

Polyherbal Antidiabetic Powder: A Natural Remedy For Diabetes Mellitus

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Abstract

Background: The increasing prevalence of diabetes in both developed and developing countries have challenged scientists to the discovery of various therapeutic agents that can be used to ensure efficient treatment and management of diabetes. Synthetic anti-diabetic drugs are widely used for the management of diabetes mellitus; but they possess several limitations that may reduce their long-term effectiveness and patient compliance.

Objective: To provide natural and cost-effective alternative for synthetic antidiabetic drugs by exploring the synergistic effect of herbs in diabetes treatment and management.

Methods: Polyherbal formulations (F1, F2, F3, F4 and F5) containing powder of *Moringa oleifera* and *Carica papaya* leaves were prepared and evaluated for physicochemical properties and in vivo antidiabetic activity.

Results: All formulations showed acceptable characteristics. Formulation F3 exhibited the best physicochemical properties, stability, and antidiabetic activity.

Conclusion: The optimized polyherbal formulation (F3) demonstrated effective antidiabetic activity and may serve as a safe and natural alternative for managing diabetes mellitus.

Keywords: Polyherbal formulation, Antidiabetic activity, *Moringa oleifera*, *Carica papaya*, Hyperglycaemia.

Introduction

Diabetes is a chronic metabolic disorder that affects lipid, carbohydrate, and proteins function, resulting in persistent hyperglycaemia, arising from abnormal insulin secretion, insulin action, or both, causing slow damage to the heart, blood vessels, eyes, kidneys and nerves.¹ Diabetes Mellitus (DM) involves various types, including type 1, type 2, and gestational; however, 95% of all diabetes cases worldwide are attributed to Type 2 Diabetes Mellitus (T2DM).² According to 2019 statistics of the International Diabetes Federation (IDF), three out of four people living with diabetes (463 million people) are of working age (i.e., aged between 20 and 64). In 2030 this number will increase to 578 million, and by 2045 to 700 million. An estimated 111 million people aged over 65 have diabetes. In this age group, one in five adults are reported to suffer from diabetes.³

A significant number of complications are associated with DM But, the most dilapidating effects of DM and its associated vascular complications are classified into four categories: nephropathy, retinopathy, neuropathy, and cardiovascular disease resistance.⁴ Synthetic antidiabetic drugs have number of serious adverse effects; the main disadvantage of current drugs such as (biguanide, sulfonylureas) is that they have to be given throughout the life and these produce serious side effects. Thus, managing diabetes without any side effects is still a challenge, therefore it is important to search for an alternative remedy such as medication from natural products.⁵

Since ancient times a number of herbal medicines have been used in the treatment of Diabetes. There is increasing demand by patients to use the natural products with antidiabetic activity.⁶ Traditional herbal medicines and functional foods are believed to improve diabetes and its associated metabolic conditions through six notable mechanism of actions including enhanced insulin secretion and sensitivity, glucose uptake by muscle cells and adipose tissues, inhibition of glucose absorption from intestine and glucose production from hepatocytes along with demonstrating anti-inflammatory properties.⁷

Polyherbal formulation is based on the principle of combining two or more medicinal plants to achieve enhanced

therapeutic efficacy through synergistic action.⁸ This approach is widely used in traditional systems of medicine, particularly for the management of complex diseases like diabetes mellitus, which involves multiple physiological pathways such as insulin resistance, oxidative stress, and inflammation.⁹ This widely diverse therapeutic spectrum makes them a promising or alternative approach to modern pharmacological interventions, especially for chronic diseases such as diabetes, and heart diseases.¹⁰ To develop and evaluate a polyherbal formulation for the treatment and management of diabetes mellitus the current investigation was conducted. The present study selected *Moringa oleifera* and *Carica papaya* because of their promising hypoglycaemic and antidiabetic properties reported in the literature.¹¹ To evaluate the polyherbal formulation as a safe and efficient natural alternative for the treatment of diabetes mellitus, their physicochemical properties, stability, and in vivo antidiabetic activity were determined.¹²

2. Herbal Components And Their Phytotherapeutic Potential

2.1 *Moringa oleifera* (Drumstick tree)

Almost every part of this plant, including leaves, seeds, pods, flowers, and roots, is used for various therapeutic purposes.¹³ *Moringa oleifera* (*M.oleifera*) leaves are particularly rich in essential nutrients such as vitamins A, C, and E, calcium, potassium, iron, and proteins. In addition to its nutritional value, the plant contains numerous bioactive compounds like flavonoids, phenolic acids, alkaloids, and glucosinolates, which contribute to its pharmacological activities.¹⁴ Its antidiabetic effect is mainly attributed to its ability to lower blood glucose levels, enhance insulin secretion, and improve glucose utilization in the body.¹⁵ Moreover, its antioxidant properties help in reducing oxidative stress, which is a major factor in the development of chronic diseases.¹⁶ Due to its wide range of health benefits and minimal side effects, *Moringa oleifera* is increasingly being explored in modern research for the development of herbal formulations and nutraceutical products.¹⁷

2.2 *Carica papaya* (Melon tree)

Carica papaya (*C.papaya*) leaf is widely recognized for its significant medicinal and therapeutic properties. Papaya leaves contain a variety of bioactive compounds such as alkaloids (carpaine), flavonoids, tannins, saponins, glycosides, and enzymes like papain and chymopapain.¹⁸ These constituents contribute to its antioxidant, anti-inflammatory, antimicrobial, and antidiabetic properties.¹⁹ The presence of strong antioxidants helps in neutralizing free radicals and reducing oxidative stress, which is a key factor in the progression of chronic diseases, including diabetes.²⁰ In diabetes, papaya leaves are known to help in lowering blood glucose levels by improving insulin sensitivity and supporting pancreatic β -cell function.²¹

3. Materials And Method

3.1 Plant materials:

The following medicinal plants were used in this study. All plants were procured from botanical garden in Malkapur, Maharashtra, and authenticated by the Department of Botany, Vidnyan Mahavidyalaya, Malkapur.

Table 3.1: Used plant materials

Sr. No	Botanical Name	Common Name	Part Used
1	<i>Moringa oleifera</i>	Drumstick tree	Leaves
2	<i>Carica papaya</i>	Melon tree	Leaves

3.2 Chemicals and reagents:

All chemicals used in the polyherbal formulation such as distilled water, ethanol, buffer solution, solvents and reagents are of Analytical Reagent (AR) grade and were procured from reliable sources to ensure the quality, purity and consistency of the formulation. The chemicals are mainly used for analytical evaluation and phytochemical screening rather than formulation. Unlike many conventional formulations, the developed polyherbal powder does not required a large number of chemicals or synthetic excipients during preparation which reduced the processing complexity and preserved the natural properties of the herbs. method

Table 3.2: Used chemicals and reagents

Sr. No	Name	Purpose / Function
1	Distilled water	Preparation of sample solution for evaluation tests
2	Buffer solutions	Used for the calibration of digital pH meter
3	Ferric chloride solution	Used for the detection of phenolic compounds and tannins
4	Glacial acetic acid	Used for the detection of glycosides
5	Dragendorff's reagent	Used for the detection of alkaloids
6	Hydrochloric acid	Used for the detection of flavonoids
7	Glucose solution	Used as a supplement for the induction of diabetes mellitus

3.3 Method

3.3.1 Collection and authentication of herbs: Fresh and healthy leaves of *Moringa oleifera* and *Carica papaya* were collected from the botanical garden during the appropriate season. The collected plant materials were authenticated by Dr. Y. P. Patil, Department of Botany, Vidyan Mahavidyalaya, Malkapur. The leaves were carefully examined for the absence of fungal infection, insect contamination, and physical damage.

3.3.2 Cleaning and washing of leaves: The selected healthy leaves were separated manually from stalks and unwanted plant parts. Then the leaves were washed thoroughly with tap water to remove adhering dust particles, soil, dirt, and other foreign contaminants. Subsequently, the leaves were rinsed with distilled water to eliminate any remaining impurities and microbial contamination. Excess surface water was removed by spreading the leaves over clean blotting cloth at room temperature. Blotting cloth is used to absorb surface water.

3.3.3 Drying of leaves: The washed leaves were subjected to shade drying under ambient room temperature in a clean and well-ventilated area. The leaves were spread uniformly in thin layers on clean cloth to ensure proper air circulation and uniform drying. The plant material was protected from direct sunlight during the drying process to prevent degradation of heat-sensitive and photosensitive phytoconstituents. The leaves were turned periodically to avoid fungal growth and ensure uniform moisture removal. Drying was continued for approximately 7–14 days until the leaves became crunchy indicating complete removal of moisture. The dried leaves were evaluated visually for absence of moisture and microbial growth before further processing.

Fig. 3.1 Dried moringa & papaya leaves



3.3.4 Grinding and pulverization: The completely dried leaves of *Moringa oleifera* and *Carica papaya* were coarsely broken manually. The dried material was pulverized separately using a laboratory blender. Grinding was carried out

carefully to obtain uniform particle size while minimizing heat generation during the process, as excessive heat may lead to degradation of active phytoconstituents.

3.3.5 Sieving: The powdered materials were passed through suitable sieve number to achieve optimum particle size and smooth texture. Sieving was carried out using manual sieving method. Oversized particles retained on the sieve were reground and sieved again to minimize particle size variation. The uniformly sieved powder was collected separately for formulation development and evaluation studies.

3.3.6 Storage of Powder: The prepared powdered material was stored in clean, dry, airtight container to protect them from environmental contamination, moisture, and oxidation. The containers were properly labelled and kept in a cool, dry place away from direct sunlight and humidity.

3.4 Procedure for the preparation of polyherbal formulation

Different formulation batches were prepared by varying concentrations of *Moringa oleifera* and *Carica papaya* leaf powder. Required quantities of both powders were weighed accurately using electronic balance. Powders were mixed geometrically in a mortar and pestle to obtain uniform blending. Sweetening agent such as stevia was added to improve palatability. Final formulations were packed in airtight containers.²²

Table 3.3 Batches of Formulation (All Ingredient Weight in Mg)

Sr. No	Batches	Ratio	M. oleifera	C. papaya	Total
1	F1	1:1	125	125	250
2	F2	1:1.5	100	150	250
3	F3	1.5:1	150	100	250
4	F4	1:2	83.3	167.7	250
5	F5	2:1	167.7	83.3	250

3.5 Evaluation of polyherbal formulation: The developed polyherbal formulation was evaluated for the following parameters:

3.5.1 Organoleptic evaluation: To determine sensory characteristics and patient acceptability the prepared formulations were evaluated for colour, odour, taste and texture.

3.5.2 Particle size analysis: Particle size of the prepared powder formulation was determined by using sieve analysis method. A clean and dry set of standard sieves was arranged in descending order of mesh size, with the coarsest sieve placed at the top and the finest sieve at the bottom. Approximately 100 grams of accurately weighed polyherbal powder formulation was placed on the uppermost sieve. The sieve assembly was fixed on a mechanical sieve shaker and shaken for 10–15 minutes. After completion of sieving, the powder retained on each sieve was carefully collected and weighed separately. The percentage of powder retained on each sieve and the percentage passing through each sieve were calculated.

3.5.3 Determination of pH: 1 g of prepared polyherbal powder formulation was weighed accurately. The weighed powder was transferred into a clean beaker containing 10 ml of distilled water. The mixture was stirred properly using a glass rod or magnetic stirrer to obtain a uniform suspension. The suspension was allowed to stand for about 5–10 minutes for complete dispersion of the powder. The digital pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2 before use. The electrode of the pH meter was immersed into the prepared suspension. The pH value was recorded once the reading became stable. The measurement was performed in triplicate, and the average value was calculated.

3.5.4 Determination of flow properties

Bulk density: An accurately weighed quantity of the prepared polyherbal powder blend was transferred carefully into a clean and dry graduated measuring cylinder without compacting the powder. The powder was allowed to settle naturally, and the initial volume occupied by the powder was noted as the bulk volume. The bulk density was calculated using the following equation:

$$\text{Bulk density} = \frac{\text{Mass of powder}}{\text{Bulk volume}}$$

Tapped density: A known quantity of the polyherbal powder blend was accurately weighed and transferred into a graduated cylinder. The initial volume was recorded. The cylinder was then fixed onto a tapped density apparatus and subjected to continuous mechanical tapping for 100 taps or until no further change in volume was observed. After completion of tapping, the final volume occupied by the powder was noted as the tapped volume. The tapped density was calculated using the following equation:

$$\text{Tapped density} = \frac{\text{Mass of powder}}{\text{Tapped volume}}$$

Angle of Repose: The angle of repose of the prepared polyherbal powder formulation was determined by the fixed funnel method. A clean and dry funnel was mounted on a stand at a suitable height above a flat horizontal surface covered with graph paper. The height of the funnel was adjusted so that the lower tip of the funnel just touched the apex of the powder heap formed during the experiment. Approximately 10 g of the polyherbal powder blend was accurately weighed and allowed to flow freely through the funnel onto the graph paper. The powder formed a conical heap on the surface. Care was taken to avoid vibration during the experiment. After complete flow of the powder, the height (h) of the heap and the radius (r) of the circular base were measured using a measuring scale. The angle of repose was calculated by using following formula:

$$\tan \theta = \frac{h}{r}$$

Where: h = height of powder cone

r = radius of powder cone

3.5.5 Zeta potential analysis: A small quantity of the prepared polyherbal formulation was accurately weighed and dispersed in distilled water to prepare a dilute suspension. Approximately 10 mg of the formulation was dispersed in 50 mL of distilled water in a clean beaker. The dispersion was stirred continuously using a magnetic stirrer for about 15–20 minutes to obtain a uniform suspension. The prepared suspension was then sonicated for 5–10 minutes using an ultrasonicator to minimize particle aggregation and ensure uniform particle dispersion throughout the medium. After sonication, the suspension was allowed to stand briefly to remove entrapped air bubbles. A clear and homogeneous sample was then carefully withdrawn using a micropipette and transferred into a clean disposable zeta potential measurement cuvette. The sample cell was placed inside the zeta potential analyzer and the measurement was carried out at room temperature. The instrument measured the electrophoretic mobility of dispersed particles under an applied electric field and automatically converted the values into zeta potential using Smoluchowski's equation.

3.5.6 Phytochemical Screening of Polyherbal Formulation: Preliminary phytochemical screening of the prepared polyherbal formulation containing *Moringa oleifera* and *Carica papaya* leaf powder was carried out to identify the presence of various bioactive phytoconstituents such as Flavonoids, Alkaloids, Glycosides, Saponins and Tannins. These phytochemicals are known to contribute significantly to the antidiabetic activity and therapeutic potential of polyherbal formulation. The phytochemical analysis was performed using standard qualitative chemical tests.

Preparation of Extract for Phytochemical Screening:

An accurately weighed quantity of the prepared polyherbal powder formulation was subjected to extraction using distilled water as solvent. About 5 g of the powder formulation was mixed with 50 ml of solvent in a conical flask and shaken continuously using a mechanical shaker. The mixture was filtered using Whatman filter paper, and the filtrate obtained was used for preliminary phytochemical screening.

Table 3.4 Phytochemical tests

Sr. No	Phytochemical Constituent	Test performed
1	Flavonoids	Shinoda test
2	Alkaloids	Dragendorff's test
3	Glycosides	Keller–Killiani test
4	Saponins	Foam test
5	Tannins	Ferric chloride test

a) Test for Flavonoids: Approximately 2 ml of the extract was taken in a test tube. A small quantity of magnesium

turnings was added followed by the addition of a few drops of concentrated hydrochloric acid. **Observation:** Development of pink, crimson red, or reddish coloration indicated the presence of flavonoids.

- b) **Test for Alkaloids:** About 2 ml of the extract was treated with a few drops of Dragendorff's reagent. **Observation:** Formation of an orange or reddish-brown precipitate confirmed the presence of alkaloids.
- c) **Test for Glycosides:** About 2 ml of the extract was treated with 1 ml of glacial acetic acid containing traces of ferric chloride solution. Concentrated sulfuric acid was carefully added along the sides of the test tube. **Observation:** Formation of a brown ring at the interface indicated the presence of glycosides.
- d) **Test for Saponins:** Approximately 2 ml of the extract was diluted with distilled water in a test tube and shaken vigorously for about 2–5 minutes. **Observation:** Formation of stable persistent froth or foam indicated the presence of saponins.
- e) **Test for Tannins:** About 2 ml of the extract was treated with a few drops of 5% ferric chloride solution. **Observation:** Formation of blue-black or greenish-black coloration indicated the presence of tannins.

3.6 In-Vivo Study

The in vivo antidiabetic activity of the prepared polyherbal formulation containing *Moringa oleifera* and *Carica papaya* leaf powder was evaluated using an experimental animal model. The study was carried out to assess the effectiveness of the formulation in reducing elevated blood glucose levels and improving diabetic conditions in laboratory animals. The animal study was conducted according to standard experimental protocols and ethical guidelines for the care and use of laboratory animals.

- **Experimental Animals:** Healthy adult male Wistar albino rats weighing approximately 150–250 g was selected for the study. The animals were procured from a registered animal breeding facility and housed in clean polypropylene cages under standard laboratory conditions for one week before initiation of the experiment. The animals were maintained under controlled environmental conditions at a temperature of 25 to 27 °C, relative humidity of 55–65%, and a 12-hour light and dark cycle. Standard pellet diet and water ad libitum were provided throughout the study period except during fasting conditions prior to blood glucose estimation. The animals were acclimatized to laboratory conditions for seven days prior to initiation of the experiment.
- **Ethical Approval:** The animal experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) constituted under the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All experimental procedures were carried out in accordance with CPCSEA guidelines to minimize animal suffering during study.
- **Induction of Diabetes Mellitus:** Diabetes was induced experimentally in rats by administration of a high-fat diet followed by glucose supplements. The animals were fed with a high-fat diet for a period of four weeks to develop insulin resistance. The high-fat diet includes carbohydrates, fats, proteins, cholesterol, and other nutritional components prepared according to standard laboratory procedures. During the induction period, glucose solution was also provided intermittently to enhance hyperglycemic conditions. After completion of the induction period, fasting blood glucose levels were measured using a glucometer by collecting blood from a tail vein. Animals showing fasting blood glucose levels above 200 mg/dL were considered diabetic and selected for the study.
- **Experimental Design:** The experimental study was designed to evaluate the antidiabetic activity of the developed polyherbal formulations containing *Moringa oleifera* and *Carica papaya* leaves powder in experimentally induced diabetic rats. The animals were randomly divided into eight groups containing six animals in each group. Diabetes mellitus was induced prior to treatment, and the prepared formulations were administered orally for the specified treatment period.

Table 3.5 Experimental Design

Group	Experimental Design	Treatment Given
Group1	Normal Control	Received normal pellet diet and distilled water

Group2	Diabetic Control	Diabetic animals received no treatment
Group3	Standard Group	Diabetic animals treated with standard antidiabetic drug
Group4	Batch F1	Treated with polyherbal formulation Batch F1
Group5	Batch F2	Treated with polyherbal formulation Batch F2
Group6	Batch F3	Treated with polyherbal formulation Batch F3
Group7	Batch F4	Treated with polyherbal formulation Batch F4
Group8	Batch F5	Treated with polyherbal formulation Batch F5

- **Preparation and Administration of Polyherbal Formulation:** The prepared polyherbal formulation was freshly suspended in distilled water before administration. The required dose was calculated according to the body weight of each animal. The formulation was administered orally using an oral feeding cannula once daily for the experimental period. The standard drug was also administered orally in the same manner to the respective group.
- **Treatment Schedule:** The treatment was continued for 28 days. During the treatment period, animals were observed daily for behavioral changes, food intake, water consumption, and general health condition. Body weight of animals was recorded at regular intervals throughout the study.
- **Estimation of Blood Glucose Level:** Fasting blood glucose levels were measured at predetermined intervals such as day 0, day 7, day 14, day 21 and day 28. Blood samples were collected from the tail vein of overnight-fasted animals and glucose levels were estimated using a digital glucometer. The reduction in fasting blood glucose levels after treatment was considered as an indicator of antidiabetic activity. At the end of the study, the obtained results were statistically analysed to determine the effectiveness of different formulation batches.

4. Results and discussion

The present study was carried out to develop and evaluate a polyherbal formulation containing *Moringa oleifera* and *Carica papaya* leaves powder for the management of diabetes mellitus. Different formulation batches (F1–F5) were prepared by varying the ratio of both herbal ingredients and evaluated for Organoleptic properties, physicochemical properties, phytochemical constituents, stability, and antidiabetic activity.

4.1 Organoleptic Evaluation: The organoleptic evaluation showed that all batches possessed acceptable color, odor, taste and texture. No lump formation or contamination was observed, which confirmed suitable storage and handling conditions.



Fig. 4.1 Optimized Polyherbal Formulation

Table 4.1 Result of Organoleptic evaluation

Sr.	Paramet	Batches
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No	er	F1	F2	F3	F4	F5
1	Color	Dark green	Dark green	Dark green	Dark green	Dark green
2	Odor	Characteri stic	Characteri stic	Characteristic	Characteri stic	Characteri stic
3	Taste	Slightly bitter	Slightly bitter	Slightly bitter	Slightly bitter	Slightly bitter
4	Texture	Fine powder	Fine powder	Fine powder	Fine powder	Fine powder

4.2 Particle size analysis: Particle size determination was performed using standard sieve analysis to evaluate the suitability of the prepared polyherbal formulations for oral administration. The results showed that batches F1, F2, F3, and F5 passed through sieve number 85 and were categorized as fine powders, while batch F4 passed through sieve number 44 and was categorized as moderately fine powder. Among all batches, F3 showed the most uniform and fine particle distribution, which contributed to its better flow properties and improved formulation performance. The particle size results indicated that all batches were suitable for oral powder formulation. However, based on better uniformity and overall physicochemical characteristics, Batch F3 was considered as the optimized batch.

Table 4.2 Result of particle size analysis

Sr. No.	Batch	Sieve number	Nature of particle
1	F1	85	Fine powder
2	F2	85	Fine powder
3	F3	85	Fine powder
4	F4	44	Moderately fine powder
5	F5	85	Fine powder

4.3 Determination of pH: The pH values of all batches were found near neutral, indicating that the formulations were suitable for oral administration and unlikely to cause gastrointestinal irritation. Batch F3 showed the most favorable pH, suggesting better formulation stability and compatibility

Table 4.3 Result of pH test

Sr. No	Batch	pH
1	F1	6.9
2	F2	7.1
3	F3	6.7
4	F4	6.8
5	F5	6.5

4.4 Determination of flow properties: The flow property evaluation demonstrated that all formulations exhibited satisfactory flow characteristics for oral powder dosage form. Parameters such as bulk density, tapped density and angle of repose were found within acceptable limits. Among all formulations, Batch F3 showed comparatively better flow properties with lower angle of repose indicating improved powder flow and packing ability. The balanced ratio of *Moringa oleifera* and *Carica papaya* leaf powder in F3 might have contributed to reduced interparticle friction and better uniformity.

Table 4.4 Flow property evaluation results

Sr. No	Batch	Bulk density (g/ml)	Tapped density (g/ml)	Angle of Repose
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1	F1	0.42	0.52	29
2	F2	0.45	0.53	30
3	F3	0.48	0.54	26
4	F4	0.43	0.51	32
5	F5	0.40	0.50	28

4.5 Stability study: The zeta potential study revealed that all formulations possessed acceptable stability. Batch F3 exhibited comparatively higher zeta potential value, indicating improved dispersion stability and

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -0.159	Peak 1: 19.0	69.9	3.68
Zeta Deviation (mV): 29.5	Peak 2: -44.6	30.1	5.51
Conductivity (mS/cm): 0.236	Peak 3: 0.00	0.0	0.00
Result quality Good			

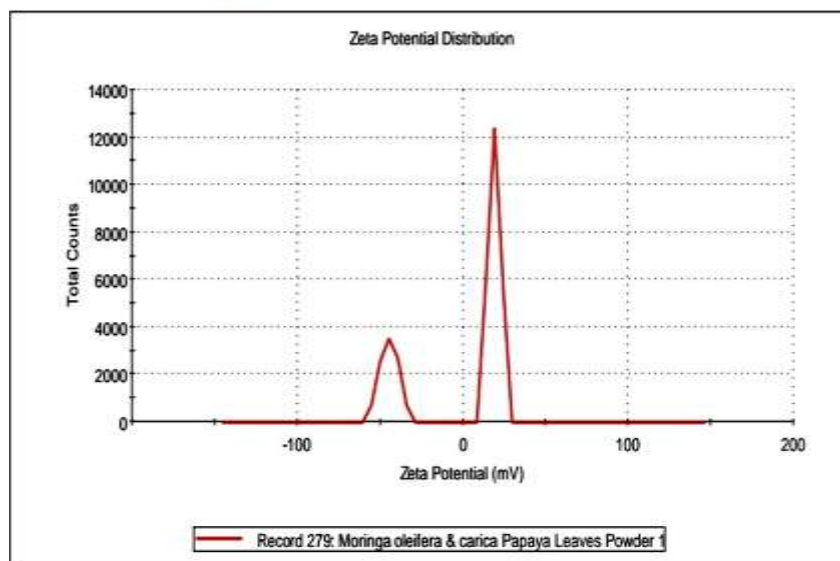


Fig 4.2 Stability Evaluation Result

lower aggregation tendency. This result suggested better physical stability of the optimized formulation. The obtained zeta potential distribution graph showed the presence of both positive and negative charged particles. The observed zeta potential value of the formulation was -0.159 mV, indicating that the overall surface charge of the particles was nearly neutral. Generally, zeta potential values greater than $+30$ mV or less than -30 mV indicate good electrostatic stability. In the present study, the formulation showed a mixed charge distribution with one major positive peak and one negative peak, suggesting the presence of different phytoconstituents contributing to the surface charge.

The zeta deviation value of 29.5 mV indicated variation in the charge distribution of particles within the formulation. This deviation may be attributed to the heterogeneous nature of different herbal constituents present in the formulation. The zeta potential distribution graph showed two peaks: The first peak at $+19.0$ mV represented the major population of particles, accounting for 69.9% of the total particle distribution. This indicated that most particles possessed a moderate positive surface charge. However, since the value was below $+30$ mV, moderate stability was indicated. The second peak at -44.6 mV represented 30.1% of the particle population and indicated strongly negatively charged particles.

Since the value was below 30 mV, this fraction of particles exhibited good electrostatic stability and lower tendency toward aggregation. Overall, the zeta potential analysis indicated that the optimized formulation (F3) possessed moderate colloidal stability with the presence of both positively and negatively charged phytoconstituent particles. The stability of the formulation may be attributed to the synergistic interaction of bioactive compounds present in the polyherbal formulation.

4.6 Evaluation of Phytochemical constituents: Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, alkaloids, tannins, saponins, glycosides, and phenolic compounds. These phytoconstituents are well known for their strong antioxidant and antidiabetic properties. Flavonoids were detected prominently in the formulation. Flavonoids and phenolic compounds may help to reduce oxidative stress and contribute significantly to antihyperglycemic activity while alkaloids and glycosides may improve glucose utilization and insulin secretion and have glucose lowering property. Among all formulations, F3 showed comparatively better and balanced phytochemical characteristics and presence of major bioactive constituents therefore it is considered as the optimized batch.

Table 4.5 Result of phytochemical screening

Phyto constituent	Test performed	F1	F2	F3	F4	F5
Flavonoids	Shinoda test	+ve	+ve	+ve	+ve	+ve
Alkaloids	Dragendorffs test	+ve	+ve	+ve	+ve	+ve
Glycosides	Keller killani test	+ve	+ve	+ve	+ve	+ve
Saponins	Foam test	+ve	+ve	+ve	+ve	+ve
Tannins	Ferric chloride test	+ve	-ve	+ve	-ve	-ve

4.7 In Vivo Evaluation of Polyherbal Formulation: The in vivo antidiabetic study was carried out to evaluate the therapeutic effectiveness of the developed polyherbal formulation using experimental diabetic rats. The study demonstrated that the formulation exhibited significant antidiabetic activity by reducing elevated blood glucose level in treated animals when compared with the diabetic control group. The diabetic control group maintained persistently high glucose levels throughout the study period, whereas treatment with the polyherbal formulation resulted in gradual and significant reduction in blood glucose concentration. Among all formulation batches, F3 showed the most pronounced antidiabetic effect and was therefore considered as the optimized batch. The significant reduction in blood glucose levels observed with F3 may be attributed to the synergistic action of various phytoconstituents present in the formulation.

Table 4.6 In vivo evaluation results

Group	Blood Glucose level mg/dl				
	0	7	14	21	28
Normal	91	105	119	115	102
Diabetic	295	305	310	325	340
Standard	275	240	205	180	125
F1	288	255	244	205	138
F2	286	270	235	210	147

F3	282	250	220	198	135
F4	290	280	245	208	155
F5	292	290	255	215	158

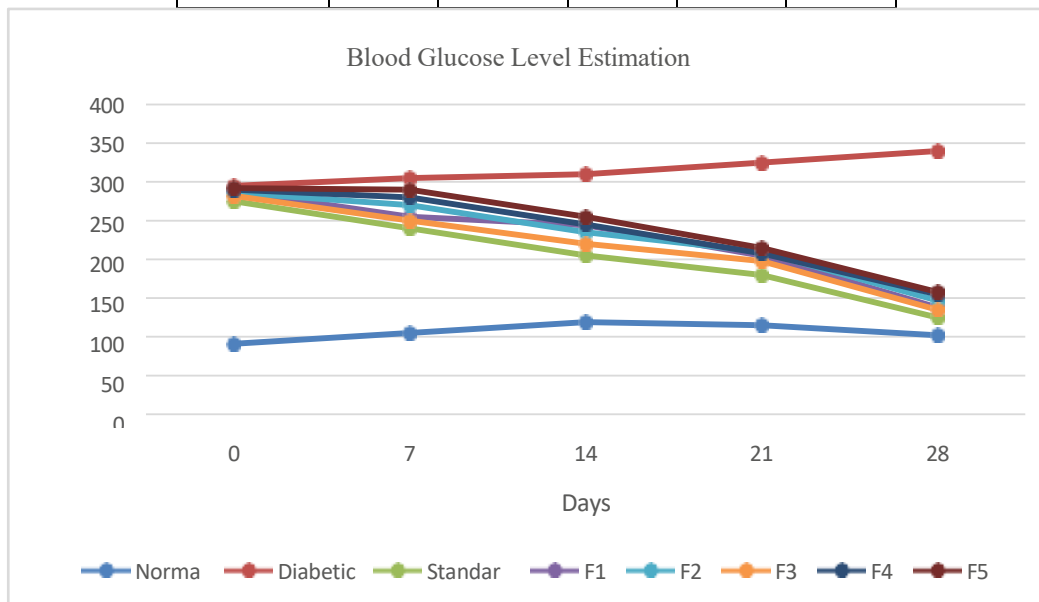


Fig 4.3 In Vivo Test Result

5. Conclusion

The synergistic polyherbal formulation of *Moringa oleifera* and *Carica papaya* showed promising antidiabetic potential due to the presence of key phytochemicals. Evaluation studies confirmed good physicochemical properties and suitability for oral use. Optimized batch demonstrated better physicochemical characteristics and uniformity. The formulation may serve as a safe, natural, and cost-effective alternative for diabetes management. Therefore, the developed polyherbal formulation may serve as a potential natural alternative for diabetes management with reduced side effects compared to synthetic drugs. The study suggests that the developed polyherbal formulation may serve as a beneficial natural therapeutic option for the treatment and management of Diabetes Mellitus. Thus, the developed polyherbal formulation can be regarded as a safe and promising treatment for diabetes mellitus treatment and management and it may investigate further through clinical testing and advanced research.

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Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

All animal experiments were conducted in accordance with the guidelines of the Ethics Committee (IAEC) of Dr. Rajendra Gode College of

Pharmacy, Malkapur, Maharashtra, India. This research did not involve human participants.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

6. References

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