SECTION : 6

INTRODUCTION, DISCUSSION AND EXPERIMENTAL TO ANTIDIABETIC ACTIVITY OF SOME MEDICINAL PLANTS AND SOME AMINOACIDS
Ancient Indian medicine, though based on terminology, different from that of modern medicine, is based on strictly logical deductions, on scientific grounds and minute observations. Medical experience and knowledge gathered since ancient times have led to a high level of differentiation and a wide spectrum of therapeutical experience.

The history of Indian Medicine can be traced back to the remote past. Ayurveda (science of life), the Indian system of medicine exists from the time immemorial. The Charaka (1500 B.C.) has said it is as 'Saswata' (eternal), while the 'Sushruta' (1000 B.C.) has mentioned its origin even before the creation of universe. Both this statements only show the existence of this branch of knowledge as early as the man descended on earth. The term 'Ayurved' is derived from the two sanskrit words 'Ayuh' meaning 'life' and 'veda' meaning 'knowledge' or 'science'. Literally, therefore, it is strictly analogous to the English term Biology (Bios-life, logos-knowledge).

There are two objects of this science of life. The first object of Ayurveda is to maintain and promote the physical,
mental and spiritual health of the individual and community, and second object is to present diseases and to treat and cure them when it appears. During the course of treatment, it prescribes the diagnostic characteristics of various diseases and ailments and their treatment or cure through the usage of drugs. These drugs are obtained through natural sources viz. plants, animals or mineral sources. Most of these drugs are clinically used in the form of compounds, preparations in which form they act best because of their combined or synergistic effect.

Diabetes (Prameha) is described in Ayurveda under the twenty types 'Madhumeha' which is one of them, is generally equated with diabetes mellitus, in which condition passing sweet urine (urine containing sugar), frequent urination, thirst and weakness are the main symptoms. Some indigenous drugs in India command repute in curing the condition of Diabetes Mellitus, formally known as 'Madhumeha' adopting oral therapy in Ayurvedic methods of treatments. A number of Indian drugs are utilized even these days by the Ayurvedic physicians for curing this disorder and command a great reputation, being easily available, cheap and for their easy mode of administration.

Many medicinal plants, individually or in combination,
in form of different formulations like powder, paste, decoction, infusion extract, pill, etc. have been recommended in various medical treatise for the treatment of Diabetes. Conflicting reports on the hypoglycaemic activity of some of the plants may be attributed to a number of variables such as botanical identity of the drugs, time and place of collection of plant material, mode of administration of the drug and the type of experimental animal. Charaka prescribes the usage of 'Triphla' (composed of fruits of Terminalia belerica, Terminalia chebula and Emblica officinalis). Neem bark used in the treatment of diabetes shows positive results.

The drugs, which have consistently shown significant hypoglycaemic activity and have low toxicity need intensive screening. It is important to note that these drugs be investigated under the conditions similar to those followed by the practitioners of indigenous system of medicine. Undue emphasis should not, however, be laid on the isolation of pure hypoglycaemic principles from the crude drugs as they may have some adverse side effects.

Few more medicinal plants of Indian origin viz. Allium cepa Linn, Ficus bengalensis Linn, petrocarpus marsupium, Vinca rosa, etc. are used in the treatment of diabetes mellitus.
In the recent years the emphasis have been to identify as many plants as possible which could have effective control of diabetes. Pharmacological screening and clinical trials, as reported by subsequent and recent workers, reveal the presence of hypoglycemic activity in some medicinal plants.

It has been found that some Indian medicinal plants have been known to possess the hypoglycaemic activity. We have studied in detail the following eight medicinal plants.
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</tr>
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</tr>
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<td>Vernonia anthelmintica Willd</td>
<td>Fruit</td>
</tr>
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<td>6</td>
<td>Holarrhena antidysenterica Wall</td>
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<td>7</td>
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<td>8</td>
<td>Vitex negundo Linn</td>
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HISTORY OF DIABETES MELLITUS:

Diabetes was known to ancient Indians as early as sixth century B.C. Charaka in his 'Charaka Samhita' has mentioned the sweetness of urine in addition to polyuria. He collected material from much earlier works of Agnivesha, who based his writings on the teachings of Atreya, who lived in sixth century B.C. 199

Diabetes is a disease known since the time of ancient Greek and Arabic physicians. They investigated it thoroughly and have prescribed treatment. The name 'diabetes' which is derived from the Greek for siphon, was given to diseases by Aretasus of cappadocia (81-138 A.D.). The adjective 'mellitus', which comes from the Latin for honey and added by the Thomas wills in 1624. Thus, 'Diabetes mellitus' not with standing its mixed percentage succinctly describes the two main sights of this disease and distinguishes it from 'Diabetes insipidus', quite different disorder which is characterised by the excretion of large quantities of urine, though tasteless (insipid). Since Diabetes mellitus is much more common than 'Diabetes insipidus', the word diabetes standing alone is sometime used to described mellitus. The honeyed state of the urine in Diabetes mellitus is due to the presence of glucose, other prominent symptoms in this disease
are thirst and wasting. 200

An Arabic diabetes is known in the name of 'ziabetes', 'maber', 'dolah', etc. In sanskrit the disease is known as 'Madhuprameh'. In 17th century, the European physicians conducted research on this disease and enriched the existing knowledge about ailment and in the year 1922 A.D. discovered 'Insulin' a polypeptide hormone moiety, involved in the regulation of metabolism, especially carbohydrate metabolism. 201

Diabetes mellitus was known to the physicians in ancient India. From the beginning of history, Ayurveda gave a good clinical description about the 'Prameh' (anomalies of urinary disorder) including 'Madhumeha' (Diabetes mellitus). According to Palaeography, the ancient Ayurvedic masters like Charaka (3000 B.C. to 1500 B.C.), Sushruta (2200 B.C. to 600 B.C.), Vegabhatta (500 B.C. to 900 A.D.) and Madhava (700 A.D.) had given a detailed clinical description and the aetiology, diagnosis and treatment of twenty varieties of 'Prameha' as well as 'Pramehapideka' can inflammatory condition in diabetes. They have also suggested that treatment of 'Pramehapideka' by antiseptic, cleanings, healing procedures or surgery. According to the intensity of the diseases and nature of the patient they adopted
'Panchkarma therapy' and advised strict diet and physical exercises.

Prameha, described in these Ayurveda texts, literally means any disease mainly having tendency of excessive and turbid urination. Of these twenty types of 'Prameha', ten fall under 'kapha', six under 'pitta' and four under 'vata'. The astiopathology of these are more or less same. Madhumeha equated with Diabetes mellitus is described as being due to vitiated vatedosha, but even other types describing under 'kapha' and 'pitta' if neglected tend to be converted to madhumeha, while the inherited diabetes (Jataprameha) and lean diabetes (Krishapruumeha) are caused by the vitiated vata independently.

The other form of Diabetes mellitus is caused by the vata vitiated by the covering of 'pitta' and/or 'kapha'. Describing the aetiology of disease, Charaka says 'vata' by its 'ruksha guna' changes the ojas which is madhur (sweet) in nature, into one of the kashaya (astringent) and transports it to the urinary apparatus leading to the causations of the condition known as 'madhumeha'.

The main symptoms of the disease mentioned are excessive thirst, more intake of water, and its rapid discharge in the urine which contains sugar. This condition is also known as
'Glycosuria', which is a mild and transitory diabetes mellitus. The patients also experiences a feeling of hotness in the back, around the waist and in the right side of the body. When the liver becomes hot, the color of the urine turns reddish while the excessive heat in the kidneys render the urine watery. The excretion of sugar renders the body weak, the muscles are degenerated and become lean and thin, pulpable and the general health is run down.

Diabetes mellitus is on the increase all over the world. Recent organised study by the United States Public Health Service in Oxford, Mass, suggests that there are now in the united states over two million diabetics, and almost half of that number are unaware that they have ailment. Approximately 4,75,000 other persons living today are potential diabetics, and about 65,000 persons become diabetic every year.

**DIABETES MELLITUS:**

Insulin deficiency is a common and serious pathologic condition in man. In animals it can be reproduced by pancreatectomy or by the administration of alloxan, a compound which is relatively toxic to the liver and kidney but which in appropriate doses causes a selective destruction
of the beta cells of the pancreatic islets. The constellation of abnormalities caused by insulin deficiency is called diabetes mellitus. Greek and Roman physicians used the term 'Diabetes' to refer to conditions in which the cardinal finding was a large urine volume, and two types were distinguished. 'Diabetes mellitus', in which the urine tasted sweet, and 'diabetes insipidus', in which the urine was tasteless. Today the term diabetes insipidus is reserved for the condition produced by lesions of the supraoptic posterior pituitary system and the unmodified word diabetes is generally used as a synonym for diabetes mellitus.

Diabetes is characterised by polyuria, polydipsia weight loss in spite of polyphagia (increased appetite), hyperglycaemia, glycosuria completed cases of diabetes may give rise to ketosis, acidosis neuritis, carbuncles and coma. There are wide spread biochemical abnormalities, but the fundamental defects to which most of the abnormalities can be traced are:

(1) a reduced entry of glucose into various 'peripheral' tissues, and

(2) an increased liberation of glucose into the circulation from the liver (increased hepatic glycogenesis).
There is, therefore, an extra cellular glucose excess and an intracellular glucose deficiency, a situation which has been called 'starvation in the midst of plenty'.

**DIABETES INSIPIDUS.**

Diabetes insipidus is the syndrome that results when antidiuretic principle deficiency develops due to disease processes in the supraoptic nuclei, the hypothalamohypophyseal tract, or the posterior pituitary gland. It also develops after surgical removal of the posterior lobe of the pituitary, but in this situation it may be temporary because the remaining supraoptic fibres recover from the trauma and begin again to secrete antidiuretic principle. The symptoms are passage of large amounts of dilute urine (polyuria) and the drinking of large amounts of fluid (polydipsia), provided the thirst mechanism is intact. It is the polydipsia that keeps these patients healthy. If their sense of thirst is depressed for any reason and their intake of dilute fluids decreases, they develop dehydration that can be fatal.
INSULIN:

As early as 1890, Mebring and Minkowski showed that the pancreas played an important role in the control of carbohydrate metabolism. Since then there has been a continuous attempt at the preparation of an effective extract of gland to alleviate the symptoms of diabetes mellitus. Credit for the discovery of insulin is given to Banting and Best, who extracted the active principle from the pancreas and demonstrated its therapeutic effects in diabetic dogs and human subjects in the years 1921 and 1922.204

The chemistry of insulin has progressed from the preparation of active extracts to the preparation of insulin in crystalline form by Abel.205

The insulin is a polypeptide hormone produced by beta-cells of islet of langerhans in the pancreas, which is necessary for the proper metabolism of glucose. Its molecular weight is of about 6000. It is a relatively small protein molecule, consists of two peptide chains, the A chain comprising 21 and the B chain 30 aminoacids. The two chains are linked by two disulphide bridges between position 7 of both chains and position 20 & 19 of the A and B chain respectively. In addition an internal disulphide bridge links the two cysteine residues A6 and A11. Human insulin differs
from that of pig, dog, sperm, whale, Finwhale and rabbit, only with respect to the C-terminal aminoacid of the B-chain ($B_{30}$). No differences in aminoacid composition have yet been found between insulins obtained from different human subjects, either diabetic or normal. All insulins were considered to be similar with respect to their biologic potency.206

The appropriate daily insulin requirement in a non-diabetic whose pancreas is removed is 40 units daily. It should not be concluded from this that normal pancreas secretes about 40 units of insulin per day (1 milligram of pure insulin measures 24 units). Alpha cells, which produce the hyperglycaemic substance, glucagon, are removed together with the beta cells. It has been estimated that even 10 percent of functioning pancreatic tissue is enough to prevent diabetes.

Insulin regulates carbohydrate, fat and protein metabolism. Insulin allows:

(1) glucose penetration into the cell membrane;
(2) synthesis of fatty acids from carbohydrates; and
(3) aminoacid transport into the cell for the formation of tissue proteins.
In the absence of insulin:

(a) the blood sugar is raised and liver glycogen is depleted;

(b) serum lipids increases and ketone bodies accumulate in the blood;

(c) the liver converts aminoacids to glucose (gluconeogenesis) with increased nitrogen excretion. This reduces protein synthesis.\textsuperscript{207}
DISCUSSION

Diabetes (Madhumeha) has been well known as a wasting disease due to insulin deficiency in human beings of all parts of the world. The ancient Indian literature of the pre-christian era have distinctly recorded the most important symptoms of this disease such as thirst, excretion of sweet urine and loss in weight.

In recent years many of these have been investigated by various workers on scientific lines both under experimental and clinical conditions. An ideal antidiabetic agent tries to make normal all aspects of metabolic disturbances in the diabetic patient, but blood glucose level being of primary importance is used as an index for evaluating these agents. In renal glycouria, the glucose may appear in urine even when the blood may show no reducing substance in urine. Hence estimation of blood sugar is the correct index of assessing carbohydrate metabolism.

Many aminoacids have been shown to stimulate insulin secretion directly from the isolated perfused pancreas, pancreatic slices or isolated islets in vitro. Most require the presence of at least some glucose but other e.g. Leucine, do not. Of those tested, Arginine is apparently the most potent. Aminoacids need not be metabolized and even non-
metabolizable aminoacids stimulate insulin secretion. It does seem, however, that there is synergism between aminoacids, glucose and hormones.  

The first intimation that food-stuffs other than glucose might stimulate insulin secretion was the observation of Cochrane et al. that mixtures of aminoacids and particularly Leucine can precipitate acute hypoglycemic attacks in children with idiopathic hypoglycemia, but also in subjects with functioning islet cell adenoma, although its effect in normal subjects appeared to be very slight. However, it has been shown that Leucine does produce a slight fall in blood sugar even in normal subjects on effect which is greater in children than in adults and which can be significantly exaggerated by exercise and particularly by pretreatment with chlorporpamide. It was subsequently shown by Berger that protein feeding produces a rise in plasma insulin that is not attributed.

The Indian indigenous antidiabetic agents can be divided into two groups according to their source of origin, viz. vegetable or herbal mineral.

Many medicinal plant remedies, individually or in combination with different formulation such as leaf powder, pastes, decoctions and infusions, pills, etc. have been
recommended in various medical treatises. Indian Materia Medica has also recorded many during items not necessarily of tried value but collected from the folklore and traditional practices. No medicine capable of giving complete radical cure of diabetes has yet been discovered.

Some of the earlier workers mentioned the use of plants and mineral preparations for the treatment of the diabetes. Mukherji has given a detail account of about a dozen of the important indigenous plants with regard to their pharmacognosy, chemistry, pharmacology and therapeutic uses. Ajgaonkar also describes potential plants for similar uses. Aiman listed 35 plants which have been tested clinically for their curative properties in the last two decades. Chaudhary and Vohara have reviewed the work done on 21 plants for their antidiabetic activity. Karnick enumerates some aspects of 16 crude Indian drug plants, frequently used as a cure for diabetes in Ayurvedic system of medicine. Mukherji did work on 24 indigenous plants used in experimental and clinical diabetes in India is reviewed.

A Glucose Tolerance Test was made amongst the diabetic patients whose diagnosis was confirmed by sugar tolerance test and whose diabetes was not controlled by strict diabetic
regime alone was done by Vad. The Glucose Tolerance Test decreases the mg percentage blood sugar between second to third hours in normal and alloxan treated albino rats. Afterwards the blood sugar returns to the normal level.

Leucine depresses blood sugar in most individuals. It inhibits hepatic output of glucose and stimulates pancreatic insulin secretion. Di George et.al shown the blood glucose depressant action of Leucine in normal individual. Our analysis showed that Leucine was considered to be the best antidiabetic agent amongst the selected aminoacids. Amongst these eight selected medicinal plants Leucine is present in five medicinal plants. Maximum amount of Leucine is also present in Alhagi camelorum Fisch, which also showed the possibility of best antidiabetic activity in normal and alloxan albino rats. Even in glucose Tolerance Test the maximum decrease of blood sugar was found to be in Alhagi camelorum Fisch, in both normal and alloxan treated albino rats.

Saviano and Franciscis showed that when Cysteine was intravenously injected, the doses were well tolerated, but in pigeons death occurred almost immediately. At lower doses of Cysteine rats were protected form the diabetogenic effect of alloxan. In our experiment Cysteine was found to be the
second best antidiabetic agent on both normal and alloxan treated albino rats. In Glucose Tolerance Test also Cysteine was found to be the second best antidiabetic agent.

Glutamic acid also had antiketogenic or glucose former property. In Insulin Glutamic acid is present in maximum amount. Our experiment showed Glutamic acid has little significant decrease in blood sugar on both normal and alloxan treated albino rats. In Glucose Tolerance Test also there was little decrease in blood sugar.

Alanine cycle is of great importance in diabetes mellitus but pure Alanine feeding on albino rats did not show any significant decrease in blood sugar on both normal and alloxan treated albino rats. Arginine, Asparagine and Aspartic acid also did not show any significant antidiabetic activity.

The results of selected eight medicinal plants did not show best significant decrease in blood sugar level on both normal and alloxan treated albino rats. The Glucose Tolerance Test of all these medicinal plants also did not show good antidiabetic results. Amongst these medicinal plants Alhagi camelorum Fisch showed the maximum decrease of blood sugar level, while Piper nigrum Linn showed minimum decrease in blood sugar level. In Glucose Tolerance Test maximum activity
was found in Alhagi camelorum Fisch, while Vitex negundo Linn showed minimum decrease in blood sugar level.

The results in detail are recorded in table X, XI and XII.
mg of sugar per 100 ml of blood

AMINOACIDS
NORMAL

Leucine L-Cysteine Glutamic Acid Asparagine
Arginine Alanine Aspartic Acid

HOURS

0 1 2 3 4 5
mg of sugar per 100 ml of blood

HOURS

○ Leucine □ L-Cysteine × Glutamic Acid ◊ Asparagine
△ Arginine + Alanine △ Aspartic Acid
GLUCOSE TOLERANCE TEST
AMINOACIDS NORMAL

Leucine □ L-Cysteine X Glutamic Acid O Asparagine
a Arginine 4. Alanine 6 A spartic Acid

mg of sugar per 100 ml of blood

HOURS

○ Leucine □ L-Cysteine X Glutamic Acid ○ Asparagine
△ Arginine + Alanine ○ Aspartic Acid
GLUCOSE TOLERANCE TEST
AMINOACIDS ALLOKAN

- Leucine
- L-Cysteine
- Glutamic Acid
- Asparagine
- Arginine
- Alanine
- Aspartic Acid

mg of sugar per 100 ml of blood

HOURS: 0 1 2 3 4 5
MEDICINAL PLANTS
NORMAL

mg of sugar per 100 ml of blood

0 1 2 3 4 5
HOURS

○ Alhagi camelorum Fisch □ Harpagos racemosus ⨯ Vernonia anthelmintha ○ Hyper nigrum Linn
△ Erythrina religiosa Hook + Swertia chirata Ham △ Holarrhena antidysenterica Θ Vitex negundo Linn
Wall
MEDICINAL PLANTS
ALLOKAN

mg of sugar per 100 ml of blood

HOURS

○ Rhipogia camelorum Fisch  □ Reparagus racemosus  X Vernonia anthelmintica  ○ Piper nigrum Linn Wills

△ Erotaeva religiosa Hook  ＋ Swertia chirata Hom  △ Holarrhena antidysenterica  ○ V. negundo Linn Wills

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GLUCOSE TOLERANCE TEST
MEDICINAL PLANTS NORMAL

- *Hilhogi camelorum* Fisch
- *Asparagus rocemosus* X
- *Vernonia anthelmintico*
- *Periploca nigrum* Linn
- *A Crataeva religiosa* Hook
- *Swertia chirata* Ham
- *Holarrhena antidysenterica*
- *Vitex negundo* Linn

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**Mg of sugar per 100 ml of blood**

**Hours**
TABLE-X

BLOOD SUGAR IN mg/100 ml AT FIXED TIME INTERVAL OF DRUGS ON FASTING ALBINO RATS.
WEIGHT : 200 gms ± 2 gms.

<table>
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<th>No</th>
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<th>100 mg/kg</th>
<th>Initial</th>
<th>1 hr</th>
<th>2 hr</th>
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<td>56.2</td>
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<td>51.4</td>
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### TABLE XI

BLOOD SUGAR IN mg/100 ml BEFORE AND AFTER GLUCOSE FEEDING
WEIGHT : 200 gms ± 2 gms.

<table>
<thead>
<tr>
<th>No</th>
<th>Aminoacids 100 gm/kg</th>
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<th>1 hr Glucose</th>
<th>1 hr</th>
<th>1½ hr</th>
<th>2 hr</th>
<th>2½ hr</th>
<th>3 hr</th>
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<td>Cysteine</td>
<td>Normal</td>
<td>58.9</td>
<td>56.9</td>
<td>2gm/kg</td>
<td>62.9</td>
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<td>Glutamic acid</td>
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<td>2gm/kg</td>
<td>68.4</td>
<td>71.4</td>
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<td>2gm/kg</td>
<td>73.6</td>
<td>76.5</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>2gm/kg</td>
<td>65.0</td>
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</tr>
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<td></td>
<td></td>
<td>Alloxan</td>
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</tr>
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<td>Asparagine</td>
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<td>56.5</td>
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<td>64.5</td>
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<td>75.5</td>
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TABLE-XII

BLOOD SUGAR IN mg/100 ml AT FIXED TIME INTERVAL OF DRUGS
ON FASTING ALBINO RATS.
WEIGHT : 200 gms ± 2 gms.

<table>
<thead>
<tr>
<th>No</th>
<th>Medicinal plant sample</th>
<th>500 mg/kg</th>
<th>Initial</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>5 hr</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>Normal</td>
<td>50.2</td>
<td>50.0</td>
<td>49.7</td>
<td>49.0</td>
<td>47.7</td>
<td>48.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alloxan</td>
<td>50.9</td>
<td>54.9</td>
<td>59.7</td>
<td>59.5</td>
<td>58.8</td>
<td>58.8</td>
</tr>
<tr>
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<tr>
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<td>Alloxan</td>
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<td>51.2</td>
<td>50.0</td>
<td>49.5</td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alloxan</td>
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<td>59.7</td>
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<td>50.3</td>
<td>50.0</td>
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<td></td>
<td>Alloxan</td>
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<td></td>
<td>Wild</td>
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<td>60.5</td>
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<tr>
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<td>52.7</td>
<td>52.7</td>
<td>52.5</td>
<td>52.0</td>
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<tr>
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<td></td>
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<td>62.5</td>
<td>62.0</td>
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<tr>
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<td>Normal</td>
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<td>53.2</td>
<td>53.0</td>
<td>52.7</td>
<td>51.7</td>
<td>51.2</td>
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<tr>
<td></td>
<td></td>
<td>Alloxan</td>
<td>53.4</td>
<td>61.5</td>
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<td>62.9</td>
<td>62.7</td>
<td>62.2</td>
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TABLE XIII

<table>
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<tr>
<th>No</th>
<th>Medicinal Plant</th>
<th>Sample</th>
<th>Weight: 200 gms ± 2 gms</th>
<th>Initial</th>
<th>1 hr Glucose</th>
<th>2 hr Glucose</th>
<th>3 hr Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>Normal</td>
<td>-</td>
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<td>-</td>
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<td>59.4</td>
</tr>
<tr>
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<td>Normal</td>
<td>-</td>
<td>50.4</td>
<td>78.1</td>
<td>75.8</td>
<td>76.4</td>
</tr>
<tr>
<td>4</td>
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<td>71.4</td>
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<td>73.8</td>
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<td>73.2</td>
</tr>
<tr>
<td>8</td>
<td>Piper nigrum Linn</td>
<td>Normal</td>
<td>-</td>
<td>51.4</td>
<td>74.5</td>
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</tbody>
</table>

<table>
<thead>
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<th>No</th>
<th>Medicinal Plant</th>
<th>Sample</th>
<th>Weight: 200 gms ± 2 gms</th>
<th>Initial</th>
<th>1 hr Glucose</th>
<th>2 hr Glucose</th>
<th>3 hr Glucose</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>Alhagi camelo-rum Fisch</td>
<td>Alloxan</td>
<td>-</td>
<td>51.8</td>
<td>57.8</td>
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</tr>
<tr>
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<td>51.6</td>
<td>58.8</td>
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<tr>
<td>4</td>
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<td>Alloxan</td>
<td>-</td>
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<td>73.0</td>
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<td>Swertia chirita Ham</td>
<td>Alloxan</td>
<td>-</td>
<td>51.4</td>
<td>73.7</td>
<td>73.2</td>
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</tr>
<tr>
<td>6</td>
<td>Vernonia anthelmintica Willd</td>
<td>Alloxan</td>
<td>-</td>
<td>51.4</td>
<td>74.0</td>
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<tr>
<td>7</td>
<td>Holarrhena antidysenterica Wall</td>
<td>Alloxan</td>
<td>-</td>
<td>52.0</td>
<td>74.5</td>
<td>73.5</td>
<td>73.0</td>
</tr>
<tr>
<td>8</td>
<td>Piper nigrum Linn</td>
<td>Alloxan</td>
<td>-</td>
<td>51.4</td>
<td>75.0</td>
<td>74.5</td>
<td>74.0</td>
</tr>
</tbody>
</table>

**BLOOD SUGAR IN mg/100 ml BEFORE AND AFTER GLUCOSE FEEDING**

**WEIGHT:** 200 gms ± 2 gms.

**Initial** | **1 hr Glucose** | **2 hr Glucose** | **3 hr Glucose**
---|---|---|---
52.0 | 58.0 | 57.8 | 57.3
51.6 | 58.8 | 58.4 | 58.2
50.4 | 60.6 | 59.9 | 59.4
50.4 | 78.1 | 75.8 | 76.4
50.7 | 73.9 | 72.7 | 72.2
52.3 | 72.8 | 71.5 | 71.0
51.4 | 72.3 | 71.8 | 71.4
52.0 | 73.8 | 73.5 | 73.1
51.4 | 74.5 | 73.5 | 73.0
Healthy, adult, albino rats of nearly 200 ± 2 gm in weight of either sex were used for the experiments. The animals were housed in an air conditioned animal house at a temperature of 26 ± 2°C.

Hypoglycaemic effect was carried out on normal albino rats of either sex weighing nearly 200 ± 2 gm. The albino rats were fasted over a period of 15 hours. Blood sample were collected from each rats from the tail vein for glucose estimation. The albino rats were divided into two groups. Normal and Treated, each group consisting of two animals.

The details about the procedure for producing diabetes in albino rats, estimation of blood sugar levels, Glucose Tolerance Test is as under.

**METHOD TO PRODUCE DIABETES IN ALBINO RATS:**

Alloxan causes chemical pancreatomy by selective necrosis in Beta cells in the islets of langerhans, thus affecting the secretion of insulin and causes permanent hyperglycaemia. Diabetes in albino rats was produced under
the following steps:

(a) Albino rats were kept on fasting for 15 hours before the experiment.

(b) Alloxan monohydrate 100 mg/kg of body weight, was injected intravenously as 2% aqueous solutions.

(c) After one hour of alloxan injection, glucose was given orally in a dose of 2.0 gm/kg of body weight.

EFFECT OF AMINOACIDS AND DRY MEDICINAL PLANTS ON ALBINO RATS FOR HYPOGLYCAEMIC EFFECT:

The work was carried out on albino rats weighing 200 ± 2 gms. The albino rats were fasted over a period of 15 hours. Blood samples were collected from tail vein for sugar estimation which was done by Folin and Wu method. Diabetes was produced in albino rats by the intravenous injection of alloxan (100 mg/kg) as 2% aqueous solution. The experiment was carried out in two groups normal and treated. The aminoacids (100 mg/kg) and medicinal plants (500 mg/kg) were given orally after a period of fasting. In the actual experiment the blood samples were collected initially and then at an interval of 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, after the drug sample has been administered.
EFFECT OF AMINOACIDS AND DRY MEDICINAL PLANTS ON ALBINO RATS
FOR GLUCOSE TOLERANCE TEST.

Albino rats were kept for fasting for a period of 15 hours. The blood samples were collected initially and estimated. In the test experiments aminoacids (100 mg/kg) and dry medicinal plant sample (500 mg/kg) were given orally to the albino rats. After one hour all the animals that is normal and treated were given glucose (2 gm/kg) orally. The time interval of one hour were given and then blood samples for sugar estimation were collected at half an hour intervals for a period of 2.5 hours.

METHOD FOR ESTIMATING BLOOD SUGAR218

Period of fasting and exercise influence on the blood sugar level considerably. Thus, these factors should, therefore be kept constant throughout the study and in all the Normal and Treated groups.

Protein free filtrate is heated with alkaline copper sulphate in a special tube (Folin's sugar tube) to prevent reoxidation. Alkaline copper sulphate is reduced by glucose to form cuprous oxide. It is treated with phosphomolybic acid. The blue colour is developed due to the reduction of
the phosphomolybic acid. It is compared with a similarly prepared standard.

The 0.5 ml of blood was collected from the albino rats tail vein, and transferred to vials containing a mixture of sodium fluoride and potassium oxalate. 0.1 ml of oxalated blood was taken in centrifuge tube. To it 3.5 ml distilled water was added. Then 0.2 ml of 10% sodium tungstate and 0.2 ml of (2/3)N sulphuric acid was added immediately, the content was allowed to stand for few minutes and centrifuged. 2 ml of the appropriate standard were taken into Folin tubes by means of pipette. To each of the Folin tubes 2 ml of the alkaline copper sulphate solution was added and placed in a boiling water bath for 8 to 10 minutes. The bulk was transferred to a beaker of cold water in one to two minutes. The phosphomolybic acid reagent (2 ml) was added. The content was mixed thoroughly allowed to stand for two minutes and diluted to 12.5 ml. The colours were compared in the photoelectric colorimeter (systronics spectrophotometer 105 MK1). The blank was carried with 2 ml of water and 2 ml of alkaline copper sulphate solution. The absorbance of solution was read on a photoelectric colorimeter at 620 μm.
Calculation:

\[ Cu = \frac{Au \times Cs}{As} \]

Cu = Concentration of unknown sample.
Cs = Concentration of standard sample.
Au = Absorbance of unknown sample.
As = Absorbance of standard sample.

The standard solution contains 0.1 mg of glucose per 2 ml of solution.

Therefore, in 100 ml of blood, the blood sugar level is

\[ Cu = \frac{Au}{As} \times 0.1 \times \frac{100}{0.5} \]

\[ = \frac{Au}{As} \times 200 \]
CONCLUSION TO THE PROJECT

In the present project, medicinal plants were investigated for different constituents and stress was laid on the relation of these plants to different types of fevers and diabetes mellitus.

The dry sample of the selected parts of these medicinal plants were used for the moisture, fat, ash, carbohydrate, protein and alkaloid content. From the Proximate Chemical analysis it can be concluded that the medicinal plants contained about 5 to 7% of moisture. Holarrhena antidysentrica Wall contained maximum and Swertio chirata Ham contained minimum amount of moisture. Piper nigrum Linn contained maximum amount and Asparagus racemosus Willd contained minimum amount of fat. Alhagi camelorum Fisch contained maximum amount and Asparagus racemosus Willd contained minimum amount of Ash. Vitex negundo Linn contained maximum amount and Vernonia anthelmintica Willd contained minimum amount of carbohydrate. Holarrhena antidysentrica Wall contained maximum amount and Asparagus racemosus Willd contained minimum amount of protein. Alkaloids were determined in Holarrhena antidysentrica Wall, Piper nigrum Linn and Vitex negundo Linn.
Studies on vitamin-C, Iron, Calcium and Copper led to the conclusion that medicinal plants were rich in vitamins and minerals. Holarrhena antidysenterica Wall contained highest amount of vitamin-A and vitamin-C. Swertia chirata Ham contained highest amount of Iron while Vernonia anthelmintica Willd contained least. Asparagus racemosus Willd contained highest amount of calcium and Holarrhena antidysenterica Wall contained least. Asparagus racemosus Willd contained highest amount of copper.

The determination of aminoacid profile by paper chromatography revealed the presence of 11 to 14 aminoacids in the medicinal plants. Histidine, Tryptophan, Valine, Glutamine and Hydroxyproline were absent in all medicinal plants. Maximum amount of essential aminoacids were present in Piper nigrum Linn. Holarrhena antidysenterica Wall contained highest percentage of aminoacids. Antidiabetic active agent Leucine was present in maximum amount in Alhagi camelorum Fisch. Asparagine, Aspartic acid, Cysteine, Glutamic acid and Proline were present in all medicinal plants.

The determination of sugars was carried out with little modification in paper chromatography method used in aminoacids. The presence of 6 to 8 sugars were revealed by
paper chromatography. Vitex negundo Linn contained highest amount of sugar while Piper nigrum Linn contained least. Glucose was present in maximum amount in Vitex negundo Linn.

From the results of antibacterial and antifungal activity tests it was concluded that Vitex negundo Linn shown maximum zone of inhibition in all micro-organisms Swertia chirata Ham shown minimum zone of inhibition in all fungi organisms. Alhagi camelorum Fisch shown minimum in all Gram-positive organisms and Gram-negative organisms.

The aminoacids and medicinal plant samples showed more or less antidiabetic activity by decreasing blood sugar. From the results of analysis it can be concluded that among aminoacids Leucine; and among eight selected medicinal plants Alhagi camelorum Fisch produced maximum decrease in mg percent blood sugar in the normal, alloxan treated and Glucose Tolerance Test on albino rats.

Finally it can be concluded that among eight selected medicinal plants Vitex negundo Linn is best antibacterial and antifungal agent. Leucine was best antidiabetic agent among seven selected aminoacids. Alhagi camelorum Fisch contained maximum amount of Leucine, thus it was concluded that Alhagi camelorum Fisch should be considered to be the best antidiabetic agent which is also confirmed by the results on
albino rats. Glucose was present in maximum amount in Vitex negundo Linn thus it should be least active as antidiabetic agent, which is also confirmed by the results on albino rats.

All other medicinal plants showed mixed activity on antibacterial, antifungal and antidiabetic properties.