International Journal of Research in Engineering, Science and Management
Volume-3, Issue-10, October-2020
journals.resaim.com/ijresm | ISSN (Online): 2581-5792 | RESAIM Publishing

Formulation of Novel Polyherbal Extract for Anti Diabetic Effect in Alloxan Induced Diabetic Rats

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***Abstract*: In present study, some plants are selected for these study in which Azadirachta Indica (Neem), Catharanthus Roseus (Vinca), and Acacia Nilotica (Babhul). Albino rats were treated with prepared polyherbal extracts at doses of 300 mg/kg, 500 mg/kg and 800 mg/kg, did not extensively lower normal blood glucose level as compared to normal control animals that is polyherbal extracts did not possess hypoglycaemic activity. Alloxan at the dose of 150 mg/kg could significantly elevate blood glucose level in all groups of animals as compared to normal control animals. 21 days treated albino rats with polyherbal extracts at doses of 300 mg/kg; 500mg/kg and 800 mg/kg significantly lower normal blood glucose level as compared to diabetic control group but not when compare with blood glucose level of group treated with standard drug. The result was bringing into being to be dose dependent and did not possess hypoglycaemic but possess antidiabetic activity.**

***Keywords*: Diabetes Mellitus (DM), Gastroparesis, PAD (peripheral arterial disease), HHNS (Hyperosmolar Hyperglycemic Nonketotic Syndrome), Azadirachta Indica (Leaves), Catharanthus Roseus (Leaves) and Acacia Nilotica (Leaves).**

1. Introduction

Diabetes mellitus is a varied metabolic disorder characterized by altered Carbohydrate, lipid and protein metabolism. The management of diabetes mellitus is considered a worldwide problem and successful treatment is yet to be discovered. The modern drugs, counting insulin and oral hypoglycaemic agents, control the blood sugar level as long as they are regularly administered and they also produce a number of unwanted effects. The treatment of diabetes mellitus has been attempt with different indigenous plants and polyherbal formulations. Predictable medicines all over the world have advocated the use of herbs to treat diabetes since time immemorial. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in frequent scientific journals. In the Ayurvedic system of medicine, as mentioned in ancient Indian books like Charak, Samhita, Mahdhav Nidan and Astang Sanghra, there are about 600 plants, which are stated to have antidiabetic property. Wide arrays of plant derived active principles representing numerous phytochemicals have demonstrated consistent hypoglycaemic activity and their possible use in the treatment of diabetes mellitus.

The meaning and origin of diabetes mellitus: Diabetes comes from Greek, and it means a “siphon”. Aretus the Cappadocian, a Greek physician during the second century A.D., named the Condition diabainein. He described patients who were passing too much water (polyuria) - like a siphon. The word became “diabetes” from the English adoption of the Medieval Latin diabetes. In 1675, Thomas Willis added mellitus to the term, although it is commonly referred to simply as diabetes. Mel in Latin means “honey”; the urine and blood of people with diabetes has excess glucose, and glucose is sweet like honey. Diabetes mellitus could literally mean “siphoning off sweet water”. In ancient China people observed that ants would be attracted to some people’s urine, because it was sweet. The term “Sweet Urine Disease” was coined.

A. Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic disorders categorized by hyperglycemia. It is related with abnormalities in carbohydrate, fat and protein metabolism and results in chronic complications including microvascular, macro vascular and neuropathic disorders. Several different types of Diabetes mellitus exist and are choices. Depending on the etiology of the Diabetes mellitus, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose consumption and increased glucose production. The metabolic deregulation associated with DM causes secondary pathophysiology changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.

B. Magnitude of the problem

According to current estimates, approximately 285 million people worldwide (6.6%) in the 20-79 years age group have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to be diabetic. The largest number will take place in the regions dominated by developing economies. International Diabetes confederation (IDF) estimates that, 50 million people suffer from diabetes in India.

C. Prevalence and Incidence

Several previous intelligence give details about the prevalence and incidence of diabetes worldwide. "Prevalence'' is the total number of people having diabetes in a population at a given time. While, "Incidence" means the number of new cases.

D. Types of Diabetes Mellitus

1) Type 1 diabetes

The body does not produce insulin. Some people may refer to this type as insulin-dependent diabetes or early-onset diabetes. Approximately 10% of all diabetes cases are type 1. Patients with type 1 diabetes will need to take insulin injections for the rest of their life. They must also ensure proper blood-glucose levels by carrying out regular blood tests & a special diet. It is known as juvenile onset diabetes because it is most commonly develops in people younger than age 20 years, although it persists throughout life IDDM appears to be an autoimmune disorder. One in which person's immune system destroys the pancreatic beta cell that occurs in genetically susceptible people. This form of diabetes is due the severe lack of insulin caused by destruction of B-Cell mass. Three interlocking mechanisms responsible for the islet cell destruction are genetic susceptibility, and environmental factors.

2) Type 2 diabetes

The body does not produce enough insulin for proper function, or the cells in the body do not react to insulin (insulin resistance). Approximately 90% of all cases of diabetes worldwide are type 2. Some people may be able to control their type 2 diabetes symptoms by losing weight, following a healthy diet, doing plenty of exercise, and monitoring their blood glucose levels. However, type 2 diabetes is typically a progressive disease - it regularly gets worse - and the patient will probably end up have to take insulin, usually in tablet form. Overweight and obese people have a much higher risk of developing type 2 diabetes compared to those with a healthy body weight. People with a lot of visceral fat, also known as central obesity, belly fat, or abdominal obesity, are especially at risk. Being overweight/obese causes the body to release chemicals that can destabilize the body's cardiovascular and metabolic systems. Being overweight, physically inactive and eating the wrong foods all contribute to our risk of developing type 2 diabetes. The scientists believe that the impact of sugary soft drinks on diabetes risk may be a direct one, rather than simply an influence on body weight. The risk of developing type 2 diabetes is also greater as we get older. Experts are not completely sure why, but say that as we age we tend to put on weight and become less actually active. Those with a close relative who had/ had type 2 diabetes, people of Middle Eastern, African, or South Asian descent also have a higher risk of developing the disease. Men whose testosterone levels are low have been found to have a higher risk of developing type 2 diabetes.

Gestational diabetes: This type affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce sufficient insulin to transport all of the glucose into their cells, resulting in increasingly rising levels of glucose. Diagnosis of gestational diabetes is made during pregnancy. The majority of gestational diabetes patients can control their diabetes with exercise and diet. Between 10 to 20 percent of them will need to take some kind of blood-glucose-controlling medications. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during child birth. [1]

E. What is prediabetes?

The vast majority of patients with type 2 diabetes initially had prediabetes. Their blood glucose levels where higher than normal, but not high enough to merit a diabetes diagnosis. The cells in the body are becoming resistant to insulin. Diabetes is a Metabolism Disorder Diabetes (diabetes mellitus) is classed as a metabolism disorder. Metabolism refers to the way our bodies use digested food for energy and growth. Most of what we eat is broken down into glucose. Glucose is a form of sugar in the blood - it is the principal source of fuel for our bodies. When our food is digested, the glucose makes its way into our bloodstream. Our cells use the glucose for energy and growth. Insulin is a hormone that is produced by the pancreas. After eating, the pancreas without human intervention releases an adequate quantity of insulin to move the glucose present in our blood into the cells, as soon as glucose enters the cells blood-glucose levels drop. A person with diabetes has a condition in which the amount of glucose in the blood is too elevated (hyperglycemia). This is because the body does not produce enough insulin, produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. So, even though the blood has plenty of glucose, the cells are not getting it for their essential energy and growth requirements. How to determine whether you have diabetes, prediabetes or neither Doctor can determine whether a patient has a normal metabolism, prediabetes or diabetes in one of three different ways (tests): [2]

- The A1C test
- At least 6.5% means diabetes
- Between 5.7% and 5.99% means prediabetes
- Less than 5.7% means normal
- The FPG (fasting plasma glucose) test

- At least 126 mg/dl means diabetes
- Between 100 mg/dl and 125.99 mg/dl means prediabetes
- Less than 100 mg/dl means normal
- An abnormal reading following the FPG means the patient has impaired fasting glucose (IFG)
- The OGTT (oral glucose tolerance test)
- At least 200 mg/dl means diabetes
- Between 140 and 199.9 mg/dl means prediabetes
- Less than 140 mg/dl means normal

F. Complications linked to badly controlled diabetes [3]

Below is a list of possible complications that can be caused by badly controlled diabetes:

- *Eye complications:* laucoma, cataracts, diabetic retinopathy, and some others.
- Foot complications - neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated.
- Skin complications - people with diabetes are more susceptible to skin infections and skin disorders.
- Heart problems - such as ischemic heart disease, when the blood supply to the heart muscle is diminished.
- Hypertension - common in people with diabetes, which can raise the risk of kidney disease, eye problems, heart attack and stroke.
- Mental health - uncontrolled diabetes raises the risk of suffering from depression, anxiety and some other mental disorders.
- Hearing loss - diabetes patients have a higher risk of developing hearing problems.
- Gum disease - there is a much higher prevalence of gum disease among diabetes patients.
- Gastroparesis - the muscles of the stomach stop working properly.
- Ketoacidosis - a combination of ketosis and acidosis; accumulation of ketones bodies and acidity in the blood.
- Neuropathy - diabetic neuropathy is a type of nerve damage which can lead to several different problems.
- HHNS (Hyperosmolar Hyperglycemic Nonketotic Syndrome) - blood glucose levels shoot up too high, and there are no ketones present in the blood or urine. It is an emergency condition. [4]
- Nephropathy - uncontrolled blood pressure can lead to kidney disease.
- PAD (peripheral arterial disease) - symptoms may include pain in the leg, tingling and sometimes problems walking properly
- Stroke - if blood pressure, cholesterol levels, and blood glucose levels are not controlled, the risk of stroke increases significantly
- Erectile dysfunction - Male impotence.
- Infections - People with badly controlled diabetes are much more susceptible to infections.
- Healing of wounds - Cuts and lesions take much longer to heal.

G. Symptoms of Diabetes [5]

People can often have diabetes and be completely unaware. The main reason for this is that the symptoms, when seen on their own, seem harmless. However, the earlier diabetes is diagnosed the greater the chances are that serious complications, which can result from having diabetes, can be avoided. Here is a list of the most common diabetes symptoms:

- Frequent urination
- Disproportionate thirst
- Intense hunger, Weight gain
- Unusual weight loss
- Increased fatigue, Irritability, Blurred vision
- Cuts and bruises don't heal properly or quickly
- More skin and/or yeast infections
- Itchy skin, Gums are red and/or swollen - Gums pull away from teeth
- Frequent gum disease/infection
- Sexual dysfunction among men
- Numbness or tingling, especially in your feet and Hands

H. Diagnosis of Diabetes [6]

Diabetes can often be detected by carrying out a urine test, which finds out whether excess glucose is present. This is normally backed up by a blood test, which measures blood glucose levels and can confirm if the cause of your symptoms is diabetes. If you are worried that you may have some of the above symptoms, you are recommended to talk to your Doctor or a qualified health professional. [9]

2. Plan of Work

The present study "Anti-diabetic effect of novel polyherbal formulation in Alloxan induced diabetic rat" was commenced. Considering the aim and objectives of present study the sequential plan of work is design as follows:

- Literature Survey from Books, National and International Journals.
- Procurement of Marketed Oral Hypoglycaemic Agents.
- Procurement of Herbal Drugs & Authentication f Herbal Drugs.
- Preparation of Polyherbal Extracts.
- Procurement of Animals (Wistar Rats).
- Acute Toxicity Testing of Novel Polyherbal Extract.
- Evaluation of Oral Glucose Tolerance Test of Novel Polyherbal Formulation

A. In Wistar Rats [7]

Induction of diabetes in rat carried out by method namely,

Alloxan Induced Diabetes:

Evaluation of Anti-Diabetic Effect of Novel Polyherbal Extract in Wistar Rats.

Statistical Analysis:

3. Plant Profile

A. *Azadirachta Indica (Neem) [8]*

Kingdom	Plantae
Super Division	Angiosperms
Division	Eudicots
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*Azadirachtaindica*

Fig. 1. Leaves of *Azadirachta indica*

Description:

Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 metres (115–131 ft). It is evergreen, but in severe deficiency it may shed most or nearly all of its leaves. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 20–25 metres (66–82 ft). The opposite, pinnate leaves are 20–40 centimetres (7.9–15.7 in) long, with 20 to 30 medium to dark green leaflets about 3–8 centimetres (1.2–3.1 in) long. The terminal leaflet often is missing. The petioles are short. [9]

Traditional medicinal use:

Products made from neem trees have been used in India for over two millennia for their medicinal properties. Neem products are believed by Siddha and Ayurvedic practitioners to be:

- Antibacterial, Sedative, Antiviral, Contraceptive,
- Antidiabetic, Anthelmintic, Antifungal.
- It is considered a major constituent in Siddha, Ayurvedic and Unani medicine and is mainly prescribed for skin diseases.
- Neem oil is also used for in good physical shape hair, to improve liver function.
- Neem leaves have been used to treat skin diseases like eczema, psoriasis, etc.

B. *Catharanthus Roseus (Vinca) [10]*

Kingdom	Plantae
Super Division	Angiosperms
Super-division	Angiosperms
Division	Eudicots
Order	Gentianales
Family	Apocynaceae
Genus	*Catharanthus*
Species	*CatharanthusRoseus*

Fig. 2. Leaves of *Catharanthus Roseus*

Description:

Vinca plants are sub shrubs or herbaceous, and have slender trailing stems 1–2 m (3.3–6.6 ft) long but not growing more than 20–70 cm (8–27.5 in) above ground; the stems frequently take root where they touch the ground, enabling the plant to spread widely. The leaves are opposite, simple broad laneolate to ovate, 1–9 cm (0.5–3.5 in) long and 0.5–6 cm (0.20–2.36 in) broad; they are evergreen in four species, but deciduous in the herbaceous V. herbacea, which dies back to the root system in winter. [11]

The flowers, produced through most of the growing season, are salver form (like those of Phlox), simple, 2.5–7 cm (0.98–2.76 in) broad, with five usually violet (occasionally white) petals joined together at the base to form a tube.

Pharmacological Activities:

Anti-cancer activity, Anti-diabetic activity, Antimicrobial activity, Anti-oxidant property, Anti-helminthic activity, Anti-ulcer property, Hypotensive Property.

C. *Acacia Nilotica (Babhul) [12]*

Kingdom	Plantae
Super Division	Angiosperms
Division	Eudicots
Order	Fabales
Family	*Fabaceae*
Genus	*Acacia*
Species	*Acacia nilotica*

Fig. 3. Leaves of *Acacia Nilotica*

Description:

Acacia nilotica (L.) Del. syn. *Acacia arabica* (Lam.) Wild. (*Mimosaceae*) is an imperative multipurpose plant. A. nilotica is a plant 5 to 20 m high with a thick spherical crown, stems and branches usually sinister to black colored, grey-pinkish slash, fissured bark, exuding a reddish low quality gum. The plant has straight, light, thin, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees

commonly without thorns. The leaves are bipinnate, with 3 to 6 pairs of pinnulae and 10 to 30 pairs of leaflets each, rachis with a gland at the bottom of the last pair of pinnulae. Flowers in globulous heads 1.2 to 1.5 cm in diameter of a bright golden-yellow colour set up either axillary or whorly on peduncles 2 to 3 cm long located at the end of the branches.

Pharmacological Activities:

- Anti-diabetic activities, Anti-bacterial and antifungal activities, Antioxidant activity.
- Anti-hypertensive and anti-spasmodic activities etc.

4. Materials and Methods

A. Collection and Authentication of Herbal Plants

The leaves of *Azadirachta Indica, Catharanthus Roseus and Acacia Nilotica* were collected from local area and the collected leaves were duly identified and authenticated from renowned botanist by Head of Department of Botany, Shivaji Sr. College, and Chikhli.

B. Preparation and Extraction of Polyherbal Formulation

Polyherbal formulation "*Azadirachta Indica* (Leaves), *Catharanthus Roseus* (Leaves) *and Acacia Nilotica* (Leaves)" were air dried for 72 hours. The sample was ground and stored in polythene container for analysis. Dried materials were coarsely ground and powdered independently before extraction. Three materials in equal ratio (w/w) were extracted by Soxhlet method using distilled water for 24 hour at room temperature. Extracts were filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator until a crude solid extracts were obtained which were then freeze-dried for complete solvent removal. [13]

C. Reagents and Drugs

Alloxan, Tab. Glibenclamide (5 mg in each tablet).

D. Instruments

Blood glucose levels were measured with the help of Glucometer, Animal weighing balance and Soxhlet Apparatus (ACP, Chikhli).

E. Procurement of Experimental Animals

Total Rats weighing 150-200 gm were procured from Animal House of Anuradha College of Pharmacy Chikhli, Dist-Bandana and were used in this study. All animals were housed in a polypropylene cage containing sterile paddy husk as bedding throughout the experiment and maintained under the controlled conditions of temperature (23± 2 ºC) and 12 hr light/dark cycle with food and water. The animal care and handling was done according to the guidelines set by the CPCSEA (F. No.25/75/2014).

F. Acclimatization and Group Allocation

All animals were acclimatized for minimum 5 days. During acclimatization animals were randomized by zigzag method and total 36 out of 46 animals were selected and allocated in different group containing 6 animals in each group for study. The animals were marked on the tail to permit individual identification, and kept in their cages for 5 days prior to closing to allow for acclimatization of the laboratory condition. [14]

G. Acute toxicity study

Extracts of polyherbal formulations were tested for their acute and short- term toxicity in Albino Wistar rats. For determining acute toxicity of a single oral administration of herbal drug, the OECD (Organization for Economic Co-operation and Development) guidelines (OECD 2001, 423, Annex 2c) were followed. Stepwise doses of extracts from 300 mg/kg b.w. up to the dose 2000 mg/kg b.w. were administered orally. Animals were kept under observation continuously for the initial 4 h and intermittently for next 6, 24, and 48 h following drug administration. Parameters like grooming, sedation, and hyperactivity, loss of righting reflex, respiratory rate and convulsion were not observed. No considerable signs of toxicity were observed in tested animals. On the basis of above study, following doses 300, 500 and 800 mg/kg were selected for present aim.

H. Methods for Evaluating Ant-diabetic Activity [15]

1) Oral Glucose Tolerance Test

Albino male rats weighing about 150-200 gm were divided into four groups containing six animals in each group. All animals were kept for overnight fasting with free access to water. Group I was kept as vehicle control which received normal saline and group II received glucose only, group III received polyherbal extract 300 mg/kg, and group IV received polyherbal extract 800 mg/kg only in a vehicle, respectively, 30 minutes after drug administration. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration, and 30, 60, 90, 120 minutes after loading glucose. Serum glucose level was measured immediately by using glucose estimation. The groups were as follows:

Table 1
Oral glucose tolerance test

Groups	Treatment	Route	Dose
A	Normal Saline	Per oral	2 ml
B	Glucose	Per oral	3gm/kg
C	Polyherbal Herbal Extract	Per oral	500 mg/kg
D	Polyherbal Herbal Extract	Per oral	800 mg/kg

2) Induction and assessment of diabetes in rats by Alloxan

Rats were made diabetic by administering Alloxan intraperitonially at a dose of 150 mg/kg. A single dose of 150 mg/kg prepared and injected intraperitonially to induce diabetes in rats. Alloxan was first weighed individually for each animal according to the body weight and then solubilised with 0.2 ml saline just prior to injection. Diabetes was confirmed after 48 hour of Alloxan injection, the blood sample was collected through tail vein and plasma glucose levels were determined to confirm the development of diabetes (>200mg/dl). Treatment with plant extract was started 72 hrs after Alloxan injection. The

groups were as follows:

Table 2
Oral glucose tolerance test

Groups	Animal No.	Treatment	Route	Dose
A	1-6	Normal Saline	I.P.	2 ml
B	7-12	Alloxan	I.P.	150 mg/kg
C	13-18	Alloxan	I.P.	150 mg/kg
D	19-24	Alloxan	I.P.	150 mg/kg
E	25-30	Alloxan	I.P.	150 mg/kg
F	31-36	Alloxan	I.P.	150 mg/kg

I.P. = Intraperitoneal

5. Experimental Design

Treatment with plant extract was started 72 hrs after Alloxan injection. Six groups of rats, six in each received the following treatment schedule: [16]

Table 3
Experimental design

Groups	Animal No.	Treatment	Route	Dose
A	1-6	Control (Normal Saline)	P.O.	2 ml
B	7-12	Diabetic Control (Normal Saline)	P.O.	2 ml
C	13-18	Polyherbal Extract	P.O.	300 mg/kg
D	19-24	Polyherbal Extract	P.O.	500 mg/kg
E	25-30	Polyherbal Extract	P.O.	800 mg/kg
F	31-36	Standard (Glibenclamide)	P.O.	5 mg/kg

P.O.=per oral

Polyherbal extracts and standard drug Glibenclamide (5 mg/kg) and saline were administered with the help of Feeding cannula. Group I serve as normal control, which received normal saline for 21 days. Group II diabetic control rats and Group III to Group VI (which previously received Alloxan) are given a fixed dose polyherbal extract (300 mg/kg, p.o), (500 mg/kg, p.o) and (800 mg/kg, p.o) and standard drug Glibenclamide (5mg/kg) for 14 consecutive days.

A. Body weight

Body weights were recorded preceding to treatment at Day 1, 7, 14, 21 and fasting body weight was recorded on Day 22 and data were expressed as Mean ± S.D.

B. Collection of blood and determination of glucose level

Animals were kept for overnight fasting 12 hrs before treatment on day 1 and on day 21. Blood samples were drawn from tail tip of rat fasting blood glucose estimation was done on Day 1 and on Day 22 by one touch electronic Glucometer using glucose test strips.

C. Statistical analysis

All the values of body weight, fasting blood sugar estimation were expressed as Mean ± SD (n=6) and data were analysed using One Way ANOVA followed by Dunnet t–test using INSTANT Graph pad.

6. Result

A. Oral glucose tolerance test

For glucose tolerance test Glibenclamide was given in the dose of 5 mg/kg showed considerable reduction in blood sugar levels. It reduced blood sugar level to basal line after 4 hours as comparison to control. The polyherbal extracts was given in the dose of 300 and 500 mg/kg showed reduction in blood sugar level, but it was not statistically significant. The polyherbal extract was given in the dose of 800 mg/kg showed significant reduction in blood sugar level. It reduced blood sugar level after 4 hours as comparison to control. But in evaluation to Glibenclamide it was not showing significant result. [17]

Table 4
Oral glucose tolerance test

Groups	Treatment	0 hr	4 hr
A	Normal Saline	90.45 ± 8.22	115.24 ± 4.28
B	Polyherbal Extract 300 mg/kg	91.70 ± 5.25	90.10 ± 4.24
C	Polyherbal Extract 500 mg/kg	93.25 ± 3.22	90.34 ± 3.49
D	Polyherbal Extract 800 mg/kg	94.35 ± 5.26	86.22 ± 2.34
E	Standard (Glibenclamide 5 mg/kg)	92.34 ± 2.63	82.42± 6.48

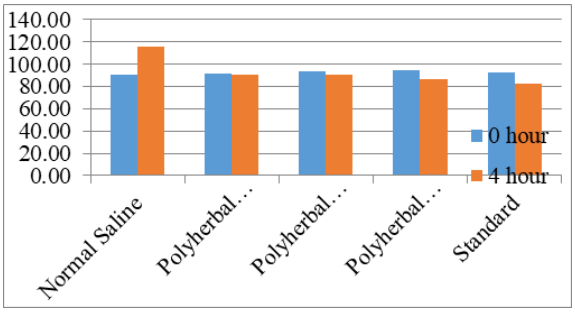

Fig. 4. Oral glucose tolerance test

B. Evaluation of hypoglycemic activity of polyherbal extract in Albino rats

Treatment of Polyherbal extract at dose of 300 mg/kg, 500 mg/kg and 800 mg/kg p.o. showed no significant changes in blood glucose level at predose (0 min), 30, 60, 90 and 120 min after dose administration as compare to normal control group. The result was found to be dose and time dependent. The obtained result are given in table 5 and illustrated in fig. 5.

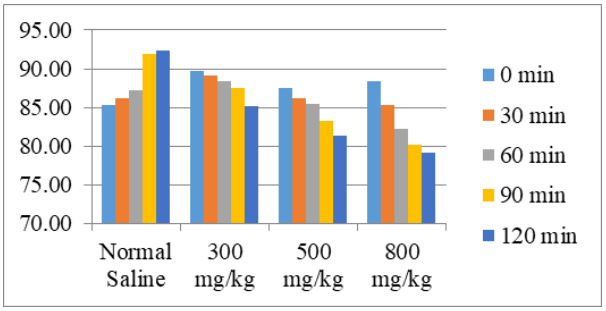

Fig. 5. Effect of polyherbal extract on Blood Glucose Level (mg/dl)

Table 5
Effect of polyherbal extract on Blood Glucose Level (mg/dl)

Treatment	0 min	30 min	60 min	90 min	120 min
Normal Saline	85.25 ± 8.21	86.14±7.21	87.19 ± 0.4	91.93±9.21	92.34±9.28
Polyherbal Extract 300 mg/kg	89.70±5.25	89.12±3.26	88.36±4.10	87.52±2.69	85.10±8.44
Polyherbal Extract 500 mg/kg	87.45±4.22	86.23±4.10	85.45±4.23	83.24±4.23	81.34±30.4
Polyherbal Extract 800 mg/kg	88.45±5.26	85.34±3.80	82.23±3.23	80.12±5.23	79.12±12.4

Table 6
Effect of Aqueous polyherbal extract on body weight

Groups	Treatment	Body Weight (gm)			
		Day 1	Day 7	Day 14	Day 21
A	Normal Control	182.5 ± 1.2	186.0 ± 2.7	190.5 ± 3.5	192.5 ± 4.6
B	Diabetic Control(Alloxan)	185.0 ± 1.3	182.0 ± 1.1	181.5 ± 0.9	180.0 ± 0.8
C	Diabetic + Polyherbal Extract (300 mg/kg)	184.5 ± 2.4	185.0 ± 2.7	187.5 ± 2.8	189.5 ± 2.7
D	Diabetic + Polyherbal Extract (500 mg/kg)	189.5 ± 2.7	191.5 ± 2.9	192.5 ± 3.2	195.0 ± 3.5
E	Diabetic + Polyherbal Extract (800 mg/kg)	186.5 ± 4.8	187.0 ± 4.5	190.5 ± 4.6	194.5 ± 4.9
F	Diabetic + Standard (Glibenclamide)	189.5 ± 3.6	191.5 ± 4.4	195.5 ± 3.4	197.5 ± 3.5

Table 7
Evaluation of Anti-diabetic activity of polyherbal extract in Alloxan induced diabetic albino rats

Treatment	Day 1	Day 7	Day 14	Day 21
Normal	85.25±9.23	84.45±8.21	86.45±10.4	87.64±11.21
Diabetic Control (Alloxan)	220.56±12.65	225.12±15.12	234.21±6.12	239.14±8.13
Diabetic + Polyherbal Extract(300 mg/kg)	222.30±7.13	219.31±14.16	215.40±11.02	204.12±12.25
Diabetic + Polyherbal Extract(500 mg/kg)	224.12±5.12	219.12±12.21	211.14±12.2	203.02±10.23
Diabetic + Polyherbal Extract(800 mg/kg)	215.45±4.23	204.14±10.2	196.12±4.5	189.16±10.25
Diabetic + Standard (Glibenclamide)	212.01±2.23	192.12±12.2	182.13±10.23	171.18±12.13

C. Effect of Aqueous polyherbal extract on body weight

Body weight of Alloxan treated animals Group II was significantly decreased as compared with Group I Normal animals. Body weight of polyherbal extract treated animals was significantly regained as compared with toxicant animals Group II. Glibenclamide treated animals were significantly recover body weight as compared with toxicant control animals. The results depicted in table 6 and illustrated in fig. 6.

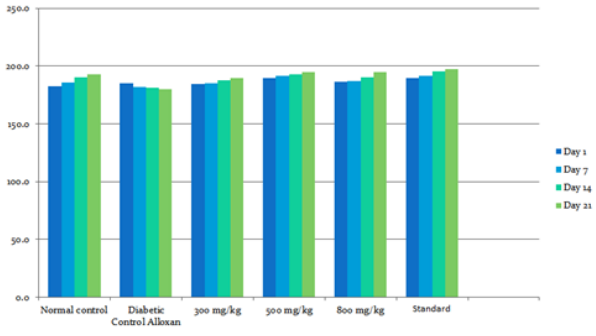

Fig. 6. Effect of aqueous polyherbal extracts on body weight

D. Evaluation of Anti-diabetic activity of polyherbal extract in Alloxan induced diabetic albino rats

Twenty-one days' treatment of polyherbal extract at dose of 300mg/kg, 500mg/kg and 800mg/kg p.o. showed decreased in blood glucose level on 1st, 7th, 14th, and 21st days as compare to diabetic control group. The effect of polyherbal extract is also compare with standard drug The result was found to be dose and time dependent. The obtained result are given in table 7 and illustrated in fig. 7.

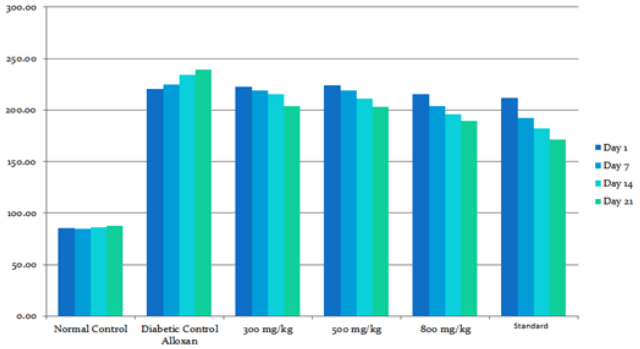

Fig. 7. Effect of polyherbal extract on Blood Glucose Level (mg/dl) of diabetic rats

7. Discussion

In the traditional system of medicinal plant, there are many herbal remedies recommended for diabetes and diabetes complications. More traditional plants used in the treatment for diabetes has been reported, although only small number of these has received scientific and medical evaluation to assess their efficacy. Currently, different types of synthetic drugs have various side effects Bigunaids (e.g. Metformin, Phenformin) have side effects sickness with alcohol, kidney problems, upset stomach, tiredness or dizziness, metal taste. Sulfonylurea's have side effects, low blood sugar, skin rash or itching, weight gain. Although all these are effective in reducing blood sugar levels but also carries many and different side effects with them which affects the normal human body functioning. So there is need to develop herbal or natural treatment for diabetes which would be free of harmful side effect. Plants have been suggested as a rich, as up till now uncultivated source of potentially useful antidiabetic drugs. However, only a few have been subjected to

detailed scientific exploration due to a lack mechanism based available in vitro assays.

8. Conclusion

Albino rats were treated with prepared polyherbal extracts at doses of 300 mg/kg, 500 mg/kg and 800 mg/kg did not significantly lower normal blood glucose level as compared to normal control animals that is polyherbal extracts did not possess hypoglycaemic activity. 21 days treatment with aqueous polyherbal extracts at a dose of 300 mg/kg, 500 mg/kg and 800 mg/kg showed significant increase in the body weight as compared to Group- B animals. Alloxan at the dose of 150 mg/kg could significantly elevate blood glucose level in all groups of animals as compared to normal control animals. Twenty-one days treated albino rats with polyherbal extracts at doses of 300 mg/kg; 500mg/kg and 800 mg/kg significantly lower normal blood glucose level as compared to diabetic control group but not when compare with blood glucose level of group treated with standard drug. The result was found to be dose dependent. The results conclude that the polyherbal extracts of AzadiractaIndica, Catharanthus Roseus and Acacia Nilotica at doses 300 mg/kg, 500 mg/kg and 800 mg/kg p.o. did not possess hypoglycaemic but possess antidiabetic activity.

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