



## Investigation Into the Antidiabetic Effects of a Developed Polyherbal Nanosuspension and Its Assessment

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 10 Oct 2023	<p><i>This study focuses on the development and evaluation of a nanosuspension containing ethanolic extracts of <i>Tinospora cordifolia</i> and <i>Syzygium cumini</i> for managing Diabetes mellitus. The main objective is to create an effective polyherbal nanosuspension by combining <i>Tinospora cordifolia</i> and <i>Syzygium cumini</i> with an optimal concentration of chitosan polymer to address Diabetes mellitus. Furthermore, both in vitro and in vivo assessments of the synthesized nanosuspensions were conducted to determine the best formulation. Methods and Findings: The ethanolic extracts of the mentioned plants were obtained using a maceration technique, followed by preliminary phytochemical screening, HPTLC analysis, and FTIR-based incompatibility assessments. The nanosuspension was prepared using the ionic gelation method by varying the chitosan polymer concentration. Comprehensive in vitro assessments were carried out, including measurements of pH, viscosity, drug content, entrapment efficiency, loading capacity, and in vitro release profiles for different formulations. The formulation with the highest drug content and optimal release characteristics was selected for further analysis of particle size, zeta potential, and surface morphology. Subsequently, the antidiabetic efficacy of the polyherbal nanosuspension was evaluated using wistar albino rats. Discussion: FTIR analysis indicated no significant interaction between the drug and the polymer. The in vitro drug release and kinetic analyses suggested that the F5 formulation exhibited superior drug release and an improved release mechanism. The particle size was determined to be approximately 420nm, and SEM imaging revealed particles that were nearly spherical in shape. Stability assessments of formulation F5 demonstrated consistent physical and chemical parameters over time.</i></p>
CC License CC-BY-NC-SA 4.0	<b>Keywords:</b> <i>Tinospora cordifolia</i> , <i>Syzygium cumini</i> , Nanosuspension, Polyherbals, Antidiabetic activity, Ionic gelation method.

### 1. Introduction

**Diabetes mellitus (DM)**<sup>1,2</sup> encompasses a collection of conditions characterized by elevated blood sugar levels, disruptions in the metabolism of lipids, carbohydrates, and proteins, as well as an increased vulnerability to complications related to vascular diseases. Recognized as a global public health challenge, diabetes mellitus is currently evolving into a widespread epidemic<sup>3</sup>. The worldwide prevalence of diabetes across all age groups was approximately 2.8% in 2000 and is projected to rise to 4.4% by the year 2030<sup>4</sup>. The total number of individuals affected by diabetes is expected to escalate from 171 million in 2000 to 366 million in 2030.

In 2013, the 'International Diabetes Federation' estimated a global prevalence of diabetes at 8.3%. At that time, 382 million individuals were already grappling with diabetes, while an additional 316 million

were at a high risk due to impaired glucose tolerance<sup>5</sup>. This number is steadily increasing and is anticipated to reach 471 million by the year 2035<sup>6</sup>.

The World Health Organization (WHO) defines diabetes mellitus as a metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both, affecting the metabolism of carbohydrates, fats, and proteins<sup>7, 8</sup>. The consequences of diabetes mellitus encompass enduring harm, malfunction, and failure of various organs<sup>9</sup>. Common symptoms of diabetes mellitus include excessive thirst, frequent urination, blurred vision, and unexplained weight loss. Individuals with diabetes are at a heightened risk of numerous complications, including cardiovascular diseases, peripheral vascular diseases, strokes, neuropathy, renal failure, retinopathy, blindness, and amputations<sup>10</sup>.

Diabetes mellitus stands as a global health crisis that persistently affects humanity, regardless of socioeconomic status or geographic location<sup>11</sup>. Approximately one person is diagnosed with diabetes every 5 seconds somewhere in the world<sup>12, 13</sup>. Its pervasive reach emphasizes the critical importance of effectively managing diabetes and its associated complications to alleviate human suffering. Scientists are fervently striving to address this incapacitating disorder, with a significant focus on exploring the potential of medicinal plants<sup>14</sup>. Herbal drugs are gaining traction due to their lesser adverse effects and popularity, especially in developing nations<sup>15</sup>. The growing interest in herbal medicines for diabetes stems from concerns about side effects associated with conventional antidiabetic drugs<sup>16</sup>. Throughout history, herbal remedies and natural products have been utilized to treat a multitude of ailments<sup>17</sup>.

According to the World Health Organization (WHO), 80% of the global population, particularly in developing countries, relies predominantly on plant-derived medicines for their healthcare needs<sup>18</sup>. These plant-based remedies, widely used in indigenous systems like Ayurveda, Siddha, and Unani, form the basis of traditional medicinal practices. Herbal formulations, referring to specific quantities of one or more herbs or processed herb(s), offer nutritional, cosmetic, and therapeutic benefits for the diagnosis, treatment, and mitigation of diseases in humans and animals<sup>19</sup>. Herbalism, deeply rooted in traditional medicinal practices and folk medicine, revolves around the utilization of plants and plant extracts<sup>20</sup>.

The concept of polyherbalism is challenging to define within modern parameters. Historically, the traditional literature, such as the "Sharangdhara Samhita," sheds light on the synergistic approach underlying polyherbal formulations<sup>21</sup>.

## 2. Materials And Methods

### Formulation Of Polyherbal Nanosuspension<sup>22</sup>

#### Preparation of Chitosan (CS) Nanosuspension<sup>23,24</sup>:

The nanosuspension was created using the ionic gelation method, which involves an ionic interaction between positively charged CS solution and negatively charged TPP solution. Chitosan nanoparticles were prepared based on a previously reported method with minor adjustments. Initially, chitosan flakes were dissolved in a 1.5% glacial acetic acid solution, resulting in a chitosan solution ranging from 0.1-0.7% w/v. Simultaneously, STPP, the cross-linker, was dissolved in distilled water at a concentration of 2mg/ml. Herbal drugs were directly dissolved in TPP solution. Next, 40ml of TPP solution (2mg/ml) containing the drug combination was carefully added drop by drop into 100ml of CS solution while employing magnetic stirring (1000rpm) at room temperature. To stabilize the suspension, 10% PEG solution was slowly introduced, and 0.05% sodium benzoate was added as a preservative. The resulting chitosan suspension was sonicated at 25°C for 90 minutes to achieve a nano-sized particle range.

**Table 1:** Working formula for nanosuspension (F1-F7)

Formulations	Extract (mg)		CS %w/v	Glacial acetic acid %v/v	STPP (mg/ml)	PEG %v/v	Water	Sodium benzoate (%w/w)	Agitation time (min)
	TC	SC							
F <sub>1</sub>	100mg	100mg	0.1	1.5	2	10	qs	0.05	90
F <sub>2</sub>	100mg	100mg	0.2	1.5	2	10	qs	0.05	90
F <sub>3</sub>	100mg	100mg	0.3	1.5	2	10	qs	0.05	90

<b>F<sub>4</sub></b>	100mg	100mg	0.4	1.5	2	10	qs	0.05	90
<b>F<sub>5</sub></b>	100mg	100mg	0.5	1.5	2	10	qs	0.05	90
<b>F<sub>6</sub></b>	100mg	100mg	0.6	1.5	2	10	qs	0.05	90
<b>F<sub>7</sub></b>	100mg	100mg	0.7	1.5	2	10	qs	0.05	90

#### SEM Analysis of Chitosan Nanoparticles<sup>25, 26</sup>:

Surface morphology of chitosan nanoparticles was analyzed using Scanning Electron Microscopy (SEM) JSM 6390 (Hitachi Pvt.). Gold-coated thin films of the sample were prepared on an aluminum grid by applying a small amount of the sample and removing excess solution with blotting paper. The SEM grid with the sample was dried under a mercury lamp for 5 minutes. SEM analysis was conducted at an accelerating voltage of 15 kV and a magnification of  $\times 20,000$  in transmission electron mode.

#### ZETA POTENTIAL Analysis<sup>27</sup>:

Zeta potential distribution was assessed using a zetasizer (Nano ZS, Malvern Instruments, UK). To obtain accurate measurements, each sample was appropriately diluted five times with filtered, distilled water and then placed into a disposable zeta cell. The zeta potential values were recorded within the zeta limits of -200 to +200 mV. Electrophoretic mobility (expressed in  $\mu\text{m/s}$ ) was converted to zeta potential using the Helmholtz-Smoluchowski equation through the inbuilt software. The zeta potential for each sample was derived from the average of three measurements.

#### PARTICLE SIZE ANALYSIS – Dynamic Light Scattering (DLS)<sup>28</sup>:

Particle size distributions of the nanoparticles were determined using a particle size analyzer (DLS), specifically the Malvern Zetasizer Nano ZS (Malvern, Worcestershire, UK). The sizes of the particles were determined by analyzing the time-dependent fluctuations in laser light scattering caused by the nanoparticles.

### PHARMACOLOGICAL EVALUATION

#### Animal Model and Details:

Animals utilized for the study were Wistar albino rats, weighing between 150-200g. The gender was not restricted, including both sexes, and a total of 27 rats were used.

#### Acute Toxicity Studies:

Acute toxicity studies were conducted in accordance with the Organisation for Economic Co-operation and Development guidelines (OECD)-No. 423 (2001) for acute toxic classic method. Three Wistar albino rats were used for each step of this study. The animals were fasted overnight with access to water only, and the polyherbal nanosuspension was orally administered at different doses: 5mg/Kg b.w, 50mg/Kg b.w, 300mg/kg b.w, and 2000 mg/kg b.w. The rats were closely monitored for 4 hours following administration and then once daily for 14 days to observe their behavior and mortality. As per OECD guidelines 423, if mortality occurred in two out of three animals at any dose, it was considered a toxic dose. However, since no mortality occurred, the procedure was repeated for a higher dose of 2000 mg/kg b.w. The maximum tolerated dose was found to be 2000 mg/kg b.w, and one-tenth of this dose (200 mg/kg b.w) was selected for evaluating the antidiabetic effect of the polyherbal nanosuspension<sup>29</sup>.

#### Antidiabetic Screening<sup>30</sup>:

#### Experimental Induction of Diabetes:

Diabetes was induced in overnight-fasted rats by administering a single intraperitoneal (i.p.) injection of freshly prepared alloxan monohydrate at a dose of 35mg/kg body weight in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg body weight. Alloxan induces diabetes by damaging the insulin-secreting cells of the pancreas, resulting in hyperglycemia. After alloxan treatment, all animals were given free access to food and water. The induction of diabetes was confirmed by measuring fasting blood glucose levels 2 days after alloxan administration. Rats with fasting blood glucose levels exceeding 200 mg/dl were considered diabetic and used for the experiment.

Experimental Design<sup>31,32</sup>:

The animals were randomly divided into four groups, each containing six animals.

Group 1: Normal control (maintained on regular rat food and drinking water)

Group 2: Diabetic control group

Group 3: Reference standard treatment group (Glibenclamide, 0.5 mg/kg bw)

Group 4: Polyherbal nanosuspension (F5 - 200mg/kg bw) administered orally at 200 mg/kg body weight. Food and water were withheld for 1 hour after drug administration.

Blood Glucose Level Testing:

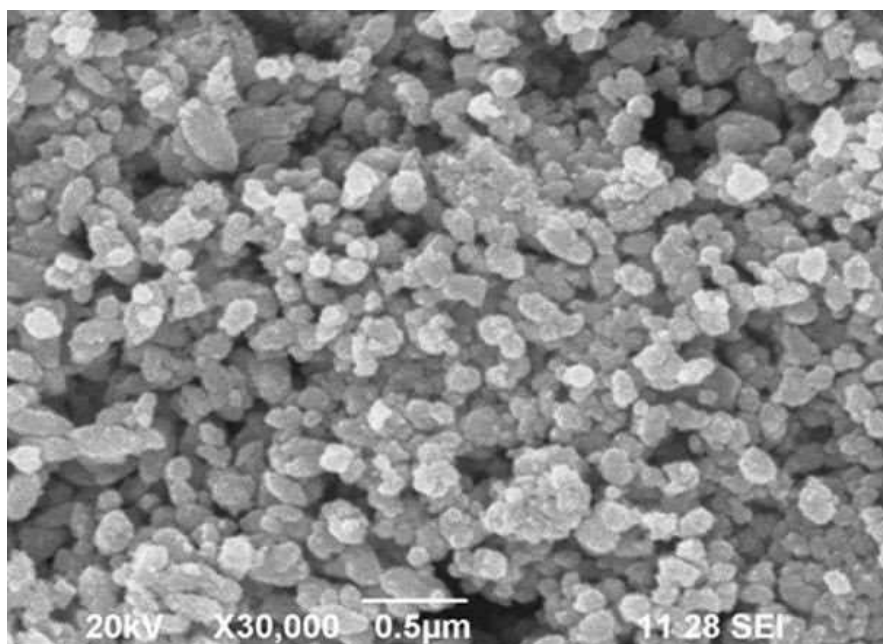
Blood samples were collected on the 0th day (before treatment), 7th day, and 14th day of the treatment through the tail vein of rats and were immediately used for blood glucose estimation with a glucometer. Results were expressed in milligrams per deciliter (dl) of blood.

Statistical Analysis<sup>33</sup>:

All values were presented as mean  $\pm$  SEM (standard error mean) for the specified number of animals. Statistical analysis was performed using PRISM software package (version 5.0). The statistical significance of differences between the control and experimental groups was assessed using One-way ANOVA followed by Dunnett's Multiple Comparison Test. A probability value less than 5% ( $P < 0.05$ ) was considered statistically significant.

### 3. Results and Discussion

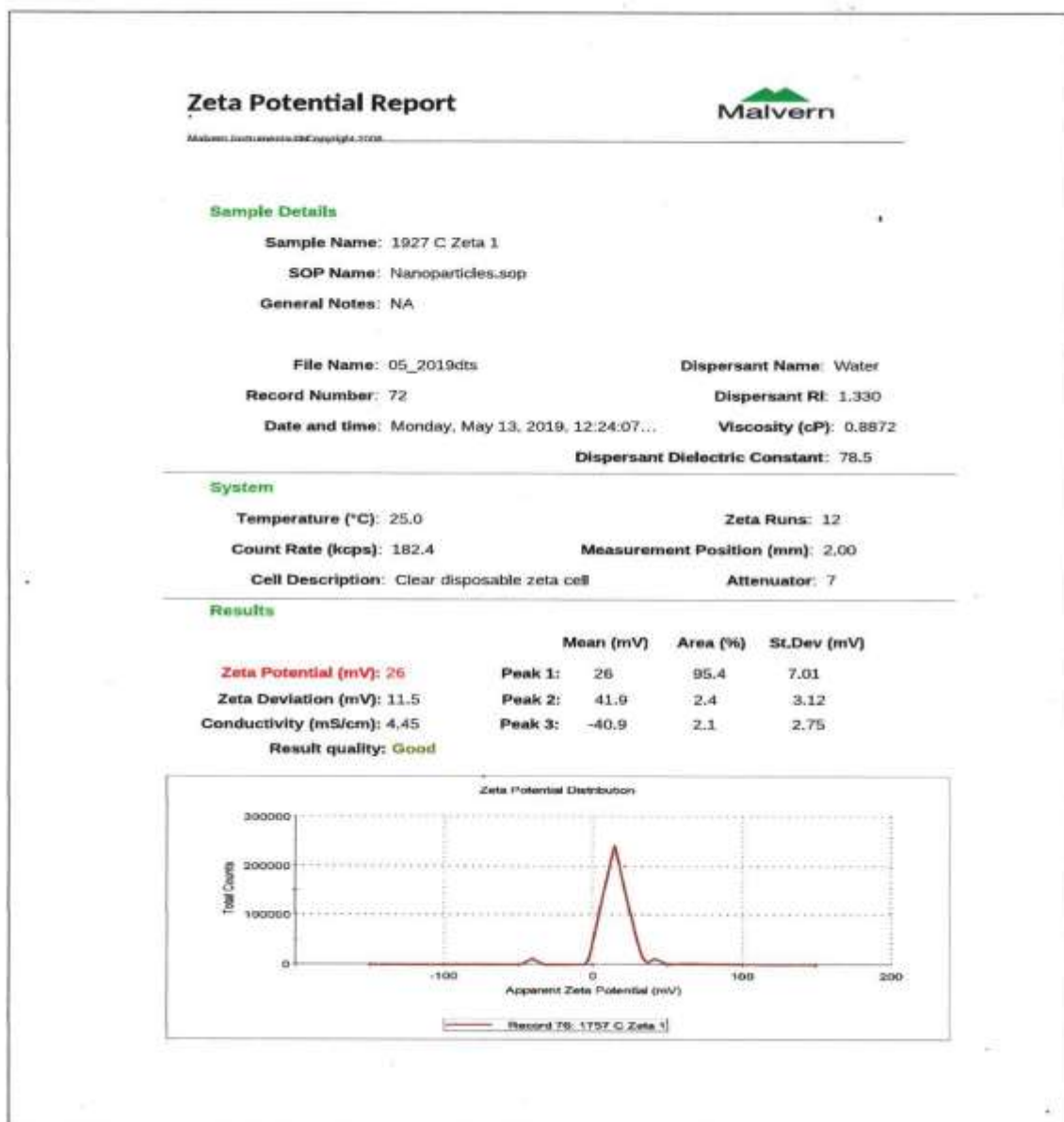
#### Surface Morphology- Sem



**Figure 1:** SEM image of formulation F5

The surface morphology of selected formulation F6 was studied using SEM.

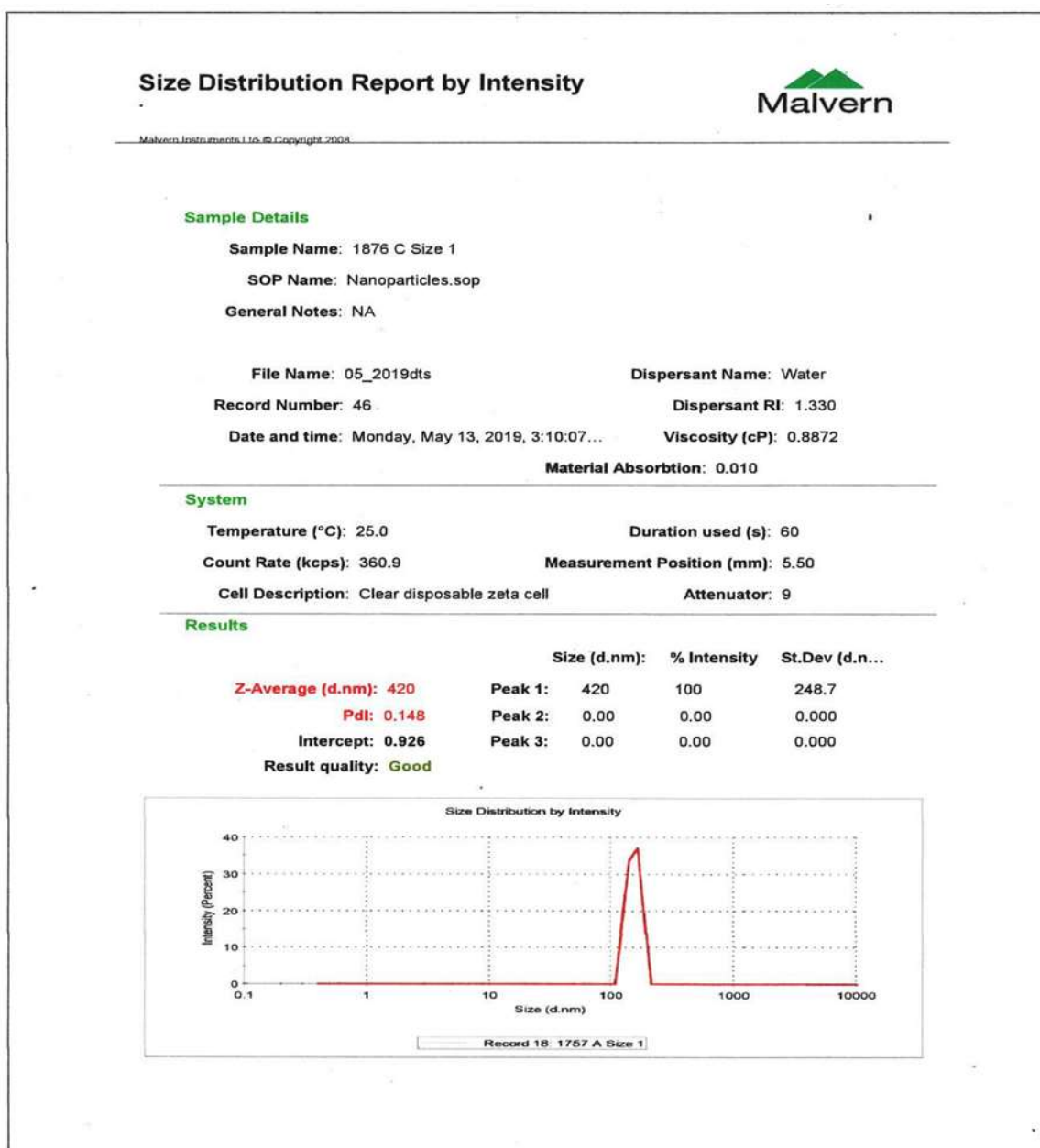
## ZETA POTENTIAL



**Figure 2:** Zeta potential report using DLS

The zeta potential of formulation F5 was found to be +26 mV. The zeta potential measures the surface charge of the particles.

## PARTICLE SIZE ANALYSIS- DLS



**Figure 3:** Size distribution report by intensity of F5

The average particle size of formulation F5 was found to be 420 nm.

### **IN VIVO STUDIES**

#### **Acute toxicity studies**

The rats administered with herbal nanosuspension up to 2000 mg/kg did not show any abnormal behaviour, during initial 4 hours after the drug administration. No mortality was observed during 14 days after the treatment. According to the OECD guidelines for the acute toxicity studies, an LD50 dose of 2000 mg/kg and the above is categorized as unclassified and hence the drug is found to be safe. Hence one by tenth of this dose was selected for the evaluation of antidiabetic effect of polyherbal nanosuspension, i.e 200 mg/kg body weight.

**Antidiabetic screening – Effect of formulation on blood glucose level of diabetic rats**

**Table 2:** Antidiabetic screening of F5

Time period (Days)	Blood glucose level (mg/dl)			
	Normal control	Diabetic control	Standard (0.5 mg/kg)	F5 (200 mg/kg)
0	84.83±4.44	290.83±8.13	289.66±3.87	286.16±5.18
7	86.66±5.00	286.16±6.33	177.5±4.76	191±3.84
14	84.83±4.16	284±7.07	112.66±4.58	126.16±5.63

Blood glucose level was initially estimated 72 hours after alloxan administration and on 7th and 14th day of treatment with formulation for all animals. Blood glucose levels were expressed as mg/dl and were given in mean. The effect of formulation on blood glucose level in alloxan induced diabetic rats has shown in table 31. The administration of alloxan led to elevation of fasting blood glucose levels, which was maintained over a period of study in diabetic control group and 14 days of daily treatment with formulation led to fall in the blood glucose level. There was a significant elevation in blood glucose level in alloxan induced diabetic control rats when compared to normal control. An oral treatment with formulation group was able to reduce blood glucose level significantly as compared to diabetic control. All values were expressed as Mean±SEM, n=6, p<0.05 Compared to control.

The extracts of *T.cordifolia* and *S.cumini* were formulated into 7 different nanosuspensions by altering the concentration of chitosan polymer and were prepared by Ionic Gelation Method. PEG was added as stabilizer, STPP as cross-linking agent and Sodium benzoate as preservative.

The selected formulation F5 was subjected to particle surface morphology using SEM, particle size and charge distribution using DLS and zeta potential.

**4. Conclusion**

Finally, the pharmacological evaluation of the selected F5 formulation showed that the antidiabetic activity was comparable with that of standard formulation. It was finally concluded that the formulation F5 was found to be more promising formulation as it shows better physicochemical characteristics and higher pharmacological activity compared to other formulations.

From the results it can be concluded that ethanolic extracts of *Tinospora cordifolia* and *Syzygium cumini* when formulated as nanosuspension shows significant improvement in Diabetes Mellitus.

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