Formulation and Evaluation of Nanoparticles Loaded with Euphorbia thymifolia Extract Against Experimentally Induced Diabetes.

***[1]K.G. Bhutkar, [1]A. Balasubramaniam, [2]V. Chamundeeswari, [3]R. Radha**

[1]School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Studies (VISTAS), Vels University, Chennai-600117, Tamil Nadu, India. [2]Principal and Professor, Department of Pharmacognosy, Faculty of Pharmacy, Shri Ramchandra University, Porur, Chennai-600016, [3]Professor and Head of Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003.

***Corresponding author**

K.G. Bhutkar

School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Studies (VISTAS), Vels University, Chennai-600117, Tamil Nadu, India.

ABSTRACT

Nanoparticles synthesized by using natural polymers have been applied to various biomedical applications. These procedures are simple, eco-friendly and serve as an alternative to complex chemical methods for the preparation of nanomaterials. In the present study, synthesis of nanoparticles, to examine their antidiabetic potential, toxicity study was focused. Ethanolic extract of *Euphorbia thymifolia* was used for the synthesis of nanoparticles. The synthesis was monitored and confirmed by Particle size & polydispersibility index, Zeta potential study and Scanning electron microscopy. The particle size of nanoparticle made from extracts of *Euphorbia thymifolia* was determined. The nanoparticle's particle size was determined to be 43.2 ± 11 and zeta potential was found to be -28.09. SEM picture of a freeze-dried nanoparticle with a longer cross-linking period, showing a tiny, spherical nanoparticle with a small size. The entrapment efficiency of *Euphorbia thymifolia* extract loaded nanoparticle was observed 81.12 %, indicating higher drug entrapment efficiency. It was discovered that as the concentration of polymer increased, the yield increased. After 12 weeks, the stability of the nanoparticles of was evaluated by analysing its absorption spectra. There were no significant changes over the storage period, and the nanoparticles did not agglomerate, indicating that they were more stable. The acute oral toxicity in rats indicated that extract of Euphorbia thymifolia was nontoxic at 2000mg/kg body weight. The formulated nanoparticles of extract of *Euphorbia thymifolia* after administration in Swiss albino mice were studied for antidiabetic activity for 1 month and found to be potent.

KEYWORDS Extraction, *Euphorbia thymifolia* Linn, Nanoparticles.

INTRODUCTION

Nanoparticles are of enormous scientific significance because they bridge the gap between atomic or molecular structures and are concerned with materials at nano level. Nanomaterials exhibit unique physical, chemical and biological attributes due to their small size, shape, composition, distribution and morphology, in contrast to micro and macro materials. Nanoparticles are termed as those particulate materials having at least one dimension and ranging from 1 to 100 nanometers (nm). Knowledge and use of plants as herbal medicines has occurred in various populations throughout human evolution, beginning when man was learning to select plants for food, and to relieve ailments and diseases. However, during the second half of the twentieth century, especially in the Western world, herbal medicines were gradually replaced by allopathic medicines. Allopathic treatments are currently more widely used than traditional medicines, especially in developed countries. However, most developing countries continue to use these natural medicines, most likely because obtaining a synthetic drug is expensive. According to the World Health Organization, 80% of people in developing countries depend on traditional medicinal practices to meet and/or supplement their basic health needs.[1-5]

Currently, despite marketing and encouragement from the pharmaceutical industry during the development of allopathic medicines, a large segment of the population in many countries continues to utilize complementary practices for their health care. Many of these practices are derived from medicinal plants. However, due to economic, political, and social changes that have occurred worldwide, the therapeutic use of these natural resources, which are mainly used by people who cannot afford different treatments, has greatly diminished.[6-8]

MATERIALS AND METHODS

Collection and authentication of plant material

The *Euphorbia thymifolia* L. aerial parts were collected from hill area of *western ghat* near Bhor city district Pune Maharashtra. Fresh plants of *E. thymifolia* were collected from Bopdev Ghat near under 10 km, area Pune Maharashtra India. and deposited the specimen for identified and authenticated at Botanical Survey of India Western Regional Office Pune. The Voucher specimens N / No. BSI/WRC/100-1/Tech./2020/102 by Scientist Mrs. Priyanka A. Ingale for Comparison and authentication of plant species *Euphorbia thymifolia* was used. [9-12]

Extraction Procedure

The selected plant material (crude drugs) *Euphorbia thymifolia* L. was collected from local place and dried in the shade. Then the dried material is pulverized in grinder. The powdered material was passed through 120 mesh sieves to remove fine powder and course powder was used for extraction. Continuous Soxhlet extraction method used for extraction. Each powered batch (200 gm. powder) loaded for extraction.[13]

Further for Preparations of Hexane extract**:** Weighed accurately crude drug course powder extracted with an adequate quantity of hexane using a soxhlet extractor at 60°-70°C. (Boiling point: 69°C) Likewise extraction was performed with Chloroform, Ethyl acetate, Ethanol and

After ethanol extraction, the air-dried marc of crude drug was macerated with distilled water containing chloroform (2.5ml/1000ml) for 48 hours with stirring. Then the resultant extract was filtered through a muslin cloth and the marc was separated. The filtrate was evaporated to dryness on hot plate at 45°C to get an aqueous semisolid extract.[14]

Qualitative analysis of Phytochemicals

Phytochemical analysis of all the solvent extracts was performed for the detection of active secondary metabolites or different constituents such as Terpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C. Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents are depending chemical methods of evaluation. [15-17]

In-Vitro Pharmacological Screening of Extracts[18-20]

Pharmacological screenings of all extracts were carried out for Antidiabetic studies**:**

Alpha-amylase activity

Alpha-amylase activity was carried out by starch iodine method. 10 µL of α-amylase solution (0.025mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing 1000µg/ml concentration. After incubation at 37°C for 10 min,100µL of starch solution (1%) was added, and the mixture was re incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5mL distilled water, the absorbance was taken at 565nm. Samples, substrate and α-amylase blank determinations were carried out under the same reaction conditions.

Inhibition of enzyme activity was calculated as

$$(\%) = (A-C)/ C \times 100$$

where, A=absorbance of the sample, and C=absorbance of control (without starch).

Formulation of Herbal nano particle on & characterization (Using Natural Polymer)

Formulation of nanoparticles[21-24]

Nano-precipitation method was followed with a slight modification for the preparation of nanosuspensions. Plant extract (2.5 g) was dissolved in 15 ml of acetone and ethanol (3:1) by sonication for 60 seconds. The resultant solution was then gradually injected (1 ml min) with a syringe connected to a thin teflon tube, into 25 ml water containing PVA 1.5% w/v with continuous magnetic stirring at 1000 rpm. The resulting emulsion obtained was then diluted in 50 ml PVA solution (0.2% w/v in water) in order to minimize coalescence and the mixture was continuously stirred (500 rpm) for 6 h at room temperature to allow solvent evaporation and nanoparticle formation. The resultant nanosuspension was consequently cooled down to 18^0C and lyophilised using lyophiliser to obtain dry powder.

CHARACTERIZATIONS OF NANOPARTICLES

1. Particle size & polydispersibility index

Particle Size The size analysis and polydispersity index of the NPs were determined using a Malvern Zetasizer Nano ZS (Malvern Instrument, Worcestershire, UK). Each sample was diluted

ten times with filtered distilled water to avoid multi-scattering phenomena and placed in disposable sizing cuvette. Polydispersity index was noted to determine the narrowness of the particle size distribution. The size analysis was performed in triplicate and the results were expressed as mean size ± SD.

2. Zeta potential study

A zeta sizer was used to examine the zeta potential (surface charge) of nanoparticles. The produced nanoparticle formulations were diluted with water (0.1ml) and put in an electrophoretic cell with a 15.5 V/cm electrical field to evaluate their zeta potential. Each sample was measured in three different ways.

3. Scanning electron microscopy

Scanning electron microscopy was used to examine the nanoparticle's morphology. In a first stage, 100l of nanoparticle formulations were applied to a 10mm glass slide and dried overnight at room temperature in a vacuum desiccator till SEM examination was done. Nanoparticles were mounted on appropriate support and coated with gold using a gold sputter module in a higher vacuum evaporator for analysis. At a voltage of 15kv, observations were made at various magnifications

4. Drug entrapment efficiency

EE in the NPs was determined after separating the NPs from the aqueous supernatant by centrifugation at 25000 rpm for 30 min (3K 30, Sigma Laboratory Centrifuge, Osterode, Germany). The supernatant was diluted with appropriate amount of distilled water and analyzed for the amount of unentrapped drug with a UV-visible spectrophotometer in this parameter. solvent was added to the pellet to dissolve the polymer and centrifuged. The supernatant was removed and the drug pellet was analyzed for the entrapped drug content.

The EE was calculated according to following formula:

$$EE\ (\%) = (TD\text{-}FD/TD) \times 100$$

Where, TD is total amount of drug added and FD is amount of drug in supernatant

Drug loading was calculated as follows,

$$\%\ \text{drug loading} = A/B \times 100$$

Where A is the drug content in the NPs and B is the weight of NPs.

The drug encapsulation efficiency was determined by using the relation in this equation.

5. Production yield of nanoparticles

The yields of nanoparticles were determined by comparing the whole of nanoparticle formed against the combined weight of the copolymer and drug.

% Yield calculation = Amount of drug X100 / Amount of drug +polymer

6. Pharmacological screenings of nanoparticle were carried out Antidiabetic studies:

1. Acute oral toxicity

Acute oral toxicity test was performed to determine the LD50 value of ethanolic extract of *Euphorbia thymifolia*. Experiments were carried out using healthy young adult albino mice

weighing 20-30g.

Acute oral toxicity studies were performed according to OECD. Albino mice (n = 6/each dose) selected by random sampling technique were used in this study. The animals were fasted for 12 hours with free access to water only. Following the period of fasting, animals were weighed and test extract was administrated orally at a dose of 1000 and 2000 mg/kg. After the administration of test extract, food for the animals were withheld for 2 hours. The mortality and clinical signs which included changes in skin, fur, eyes and mucous membranes were noted for the first 4 hours subsequently for 72 hours and thereafter for 7 days of test drug administration. For complete 7 days, the gross behaviors like body positions, locomotion, rearing, tremors and gait were observed and also the effect of plant extract on grip strength, pain response and righting reflex were noted. In addition, the intake of food and water behavior was monitored.

Animals required

- **a. Species and Strain:** Swiss albino mice
- **b. Age and Weight:** Weight range: 20-30 g.(12-16 weeks)
- **c. Gender:** Either sex

Table 1. Acute toxicity study animal groups.

Sr. No	**Name of group Table 1.** Acute toxicity study animal groups.	**Treatment** (mg/kg)	**No. of animals**
1	Acute Toxicity	1000	6
		2000	6

2. Glucose tolerance Test:

The oral test method for glucose tolerance was used to determine the anti-hyperglycemic activity. Mice were taken as the study subjects and were injected with different doses of the nanoparticles of *Euphorbia thymifolia*, followed by glucose. After 1 hour of administration serum glucose levels were examined. Different doses were administered in 50, 100, 200 and 400 mg/kg of body weight in mice to determine the glucose level reduction in the serum. The dose level of 400mg/kg showed the maximum glucose reduction level in the serum when compared with the anti-hyperglycemic drug; glibenclamide which has a dose level of 10mg/kg body weight which showed glucose reduction.

3. Oral glucose tolerance test (OGTT):

The overnight starved normal healthy Wistar rats were utilized to perform OGTT (Bonner-Weir, 1988). The rats were allocated into six groups (n=6). Group I to III administered orally with 0.2% Carboxymethylcellulose (CMC) solution, Nanoparticles of ethanol extracts of EUT (500 mg/kg suspended in 0.2% CMC) and standard drug glibenclamide (5 mg/kg suspended in 0.2% CMC), respectively. After treatment with extracts blood samples were withdrawn at 0, 30, 60 and 120 min by retro-orbital puncture under diethyl-ether anaesthesia. The fasting blood glucose (FBG) concentration was measured by diagnostic strips (Accu-check, Roche Diagnosis, USA).

4. Blood Glucose Lowering Activity:

Male Wistar rats weighing 180–240 g are kept on standard diet. Groups of 6 non-fasted animals are treated orally or intraperitoneally with various doses of the test compounds suspended in 0.4% starch suspension. One control group receives the vehicle only. Blood is withdrawn from the tip of the tail immediately before, and 1, 2, 3, 5, and 24 h after administration of the test compound. Blood glucose is determined in 10 µl blood samples with the hexokinase enzyme method.

5. **In vivo streptozotocin induced antidiabetic activity:**

The animal used for experiment will be 8-week old Swiss albino mice with weights ranging from 20 to 30 gram. The animals will be kept for 1 week in advance to adapt to the conditions of the cage before being treated. The mice will be placed in a cage that is given a husk base to absorb dirt from mice. During the adaptation period, the mice will be fed, watered and weighted daily. The mice are considered healthy when their weight increases or remains the same or decreases no more than 10%. The mice used for experiment were divided into 8 groups, containing 10 animals in each group. Before treatment, the mice fasted for 18 hours (ad libitum). Blood samples were drawn from each mouse‘s tail for measuring the first blood glucose levels (baseline). All mice then will treat intra peritoneally with 25mg/kg BW of streptozotocin, to elevate their blood glucose levels. Blood samples were collected from the tail vein of the overnight (12-15h) fasted mice and blood glucose level was determined on 0th, 5th, 10th and 15th day along with body weight and body temperature. If the blood glucose levels of mice > 200 mg/dL then the mice are considered to have hyperglycemia.

Animals required

- **a. Species and Strain:** Swiss albino mice
- **b. Age and Weight:** Weight range: 20-30 g.(12-16 weeks)
- **c. Gender:** Either sex

In vivo streptozotocin induced antidiabetic activity:

Table 2. antidiabetic activity study animal groups.

Sr. No	Name of group	Treatment	No. of animals
1	Normal group	Tween 80	6
2	Diabetic control	-	10
3	Standard	Drug	10
4	Ethanolic Extract of EUT Nanoparticle Formulation	Formulations	10

The histopathological analysis of pancreatic tissue

The pancreas tissue samples, which were taken for histopathological analysis, were fixed in 10% formalin solution for 48 hrs. and then were washed under running water for 8 hrs. They were treated with alcohol (70°, 80°, 90°, 96°, and 100°) and a series of xylene during the routine tissue control period and then were blocked in paraffin. The samples were prepared on the slides by

taking 4-µm sections from each block. They were prepared for the histopathological analysis and stained with hematoxylin–eosin (HE) staining. The relevant areas were photographed and analyzed using a light microscope (Zeiss Axioscope 2 plus, USA). The tissues were rated as negative (−), slight (+), moderate (++), or severe (+++) according to the histopathological findings.

RESULTS AND DISCUSSION

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction.

Table 3. Successive extractive values of the powdered leaves of *Euphorbia thymifolia* L

Sr. No.	Extraction solvent used	Yield%
1.	Hexane extract	08.61 %
2.	Chloroform extract	15.15 %
3.	Ethyl acetate extract	07.47%
4.	Ethanol extract	17.16 %
5.	Aqueous (Water) extract	03.22%

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1.

Table 4. Preliminary phytochemical screening of extracts

Extracts	Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
Tests for carbohydrates					
Molish Test	-	+	-	-	-
Fehling Test	-	+	-	-	-
Benedict Test	-	+	-	-	-
Test for Monosaccharide					
Barfoed's Test	+	-	-	-	-
Test for Non-reducing polysaccharides					
Iodine Test	-	-	-	-	-
Test for Proteins					
Biuret test	-	+	-	-	-
Millions test	-	-	-	-	-
Tests for Steroids					
Salkowaski reaction	-	+	+	+	-

Libermann Burchard reaction	+	-	-	+	-
Libermann reaction	-	-	-	-	-
Tests for Terpenoids	+	+	+	+	+
Test for Glycosides					
Borntrager's Test	-	-	-	+	-
Killer- Killani Test	-	+	-	-	-
Test for Saponin					
Foam test	-	-	-	+	+
Tests for Flavonoids					
Shinoda test	-	-	+	+	-
Lead acetate Test	-	-	-	+	-
Sod-hydroxide Test	-	-	+	-	-
Tests for Alkaloids					
Meyers Test	-	+	-	+	-
Wagner's Test	-	-	-	-	+
Hager's Test	-	-	-	-	-
Dragendorff Test	-	+	+	+	-
Test for Tannins & Phenolic compounds					
$FeCl_3$	-	+	-	-	-
Lead acetate	-	+	-	+	-

+ Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

The all extracts were screened for the presence of various constituents. The result of this preliminary phytochemical examination is shown in above.

The result of phytochemical study on *Euphorbia thymifolia L.* revealed presence of primary metabolites as well as secondary metabolites such as carbohydrates, lipids, alkaloids, steroids, flavonoids, tannins and terpenoids.

In-Vitro Pharmacological Activity

Alpha Amylase Inhibitory Assay

Table 5: The percent inhibition of extracts of plants on alpha amylase inhibitory assay.

Sr. No.	Sample Code	Conc. µg/ml	OD	%inhibition of alpha amylase
1	Blank		1.20	-
2	Standard – Acarbose	100	0.43	64.16
	Standard – Acarbose	500	0.31	74.16
	Standard – Acarbose	1000	0.11	**90.83**

3	EAQ	100	0.99	17.50
		500	0.95	20.83
		1000	0.62	**48.33**
4	EHX	100	0.68	43.33
		500	0.48	60.00
		1000	0.36	**70.00**
5	ECH	100	0.88	26.66
		500	0.81	32.50
		1000	**0.60**	**50.00**
6	**EET**	**100**	**0.45**	**62.50**
		500	**0.43**	**64.16**
		1000	**0.32**	**73.33**
7	**EEA**	100	0.65	42.50
		500	0.63	44.16
		1000	0.62	43.33

After evaluation active extracts—Ethanol further proceed for TLC analysis to observe responsible potent phytochemicals.

CHARACTERIZATIONS OF NANOPARTICLES

The prepared nanoparticle of extract of *Euphorbia thymifolia* was subjected for different evaluations parameters.

Particle size determination by Zeta sizer

The particle size of nanoparticle made from extracts of *Euphorbia thymifolia* was determined. The nanoparticle's particle size was determined to be between a range of 1-100nm. 43.2 ± 11

-28.09.

Table 6: Particle size and zeta potential of extract of *Euphorbia thymifolia*

Sr. No.	Sample	Nanoparticle Size (nm)	Zeta Potential (mV)
1	Extract of *Euphorbia thymifolia*	43.2 ± 11	-28.09

Values are shown as the mean ± standard deviation; n=5.

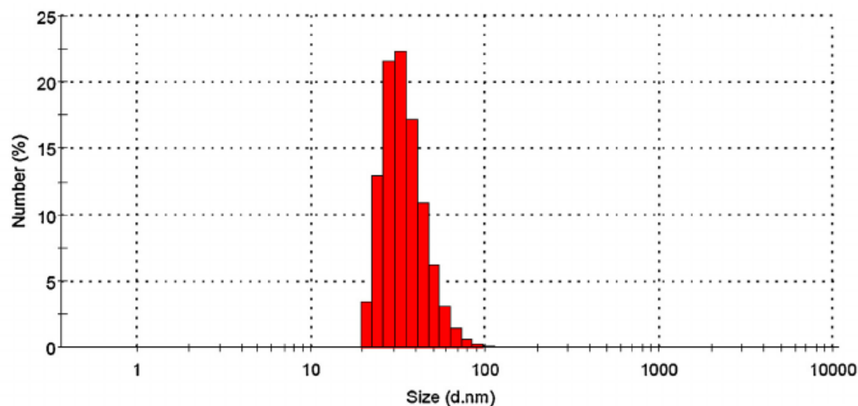


Figure 1. Particle Density Index of extracts derived extract of *Euphorbia thymifolia* loaded nanoparticle.

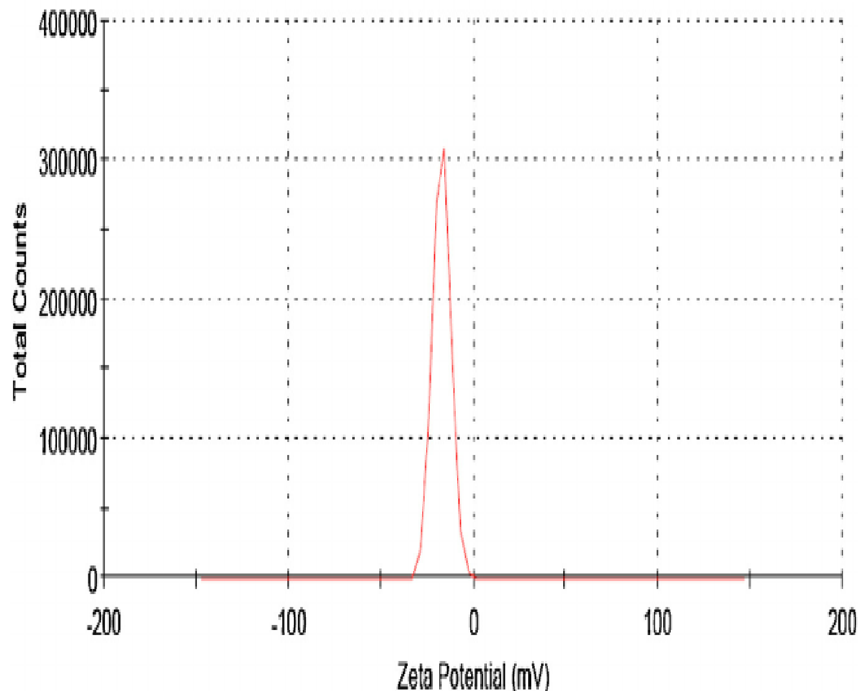


Figure 2. Zeta particle size distribution peak of nanoparticles of extract of *Euphorbia thymifolia.*

Scanning electron microscopy

SEM picture of a freeze-dried nanoparticle with a longer cross-linking period, showing a tiny, spherical nanoparticle with a small size.

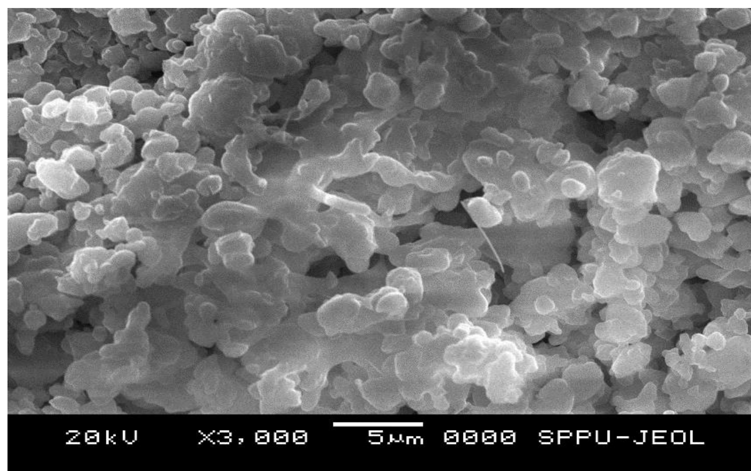


Figure 3. Scanning electron micrograph of nanoparticles obtained by extract of *Euphorbia thymifolia*

Drug entrapment efficiency

The entrapment efficiency of *Euphorbia thymifolia* extract loaded nanoparticle was observed 81.12 %, indicating higher drug entrapment efficiency.

Table 7. % Entrapment efficiency of Euphorbia thymifolia extract loaded nanoparticle

Formulations	**Entrapment efficiency (%)**
Extract of *Euphorbia thymifolia*	81.12

Production yield of nanoparticles

It was discovered that as the concentration of polymer increased, the yield increased.

Table 8. Production yield of Euphorbia thymifolia extract loaded nanoparticle

Formulation	**Production yield (%)**
NF	72.41

Transmission electron microscopy

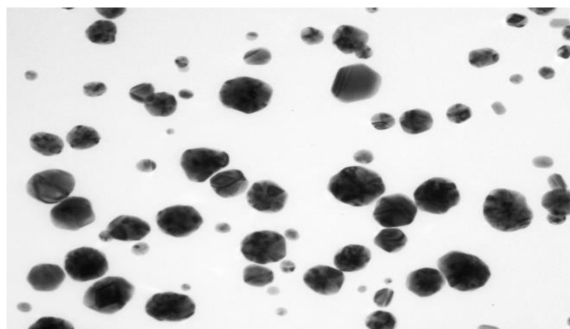


Figure 4 - TEM of Euphorbia thymifolia extract loaded nanoparticle

Stability of Nanoparticles-:

After 12 weeks, the stability of the nanoparticles of was evaluated by analysing its absorption spectra. There were no significant changes over the storage period, and the nanoparticles did not agglomerate, indicating that they were more stable.

The pattern of change in entrapment efficiency, particle size and zeta potential were same for prepared nanoparticles. There is slight increase in the size of the nanoparticles (1.7%) was observed after three months of storage at 4°C. The entrapment efficiency of nanoparticles was decreased by about 1-3%, whereas zeta potential was found to be decreased by 5%. The changes observed during the storage are negligible.

Table 9. Effect of storage on particle size, zeta potential and entrapment efficiency of Nanoparticles (n=3). Values are expressed as mean ±SD

Storage time	**"0" Month**	**"1" Month**	**"2" Month**	**"3" Month**
Particle size (nm)	-51.2	-47.74	-48.68	-42.25
Zeta potential (mV)	-27.54	-25.78	-21.27	-19.26
Entrapment efficiency (%)	78.23	79.25	78.48	76.29

(n=3). Values are expressed as mean ±SD

Anti-diabetic study

Acute Toxicity

The acute oral toxicity in rats indicated that extract of Euphorbia thymifolia was nontoxic at 2000mg/kg body weight.

Table 10. Acute toxicity of extract of Euphorbia thymifolia

Formulation/Group	**No. of animals in group**	**Dose (mg/kg)**	**Results**
Ethanolic Extract of EUT Nanoparticle Formulation	6	2000	No toxic sign
Ethanolic Extract of EUT Nanoparticle Formulation	6	2000	No toxic sign

In vivo streptozotocin induced antidiabetic activity:

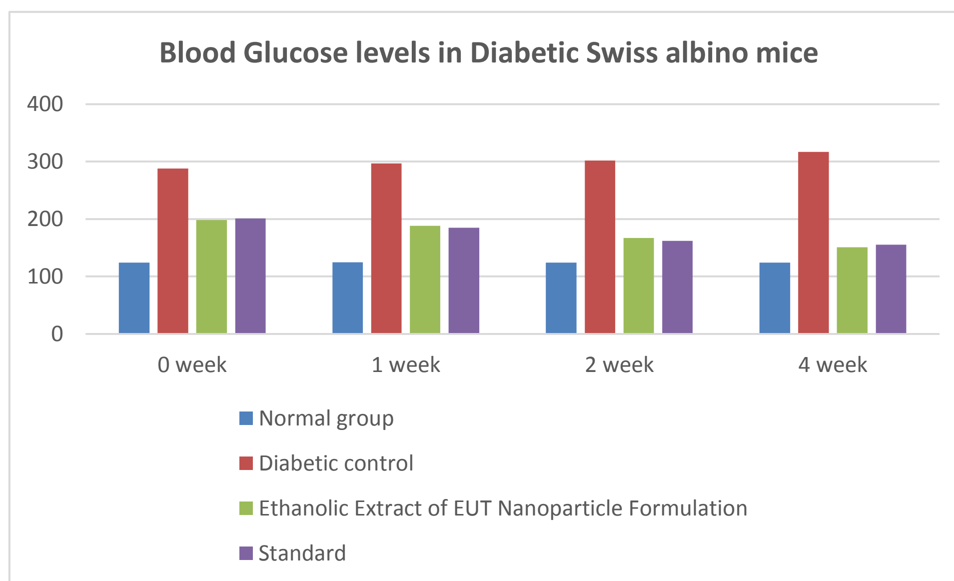
Hypoglycemic study

Effect of Ethanolic Extract of EUT Nanoparticle Formulation on Blood Glucose levels in Diabetic rabbits

The formulated nanoparticles of extract of *Euphorbia thymifolia* after administration in Swiss albino mice were studied for antidiabetic activity for 1 month.

Table 11. Blood Glucose levels in Diabetic Swiss albino mice

Groups	**0 week**	**1 week**	**2 week**	**4 week**
Normal group	124	124.5	124	124
Diabetic control	288	297	302	317
Ethanolic Extract of EUT Nanoparticle Formulation	198	188	167	151
Standard	201	185	162	155



Graph 1. Blood Glucose levels in Diabetic Swiss albino mice

The pancreatic histopathology

Figure 5 and Table 12 show the histopathological structure of the pancreatic tissue, and its analysis, effectively. It was observed that the pancreatic β-cells of the diabetic rats were completely damaged, due to streptozotocin induction compared to the control group. It was revealed that the atrophy of the langerhans islets, degeneration, and necrosis in the β-cells decreased significantly and the pancreatic structure reformed in the diabetic rats after the EUT and STD administrations. EUT did not cause damage in the pancreatic β-cells of the healthy rats that were treated with EUT.

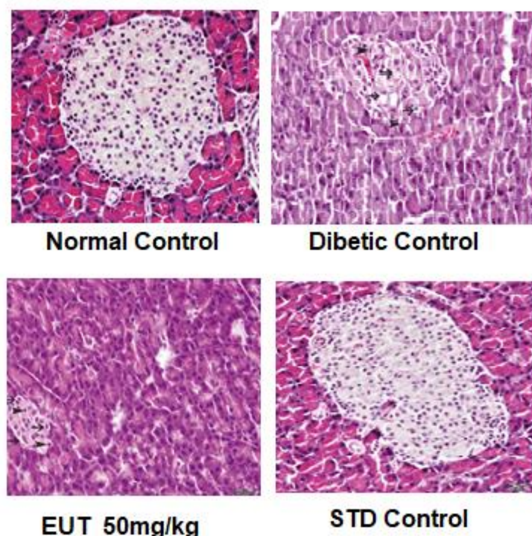


Figure 5: Histopathology of the pancreatic tissue of control and treatment groups of extract of *Euphorbia thymifolia.*

Table 12: Rating the histopathology effect of extract of *Euphorbia thymifolia* on pancreatic tissue of control and treatment groups.

	Group NC	Group DC	Group EUT	Group STD
Atrophy in the islets of Langerhans	−	+++	−	−
Hydropic degeneration in β-cells	−	+++	+	++
Necrosis in β-cells	−	++	−	+

The sections examined in the light microscope were evaluated as negative (−), slight (+), moderate (++) and severe (+++) according to the lesions.

CONCLUSION

In the current investigation, the extract of *Euphorbia thymifolia* was used in development of nanoparticle and evaluation. The herbal drugs available in the market at present may contain a single herb or combinations of several different herbs because it is believed to have synergistic and/ or complementary effects. Animal products and minerals are also the contents of some herbal products, including many traditional medicine formulations. Herbal products are sold as either raw plants, extracts or as a dosage forms like tablets, capsules, syrup etc. In the present work, formulations containing extracts of *Euphorbia thymifolia* was selected on the basis of their literature claim for hypoglycemic/antidiabetic action & practical findings from their extractive yields. Present research work deals with formulation and evaluation of the Nanoparticles as a novel dosage form from extracts. Hence, these investigations provide strong support for the selected medicinal plants for this research work and which also ascertain its folk claims. The morphology of the

Langerhans islets was observed to deteriorate in the STZ-induced mice. In EUT-treated diabetic groups, the histopathological changes in the Langerhans islets improved in parallel with the increase in dosage. It has been stated that the regeneration in the β-cells may be due to the decreasing of the oxidative stress by plant extract. The present research work may be helpful in development of efficacious and nanoparticle in diabetes mellitus treatment. Also this research work may prove as an important tool for detection of possible mechanism of action of the herbal drugs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Zheng, M., Jia, H., Wang, H., Liu, L., He, Z., Zhang, Z., Yang, W., Gao, L., Gao, X., & Gao, F. (2021). Application of nanomaterials in the treatment of rheumatoid arthritis. *RSC Advances*, *11*(13). (https://doi.org/10.1039/d1ra00328c

2. Kumari B. 2018 A Review on Nanoparticles: Their Preparation method and applications. Ind Res J Pharm Sci.;5(2):1420.

3. Haroon Anwar, S. (2018). A Brief Review on Nanoparticles: Types of Platforms, Biological Synthesis and Applications. *Research & Reviews Journal of Material Sciences*, *06*(02). https://doi.org/10.4172/2321-6212.1000222

4. Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. In *Arabian Journal of Chemistry* (Vol. 12, Issue 7). https://doi.org/10.1016/j.arabjc.2017.05.011

5. Golhani, D. K., Krishna, B. G., Khare, A., & Zaidi, S. A. H. (2018). Techniques for Nanoparticle Synthesis. *International Journal of Advance Research, Ideas and Innovations in Technology*.

6. Husni, P., & Ramadhania, Z. M. (2021). Plant Extract Loaded Nanoparticles. *Indonesian Journal of Pharmaceutics*, *3*(1). https://doi.org/10.24198/idjp.v3i1.34032

7. Kalakotla, S., Gottumukkala, K. M., Sandhya Rani, M., & Pravallika, P. L. (2015). Herbal drugs and herbal mediated silver nano particles as anti diabetics: A new horizon. *International Journal of Pharmaceutical Sciences Review and Research*, *31*(2).

8. Arulanandraj, N., Dhivya, S., & Gopal, V. (2018). A review on Herbal Nanoparticles. *Pharmatutor*, *6*(5). https://doi.org/10.29161/pt.v6.i5.2018.3

9. Ozougwu, O. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology*, *4*(4). https://doi.org/10.5897/jpap2013.0001

10. Banday, M. Z., Sameer, A. S., & Nissar, S. (2020). Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine*, *10*(04). https://doi.org/10.4103/ajm.ajm_53_20

11. Plows, J. F., Stanley, J. L., Baker, P. N., Reynolds, C. M., & Vickers, M. H. (2018). The pathophysiology of gestational diabetes mellitus. In *International Journal of Molecular Sciences* (Vol. 19, Issue 11). https://doi.org/10.3390/ijms19113342

12. Kalakotla, S., Gottumukkala, K. M., Sandhya Rani, M., & Pravallika, P. L. (2015). Herbal drugs and herbal mediated silver nano particles as anti diabetics: A new horizon. *International Journal of Pharmaceutical Sciences Review and Research*, *31*(2).

13. (An Overview on Euphorbiales; Family—Euphorbiaceae harika gupta)

14. (2013). A review on phyto-pharmacological potentia of Euphorbia thymifolia L. *Ancient Science of Life*, *32*(3). https://doi.org/10.4103/0257-7941.123001)

15. Raveendran, P., Fu, J., & Wallen, S. L. (2003). Completely “green” synthesis and stabilization of metal nanoparticles. *Journal of the American Chemical Society*, *125*(46), 13940-13941.

16. Petit, C., Lixon, P., & Pileni, M. P. (1993). In situ synthesis of silver nanocluster in AOT reverse micelles. *The Journal of Physical Chemistry*, *97*(49), 12974-12983.

17. Ahmad, A., Senapati, S., Khan, M. I., Kumar, R., Ramani, R., Srinivas, V., & Sastry, M. (2003). Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. *Nanotechnology*, *14*(7), 824.

18. Furube, A., Du, L., Hara, K., Katoh, R., & Tachiya, M. (2007). Ultrafast plasmon-induced electron transfer from gold nanodots into TiO2 nanoparticles. *Journal of the American Chemical Society*, *129*(48), 14852-14853.

19. Ahmad, M., King, T. A., Ko, D. K., Cha, B. H., & Lee, J. (2002). Performance and photostability of xanthene and pyrromethene laser dyes in sol-gel phases. *Journal of Physics D: Applied Physics*, *35*(13), 1473.

20. Kaithwas, G., Gautam, R., Jachak, S. M., & Saklani, A. (2012). Antiarthritic effects of Ajuga bracteosa Wall ex Benth. in acute and chronic models of arthritis in albino rats. *Asian Pacific journal of tropical biomedicine*, *2*(3), 185-188.

21. Nair, B., & Pradeep, T. (2002). Coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. *Crystal growth & design*, *2*(4), 293-298.

22. Shahverdi, A. R., Fakhimi, A., Shahverdi, H. R., & Minaian, S. (2007). Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. *Nanomedicine: Nanotechnology, Biology and Medicine*, *3*(2), 168-171.

23. Sastry, M., Ahmad, A., Khan, M. I., & Kumar, R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Current science*, 162-170.

24. Ahmad, A., Senapati, S., Khan, M. I., Kumar, R., Ramani, R., Srinivas, V., & Sastry, M. (2003). Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. *Nanotechnology*, *14*(7), 824.

25. Daniel, M. C., & Astruc, D. (2004). Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical reviews*, *104*(1), 293-346.

26. Spatz, J. P., Mössmer, S., Hartmann, C., Möller, M., Herzog, T., Krieger, M., ... & Kabius, B. (2000). Ordered deposition of inorganic clusters from micellar block copolymer films. *Langmuir*, *16*(2), 407-415.

27. Mallick, K., Witcomb, M. J., & Scurrell, M. S. (2005). Self-assembly of silver nanoparticles in a polymer solvent: formation of a nanochain through nanoscale soldering. *Materials chemistry and physics*, *90*(2-3), 221-224.