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FORMULATION AND EVALUATION OF ROTENOIDS (BOERHAVIA DIFFUSA) NANOPARTICLES IN THE TREATMENT OF CHRONIC KIDNEY DISEASE

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ABSTRACT

Chronic Kidney Disease (CKD) is a progressive and irreversible condition that requires long-term therapeutic management to delay renal deterioration and associated complications. Limitations of conventional therapy, including poor patient compliance and systemic side effects, have encouraged the exploration of novel drug delivery systems using herbal therapeutics. Punarnava (Boerhavia diffusa Linn.), a traditionally acclaimed medicinal plant, exhibits significant nephroprotective, diuretic, antioxidant, and anti-inflammatory activities, making it a promising candidate for CKD management. The present study aims to formulate and evaluate Punarnava extract-loaded nanoparticles to enhance bioavailability, improve renal targeting, and achieve sustained drug release. Nanoparticles were designed using a suitable biodegradable polymer to protect active phytoconstituents such as punarnavoside and flavonoids from degradation while enabling controlled release. The formulated nanoparticles were characterized for particle size, surface morphology, entrapment efficiency, and in-vitro drug release behaviour. The nanoparticle-based delivery system is expected to maintain prolonged therapeutic concentrations, reduce dosing frequency, and minimize adverse effects. This approach demonstrates the potential of Punarnava nanoparticles as a novel, effective, and safer strategy for the management of Chronic Kidney Disease.

Keywords: *Boerhavia diffusa, Nanoparticles, Chronic Kidney Disease, Novel, Controlled release.*

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INTRODUCTION

Chronic kidney disease is the progressive destruction of renal tissue due to fibrosis and scarring of functioning nephrons, leading to an irreversible decrease in renal functions [1]. In early stage of chronic kidney failure becoming clinically apparent as renal insufficiency, evidenced by azotaemia and possibly poly urea and nocturia resulting from impaired tubular transport and concentration of urine [2]. Chronic renal failure represents progressive and irreversible destruction of renal structures, leading to a build-up of metabolic products, drugs, and poisons and disorders of water, electrolyte, acid-base balance, and renal endocrine functions [3,4]. Chronic kidney disease is a major global public health problem with ever-growing prevalence, particularly in developing countries. The main causes of CKD include diabetes mellitus, hypertension, glomerulonephritis, chronic pyelonephritis, and prolonged exposure to nephrotoxic drugs. Genetic disorders such as polycystic kidney disease also cause the disease [5].

Punarnava is a well-known medicinal plant that has been used in Ayurveda since ancient times. The name "Punarnava" means "one that rejuvenates or renews the body." It is highly valued for its beneficial effects on the kidneys, liver, and maintenance of fluid balance in the body [6,7]. Due to its wide range of therapeutic applications, Punarnava is commonly included in many Ayurvedic formulations. It is reported to be used as Diuretic, Hepatoprotective agent for liver disorders, Effective in the management of Jaundice, Oedema & swelling cause by kidney or heart problems, Anti-inflammatory activity, an expectorant [8].

Fig 1: Punarnava

MATERIALS AND METHODS

Collection of the plant

The whole plant of *Boerhavia diffusa* (Punarnava) was collected, authenticated, washed to remove soil, and shade dried at room temperature (25–30°C) for 7–10 days to prevent degradation of sensitive phytoconstituents. Shade drying was chosen over oven drying to keep bioactive compounds like flavonoids, alkaloids, glycosides, and phenolic constituents intact.

Extraction of the plant material

The dried plant material was coarsely ground using a mechanical grinder and sifted through 40 mesh to achieve a uniform particle size. This uniformity improves solvent penetration and extraction efficiency. About 25g of the powdered plant material was subjected to soxhlet extraction with 250 mL of 70% acetone, which was made by mixing 175 mL of acetone with 75 mL of distilled water. The hydro alcoholic solvent system was selected for its intermediate polarity, allowing for the extraction of both polar and moderately non-polar phytoconstituents. Soxhlet extraction was performed at 55–60°C for 24-48 hours using a heating mantle equipped with a condenser. The continuous boiling and condensation ensured efficient solvent recycling and thorough extraction of active constituents. The filtrate was concentrated with a water bath set at 40–45°C to prevent degradation of heat-sensitive phytochemicals [9].

Formulation of Punarnava Nanoparticles by Ultra Sonication

Ultrasonication method was used to prepare nanoparticles. The polymer–drug mix was taken in a beaker and set in an ice bath. The ultra sonicator was fixed at about 1–2 cm into the solution and set to run at 40% amplitude. The whole thing took 15 minutes, using a pulse setting: 5 seconds on, 5 seconds off. While the ultrasonicator runs, it sends sound waves into the liquid, making tiny bubbles that burst with a lot of force. When those bubbles collapse, it creates shock waves and strong shear forces. This action breaks up the bigger particles and leaves you with nanoparticles. The pulse mode stops the solution from overheating, keeps the polymer structure safe, and helps break everything down to a more even size. Once sonication finished, the solution turned a bit opalescent [10].

Table 1: Formulation Design: Formulation 1

Ingredients	S1	S2	S3	S4	S5	S6
Punarnava extract	300 mg	300 mg	300 mg	300 mg	300 mg	300 mg
Gelatine	0.3 g	0.4 g	0.5 g	0.6 g	0.7 g	0.8 g
Tween 80 (0.5% v/v)	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Distilled water	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml

Table 2: Formulation Design: Formulation 2

Ingredients	S1	S2	S3	S4	S5	S6
Punarnava extract	300 mg	300 mg	300 mg	300 mg	300 mg	300 mg
Sucrose	0.3 g	0.4 g	0.5 g	0.6 g	0.7 g	0.8 g
Span 80 (0.5% v/v)	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Distilled water	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml

Table 3: Formulation Design: Formulation 3

Ingredients	S1	S2	S3	S4	S5	S6
Punarnava extract	300 mg	300 mg	300 mg	300 mg	300 mg	300 mg
Gelatine	0.3 g	0.4 g	0.5 g	0.6 g	0.7 g	0.8 g
Span 80 (0.5% v/v)	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Ethanol	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Distilled water	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml	q.s 50 ml

Evaluation of Punarnava Nanoparticles

i. Particle Size Analysis

Particle size really matters when it comes to how stable nanoparticles are, how they release drugs, how well the body can absorb them, and how effective they are overall. Here, the size of gelatine–Tween 80 nanoparticles was evaluated using Dynamic Light

Scattering (DLS). To make sure particles didn't clump together or mess up the results, dilute the formulation with distilled water. Then put the diluted sample in a clean cuvette and kept the temperature steady at 25°C during analysis with a Zetasizer. The instrument gave the average particle size in nanometres and produced an intensity distribution curve. Smaller particles actually have a bigger surface area, dissolve faster, and let drugs pass through more easily, which boosts their therapeutic effect [11].

ii. Polydispersity Index (PDI)

The Polydispersity Index, analyses the uniformity of nanoparticles in size. It's a key measure for checking if your formulation will stay stable over time. A low PDI means the particles are mostly the same size, which is what you want.

PDI is calculated using this formula: PDI = $\Delta d/d_{avg}$

Here, Δd is the standard deviation of the particle size, and d_{avg} is the average particle size. If your PDI falls between 0.05 and 0.3, your particles are pretty uniform. Higher numbers mean there's more size variation, which can lead to clumping. Keeping the size distribution narrow helps ensure you get consistent results and predictable drug release each time [11].

iii. Zeta Potential

Zeta potential explains about the surface charge of your nanoparticles. It was evaluated using electrophoretic light scattering. Basically, when an electric field is applied, the charged particles move toward the opposite electrode. The instrument measures how fast they move (electrophoretic mobility) and calculates the zeta potential in millivolts.

When the absolute zeta potential is higher than ±30 mV, particles repel each other pretty well, so they don't clump together—this means good stability. If it's lower, particles tend to aggregate over time. The surface charge also affects how nanoparticles interact with biological membranes and can improve their mucoadhesive properties [12].

iv. Shape and Surface Morphology

Scanning Electron Microscopy (SEM) was used to analyse the morphology and shape of optimized Tween 80/gelatine nanoparticles. The lyophilized nanoparticle sample was mounted onto an aluminium stub and then gold-coated before SEM imaging was performed using appropriate magnification levels for the analysis of the surface morphological properties of the nanoparticles.

Micrographs of the nanoparticles obtained using SEM revealed they were uniformly and predominantly spherical in shape with a smooth surface morphology and very little aggregation; demonstrating the successful coat ability of the polymers, as well as stability of structure (smooth). The size of the particles measured using SEM was closely correlated with sizes measured using Dynamic Light Scattering analysis, thus confirming that stable gelatine-based nanoparticle systems were achieved [12].

v. Percentage Entrapment Efficiency (%EE)

Entrapment efficiency shows how much drug actually gets trapped inside the gelatine nanoparticle. High efficiency means more drug loading, losing less during prep, and getting better sustained release. 1 mL of the nanoparticle mix was taken in a centrifuge at 4000 rpm for 15 minutes, and separated out the supernatant (which holds the free, unentrapped drug). After diluting, measure the amount of free drug with a UV–Visible spectrophotometer at the λmax of Punarnava extract. A higher percentage here means the drug is interacting well with the gelatine matrix.

vi. Drug Content Determination

Drug content analysis was performed to determine the uniform distribution of Punarnava extract within the prepared gelatine-based nanoparticle formulations (S1–S6). The accurately weighed quantity of nanoparticle formulation equivalent to 100 mg was dissolved in suitable solvent, sonicated for complete drug extraction, filtered, and analysed spectrophotometrically at the predetermined λmax [13].

The percentage drug content was calculated using the formula:

Drug Content (%) = Actual drug present/ Theoretical drug amount ×100

vii. *In-Vitro* Drug Release Study

Punarnava extract release pattern from gelatine–Tween 80 nanoparticles over time was evaluated using a USP Type II (Paddle type) dissolution setup. The test ran in 900 mL of phosphate buffer at pH 7.4, kept steady at 37°C. The paddle was set to spin at 50 rpm. 5 mL samples were withdrawn at 1, 2, 4, 6, and 8 hours, swapping each one out for fresh buffer to keep conditions stable. After filtering, the samples were checked with a UV spectrophotometer. Then the amount of drug release over time was calculated and plotted the results [14].

viii. Drug Release Kinetics

The release data of the gelatine nanoparticles was studied. the correlation coefficient (R^2) for each one was analysed, then picked the model with the highest R^2 as the best fit. Most gelatine nanoparticle systems let the drug out mainly by diffusion. In many cases, the release follows the Higuchi or korsmeyer–peppasmodels, because of how the matrix lets the drug move and the polymer relaxes [15].

ix. Stability Studies

The optimized gelatine–Tween 80 nanoparticles stability over time was evaluated. The formulation was stored at 30°C and 65% relative humidity, following ICH guidelines. At regular intervals, particle size, PDI, zeta potential, entrapment efficiency, and how the drug released was checked. No major changes in were observed during storage. Stable nanoparticles means the product lasts longer and delivers the drug consistently when it's needed [12].

RESULTS AND DISCUSSION

The present study successfully developed and evaluated Punarnava (*Boerhavia diffusa*)-loaded nanoparticles, and the obtained results clearly demonstrate the effectiveness of the formulation

strategy in improving drug delivery characteristics for Chronic Kidney Disease (CKD) management.

i. Particle Size Analysis

Particle size analysis revealed nanoscale dimensions (~265.7 nm), which are considered optimal for enhanced cellular uptake and renal targeting.

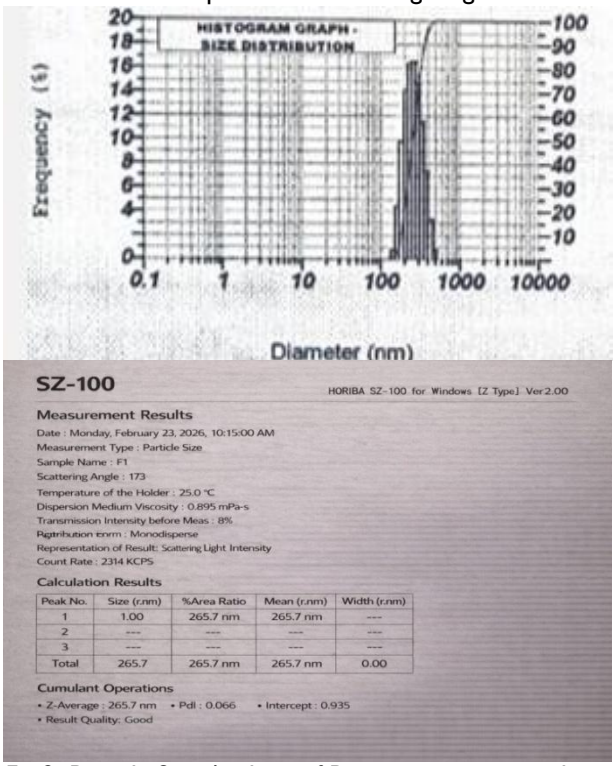

Fig 2: Particle Size Analysis of Punarvana nanoparticles

ii. Polydispersity Index (PDI)

The relatively low Polydispersity Index (PDI) indicated a narrow size distribution, confirming uniformity of the nanoparticle system. Such homogeneity is crucial for predictable drug release and formulation stability.

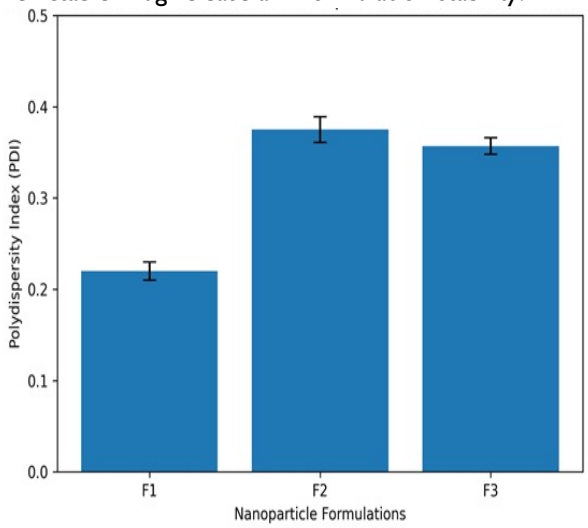

Fig 3: Polydispersity Index (PDI) of Punarvana nanoparticles

iii. Zeta Potential

The zeta potential value (–26.8 mV) suggested moderate electrostatic stability, preventing aggregation and ensuring prolonged shelf life.

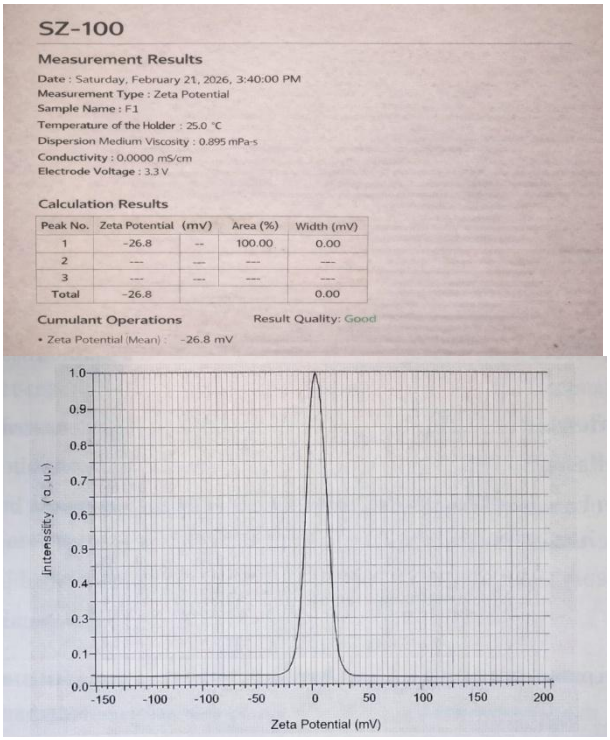

Fig 4: Zeta Potential of Punarvana nanoparticles

iv. Percentage Entrapment Efficiency (%EE)

Entrapment efficiency (%EE) showed a progressive increase from S1 to S6, reaching a maximum of 89.36 ± 1.47%. This trend can be attributed to the increased polymer concentration, which enhances matrix density and promotes better encapsulation of phytoconstituents. Similarly, drug content analysis across all formulations demonstrated high and consistent drug incorporation, particularly in higher polymer concentrations, indicating efficient formulation design and minimal drug loss.

Table 4: Entrapment Efficiency Percentage (%EE) of Punarvana nanoparticles

Formulation code	Drug Content (%)	Entrapment Efficiency (%)
S1	97.85 ± 0.28	55.23 ± 0.31
S2	98.12 ± 0.35	61.00 ± 0.27
S3	97.68 ± 0.22	66.50 ± 0.25
S4	98.45 ± 0.30	71.50 ± 0.29
S5	98.73 ± 0.41	76.50 ± 0.26
S6	98.96 ± 0.33	89.36 ± 1.47

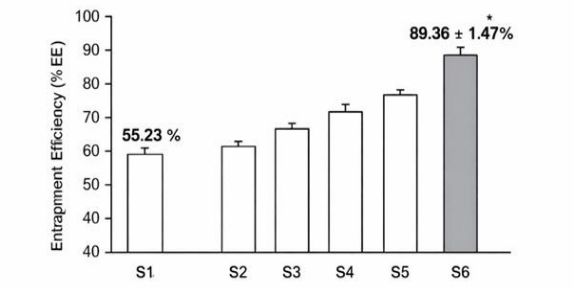

Fig 5: Entrapment Efficiency of Punarvana nanoparticles

v. Shape and Surface Morphology

SEM analysis confirmed spherical morphology with smooth surfaces and minimal aggregation, supporting the successful formation of stable nanoparticles. Morphological uniformity further contributes to controlled drug release behavior.

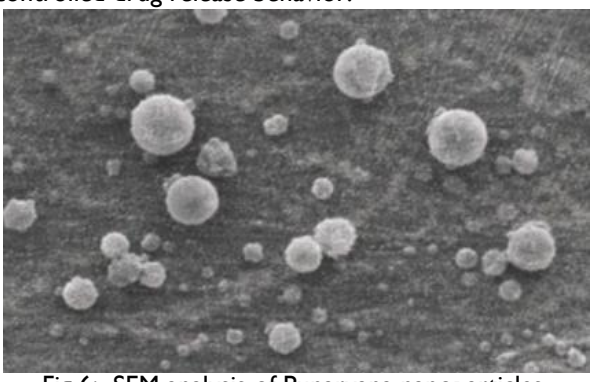

Fig 6: SEM analysis of Punarvana nanoparticles

vi. Drug Content Determination

In-vitro drug release studies exhibited a biphasic pattern with an initial burst release followed by sustained release up to 24 hours. Among all formulations, F3 demonstrated the highest cumulative release (68.5%), suggesting an optimal polymer–surfactant combination. The initial burst effect may be due to surface-associated drug, while the sustained phase reflects diffusion from the polymeric matrix.

Table 5: Drug Content of Punarnava nanoparticle Formulation 1

Formulation 1	Gelatine (g)	Drug Content (%)
S1	0.3 g	80.74
S2	0.4 g	83.65
S3	0.5 g	86.95
S4	0.6 g	90.21
S5	0.7 g	93.78
S6	0.8 g	96.94

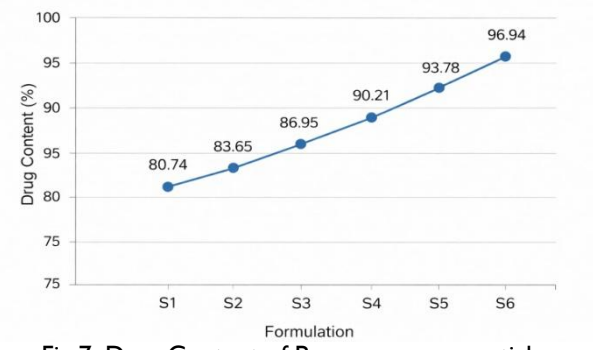

Fig 7: Drug Content of Punarnava nanoparticle (Formulation 1)

Table 6: Drug Content of Punarnava nanoparticle Formulation 2

Formulation 2	Sucrose (g)	Drug Content (%)
S1	0.3 g	81.12
S2	0.4 g	84.08
S3	0.5 g	87.36
S4	0.6 g	90.74
S5	0.7 g	94.02
S6	0.8 g	96.58

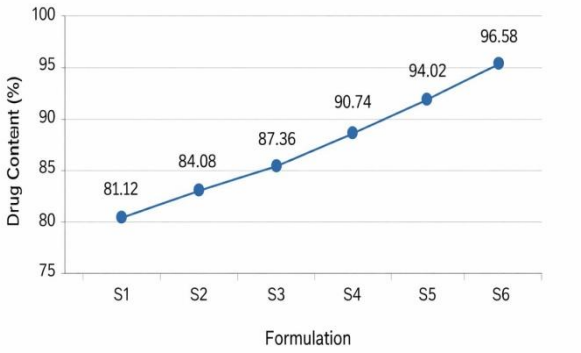

Fig 8: Drug Content of Punarnava nanoparticle (Formulation 2)

Table 7: Drug Content of Punarnava nanoparticle Formulation 3

Formulation 3	Gelatine (g)	Drug Content (%)
S1	0.3 g	80.92
S2	0.4 g	83.74
S3	0.5 g	86.48
S4	0.6 g	89.96
S5	0.7 g	93.21
S6	0.8 g	96.12

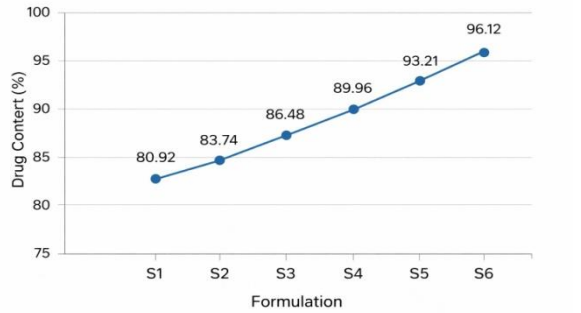

Fig 9: Drug Content of Punarnava nanoparticle (Formulation 3)

vii. *In-Vitro* Drug Release Study

Table 8: *In-vitro* percentage of drug release of Punarnava nanoparticle

Time (Hours)	F1 (%) Drug release (Gelatine+ Tween 80)	F2 (%) Drug release (Sucrose+ Span 80)	F3 (%) Drug release (Gelatine+ Span 80)
0	0.0	0.0	0.0
1	19.3	16.0	18.1
2	29.5	20.0	26.8
4	38.8	29.7	34.8
6	44.7	38.9	44.9
8	50.6	44.0	51.7
12	56.6	49.2	60.1
24	67.4	54.5	68.5

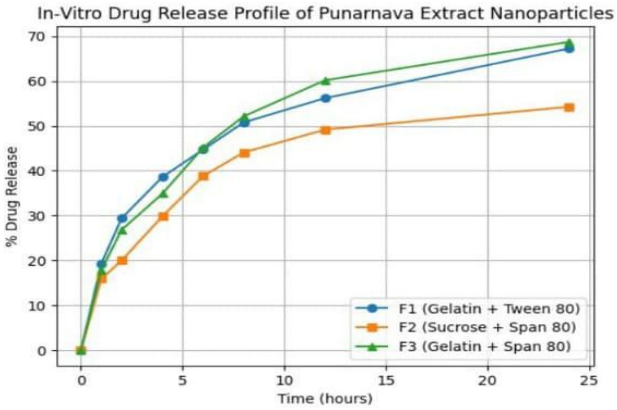

Fig 10: *In-vitro* drug release of Punarnava nanoparticle

viii. Drug Release Kinetics

Kinetic modeling indicated that drug release followed the Korsmeyer–Peppas model with R^2 values close to unity and 'n' values between 0.54–0.65, confirming non-Fickian (anomalous) diffusion. This suggests that drug release is governed by both diffusion and polymer relaxation mechanisms.

Table 8: Kinetic Model Fitting of Punarnava Nanoparticle Formulation

Model	F1	F2	F3
Zero Order (R^2)	0.928	0.910	0.934
First Order (R^2)	0.952	0.938	0.961
Higuchi (R^2)	0.981	0.972	0.986
Korsmeyer-Peppas (R^2)	0.989	0.978	0.993
n Value	0.61	0.54	0.65

The results confirm that the formulated nanoparticles significantly enhance drug encapsulation, stability, and controlled release of Punarnava extract. These findings support the potential of this nanoformulation as an effective and sustained drug delivery system for CKD therapy, improving therapeutic efficacy while minimizing dosing frequency and side effects.

CONCLUSION

The study successfully formulated Punarnava (*Boerhavia diffusa*) extract-loaded gelatine nanoparticles using Tween 80 and Span 80, exhibiting desirable physicochemical properties. The optimized formulation showed a particle size of 265.7 nm, low PDI, and a zeta potential of –26.8 mV, indicating good stability and uniformity. SEM analysis confirmed spherical and smooth nanoparticles, while high entrapment efficiency (89.36%) and improved drug content demonstrated effective drug incorporation. In-vitro release studies showed a biphasic pattern with sustained release up to 24 hours, with F3 showing maximum drug release (68.5%). Drug release followed the Korsmeyer–Peppas model with non-Fickian diffusion. Stability studies confirmed good stability under refrigerated conditions. Overall, the formulation shows strong potential as an effective drug delivery system to treat of Chronic Kidney Disease (CKD) using herbal therapeutics.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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