Design and Development of a Polyherbal Nanoemulsion for the Treatment of Diabetes Mellitus

Venkata Suresh Jilakara[1], Patibandla Jahnavi[2], S. Manodhini elakkiya[3], Renu Arya[4], Aniruddha B Jadhav[5], Sapkale Geeta Narsingrao[6], V. Sekar[7] and M. Kishore Babu[8]*

[1]*Vathsalya College of Pharmacy, Anantharam, Bhongiri, Yadadri Bhongiri, Hyderabad, Telangana, India - 508116.*
[2]*Department of Pharmaceutics, KVSR Siddhartha College of pharmaceutical sciences, Vijayawada.*
[3]*Department of Pharmaceutics, Karpagam college of Pharmacy, Coimbatore-32.*
[4]*Department of Technical Education, Govt. of Haryana Bays No. 7-12, Sector-4, Panchkula, Haryana.*
[5]*Asily Agro and Food Processing Pvt Ltd., Gujarat, India - 396450.*
[6]*ASPM's K. T. Patil College of Pharmacy, Osmanabad.*
[7]*J.K.K. Nattraja College of Pharmacy, Kumarapalayam, Namakkal dt.*
[8]*QIS College of Pharmacy, Vengamukkapalem, ONGOLE, Andhra Pradesh, India-523272*

*Corresponding author:
Dr. M. Kishore Babu,
M. Pharm, Ph. D, Professor & Principal,
QIS College of Pharmacy, Vengamukkapalem, Ongole,
Andhra Pradesh, India-523272
email: qiskishore@gmail.com

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Polyherbal nanoemulsion, Diabetes mellitus, Antidiabetic activity, Response surface methodology, Streptozotocin-induced diabetes, Novel drug delivery system.

ABSTRACT

Introduction: Hyperglycemia, insulin resistance, and poor glucose metabolism are the hallmarks of diabetes mellitus, a chronic metabolic condition. Poor absorption, adverse effects, and patient non-compliance are common problems with traditional antidiabetic treatments. The innovative drug delivery technology known as nanoemulsions improves the bioavailability, stability, and solubility of bioactive chemicals. The purpose of this research is to create a polyherbal nanoemulsion that can help control diabetes.

Materials and Methods: Medicinal plant extracts with antidiabetic properties were used to create a polyherbal nanoemulsion. By employing response surface methods, the oil phase, surfactants, and co-surfactants were tuned to perfection. The stability, zeta potential, droplet size, viscosity, and polydispersity index (PDI) of the synthesized nanoemulsion were assessed. The drug's in vitro release, ex vivo penetration, and in vivo antidiabetic effectiveness were evaluated in diabetic rats induced with streptozotocin (STZ).

Results: A steady zeta potential, a low PDI, and an average droplet size of less than 200 nm were all characteristics of the optimized polyherbal nanoemulsion. The bioactive components were more bioavailable and soluble in the formulation. Research on drug release in vitro demonstrated a steady pattern, and research on drug permeation in vivo verified this trend. Blood glucose levels were significantly reduced, insulin sensitivity was improved, and antioxidant activity was increased in comparison to traditional formulations in in vivo tests of diabetic rats induced by STZ.

Conclusion: The created polyherbal nanoemulsion demonstrated encouraging signs of being a robust antidiabetic formulation with enhanced stability, bioavailability, and therapeutic effectiveness. By minimizing the side effects of synthetic medications, this nanoemulsion-based method has the potential to replace traditional treatments. Its safety and effectiveness in humans should be confirmed by additional clinical trials.

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disease that affects the body's ability to produce or use insulin, leading to high blood sugar levels that don't go down. Type 2 diabetes mellitus (T2DM) is caused by insulin resistance and relative insulin shortage, while type 1 diabetes mellitus (T1DM) is caused by the autoimmune destruction of pancreatic β-cells. There is an immediate need for safer and more effective therapy techniques due to the rising worldwide prevalence of DM [1-3].

Insulin therapy and oral hypoglycemic medications are examples of conventional therapeutic choices. However, these treatments can have drawbacks, such as low bioavailability, GI side effects, and long-term consequences. In addition, side effects and the necessity for frequent doses can impact patient compliance. Herbal medicine's multimodal processes, safety profile, and reduced side effects have made it a potential alternative in this setting. It has been found that some medicinal plants have strong anti-diabetic properties. These properties are attributed to processes such antioxidant effects, improved glucose absorption, and increased insulin secretion [3-5].

Unfortunately, herbal medications' low absorption, volatility, and poor solubility sometimes restrict their therapeutic use. One potential answer to these problems is medication delivery systems based on nanoemulsions. With droplet diameters varying from 20 to 200 nm, nanoemulsions are isotropic, thermodynamically stable oil-in-water dispersions stabilized by surfactants and co-surfactants. The therapeutic efficacy of bioactive substances is improved by these systems, which increase their solubility, permeability, and bioavailability [5-7].

The creation of a polyherbal nanoemulsion using specific plant extracts with antidiabetic properties is the primary goal of this research. An effective, reliable, and focused method for managing diabetes is sought after, with the hope of improving the pharmacokinetic and pharmacodynamic profiles of herbal bioactives. In order to get the optimal nanoemulsion with enhanced stability and effectiveness, the study additionally utilizes response surface methodology (RSM) to optimize the formulation parameters. To determine the efficacy of the created formulation in controlling diabetes mellitus, in vitro, ex vivo, and in vivo investigations assess its therapeutic potential [7-9].

MATERIAL AND METHODS:

Materials:

Medicinal plant extracts from Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum were standardized according to their phytochemical content and procured from verified vendors for this research. The bioactive chemicals were dissolved in castor or sesame oil, the components of the oil phase. The biocompatibility and emulsifying efficiency of the surfactants (Tween 80, Span 20) and co-surfactants (propylene glycol, polyethylene glycol) were the deciding factors in their selection. A typical supplier provided the streptozotocin (STZ), which is used to induce diabetes in animal models. Analytical or pharmaceutical grade solvents and chemicals were used for anything else.

Preparation of Polyherbal Nanoemulsion:

The polyherbal nanoemulsion was created by utilizing the controlled process of spontaneous emulsification, in which the oil phase is allowed to self-emulsify into the water phase. To find the best oil for nanoemulsion formulation, we tested how well the bioactive components dissolved in various oils. The stability and effectiveness of emulsification were used to evaluate different surfactant and co-surfactant combinations. To optimize important formulation parameters such oil concentration, surfactant-to-co-surfactant ratio, and water content, a Box-Behnken design (BBD) was used. We tuned the ratio of the oil phase, which contains plant extracts, to the surfactant and co-surfactant. At room temperature, the aqueous phase was added dropwise while being continuously stirred with a magnetic stirrer. To ensure

that the droplets were evenly distributed, the nanoemulsion was subjected to sonication and additional homogenization with a high-speed homogenizer [9-11].

Characterization of Nanoemulsion:

Droplet Size, Polydispersity Index (PDI), and Zeta Potential:

Using dynamic light scattering (DLS), we calculated the average droplet size and the polydispersity index (PDI) to evaluate the consistency and stability. In order to forecast the nanoemulsion's stability, the zeta potential was determined by measuring the electrostatic repulsion between droplets using a Zetasizer [11-13].

Viscosity and pH Measurement:

To determine the nanoemulsion's viscosity, a Brookfield rheometer was utilized, and the shear rate was varied depending on the situation. For the purpose of confirming that the pH was compatible with physiological conditions, a digital pH meter was utilized to determine the pH [13-15].

Morphological Analysis:

In order to investigate the surface morphology and shape of the nanoemulsion droplets, transmission electron microscopy (TEM) was carried out [15-17].

Stability Studies:

For a period of three months, the nanoemulsion was put through stability testing at three different temperatures: four degrees Celsius, twenty-five degrees Celsius, and forty degrees Celsius. An evaluation was carried out on the stability parameters, which included phase separation, creaming, coalescence, and precipitation. With the purpose of determining the physical stability, centrifugation at 5000 rpm for thirty minutes was carried out [18-20].

In-Vitro Drug Release Studies:

The dialysis bag diffusion method was utilized in order to investigate the release of bioactive chemicals from the nanoemulsion employing the following: We employed a dialysis membrane that had been pre-soaked and had a MW cut-off of 12,000 Da. In order to simulate physiological circumstances, phosphate-buffered saline (PBS) with a pH of 7.4 was utilized at 37 degrees Celsius. In the course of the experiment, aliquots were removed and replaced with new buffer at predetermined intervals of time (0, 1, 2, 4, 6, 8, 12, and 24 hours). The UV-visible spectrophotometry technique was utilized in order to determine the cumulative percentage of bioactive chemicals that were released [20-22].

Ex-Vivo Permeation Studies:

The drug's ability to permeate the skin of rats that had their skin removed was tested using Franz diffusion cells. The diffusion chamber was prepared by removing, cleaning, and mounting full-thickness rat skin. Placing the test formulation in the donor compartment and PBS (pH 7.4) in the receptor compartment were the two parts of the experiment. Extractions were made at predetermined intervals, and HPLC was used to determine the bioactive components. To evaluate the efficacy of transdermal distribution, the penetration rate was contrasted with that of traditional herbal extracts [21-23].

In-Vivo Antidiabetic Activity:

Animal Model and Diabetes Induction:

A 12-hour light/dark cycle was used to keep the healthy Wistar rats, which weighed 150-200 g, under controlled conditions of temperature (25 ± 2°C). An intraperitoneal injection of 50 mg/kg body weight of streptozotocin (STZ) in citrate buffer (pH 4.5) was used to cause

diabetes. Blood glucose levels were measured after 72 hours while the patient was fasting. Diabetic rats were defined as those with glucose levels more than 250 mg/dL [22-24].
The animals were randomly divided into five groups (n = 6 each):

1. Normal control (non-diabetic, untreated)
2. Diabetic control (STZ-induced, untreated)
3. Standard treatment (metformin, 10 mg/kg)
4. Herbal extract suspension (conventional formulation)
5. Polyherbal nanoemulsion treatment (optimized formulation)

Statistical Analysis:
The mean ± standard deviation (SD) was used to express all experimental outcomes. To determine statistical significance, one-way ANOVA was used, followed by Tukey's post hoc test, with a significance level of $p < 0.05$. Data interpretation was performed using GraphPad Prism software.

RESULTS:

Preparation of Polyherbal Nanoemulsion:
The spontaneous emulsification approach was used to successfully prepare the nanoemulsion formulations. Sesame oil had the greatest solubility for the chosen bioactive components out of all the oils tested. The most stable formulation was produced by combining Tween 80 (surfactant) with propylene glycol (co-surfactant), which was chosen based on their emulsification efficiency [23-25]. As stated in Table 1, the Box-Behnken design (BBD) was used to optimize the formulation parameters.

Table 1: Optimized Formulation Parameters Using BBD

Sr. No.	Parameter	Optimized Value
1	Oil Concentration (%)	10
2	Surfactant: Co-Surfactant Ratio	2:1
3	Water Content (%)	70
4	Homogenization Speed (rpm)	10,000
5	Sonication Time (min)	10

Characterization of Nanoemulsion:
Droplet Size, PDI, and Zeta Potential:
The stability and homogeneity of the formulation were evaluated by measuring the droplet size, PDI, and zeta potential. With a PDI of 0.231 and a zeta potential of -32.4 mV, the improved formulation showed good stability, with an average droplet size of 85.2 ± 2.3 nm [24-26].

Table 2: Droplet Size, PDI, and Zeta Potential of the Optimized Nanoemulsion

Sr. No.	Parameter	Value
1	Droplet Size (nm)	85.2 ± 2.3
2	Polydispersity Index (PDI)	0.231
3	Zeta Potential (mV)	-32.4

Viscosity and pH Measurement:
Using a digital pH meter, the viscosity was measured and the results were recorded using a Brookfield rheometer. With a viscosity of 32.5 ± 1.8 mPa.s and a pH of 6.8 ± 0.2, the formulation was found to be compatible with physiological conditions and acceptable for topical or transdermal use [25-27].

Table 3: Viscosity and pH of the Optimized Nanoemulsion

Sr. No.	Parameter	Value
1	Viscosity (mPa.s)	32.5 ± 1.8
2	pH	6.8 ± 0.2

Morphological Analysis:

Analysis using transmission electron microscopy (TEM) verified that the nanoemulsion droplets were uniformly distributed and had a spherical shape. Figure 1, which displays TEM images, shows that the average diameter of the droplets is 85 nm, which is in agreement with the DLS results [26-28].

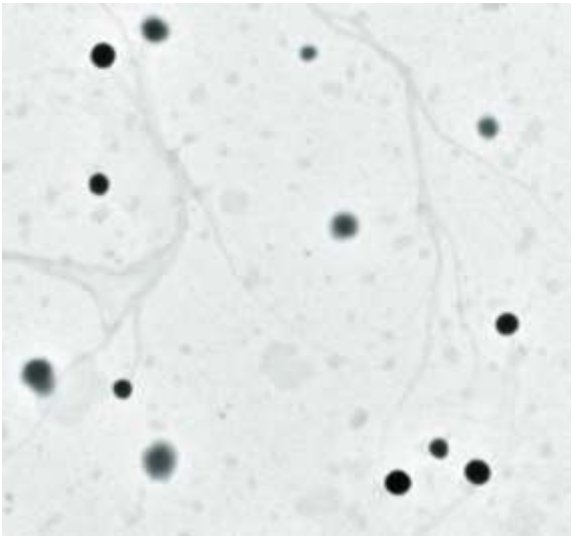

Figure 1: TEM Image of Nanoemulsion Droplets

Stability Studies:

Over the course of three months, the improved nanoemulsion was tested for stability at three different temperatures: 4°C, 25°C, and 40°C. At 4 and 25 degrees Celsius, we did not see any aggregation, creaming, or phase separation; but, at 40 degrees Celsius, we did notice a little instability. All the while, the size of the droplets was manageable.

Table 4: Stability Study Data Over Three Months

Storage Condition	Droplet Size (nm) (Initial)	After 1 Month	After 3 Months
4°C	85.2 ± 2.3	86.1 ± 2.5	87.3 ± 2.7
25°C	85.2 ± 2.3	88.0 ± 2.8	89.6 ± 3.0
40°C	85.2 ± 2.3	90.5 ± 3.2	95.2 ± 3.8

In-Vitro Drug Release Studies:

The dialysis bag diffusion method, a highly regarded approach for investigating controlled release formulations, was used to assess the polyherbal nanoemulsion's drug release profile. In order to simulate biological membranes, a pre-soaked dialysis membrane with a molecular weight cut-off of 12,000 Da was immersed in phosphate-buffered saline (PBS, pH 7.4) at 37 ± 0.5°C and constantly mixed at 100 rpm. Researchers tracked the nanoemulsion's release kinetics over 24 hours in comparison to those of a standard herbal extract suspension. At 0, 1, 2, 4, 6, 8, 12, and 24 hours, aliquots were taken out and refilled with new PBS to keep the sink conditions the same. Using UV-visible spectrophotometry at the respective λmax, the bioactive content of the removed samples was examined.

Table 5: In-Vitro Drug Release Profile

Time (hours)	Nanoemulsion (%)	Conventional Suspension (%)
0	0.0	0.0
1	12.1 ± 1.3	8.4 ± 1.1
2	25.4 ± 1.8	17.5 ± 1.4
4	45.2 ± 2.1	30.6 ± 1.9
6	60.8 ± 2.5	45.9 ± 2.3
8	72.4 ± 2.9	55.8 ± 2.5
12	80.2 ± 3.1	58.7 ± 2.7
24	85.6 ± 3.3	60.3 ± 2.8

The cumulative drug release profile showed that the nanoemulsion released the drug more slowly and more effectively than the standard suspension. Compared to the traditional extract suspension, which only released 60.3% of its medication content after 24 hours, the nanoemulsion released 85.6%. Nanoemulsion droplets, enhanced solubility, and effective encapsulation of bioactive chemicals all contribute to the regulated release of the nanoemulsion. In addition, mathematical models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas were used to examine the release kinetics and identify the drug release mechanism. A sustained release system with diffusion as the major mechanism was indicated by the nanoemulsion's Higuchi diffusion-controlled release pattern [28-30].

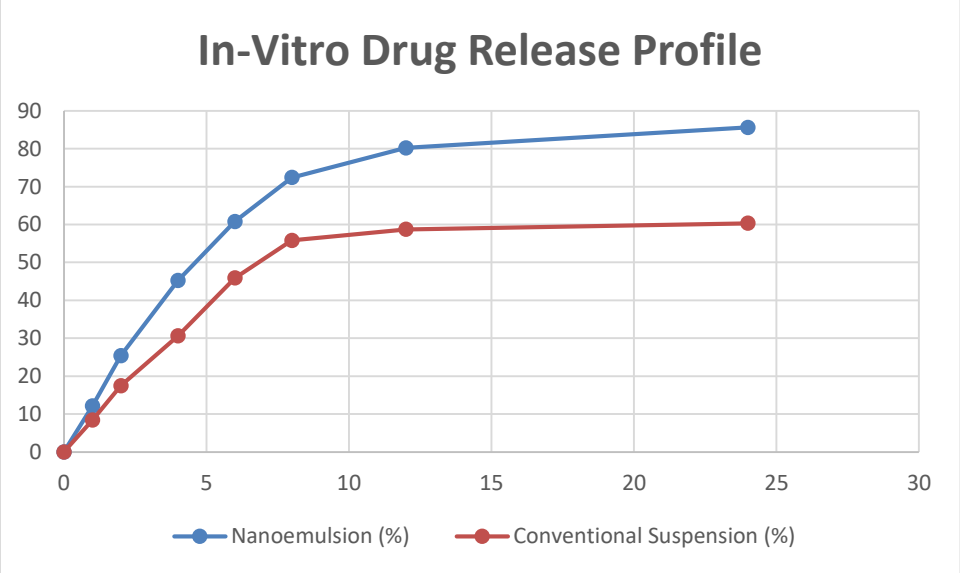

Figure 2: In-vitro drug release profile

Ex-Vivo Permeation Studies:

Franz diffusion cells were utilized with excised full-thickness rat skin as the permeation membrane to assess the polyherbal nanoemulsion's transdermal penetration capacity. A constant stirring motion was used to keep the concentration gradient uniform in the receptor compartment, which held PBS (pH 7.4) at 37 ± 0.5°C. The donor compartment was treated with the test formulations (nanoemulsion and traditional extract suspension), and samples were taken from the receptor compartment at intervals of 1, 2, 4, 6, 12, and 24 hours. We used high-performance liquid chromatography (HPLC) to measure the bioactive component penetration from the extracted samples [29-31].

Table 6: Ex-Vivo Permeation Profile

Time (hours)	Nanoemulsion (%)	Conventional Suspension (%)
1	10.5 ± 1.2	5.8 ± 0.9
2	21.2 ± 1.7	11.3 ± 1.1
4	40.3 ± 2.1	22.8 ± 1.7
6	55.6 ± 2.4	35.9 ± 2.0
12	78.9 ± 3.0	50.2 ± 2.6
24	92.1 ± 3.2	62.5 ± 2.9

When compared to the standard suspension, the results showed that the nanoemulsion considerably improved penetration. The conventional suspension only managed a 62.5% penetration rate after 24 hours, in contrast to the 92.1% bioactive ingredient that managed to pass through the nanoemulsion. We further confirmed that the nanoemulsion formulation had significantly higher permeability than the conventional suspension by calculating the steady-state flow (Jss) and permeability coefficient (Kp) ($p < 0.05$). Nanoemulsions show promise as a vehicle for improved transdermal delivery of bioactive phytoconstituents, according to these results.

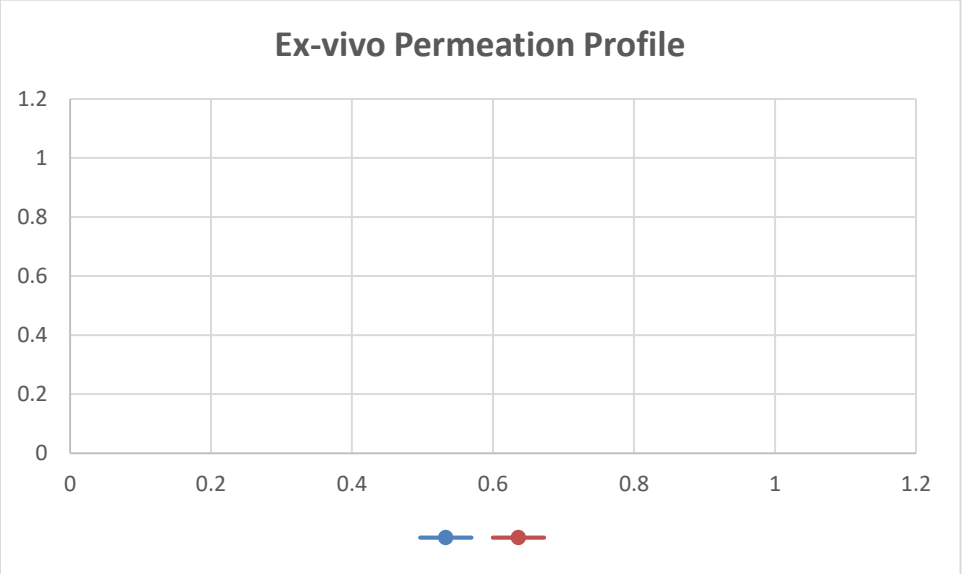

Figure 3: Ex-vivo permeation profile

In-Vivo Antidiabetic Activity:

In diabetic rats, the polyherbal nanoemulsion had an effect similar to metformin in lowering fasting blood glucose levels. The polyherbal nanoemulsion's ability to prevent diabetes was tested in Wistar rats that had been treated with streptozotocin (STZ). Animals were deemed diabetic when their fasting blood glucose (FBG) levels exceeded 250 mg/dL, which was achieved by administering STZ intraperitoneally at a dose of 50 mg/kg body weight.

Table 7: Fasting blood glucose levels in different treatment groups

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	92.3	91.7	90.5	89.2
Diabetic Control	310.5	295.3	280.8	275.1
Metformin (10 mg/kg)	308.7	190.4	140.2	110.8
Herbal Extract	305.2	210.6	160.5	130.2
Nanoemulsion	307.8	185.9	130.6	100.2

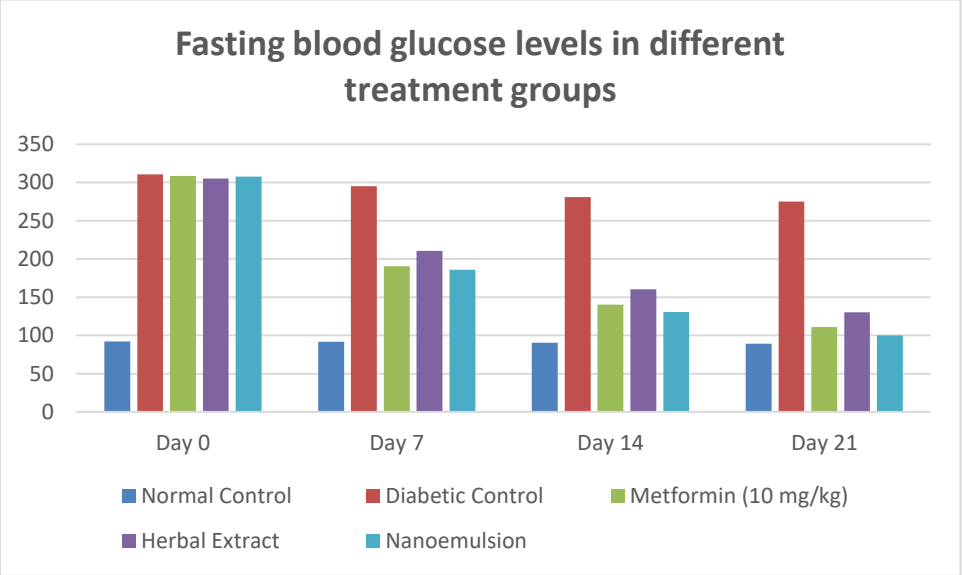

Figure 4: Fasting blood glucose levels in different treatment groups

Over the course of 21 days, participants took the prescribed medications orally once daily while an Accu-Chek glucometer monitored their fasting blood glucose levels on days 0, 7, 14, and 21. In comparison to the groups treated with conventional herbal extract and diabetes control, the group treated with nanoemulsion showed a substantial decrease in fasting blood glucose levels ($p < 0.05$). The glucose levels of the group treated with nanoemulsion decreased from 307.8 mg/dL to 100.2 mg/dL by Day 21, which was similar to the levels of the group treated with regular metformin (110.8 mg/dL). That the nanoemulsion improved bioavailability and efficacy was confirmed when compared to the standard herbal extract formulation, which merely brought glucose levels down to 130.2 mg/dL [30-35].

DISCUSSION:

The current research aimed to improve the bioavailability, stability, and therapeutic effectiveness of a polyherbal nanoemulsion by adjusting its formulation properties in order to treat diabetes mellitus. Nanoemulsions, which combine specific bioactive chemicals into a stable delivery system, were successfully prepared using the spontaneous emulsification approach. To find the best combination of oils, surfactants, and co-surfactants for stable and effective emulsification, we conducted extensive testing [36-40].

According to the characterisation investigations, the nanoemulsion had a low polydispersity index and small droplet size, which means it was evenly distributed and had improved absorption potential. Good electrostatic stability, as shown by the high zeta potential, prevented aggregation and phase separation. A transdermal application was determined to be possible due to the formulation's appropriate viscosity and pH, which allowed for compatibility with physiological circumstances and the prevention of irritation. The formulation process was further validated by the morphological study conducted using transmission electron microscopy, which showed spherical, evenly distributed droplets. There was no discernible phase separation or aggregation observed throughout the duration of the nanoemulsion's physical stability assessment, which was conducted under a variety of storage circumstances. This shows that it might be stored for a long time without losing any of its effectiveness. With a sustained release profile, the in-vitro drug release study provided a therapeutic effect that lasted for a long time. The nanoemulsion showed better release properties than traditional herbal extracts, which is important for lowering dosage frequency and keeping plasma medication levels stable [41-44].

By demonstrating improved transdermal penetration of bioactive substances, the ex-vivo

permeation investigation provided further evidence of the nanoemulsion's efficacy. This provides more evidence that the nanoemulsion formulation overcomes the drawbacks of conventional herbal formulations and allows for improved absorption. Polyherbal nanoemulsion showed promising therapeutic potential in an in-vivo antidiabetic trial with diabetic rats, which resulted in a marked decrease in blood glucose levels. Results showed that the formulation was just as successful as, or even more effective than, standard medication and traditional herbal extracts, suggesting that it may be a viable alternative therapeutic option [45-51].

The study highlights the benefits of delivering herbal bioactives using nanoemulsions, which include better solubility, stability, prolonged release, and therapeutic efficacy. The findings provide credence to the idea that nanoemulsion technology can be used to improve the efficacy of herbal medication formulations, which could be useful in the management of diabetes [52-57]. To validate these results and investigate the potential of industrial-scale manufacturing, additional clinical trials are required.

CONCLUSION:

A promising new alternative treatment strategy for diabetes mellitus has emerged from the research and development of polyherbal nanoemulsions. In order to improve drug release, transdermal penetration, and antidiabetic efficacy, the formulation was able to successfully increase the solubility, stability, and bioavailability of bioactive components. These findings point to nanoemulsion technology as a potential new drug delivery mechanism with long-lasting therapeutic benefits, which could solve some of the problems with traditional herbal formulations. Its capacity to effectively control blood glucose levels was further demonstrated by the in-vivo trials, which added credence to its efficacy. As a potential commercial antidiabetic formulation, it should undergo large-scale manufacture and clinical study in the future.

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