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RESEARCH ARTICLE
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COMPARATIVE EVALUATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF CLEMATIS TRILOBA AND THEIR SMEDDS FORMULATION IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

Objectives: The present study aimed to evaluate and compare the antidiabetic activity of ethanolic leaves extract of *Clematis triloba* and their SMEDDS formulation with Glibenclamide (GL) by α-amylase, α-glucosidase enzyme inhibitory assay and streptozotocin induced diabetic rats.

Methods: Using the 3,5-dinitrosalicylic acid method and 4-Nitrophenyl--D-glucopyranoside, respectively, the α-amylase inhibition and α-glucosidase enzyme inhibitory activities of *Clematis Triloba* extracts were assessed. In several animal models, including hypoglycemic, oral glucose-loaded, and streptozotocin-induced diabetic rat models, the extract and SMEDDS formulation ability to reduce levels of blood glucose was investigated.

Results: The enzymes α-amylase and α-glucosidase were significantly inhibited by the *clematis triloba* ethanolic extract in a dose-dependent manner. When compared to an ethanolic extract, the SMEDDS formulation of *clematis triloba* showed greater antidiabetic efficacy.

Conclusion: The current research suggests that *clematis triloba's* ethanolic leaf extracts have *in vitro* and *in-vivo* anti-diabetic action. This study showed that these herbs have great therapeutic efficacy in treating diabetes mellitus and its accompanying problems, hence proving their traditional applications in Indian traditional medicine.

Keywords: - antidiabetic, α-glucosidase, streptozotocin, SMEDDS, *Clematis Triloba,* α-Amylase.

INTRODUCTION

One of the most common endocrine illnesses, diabetes mellitus is characterized by chronic hyperglycemia with changes in protein, carbohydrate, and lipid metabolism as well as glycosuria, ketosis, and acidosis as a result of deficiencies in insulin production or action, or both.

People are affected by diabetes mellitus (DM) and its effects, which have a significant socioeconomic impact, in both developing and developed countries. A severe, ongoing, and complicated metabolic disease with several aetiologias is diabetes mellitus (DM). Diabetes can also be referred to simply just diabetes. According to estimates, this disease affects 25% of the world's population.[1] Diabetes is a complex aetiology that involves both hereditary and environmental

factors. When diabetes is developing, the body's cells are unable to metabolise sugar in an efficient manner because insulin (a peptide hormone that controls blood glucose) does not operate on target tissues effectively or is insufficiently sensitive to them.[2,3] Diabetes can be identified by chronic hyperglycaemia and modifications in macromolecule metabolism brought on by deficiencies in insulin synthesis, action, or both. If one or both of these signs appear, diabetes can be distinguished from other diseases. Diabetes can cause long-term harm to a variety of organ systems, including the heart, blood vessels, eyes, kidneys, and nerves, which can lead to disability and early death.[4] The degree of the disease that hyperglycaemia causes to the various organ systems may be related to the illness's severity and the extent of therapy. Low body weight, dry lips, frequent urination, hazy vision, and frequent urination are all signs of diabetes.[5,6]

Along with the rise in diabetes diagnoses, the incidence of risk factors for the disease, such as being overweight or obese, is rising. The World Health Organisation (WHO) estimates that diabetes will overtake heart disease as the sixth biggest cause of death by 2030, which only makes the situation worse. Despite extensive study, the incidence and prevalence of diabetes have been rising worldwide, with tropical developing countries suffering a disproportionately heavy burden.[7] According to population studies, developing nations will have 82 million more adults aged 64 and older with diabetes by 2030 than industrialised nations.[8] 90% of people with diabetes have Type 2 DM, which is easily avoidable and controlled compared to Type 1 DM, which cannot be avoided with the knowledge we presently possess. Among people who have the condition, Type 2 DM accounts for the majority of instances. Due to the complexity and interdisciplinary nature of diabetes care, the emphasis on primary diabetes prevention should be placed on the promotion of a healthy diet and way of life (such as exercise). Two crucial therapeutic facets that are crucial in the management of type 2 diabetes are diet and exercise. The aim of treating normolipidemic and normoglycemic conditions may be attained and maintained by using these together.

It is vital to utilise herbal products with antidiabetic potential since oral hypoglycaemic drugs (OHAs) occur in a variety of forms and the cells might develop resistance to them.[9] Numerous Phyto molecules from plants have been found and their potential to treat diabetes studied in order to achieve this. In vivo, certain Phyto molecules may not function at all or only partially, while having considerable therapeutic efficiency in vitro. It could not be able to cross the biological membrane or have poor solubility/dispersion in the gastrointestinal tract (GIT), leading to poor bioavailability.[10] Clematis *triloba* is a sprawling climber, characterized by leaves that are 1-2 inches in size, possessing a small, silky texture, and arranged in a simple or one-ternate pattern and a member of the Ranunculaceae family. The *clematis triloba* plant is commonly distributed in the Deccan region and is also prevalent in areas such as Konkan, Kartrizghat near Pune, Dongargaon near Ahmednagar, hills near Pune, and the Mawal and Nashik districts of Maharashtra. The dried stem of *clematis triloba* is characterized by a light brown colour, displaying ridges and furrows along with the presence of bark and a fibrous interior. According to studies, the Phyto molecules in *Clematis triloba* have a low water solubility, which has a negative impact on their oral bioavailability, causes a high level of inter- and intra-subject variability, and prevents them from being dose-proportional. Additionally, it stimulates GLUT4, which helps the cells absorb and use glucose. Formulation techniques need to be emphasised in order to increase the bioavailability of inert compounds with poor water solubility and, moreover, to produce some more potent therapeutic effects. Self-micro emulsified formulation is a technique for increasing the absorption of poorly soluble Phyto molecules due to their lipidic content and small particle size.[11,12]

SMEDDS

The components of a self-micro emulsified formulation, which can be solid or liquid, are co-surfactant, oil/lipid, surfactant, and co-soluble extract or Phyto molecule. When substances are ingested, the gastrointestinal system dilutes them in aqueous media to produce oil-in-water microemulsions and nano emulsions with particle sizes ranging from 100 to 500 nm.[13-15]

One of the difficulties a formulator faces when developing an oral dosage form is getting the medication to dissolve in the GI system. SMEDDS quickens and broadens drug absorption. The SMEDDS method is used to treat drugs in BCS Class II that have low water solubility and bioavailability.[16,17] The delivery of these drugs as lipids enhances their bioavailability by skipping the absorptive barrier of lower water solubility and shifting to the bile-salt mixed micellar phase, where absorption happens swiftly. The medication's characteristics, such as its log P and water solubility, are unsatisfactory because they cannot be utilized to predict the effects in vivo. In the SMEDDS formulation, little, positive, or even negative free energy may be needed for the production of an emulsion. Emulsification consequently happens suddenly. For emulsification to take place, the interfacial structure must not exhibit any resistance to surface shearing. The simplicity of emulsification may be due to water's ease of penetration into different liquid crystalline or gel phases on the droplet surface.[18-21]

In this study, the anti-diabetic effectiveness of a self-micro emulsifying formulation of *Clematis triloba* extract (SMEDDS CTLE) was compared to that of pure CTLE extract, and an animal model of diabetes caused by streptozotocin was used for evaluation. The enhanced formulation's pharmacological effectiveness in treating diabetes in test animals was evaluated.

METHODOLOGY

Collection and Authentication Plant Materials

The Fresh leaves of the *Clematis triloba* naturally growing plant belonging to the *Ranunculaceae* family was extensively collected from the Nashik District of Maharashtra, and Dr. Sayyad I.G., Head of Botany department, Gandhi college, Kada, Maharashtra, India, recognized and authenticated the plant material. The herbarium sheet of plant was submitted in the department. Shade drying was precautionary measure followed to avoid the destruction of active principles. For further investigation, the plant samples were air dried at room temperature.[22]

Preparation of plant extract

Fresh *clematis triloba* plant leaves were washed three times, once with tap water, once with distilled water, before being air dried for 15 to 20 days at room temperature in the shade. The dried leaves that had been mechanically pulverized were sieved using sieve No. 10/44. A batch of 250g of the powdered *Clematis triloba* leaf material was extracted for 72 hours with 80% ethanol after being placed individually in a one-liter thimble of a Soxhlet device. Each and every one of the extracts was vacuum-filtered and concentrated using a rotary evaporator. Before being completely dried in desiccators, the residual solvent was properly removed on a water bath.[22]

Qualitative phytochemical analysis

The obtained *Clematis Triloba* leaves extracts were submitted to conventional preliminary phytochemical analytical procedures. The extract was tested for the presence or absence of a number of active ingredients, including tannins, phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, and alkaloids.[22-29]

Animal care and selection

Healthy albino Wister rats, weighing between 150-200 g and aged 8-12 weeks, of both sexes and approximately the same age, were sourced from Yashoda Technical Campus, Animal House, Satara, MH, India. The animals were housed in polypropylene cages, maintained under standard conditions with a 12-hour light-dark cycle, and provided ad libitum access to pellet food and water. Prior to the commencement of the experiment, the rats were acclimatized to the laboratory environment following random group assignments.

Conforming to the worldwide criteria set by the Organization for Economic Cooperation and Development (OECD), the rats received care in accordance with ethical guidelines. A minimum fasting period of 12 hours was observed before the initiation of each activity. Approval for the

research protocol was granted by the Institutional Animal Ethics Committee (YSPM/YTC/PHARMA-IAEC/2021-22/16) after thorough scrutiny, following the guidelines of the committee for the purpose of control and supervision of experimental animals (CPCSEA).

Acute toxicity study

The acute toxicity studies were conducted following the guidelines of OECD recommendation 423, utilizing the Acute Toxic Class technique. Prior to the administration of the test medication, animals underwent an overnight fasting period. Ethanol extract of Clematis triloba was orally administered at doses of 50 mg/kg, 300 mg/kg, 500 mg/kg, and 2000 mg/kg. Subsequently, food deprivation was enforced for 3 to 4 hours. The experimental rats were observed during the first 30 minutes post-treatment, followed by a 4-hour interval, and then daily throughout the 14-day trial period. The rats were closely monitored for behavioral changes and potential signs of mortality.[30,31]

In-vitro Antidiabetic Activity

Assessment of α-Amylase Inhibitory Activity

Using the 3,5-dinitrosalicylic acid (DNSA) technique, the α-amylase inhibition test was carried out. Amylase (10 mL), phosphate buffer (50 mL, 100 mM, pH=6.9), and plant extract (20 mL, 20–100 µg/mL) at varied quantities are combined. The substrate was then added, and the mixture was incubated at 37°C for 30 minutes with 20 µL of 1 percent soluble starch (in phosphate buffer, 100 mM, and pH 6.8). Following that, 100 µL of di-nitro salicylic acid (DNSA) reagent was added to the mixture, and it was then given 10 minutes to boil. Acarbose absorbance at 540 nm was measured using a UV-visible spectrophotometer at the same concentration range of 20-100 µg/ml.[32] The following equation was used to determine the amount of α-amylase inhibition, which was represented as a percentage of inhibition:

Percentage inhibition (%) = $A_{control} - A_{extract} / A_{control} \times 100$

Where A extract is the absorbance of extract and A control is the absorbance of control at 540 nm. The IC_{50} values were calculated from the graph by plotting the percentage of α-amylase inhibition against the extract concentration.

Assessment of α- glucosidase Inhibition Activity

Ethanolic leaves extract of *clematis triloba* was tested for α-glucosidase inhibitory action as standard procedure. In sodium phosphate buffer (pH 6.9), a reaction mixture containing 2.9 mM 4-Nitrophenyl--D-glucopyranoside (pNPG), 0.25 ml of each sample, and 6U/ml α-Glucosidase was created. 20 minutes at 37^0C were spent incubating this reaction mixture. After 5 minutes, the absorbance was measured at 405 nm.[33] In the absence of the test ingredient, the solution was set up as the control (blank), and each absorbance was measured in triplicate using the reference medication acarbose (20–100 µg/ml). The % inhibition was calculated using the calculation shown below:

Percentage inhibition (%) = $A_{control} - A_{extract} / A_{control} \times 100$

Where, $A_{control}$ is the absorbance of control and $A_{extract}$ is the absorbance of extract at 405 nm.

The inhibitory concentration (IC50) value is the concentration of an inhibitor needed to block 50% of the activity of the drug being tested. The results, which were all calculated in triplicate, are all provided as the mean standard error of the mean. Additionally, an IC_{50} value is provided for the result. To calculate the IC_{50}, regression analysis was employed.

In-vivo Antidiabetic Activity

Grouping and dosing of animals

Male rats were chosen for all rat models (hypoglycemic, oral glucose-loaded, and STZ-induced diabetes model) since they are more susceptible to STZ than female rats. Rats (n = 6) were divided randomly into 6 groups of 6 rats each.

A negative control group (group I), which received distilled water; a positive control group (group II), which received glibenclamide 5 mg/kg (GL 5 mg/kg); and groups III and IV, which received two different doses of *clematis triloba* ethanolic extract (CTLE) were used in the hypoglycemic and oral glucose loaded animal models. Group V and VI received 2 different doses of SMEDDS formulation of CTLE.

In accordance with OECD guidelines, 1 mL/100 g of the rat's body weight was delivered as the extract dosages, which were chosen based on an acute toxicity study. For this investigation, a lower dose of 100 mg/kg was used; the greater dose was 200 mg/kg, which was twice as much as the lower amount. Based on past trials, glibenclamide was chosen as the study's standard medication. Due to the fact that individuals often consume plant materials by oral means, the study was carried out utilizing this method of delivery.[34-36]

Blood Glucose Level of Hypoglycemic Rat

A well-fed and typical rat underwent a 14-hour period of food deprivation while having unrestricted access to water. Subsequently, the rat was randomly allocated into six groups, each consisting of six rats. Oral gavage was employed to administer both vehicles and medications. Blood samples were aseptically collected from the tail tips of each rat to assess baseline blood glucose levels (BGL) immediately before treatment (at 0 hours) and at 1-, 2-, and 3-hours post-treatment.[37]

Blood Glucose Level After Oral Glucose Tolerance Test (OGTT)

Rats, who exhibited better insulin sensitivity (insulin-stimulated glucose utilization) than humans and were used for the oral glucose tolerance test (OGTT) after a 14-hour fast, were used in the study. After the rats were split into groups and received the aforementioned treatments, the initial blood glucose level was determined. Each mouse was given a 2 g/kg oral glucose solution following a 30-minute extract administration. Immediately prior to treatment (at 0 minutes) and at 0-, 30-, 60-, and 120-minutes following glucose injection, each animal's blood sugar level was measured as a baseline.[38]

Induction of Experimental Diabetes

Rats were treated with an intraperitoneal injection of STZ, 50 mg/kg body weight, diluted in 0.1 M sodium citrate buffer, pH 4.5. They are free to acquire food and water following the injection. In order to reduce hyperinsulinemia-related mortality, rats were given 1 ml/kg of 5% glucose to drink. After 48 hours from the streptozotocin injection, the onset of diabetes was established. Animals were chosen for this investigation if their fasting blood glucose levels were greater than 250 mg/dl but less than 350 mg/dl after receiving STZ for 48 hours.[39-46]

Experimental design

For the study, a total of 7 groups of animals (n = 6 animals) were chosen.:

1. Group I: Normal Control Group (Saline)
2. Group II: Diabetic Control (Streptozotocin, 50 mg/kg, i, p.)
3. Group III: Positive Control (STZ + Glibenclamide treated group)
4. Group IV: STZ + CTLE (100 mg/kg)
5. Group V: STZ + CTLE (200 mg/kg)
6. Group VI: STZ + SMEDDS formulation of CTLE (100 mg/kg)
7. Group VII: STZ + SMEDDS formulation of CTLE (200 mg/kg).

Statistical data analysis

The data were analyzed using one-way analysis of variance (ANOVA), and Dunnett's test was carried out using GraphPad Prism (GraphPad Software). The data was shown as mean ± SEM. The P value was calculated for determining statistical significance.

RESULT AND DISCUSSION

study on phytochemicals of the ethanolic leaves extract of *clematis triloba* (CTLE).

The phytochemical investigation of *clematis triloba* plant by ethanol extract showed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, saponins and triterpenoids.

Table 1: Phytochemical studies of CTLE.

Sr. No.	Phyto-constituents	Tests	CTLE
1	**Carbohydrates and Glycosides**	Fehling's test	+
		Molisch's test	+
		Borntrager's test	-
		Barfoed's test	+
2	**Proteins and Amino Acids**	Biuret Test	-
		Million's test	-
		Ninhydrin test	-
3	**Alkaloids**	Dragendroff's test	+
		Mayer's test	+
		Wagner's test	+
		Hager's test	-
4	**Sterols**	Liebermann-Burchard test	-
		Salkowski's test	-
5	**Tri-terpenoids and saponins**	Liebermann-Burchard test	+
		Foam test	+
6	**Flavones and flavonoids**	Aq. NaOH	+
		Conc.H_2So_4	+
		Shinoda test	-
7	**Phenols and Tannins**	$FeCl_3$ test	+

+ Indicates Presence, - Indicates Absence

In-vitro Pharmacology studies

α-Amylase Inhibitory action of CTLE

Clematis triloba ethanolic leaf extract exhibits the suppression of the α-amylase enzyme. Comparing the IC_{50} value of the plant extract to the reference (acarbose) demonstrates the considerable effect (Table 2). According to the current observation, the plant may be helpful in the treatment of type 2 diabetes.

Table 2: Inhibitory action of CTLE by α-Amylase Inhibitory activity.

Sr. No.	Concentration (µg/ml)	Percentage Inhibition at different conc. (µg/ml)	
		CTLE	Acarbose (Standard)
1.	20	12.25±0.58	15.48±0.32
2.	40	21.18±1.96	26.67±1.07
3.	60	30.63±0.27	38.44±1.41
4.	80	41.26±1.79	50.18±0.68
5.	100	50.74±1.43	62.37±1.09
6.	**IC_{50} (µg/ml)**	79.38±1.57	98.71±1.31

Values are expressed in mean± S.D.; each test was tested in triplicate (n =3).

The activity of CTLE over inhibition of α- amylase enzyme was in depended over the concentration as it increases the action also increases up to maximum limit (as shown in Table 2).

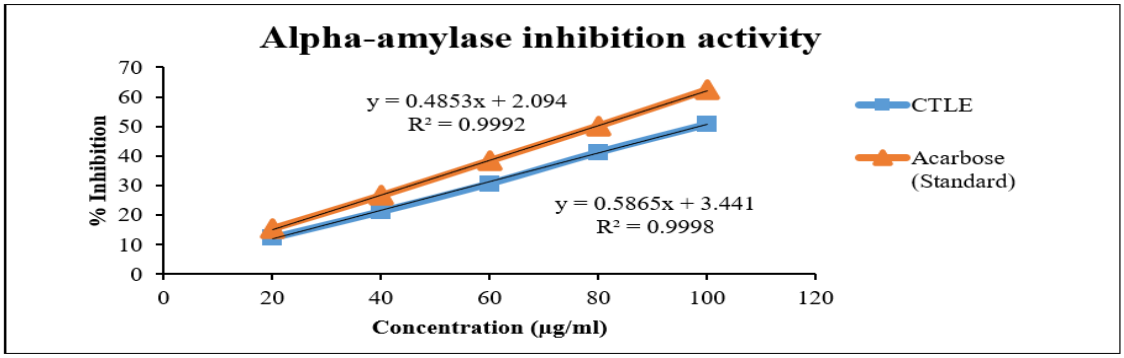

Figure 1: Percentage inhibition in α-Amylase Inhibition activity of CTLE at different concentration.

α-Amylase inhibitors are crucial in lowering the rise in blood sugar levels after meals. Acarbose, one of its inhibitors, prevents the release of glucose into the blood, preventing diabetes. At a dose of 100 g/ml, the percentage inhibition of α -amylase inhibitory for CTLE is 50.74±1.43%, which is low compared to the conventional acarbose (62.37±1.09%) (figure 1). This demonstrates that CTLE inhibits α-amylase less effectively than acarbose.

Alpha-glucosidase inhibitory activity of CTLE

Table 3 illustrates the % inhibition of α-glucosidase inhibitory action of *clematis triloba* (CTLE) Standard and ethanolic leaves extract. When compared to the ethanolic leaf extract of *Clematis triloba*, which had a percentage of α-glucosidase inhibition of 64.28±0.85%, the standard (acarbose) had a higher percentage at 71.24±1.38%. The calculated IC50 values are 73.68±1.89 for CTLE and 60.59±1.76 for acarbose. It has been observed that clematis plant extract exhibited more IC50 value as compared to Standard (Fig 2). CTLE has significantly more anti-diabetic potential than the anti-diabetic medicine acarbose, which may suggest using plants as possible natural anti-diabetic drugs.

Table 3: Inhibitory action on α-glucosidase by CTLE.

Sr. No.	Concentration (µg/ml)	% Inhibition at different conc. (µg/ml)	
		CTLE	Acarbose (Standard)
1.	20	20.42±0.37	29.44±0.72
2.	40	31.96±1.45	39.28±1.24
3.	60	43.21±1.24	49.18±0.37
4.	80	52.93±0.96	59.32±0.29
5.	100	64.28±0.85	71.24±1.38
6.	IC_{50} (µg/ml)	73.68±1.89	60.59±1.76

Values are expressed in mean± S.D.; each test was tested in triplicate (n =3).

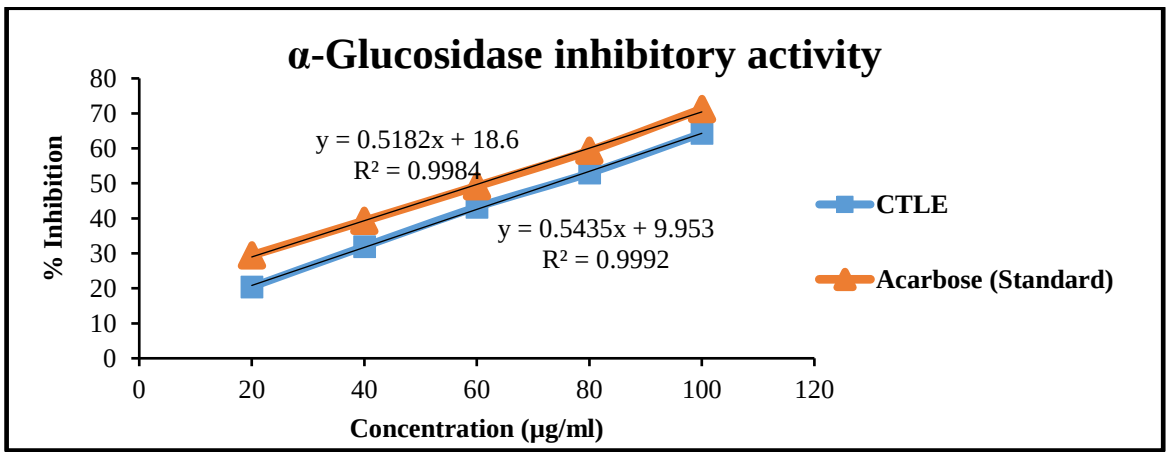

Figure 2: Percentage inhibition in α-glucosidase Inhibition activity of CTLE at different concentration.

From above observation it was clearly indicated that ethanolic leaves extract of *clematis triloba* has α-glucosidase inhibitory activity.

In-Vivo Pharmacological Studies

Acute toxicity studies

No morbidity or death were recorded at the end of the 14-day acute toxicity investigation. The animals were in good condition and showed no symptoms of poisoning, such as tremors, convulsions, or behavioral abnormalities. A change in body weight was also expected. The outcomes are presented below.

Table 4: Observation table of Acute Toxicity Study of leaves extracts of *Clematis triloba*.

Sr.No.	Number of animals	Treatment/dose in mg/kg	Observation	Result
1.	3	50 mg/kg	No symptom	No death
2.	3	300 mg/kg	No symptom	No death
3.	3	500 mg/kg	No symptom	No death
4.	3	2000 mg/kg	No symptom	No death
LD_{50}				2000 mg/kg

The findings indicated that 2000mg/kg was the highest dosage that could be tolerated. In order to assess its in vivo anti-diabetic effectiveness, its ED50, which was found to be 100 mg/kg, was used. A higher dose of 200 mg/kg was also used for pharmacological tests in order to assess the dose-dependent activity.

Hypoglycemic study in normal fasted rats

In this experiment, the hypoglycemic activity of the ethanolic leaves extract of *Clematis triloba* and self-micro emulsifying drug delivery system (SMEDDS) formulated CTLE *(Clematis triloba leaves extract)* was investigated in normal rats. The study spanned a 3-hour period, during which the ethanolic extract demonstrated significant hypoglycemic effects, reducing blood glucose levels to 57.24 mg/dl and 51.27 mg/dl at doses of 100 and 200 mg/kg, respectively. The SMEDDS formulated CTLE also exhibited notable hypoglycemic activity, with glucose levels decreasing to 49.96 mg/dl and 46.78 mg/dl at the same respective doses. In comparison, the standard drug Glibenclamide, administered at 5 mg/kg, resulted in a blood glucose level of 43.27 mg/dl over the same study duration. The observed trend in the change in blood glucose levels from 0 to 3 hours revealed a consistent reduction, indicating the potential effectiveness of both the ethanolic extract and the SMEDDS formulation in lowering blood glucose levels in normal rats.

Table 5: Hypoglycemic effect of CTLE on blood glucose level in normal rats

Group	Treatment	Blood glucose level (mg/dL)			
		0 hr	1 hr	2 hr	3 hr
I	Normal control	76.34±01.32	74.61±01.43	77.36±01.57	78.42±01.04
II	Standard control Glibenclamide (5 mg/kg)	78.23±02.27*	67.15±02.09*	56.42±03.24**	43.27±2.06**
III	CTLE (100 mg/kg)	77.83±01.43*	64.42±03.03*	58.32±02.14*	57.24±03.78*
IV	CTLE (200mg/kg)	76.25±02.17**	63.25±1.16**	56.98±02.02**	51.27±01.23**
V	SMEDDS CTLE (100 mg/kg)	75.31±02.13**	64.33±3.4**	60.93±01.63**	49.96±02.78**
VI	SMEDDS CTLE (200 mg/kg)	74.69±02.07**	61.45±2.04**	56.37±01.29**	46.78±01.06**

Abbreviations: - CTLE, Ethanolic leaves extract of *clematis triloba*. SMEDDS, Self-micro-emulsifying drug delivery system

In each group, all values are given as the mean±SEM with n=6.

Dunnett's multiple comparisons test followed by ONE WAY ANOVA was used to analyse the data. *P<0.05; **P<0.01; ***P<0.001. as compared to the appropriate diabetes control.

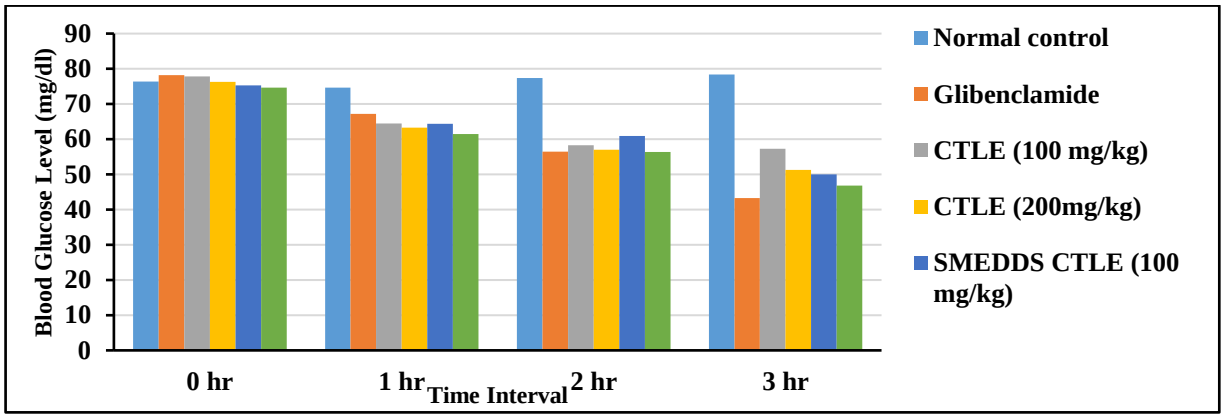

Figure 3: The Hypoglycemic effect of CTLE and SMEDDS CTLE in normal rats.

Oral Glucose Tolerance Tests (OGTT)

Following a 30-minute period of glucose loading, normal rats exhibited a considerable elevation in blood glucose levels after the administration of 2 gm/kg of glucose. The blood glucose levels in rats treated with the extract, self-microemulsifying drug delivery system (SMEDDS) formulation, and the standard drug peaked at 30 minutes and returned to baseline levels after 120 minutes. The maximum anti-hyperglycemic effects of *Clematis triloba* leaves extract (CTLE) at doses of 100 and 200 mg/kg were found to be 83.45±3.25 to 84.93±2.97 and 79.32±4.28 to 81.13±3.93, respectively. Similarly, the SMEDDS formulated CTLE at the same doses exhibited anti-hyperglycemic effects with values ranging from 76.52±3.85 to 79.41±3.43 and 77.93±5.63 to 74.48±5.14. Administration of both CTLE and SMEDDS formulated CTLE at doses of 100 and 200 mg/kg resulted in significantly lower glucose values compared to normal control rats. The oral glucose tolerance test revealed that the most substantial improvement in glucose tolerance occurred at a dosage of 200 mg/kg. These findings suggest that both CTLE and its SMEDDS formulation possess promising anti-hyperglycemic effects, particularly at higher doses, as indicated by their impact on glucose tolerance during the experimental period.

Table 6: Blood Glucose (mg/dl) Analysis in Oral Glucose Tolerance Test

Groups	Treatment/Group	Blood sugar levels at various time intervals (in mg/dl)				
		0 min	30 min	60 min	90 min	120 min
I	Normal control	77.28±03.48	76.17±7.16	77.49 ± 6.74	79.63±4.28	78.82±5.87
II	Standard control (glibenclamide 5 mg/kg)	75.23±1.03	83.98±2.05	122.46±10.82	96.57±4.96**	89.78±4.08**
III	CTLE (100 mg/kg)	83.45±3.25*	92.59±4.16*	89.43±5.86*	87.28 ± 6.51	84.93±2.97*
IV	CTLE (200 mg/kg)	79.32±4.28**	84.56±6.23**	83.98±8.54*	82.73±1.78**	81.13±3.93**
V	SMEDDS CTLE (100 mg/kg)	76.52±3.85*	78.37±1.29**	82.33±5.71**	80.48±9.72*	79.41±3.43*
VI	SMEDDS CTLE (200 mg/kg)	77.93±5.63**	76.97±2.12**	76.12±2.45**	75.68±6.74**	74.48±5.14**

Abbreviations: - CTLE, Ethanolic leaves extract of *clematis triloba*. SMEDDS, Self-micro-emulsifying drug delivery system

In each group, all values are given as the mean±SEM with n=6.

Dunnett's multiple comparisons test followed by ONE WAY ANOVA was used to analyse the data. *P<0.05; **P<0.01; ***P<0.001. as compared to the appropriate diabetes control.

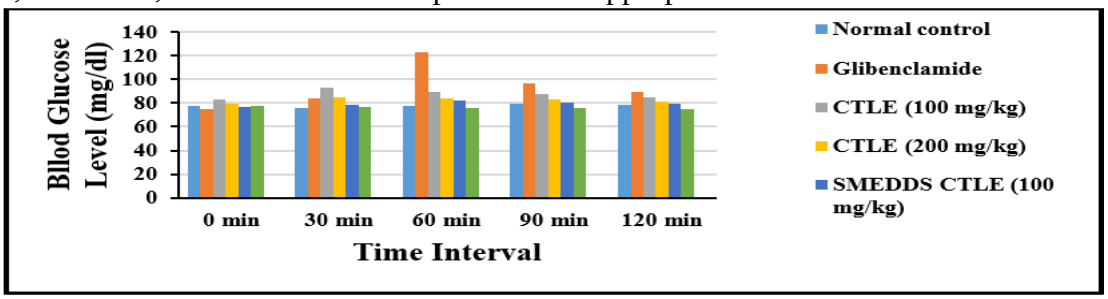

Figure 4: The Results of Oral Glucose Tolerance Test (OGTT) in normal rats.

Effect of CTLE and SMEDDS formulated CTLE on blood glucose level in diabetic rats.

The study aimed to assess the impact of repeated oral administration of Clematis triloba leaves extract (CTLE) and CTLE formulated with self-microemulsifying drug delivery system (SMEDDS) on fasting blood glucose levels in streptozotocin (STZ)-induced diabetic rats. The evaluation was conducted at different time points: initially and at 1st, 3rd, 6th, and 8th hours after treatment. The diabetic control group exhibited a significant increase in blood glucose levels from 253.72±6.58 to 290.57±3.89 mg/dl. The standard drug glibenclamide demonstrated a noteworthy reduction in blood glucose levels from 272.46±3.21 to 83.49±2.95 mg/dl at the end of the 8th hour. Treatment with CTLE and SMEDDS formulated CTLE at doses of 100 mg/kg and 200 mg/kg resulted in a decrease in blood glucose levels from 257.31±2.87 to 133.67±3.49, 259.53±6.28 to 117.25±4.76, 279.48±5.47 to 110.48±4.25, and 273.89±4.78 to 93.87±2.92, respectively. These findings suggest that both CTLE and its SMEDDS formulation exhibit anti-hyperglycemic effects, as evidenced by the significant reduction in blood glucose levels in STZ-induced diabetic rats over the course of the study period.

Table 7: Effect of CTLE and SMEDDS formulation of on blood glucose level on STZ induced diabetic rats after single dose treatment.

Group	Treatment	Blood glucose level (mg/dl) at various time interval				
		0 hr	1 hr	3 hr	6 hr	8 hr
I	Normal control	81.47±1.24	86.58±3.57	85.98±1.63	84.43±3.02	83.76±1.93
II	Diabetic control	253.72±6.58#	272.16±4.43#	278.27±3.59#	292.69±5.24#	290.57±3.89#
III	Standard control (glibenclamide 5 mg/kg)	272.46±3.21	179.63±5.65**	155.58±4.78**	127.44±6.14***	83.49±2.95***
IV	CTLE (100mg/kg)	257.31±2.87	226.72±3.18**	194.42±5.32**	171.59±4.78***	133.67±3.49***
V	CTLE (200 mg/kg)	259.53±6.28	201.37±6.59**	179.56±5.93**	155.78±4.87***	117.25±4.76***
VI	SMEDDS CTLE (100 mg/kg)	279.48±5.47	225.14±5.63**	209.57±5.23**	184.37±3.78***	110.48±4.25***
VII	SMEDDS CTLE (200 mg/kg)	273.89±4.78	209.45±2.96**	187.29±3.29**	141.53±4.16***	93.87±2.92***

Abbreviations: - CTLE, Ethanolic leaves extract of *clematis triloba*. SMEDDS, Self-micro-emulsifying drug delivery system

In each group, all values are given as the mean ± SEM with n=6.

Dunnett's multiple comparisons test followed by ONE WAY ANOVA was used to analyses the data.

*P<0.05; **P<0.01; ***P<0.001. as compared to the appropriate diabetes control.

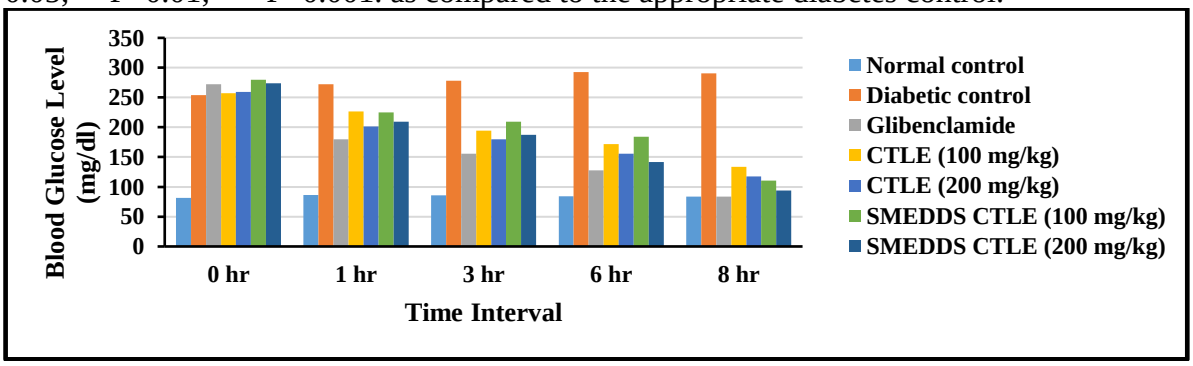

Figure 5: The effect of CTLE and SMEDDS CTLE on blood glucose levels at different time intervals in normal and diabetic rats.

CONCLUSION

According to the results of this study's phytochemical screening, the ethanolic extract of *Clematis triloba* includes possible secondary metabolites. The blood glucose levels of diabetic rats and oral glucose-loaded experimental animals were significantly lowered by *clematis triloba* ethanolic extract. The results also revealed that the ethanolic fraction has low blood glucose reduction in

streptozotocin-induced rats and also having a α-Amylase, α-glucosidase inhibition activity. The use of SMEDDS to increase the bioavailability of lipid soluble medicines is gaining popularity. The study's findings indicate that an improved SMEDDS formulation has stronger pharmacodynamic efficacy than CTLE extract without formulation.

The observations showed that streptozotocin injection caused hyperglycemia, polydipsia, polyphagia, body loss, dyslipidemia, and oxidative stress. STZ-diabetic rats were given CTLE and SMEDDS formulation (100 and 200 mg/kg) after 8 hours of therapy to lower blood glucose levels. As a result, SMEDDS has a stronger anti-diabetic and protective impact when compared to CTLE of pancreatic cells.

Blood glucose levels were reduced by SMEDDS formulation by almost two times more than by CTLE extract taken alone. The self-micro-emulsified formulation creates a micro emulsion that has a stronger pharmacodynamic response after oral delivery. The solubilized extract presentation and the larger interfacial area for absorption and dissolution that smaller particles offer may both contribute to this reaction. Other potential contributing elements include the size of the particles. The presence of surfactant and co-surfactant means that SMEDDS is essential for promoting permeability across the intestinal membrane. Further chemical and pharmacological investigations are required to elucidate the exact mechanism of action of *clematis triloba* extract.

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DISCLOSURE OF INTERESTS

The author declares that they have no conflict of interest.

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