic response of the anterior pituitary extract in glucose fed animals. Mukherjee et al

(1972) isolated a quaternary base of alkaloid and proved hypoglycemic activity in alloxan-diabetic rats.

Cyamopsis tetragonolobus (Indian cluster bean) has yielded the polysaccharide, *guaran*, which is now a recognized fibre supplement and food stabilising agent (Pillai et al, 1980; Day, 1986). This galactomanan, like other fibres may benefit in the diabetic condition by reducing postprandial hyperglycemia.

The hypoglycemic and hypolipaeamic effects of guar in normal and diabetic guinea pigs were studied by Srivastava et al (1987). Esters and glycosides of castanospermine have been proposed as antidiabetic (Liu et al, 1989) and the derivatives of this alkaloid were reported to act as α -glucosidase inhibitors (Yoshiaki et al, 1989). Some weakly hypoglycemic alkaloids have been isolated from *Catharanthus roseus* (periwinkle) e.g. catharanthine, leurosine, vindoline and vindolinine of other effects of these alkaloids preclude their safe use in diabetes (Farnsworth and Segelman, 1971). Gupta et al (1967) observed hypoglycemic action of *Casaria esculenta* in rats and rabbits.

The hypoglycemic β -sitosterol-D-glucoside was isolated (Ambike et al, 1967; Varshney et al, 1965, Modak et al, 1966) from *Ficus religiosa*. A glycoside, bengalenoside from *Ficus bengalensis* (Banyan tree) has been reported to exert a mild hypoglycemic effect in normal fasting rabbits (Brahmachari and Augusti, 1964).

The slowly generated but sustained glucose lowering effect of *Gymnema sylvestre* (Gurmar) was reported by Shanmugasundaram et al, (1981, 1983).

The active constituent, paniculatan was isolated from the inner bark of *Hydrangea paniculata* and its hypoglycemic effect in normal mice was studied (Tomoda et al, 1987).

Momordica charantia (Karela)

Fresh juice or tablets prepared from the fruit of *Momordica charantia* showed remarkable fall in blood sugar levels of diabetic patients (Vad, 1961). Later on, charantin, a non-nitrogenous principle was isolated and its hypoglycemic effect was confirmed in normal rabbits (Lotlikar et al, 1966). More work has been carried out on this plant (Pabrai et al, 1961; Akthar et al, 1981, Sharma et al, 1960).

Moran A, a glycoprotein was isolated from the root bark of *Morus alba* (Hikino et al, 1985), Hikino et al (1988) successfully isolated four glycans and oryzabran A,B,C,D from brans of *Oryza sativa* and their remarkable hypoglycemic effects were observed in normal and alloxan-induced hyperglycemic mice.

Pterocarpus marsupium

The hypoglycemic activity of this plant was reported by Rajasekharan (1976). The flavonoid, epicatechin, has been isolated from the heartwood of *Pterocarpus marsupium* but epicatechin have not been substantiated during rigorous tests (Kulb et al, 1982; Sheehan et al, 1983; Ryle et al, 1984). Although epicatechin stimulates insulin release from normal isolated islets (Hii and Howel, 1984) or increases cAMP content and convert proinsulin to insulin in immature and mature rat islets in vitro (Ahmad Faiyaz et al, 1991), neither an aqueous extract of *P. marsupium* nor

epicatechin were effective in improving glycemic control in streptozotocin diabetic mice (Bailey et al, 1986). But Chakravarty et al (1981) administered (-) - epicatechin to albino rats for 2 days prior to alloxan and it was able to protect the animals against the diabetogenic action of alloxan. An alkaloid fraction was isolated from alcoholic extract of *Pterocarpus marsupium* which produced marked hypoglycemic activity in rabbits (Saifi et al, 1971). Two flavonoids from *Phyllanthus fraternus* have been reported in alloxan-diabetic rats (Hukeri et al, 1988).

On the occasion of "World Congress On Biotechnological Developments in Medicinal substances of plant and Marine Origin" at Lucknow, hypolipidemic and hypoglycemic efficacy of *Psyllium hydrophilic* mucilloid in NIDDM patients was reported by Gupta et al (1995).

Puererin an active constituent from the *Pueria tuberosa* DC was isolated and studied by Nikam (1981) its hypoglycemic activity in different hyperglycemic conditions.

The polysaccharides saccharan A-F were isolated from the stalks of *Saccharum officinarum* (Takahashi et al, 1985) and their hypoglycemic effects were observed. Kohli (1983) had shown the hypoglycemic effect of *Syzizium cumini*.

Tecomine and Tecostanine, two alkaloids isolated from *Tecoma stans*, also exert weak hypoglycemic activity (Hammouda & Amer, 1966; Hammouda & Khallafallah 1971). Some alkaloids like trigonelline (N-methyl nicotinic acid) (Varsney et al, 1968; Hardman et al, 1972) coumarine, nicotinic acid (Shami et al, 1974) were isolated and their hypoglycemic study was carried out. A new furostanol glycoside,

trigonelloside, was isolated and characterized by Hardman et al (1980) from the seeds of fenugreek (*Trigonella foenum graecum*). The hypoglycemic and insulinotropic activity of *Tinospora crispa* was studied by Noor et al (1989) with human and rat islets and HIT - T15 B cells. The blood sugar lowering effect of *Tinospora cordifolia* was noted by Raghunathan (1969) in adrenaline induced hyperglycemic rabbits.

Recently, it was found that water fraction of alcoholic extract of the leaves of *Vinca rosea* led to marked lowering of blood glucose in normal and streptozotocin-induced diabetic rats (Chattopadhyay et al, 1991). Padmini et al (1990) showed the insulin releasing effects of Siddha drug called Vatharasavangam in rabbits. The active principle from *Vaccinium myrtillus* (bilberry) which reduced the insulin requirement in IDDM patients was reported to be a glucoside known as neomyrtillin (Allen, 1927).

Singh et al (1972) claimed that indigenous drugs apart from their hypoglycemic activity may also be correcting the metabolic abnormalities simultaneously.

Sadhukhan et al (1994) clinically evaluated a herbal antidiabetic product containing four different crude drugs in 67 diabetic patients and 12 normal subjects.

Aims and Objectives

AIMS AND OBJECTIVES

Diabetes mellitus is characterized by both fasting hyperglycemia and exaggerated postprandial increases in the blood glucose level. Understanding the causes of these abnormalities is important for the rational use of current treatments and for developing more effective therapy. To control hyperglycemia, remained the main goal in the treatment of diabetes mellitus.

However, this disease affecting a significant proportion of the population has been known since the dawn of civilisation but its treatment with insulin has started only since 1925. The insulin therapy has certainly reduced the mortality and morbidity and still the difficulties of frequent injections have not been solved completely.

There are two main categories of oral hypoglycemic compounds - the sulphonylureas and biguanides. Among the sulphonylureas, tolbutamide, chlorpropamide, glibenclamide etc. and phenformin of biguanide derivatives are used by diabetic patients. These antidiabetic drugs are also not free from side effects during prolonged treatment (Cader and Marble, 1984).

The plants used in traditional medicine can serve as a source of novel therapeutic agents. The practitioners of traditional systems of medicine in India are believed to rely on at least 2000-plant based substances. The plant derived drugs being economically affordable and ecofriendly, have less side effects and renewable sources. About 120 biologically active chemicals are already in use in medicine.

According to pharmacology specialists, atleast 70 of these owe their discovery to follow up research that aimed to verify tips obtained from traditional medicine.

During last few decades number of scientists were engaged to investigate useful drugs from medicinal plants and they have achieved their goal successfully by producing remarkable drugs for the treatment of malaria, vascular diseases, cancer such as quinine digoxin, serpentine, vincristine, taxol etc. Unfortunately, not a single drug derived from medicinal plant which could replace insulin or synthetic drug therapy for diabetic patients. According to the report produced by the Royal Botanic Gardens, still 95% plants have not been exposed for further research work through out the world (Roger, 1995). Therefore, there is a wide scope to search and exploit useful medicinal plants for the treatment of diabetes mellitus.

In this investigation about 12 medicinal plants (Table 1) have selected for the evaluation of hypoglycemic activity.

Table 1
LIST OF HYPOGLYCEMIC PLANTS

Sr. No.	BOTANICAL NAME	COMMON NAME	PART USED
1.	<i>Trigonella foenum-graecum</i>	Methi	Seeds
2.	<i>Zea mays</i>	Maka	Styles
3.	<i>Carum carvi</i>	Shahjira	Seeds
4.	<i>Cyamopsis tetragonolobus</i>	Guar	Seeds
5.	<i>Trichosanthes dioica</i>	Padval	Whole fruit
6.	<i>Guazuma tomentosa</i>	Nagarmotha	Tubers
7.	<i>Helecters isora</i>	Murudshenga	Whole fruit
8.	<i>Alpinia galanga</i>	Kostha kulinjan	Roots
9.	<i>Centella asiatica</i>	Brahmi	Leaves
10.	<i>Eleusine coracana</i>	Nachni	Seeds
11.	<i>Azadirachta indica</i>	Neem	Bark
12.	<i>Hygrophila spinosa</i>	Gokhru	Roots

The protocol for further detailed study was planned as follows :-

1. Preparation and screening of plant extracts for hypoglycemic activity.
2. Isolation of active principle from crude extract.
3. Hypoglycemic activity of active substance in experimental animals.
4. Hypoglycemic activity of active substance in orally glucose fed and alloxan induced hyperglycemic animals.
5. Sub-acute toxicity study of active substance.

Materials and Methods

MATERIALS AND METHODS

1. Collection of plant material

The plant material was collected from the local market, identified, cut into small pieces and dried at room temperature under the shade. Then the material was powdered to a uniform mesh (40 mesh).

2. Preparation of plant extracts

The usual method of preparing the extract is by continuous extraction of powdered plant material in Soxhlet apparatus with different solvents as described by Davis et al (1950).

Soxhlet Extraction

Five hundred grams of powdered plant material was extracted with methanol in the Soxhlet apparatus, till the last portion of the extract was colourless. Total methanolic extract was concentrated by distillation and thus the residue was prepared methanol free.

Water Extraction

After complete methanolic extraction of plant material, powder was again extracted with hot water (60-70°C). The material was filtered and filtrate was

concentrated under reduced pressure to get the residue. Same was tested for hypoglycemic activity.

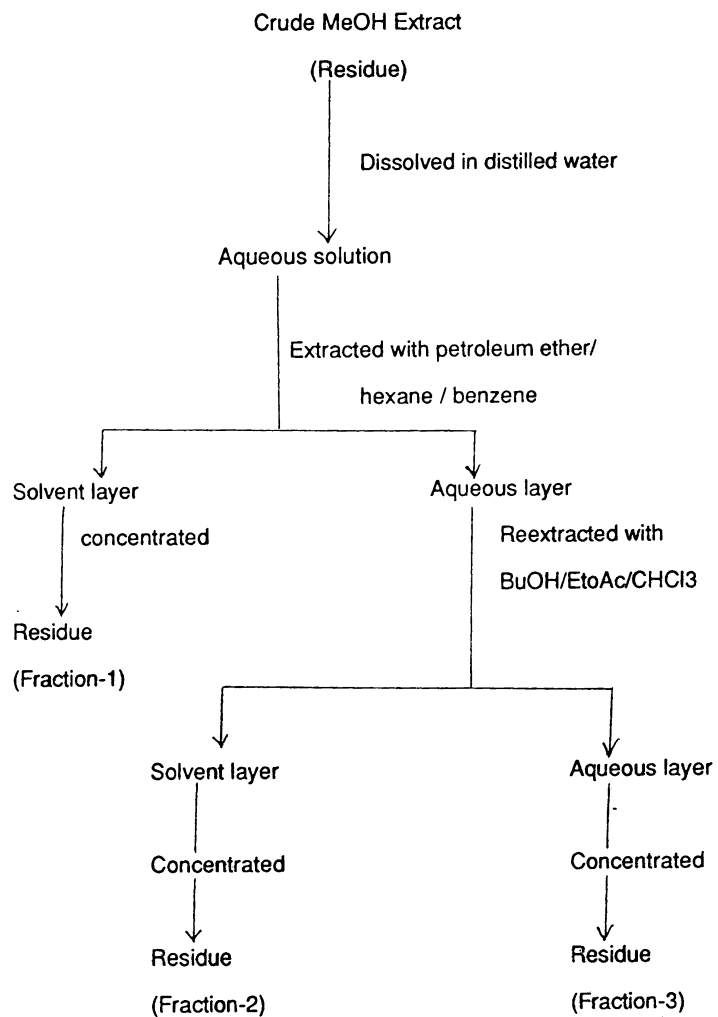
3. Isolation of an active principle

It was achieved by two solvent extraction method (Alders, 1955). The principle feature of this method is the use of two solvents whose inter solubility is low and in either of which one of the two substances (or groups of substances) to be separated is preferentially soluble.

The crude methanolic extract was suspended in hot water (60-70°C) and filtered out. The filtrate was cooled and extracted repeatedly with petroleum ether, or benzene or hexane in separating funnel, in order to remove oily or fatty substances. The solvent layer was taken out, pulled together and solvent was recovered at its boiling point. The residue (Fraction-1) was tested for hypoglycemic activity.

Aqueous layer was re-extracted with butanol or ethyl acetate and then both the layers were separated out and the solvents were distilled off at their corresponding boiling points. The residues obtained were labelled as Fraction-2 and Fraction-3 respectively.

Flow sheet for isolation of an active principle



4. Chemical characterization of an active extract

The hypoglycemic activity of an active extract is often attributed to the presence of certain chemical groups such as glycosides, alkaloids, phenolic compounds and terpenoids. Therefore, phytochemical screening of the crude extracts for detecting the presence of these chemical compounds may be correlated to the hypoglycemic activity. The presence of these compounds in active crude extract was detected by phytochemical methods described by Harborne (1984). The presence of different chemical groups was identified as follows :

a) Detection of glycosides

Glycosides are organic compounds associated with moiety monosaccharide. The presence of glycoside was detected by adding 3.0 ml of 1% alcoholic naphthol solution and 2.0 ml concentrated sulphuric acid to 5.0 ml of methanolic extract (3%). Development of red violet ring at the junction indicated the presence of glycosides.

b) Detection of phenolic compounds

Phenolic compounds are more polyhydroxy organic compounds which react with Ferric chloride (FeCl_3) and give bluish green colour. To 5.0 ml of 3% solution of extract in methyl alcohol 0.5 ml of 1% Ferric chloride solution in 0.5 N Hydrochloric acid (HCl) was added. Development of bluish green colour indicated the presence of phenolic compounds.

c) Detection of terpenoids

These compounds contain isoprene molecule $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CH}=\text{CH}_2$ i.e. their carbon skeleton is made up of two or more isoprenes. The presence of terpenoids was detected by Liebermann-Burchard reaction where in 5.0 ml of 3% hot methanolic solution of the extract was allowed to react with 5.0 ml of solution containing acetic anhydride and conc. sulphuric acid (20:1). Development of red or violet red colour showed the presence of terpenoids.

d) Detection of alkaloids

Alkaloids are basic substances which contain one or more nitrogen atoms joined in cyclic systems.

The presence of alkaloids was detected by adding 5.0 ml of Mayer's reagent to equal volume of acidified hot 3% methanolic solution of the extract. Formation of white precipitate at the interface showed the presence of alkaloids.

Mayer's Reagent

Dissolve 1.36 gms of Mercuric chloride in 60.0 ml distilled water. 5.0 gms of Potassium iodide is dissolved in 10.0 ml of distilled water. Mix these two solutions and then dilute it to 100 ml volume using distilled water.

5. Hypoglycemic effect of various plant extracts in normal fasting mice

Experimental procedure

Normal healthy male (Haffkine strained) mice weighing between 18-20 grams were used for the experiments. Mice were fasted for 18 to 20 hours prior to the experiment and water was allowed *ad libitum* during experimental period. Control group consisting of 5 mice were fed 0.5% Tween-80 by feeding tube whereas the extract was administered orally at the dose of 500 mg/kg b.w. to the experimental group simultaneously. The fasting blood samples were drawn after 0 hr, 2 hours and 4 hours from the control group as well as experimental group.

6. Hypoglycemic effect of plant extracts in normal fasting rats

Experimental procedure

Six normal healthy male Haffkine strained albino rats weighing between 150-170 grams maintained on a standard laboratory diet, were used for the experiment. Animals were fasted for 18 to 20 hours prior to the commencement of the experiment water being allowed *ad libitum* during fasting and experimental period. Fasting blood samples (0 hr) were collected from the orbital sinus using a 50 µl graduated capillary. The extract was administered orally at the dose of 100 and 500 mg/kg b. w. with a suspension in Tween-80 by stomach tube and the same amount of vehicle was administered to the control group. Blood samples were drawn after 2 hours and 4 hours from both the groups during the experiment.

7. Hypoglycemic effect of an active extracts in hyperglycemic mice

a) Glucose fed hyperglycemia

Glucose Tolerance Test (GTT) can be studied by administration of glucose either orally or intravenously. Glucose tolerance test is the capacity and the magnitude of the rise in blood glucose concentration which results from the administration of exogenous glucose. During this experiment, two mechanisms are possible:

- (i) It depends upon speed of the peripheral tissues for the assimilation of glucose and
- (ii) The ability of the liver to reduce its net output of glucose (Duncan et al. 1960)

Experimental procedure

Normal healthy Haffkine strained mice weighing between 18 to 20 grams were used for this study. Mice were checked for their hyperglycemic response produced by oral glucose load. Selected mice were divided into two groups in such a way that, they were similar in body weight and showing similar glucose tolerance curve. Each group consisted of 6 mice. Fasting blood samples ('0' hours) of the animals were taken and immediately the extract was administered as usual. After 3½ hours of administration of the extract, glucose (1.5 gm/kg b.w.) was fed orally to these animals. Blood samples were collected at the intervals of 30 mins, 1 hour and 2 hours. Control groups were fed only 0.5% Tween-80 orally.

b) Alloxan-induced hyperglycemia

Alloxan and streptozotocin are the diabetogenic compounds. Dunn et al. (1943) and Bailey et al. (1943) have shown the diabetic condition produced by injection of alloxan in experimental animals. Alloxan causes necrosis of β -cells and produces more or less permanent diabetes. The β cell toxicity of alloxan was discovered serendipitously in 1943 while testing the nephrotoxicity of uric acid derivatives in rats and rabbits (Mcletchie, 1982). The animals which received alloxan showed hyperglycemia alongwith other symptoms of diabetes mellitus.

Procedure for inducing diabetes in mice

The normal healthy Haffkine strained male mice weighing between 22-24 grams were used for this experiment. They were kept under observation for a period of 7 days on standard diet. The mice were fasted for 12 hours. This preliminary starvation makes the animal more susceptible to alloxan. After collection of fasting blood samples ('0' hr), alloxan (50 mg/kg b.w.) was injected intravenously. Then blood samples were drawn after 24, 48, 72, 74 and 76 hours from control group.

It was found that about 30% of the animals died following the administration of alloxan. The symptoms of hypoglycemia were noticed in all the mice between 12-18 hours after alloxan. At this stage, 5% glucose was injected (i.m.) to all animals because due to hypoglycemic shock the animal may die. The administration of glucose promptly regain the blood sugar level and subsequently the animals develop hyperglycemia.

8. Hypoglycemic activity of active extract in alloxan-induced diabetic mice

Alloxan-induced diabetic mice having blood sugar range from 300 to 350 mg% were divided into two groups, six mice in each group (Alloxan control and experimental group). In experimental group, the extract was administered (500 mg/kg b.w.) orally and blood samples were drawn thereafter 2 and 4 hours (74 and 76 hours after alloxan-treatment) during the experimental period.

9. Sub-acute toxicity study of active extract in normal rats

Toxicity test in animals is often carried out before a new drug is taken up for clinical trials. There are many drugs which are required to be administered for a long period (e.g. hypoglycemic drugs, antituberculous drugs). Therefore, the main objective of toxicity study of an active extract is to determine its adverse effects in experimental animals.

Experimental procedure

Two groups (control and experimental) consisted of 6 normal healthy albino rats (150-170 gms b.w.) were taken for this study. Initially all rats were raised on standard diet for 7 days. Initial body weights, fasting blood sugar levels and food intakes were recorded.

Drug was administered (100 mg/kg b.w.) orally daily for 4 weeks to experimental group and vehicle to the control group. Daily food intakes of both the groups were noted. The fasting blood sugar levels and body weights were recorded

every week end. The physical abnormalities, mortality and side effects due to extract were also observed during experimental period. At the end of 4th week, rats were sacrificed to see if there were any abnormal biochemical changes in important organs.

Following general biochemical parameters were estimated in order to see the effect of extract on carbohydrate, fat and protein metabolism.

- A) Blood sugar
- B) Serum cholesterol
- C) Serum total lipids
- D) Serum total proteins
- E) Serum triglycerides
- F) SGPT
- G) SGOT

10. Estimation of biochemical parameters

a) Estimation of blood sugar (Cooper et al, 1970)

Blood sugar estimation was done by O-Toluidine method as described below.

Principle

Glucose reacts with aromatic amines such as O-Toluidine in hot acetic acid solution to form a glycosylamine which undergoes condensation to form a green coloured product. The green colour which is proportional to glucose concentration compared with that of standard gives concentration of the sample.

Reagents

- 1) **Trichloroacetic acid (TCA)** : 3% TCA in distilled water.
- 2) **O-Toluidine reagent** : Pure thiourea (1.5 gms) was dissolved in about 200 ml glacial acetic acid with gentle warming. This was allowed to cool and transferred to a one litre volumetric flask. Then 60 ml O-Toluidine (E.Merck) was added dropwise with constant stirring to dissipate the heat. The total volume was made to one litre with glacial acetic acid. This was stored in an amber coloured bottle. It has to be kept two overnights in dark before use.
- 3) **Stock standard** : Glucose 100 mg% in 0.25% benzoic acid made in distilled water.
- 4) **Working standard** : Stock standard was diluted 1:10 in distilled water to make 100 µg/ml.

Procedure

Blood (0.05 ml) was drawn from the orbital sinus of the mouse or rat, using a 50 µl graduated capillary. After blotting off excess blood, it was added to 0.95 ml of 3% TCA. After 10 minutes, it was centrifuged at 1000 rpm for 15 minutes and 0.5 ml of the protein free supernatant was added in the sample tube. Standard concentrations (20-80 µg/ml) were taken in 0.5 ml volumes. Then 2.5 ml O-Toluidine reagent was added in all tubes. After plugging with non-absorbent cotton, the tubes

were placed in a boiling water bath for 20 mins. After cooling, the absorbance was read at 630 nm, against distilled water blank.

b) Estimation of serum cholesterol (Zurkowski, 1964)

When serum is added to a mixture of acetic anhydride and concentrated sulphuric acid, green colour develops (Liebermann-Burchard reaction). Zurkowski modified this method in which protein precipitation step was eliminated. The colour produced was measured at 610 nm on "Shimadzu".

Reagents

- i) Reagent A : 5% sulphosalicylic acid in glacial acetic acid
- ii) Reagent B : Acetic anhydride
- iii) Reagent C : Concentrated Sulphuric acid
- iv) Standard : 250 mg% cholesterol

Cholesterol reagent : 3.5 ml (A) + 6.5 ml (B) + 1.0 ml (C).

Calculations

$$\text{Serum total cholesterol in mg/dl} = \frac{\text{Optical Density of Test}}{\text{Optical Density of standard}} \times \text{concentration of standard}$$

Standardization and experimental procedure

This method is a one-stop method and is quite rapid and accurate as compared to the other methods for the estimation of cholesterol.

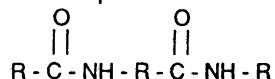
Pure cholesterol was used as standard. The different concentrations of standard cholesterol (100-400 mg/dl) were run simultaneously along with samples. Cholesterol reagent - a mixture of sulphosalicylic acid in glacial acetic acid (A), acetic anhydride (B), concentrated sulphuric acid (C) was prepared fresh each time and was added to the cuvettes containing serum, standard and distilled water (0.1 ml each).

After allowing the solutions to stand for 12 minutes, the green colour developed and its absorbance was read at 610 nm on "Shimadzu". The concentration of cholesterol of an unknown sample was read from the standard graph.

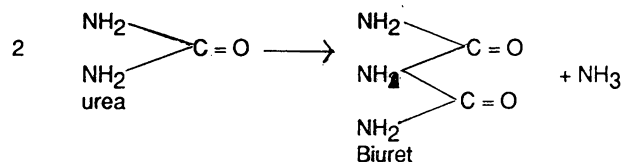
c) Estimation of serum total proteins (Weichselbaum, 1969)

Principle

The compounds with atleast two peptide bonds



react with copper salts in an alkaline solution to give a violet colour. The simplest compound which gives this reaction is biuret which is prepared by heating urea.



The biuret reaction is specific for peptides, polypeptides and proteins. The absorption maximum for proteins is at 545 nm. The colour intensity is directly proportional to the number of peptide bonds.

Reagents

i) Stock biuret reagent of Wieschelbaum one litre solution in 0.2N NaOH contains:

1. Potassium sodium tartarate	45.0 gms
2. Copper sulphate	15.0 gms
3. Potassium iodide	5.0 gms

ii) Dilution solution : Potassium iodide (5 gms) dissolved in 0.2N NaOH and the volume made upto one litre.

iii) Biuret reagent : Stock solution (20 ml) was diluted to 100 ml with diluent.

iv) 0.154 M (0.9%) Sodium chloride.

Experimental procedure

For the estimation of total proteins serum samples were run along with standards.

Test (T) : Serum (0.02 ml) sample and biuret (1.0 ml) were taken in a test tube.

Test Blank (TB) : One ml NaCl solution was treated with serum (0.02 ml).

Reagent Blank (RB) : Test tube contained biuret (1.0 ml) and NaCl solution (0.02 ml).

Standard (S) : Biuret (1.0 ml) was added to the test tube containing standard (0.02 ml).

After 30 minutes, readings were taken against distilled water at 545 nm on "Shimadzu". The concentration of serum total proteins was calculated by using the formula :

$$\text{Total proteins} = \frac{A(T) - A(TB) - A(RB)}{A(S) - A(RB)} \times C(S) \text{ in gms/100 ml}$$

d) Estimation of serum total lipids (Frings et al. 1972)**Principle**

The lipids react with vanillin in a medium of sulphuric acid and phosphoric acid to form a chromogen. Frings et al. (1972) reported a simple method for determining serum total lipids.

Reagents

- i) Vanillin : 6 gms/litre
- ii) Phosphate vanillin : Vanillin reagent (350 ml) + 50 ml distilled water + 600 ml of concentrated phosphoric acid.
- iii) Concentrated sulphuric acid
- iv) Standard = Olive oil.

Standardization and experimental procedure

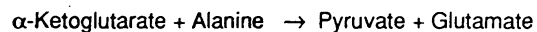
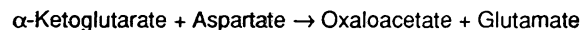
Working standard (triolein) was prepared by dissolving in alcohol and various concentrations ranging from 100 to 800 mg% were taken for the standard curve. Working standard, serum and distilled water (0.02 ml) were taken into cuvettes. Concentrated sulphuric acid (0.2 ml) was added and the contents were mixed on a vortex type mixture. All the cuvettes were placed in boiling water bath for 10 minutes (± 1 min) and then cooled them in ice cold water for about 5 minutes. Freshly prepared phosphovanillin reagent (10.0 ml) was added to each cuvet, mixed well on a vortex type mixture and then incubated at 37°C ($\pm 2^\circ\text{C}$) in a water bath for 15

minutes. The samples were cooled and absorbance was measured within 30 minutes at 540 nm by using "Shimadzu".

e) Estimation of SGPT and SGOT : (Reitman et al. 1957) :

Principle

The enzymes (transaminases), present in the human tissues including serum, catalyse the reactions as follows :



The present method for the assay of Glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminases, especially designed for use as a routine laboratory procedure. In this method, the amount of oxaloacetate or pyruvate formed is measured spectrophotometrically by the formation of a 'hydrazone' at the wavelength of 505 nm. The increase in the absorbance is proportional to the pyruvate or oxaloacetate that is produced.

Reagents

- 1) GOT substrate (pH = 7.5)
 - DL - Aspartic acid 13.3 gms
 - α -Ketoglutaric acid 0.146 gms

Above chemicals were dissolved in minimum amount of 1 N NaOH and about 90 ml of phosphate buffer was added. The volume was made upto 500 ml with phosphate buffer.

- 2) GPT substrate (pH = 7.5)
- | | |
|-----------------------------|-----------|
| DL-Alanine | 8.9 gms |
| α -Ketoglutaric acid | 0.146 gms |

Both the chemicals were dissolved in 1 N NaOH and volume was made 500 ml with phosphate buffer.

- 3) Phosphate buffer (pH = 7.5)
- One litre solution contained anhydrous Disodium hydrogen phosphate (11.3 gms) and anhydrous Potassium dihydrogen phosphate (2.7 gms)

- 4) 2,4-Dinitrophenyl hydrazine (DNPH)
- DNPH (19.8 mg) was dissolved in 10 ml of hydrochloric acid and diluted to 100 ml with D/W.

- 5) 0.4 N NaOH

- 6) Pyruvate std. (20 mM) : pH 7.5
- Sodium pyruvate (220 mg) dissolved in 100 ml of phosphate buffer.

- 7) Working pyruvate std. (4 mM)
- One ml of stock pyruvate standard was diluted to 5 ml with phosphate buffer.

Experimental procedure

Serum GPT and GOT activities were estimated by this method in which the procedure can be described as follows :

Test (T) : Substrate for GPT or GOT (0.5 ml) was taken and warmed in water bath at 37°C for 3 minutes. Serum (0.1 ml) was added and incubated for 30 and 60 minutes in water bath for the estimation of SGPT and SGOT respectively. 2,4-Dinitrophenyl hydrazine (DNPH) reagent (0.5 ml) was added immediately.

Test control (C) : Desired substrate (0.5 ml) was mixed with 0.5 ml of DNPH solution and 0.1 ml of serum.

Standard (S) : One ml of stock pyruvate standard (20 mM) was diluted to 5 ml with phosphate buffer and used as working standard. To the working standard (0.1 ml), substrate (0.4 ml) and distilled water (0.1 ml) and DNPH (0.5 ml) was added.

Reagent blank (RB)

Desired substrate (0.5 ml) was mixed with 0.5 ml of water and 0.5 ml of DNPH reagent.

All the tubes were kept at room temperature for 20 minutes for the reaction of DNPH and sodium hydroxide (5 ml) was added and mixed thoroughly. The optical density was measured after 20 minutes the addition of sodium hydroxide but before 30 minutes on "Shimadzu" at 510 nm against distilled water.

The values of serum pyruvate are expressed in micromoles of pyruvate/minute/litre of serum and calculations were carried out by using the formulae as given below :

Calculation for SGPT

$$\frac{A(T) - A(C)}{A(S) - A(B)} \times 133 \text{ micromoles}$$

Calculation for SGOT

$$\frac{A(T) - A(C)}{A(S) - A(B)} \times 67 \text{ micromoles}$$

where A = Absorbance

f) Estimation of serum triglycerides (Mendez et al, 1975)

Principle

Serum triglycerides were extracted in the presence of sodium metaperiodate; it produces formaldehyde which is then condensed with acetylacetone and forms a yellow colour. The colour was measured spectrophotometrically.

Reagents**i) Saponification reagent**

Dissolve 10.0 gm of KOH in 75 ml of water and 25 ml of isopropanol. This solution is stable for at least two months at room temperature in a brown glass bottle.

ii) Sodium metaperiodate reagent

One litre solution contained.

Ammonium acetate (anhydrous)	77.0 gms
Glacial acetic acid	60.0 ml
Sodium metaperiodate	0.650 gms

iii) Acetylacetone reagent

0.4 ml of 2,4-pentanedione was added to 100 ml of isopropanol. The reagent is stable for at least two months in a brown glass bottle.

iv) Standard stock Triolein

1 gm/dl of isopropanol

Standardization of triglyceride

Working standard of triolein was prepared from the stock standard with isopropanol and its concentration were taken from 50 to 200 mg/dl. to the tubes containing standard, followed by heptane (2.0 ml), isopropanol (3.0 ml) and diluted -

sulphuric acid (1.0 ml). The contents were mixed on vortex type mixer for 30 seconds and allowed to stand for few seconds.

The upper heptane layer (0.2 ml) was pipetted and transferred to another centrifuge tube containing isopropanol (2.0 ml). To each tube, saponification reagent (KOH, 0.6 ml) was added and mixed well and allowed to stand for 5 minutes at room temperature. After the addition of sodium metaperiodate reagent and acetyl acetone reagent (1.5 ml each) tubes were kept in water bath at 65-70°C for 15 minutes. The intensity of the colour was read at 415 nm within 45 mins. using water as blank on "Shimadzu".

Experimental procedure

Serum sample (T) (0.5 ml) was pipetted into a stoppered centrifuge tube, Isopropanol (3.5 ml) was added to the serum sample. The earlier mentioned procedure was followed for the estimation of triglycerides.

Calculation

$$\text{Serum triglycerides} = \frac{A(T)}{A(S)} \times C(S) \quad \text{where A = Absorbance}$$

Results

RESULTS

1. Hypoglycemic activity of crude extracts

Tween-80 and gum acacia (0.50%) were used as vehicle for control groups.

Fasting blood sugar level of mice found between 60.60 to 86.42 mg%. Blood sugar reduction varied from +3.36 to 9.17% of individual mouse in control group.

In the case of rats, the fasting blood sugar was observed between 62.74 to 80.85 mg%. Blood sugar reduction varied from 1.07% to 9.75% of individual rat in control group.

Methanolic extracts of 12 indigenous medicinal plants were tested for hypoglycemic activity in normal fasting mice at the dose level of 500 mg/kg b.w. p.o (Table 2).

The remarkable hypoglycemic activity was shown by methanolic extract of *Zea mays* (maka styles), *Carum carvi* (shahjira seeds), *Guazuma tomentosa* (Nagarmotha tubers) 28.66% ($p<0.01$), 18.44% ($p<0.01$) and 26.03% ($p<0.01$) in normal fasting mice at the end of 4 hours respectively.

Table 2
Hypoglycemic effect of methanolic extracts of various plants
in normal fasting mice (n=5)
(Dose of extract = 500 mg/Kg b.w.p.o.)

Sr. No.	Common name (part used) of plant	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
*	Control (Tween-80) (0.5%)	70.34 ± 5.03	69.25 ± 3.15	68.32 ± 2.98	1.87 ± 2.10	3.16 ± 2.96
1.	Methi (Seeds)	69.10 ± 3.70	62.18 ± 3.35	57.32 ± 3.89	9.92 ± 1.30 (p < 0.01)	17.00 ± 4.18 (p < 0.01)
2.	Maka (styles)	70.23 ± 6.85	56.35 ± 5.03	49.91 ± 4.77	19.31 ± 8.43 (p < 0.01)	28.66 ± 6.54 (p<0.01)
3.	Nachni (seeds)	70.67 ± 6.78	64.35 ± 5.35	63.15 ± 6.22	8.81 ± 2.59 (p < 0.01)	10.61 ± 4.03 (N.S.)

(Contd....)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

Sr. No.	Common name (part used) of plant	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
4.	Shahjira (seeds)	64.58 ± 4.22	57.89 ± 5.54	52.74 ± 6.83	10.44 ± 4.54 (p < 0.01)	18.44 ± 7.28 (p < 0.01)
5.	Guar (seeds)	66.56 ± 5.10	63.84 ± 2.75	63.19 ± 4.21	4.70 ± 4.96 (N.S.)	5.52 ± 3.71 (N.S.)
6.	Neem (bark)	67.87 ± 3.49	66.05 ± 3.53	64.84 ± 4.31	2.88 ± 2.48 (N.S.)	4.68 ± 3.97 (N.S.)
7.	Brahmi (Leaves)	74.23 ± 6.60	69.39 ± 4.80	68.17 ± 4.14	6.30 ± 4.92 (N.S.)	7.92 ± 4.11 (N.S.)
8.	Padval (Whole fruit)	68.41 ± 5.94	59.69 ± 6.02	56.98 ± 3.65	12.74 ± 4.44 (p < 0.01)	16.56 ± 1.99 (p < 0.01)

(Contd....)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

Sr. No.	Common name (part used) of plant	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
9.	Koshta-kulinjan (Tubers)	70.63 ± 4.26	75.31 ± 3.59	76.69 ± 3.27	N.A.	N.A.
10.	Nagarmotha (Tubers)	70.81 ± 6.76	57.90 ± 8.43	53.69 ± 6.39	17.31 ± 7.61 (p < 0.01)	26.03 ± 6.95 (p < 0.01)
11.	Gokhru (Tubers)	77.06 ± 10.68	69.08 ± 8.91	68.72 ± 7.81	9.29 ± 5.39 (p < 0.05)	10.56 ± 5.50 (N.S.)
12.	Murudshenga (Whole fruit)	66.96 ± 4.98	64.23 ± 3.27	63.32 ± 3.49	3.92 ± 3.14 (N.S.)	5.28 ± 4.14 (N.S.)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05; N.A. = Not Active

Water extracts of same medicinal plants were tested for hypoglycemic activity in normal fasting mice at the dose level of 500 mg/kg b.w. p.o (Table 3).

It was found that water extracts of *Zea mays* (maka styles), *Cyamopsis tetragonolobus* (Guar seeds), *Helecteres isora* (Murudshenga-whole fruit), *Centella asiatica* (Brahmi leaves) & *Carum carvi* (Shahjira seeds) showed 22.30% ($p < 0.01$), 21.02% ($p < 0.01$), 31.26% ($p < 0.01$), 24.68% ($p < 0.01$), 22.00% ($p < 0.01$) reductions respectively in blood sugar at the end of 4 hours.

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Table 3
Hypoglycemic effect of water extracts of various plants
in normal fasting mice (n = 5)
(Dose of extract = 500 mg/Kg b.w.p.o.)

Sr. No.	Common name (part used) of plant	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
*	Control (Tween-80) (0.5%)	66.97 ± 4.43	66.50 ± 4.04	66.19 ± 4.70	1.12 ± 1.29	1.84 ± 1.72
1.	Methi (seeds)	68.82 ± 6.09	63.08 ± 6.63	59.31 ± 6.07	8.37 ± 4.02 (p < 0.01)	13.88 ± 2.17 (p < 0.01)
2.	Maka (styles)	68.10 ± 2.85	56.36 ± 6.63	52.96 ± 5.63	17.21 ± 6.84 (p < 0.01)	22.30 ± 6.26 (p<0.01)
3.	Nachni (seeds)	66.61 ± 3.07	62.04 ± 4.45	57.33 ± 3.38	6.88 ± 4.40 (p < 0.02)	13.82 ± 5.49 (p < 0.01)

(Contd....)

Key :- All values (Mean) ± S.D.

Sr. No.	Common name (part used) of plant	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
4.	Shahjira (seeds)	67.11 ± 3.53	59.69 ± 3.52	52.57 ± 4.06	10.96 ± 5.23 (p < 0.01)	22.00 ± 2.07 (p < 0.01)
5.	Guar (seeds)	67.21 ± 5.00	59.61 ± 7.87	54.14 ± 6.13	13.60 ± 2.77 (p < 0.01)	21.02 ± 1.57 (p < 0.01)
6.	Neem (bark)	72.59 ± 7.11	69.58 ± 5.10	68.52 ± 4.69	4.39 ± 2.81 (N.S.)	6.51 ± 5.84 (N.S.)
7.	Brahmi (Leaves)	69.64 ± 6.06	56.21 ± 7.20	52.50 ± 6.20	19.40 ± 5.11 (p < 0.01)	24.68 ± 4.05 (p < 0.05)
8.	Padval (Whole fruit)	68.48 ± 5.31	67.41 ± 4.76	65.90 ± 4.99	1.72 ± 1.42 (N.S.)	4.55 ± 3.29 (N.S.)

(Contd....)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

Sr. No.	Common name (part used) of plant	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
9.	Koshta-kulinjan (Tubers)	66.35 ± 4.63	66.20 ± 4.62	65.14 ± 3.67	1.57 ± 1.91 (N.S.)	3.12 ± 3.43 (N.S.)
10.	Nagarmotha (Tubers)	69.35 ± 4.10	67.37 ± 1.58	65.54 ± 1.65	2.91 ± 3.38 (N.S.)	6.14 ± 4.90 (N.S.)
11.	Gokhru (Tubers)	67.96 ± 3.89	64.65 ± 3.64	61.50 ± 4.45	4.92 ± 2.51 (N.S.)	9.52 ± 3.86 (N.S.)
12.	Murudshenga (Whole fruit)	68.83 ± 3.83	57.89 ± 5.96	45.44 ± 6.90	12.25 ± 5.96 (p < 0.01)	31.26 ± 6.53 (p < 0.01)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

2. Dose response study of crude extracts

The dose response study of active extracts was carried out. The crude extract was administered orally at the dose of 500, 250, 100 and 50 mg/kg b.w. to normal fasting mice.

It was found that methanolic extract of maka styles has shown 9.27% ($p < 0.05$) reduction at the dose of 50 mg/kg b.w. While at the dose of 100 and 250 mg/kg b.w. it has shown 15.52% and 18.82% depression in blood sugar levels (Table 4).

The methanolic extract of shahjira seeds and methanolic extract of nagarmotha tubers produced 18.12% (Table 5) and 17.02% (Table 6) reductions in blood sugar levels respectively at the dose of 250 mg/kg b.w. The maximum and promising blood sugar lowering effect was given by methanolic extract of maka styles at the studied dose levels.

Table 4
Dose response study of methanolic extract of maka styles
in normal fasting mice (n = 5)

Sr. No.	Group/Dose in mg/kg b.w.	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
*	Control (Tween-80) (0.5%)	70.34 ± 5.03	69.25 ± 3.15	68.32 ± 2.98	1.87 ± 2.10	3.16 ± 2.96
1.	(500)	70.23 ± 6.85	56.35 ± 5.03	49.91 ± 4.77	19.31 ± 8.43 (p < 0.01)	28.66 ± 6.54 (p < 0.01)
2.	(250)	69.73 ± 4.70	62.93 ± 6.67	56.60 ± 3.88	9.86 ± 5.43 (p < 0.01)	18.82 ± 1.44 (p < 0.01)
3.	(100)	69.95 ± 5.01	63.87 ± 6.56	59.20 ± 5.83	8.80 ± 4.41 (p < 0.01)	15.52 ± 2.93 (p < 0.01)
4.	(50)	68.41 ± 4.92	64.65 ± 3.86	61.95 ± 3.38	5.39 ± 2.62 (N.S.)	9.27 ± 4.57 (p < 0.05)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

Table 5
Dose response study of methanolic extract of shahjira seeds
in normal fasting mice (n = 5)

Sr. No.	Group/Dose in mg/kg b.w.	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
*	Control (Tween-80) (0.5%)	72.02 ± 9.62	69.37 ± 7.03	68.43 ± 8.27	3.77 ± 3.87	5.47 ± 3.41
1.	(500)	64.58 ± 4.22	57.89 ± 5.54	52.74 ± 6.83	10.44 ± 4.54 (p < 0.01)	18.44 ± 7.28 (p < 0.01)
2.	(250)	71.09 ± 5.29	66.10 ± 3.72	58.10 ± 3.65	6.85 ± 3.98 (p < 0.05)	18.12 ± 4.30 (p < 0.01)
3.	(100)	70.29 ± 5.26	69.23 ± 4.68	67.97 ± 3.05	2.10 ± 2.24 (N.S.)	3.97 ± 3.87 (N.S.)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

Table 6
Dose response study of methanolic extract of nagarmotha tubers
in normal fasting mice (n = 5)

Sr. No.	Group/Dose in mg/kg b.w.	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
*	Control (Tween-80) (0.5%)	68.48 ± 6.62	67.07 ± 6.25	66.45 ± 6.99	2.23 ± 2.09	3.64 ± 3.20
1.	(500)	70.81 ± 6.76	57.90 ± 8.43	53.69 ± 6.39	17.31 ± 7.16 (p < 0.01)	26.03 ± 6.95 (p < 0.01)
2.	(250)	71.69 ± 5.57	68.07 ± 6.36	59.61 ± 6.91	5.13 ± 1.86 (N.S.)	17.02 ± 3.58 (p < 0.01)
3.	(100)	67.72 ± 4.98	66.96 ± 6.55	66.81 ± 7.44	1.86 ± 2.14 (N.S.)	2.33 ± 2.84 (N.S.)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

3. Hypoglycemic effect of plant extracts in normal fasting rats

The extracts which possessed promising hypoglycemic activity in mice were tested in normal rats, at the dose level of 100 and 500 mg/kg b.w. It can be seen from (Table 7), that methanolic extract of maka styles showed 20.58% and 22.42% reduction in blood sugar level at the dose level of 100 and 500 mg/kg respectively. Whereas, methanolic extract of Nagarmotha tubers and shahjira seeds produced 21.35% and 22.03% reduction in blood sugar level at the dose of 500 mg/kg b.w.

4. Hypoglycemic activity of isolated fractions from crude extracts

Three fractions were isolated using two-solvent extraction methods and the fractions were tested for hypoglycemic activity. These fractions were administered orally at a dose level of 500 mg/kg b.w. to normal fasting mice. The results shown in Table 8 indicate that fraction-2 (butanolic layer) isolated from crude methanolic extract of maka styles has given 23.02% reduction in blood sugar at the end of 4 hours. While other fractions were not found hypoglycemic (Table 8). The fraction-1 (petroleum ether layer) and fraction-3 (aqueous layer) from crude methanolic extract of shahjira seeds have shown 16.01% and 11.36% reductions in blood sugar at the end of 4 hours (Table 9).

5. The phytochemical screening of active extract shows the presence of glycosides.

Table 7
Hypoglycemic effect of methanolic extracts of various plants
in normal fasting rats (n=6)

Sr. No.	Common name (part used) of plant in mg/kg b.w.	Blood sugar in mg % $\pm$ S.D. after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
*	Control (Tween-80) (0.5%)	66.80 $\pm$ 2.66	61.06 $\pm$ 3.26	62.13 $\pm$ 3.45	7.51 $\pm$ 4.67	7.56 $\pm$ 2.20
1.	Methi Seeds (0.5%)	71.06 $\pm$ 6.61	59.08 $\pm$ 4.09	57.66 $\pm$ 2.09	16.33 $\pm$ 8.86 (p < 0.05)	17.61 $\pm$ 2.67 (p < 0.05)
2.	Maka Styles (500)	67.60 $\pm$ 4.07	56.66 $\pm$ 3.29	52.04 $\pm$ 2.71	16.10 $\pm$ 3.32 (p < 0.01)	22.42 $\pm$ 2.40 (p<0.01)
	(100)	68.25 $\pm$ 3.46	56.95 $\pm$ 2.37	54.19 $\pm$ 3.04	16.48 $\pm$ 2.72 (p < 0.01)	20.58 $\pm$ 2.20 (p < 0.01)

(Contd....)

Key : S.D. = Standard Deviation

Sr. No.	Common name (part used) of plant in mg/kg b.w.	Blood sugar in mg % $\pm$ S.D. after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
3.	Nagarmotha tubers (500)	68.74 $\pm$ 5.71	63.62 $\pm$ 4.09	54.04 $\pm$ 00.00	7.30 $\pm$ 3.13 (N.S.)	21.35 $\pm$ 5.24 (p<0.01)
	(100)	67.67 $\pm$ 2.60	56.95 $\pm$ 2.37	61.45 $\pm$ 4.48	7.78 $\pm$ 2.95 (N.S.)	9.23 $\pm$ 4.46 (N.S.)
4.	Shahjira seeds (500)	69.14 $\pm$ 2.17	59.54 $\pm$ 1.95	53.79 $\pm$ 3.54	13.82 $\pm$ 4.03 (p < 0.05)	22.03 $\pm$ 6.86 (p<0.01)
	(100)	68.62 $\pm$ 2.52	59.73 $\pm$ 1.72	56.98 $\pm$ 2.07	14.39 $\pm$ 2.11 (p < 0.01)	16.83 $\pm$ 1.26 (p < 0.01)

Key : S.D. = Standard Deviation; N.S. = Not Significant

Table 8
Hypoglycemic effect of isolated fractions from
methanolic extract of maka styles
(Dose = 500 mg/kg b.w. p.o.)

Sr. No.	Crude Extract/ Isolated fraction	Blood sugar in mg % $\pm$ S.D. after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
1.	Crude MeOH Extract	70.23 $\pm$ 6.85	56.35 $\pm$ 5.03	49.91 $\pm$ 4.77	19.35 $\pm$ 8.43 (p < 0.01)	28.66 $\pm$ 6.54 (p < 0.01)
2.	Fraction - 1 (Pet. ether layer)	73.32 $\pm$ 9.87	74.02 $\pm$ 9.51	74.29 $\pm$ 9.25	1.01 $\pm$ 0.59 (N.S.)	0.87 $\pm$ 2.47 (N.S.)
3.	Fraction - 2 (BuOH layer)	65.84 $\pm$ 3.67	55.03 $\pm$ 4.33	49.74 $\pm$ 5.24	16.01 $\pm$ 3.95 (p < 0.01)	23.02 $\pm$ 3.52 (p < 0.01)
4.	Fraction - 3 (Aeucous layer)	78.39 $\pm$ 2.44	78.58 $\pm$ 2.23	78.38 $\pm$ 3.97	1.89 $\pm$ 0.76 (N.S.)	1.82 $\pm$ 0.72 (N.S.)

Key : S.D. = Standard Deviation; N.S. = Not Significant i.e. p > 0.05

Table 9
Hypoglycemic effect of isolated fractions from
methanolic extract of shahjira seeds
(Dose = 500 mg/kg b.w. p.o.)

Sr. No.	Crude Extract/ Isolated fraction	Blood sugar in mg % $\pm$ S.D. after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
1.	Crude MeOH Extract	73.24 $\pm$ 5.03	63.11 $\pm$ 6.94	58.53 $\pm$ 4.68	14.93 $\pm$ 4.08 (p < 0.01)	21.35 $\pm$ 4.56 (p < 0.01)
2.	Fraction - 1 (Pet. ether layer)	76.68 $\pm$ 8.49	67.52 $\pm$ 8.68	64.59 $\pm$ 9.54	12.05 $\pm$ 3.96 (p < 0.05)	16.01 $\pm$ 4.05 (p < 0.01)
3.	Fraction - 2 (BuOH layer)	70.70 $\pm$ 8.25	70.84 $\pm$ 8.84	69.40 $\pm$ 9.64	1.26 $\pm$ 1.92 (N.S.)	4.01 $\pm$ 4.75 (N.S.)
4.	Fraction - 3 (Aeueous layer)	79.69 $\pm$ 5.61	74.89 $\pm$ 3.22	70.56 $\pm$ 4.33	7.35 $\pm$ 4.06 (p < 0.05)	11.36 $\pm$ 2.81 (p < 0.01)

Key : S.D. = Standard Deviation; N.S. = Not Significant i.e. p > 0.05

6. Comparision of hypoglycemic activity of active extract with tolbutamide

In normal fasting mice, the methanolic extract of maka styles (500 mg/kg b.w.) has given 87.19% of the hypoglycemic activity of tolbutamide (50 mg/kg b.w.p.o.) at the end of 4 hours (Table 10).

In normal fasting rats, the methanolic extract of maka styles at the dose level of 100 and 500 mg/kg b.w. p.o. has shown 20.58% and 22.42% (56.86% that of tolbutamide) reductions in blood sugar at the end of 4 hours (Table 11).

Table 10
Comparision of hypoglycemic activity of methanolic extract of
maka styles with tolbutamide in normal fasting mice (n = 5)

Sr. No.	Group/Dose in mg/kg b.w.	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
1.	Control (Tween-80) (0.5%)	70.34 ± 5.03	69.25 ± 3.15	68.32 ± 2.98	1.87 ± 2.10	3.16 ± 2.96
2.	Treated (Extract) (500 mg/kg b.w.)	70.23 ± 6.85	56.37 ± 5.03	49.91 ± 4.77	19.31 ± 8.43 (p < 0.01)	28.66 ± 6.54 (p < 0.01)
3.	Control (Gum acacia) (0.5%)	75.62 ± 6.98	70.22 ± 5.66	69.31 ± 6.34	3.17 ± 2.43	4.50 ± 1.99
4.	Treated (Tolbutamide) (50 mg/kg b.w.)	73.91 ± 4.17	61.74 ± 5.02	49.42 ± 2.40	16.49 ± 3.96 (p < 0.01)	32.87 ± 6.16 (p < 0.01)

Key :- All values (Mean) ± S.D.

Table 11
Comparison of hypoglycemic activity of methanolic extract of
maka styles with tolbutamide in normal fasting rats (n = 6)

Sr. No.	Group/Dose in mg/kg b.w.	Blood sugar in mg % after			% Reduction in blood sugar after	
		0 hr	2 hrs	4 hrs	2 hrs	4 hrs
1.	Control (Tween-80) (0.5%)	66.80 ± 2.66	61.06 ± 3.26	62.13 ± 3.45	7.51 ± 4.67	7.56 ± 2.20
2.	Extract (500)	67.60 ± 4.70	56.66 ± 3.29	52.04 ± 2.71	16.10 ± 3.32 (p < 0.01)	22.42 ± 2.40 (p < 0.01)
3.	Extract (100)	68.25 ± 3.46	56.95 ± 2.37	54.19 ± 3.04	16.48 ± 2.72 (p < 0.01)	20.58 ± 2.20 (p < 0.01)
4.	Control (Gum acacia) (0.5%)	73.34 ± 5.95	71.30 ± 5.18	70.36 ± 4.38	2.75 ± 1.02	3.24 ± 1.59
5.	Tolbutamide (50)	72.89 ± 3.99	47.99 ± 4.63	44.00 ± 4.68	33.99 ± 7.29 (p < 0.01)	39.43 ± 7.64 (p < 0.01)

Key :- All values (Mean) ± S.D.

7. Hypoglycemic effect of active extract in oral glucose tolerance test

Table 12 shows the effect of single dose of 500 mg/kg b.w.p.o. of different extracts on blood sugar level in glucose fed mice compared with control group.

The glucose fed group showed a peak rise in blood sugar (194.94 mg%) at the end of 1/2 hour which returned to about normal fasting blood sugar level at the end of 2 hours. In the case of drug treated group, methanolic extract of maka styles significantly inhibited the hyperglycemia produced by oral glucose load (Table 12). The maximum blood sugar lowering effect was shown by methanolic extract of maka styles,

In normal fasting rats, methanolic extract inhibited hyperglycemia produced by oral glucose load (Table 13).

These results show that the methanolic extract of maka styles remarkably inhibited elevated blood sugar levels in normal fasting mice as well as rats.

Table 12
Effect of plant extracts on hyperglycemia in orally
glucose fed mice (n = 6)
(Dose of plant extract = 500 mg/kg b.w.)

Sr. No.	Treatment	Mean blood sugar in mg % $\pm$ S.D. after			
		'0' hr	'½' hr	'1' hr	'2' hr
*	Control group	72.30 $\pm$ 4.45	72.27 $\pm$ 3.30	70.81 $\pm$ 4.34	72.40 $\pm$ 9.55
1.	Glucose fed group (1.5 gm/kg b.w.)	68.47 $\pm$ 5.55	194.94 $\pm$ 13.85 (p < 0.01)	147.85 $\pm$ 9.12	86.23 $\pm$ 8.82
2.	Glucose $\pm$ Methanolic Extract of Shahjira seeds	68.57 $\pm$ 4.98	193.60 $\pm$ 11.75 (N.S.)	139.71 $\pm$ 11.17	82.49 $\pm$ 7.53
3.	Glucose $\pm$ Methanolic extract of nagarmotha tubers	70.09 $\pm$ 4.21	171.49 $\pm$ 13.70 (p < 0.05)	122.64 $\pm$ 12.99	65.38 $\pm$ 4.54
4.	Glucose $\pm$ Methanolic extract of maka styles	73.68 $\pm$ 7.09	157.08 $\pm$ 13.93 (p < 0.01)	129.24 $\pm$ 9.30	70.11 $\pm$ 6.06

Key :- S.D. = Standard deviation; N.S. = Not Significant i.e. p > 0.05

Table 13
Effect of methanolic extract of maka styles on hyperglycemia
in orally glucose fed rats (n = 6)
(Dose of extract = 500 mg/kg b.w.p.o.)

Sr. No.	Treatment	Mean blood sugar in mg% $\pm$ S.D. after			
		'0' hr.	$\frac{1}{2}$ hr.	1 hr.	2 hrs.
1.	Control (Tween-80)	72.59 $\pm$ 5.36	72.72 $\pm$ 6.60	72.24 $\pm$ 6.47	70.70 $\pm$ 6.63
2.	Glucose fed group (1.5 gm/kg b.w.)	70.82 $\pm$ 5.74	174.49 $\pm$ 13.78 (p < 0.01)	135.33 $\pm$ 7.85	80.04 $\pm$ 11.38
3.	Glucose $\pm$ Extract	71.44 $\pm$ 6.08	130.05 $\pm$ 8.38 (p < 0.01)	110.81 $\pm$ 6.42	76.47 9.48

Key :- All values (Mean) $\pm$ S.D.

8. Hypoglycemic effect of active extract in alloxan-induced hyperglycemia

In alloxan-treated mice, the blood sugar levels were significantly elevated upto 319.99 mg% at the end of 72 hours. In drug treated group, the methanolic extract of maka styles was administered orally at the dose of 500 mg/kg b.w. after 72 hours. It was observed that the extract has lowered the elevated blood sugar levels by 40.49% ($p < 0.01$) at the end of 76 hours (Table 14).

9. Sub-acute toxicity study

The methanolic extract of maka styles was administered orally at a does level of 100 mg/kg b.w. daily for 4 weeks. During this study, (a) food intake (b) gain in the body weight (c) fasting blood sugar levels were recorded weekly. The results are presented in Table 15,16,17 and Fig. 4 to 6 respectively.

a) Food intake

The food intake of rats, treated with methanolic extract of make styles was almost similar to that of control group. The consumption of food during the first week by control rat was 16.62 gm/day and this was increased to 20.26 gm/day at the end of 4 weeks. Similarly, food consumed by extract treated rats was 17.65 gm/day (in first week) and it was increased to 21.17 gm/day/rat (at the end of 4 weeks) (Table 15).

b) Body weight

The comparison of gain in body weight of extract treated group with control group is presented. Average body weight of individual rat in the control group increased at the end of 4 weeks. The initial body weight of control rat was 160.43 gms which increased to 211.45 gms (21.80%) at the end of 4 weeks. Whereas, in extract treated group, average gain in body weight after 4 weeks by rat from 162.13 gms to 217.09 gms (33.89%) was recorded.

c) Weekly fasting blood sugar level

Initial mean fasting blood sugar level of control groups was 73.99 ± 5.06 mg%. There was 5.06% reduction in blood sugar levels in control group after '4' weeks. The initial mean fasting blood sugar level of extract treated gradually decreased from 73.05 mg% to 56.55% after '4' weeks (Table 17). In other words, 25.21% reduction in blood sugar levels was noted which was quite significant (i.e. $p < 0.01$) at the end of 4 weeks

10. Effect of methanolic extract of maka styles on different biochemical parameters

The effect of oral administration of methanolic extract of maka styles on different biochemical parameters was studied (Table 18).

Serum cholesterol levels were decreased from 122.83 mg% to 104.58 mg% after '4' weeks in extract treated group. Therefore, there was 14.85% depression in cholesterol levels which was quite significant ($p < 0.01$).

Serum triglycerides levels were depressed by 6.94% ($p < 0.01$) in extract treated group.

Even serum total lipid levels were also decreased from 318.80 mg% to 283.19 mg% in extract treated group after '4' weeks. The decrease profound to be significant at $p < 0.01$.

There was no significant change in other biochemical parameters.

Table 14
Effect of oral administration of methanolic extract of maka styles on elevated blood sugar
in alloxan-induced diabetic mice (n=6); (Dose of extract = 500 mg/kg b.w.)

Sr. No.	Treatment	Mean blood sugar in mg % $\pm$ S.D. after						% Reduction in blood suger after '4' hrs
		'0' hr	24 hrs	48 hrs	72 hrs	74 hrs	76 hrs	
*	Saline Control	73.64 $\pm$ 6.60	76.35 $\pm$ 6.93	73.91 $\pm$ 5.59	73.91 $\pm$ 5.59	74.34 $\pm$ 3.63	73.01 $\pm$ 4.02	3.60 $\pm$ 1.73
1.	Alloxan Control	75.59 $\pm$ 10.01	268.68 $\pm$ 8.87	309.05 $\pm$ 12.98	319.99 $\pm$ 16.95	314.47 $\pm$ 12.14	318.87 $\pm$ 14.06	1.80 $\pm$ 1.41
2.	Alloxan $\pm$ Extract	77.06 $\pm$ 6.75	272.79 $\pm$ 9.82	327.38 $\pm$ 19.99	311.18 $\pm$ 19.88	254.45 $\pm$ 20.46	185.22 $\pm$ 13.51	40.49 $\pm$ 1.68 (p < 0.01)

Key :- S.D. = Standard deviation

Table 15
Effect of oral administration of methanolic extract of maka styles (for 4 weeks)
on food intake in normal rats (n = 6)

Sr. No.	Group	Mean food intake in gms/day/rat after			
		1 week	2 weeks	3 weeks	4 weeks
1.	Control	16.62	19.54	19.92	20.26
2.	Treated	17.65	19.77	19.83	21.17

Table 16
Effect of oral administration of methanolic extract
of maka styles on body weight of normal rats (n = 6)

Sr. No.	Group	Initial	Body weight in gms. $\pm$ S.D. after			
			1 week	2 weeks	3 weeks	4 weeks
1.	Control	160.43 $\pm$ 7.63	182.83 $\pm$ 10.15	198.75 $\pm$ 14.35	210.47 $\pm$ 11.92	211.45 $\pm$ 5.83
2.	Treated	162.13 $\pm$ 8.60	184.53 $\pm$ 9.96	196.63 $\pm$ 11.79	202.66 $\pm$ 14.03	217.09 $\pm$ 13.45

Key :- S.D. = Standard deviation

Table 17
Effect of oral administration of methanolic extract of maka styles (for 4 weeks)
on fasting blood sugar in normal rats (n = 6)

Sr. No.	Group	Initial	Mean blood sugar in mg % $\pm$ S.D. after				% Reduction in blood sugar $\pm$ S.D. after '4' weeks
			1 week	2 weeks	3 weeks	4 weeks	
1.	Control Group	73.99 $\pm$ 5.06	74.58 $\pm$ 5.60	72.51 $\pm$ 4.20	72.55 $\pm$ 5.74	71.40 $\pm$ 5.85	5.06 $\pm$ 3.74
2.	Treated Group	73.05 $\pm$ 4.88	75.13 $\pm$ 2.82	66.02 $\pm$ 3.50	58.04 $\pm$ 2.94	56.55 $\pm$ 4.84	25.21 $\pm$ 9.74 (p < 0.01)

Key :- S.D. = Standard Deviation

Table 18
Effect of oral administration of methanolic extract of maka styles
on different biochemical parameters in rats (n = 6)

Estimation of serum								
Sr. No.	Group		Cholesterol (mg %)	Triglycerides (mg %)	Total lipids (mg %)	Total proteins (mg %)	GPT (I.U./Lit.)	GOT (I.U. /lit)
1.	Control Group		121.16 ± 7.67	214.50 ± 25.15	316.28 ± 7.49	6.68 ± 0.35	37.68 ± 1.88	95.68 ± 1.89
2.	Treated Group	Initial	122.83 ± 8.82	212.88 ± 28.29	318.80 ± 11.83	7.03 ± 0.45	39.56 ± 1.96	93.95 ± 4.28
		After '4' weeks	104.58 ± 6.43 (p < 0.01)	198.10 ± 22.98 (p < 0.01)	283.19 ± 10.13 (p < 0.01)	7.10 ± 0.46	34.60 ± 1.76	93.52 ± 3.46

Key = All values (Mean) ± S.D.
S.D. = Standard Deviation

Discussion

DISCUSSION

Since ancient time, diabetics have been treated orally with plant extracts based on folk medicine. Though considerable work has been carried out for the evaluation of hypoglycemic activity of medicinal plants, still there is a scope for searching effective remedy for diabetics. The present investigation was initiated to confirm the hypoglycemic activity of different plant materials. Attempt was also initiated to isolate the active constituent from a plant extract.

Menczel et al (1962) have reported 25.00% reduction in fasting blood sugar in normal rabbits after subcutaneous administration of extract of 2 gm *Zea Mays* (Maka) styles per kg. body weight. In the present investigation, the crude methanolic extract of maka styles lowered the fasting blood sugar by 28.66% (Table 2) and 22.42% in normal mice and rats respectively at the dose level of 500 mg / kg b.w. p.o (Table 7).

The hypoglycemic effect of aqueous suspension of *Carum carvi* (shahjira) seeds in normal glucose fed hyperglycemic and alloxanated diabetic rats at the dose of 2 gm / kg b.w. was reported by *Kalia* et al (1992). In the present study, the methanolic extract of shahjira seeds produced 18.44% (Table 2) and 22.03% (Table 7) blood sugar reduction in normal fasting mice and rats respectively at the end of 4 hours at the dose level of 500 mg/kg b.w. p.o.

According to *Singh* et al (1976), the experimental diets of *Acacia catechu*, *Acacia suma* and *Albizzia odoratissima* seeds caused a lowering of blood sugar level by 20.4 %, 25.6 % and 22.5 % respectively in normal rats. When experimental diets

of *Acacia arabica*, *Acacia benthami* and *Acacia modesta* seeds were fed to normal rats, 29.99%, 27.68% and 25.05% lowering in blood sugar levels respectively observed by Singh et al (1975). Pillai et al (1980) found 14.40% reduction in fasting blood sugar at the end of 5 hours when decoction of *Cyamopsis tetragonolobus* (guar) seeds were administered to normal rabbits. While in the present investigation water extract of guar seeds lowered the fasting blood sugar by 21.02% in normal fasting mice at the end of 4 hours (Table 3). The methanolic extract of guar seeds was not hypoglycemic.

During dose response study, it was observed that the hypoglycemic activity of methanolic extract of maka styles was elevated as the dose was increased (Table 4). Pillai et al (1980) found that when the dose of guar whole fruit was increased from 10, 20 to 40 gm/kg b.w. the hypoglycemic activity was changed from 16.31%, 24.95% to 11.03% respectively in normal fasting rabbits.

The methanolic extract of shahjira seeds and nagarmotha tubers showed 18.44%, 18.12% and 26.03%, 17.02% reduction in fasting blood sugar at the dose level of 500 and 250 mg/kg b.w. respectively in mice (Table 5 & Table 6). In normal fasting rats, the hypoglycemic activity of methanolic extract of methi seeds, maka styles, nagarmotha tubers and shahjira seeds was confirmed (Table 7).

The fraction-2 (n-butabolic layer) isolated from crude methanolic extract of maka styles has shown 23.02% reductions in blood sugar level which was found. Comparatively less than that of its crude methanolic extract 28.66% (Table 8). The fraction-1 (petroleum ether layer) and fraction-3 (aqueous layer) isolated from methanolic extract of shahjira seeds showed 16.01% and 11.36% reduction in

fasting blood sugar which were found comparatively less than that of crude methanolic extract i.e. 21.35% (Table 9).

The light petroleum ether extract and ethyl ether extract of the dried powder of *Allium cepa* (onion) bulbs have shown 15.5% and 19.2% reduction in fasting blood sugar levels respectively after 4 hours at the dose level of 0.5 gm/kg b.w. (Brahmachari et al, 1961).

Desai et al (1985) reported that alcoholic extract of *Hamiltonia suaveolens* roots (500 mg/kg b.w. p.o.) caused 45.00% reduction in blood sugar in normal rats at the end of 5th hour. Similar reduction was observed in normal rabbits, dogs and monkeys. Among other fractions isolated from crude extract, maximum hypoglycemic activity was obtained with ethyl acetate extract which led to 40 percent reductions in blood sugar at a dose level of 50 mg/kg b.w.

The two orally effective hypoglycemic principles were extracted from *Allium sativum* (garlic) and *Ficus religiosa* (Brahmachari et al, 1962). The total ethyl ether extract from 50 gm dry garlic powder and water extract from 50 gm dry root bark powder of *Ficus religiosa* produced 14.75% and 18.97% reduction in blood sugar levels after 4 hours in normal fasting rabbits.

The steroidal glycoside (Kato et al, 1995) PO-2, isolated from *Polygonati Rhizoma* (Fam-Liliaceae) lowered the blood sugar level from 202 ± 5 to 109 ± 5 mg % and 197 ± 6 to 147 ± 6 mg % at the dose of 10 and 50 mg/kg b.w.i.p. in normal mice. However, a steroid, diosgenin did not affect the blood glucose level in either

normal or streptozotocin-diabetic mice, indicating that the branching units are essential for the biological activity.

A glycoside, leucoanthocyanin, isolated from the ethanolic extract of the bark of *Ficus bengalensis* showed 22.00% and 21.10% reduction in fasting blood sugar in normal rabbits (Brahmachari et al, 1964). However, they found that the aqueous crude extract was more active than the pure glycoside.

The n-butanolic extract of rhizomes of *Ophiogon japonicus* (BM) (Kako et al, 1995) at the dose of 100 mg/kg b.w. reduced the blood glucose of normal mice from 201 ± 13 to 151 ± 7 mg % 4 hours after intraperitoneal administration and also significantly lowered the blood glucose of streptozotocin-induced diabetic mice. BM also tended to suppress epinephrine-induced hyperglycemia in mice.

It was observed that the methanolic extract of maka styles produced 87.19% and 56.86% hypoglycemic activity in mice and rats as compared with the hypoglycemic activity of tolbutamide (Table 10 & Table 11). The three flavonoid compounds A, B and C isolated from the ethanolic extract of the bark of *Ficus bengalensis* have shown 36.4%, 48.5% and 23.63% hypoglycemic activity compared with that of tolbutamide (Brahmachari et al, 1964).

The oral administration of methanolic extract of maka styles lowered the blood sugar level from 194.94 ± 13.85 to 157.08 ± 13.93 (Table 12) after ½ hour in orally glucose (1.5 gm/kg b.w.) fed mice. The same methanolic extract of maka styles significantly lowered the elevated blood sugar level i.e. from 174.49 ± 13.78 to 130.05 ± 8.38 after ½ hour when compared with control group in orally glucose fed

rats (Table 13). *Narayanan et al* (1984) found that when alcoholic extract of leaves of *Bougainvillea spectabilis* was administered orally to glucose fed mice, after 30 mins. The blood sugar level decreased from 154.6 to 135.00 mg %.

The diabetogenic effect of alloxan has been confirmed adequately in various species of animals like rabbits, rats, dogs, monkeys etc. In the present study, normal Haffkine strained mice were used for the development of diabetic condition. Alloxan is known to produce selective necrosis of β -cells of islets of Langerhans in pancreas and thus, removes the source of endogenous insulin (*Dunn et al*, 1943). The significant hypoglycemic effect i.e. 40.49% ($p < 0.01$) was observed in alloxan induced diabetic mice with a single administration (500 mg/kg b.w. p.o.) of methanolic extract of maka styles when compared with the control group. The blood sugar level after 72 hours i.e. 311.18 ± 19.88 mg% was depressed to 185.22 ± 13.51 mg% (Table 14).

According to *Pillai et al* (1980) the whole guar decoction (40 gm/kg b.w.) decreased the elevated blood sugar level from 324.60 ± 28.60 mg % to 199.20 ± 16.23 mg % ($p < 0.01$) at the end of 5 hrs. in alloxan-diabetic rabbits. It was pointed out that the mechanism of hypoglycemic action of guar is not due to pancreatic β -cell stimulation and that an extra-pancreatic site for the hypoglycemic effect of guar was involved.

The antidiabetic effect of two alkaloids isolated from the leaves of *Tecoma stans*, tecomine and tecostamine, was studied in alloxan diabetic rabbits by *Hammouda et al* (1966). They have reported that both the alkaloids in the daily dose of 20 mg/kg were effective in reducing the hyperglycemia to near normal blood sugar

levels. From the mechanistic point of view, the alkaloids seem to need a minimum of active B-cells of the pancreas for action and in this respect resemble other orally active antidiabetic drugs i.e. sulphonylureas.

Toxicological Studies of an active extract :

An active extract (methanolic extract of *Maka styles*) was administered orally in single dose ranging from 500 to 5000 mg/kg the normal healthy mice and rats for acute toxicological studies. No untoward reaction or death was observed upto seven days, suggesting the safety of the drug.

In sub-acute toxicity study, an active extract did not show any death or toxic symptoms upto the period of 4 weeks in normal rats suggesting thereby that this extract is free from toxic effects on prolonged treatment. Further this extract did not show any decrease in the body weight as well as in food intake of the animals.

Tolbutamide in long term treatment did not affect loss of body weight whereas loss of appetite and body weight following treatment with phenformin has been reported (*Campbell et al, 1969*). But this active extract did not show any side effects during experimental period.

The blood sugar lowering effect of active extract was gradually increased every week end (Table 17). Serum cholesterol triglycerides and total lipid levels were also decreased by 14.85%, 6.94% & 11.10% respectively. These results suggest that methanolic extract of *Maka styles* besides being hypoglycemic, has hypocholesterolemic effect in normal rats (Table 18).

Sharma et al (1988) reported the decrease in blood sugar, serum cholesterol, triglycerides and phospholipids i.e. 15.54%, 22.35%, 24.02% and 29.98% respectively after 4 weeks daily feeding of *Trichosanthes dioica* (padval) seeds at the dose of 1 gm percent in normal rabbits. Some of the plants like *Cyamopsis tetragonolobus* (*Srivastava et al, 1987*) and *Trichosanthes dioica* (*Sharma et al, 1992*) were reported as hypocholesterolemic as well as hypotriglyceridaemic besides having hypoglycemic activity. Similarly, remarkable fall in cholesterol and triglyceride levels were observed in long term studies of glibenclamide (*Caness et.al, 1971*). In the case of biguamide (phenformin) there was a fall in the levels of plasma triglycerides, free fatty acids and cholesterol levels also (*Schwartz et al, 1966*).

An active extract did not show any significant change in serum total proteins, GPT and GOT levels when compared with the control group during the 4 week study (Table 18).

The methanolic extract of maka styles may prove to be a simple and effective hypoglycemic agent in diabetes mellitus.

Summary and Conclusions

SUMMARY AND CONCLUSIONS

The screening of 12 medicinal plants for the evaluation of hypoglycemic activity was carried out in normal fasting mice.

The definite and potent hypoglycemic activity was confirmed in normal fasting rats.

The methanolic extract of maka (*Zea mays*)- styles (active extract) showed potent hypoglycemic effect in normal fasting mice as well as rats.

An active extract inhibited elevated blood sugar levels in orally glucose fed mice and rats.

An active extract showed remarkable hypoglycemic activity in normal fasting mice and rats when compared with standard hypoglycemic drug i.e. tolbutamide.

In alloxan-induced diabetic mice, a single dose administration of an active extract produced remarkable and significant reduction in blood sugar levels.

Isolated fractions did not yield more hypoglycemic effect as compared to the crude methanolic extract.

In toxicological studies, an active extract did not show any toxic effects or mortality upto 5000 mg/kg b.w. p.o. in normal healthy animals.

In sub-acute toxicity study, an active extract was found to be free from toxic symptoms and no death of animals was observed. In addition, following observations were noted :

1. An active extract did not affect the body weight and food intake in normal rats.
2. An active extract produced significant reduction in blood sugar level during the experimental period.
3. An active extract significantly lowered serum cholesterol, triglyceride and total lipid levels at the end of 4 weeks.

Thus, from this investigation it can be concluded that methanolic extract of *Zea mays* (maka) styles may yield a pioneering molecules as an oral hypoglycemic agent.

Bibliography

BIBLIOGRAPHY

- Ahmad Faiyaz; Khan, M.M; Rastogi, A.K; Chaubey, M. and Kidwai, J.R. (1991).
Ind. J. Expl. Bio; 29 : 516.
- Aiman, R. (1970).
Ind. J. Physiol. Pharmacol; 14 : 65.
- Akhtar, M.S; Akhtar, M.A. and Yaquals, M. (1981).
J. Med. Plant Res; 42 : 205.
- Alders L. (1955).
"Liquid-liquid Extraction, Theory and Laboratory Experiments"; Elsevier Publishing Company, Pp. 136.
- Allen, F.M. (1927).
J. Am. Med. Assoc; 89 : 1577.
- Ambike, S.H. and Rajarama Rao, M.R. (1967).
Ind. J. Pharm; 29 : 91.
- Augusti, K.T. and Benaim, M.E. (1975).
Clin. Chim. Acta, 60 : 121.
- Augusti, K.T. and Mathew, P.T. (1975).
Experientia, 31 : 148.
- Bailey, C.C. and Bailey, O.T. (1943).
J. A. M. A; 122 : 1165.
- Bailey, C.J; Day, C. and Leatherdale, B.A. (1986).
Prac. Diabetes; 3 : 190.
- Banting, F.G; Best, C.H; Collip, J.B; Campbell, W.R. and Fletcher, A.A. (1922b).
Canad. Med. Assoc. J; 12 : 141.
- Belfiore, F. (1980).
"Enzyme Regulation and Metabolic Diseases", Karger, New York, Pp. 180.
- Bennett, P.H. (1991).
"Text book of Diabetes", Vol. I, ed. by John C. Pickup and Gareth Williams, Blackwell Scientific Group, Oxford, P. 37.

Bernard, C. (1949).

Quoted from "A Handbook on Diabetes mellitus and Modern Treatment", Ed. Bose, J.P.; 4th edition; U.N. Dhar and Sons Ltd; Calcutta, P.2.

Bliss, M. (1982).

"The discovery of insulin", University of Chicago Press, Chicago.

Brahmachari, H.D. and Augusti, K.T. (1961).

J. Pharm. Pharmacol; 13 : 128.

Brahmachari, H.D. and Augusti, K.T. (1962).

J. Pharm. Pharmacol; 14 : 254.

Brhmachari, H.D. and Augusti, K.T. (1964).

Ind. J. Physiol. Pharmacol; 8 : 60.

Cader Asmal, A. and Marble, A. (1984).

Drugs; 28 : 62.

Campbell, G.D. (1969).

"Oral hypoglycemic agents - Mediinal Chemistry", A series of monographs, Vol.9, Pp. 40.

Campbell, I.W. (1990).

Quoted from "New antidiabetic drugs; ed. Bailey, C.J. and Flatt, P.R. and Smith-Gordon, Pp. 35.

Caness, I; Valiente, S; Artega, A; Gracia, M; Mella, I and Ferrel, E. (1971).

Quoted from Drugs, 1 : 116.

Chakravarty, B.K; Gupta, S; Gambhir, S.S. and Gode, K.D. (1981).

Life Sciences, 29(20) : 2043.

Chandalia, H.B. and Pathi, D. (1990).

Bombay Hospital Journal, 32(1) : 89.

Chattopadhyay, P.R; Sarkar, S.K; Ganguly, S; Banerjee, B.W. and Basu, T.K. (1991).

Ind. J. Physiol. Pharmacol; 35(3) : 145.

Cooper, S.R. and McDaniel, V. (1970).

"Std. Methods of Clin. Chem." edited by R.P. McDonald, Vol.6 : 159. Academic Press.

Davis, H; Partridge, M.W. and Robinson, A.I. (1950).
In "Bentley's Textbook of Pharmaceutics", Published by Tindal and Co. (London), 5th
Edition, Pp. 248.

Day, C. (1986).
Industr. Biotechnol. 6 : 137.

Day, C. and Bailey, C.J. (1986).
Diabetic Med; 3 : 361A.

De, U.N. and Mukerjee, B. (1953).
Ind. J. Med. Sci. 7 : 665.

Desai, A.C. and Bhide, M.B. (1985).
Ind. J. Med. Res; 81 : 86.

Dhar, H.L; Dhirwani, M.K. and Sheth, V.K. (1975).
Br. J. Clin. Pract; 29 : 119.

Duncan, L.J.P. and Baird, J.D. (1960).
Pharma. Rev; 12 : 91.

Duncan, C.G. (1964).
"Diseases of Metabolism", Ed. Duncan, C.G; Saunders, W.B; Co; Philadelphia and
London; 5th edition, Pp. 921.

Dunn, J.S. and McLetchie, N.G.B. (1943).
Lancet; 1 : 484.

Ezzele, J.W; Dobrogosz, W.T; Kloos, W.E and Manclark, C.R. (1981).
J. Infect. Disea; 143 : 562.

Farnsworth, N.R; Segelman, A.B. (1971).
Hypoglycemic Plants, Tile & Till, 57 : 52.

Frings, C.S; Fendley, T.W; Dunn, R.T. and Queen, C.A. (1972).
Clinical Chemistry, 18(7) : 673.

Goeddel, D.V; Kleid, D.G; Bolivar, F; Heynekar, H.L; Yansura, D.G; Crea, R; Hirose,
T; Kraszewski, A; Italeura, K. and Riggs, A.D. (1979).
Proc. Natl. Acad. Sci; USA, 76 : 106.

Gupta, R.K; Gupta, S. and Samuel, K.C. (1977).
Ind. J. Expl. Biol; 15 : 113.

Gupta, R.R; Agarwal, C.G; Singh, G.P. and Ghotak, A. (1995).
 Proceeding of "World Congress On Biotechnological Developments in Medicinal
 Substances of Plant and Marine Origin", Lucknow.

Gupta, S.S. (1963).
 Ind. J. Med. Res. 51 : 716.

Gupta, S.S; Verma, C.L; Gang, V.P. and Khandelwal, P. (1967).
 Ind. J. Med. Res; 55, 754.

Hammouda Y and Amer, M.S. (1966).
 J. Pharm. Sci; 55 : 1452.

Hammouda, Y. and Khallafallah, N. (1971).
 J. Pharm. Sci; 60 : 1142.

Harborne, J.B. (1984).
 "Phytochemical Methods : A guide to Modern Tech. of Plant Analysis: Chapman and
 Hall Publishers, 2nd edition.

Hardman, R. and Fazli, F.R.Y. (1972).
 Planta Medica; 21 : 131, 188, 322.

Hardman, R; Kasuji, J. and Parfit, R.T. (1980).
 Phytochemistry, 19 : 698.

Hii, C.S.T. and Howel, S.L. (1984).
 Diabetes, 33 : 291.

Hikino, H; Mizuro, T; Oshima, Y. and Konno, C. (1985).
 Planta Med; 51 : 159.

Hikino, H; Takashi, M; Oshima, Y. and Konno, C. (1988).
 Planta Med; 54 : 1.

Horwitz, D.L. (1990).
 "New antidiabetic drugs", ed. Bailey, C.J. and Flatt, P.R; Smith-Gordon; Pp. 53.

Hukeri, V.I; Kalyani, G.A. and Kakrani, H.K. (1988).
 Fitoterapia; 59 : 68.

Kako, M; Miura, T; Usami, M; Kato, A. and Kadowaki, S. (1995).
 Biol. Pharm. Bull; 18(5) : 785.

Kalia, A.N; Agarwal, D.K; Saini, A.. and Harbans Lal (1992).
 Ind. J. Clin. Biochem; 7 : 49.

Karawya, M.S; Abdel Wahab, S.M; El-Olemmy, M.M. and Parag, N.M. (1984).
J. Natl. Prod; 47 : 775.

Kato, A; Miura, T. and Fukunaga, T. (1995).
Biol. Pharm Bull; 18(1) : 167.

King, K. and Rewers, M. (1991).
Bulletin of the world Health Organization, 69(6) : 643.

Knauff, R.K; Fajans, S.S; Ramirez, E. and Conn, J.W. (1959).
Metabolism, 8 : 606.

Koholi, K.R. (1983).
Quoted from J. Sci. Res. Plants and Med. VI (1985(1-4) : 21.

Kulb, H; Kiesel, V; Greolich, B; Vander Bosuch, J. (1982).
Lancet (I) : 1303.

Liu, P.S; Daniel, J.K.and Rhinehort, B.L. (1989).
Chem. Abs. 111 : 7569.

Lotlikar, M.M. and Rajarama Rao, M.R. (1966).
Ind. J. Pharm; 28 : 129.

Manchester, K.L. (1972).
Diabetes, 21, Suppl. 2, 447.

Mann, J. (1987).
Am. J. Clin. Nutri; 45 : 1202.

Marble, A. (1971).
"Joslin's Diabetes Mellitus", Ed. Marble, et al; 11th edition (Indian) K.M. Verghese
Co., Bombay, P. 1.

Mcletchie, N.G.B. (1982).
Diabetologia, 25 : 360.

Menczel, E. and Sulman, F.G. (1962).
Proc. Soc. Exp. Biol. Med; 110 : 178.

Mendez, J; Franklin, B. and Gahagan, H. (1975).
Clin. Chem; 21(6) : 768.

Mirsky, I.A; Diengott, G. and Perisutti, G. (1956).
Endocrinology, 59 : 715.

Modak, A.T. and Rajarama Rao, M.R. (1966).
Ind. J. Pharm. 28 : 105.

Mukherjee, B. (1957).
J. Sci. Industr. Res; 16A, 10, Pp. 1.

Mukherjee, K; Patra, B; Sikandar, S. and Dasgupta, S.K. (1972).
Ind. J. Pharmacol; 4 : 114.

Mutalik, G.S; Mitra, D.K; Oak, V.R. and Nayak, N.J. (1972).
J. A. P. I; 20 : 229.

Nadkarni, A.K. (1976).
"Indian Materia Medica", 2nd Edition, Popular Prakashan, Bombay.

Narayanan, C.R; Joshi, D.D. and Mujumdar, A.M. (1984).
Current Science, 53(11) : 579.

Narayanan, C.R; Joshi, D.D; Mujumdar, A.M. and Dhekhe, V.V. (1987).
Current Science, 56(3) : 130.

Nikam, S.T. (1981).
Ph.D. Thesis submitted to the University of Bombay.

Noor H; Hammonds, T; Sutton, R. and Asheroft, S.J.H. (1989).
Diabetologia, 32 : 354.

Oliver Bever B. and Zahad, G.R. (1979).
Quat. J. Crude Drug Res; 17 (3-4) : 139.

Padmini, E; Meenakshi, C.E. and Motlog, D.B. (1990).
Ind. J. Med. Res; 92(B) : 178.

Perleg, M. and Kipnis, D.K. (1966).
Diabetes, 15 : 867.

Pillai, N.R; Seshadri, C. and Santhakumari, G. (1980).
Ind. J. Med. Res; 72 : 128.

Raghunathan, K. and Sharma, P.V. (1969).
J. Res. Ind. Med; 4(1) : 59.

Rajasekharan, S. and Tuli, S.N. (1976).
J. Res. Ind. Med. Yoga and Homeo; 11(2) : 9.

Reitman, S. and Frankel, S. (1957).
Am. J. Clin. Pathol; 28 : 56.

Recant, L. (1955).
Am. J. Med; 19 : 610.

Roger Dobson (1995).
Quoted from "Bombay Times", Complimentary to the Times of India, Bombay, P. 6,
dated May 30, 1995.

Roman Ramos, R; Alarcon Aquilar, E; Lara Lemus, A. and Flores Saeuz, J.L.
(1992).
Arch. Med. Res; 23(1) : 59.

Ryle, P.R; Barker, J; Gaines, P.A; Thomson, A.D. and Chakraborty, J. (1984).
Life Sci; 34 : 591.

Sadhukhan, B; Roychowdhary, U; Banerjee, P. and Sen S. (1994).
J. Ind. Med. Assoc; 92(4) : 115.

Saifi, A.Q; Shinde, S; Kaviswar, W.K. and Gupta, S.R. (1971).
J. Res. Ind. Med; 6 : 205.

Sanger, F. (1959).
Sciences, 129 : 1340.

Schwartz, M.J; Mirsky, S. and Schaefer, L.E. (1966).
Metabolism, 15 : 808.

Shami, J.A; Goldschmied, B; Joseph, Z; Ahronson and Sulman, F.G. (1974).
Arch. Int. Pharmacodyn. Ther; 210 : 27.

Shanmugasundaram, K.R; Paneer-selvam, C; Samudram, P; and
Shanmugasundaram, E.R.B. (1981).
Pharm. Res. omm; 13 : 475.

Shanmugasundaram, K.R; Paneer-selvam, C; Samudram, P; and
Shanmugasundaram, E.R.B. (1983).
J. Ethnopharmacol; 7 : 205.

Sharma, G. and Pant, M.C. (1988).
Ind. J. Med. Res; 87 : 398.

Sharma, G. and Pant, M.C. (1992).
Ind. J. Clin. Biochem., 7 : 53.

Sheehan, E.W; Stiff, D.D; Duah, F; Slatkin, D.J; Schiff Jr., P.L. and Zemaitis, M.A.
(1983).
Life Sci. 33 : 593.

Singh, K.N; Chandra, V. and Borthwal, K.C. (1975).
Ind. J. Physiol. Pharmacol; 19 : 167.

Singh, K.N; Mittal, R.K. and Borthwal, K.C. (1976).
Ind. J. Med. Res; 4 : 754.

Singh, R.S. and Tripathy, S.N. (1972).
Quoted from J. Res. Ind. Med. Yoga and Homeo. (1978); 13(4) : 12.

Smith, D.L; VeEchio, J.J. and Forist, A.A. (1965).
Metabolism, 14 : 229.

Srivastava, A; Longia, G.S; Singh, S.P. and Joshi, L.D. (1987).
Ind. J. Physiol. Pharmacol. 21(2) : 77.

Takahashi, M; Konno, C; Hikino, H. (1985).
Planta Med; 51 : 258.

Tomoda, M; Shimizu, N; Oshima Y; Takahashi, M; Murakami, M. and Hikino, H.
(1987).
Planta Med; 53 : 8.

Tripathi, S.N; Tiwari, C.N; Upadhyay, B.N. and Singh, R.S. (1979).
J. Res. Ind. Med. Yoga and Homeo; 14(3-4) : 160.

Vad, B.G. (1961).
Ind. J. Pharm; 23 : 115.

Varsney, I.P; Sharma, S.C. (1968).
J. Ind. Chem. Soc. 43(8) : 564.

Varsney, I.P. and Bhatnagar, S.P. (1965).
Ind. J. Pharma; 27 : 231.

Waters, A.K; Morgan, D.B. and Wales, J.K. (1978).
Diabetologia, 14 : 95.

Weichselbaum, T.E. (1969).
Clinical Chemistry, Theory and Practice, Ed. Richterich R; Academic Press, New
York P. 245.

Yoshiaki, Y; Yoji, E. and Takashi, O.S; Karuya, M; Masayoshi, W. and Hiroshi, E.
(1989).
Chem. Pharm. Bull; 37 : 106.

Zurkowski, P. (1964).
Clin. Chem; 10 : 451.

Synopsis

INTRODUCTION

Diabetes mellitus is a common disease affecting a significant proportion of the population. It is a disorder of metabolism where insulin deficiency may be absolute or relative leading to a chronic state of hyperglycaemia with or without glycosuria.

According to the classification system devised by the National Diabetes Data Group, diabetes mellitus is divided into two main classes :-

- 1) Insulin-dependent diabetes mellitus (IDDM).
- 2) Non-insulin dependent diabetes mellitus (NIDDM).

Diabetes mellitus has been treated orally with herbal remedies based on folk medicine since ancient times. The discovery of insulin in 1921 followed by oral hypoglycemic agents such as sulphonylureas and biguanides has rationalised and simplified the treatment of diabetes¹. In spite of such uses control of hyperglycemia is not always satisfactory and moreover, prolonged uses of such synthetic drugs may frequently be associated with some undesirable effects like anorexia, nausea, vomiting, diarrhoea, etc.

The traditional system of medicine provides information that diabetes mellitus could be managed successfully with the use of some indigenous herbs. Sushruta prescribed a mixture of herbs including *Gymnema sylvestre* (Gurmar) which is still used in Asia as a treatment for diabetes mellitus². More than 400 plants are known to have been recommended and recent investigations have affirmed the potential value of some of these treatments³. Now, W.H.O. also appreciates the use of traditional drugs in different therapies⁴.

In last few years, hypoglycemic activity has been reported in a number of plants viz., *Allium cepa*⁵, *Momordica charantia*⁶, *Casseria esculenta*⁷, *Pterocarpus marsupium*⁸, *Triganella foenum-graecum*⁹. Nevertheless, no medicine capable of eliciting a radical cure for diabetes has yet been discovered. Doubts about the efficacy and safety of present day oral hypoglycemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes. Traditional herbal remedies which are naturally free from side effects are still in use by diabetic patients

may therefore, represent new avenues in the search for alternative hypoglycemic drugs. In this present investigation indigenous plants were studied for hypoglycemic activity.

AIMS AND OBJECTIVES

1. Preparation of plant extracts and screening for hypoglycemic activity.
2. Isolation of active principle from crude extract.
3. Hypoglycemic activity of active substance in experimental animals.
4. Subacute toxicity study of active substance.

MATERIALS AND METHODS

1. Preparation of Plant Extracts :

Selected plant materials were purchased from local market, cut into pieces and dried at room temperature. Then plant material was finely powdered and used for extraction.

Soxhlet extraction

Five hundred grams dry powder was extracted with methanol in the Soxhlet apparatus, till the last portion of extract was colourless. Total methanolic extract was concentrated on water-bath and thus the residue was methanol free.

Water extraction

After complete extraction of plant material with methanol, powder was again extracted with hot water (60-70°C). Water extract was concentrated under reduced pressure.

2. Estimation of blood sugar :

Blood samples (0.05 ml) were drawn from retro-orbital sinus and blood sugar level was estimated by O-Toluidine method¹⁰.

3. Hypoglycemic Activity of various Extracts in Experimental Animals :

Mice : Five normal healthy (Haffkine strained) mice weighing between 18-20 grams were used. Mice were fasted for 1 to 20 hours prior to the experiment. Fasting blood samples were drawn and then drug was administered orally (500 mg/kg body weight). Gum acacia (0.5%) or Tween-80 (0.5%) was fed to control group of mice.

Rats : Six healthy albino rats weighing 150-170 grams were used. Drug was administered orally (500 mg/kg and 100 mg/kg body weight) to overnight fasted rats, after withdrawal of fasting blood samples. Blood samples were collected after regular

intervals of 2 hours and 4 hours. Control groups of rats were also kept simultaneously.

4. Isolation of active principle :

Active extract was dissolved in distilled water and extracted with non-polar solvent such as petroleum ether or benzene in separating funnel, in order to separate out fatty or oily substances. Petroleum ether or benzene layer was taken out and solvent was recovered. Residue (Fraction-I) was tested for hypoglycemic activity.

Aqueous layer was re-extracted with butanol or ethyl acetate and then both the layers were separated out and concentrated on water bath and labelled as Fraction-2 and Fraction-3 respectively.

5. Hypoglycemic Effect of Active Extracts in Hyperglycemic Mice :

(A) Glucose induced hyperglycemia

Hyperglycemia was produced by giving a glucose load (1.5 gm/kg body weight) to overnight fasted mice. Drug was administered orally 3½ hours prior to glucose load. Blood samples were collected after ½, 1 and 2 hours. Hypoglycemic activity was compared with control group.

(B) Diabetes Induced by Alloxan

After collection of fasting blood samples from normal healthy mice, alloxan (50 mg/kg body weight) was injected intravenously. The blood samples were drawn after 24, 48, 72, 74 and 76 hours of control group. Drug was administered to experimental group (diabetic mice) after collection of 72 hrs blood samples. Thereafter, 2 and 4 hours (i.e. after 74 and 76 hours) blood was collected from the same group.

6. Sub-acute Toxicity Study of Active Extract :

Two groups consisted of 10 normal healthy albino rats (150-170 gms body weight) were taken for this study. Initial body weight, fasting blood sugar levels and food intake were recorded.

Drug was administered (100 mg/kg body weight) orally daily for 4 weeks to experimental group. Daily food intake of both the groups were observed and fasting blood sugar levels and body weights were recorded every week end. Physical abnormalities were also observed during experimental period. At the end of 4th week, rats were sacrificed to see if there were any abnormal biochemical changes and adverse effects in important organs.

7. Statistical Calculations :

The statistical data was analysed by using "Mann and Whitney U test"¹¹.

RESULTS AND DISCUSSION

Methanolic and water extracts of 12 indigenous medicinal plants were screened for hypoglycemic activity in normal fasting mice.

Methanolic extracts (500 mg/kg. body weight p.o.) of *Zea mays* (Maka styles), *Carum carvi* (Shahjira seeds), and *Guazuma tomentosa* (Nagarmotha tubers) have produced 28.66% ($p < 0.01$), 18.44% ($p < 0.01$) and 26.03% ($p < 0.01$) reductions in blood sugar in normal fasting mice at the end of 4 hours (Table 1).

Water extracts (500 mg/kg. body weight p.o.) of *Zea mays* (Maka styles), *Helecters isora* (Murud shenga fruit) and *Centella asiatica* (Brahmi leaves) showed 22.30% ($p < 0.01$), 31.26% ($p < 0.01$) and 24.68 ($p < 0.01$) reductions in blood sugar at the end of 4 hours (Table 2).

Hypoglycemic activity of methanolic extracts of Shahjira seeds, Maka styles and Nagarmotha tubers were also seen in normal fasting rats at the dose level of 100 mg/kg b.w. and 500 mg/kg b.w. (Table 3).

Isolated fractions from the methanolic extract of Shahjira seeds and Maka styles did not show considerable hypoglycemic effect than its crude methanolic extract.

Methanolic extract of Maka styles significantly inhibited hyperglycemia induced by oral glucose load (Table 4).

The potent and significant blood sugar lowering effect was shown by methanolic extract of Maka styles in alloxan-induced diabetic mice (Table 5).

In subacute toxicity study, methanolic extract of Maka styles did not show any mortality or toxic symptoms upto the period of 4 weeks. The drug did not affected the regular food intake and gain in body weight as compared with control group. The blood sugar lowering effect was gradually increased by every week end (Table 6).

**TABLE 1 : Hypoglycemic Effect of Methanolic Extracts of Plant Material
in Normal Fasting Mice (n=5)
(Dose of extract = 500 mg/Kg b.w.p.o.)**

Sr. No.	Group	Fasting blood Sugar in mg % at '0' hr.	Blood sugar in mg % after '2' hrs.	% Reduction in blood sugar after '2' hrs.	Blood sugar in mg% after '4' hrs.	% Reduction in blood sugar after '4' hrs.
1.	Control Treated (Nagarmotha tubers)	68.48 ± 6.62 70.81 ± 6.76	67.07 ± 6.25 57.90 ± 8.43	2.23 ± 2.09 17.31 ± 7.16 (p < 0.01)	66.45 ± 6.99 53.69 ± 6.39 (p < 0.02)	3.64 ± 3.20 26.03 ± 6.95 (p < 0.01)
2.	Control Treated (Maka styles)	70.34 ± 5.03 70.23 ± 6.85	69.25 ± 3.15 56.37 ± 5.03	1.87 ± 2.10 19.31 ± 8.43 (p < 0.01)	68.32 ± 2.98 49.91 ± 4.77 (p < 0.01)	3.16 ± 2.96 28.66 ± 6.54 (p < 0.01)
3.	Control Treated (Shahjira seeds)	72.02 ± 9.62 64.58 ± 4.22	69.37 ± 7.03 57.89 ± 5.54	3.77 ± 3.87 10.44 ± 4.54 (p < 0.01)	68.43 ± 8.27 52.74 ± 6.83 (p < 0.05)	5.47 ± 3.41 18.44 ± 7.28 (p < 0.01)
4.	Control Treated (Gokhru tubers)	70.77 ± 3.52 77.06 ± 10.08	69.99 ± 4.30 69.80 ± 8.91	1.54 ± 1.22 9.29 ± 5.39 (p < 0.05)	68.90 ± 5.39 68.72 ± 7.81 (N.S.)	3.34 ± 2.79 10.56 ± 5.50 (N.S.)

Key :- All values (Mean) ± S.D.; N.S. = Not significant i.e. p > 0.05

**TABLE 2 : Hypoglycemic Effect of Water Extracts of Plant Material
in Normal Fasting Mice (n=5)
(Dose = 500 mg/Kg b.w.p.o.)**

Sr. No.	Group	Fasting blood Sugar in mg % at '0' hr.	Blood sugar in mg % after '2' hrs.	% Reduction in blood sugar after '2' hrs.	Blood sugar in mg% after '4' hrs.	% Reduction in blood sugar after '4' hrs.
1.	Control Treated (Murudshenga fruits)	66.97 ± 4.43 68.83 ± 3.83	66.50 ± 4.04 57.89 ± 6.66	1.12 ± 1.29 12.25 ± 5.96 (p < 0.01)	66.19 ± 4.70 45.44 ± 6.90 (p < 0.01)	1.84 ± 1.72 31.26 ± 6.53 (p < 0.01)
2.	Control Treated (Maka styles)	64.99 ± 3.84 68.10 ± 2.85	64.68 ± 2.78 56.36 ± 5.18	1.37 ± 1.44 17.21 ± 6.84 (p < 0.01)	64.99 ± 2.94 52.96 ± 5.63 (p < 0.05)	1.85 ± 1.87 22.30 ± 6.26 (p < 0.01)
3.	Control Treated (Brahmi)	68.05 ± 4.59 69.64 ± 6.06	66.81 ± 5.29 56.21 ± 7.20	2.32 ± 1.95 19.40 ± 5.11 (p < 0.01)	66.50 ± 6.33 52.50 ± 6.20	3.25 ± 2.62 24.68 ± 4.05 (p < 0.01)

Key :- All values (Mean) ± S.D.

TABLE 3 : Hypoglycemic Effect of Plant Extracts in Normal Fasting Rats (n = 6)

Sr. No.	Name of the Plant and part used	Dose in mg/kg b.w.p.o.	Mean blood sugar in mg. % $\pm$ S.D.			% Reduction in blood sugar $\pm$ S.D.
			0 hr	After 2 hrs	After 4 hrs	
*	Control (Tween-80) (0.5%)	-	66.80 $\pm$ 2.66	61.06 $\pm$ 3.26	62.13 $\pm$ 3.45	7.56 $\pm$ 2.20
*	Control (Gum acacia) (0.5%)	-	73.34 $\pm$ 5.95	71.30 $\pm$ 5.48	70.36 $\pm$ 4.38	3.24 $\pm$ 1.59
1.	Methi seeds (M.E.)	500	71.06 $\pm$ 6.61	59.08 $\pm$ 4.09	57.66 $\pm$ 2.09 (p < 0.05)	17.61 $\pm$ 2.67
2.	Maka styles (M.E.)	500	67.60 $\pm$ 4.07	56.66 $\pm$ 3.29	52.04 $\pm$ 2.71 (p < 0.01)	22.42 $\pm$ 2.40
		100	68.25 $\pm$ 3.46	56.95 $\pm$ 2.37	54.19 $\pm$ 3.04 (p < 0.05)	20.58 $\pm$ 2.20
3.	Nagarmotha (tubers) (M.E.)	500	68.74 $\pm$ 5.71	63.62 $\pm$ 4.09	54.04 (p < 0.05)	21.35 $\pm$ 5.24
		100	67.67 $\pm$ 2.60	62.35 $\pm$ 1.78	61.45 $\pm$ 4.48 (N.S.)	9.23 $\pm$ 4.46

(Contd...)

Key : M.E. = Methanolic extract
N.S. = Not Significant

S.D. = Standard deviation

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Sr. No.	Name of the Plant and part used	Dose in mg/kg b.w.p.o.	Mean blood sugar in mg. % $\pm$ S.D.			% Reduction in blood sugar $\pm$ S.D.
			0 hr	After 2 hrs	After 4 hrs	
4.	Shahjira seeds (M.E.)	500	69.14 $\pm$ 2.17	59.54 $\pm$ 1.95	53.79 $\pm$ 3.54 (p < 0.01)	22.03 $\pm$ 6.86
		100	68.62 $\pm$ 2.52	59.73 $\pm$ 1.72	56.98 $\pm$ 2.07 (N.S.)	16.83 $\pm$ 1.26
5.	Tolbutamide (M.E.)	50	72.89 $\pm$ 3.99	47.99 $\pm$ 4.63	44.00 $\pm$ 4.68 (p < 0.01)	39.43 $\pm$ 7.64

Key : M.E. = Methanolic extract
N.S. = Not Significant
S.D. = Standard deviation

TABLE 4 : Effect of Plant Extracts on Hyperglycemia in orally Glucose Fed Mice (n = 6)

Sr. No.	Treatment	Mean blood sugar in mg. % $\pm$ S.D. after			
		0 hr.	1½ hr.	1 hr.	2 hrs.
1.	Control Group	72.30 $\pm$ 4.45	72.27 $\pm$ 3.30	70.81 $\pm$ 4.34	72.40 $\pm$ 9.55
2.	Glucose fed group (1.5 gm/kg.b.w.)	68.47 $\pm$ 5.55	194.94 $\pm$ 13.85 (p < 0.01)	147.85 $\pm$ 9.12	86.23 $\pm$ 8.82
3.	Glucose + Methanol extract of Shahjira seeds	68.57 $\pm$ 4.98	193.60 $\pm$ 11.75 (N.S.)	139.71 $\pm$ 11.17	82.49 $\pm$ 7.53
4.	Glucose + Methanol extract of Nagarmotha tubers	70.09 $\pm$ 4.21	171.49 $\pm$ 13.70 (p < 0.05)	122.64 $\pm$ 12.99	65.38 $\pm$ 4.54
5.	Glucose + Methanol extract of Maka styles	73.68 $\pm$ 7.09	157.08 $\pm$ 13.93 (p < 0.01)	129.24 $\pm$ 9.30	70.11 $\pm$ 6.06

Key : S.D. = Standard Deviation
N.S. = Not Significant (i.e. p > 0.05)

TABLE 5 : Effect of Oral Administration of Methanolic Extract of Maka Styles on elevated Blood Sugar in Alloxan-induced Diabetic Mice (n = 6)

Sr. No.	Treatment Control/ Drug	Mean blood sugar in mg. % $\pm$ S.D. after						% Reduction in blood sugar after 4 hrs.
		0 hr	24 hrs	48 hrs	72 hrs	74 hrs	76 hrs	
1.	Saline Control	73.64 $\pm$ 6.60	76.35 $\pm$ 6.93	73.91 $\pm$ 5.59	74.34 $\pm$ 4.37	72.02 $\pm$ 3.63	73.01 $\pm$ 4.02	3.60 $\pm$ 1.73
2.	Alloxan Control (50 mg/kg.b.w.)i.v.	75.59 $\pm$ 10.01	268.68 $\pm$ 8.87	309.05 $\pm$ 12.98	319.99 $\pm$ 16.95	314.47 $\pm$ 12.14	318.87 $\pm$ 14.06 (p < 0.01)	1.80 $\pm$ 1.41
3.	Alloxan + Extract (500 mg/kg. b.w.)p.o.	77.06 $\pm$ 6.75	272.79 $\pm$ 9.82	327.38 $\pm$ 19.99	311.18 $\pm$ 19.58	254.45 $\pm$ 20.46	185.22 $\pm$ 13.51 (p < 0.01)	40.49 $\pm$ 1.68 (p < 0.01)

TABLE 6 : Effect of Oral Administration of Drug (for 4 weeks) on Fasting Blood Sugar in Normal Rats (n = 6)

Sr. No.	Group	Initial	Mean blood sugar in mg. % $\pm$ S.D. after				% Reduction in blood sugar $\pm$ S.D. after 4 weeks
			1 week	2 weeks	3 weeks	4 weeks	
1.	Control Group	73.99 $\pm$ 5.06 (6.83)	74.58 $\pm$ 5.60 (7.50)	72.51 $\pm$ 4.20 (5.79)	72.55 $\pm$ 5.74 (7.91)	71.40 $\pm$ 5.85 (8.19)	5.06 $\pm$ 3.74
2.	Treated Group	73.05 $\pm$ 4.88 (6.88)	75.13 $\pm$ 2.82 (3.75)	66.02 $\pm$ 3.50 (5.30)	58.04 $\pm$ 2.94 (5.06)	56.55 $\pm$ 4.84 (8.55)	25.21 $\pm$ 9.74 (p < 0.01)

Key : S.D. = Standard Deviation

Figures in parentheses indicate coefficient of variation in %.

SUMMARY

Six plant extracts have shown promising hypoglycemic activity in normal fasting mice.

Methanolic extract of *Zea mays* styles has potent hypoglycemic effect in orally glucose fed as well as in alloxan-diabetic mice. No adverse effects were seen in sub-acute toxicity study.

REFERENCES

1. Asmal, C.A. and Marble, A. (1984).
Drugs, 28, 62-78.
2. Shanmugasundaram, K.R., Paneerselvam, C., Samudram, P. and
Shanmugasundaram, E.R.B. (1981).
Pharm. Res. Comm., 13, 475-486.
3. Bailey, C.J. and Day, C. (1978).
Diabetes Care, 12, 553-564.
4. World Health Organization (1978).
W.H.O. Tech. Report Ser. No. 622, 13-22.
5. Gupta, R.K., Gupta, S. and Samuel, K.C. (1977).
Ind. J. Exp. Biol., 15, 113-117.
6. Akhtar, M.S., Akhtar, M.A. and Yaqab (1981).
J. Med. Plant Res., 42, 205-211.
7. Gupta, S.S. (1967).
Ind. J. Med. Res., 55, 755-763.
8. Chakravarty, B.K., Gupta, S., Gambhir, S.S. and Gode, K.D. (1981).
Life Sciences, 29, 2043-2047.
9. Nadkarni, A.K. (1976).
"Indian Materia Medica", Vol. II, Popular Prakashan, Bombay, pp. 344-345.
10. Cooper, G.R. and McDaniel, V. (1973).
Crit. Rev. Lab. Sci., 4, 101-105.
11. Goldstein, A. (1964).
"Biostatistics - An Introductory Text", The Macmillan Company, New York,
pp.55-59.

