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Formulation and Evaluation of Biosynthesized *Terminalia Arjuna* Extract Loaded Polymeric Nanoparticles for the Treatment of Hyperlipidemia

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ABSTRACT

The work aimed to formulate and characterize the Terminalia arjuna bark extract-loaded polymeric nanoparticles used to treat hyperlipidemia. To study the in vitro drug release from the prepared nanoparticles by static diffusion method and to carry out the in vivo anti-hyperlipidemic activity in the Wistar rat model. The Terminalia arjuna bark was dried and powdered, further through Soxhlet apparatus ethanolic extraction was carried out and fine powder was formed.Phytochemical screening and quantitative analysis wear performed.Terminalia arjuna bark extract loaded nanoparticles were prepared by polymerisation i.e., solvent evaporation method.The obtained sample of EETA (ethanolic extraction of Terminalia arjuna bark extract) was estimated for UV and IR studies.Prepared polymeric nanoparticles were characterised for FT-IR,Particle size, PDI, Zeta potential,% DEE, SEM, TEM and antioxidant activity.In vitro and In vivo drug release studies and Stability studies were also evaluated.The FT-IR study showed that drugs and polymers are compatible with each other.Particle size was found to be in the range of 188nm to 220nm.The PDI for methods was found to be 0.335 to 1.000 respectively.The % DEE of TANP4 was found to be 88%.The TEM showed that the polymeric nanoparticles prepared were found to be granular, elliptical in shape and crystalline in nature.The SEM showed irregularity of shape with smooth texture.The antioxidant activity of TANP4 showed good antioxidant property as compared to standard ascorbic acid.After 12 hours, the in vitro release research demonstrated sustained activity; a higher polymer led to a lower drug release. The produced polymeric nanoparticles exhibit notable hyperlipidemic activity, according to the in vivo investigation.According to stability studies, formulations remain stable up to three months.Terminalia arjunabark extract loaded polymeric nanoparticles was successfully prepared by solvent evaporation method.The formulated nanoparticles were characterized for various parameters and fruitfully performed in vivo activity.

Keywords: *Polymeric nanoparticles, a solvent evaporation method, anti-hyperlipidemic activity, ethanolic extraction*

Introduction

Oral drug delivery remains the most widely preferred route of administration due to its non-invasive nature, ease of use, cost-effectiveness, and compatibility with mass production. Compared to parenteral methods such as intravenous, intramuscular, and subcutaneous injections—or even inhalational therapies—oral formulations offer significantly greater patient compliance and convenience [1].In the pursuit of patient-friendly, needle-free delivery options, oral administration of biopharmaceuticals—including proteins, vaccines, and anti-cancer agents—has emerged as a promising alternative. This advancement is being driven by the use of nanocarrier-baseddelivery systems, which utilize both natural and synthetic polymers to enhance drug delivery efficiency. Various nanocarrier platforms have been explored, including nanoparticles, liposomes, micelles, nanocrystals, dendrimers, nano-emulsions, carbon nanotubes, and nanospheres [2].In these systems, therapeutic agents can be dissolved, encapsulated, adsorbed, or covalently linked to the nanocarrier matrix composed of colloidal particles. The choice of preparation method depends on the physicochemical characteristics of both the drug and the polymer matrix.

Nanoparticles are especially advantageous in oral drug delivery because they can protect drugs from enzymatic degradation and harsh gastrointestinal (GI) conditions, prolong residence time in the gut through mucoadhesive interactions, and enhance absorption and bioavailability [3]. As such, nanotechnology has opened new frontiers in improving the oral delivery of complex and sensitive drug molecules.When taken orally, these medications encapsulated nanoparticles have a higher bioavailability.The optimum mix of qualities can be found in polymer materials, which are stable, permit large loading of several agents, give control over drug release kinetics, are easily adaptable to display a wide range of surface-attached ligands, and many polymers have a long history of being safe for human use.Polymeric nanoparticles for oral medication administration have been created using a range of biodegradable and non-biodegradable polymers[4].

Nanocarriers can enhance drug absorption through the gastrointestinal (GI) mucosa by either increasing membrane permeability for the drug or its carrier system, or by inhibiting efflux transporters that actively expel drugs from the GI epithelium.

Additionally, the use of bio adhesive polymers can prolong the residence time of the drug in the GI tract, promote closer contact with the absorption site, and facilitate sustained or targeted release of the therapeutic agent. These mechanisms collectively improve drug bioavailability and therapeutic efficacy.However, despite their advantages in enhancing solubility, activity, and systemic distribution of drugs, nanocarrier-based drug delivery systems still face several critical challenges. These include quality control, physicochemical stability, storage requirements, scalability for large-scale manufacturing, and precision in formulation technologies. Furthermore, concerns related to *in vivo* metabolism, excretion, as well as potential acute and chronic toxicities must be rigorously addressed. The clinical and commercial translation of nanocarrier systems thus hinges on robust and comprehensive research aimed at resolving these limitations and establishing safety and efficacy benchmarks [2].

Recent research has shown that the stability and nanoscale size of polymeric nanoparticles have led to the manifestation of several novel features and activities.Polymeric nanoparticles which range in size from 1 to 1000 nm, can have active substances embedded within them or surface absorbed onto their core[14].

The use of biopolymer formulated nanoparticles in pharmaceutical formulations is becoming more and more popular.Polysaccharide nanoparticles are of particular interest because of their numerous uses, nontoxicity and biocompatibility.They enhance the permeability and solubility of many potent medications since they typically serve several purposes.Drugs coated with polysaccharides are more effective because of their biocompatibility and ability to penetrate tissues[15].

Terminalia Arjuna or Arjun a is a member of the Combretaceae family.Based on centuries of observations by ancient physicians, its bark decoction is used in the Indian subcontinent to treat angina pain, hypertension, congestive heart failure, and dyslipidemia[16].

It is a large evergreen deciduous tree with a height 60-80 feet in height. leaves are simple sub-opposite, oblong or elliptical, chordate, shortly acute or obtuse at the apex, 5-25, 4- 9 cm.The base is spherical, or occasionally cordate.Short (2-4cm) sericeous and featuring two (or one) conspicuous glands at the petiole tip, just beneath the leaf, is the petiole.This character stands for Terminalia arjuna's distinct pharmacognostic characteristic.The bark is smooth, pinkish-grey from the outside and fakes off in large, curved and rather flat ,pieces[17].

The hyperlipideHyperlipidemialy defined as conditions in which the concentration of plasma lipids including cholesterol, triglycerides, cholesterol esters and phospholipids and plasma lipoproteins like VLDL and LDL in plasma exceeds an arbitrary normal limit and reduces HDL limit. [18-19].

Materials and Methods

Gumrosin (Himalaya Terpenes Pvt Ltd), Guar gum(SD fine chemicals, Mumbai),Sodium lauryl sulphate (SD fine chemicals, Mumbai),Sodium hydroxide (Fischer scientific, Mumbai),Hydrochloric acid (SD fine chemicals, Mumbai),Acetic acid (Fischer scientific, Mumbai),Potassium dihydrogen orthophosphate (Qualigens chemicals, Mumbai)Ethanol (SDfine chemicals, Mumbai).

Soxhlet ethanolic extraction of *Terminalia arjuna* bark

The Soxhlet device allows the desired content to be isolated when other compounds or contaminants are insolublein the solvent.The glass Soxhlet apparatus is composed of a condenser at the top, a syphon tube, and extraction chamber, and a flash with spherical bottom.All the *Terminaliaarjuna* barks are shade dried for 15 days, and they are coarsely powdered using a pulveriser.The plant material is enclosed in a cotton thimble, a tightly sealed permeable pouch.Extracting secondary metabolites with Ethanol 99%.The solvent leaking out of the extraction chamber will no longer leave any trace when the bioactive ingredients have been eliminated.This process is repeated for roughly 15-20 cycles and subjected to evaporation in a Rota evaporator and the powered form of *Terminalia arjuna* is collected[15].

Phytochemical investigation of *Terminalia arjuna* extract

A. Test for Carbohydrates

Molisch's Test: To detect the presence of carbohydrates, 2–3 ml of the plant extract was placed in a clean test tube. A few drops of α-naphthol solution in alcohol were added and the mixture was gently shaken. Subsequently, concentrated sulfuric acid was carefully added along the inner wall of the test tube without disturbing the mixture. The formation of a violet or purple-colored ring at the interface of the two liquids confirmed the presence of carbohydrates**.**

Fehling's test: To 1 ml of extract, add equal proportion of Fehling's A and Fehling's B solution was added and slightly heated, resulting in a brick-red precipitate, indicating the presence of carbohydrate.

Benedict's test: Equal volume of plant extract and Benedict's reagent was mixed.Test tube was heated in boiling water bath for 5 min, resulting in a red precipitate[20].

B. Test for Monosaccharides

Barfoed's Test: To test for the presence of monosaccharides, an equal volume of Barfoed's reagent and the plant extract was mixed in a test tube. The mixture was then heated in a boiling water bath for 1–2 minutes and allowed to cool. The formation of a red precipitate indicated the presence of monosaccharides, due to the reduction of copper(II) acetate to copper(I) oxide under acidic conditions.

C. Test for Proteins

Biuret test: Add 3ml extract to 4% sodium hydroxide and a few drops of 1% copper sulphate solution.The appearance of purple colour indicates presence of proteins.

Millon's test: Add 3 ml of Millon' reagent and heated on water bath.Pink or red precipitate shows the presence of proteins.

D. Test for Amino Acids

Ninhydrin Test: To detect the presence of proteins or free amino acids, 3 ml of the plant extract was mixed with 3 drops of 5% Ninhydrin reagent in a test tube. The mixture was then heated in a boiling water bath for 10 minutes. A blue to purple coloration indicated a positive result, confirming the presence of proteins or free amino acids, due to the reaction of ninhydrin with amino groups forming a colored complex.

E. Test for Steroids

1. Salkowski Reaction:

To 2 ml of the plant extract, add 2 ml of chloroform followed by 2 ml of concentrated sulfuric acid. Shake the mixture gently. The formation of a bluish-green coloration indicates the presence of steroids.

2. Liebermann-Burchard Reaction:

Mix 2 ml of the extract with chloroform, then add 1–2 ml of acetic anhydride. Carefully add 2 drops of concentrated sulfuric acid along the side of the test tube. The appearance of a red color, changing to blue and finally to green, confirms the presence of steroids and triterpenoids.

3. Liebermann Reaction:

To 3 ml of the extract, add 3 ml of acetic anhydride. Heat the mixture gently, then cool it. Add a few drops of concentrated sulfuric acid. The development of a blue coloration indicates a positive result for steroids.

F. Test for Flavonoids

Shinoda test: Mix aqueous extract, 95% ethanol, few drops of 10% hydrochloric acid, and 0.5 gmMagnesium turnings.Orange, pink, red to purple colour appears.

lead acetate solution: Aqueous extract was shaken with led acetate solution. Yellow colour precipitate formed[22].

G. Test for Glycosides

Borntrager's Test: Take 3 ml of the aqueous extract and add a small amount of dilute sulfuric acid. Boil the mixture gently for a few minutes, then filter it. To the cooled filtrate, add an equal volume of chloroform and shake well. Allow the layers to separate. Transfer the chloroform (organic) layer to a new test tube and add an equal volume of ammonia solution. A pink or red coloration in the ammoniacal layer indicates the presence of anthraquinone glycosides.

Libermann reaction: Mix 3 ml extract with 3 ml acetic anhydride.Heat and cool, add few drops of concentrated sulfuric acid.The blue colour appears.

Legal's test: To 2 ml extract, add 1 ml pyridine and 1 ml sodium nitroprusside.Pink to red colour appears.

Baljet's test: An aqueous extract shows yellow to orange colour with sodium picrate.

H. Test for Saponin Glycosides

Foam Test: Shake the drug extract vigorously with distilled water in a test tube. Persistent and stable foam formation that lasts for more than 10 minutes indicates the **presence of saponin glycosides**.

I. Test for Tannins and Phenolic Compounds

1. Ferric Chloride Test: Add a few drops of 5% ferric chloride ($FeCl_3$) solution to 2 ml of extract. A deep blue-black coloration indicates the presence of phenolic compounds or tannins.

2. Lead Acetate Test: To 2 ml of the extract, add a few drops of lead acetate solution. A white precipitate confirms the presence of phenolic compounds.

3. Acetic Acid Test: Add a few drops of acetic acid to 2 ml of the extract. The appearance of a red coloration suggests the presence of phenolic compounds.

4. Potassium Dichromate Test: Add a few drops of potassium dichromate solution to 2 ml of the extract. A red precipitate indicates the presence of tannins.

5. Dilute Nitric Acid Test: Add a few drops of dilute nitric acid to 2 ml of the extract. A reddish to yellow coloration indicates the presence of phenolic groups.

6. Dilute Potassium Permanganate Test: Add a few drops of dilute potassium permanganate solution to 2 ml of extract. Decolorization of the solution indicates the presence of phenolic compounds due to their reducing nature.

J. Test for Alkaloids

Dragendroff's test: 2 mlextract, add few drops of dragendroff reagent.The orange brown precipitate is formed.

Mayer's test: 2ml extract,add few drops of Mayer's reagent gives precipitate.

Hager's test: 2 ml extract, add few drops Hager reagent which gives yellow precipitate.

Wagner's test: 2 ml extract, add few drops Wagner reagent which gives reddish brown precipitate.

Tannic acid test: 2 ml extract, add few drops of tannic acid solution gives buff colour precipitate[5].

Quantitative phytochemical evaluation of Terminalia arjuna EthanolicExtract

Assessment of total quantitative polyphenolic content

The Folin Ciocalteaureagent by Singleton and Rosie was employed to measure the total phenolic contents in aqueous extract of *Terminaliaarjuna* bark. 5 ml of tenfold dilutedFolin Ciocalteau reagent and 4 ml of sodium carbonate solution was mixed with 1 mlaliquots of 50, 100, 150, 200, 250, 300, 350 and 400 mg/ml of aqueous extract solution.The absorbance at 765 nm was measured 30 minutes later.Using this procedure, the absorbance was tested 1 hrlater to ascertain the extracts total phenol phenolic content. [24]

$$C = C1 \times V/m$$

Where:

- **C** = Total phenolic content expressed as mg of **gallic acid equivalent (GAE)** per gram of extract (mg/g GAE)
- $\mathbf{C_1}$ = Concentration of gallic acid obtained from the calibration curve (mg/mL)
- **V** = Volume of the plant extract used (mL)
- **m** = Weight of the plant extract (g)

Assessment of Total Flavonoids: The method used to determine the flavonoids relies on the development of a flavonoid-aluminium combination,which exhibits a maximum absorbance at 430 nm. 50 ml of sodium nitrate (5% w/v) and 1 ml of distilled water were combined with 200 ml of the tested extracts (0.5 mg/ml).The reaction mixture was incubated for 6 minutes at room temperature before 120 ml of 10%w/vAlCl3 was added.

The combination was then incubated for a further 5 minutes or so under the same conditions. 400 ml of 1MNaOH were added.At 430 nm, the absorbances were measured in comparison to the blank.The flavonoid concentration was calculated as rutin equivalents and reported as mg/rutin mg of plant extract, with quercetin serving as the standard to create the calibration curve. Each of the determinants has been made three times.[6]

$Y = 0.0014 X – 0.0033, R^2 = 0.997$
Were,
Y = absorbance
X= flavonoid content in µg/g

FT – IR method: A small amount of TA Extract was taken and compressed with KBr to obtain the pellet. This pellet was subjected to estimation using an IR instrument (Bruker Alpha 2). The obtained graph was compared with the standard graph of EETA.

Development of UV spectroscopic methods
Determination of Absorption Maxima
Absorption maxima of an active pharmaceutical ingredient are been an important parameter in proceedings of further preparations as well as evaluations. Since absorption maxima is unique for API it would be easier to determine the presence of drug and even its concentrations.

Preparation of standard stock solution
A precisely weighed 100 mg sample of Terminalia arjuna extract was extracted and diluted in a little amount of 7.4 pH phosphate buffer in a 100 ml volumetric flask.Then, using the same solvent, the volume was increased to the 100 ml mark; this is known as the stock I solution.Next, 10 ml of the stock solution mentioned above was pipetted out and put into a second 100 ml volumetric flask.Using the same solvent, the volume was increased to the 100 ml mark. This solution known as stock II solution, is used to find absorption maxima.

Determination of absorption maxima
Thus, prepared stock II solution was a typical solution to determine absorption maxima since it is within the beers range. The wavelength range in UV spectrophotometer was adjusted from 200 nm to 400 nm and base-line correction was carried out using blank 7.4 pH buffer to eliminate any kind of absorbance. Then in sample cuvettes, the TA extract stock II solution was transferred and spectrum was analyzed. The maximum absorbance at particular wavelength was reported as its absorption maxima. [7]

Development of Standard calibration curve
Stock I: After being accurately weighed, 100 mg of Terminaliaarjuna were put into a 100 ml volumetric flask and dissolved in a small amount of phosphate buffer with a pH of 7.4.Using the same buffer, the volume was adjusted up to the mark.

Stock II: 10ml of the stock I solution mentioned above were pipetted out and put into a 100 ml volumetric flask.The volume was adjusted to the 100 ml mark using buffer.
From above stock II solution appropriate aliquots of the sample were with-drawn and transferred to different volumetric flask and volume was made up to the mark with 7.4 pH buffer to produce 5, 10, 15, 20, 25, 30, 35 and 40µg/ml concentrations.

These solutions were scanned in UV spectrophotometric between the range of wavelength 200nm to 400nm and the absorbance at 278 nm is been reported. Further, the standard calibration graph of concentration v/s absorbance was developed. Slope, intercept and regression coefficients were reported. [8]

Preparation of polymeric nanoparticles

Table No 01. The formula for the preparation of polymeric nanoparticle by solvent evaporation method

Ingredients	Batch Code			
	F1	F2	F3	F4
Terminalia arjuna bark extract	100	100	100	100
Guar gum	300	400	-	-
Gum rosin	-	-	300	400
Sodium lauryl sulphate (mg)	500	500	500	500
Ethanol	10	10	10	10
Deionized water (ml)	100	100	100	100

Solvent evaporation method
Terminalia arjuna extract and gums were accurately weighed and diluted in ethanol to produce solution I. Solution II was created by simultaneously dissolving sodium lauryl sulphate in distilled water. The solution I was added to solution II then it was homogenized for 10 minutes at 15,000 rpm the dispersion was sonicated for 5 minutes with (30 sec pause).Each formulation was stirred on a magnetic stirrer for 6 hours at 100 rpm to evaporate methanol, then centrifuged for 20-30 mins at 15,000 rpm and the supernatant liquid was removed to form pallets.The pellet was pre-frozen at 40°C in a pre-glycolic route after being redispersed in a cryoprotectantsolution containing 0.5% mannitol.It was lyophilized for four hours at -80°C (primary drying)and 24 hrs at -40°C (secondary drying).To prevent moisture,the resulting spongy nanoparticle was kept in an airtight container.[7]

Characterization
Compatibility Studies
Attenuated total reflectance- Fourier transform infrared spectroscopy was carried for individual excipients and a formulation from each method.The study was carried out to identify any kind of drug exception interactions.Any changes or shifts in the peaks of FTIR indicates the changes within functional groups.To ensure the drug excipients interactions at ATR-FTIR study was carried out.Excipients and formulations are kept separately in Bruker alpha at ambient temperature of 25 + 0.5°C. The analytical procedure was simple and did not need any special sample preparation.The various functional groups of the samples were analysed after the spectra were captured by positioning the separate excipients and scanning the samples in the 4000-400 cm^{-1} range.

Solubility study of polymeric nanoparticles
The polymeric nanoparticle made by the solvent evaporation process was put through a solubility test using distilled water, methanol, and DMF in accordafollowingle of Terminalia arjuna bark extract. After dissolving 1 mg of the corresponding sample in 10 ml of solvent, the mixture was sonicated for two minutes.

% Drug content (%DC)
10 mg of *Terminaliaarjuna*bark extract containing nanoparticles was equivalently weighed and dissolved in 7.4 pH phosphate buffer followed by continuous stirring, then the solution was sonicated for 10 min and the solution was diluted to appropriate beer's range.

Absorbance was observed at 278 nm by using UV spectrophotometer.%DC was calculated by using the formula:

$$\%Drug\ content = \frac{x\ X\ dilution\ factor\ X\ 100}{1000}$$

Drug entrapment efficiency

The amount of *Terminaliaarjuna* entrapment efficiency was determined by the UV analysis (Shimadzu UV-1800) at 276 nm.Following centrifugation and evacuation of the first pellet, the supernatant was UV monitored at a wavelength of 278 nm, and the associated absorption maxima were noted.In order to analyze the samples indirect estimate of encapsulation effectiveness and percentage yield, a generic calibration curve was created using varying doses of methanol extract (1-10 mg in 10 ml water) vs. total supernatant absorption, as revealed by UV monitoring.The efficiency and encapsulation %were calculated using the following formula [9].

$$\%DEE = \frac{Amount\ of\ drug\ actual\ present * 100}{Theoretical\ drug\ load\ expected}$$

Fourier transform infrared spectroscopy (FT-IR) analysis

Following a baseline adjustment using dried potassium bromide to verify compatibility,FT-IR analysis was performed on the purified drug and nanoparticles mixed with Potassium (KBr). For this analysis, pellets were formed in an evacuated die, subjected to a pressure of approximately 5 x 106 Pa to create clear, transparent discs measuring 2 cm in diameter and 0.2 cm in thickness.Spectra were recorded at room temperature using an integrated.Fourier transform spectrometer (Bruker Alpha2 Germany), covering a range from 4000 cm^{-1} to 400 cm^{-1}.

Particle size, PDI and Zeta potential

A particle size analyzer (Nano ZS, Malvern equipment,UV) was used to measure the mean particle size,PDI and zeta potential of the produced nanoparticles.DLS was used for size analysis, and LDV and PALS were combined for zeta potential both of these values provide PDI[7].

Transmission electron microscopy studies (TEM)

The polymeric nanoparticle from formulation F6 was subjected to high-resolution transmission electron microscopy using JEOLJEM 2010FUHR running at 200 kV.After that, the polyol-suspended nanoparticles were put on the transmission electron microscopy grid's amorphous carbon membrane and let to evaporate at room temperature.The grid underwent further heat treatment at 150°C under an ultrahigh vacuum to get rid of the majority of the organic compounds.This treatment significantly increased high-resolution photos without changing the crystallinity of the particles.To ascertain the particle size distribution, we examined the TEMpictures using a digital camera and the SAISAM and TAMI software (Microvision Instruments)[10].

Scanning electron microscopy studies (SEM)

After sputtering a thin layer of gold onto the produced nanoparticles from F2, F4, and F6, the microstructure was examined using a scanning electron microscope equipped with an EDAX equipment (JEOL 6390LA/OXFORD XMX N) that ran at a 20kV acceleration voltage[11].

***In vitro* drug release study**

In vitro drug release from TA-loaded nanoparticles was tested using the dialysis procedure, which involves placing 10 mg of the nanoparticles in a dialysis bag, sealing it and then immersing it in release media (20 ml of phosphate buffer saline at pH 7.4).Samples were obtained for analysis from the dialysis membrane,where the medication released from the TA-loaded nanoparticles diffuses to the outer release media. The whole assembly was kept at room temperature and a magnetic stirrer set at rpm of 100.At the given time intervals 1 ml of solution (2,4,6,8,10…24 hr) has been withdrawn and replaced with a fresh buffer.The samples collected were diluted with phosphate buffer, and UV spectrophotometrically analysed at 278 nm to quantify the amount of drug released.Drug release experiments were also performed and compared with the synthesized nanoparticles for *Terminalia arjuna*(ethanol extract).The studies were replicated three times, and the mean ± standard deviation expressed all the results[11].

Antioxidant Activity

The antioxidant potential of NP1 and the plant extract was evaluated using the DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay. Briefly, 0.1 mM DPPH solution in ethanol was mixed with various concentrations of the plant extract to achieve final concentrations of 10, 20, 40, 80, and 160 µg/mL.Ascorbic acid was used as the standard antioxidant for comparison.

After mixing, the reaction mixtures were incubated at room temperature for 30 minutes in the dark, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

The antioxidant activity was expressed as the percentage of DPPH radical scavenging, calculated using the formula:

$$Percentage\ of\ DPPH\ radical\ scavenging\ activity = \frac{Ac - As}{Ac}$$

Where:

- AsA_sAs = Absorbance of the test sample
- AcA_cAc = Absorbance of the control (DPPH solution without sample)

***In vivo* antihyperlipidemic drug action in Wistar albino rats**

The PNPTANP4 was selected for the *in vivo* activity because it demonstrated excellent *in vitro* drug release.The plasma lipid profiles of both sexes of healthy albino rats weighing 200-300 grams were measured.Throughout the investigation the animals had unrestricted access to food and water.The animals were split into four groups TANP4, standard, control and normal.The treatment lasted 21 days. The standard group was given Terminalia arjuna bark extract (2 mg/kg body weight) once a day, the control group received pure 2 ml coconut oil, and the normal group received regular diet pellets. The polymeric nanoparticle solution (15 mg/kg body weight) prepared in distilled water was supplied to test group TANP4.Daily dosing continued for 28 days.A blood sample was taken using a retroorbital device on days 0, 7, 14 and 21.It was centrifuge for 20 min at -20°C at 3000 rpm.Using testing kits (Ebra Ltd. India), the plasma levels of cholesterol, triglycerides, HDL and LDL were estimated.For the reference, fixed volumes specified in the standard kit's leaflets were prepared, and working reagent was added to each group's plasma and left for 10 minutes.At the same time, a blank was made by removing the sample, which was then absorbed spectrophotometrically for cholesterol at 505 nm and triglycerides at 546 nm, respectively[12].

Comparative pharmacokinetic drug release study

Higuchi's model and Korsmeyers and Peppa's model are used in drug release studies.

Higuchi's Model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

Q = [DЄ /ι (2A – ЄCs) Cst] ½

Were,

Where:

- **Q** = Amount of drug released at time ttt
- **D** = Diffusion coefficient of the drug in the matrix
- **A** = Surface area of the drug matrix
- **C_s** = Solubility of the drug in the matrix
- **C** = Concentration of the drug at time ttt
- **\varepsilon** = Porosity of the matrix
- **t** = Time
- **T** = Time (often used as ttt in standard equations, not different in this case)

This equation can describe drug release based on diffusion and the solubility of the drug in the matrix, considering the release profile over time.

You're absolutely correct! When simplifying the equation under the assumption that the diffusion coefficient (D), solubility (C_s), and surface area (A) are constant, the drug release equation can be reduced to the following form:

Q = Kt1/2

Where:

- **Q** = Amount of drug released at time **t**
- **K** = Constant (related to the diffusion rate)
- **t** = Time (in hours, or any chosen time unit)
- The term t1/2t^{1/2}t1/2 (square root of time) represents the time-dependent nature of the drug release process under diffusion-controlled mechanisms.

Explanation:

- When the drug release follows Fickian diffusion (meaning it is diffusion-controlled), the cumulative drug release is proportional to the square root of time.
- By plotting the cumulative drug release (Q) against t1/2t^{1/2}t1/2, you should get a straight-line graph.
- The slope of this line will be equal to the constant K, which is a function of the diffusion properties of the system.

This simplified model indicates that the release mechanism is diffusion-driven, which is a common scenario for many matrix-based drug delivery systems, especially when the drug release is slow and controlled.

Next Step:

To verify this model:

1. Plot the cumulative drug release (QQQ) on the y-axis.

2. Plot the square root of time (t1/2t^{1/2}t1/2) on the x-axis.

3. Check for a linear correlation. If the plot is linear, the drug release is diffusion-controlled, and you can calculate the slope (K).

If you'd like to do the actual analysis or need further help with plotting or understanding how to calculate K, feel free to share your data!

Korsmeyers Equation / Peppas Model

The Korsmeyer-Peppas model, commonly used to describe the release kinetics of drugs from polymeric systems, can be expressed using the following equation:

Mt / Mα = Ktn

Where:

- **M_t / M_\alpha** = Fraction of the drug released at time **t** (fraction of the total drug released).
- **K** = Constant that incorporates the structural and geometrical characteristics of the drug or polymer system.
- **n** = Diffusion exponent, which helps to identify the mechanism of drug release.
- **t** = Time at which the fraction of the drug has been released.

Simplifying the Equation:

By applying a logarithm to both sides of the equation, it can be linearized:

Above equation can be simplified as follows by applying long on both sides,

Log Mt / Mα = Log K + n Log t.

Using KinetDS3 software or Excel, you can fit your release data to the Korsmeyer-Peppas model and analyze the release mechanism based on the value of n. This method helps to better understand the underlying mechanisms (whether diffusion-controlled, swelling-controlled, or a combination of both) for the drug release from liposomal systems or other drug delivery systems [13].

Stability studies

Stability studies were conducted to quantify drug loss from polymeric nanoparticles. A stability analysis was conducted for the improved formulation TANP4.Formulation was divided into three sets of samples and stored at -2°C and room temperature (29°C).Stability of the formulations was predicted from the results obtained for entrapment efficiency and *in vitro*drug release study after storing at room temperature and -2°C samples were estimated for one month interval for 3 months.[11]

Results and Discussion

Estimation of Terminalia arjuna Bark Extract

UV- visible Spectrophotometric method

The sample which was subjected to UV- visible Spectrophotometric method showed λmax at 278 nm by using ethanol as solvent.

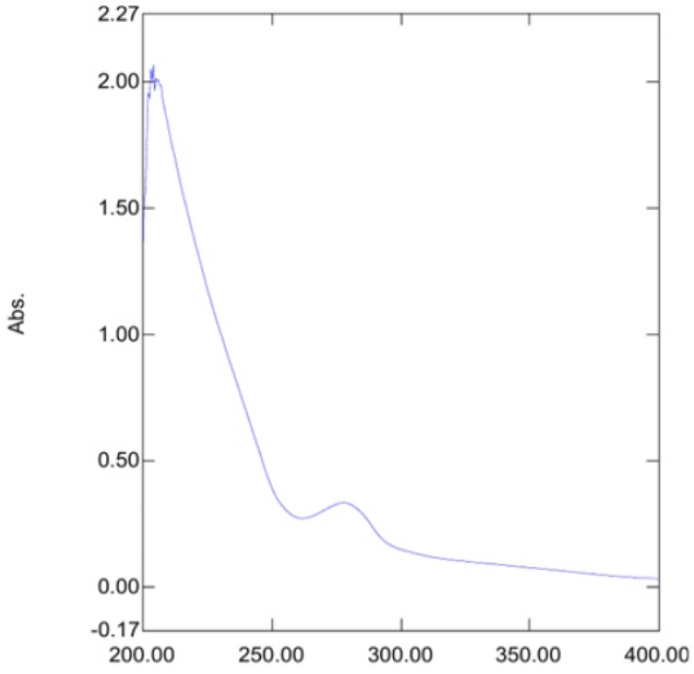


Fig No 01. max of Terminalia arjuna Extract at 278 nm (Arjunolicacid)

Estimation of the *Terminaliaarjuna*bark extract sample was performed by UV visible Spectrophotometric method; described in section Table No.02.The absorption maxima λmax was found to be 278 nm(Arjunolic acid) as shown in Fig.01.

Standard calibration curve of DST in Methanol

Table 02: Absorbance data for the standard calibration curve of EETA

Sl. No	Con in µg/ml	Abs at 278nm
01	5	0.087
02	10	0.146
03	15	0.328
04	20	0.362
05	25	0.431
06	30	0.553
07	35	0.636
08	40	0.734

**(All the samples were taken in triplicate. n = 3 ± SD)*

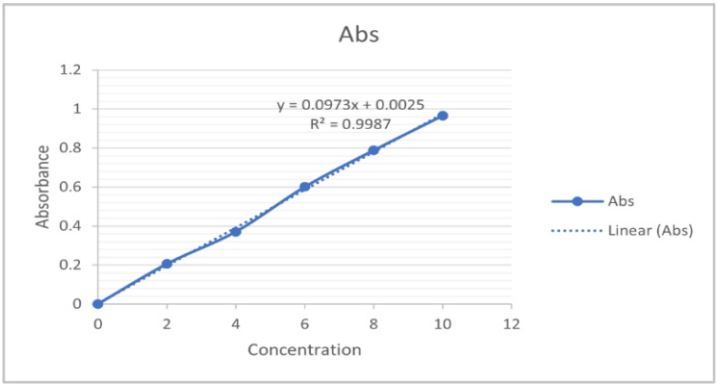


Fig No 02. Standard calibration curve of Terminalia arjuna Extract in ethanol

Table 03: Statistical data for calibration curve.

Sl. No	Parameters	Value
01	λmax(nm)	278nm
02	Beer's law limits.	02–40µg/ml
03	Slope	0.010
04	Constant	0.0025
05	R2	0.9987

The standard calibration was executed according to the procedure in methanol described in section Table No 02. And obtained values are depicted in the Table No 03. The slope of the curve was found to be 0.0957 and R2 value was found to be 0.998

Solubility of nanoparticles

Table 04: Solubility of nanoparticles in different solvents

Solvent Name	Nanoparticles solubility
Distilled water	Sparingly soluble
Methanol	Soluble
Ethanol	Soluble

The obtained nanoparticles were subjected to solubility test according to drug profile of *Terminalia arjuna* bark extract described in Table No 03 and results were given in Table No 04. The obtained polymeric nanoparticles were soluble in methanol, ethanol but TANPs were sparingly soluble in water.

Phytochemical Investigation

Table No 05: Phytochemical Analysis of Ethanolic Extract of Terminalia arjuna Bark

Phytoconstituents	Tests	Conclusion
Phytosterols	Salkowski reaction	++
Triterpenoids	Libermann-Burchard's test	+
Saponins	Foam test	+
Carbohydrates	Molisch's test	+
Flavonoids	Lead acetate test	++
Lactones Phenolic compounds an Tannins	Legal's test 5% $FeCl_3$ Test	++ ++
Proteins	Ninhydrin test	+
Glycosides	Keller-Killian test	++

Ethanolic Terminalia arjuna bark extract with all phytochemical investigation result showed presence of triterpenoids, flavonoids, phenols, tannins etc. which are described in the Table No 05.

Quantitative phytochemical evaluation

Table No 06: Quantitative phytochemical evaluation of Ethanolic extract of Terminalia arjuna bark extract

Phytochemicals	Concentration
Triterpenoids	129.2mg/g
Flavonoids	116.5mg/g
Polyphenols	57.2 mg/g

Quantitative analysis of ethanolic terminalia arjuna bark extract for triterpenoids, flavonoids and phenols resulted in 129.2 mg/g, 116 mg/gm, and 57.2 mg/g respectively.Which described in Table No 06.

FT-IR analysis

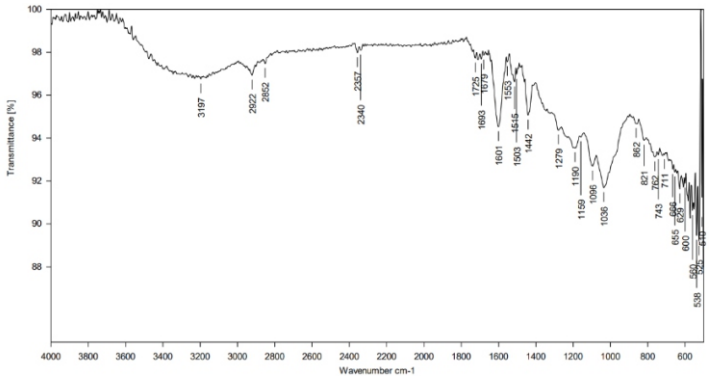


Fig No 03. FT-IR Spectrum of EETA active ingredient

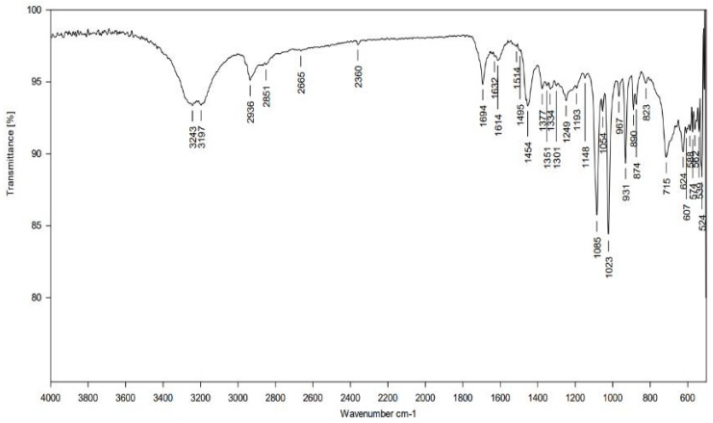


Fig No 04. FT-IR Spectrum of formulation TANP2

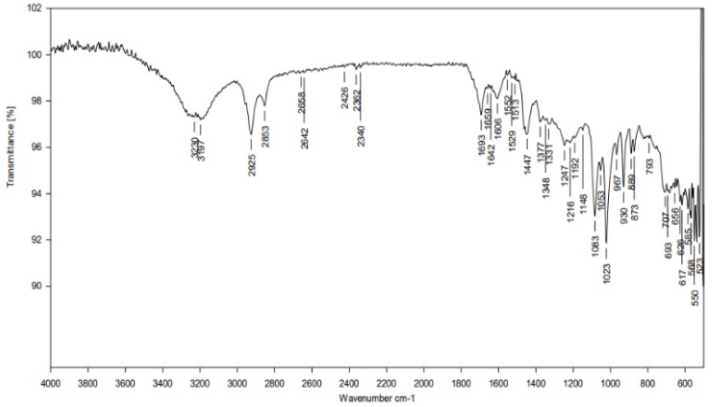


Fig No 05. FT-IR Spectrum of formulation TANP4

Table 07: FT-IR interpretation of drug nanoparticles.

Sl. No	Compound	Functional groups	Frequency range cm^{-1}	Peak cm^{-1}	Vibration
01	Terminalia arjuna	OH	3600-3200	3197	Stretching
		COOH	3600-2500	2852	Stretching
		C=C	1680-1600	1679	Stretching
		O=C-OR	1750-1720	1725	Stretching
		C-OC	1250-1050	1095	Stretching
02	Terminalia arjuna + Guar gum	OH	3800-3600	3625	Stretching
		C-H	3000-2800	2905	Stretching
		C=O	1800-1600	1713	Stretching
		OH	3800-3600	3625	Stretching
03	Terminalia arjuna + Gum rosin	C-H	1550-1500	1507	Bending
		OH	3580-3700	3592	Stretching
		C-H	2840-3000	2871	Stretching
04	TANP1	COOH	3600-2500	3243	Stretching
		C=C	1680-1600	1694	Stretching
		C-OC	1250-1050	1085	Stretching
05	TANP2	C-H	2840-3000	2925	Stretching
		C=O	1800-1600	1642	Stretching
		COOH	3600-2500	3175	Stretching
		C=C	1680-1600	1693	Stretching

The FT-IR spectrum estimation was performed to verify for a pure sample of *Terminalia arjuna* bark extract, physical mixture of pure drug, and polymers studies carried out for all the formulations.The method has described in Fig 03, 04, 05 and the result is exhibited in Table No 07. The peak values which were present in the Terminaliaarjuna bark extract graph was approximately appeared in the formulationTANP1& TANP2.From the interpretation of spectra's, principle peak of drug and polymer were not altered hence there was no interaction between drug and polymer.

Determination of particle size, PDI, Zeta potential and DEE

Table 08: Measurement values of particle size, PDI,Zeta potential and DEE

Sl.No	Formulation	Size in nm	PDI	Zeta potential in mV	% of DEE of Efficiency
01	TANP1	220.4	0.349	-12.7	72.7%
02	TANP2	198.8	0.304	-24.3	75%
03	TANP3	209.1	0.453	-21.6	70%
04	TANP4	188	0.348	-24.0	84%

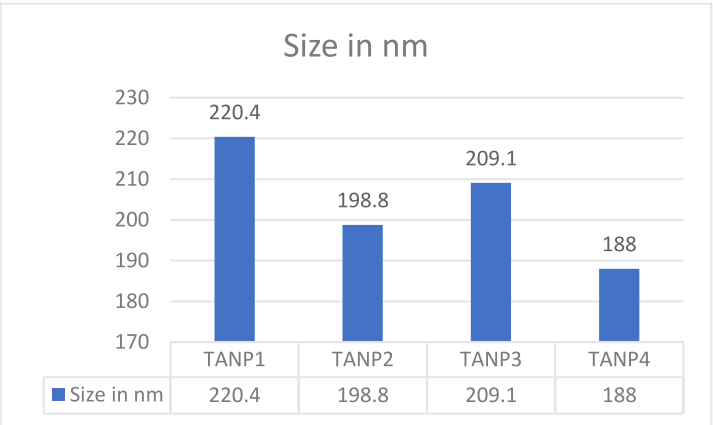


Fig No 06. Particle size distribution of all the formulations

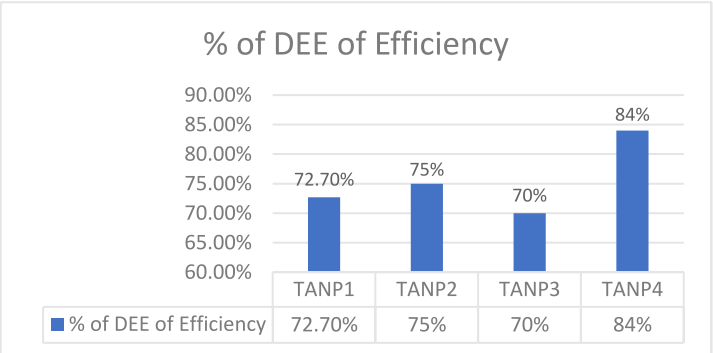


Fig No 07. %DEE of TANP polymeric nanoparticles

Table No. 8 describes the procedures for measuring DEE, particle size, PDI, and zeta potential. The drug entrapment effectiveness of polymeric nanoparticles was reported to be between 70% and 84%. This range indicates that drug entrapment increased as polymer concentration rose. It demonstrates that when the polymer concentration increased, better entrapment was found, as illustrated in Figure 7.

Particle size and PDI of polymericnanoparticles was found to be in the range of 188 to 220 nm and 0.3492 to 0.453 respectively.

The surfacecharge of particles can be signified by zeta potential.The zeta potential value of the polymeric nanoparticles was found to be in the range of-12.7 to -24.6.Table 08 shows the values for DEE, particle size, PDI, and zeta potential.

Drug Content

Table 09: Percentage drug content of all the formulations

Formulations	Drug content
TANP1	68%
TANP2	74%
TANP3	80%
TANP4	88%

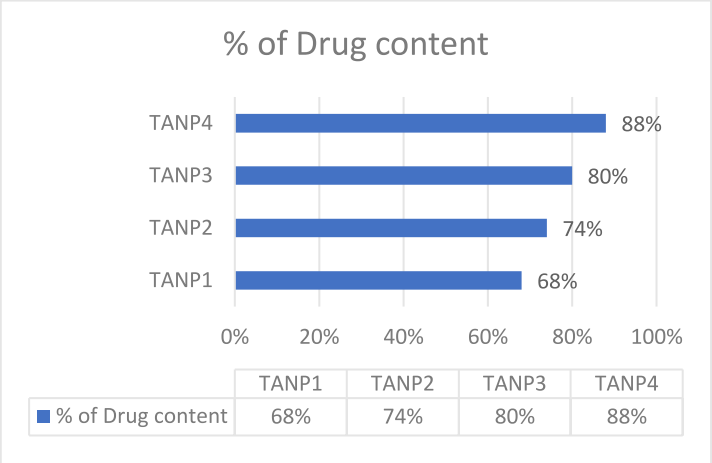


Fig No 08. %Drug Content of TANP Polymeric nanoparticles

Drug content results of all formulations were shown in Table No 09 and bar graph of polymericnanoparticle was shown in Fig08.As the polymer concentration increases drug content decreases.

Transmission electron microscopy

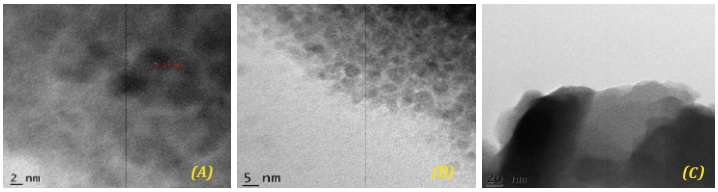


Fig No 09. A, B & C. TEM photographs of formulation TANP4

HR-TEM was used to characterize the TANP4 polymeric nanoparticles.The method has been outlined in Fig 09. The nanoparticles are arranged in the eccentric pattern which gives the proof of crystallinity in nature.

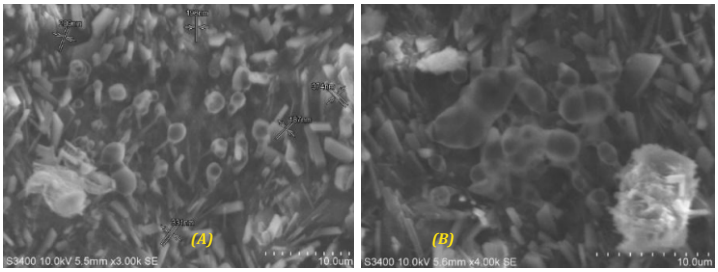


Fig No 10. A & B SEM photographs of formulation TANP4

TANP4 nanoparticles were characterized by FE-SEM with EDX,the nanoparticles were found to be irregular in shape as shown in Fig 10.The texture was found to be smooth and the particles are non-porous.

In-vitro Drug release study

Table 10: In-vitro diffusion profile of formulation TANP1 to TANP4 in 7.4 pH buffer

Time in Hrs	TANP1	TANP2	TANP3	TANP4
01	4.15	5.975	4.34	6.78
02	14.18	16.47	15.88	18.82
03	19.25	21.92	20.99	25.97
04	24.26	27.18	26.93	30.08
05	30.82	33.11	31.47	37.97
06	33.74	39.22	35.74	41.27
07	40.28	43.42	42.95	47.17
08	53.12	57.40	55.17	60.18
10	65.18	68.76	66.92	73.83
12	82	88.02	85.13	90.75

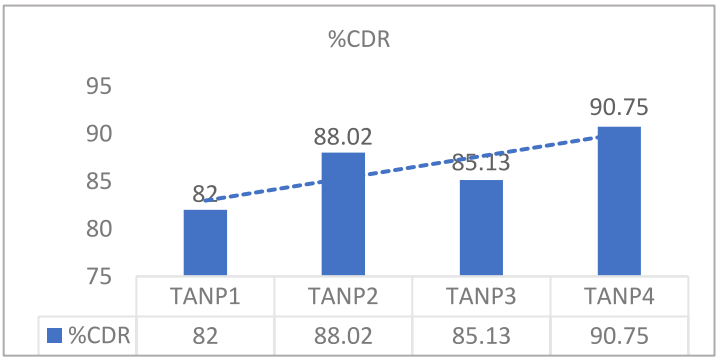


Fig No 11. Graph of % cumulative drug release for formulation TANP1-TANP4

*In vitro*diffusion studies were performed by dialysis membrane equilibrium method in 7.4 pH buffer up to the end of the study and the result has been shown in Table No 10.% cumulative drug release of TANP1, TANP2, TANP3 and TANP4 was found to be in the range of 82% and 90.75% as shown in Fig11.The results showed that increasing the polymer causes slower drug release.

Antioxidant activity
DPPH % Scavenging Activity

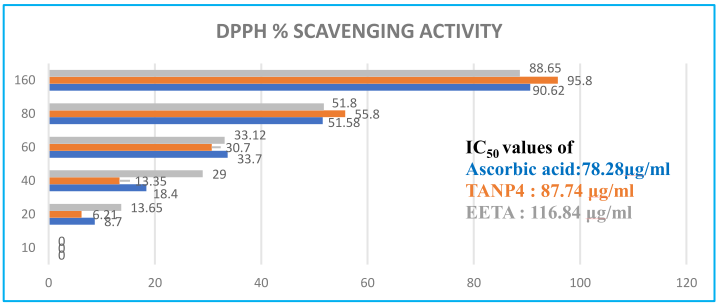
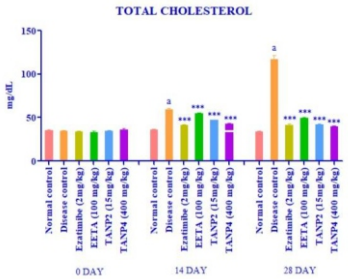


Fig No 12. % Scavenging activity

The DDPH method,a more stable and well-known free radical based on the reduction of absorbing hydrogen or electrons from donors,was used in this work to examine the antioxidant activity of EETA and TANP4.The TANP4sDPPH reducing stability was evaluated by color change, while the control showed no color change.The DPPH solution changed color when TANP4 was added.This is because TANP4 scavenges DPPH by donating a hydrogen atom to stabilize the molecule, which is what causes the absorbance of 517 nm.The functional group attached to the surface of the nanoparticles,which were made from the ethanolic extract of Terminalia arjuna bark, may be responsible for TANP4s antioxidant capacity. The percentage inhibition of DPPH in the ethanolic extract of *Terminalia arjuna*bark and TANP4 are standard are shown in the Fig 12.The IC_{50} values were observed as 116.84 µg/ml of EETA, 87.74µg/ml of TANP4, 78.28 µg/ml of standard ascorbic acid.The result confirmed that the EETA showed 62%, TANP4 showed 72%, and standard showed 85% of antioxidant activity.

In-vivo studies:



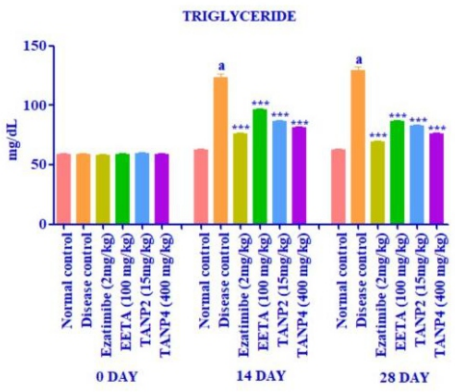


Fig No 13. Serum Cholesterol & triglycerides of rats group at different time intervals

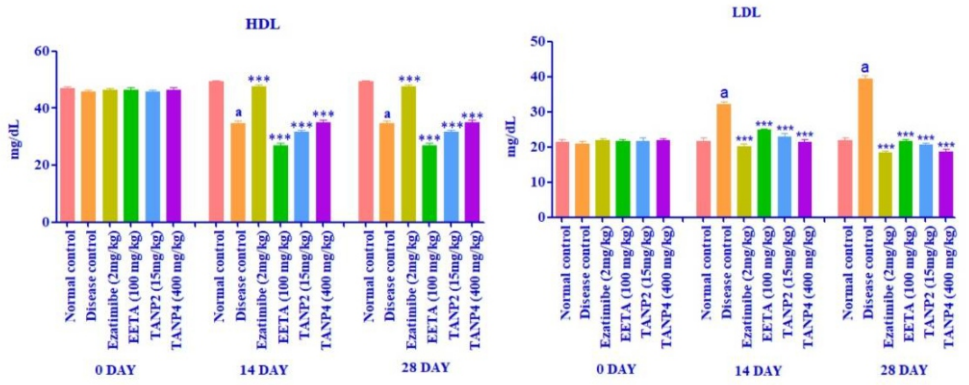


Fig No 14. Serum HDL & LDL of rats group at different time intervals

Table 11: Serum cholesterol, Triglycerides, HDL & LDL of rats group at 28th Day time intervals

Sl. No	Treatment group	Cholesterol	Triglycerides	HDL	LDL
01	Normal control	33.83±1.10	62.69±1.14	49.44±0.17	22±0.73
02	Disease control	117.1±4.30	129.2±3.12	34.83±0.83	39.33±0.98
03	Ezetimibe (2mg/kg)	41.13±1.50	69.47±1.14	47.67±0.61	18.33±0.61
04	EETA (100mg/kg)	49.33±0.84	87±0.85	27±0.8583	21.67±0.61
05	TANP2	42.33±0.61	82.83±0.83	37.67±0.61	60.73±0.47
06	TANP4	39.67±0.61	76.22±0.41	35±0.85	18.67±0.84

The antihyperlipidemic study was executed in Wistar albino rats containing four animals in each group and four groups were selected, detailshave been given in Table no 11 and shown in Fig 13 & 14. Over a 28-day period, the groups were assessed for their total serum triglyceride and cholesterol levels.On0th day the total serum triglycerides, cholesterol, HDL & LDL of the normal group were found to be more than that of the 7th, 14th, and 28th day.Meanwhile, the control and treatment groups had greater triglycerides, cholesterol, HDL, and LDL groups on the 28th day, which was attributable to random sampling of the animals.On the 28th day TANP4 showed a significant decrease in Triglycerides,Cholesterol, HDL and LDL level than standards.

Pharmacokinetic study of polymericnanoparticles

Table 12: Pharmacokinetic study of polymeric nanoparticles

Formulation code	Zero order (R2) value	Higuchi (R2)	Korsmeyer Peppas (R2 value)	Best fit model
TANP1	0.9744	0.9366	0.9743	Both zero order and korsmeyer peppas
TANP2	0.9895	0.8522	0.9939	Korsmeyer peppas
TANP3	0.931	0.8389	0.9902	Korsmeyer peppas
TANP4	0.9968	0.9563	0.9852	Zero order

The best fitting model was confirmed by the kinetic investigation.According to Table No.12.Using KinetdS3 software, the release data was submitted to the Korsmeyer Peppas and Higuchi model. Values for the regression coefficients were acquired.The Korsmeyer-Peppas model was determined to be the best.The optimized equation TANP4 had anR2 value of 0.9902.

Stability studies

Table 13: Stability studies of polymeric nanoparticles at 25oC and -2oC

		ROOM TEMPERATURE 25° C				-2°C			
	SI No	0th Day	30th Day	60th Day	90th Day	0th Day	30th Day	60th Day	90th Day
% of DEE	TANP1	77.7	77.1	76.8	76.02	77.7	77.4	76.2	75.50
	TANP2	73.7	73.03	71.5	70.2	73.7	73.4	72	71.05
	TANP3	65	64.08	63.01	61.09	65	64.07	64.02	63.04
	TANP4	84.1	83.08	82.01	81.09	84.1	83.09	83.02	82.01
% of Cumulative drug release	TANP1	83.05	82.01	80.24	79.9	83.05	83.01	81.37	79.20
	TANP2	79.02	78	77.02	76.09	79.02	78.08	77.2	75.42
	TANP3	91.13	90.21	88.61	87.99	91.13	90.22	87.33	86.23
	TANP4	69.75	67.66	66.32	64.21	69.75	68.3	66.2	65.23

The findings of accelerated stability investigations, which involved storing the nanoparticle suspension or solution at room temperature and -2°C for three months, are shown in Table No. 13. It was discovered that the formulations%DEE ranged from 61.09 to 84.1% at normal temperature and from 63.04 to 84.1% at -2°C. It was discovered that the formulations' *in vitro* drug release ranged from 64.21 to 91.13% at normal temperature and from 65.23 to 91.13% at -2°C.

Conclusion

Terminalia arjuna extract loaded polymeric nanoparticles were successfully prepared by solvent evaporation method using guar gum and gumrosin as polymers.The formulated nanoparticles were characterised for various parameters and fruitfully performed the *in vivo* activity.

From the present work it is concluded that

- FT-IR studies were carried to observe the possible interaction between the selected drug and polymer.The results of the FT-IR analysis show that the drug and polymer did not interact.
- All formulations showed high drug entrapment efficiency.Among the different formulations, TANP2 and TANP4 formulations were showed maximum drug entrapment efficiency.
- Particle size of prepared nanoparticles obtained in the range between 188 nm to 220.4 nm.TANP4 has showed a least particle size.
- Polydispersity index of all the formulations was found to be less than 1.
- Surface morphology of prepared polymeric nanoparticle obtained from SEM has showed a smooth with irregular shape.
- It was determined from TEM study that TANP4 nanoparticles contained crystallineparticles.
- According to the results of an *in vitro* diffusion research, produced nanoparticles are a sustained release drug delivery system.
- The antioxidant study revealed that polymeric nanoparticles of (TANP4) which has good antioxidant property as compared to *Terminalia arjuna* loaded polymeric nanoparticle.
- The release kinetic shows that it follows Korsmeyer Peppas model.
- From the results of*in vivo* release studies of TANPs, significant decrease in Triglycerides,Cholesterol, HDL and LDL levels was observed when compared with the results of normal group and control group, which was carried in animals (Wistaralbino rats).
- Stability experiments revealed that there were not many changes in the parameters during three months.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

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