Formulation and Evaluation of Herbal Anti-diabetic Formulation Containing *Eugenia jambolana*, *Gymnema sylvestre*, *Tinospora cordifolia*, *Pterocarpus marscium*, *Terminalia bellerica* & *Emblica officinalis*

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Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population). It is caused by the deficiency or ineffective production of insulin by pancreas which result in increase or decrease in concentration of glucose in the blood. Due to various drawbacks of synthetic antidiabetic drugs there is a continuous search for alternative therapy in diabetes. Many herbal plants with hypoglycemic properties are known for a long time and they are used traditionally in India. Present study was conducted to formulate a suitable dosage form and used traditionally such as *Eugenia jambolana*, *Gymnema sylvestre*, *Tinospora cordifolia*, *Pterocarpus marscium*, *Terminalia bellerica* & *Emblica officinalis* are most of the effective and the most commonly studied Indian plants in relation to diabetes.

**Keyword:** Diabetes Mellitus, Hypoglycaemic, Herbal Medicines, Allopathic Drugs, Ayurveda.

1. Introduction
The word diabetes was coined by the Greek physician Aretaeus in the first century AD. Diabetes mellitus has been known since ages and sweetness of urine has been mentioned in Ayurveda by Sushruta. Its pharmacotherapy is 80 year old. The presence of sugar in the urine of diabetics was demonstrated by Dobson in 1755[1].

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at cellular level. It is the most common endocrine disorder, affecting 100 million individuals worldwide[2].

Insulin is polypeptide hormone produced by the β-cells of islets of Langerhans of pancreas and is main key for metabolism of carbohydrate, fats and proteins. It is anabolic hormone and promotes synthesis of glycogen, tricylglycerols and proteins. Human insulin has molecular weight 5734 and contains 51 amino acid in two polypeptides chains. The chain A has 21 amino acids and chain B has 30 amino acids. These chains held together by two inter chain disulfide bridges[3]. Acute complications include diabetic ketoacidosis, nonketotic hyperosmolar coma, and diabetic coma. In case of chronic complication, chronic elevation of blood glucose level leads to damage to blood vessels. In diabetes, the resultant problems are grouped under "micro vascular disease" (due to damage to small blood vessels) and "macro vascular disease" (due to damage to the arteries)[4].

There are different approaches to the treatment of diabetes, like insulin treatment in type 1 diabetes:
Sulphonylureas, which release insulin from pancreas by blocking the ATP-sensitive potassium channels\(^5\): Biguanides which decrease the insulin resistance; Thiazolidinediones, which increase the insulin sensitivity; alpha-glucosidase inhibitors like acarbose which decrease glucose absorption from intestine, thereby decreasing postprandial hyperglycemia; meglitinides like rapaglinide which are insulin secretogoues. Traditional herbal mineral plays an important part in the treatment of diabetes. If research was able to even identify some 5-6 herbal drugs that can reduce dose of insulin by increasing resistance, reducing insulin resistance, then it is possibly contributes in the treatment of diabetes. Herbal medicines are often used as therapeutic remedies in combination with allopathic drugs\(^6\).

1.1 Etiology of Diabetes, Cure and Strategy
Diabetic patients are diagnosed by blood or urinary glucose measurement through different techniques. On the basis of etiology DM (Diabetes mellitus) are categories mainly two types viz:

1. Primary Diabetes (Type I or Insulin Dependent Diabetes Mellitus).
2. Secondary Diabetes (Type II or Non insulin Dependent Diabetes Mellitus).

Primary DM (Diabetes mellitus) clinically dependent on insulin due to there is decrease in the number of \(\beta\)-cells in the islets of Langerhans and thus there is absolute deficiency of insulin hence this is known as Insulin Dependent Diabetes Mellitus (IDDM) or Type I. The main treatment for this Type I of DM (Diabetes mellitus) is insulin.

Secondary DM (Diabetes mellitus) is referred as Type II or Non insulin Dependent Diabetes Mellitus (NIDDM) because these types of patients are insulin resistant as well as loss of insulin secretion contributes to the onset of disease. The patients are usually obese and the treatment is usually dietary, through supplementary oral hypoglycemic drugs\(^7\).

1.2 Strategy for Treatment of Diabetes
Basic therapeutic approach to treat diabetes may be to inhibit the absorption of glucose by retarding the action of gastrointestinal enzymes such as \(\alpha\)-glucosidase and \(\alpha\)-amylase. Because the complication of disease is mainly due to the higher glucose level in blood which dysfunction the other organs of body. Thus we can say that the effective \(\alpha\)-glucosidase inhibitors may serves as chemotherapeutic agents for clinic use in the treatment of diabetes and obesity\(^8\).

1.3 Medicine for Treatment of Diabetes Mellitus
1.3.1. Insulin
Insulin increases glucose uptake in cells by stimulating the translocation of the glucose transporter GLUT4 from intracellular sites to the cell surface\(^9\). Insulin circulates in blood as the free monomer and its half life in plasma is about 5 - 6 min in normal subjects. Although glucose is the principal stimulus to insulin secretion in human beings, this process is tightly regulated by the coordinated of nutrients, gastrointestinal and pancreatic hormones and autonomic neurotransmitters\(^10\). The main drawback of insulin is taken through injection.

1.3.2. Oral Hypoglycemic Drugs
Oral Hypoglycemic drugs are those drugs that lower blood glucose level and taken orally. These drugs are synthetic and complex organic substances. Hence the search for oral active drugs is in demand.

1) Sulphonylureas Drugs
   i. First Generation Drugs
      a. Tolbutamide;
      b. Chlorpropamide;
   ii. Second Generation Drugs
      a) Glibenclamide;
      b) Glipizide;
      c) Gliclazide.

2) Biguanides
   a) Phenformin;
   b) Metformin.

3) Meglitinide / Phenyl alanine analogues
   a) Repaglinide
   b) Nateglinide
4) Thiazolidinediones
   a) Rosiglitazone
   b) Pioglitazone
5) alpha-glucosidase inhibitors
   a) Acarbose;
   b) Miglitol.

These drugs are effective in diabetes but having some limitations such as hypoglycemia occurs with regular use of sulfonylurea compounds but occurrences are much fewer than with insulin therapy. It is prescribed by doctors that biguanids should not use in patients with renal diseases. On the other hand the main side effect of Acarbose is flatulence.[11]

1.3.3. Herbal Drugs
There are many herbal products/herbal extracts are reported to treat the diabetes mellitus, we can classify these drugs according to their mode of action as:

1.3.3.1. Extracts/Drugs Act as α-Glucosidase or α-Amylase Inhibitor
These types of drugs/extracts are able to reduce the blood glucose level by inhibiting the gastric enzymes which is obligatory for the break the polysaccharides in to the simple sugar.

The aqueous and methanolic extract of Syzygium cumini (seed) and Pisidium guajava (leaves) shows α-amylase inhibition[12]; while Rhus verniciflora stem screened for α-glucosidase inhibition effect mixture of methanol and ethanol extract shows the potent inhibition of α-glucosidase enzyme[13]. There are large number of plants which have the capability to inhibit the α-glucosidase and α-amylase activity and may be used as treatment of diabetes Type I and Type II.

1.3.3.2. Extracts/Drugs Increases Insulin Secretion or β-Cell Regeneration
These types of drugs are directly concern with the Type I or IDDM diabetes which are disable to secreting the less or few amount of insulin.

Radix of Acorus calamus is used as in the therapy of diabetes in traditional folk medicine of America and Indonesia, this sensitizes the insulin activity of its ethyl acetate extract[14]. Moreover aqueous extract of Gymnema sylvestre leaves stimulate β-cell regeneration by proliferation of its precursor or cells in the pancreatic duct[15].

1.3.3.3. Extracts/Drugs Act as Hypoglycemic, Anti-hyperglycemic or Antidiabetic Effect
These classes of herbal drugs reduce the blood glucose level directly, this may be also used to treat the both type of diabetes mellitus (IDDM and NIDDM). Mangifera indica Linn. (Locally known as mango tree) has antidiabetic property, ethanolic and water extract of leaves and stem bark of Mangifera indica shows significant anti-hyperglycemic effect[16]. Tinospora cordifolia stem extract show antidiabetic and ameliorative activity[17].

1.3.3.4. Extracts/Drugs Dealing with the Complications of Diabetes Mellitus
Diabetes mellitus is metabolic syndrome characterized by deregulation in carbohydrate metabolism associated with defect in insulin secretion or action by which glucose level of blood increases, the different type of complication occurred. To treat these type of problem many herbal drugs/extract may play a key role.

The aqueous extract of fruit of Terminalia bellerica Roxb reduces oxidative stress in Type II diabetes mice model[18]. Oral administrations of Coccinia indica leaf extract (200 mg/kg body weight) for 45 days significantly reduce the thiobarbituric acid reactive substances and hydroperoxides. The extract also causes a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in liver and kidney of streptozotocin diabetic rats, which clearly shows the antioxidant property[19].

2. Materials and Methods
Dried herbal plants were collected from the Gaumukh Pharmaceuticals Sonepat, Haryana, India. Prior to the commencement of the experiment, all the drugs were placed to the new environmental condition for a period of one week to remove moisture from the herbal drug. During
the experimental period, the drugs were kept in a well ventilated laboratory at room temperature of 25°C.

2.1 Plant Materials and Excipients used in the formulation

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical name</th>
<th>Company name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Amla (<em>Emblica officinalis</em>)</td>
<td>Gaumukh Pharmaceuticals, Sonepath</td>
</tr>
<tr>
<td>2.</td>
<td>Gymnema (<em>Gymnema sylvestre</em>)</td>
<td>Gaumukh Pharmaceuticals, Sonepath</td>
</tr>
<tr>
<td>3.</td>
<td>Giloe (<em>Tinospora cordifolia</em>)</td>
<td>Gaumukh Pharmaceuticals, Sonepath</td>
</tr>
<tr>
<td>4.</td>
<td>Baheda (<em>Terminalia bellerica</em>)</td>
<td>Gaumukh Pharmaceuticals, Sonepath</td>
</tr>
<tr>
<td>5.</td>
<td>Jamun (<em>Eugenia jambolana</em>)</td>
<td>Gaumukh Pharmaceuticals, Sonepath</td>
</tr>
<tr>
<td>6.</td>
<td>Pterocarpus (<em>Pterocarpus marsupium</em>)</td>
<td>Gaumukh Pharmaceuticals, Sonepath</td>
</tr>
<tr>
<td>7.</td>
<td>Aspartame</td>
<td>CDH Laboratory Reagent</td>
</tr>
<tr>
<td>8.</td>
<td>Citric acid monoanhydrate</td>
<td>CDH Laboratory Reagent</td>
</tr>
<tr>
<td>9.</td>
<td>Sodium phosphate, dibasic</td>
<td>Fisher scientific</td>
</tr>
<tr>
<td>10.</td>
<td>Benzoic acid</td>
<td>Rankem Laboratory Reagent</td>
</tr>
<tr>
<td>11.</td>
<td>Sodium benzoate</td>
<td>Rankem Laboratory Reagent</td>
</tr>
<tr>
<td>12.</td>
<td>HPMC</td>
<td>CDH Laboratory Reagent</td>
</tr>
</tbody>
</table>

2.2 Experimental Formulation Design:
2.2.1. Preparation of Formulation

Formulation is a process to access the route for the preparation leads to come out a product named as formulation. In the formulation, the concentrations of the ingredients are well defined in the documentation to prevent the errors and weigh in the quantity as required or specified.

There are various steps involved in the formulation of syrup as follows:
1. Extraction of crude drug
2. Preparation of citrate-phosphate buffer pH 4.2
3. Preparation of syrup base
4. Preparation of final formulation.

1. Extraction of Crude Drugs by using Percolation method

Step 1. (Size Reduction):- The drug was subjected to suitable degree of size reduction, usually from coarse powder to fine powder, increased the surface area of drug, for uniform packing of the percolator, to slow down the movement of the menstruum.

Step 2. (Imbibation):- During imbibation the powdered drug was moistened with a suitable amount of menstruum and allowed standing for some hours in a closed container. During this period the drug swelled up and menstruum penetrated the cell walls. After the lapse of time, the moistened drug was passed through a coarse sieve to remove the lumps and mixed the dry powder, if any.

Step 3. (Packing):- After imbibation the moistened drug was evenly packed into a percolator. In a percolator, glass wool was placed on false bottom to support the column of the drug and help in the escape of the percolate. A small amount of moistened drug was introduced in the percolator and pressed lightly with glass rod to give even compression. Similarly, more of moistened drug was introduced and pressed till whole of the drug packed in the percolator. After suitable packing of the drug a piece of filter paper was placed over top of it on which small quantity of purified sand was placed to prevent disturbances of the packed material.

Step 4. (Maceration):- After packing the column, sufficient menstruum was added to saturate the material and top of the percolator covered with a lid. The tap fitted at the bottom of percolator was closed. More amount of menstruum was allowed to add at the top to maintain a layer of menstruum over the drug. The
percolator kept aside for 24 hours to macerate the drug.

Step 5. (Percolation):- After 24 hours maceration of the drug, the lower tap of the percolator was opened and liquid was collected in a container until 3/4th volume of the finished product is obtained. Marc was allowed to press for the complete removal of menstruum and placed in a closed container.

Drying of extract- The aqueous extract obtained from the process of extraction i.e. percolation was concentrated by allowing the solvent evaporation of the extract using water bath. After complete evaporation, dried drug extract was stored in a well closed container to prevent the growth of moulds and microorganisms[20].

2. Preparation of citrate-phosphate buffer pH 4.2:- Citrate phosphate buffer was prepared by mixing the freshly prepared standard solution of citric acid (0.1M) and dibasic sodium phosphate (0.2M) as specified in the table below as per pH required.
   (a) 0.1 M Citric acid; 19.21 g/l (M.W. 192.1)
   (b) 0.2 M Dibasic sodium phosphate; 35.6 g/l (dihydrate; M.W. 178.0)
Mix citric acid and sodium phosphate solutions in the proportions indicated and adjusted the final volume to 100 ml with deionized water. The final pH was adjusted using a sensitive pH meter[21]. Prepared citrate-phosphate buffer pH 4.2 was sterilized in autoclave and placed in well closed container until used.

3. Preparation of syrup base- Syrup base is a mixture of ingredients containing inert substances (other than the drugs having therapeutic action) i.e. sweeteners, preservatives, flavors, viscous agents, etc… Firstly, buffer of 40mL was freshly prepared and viscosity enhancer such as HPMC (5%w/v) was dispersed with vigorous shaking. This mixture solution of HPMC was allowed to sterile in autoclave.

4. Preparation of syrup- All the active ingredients, sweeteners and preservatives weighed as specified in the formulation table and allowed to dissolve in 10mL distilled water with vigorous shaking until the clear solution not prepared. Then, 40mL of buffer solution and 10mL of aqueous solution was mixed properly. The prepared solution was used to formulate and got sterile in the autoclave.

2.2.2 Formulations:
Three formulations were prepared according to their concentration specified in the table below and named as F1, F2 & F3.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Ingredients</th>
<th>Concentration (per 50mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>1.</td>
<td>Giloe</td>
<td>100mg</td>
</tr>
<tr>
<td>2.</td>
<td>Gymnema</td>
<td>100mg</td>
</tr>
<tr>
<td>3.</td>
<td>Jamun</td>
<td>75mg</td>
</tr>
<tr>
<td>4.</td>
<td>Pterocarpus</td>
<td>75mg</td>
</tr>
<tr>
<td>5.</td>
<td>Baheda</td>
<td>35mg</td>
</tr>
<tr>
<td>6.</td>
<td>Amla</td>
<td>40mg</td>
</tr>
<tr>
<td>7.</td>
<td>HPMC</td>
<td>2.5gm (5%)</td>
</tr>
<tr>
<td>8.</td>
<td>Sodium benzoate</td>
<td>100mg (0.2%)</td>
</tr>
<tr>
<td>9.</td>
<td>Benzoic acid</td>
<td>50mg (0.1%)</td>
</tr>
<tr>
<td>10.</td>
<td>Aspartame</td>
<td>250mg (0.5%)</td>
</tr>
<tr>
<td>11.</td>
<td>Syrup base</td>
<td>q.s. to 50mL</td>
</tr>
</tbody>
</table>
3. Result & Discussion
All formulations showed dark color due to plant extract. The pH of formulation (F1, F2 and F3) varies from 4.13–4.25 may be due to varied percentage of acidic or basic compounds present in the aqueous extract. The % sedimentation of each formulation (F1, F2, and F3) varies from 1-1.5% may be due to difference in the concentration of different aqueous extracts. Microbial test was conducted for all three formulations and results inferred that the formulations were sterile, No growth were seen thus passed the test. The viscosity of formulation (F1, F2 and F3) varies from 50.7-56.6.

<table>
<thead>
<tr>
<th>Formulation/Parameter</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Odour</td>
<td>pungent</td>
<td>pungent</td>
<td>pungent</td>
</tr>
<tr>
<td>pH</td>
<td>4.13</td>
<td>4.21</td>
<td>4.25</td>
</tr>
<tr>
<td>% Sedimentation</td>
<td>1%</td>
<td>1%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Microbial Test</td>
<td>pass</td>
<td>pass</td>
<td>pass</td>
</tr>
<tr>
<td>Viscosity</td>
<td>52.6 mPa</td>
<td>51.3mPa</td>
<td>50.7mPa</td>
</tr>
</tbody>
</table>

4. Stability study- The results inferred that the formulation F1 was failed in the sterility test while other formulations were remained unchanged at 40°C. Thus the effective concentration of preservative must be NLT 0.3%w/v.

5. Conclusion
The present worker concluded that the formulations containing multiple aqueous herbal extracts using aspartame as sweetener for diabetic patients was successfully prepared and found stable at 40°C. The formulations prepared are unique in it containing natural anti-oxidants for the oxidizable part of extracts. The drugs using in the formulation i.e. Emblica officinalis, Tinospora cordifolia, Gymnema sylvestre, Terminalia bellercia, Eugenia jambolana, Pterocarpus marscipum may exhibit anti-diabetic activity on alloxan induced diabetic rat as per given literature. There is increasing demand by patients to use the natural products with antidiabetic activity. In recent times there has been renewed interest in the plant remedied. Plants hold definite promises in the management of diabetes mellitus. Here the present worker creates a thrust for the future researchers to standardize the selected formulation for better optimization that can play a significant role in improving the hypoglycemic action.

6. Acknowledgement:
I would like to express my deep gratitude to Mr. Suresh Jain, Chancellor, TMU and Prof. R. K. Mittal, Vice-Chancellor for providing the required facility in campus, for their guidance, enthusiastic encouragement and useful critiques of this research work. I would also like to extend my thanks to the technicians of the laboratory of the Pharmaceutics department for their valuable help. Finally, I wish to thank my parents for their support and encouragement throughout my study.

7. References
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